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AN ENDEAVOR TO OUTSTRIP PATRIARCHY: MANJU KAPOOR'S '*DIFFICULT DAUGHTERS*'

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Abstract
The women characters in Manju Kapur's novels seem to be left without the freedom to act and they remain solely in the field of hallucination, mere fantasy to be dreamt and loved. Manju Kapur in *Difficult Daughters* projects the image of the rebellious, but stoic women ultimately breaking the customary confines in the backdrop of conformist narrative thread. Manju Kapur in her works presents women who try to establish their own self. In *Difficult Daughters*, Virmati, in her pursuit of identity, who is also the focal character of the novel, revolts against convention? The very name of the Manju Kapur is one of the best known celebrated post-independence writers exploring sociological and psychological sensitive issues. Kapur tries to explore the insight or human psyche of her protagonist Virmati, torn between desire for love and duty towards family. Thus, the conflict of internal and external experiences, pressures and expectation produce worries. The novel "*Difficult Daughters*'' is a connotation to a point that a woman, who tries to search for self, is recognized as a difficult daughter in the family and the society. A woman is "new" if her basic concerns are deeper than purely seeking equality with men, asserting her own persona and insisting upon her own rights as a woman. The woman today has her own quest for self-discovery and self-fulfillment. A woman is trying now to be her own gravitational force, beyond the pull of patriarchy. Manju Kapur's novel *Difficult Daughters* is a story of a daughter's journey back into her mother's aching past. It spans the genres of literature and history and falters in both. *Difficult Daughters* is a story of three generations of women: Ida, the narrator, who is a divorcee. Virmati, her mother, who marries an already married professor for love, and Kasturi, her grandmother, who come to terms with a difficult daughter, Virmati. This was not a fictional family, but the story of a real, middle class home with fathers, mothers and brothers and sisters that one had seen and lived with.

Key Words: family, generation, identity, marriage, men, patriarchy, society, values, women

Introduction

Patriarchy is an institutionalized social system in which men dominate over others, but can also refer to dominance over women specifically; it can also extend to a variety of manifestations in which men have social privileges over others to cause exploitation or

oppression, such as through male dominance of moral authority and control of property. - Sylvia Welby

Manju Kapur is an outstanding women novelist in the field of Indian literature. In all her writings she deals with the silent suffering of Indian middle class women either as a victim of tradition or patriarchy or searching for identity. She usually sketches her thoughts and ideas, around women issues and voices out through her female protagonist.

Kapur's perception of women liberation is deeply stretched with social and cultural matters. Her novels play a significant role to insist the self-development of every woman for the furtherence of Indian society. She is the author of six novels. Her first novel *Difficult Daughters* wins the commonwealth prize in the year 1999. Other works of Manju Kapoor are *A Married Women* (2002), *Home* (2006), *The Immigrant (* 2008), *Custody* (2011) and *Brothers (2016). Difficult Daughters* is the story of a woman tattered amid family duty, the aspiration for education, and illegal love. Virmati, a young woman born in Amritsar into a stern and didactic family, falls in love with a neighbour, the Professor--a man who is already married.

That the Professor ultimately marries Virmati, installs her in his home and helps her towards further studies in Lahore. It is a small consolation to her offended family or even to Virmati, who ascertains that the scuffle for her own liberty has created irretrievable lines of partition and pain around her.

Difficult Daughters is about the chronicle of three generations of women and how intergenerational trauma affects them. It also picturises the quest of women for identity in a patriarchal system. The family compromises of Lala Diwan Chand who has two sons Suraj Prakash and Chander Prakash. Suraj is married to Kasturi and Chander Prakash is married to Lajwanti. Essentially the story is of three generations- Kasturi, the mother of Virmati, Virmati (the main protagonist), and Ida, the daughter of Virmati. Ida who belongs to the third generation is the protagonist of the book. Virmati is the 'difficult daughter' in the prosperous merchant family of Lala Diwan Chand. Ruby Mihoutra comments in her article "Existential Images of Women in Manju Kapur's Difficult Daughters" that 'While in the generation of Kasturi, woman's role was confined to childbearing and kitchen work, the generation of Virmati …..breaks away from the tradition bound limits of Indian women' (Milhoutra. P.164. 2005). Fortunately she is saved and Indumati is married off to Inderjeet in the name of the family name and honour. Virmati then is sent to Lahore for further studies. As Jaideep Rishi points out in his essay: "Mother–Daughter Relationship in Manju Kapur's Difficult Daughters: "Kasturi unknowingly becomes the voice of patriarchy. She holds those values as ideals which patriarchy has taught her to be so and when her daughter rebels against such values she takes it to be a rebellion against her own self." She deems the patriarchal postulations about the superiority of male in the family as well social system. Kasturi's believes that marriage is her destiny. After her graduation, her education continued at home.

Lalaji, the grandfather of Virmati the great supporter of patriarchal system, has a jewelry shop and a mill and he hoped that his sons would continue his work. "Lala Diwan Chand was vehemently opposed to any kind of division in the family…….his property that he refused to divide. He had worked all his life to make it grow, and he was not about to halve and quarter it now" (DD,25). Even after the division of the family into two units Lalaji had given clear instructions that his sister would be looked after 'with the dignity and respect that was her due' (DD, 28). His sister was clearly thankful for it was her brother who had given

her a home after she was widowed at the age of fourteen. Indeed the joint family structure can be a blessing for the old who are looked after and get companionship, the children who have the support and help of both the elders and the youngsters and the ill and sick are looked after. Following the customs he postpones Virmati's marriage to Inderjeet due to sudden deaths in the family. Rather than sit at home, she joins college. There she is inextricably drawn to the Professor who woes her on the pretext that his wife is not his companion and he yearns for an invigorating scholarly partner. Virmati is caught between familial and romantic love. Her family is opposes this match because Harish is already married.

Virmati takes the step to commit suicide to avoid marriage with Inderjeet. Kasturi considers this as an insult to the reputation of the family. She cannot understand why her daughter does not want to get married and have a family. She believes "A woman's shaan is in her home." In this context Dr. Ruby Milhoutra opines: "Her mother tried to ensure her future happiness by the impeccable nature of her daughter's qualifications.

She was going to please her in- laws… "(DD,57). Virmati revolts against deep rooted family traditions and marries the professor. She prepares herself to stay with the professor's family, which compromises of his first wife, mother-in-law, sister-in-law and children. Although Virmati succeeds in marrying the Professor, it proves to be a disaster. She has to live as a second wife and under the hostile gaze of Ganga, her husband's first wife without identity. Only her mother-in-law accepts her to some extent and that too at the behest of her son. During her conjugal life Virmati feels that it would have been better if she had not married Harish. "I should never have married you" (DD, 212).

After some time she suffers a miscarriage. Sometimes Virmati blames herself to be responsible for the destruction of Ganga's life. Dr. Arpita Ghosh comments: An "intensive education" fails to teach Virmati that resistance to patriarchy to forge an identity and ensure independence is not equivalent to trespassing into another woman's domain. Marrying a man of her choice was not an issue for her family but marrying a family-man who was already married with children was objectionable. She failed to realize the gravity of the situation.

Virmati blooms into a type of 'new woman' as Irish writer Sarah Grand (1854–1943) used the term that refers to independent women seeking radical change. She displays her strength of mind in overcoming her dejection. She is "strong to bear the pain, silently, without anyone knowing" (DD, 91). She is still stressed to institute her Self and to gain belligerence. She embodies the modern woman has frayed between craving for Self and her vulnerability. After her first sex encounter with Harish, she tries to justify by saying that there was no point in foolishly denying it on the basis of an "outmoded morality" (DD, 114). The novel also explores the problems of women in a male dominated society. Born out of typical Indian family, Virmati is caught between tradition and modernity. It results only in self-alienation and she becomes a symbol of female imagination. Responding to the pressures and the family structure at the Professor's house, she struggles to get the Professor's love and attention. Though she considers herself as a new woman She is amazed at her former roommate Swarna Lata's efforts to participate and help to bring about alteration in her life and be a part of the change in patriarchal system. With Swarna Lata's help she underwent abortion in the hands of a proper doctor at Mohini Dutta's guest room before marriage. Swarna asks Virmati to come and demonstrate against the Hindu code bill "Men don't want family wealth to be divided among women. Say their sisters get dowry, that's their share, and the family structure will be threatened, because sisters and wives will be seen as rivals,

instead of dependents who have to be nurtured and protected. As a result women will lose their moral position in society! Imagine!"(DD, 232).

Ida, Virmati's daughter and the narrator, understands their family structure. She thinks that 'when I grow up I should be very careful to tailor my needs to what I knew I could get. That is my female inheritance. That is what she tried to give me. Adjust, compromise, adapt, assertion, though difficult to establish, is easy to remember' (DD, 236). Manju Kapoor remarks: "conflict between mother and daughter is inevitable and I suppose I was a difficult daughter. The conflict carries on through generation because mothers want their daughters to be safe. We want them to make the right choices- right in the sense that they are socially acceptable. My mother wanted me to be happily married; I want my daughters to have good jobs."

Ida becomes the typical daughter of a 'difficult daughter' Virmati. She could not grow an understanding with her mother during her lifetime and after Virmati's death this realization engulfs her with guilt. Ida sets on a journey into her mother's past in search of a woman she could know and understand She rebels against Virmati, rejects her own womanhood and follows her own whims, even though she experiences a strong bond with her mother, "without her I am lost, I look for ways to connect" (DD 3). The story of Virmati is basically a story of Manju Kapur's own mother. She acclaimed that the heroine of her writing is her mother. In an interview with Jo Stimpson she asserts: that 'I based my first novel on her. I admire her fighting spirit, her generosity, her capacity to endure. She irritated me when she was alive, but now I see these things more clearly. I think of her every day'.

The act of setting out a quest and then to write out the mother's life keeps Ida connected with her mother. It would be appropriate to say that Ida longs for her mother even after her death. Thus through the voice of Ida the writer narrates the gripping tale of three women, Virmati, Shakuntala and Swarna, set in the pre-independence era who choose to not conform to society's standards, and to rise above expected domestic ambitions; they "thought to be something other than a wife."

Kritika Agarwal comments in the book review: "They think for themselves, prioritise their life before others, go for higher studies, participate in the Satyagraha movement, and choose not to ever get married. From the Hindu code bill, dowry, to abortion, and property rights, Virmati, Shakuntala and Swarna lay the foundation of the rights that women today enjoy. Set around the time of partition, Kapur traces the life of her mother in undivided Punjab. This novel mirrors the society's obsession with its conservative ideas of women, superstitions, male child, and the family's sacred duty of marriage."

Manju kapoor brilliantly portrays the desire, struggle, and effort to emerge out of patriarchal system of the society by Virmati. The novel sings the struggles of women who despite facing their personal battles, significantly contribute to India's independence; but is equally relatable today's society and the woes that still fight to outgrow.

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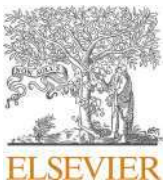

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Magnetic studies of Mn^{2+}substituted Zn-ferrite nanoparticles: Role of secondary phases, bond angles and magnetocrystalline anisotropy

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ABSTRACT

Magnetic studies of $Mn_xZn_{1-x}Fe_2O_4$ ($x = 0.5, 0.6, 0.7$) nanoferrite particles prepared by sol-gel auto combustion method are presented. The deviation of the oxygen positional parameter (U) from the ideal value of 0.375 is an indicator for the cation redistribution in the present ferrite systems. The variation of bond angles estimated from the cation distribution seemed to be strengthening of A–B superexhange interaction. The nature of M − H loops revealed that the present ferrite nanoparticles are in the single domain state showing superparamagnetism. The saturation magnetization (M_s) increases while coercivity (H_c) decreases with the substitution of Mn^{2+} ion concentration. The highest value of $M_s = 25$ emu/g was reported for the composition $x = 0.7$. It was observed that the blocking temperature (T_B) is decreasing and again increasing with the doping level of Mn^{2+}. From FC-ZFC curves, the uneven variation of magnetization (M) at 10 K can be found. It attributes to the cation distribution in the ferrite samples at 10 K is different from those of at 300 K. The Mössbauer spectra revealed that the ferrite nanoparticles exhibited both quadruple and hyperfine interactions can be found in the composition $x = 0.7$ while the ferrite nanoparticles exhibited that only quadruple interaction can be in the compositions $x = 0.5$ and 0.6. The isomer shift (δ) values showed the presence of high spin Fe^{3+} ions only. The paramagnetic doublets having higher value of quadruple splitting (Δ) are assigned to the core structure while doublets lower value of quadruple splitting (Δ) is assigned to shell structure. The present results are incorporated in terms of cation distribution, magnetocrystalline anisotropy considering the core-shell morphology of ferrite nanoparticles.

1. Introduction

The soft magnetic nature of spinel ferrites under nanoscale dimensions has been touted for various scientific and engineering applications [1–4]. Among different spinel ferrites, Mn–Zn ferrite attracted much attention due to excellent magnetic properties as well as mechanical and chemical stabilities. However, the chemical stability of Mn–Zn ferrite is questionable due to easier transformation of Mn^{2+} at octahedral site (B) into Mn^{3+}, resulting to formation of the secondary phases. From the literature reports [5,6], it can be observed that the inert gas atmosphere was provided during sintering process in order to avoid the formation of these secondary phases. It was well established that the preparation methods, sintering temperature, concentration of dopants can affect the solid state properties of spinel ferrites [7–10].

Spinel ferrites can exhibit magnetic and electric properties together and therefore these are suitable candidates for microwave frequency applications [11,12]. Apart from the engineering applications, the superparamagnetic ferrite nanoparticles are adequately suitable for

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bio-medical applications [13]. The magnetization of the spinel ferrites is the resultant magnetic moments of octahedral (*B*) and tetrahedral (*A*) interstitial sites. It is popularly known as *A*–*B* superexchange interaction [14]. Generally, Neels' sub-lattice model was adopted to evaluate the magnetic moment of spinel ferrite [14]. In ultrafine ferrite nanoparticles, the magnetic anisotropic energy (*KV*, where *K* = magnetic anisotropy constant, *V* = volume of the nanoparticle) no longer keep the collinear arrangement of magnetic spins at *B*– site, but canted at some angles impeccable to spin glass behaviour. In such a case Neels' sub-lattice model is not so adequate. Different researchers adopted various models to probe the magnetic behaviour of ultrafine ferrite nanoparticles.

Sun et al. [15] synthesized the Mn–Zn ferrite via sol-gel method and studied the magnetic behaviour of ferrite samples with concentration of Zn^{2+} and sintering temperature. They observed an increase in M_s with the increase of Zn^{2+} ion concentration up to $x = 0.4$ and after there is steady decrease in M_s. They explained this behaviour based on cation distribution following the Neels' sub-lattice model. They reported a value of saturation magnetization $M_s = 77.3$ emu/g of $Mn_{0.6}Zn_{0.4}Fe_2O_4$ sintered at 1100 °C.

Amirabadizadesh et al. [16] explained the magnetic behaviour of co-precipitated Mn–Zn ferrite nanoparticles based on the possibility of hard rotation of spin due to induced anisotropy.

Kareem et al. [17] studied the effect of precipitation temperature on PEG decorated $Mn_{0.8}Zn_{0.2}Fe_2O_4$ nanoparticles. They observed a steady increment in M_s as the precipitation temperature is increasing from 25 °C to 75 °C and after a slight decrement in M_s for a precipitation temperature 100 °C. They reported that the increase in M_s is due to increase in crystallite and particle size as observed from XRD and FE-SEM studies. They also reported that the decrease in M_s can be expected due to saturation minimizing the surface spin effect.

Maleknejad et al. [18] reported that the effect of the amount of fuel on the values of M_s and permeability (μ) of Mn–Zn ferrite synthesized by the microwave–induced combustion method. The value of M_s is gradually increasing with the crystallite size, whereas its coercivity is decreasing with the crystallite size. They explained this typical behaviour of magnetic properties based on Herzer's random anisotropy model.

In our research laboratory we prepared a series of $Mn_xZn_{1-x}Fe_2O_4$ (x = 0.5, 0.6, 0.7) ferrite nanoparticles using sol-gel auto combustion and co-precipitation methods. We observed that the secondary phases were found in the samples prepared by sol-gel auto combustion method while they are absent in the samples prepared by co-precipitation method. A part of structural, elastic, vibrational and electron spin resonance (ESR) studies of Mn–Zn ferrites prepared by sol-gel auto combustion method were reported [19]. It was observed that the presence of secondary phases in these samples showed the effect on their structural and microscopic magnetic behaviour. Cation redistribution is also stood as one of the factors for their structural and elastic properties. Generally, doping of metal ions in the spinel structure leads to the expansion of *A* and *B* sites. Oxygen positional parameter (*U*) encounters the measure of their expansion difference. When the metal ions occupied their preferential sites, the ideal value of *U* is 3/8 = 0.375. In the case where the measured value of *U* is greater than 0.375, then it can be expected due to cation redistribution against to their normal occupancy. It is well known that study of magnetic behaviour of spinel ferrites to understand their electromagnetic property is very important for a particular application [20,21]. Herein, we reported the magnetic studies of Mn^{2+} substituted Zn-ferrite nanoparticles. The results are obtained from vibrating sample magnetometer (VSM), superconducting quantum interference device (SQUID) and Mössbauer spectroscopy. The results are incorporated in terms of cation redistribution and magnetocrystalline anisotropy presuming the core-shell morphology of ferrite particles.

2. Synthesis and characterization techniques

2.1. 1 synthesis

Analytical grade manganese sulfate ($MnSO_4{\cdot}7H_2O$, 99% purity, HIMEDIA), zinc sulfate ($ZnSO_4{\cdot}7H_2O$, 99% purity, HIMEDIA) and ferric nitrate ($Fe(NO_3)_3{\cdot}9H_2O$, 98% purity, HIMEDIA) are used as precursors. Citric acid ($C_6H_8O_7H_2O$, 99% purity, HIMEDIA) is used fuel for auto combustion at a molar ratio of metal ions to citric acid as 1:1. The detailed description about the synthesis process can found from our earlier publication [9,19].

2.2. Characterization techniques

Room temperature magnetization and field cooling measurements have been done using SQUID. Mössbauer spectra were recorded at room temperature (27 °C) using a Mössbauer spectrometer operated in constant acceleration mode (triangular wave) in transmission geometry employing a Co-57 in Rh matrix of strength 50 mCi. The calibration of the velocity scale is done by using α-Fe metal foil. The outer line width of calibration spectra is 0.30 mm/s. Mössbauer spectra were fitted by WINNORMOS site fit program assuming Lorentzian line shapes. The isomer shifts were measured relative to α -Fe metal foil.

3. Results

3.1. Inter-ionic bond lengths and bond angles

Generally, the complete description about the structural and magnetic studies have been given through the calculations of metal ion-metal ion (Me–′Me′′) and metal ion-oxygen ion (Me–O) distances as well as bond angles. In order to estimate these parameters, the cation distributions have been made following the Bertaut approach [22] as well as the procedure reported by Priyadarshini et al. [23]. The estimated cation distributions are used to calculate the tetrahedral and octahedral bond lengths (R_A and R_B) as well as theoretical lattice parameter (a_{th}) employing the following relations.

Tetrahedral bond length $R_A = r_A + r\ (O^{2-})$ and octahedral bond length $R_B = r_B + r\ (O^{2-})$.Where $r\ (O^{2-}) = 1.32$ Å is the radius oxygen anion. r_A and r_B = radii of tetrahedral and octahedral sites. These are estimated using the following relations.

$$r_A = C_{Zn}(A)\cdot r\left(Zn^{2+}\right) + C_{Fe}(A)\cdot r\left(Fe^{3+}\right) + C_{Mn}(A)\cdot r\left(Mn^{2+}\right) \quad (1)$$

$$r_B = \frac{1}{2}\cdot\left[C_{Zn}(B)\cdot r\left(Zn^{2+}\right) + C_{Fe}(B)\cdot r\left(Fe^{3+}\right) + C_{Mn}(B)\cdot r\left(Mn^{2+}\right)\right] \quad (2)$$

where C_M = concentration and r(M) = radius of the metal ion at *A* or *B* site. The values used in the present calculation are $r\ (Zn^{2+}) = 0.74$ Å, $r\ (Fe^{3+}) = 0.67$ and $r\ (Mn^{2+}) = 0.82$ Å respectively [24].

$$a_{th} = \frac{8}{3\sqrt{3}}\left[R_A + \sqrt{3}\,R_B\right] \quad (3)$$

The values of R_A is used to find out the oxygen positional parameter (*U*) using the following relation [24].

$$U = \left[\frac{R_A}{\sqrt{3}a} + \frac{1}{4}\right] \quad (4)$$

The bond lengths and bond angles have been calculated following the procedures described in the literature [25,26].

3.2. Me′ – Me′′ distances

$$p = a\left[\frac{5}{8} - U\right], \quad (5a)$$

$$q = \sqrt{3}a\left[U - \frac{1}{4}\right], \tag{5b}$$

$$r = \sqrt{11}a\left[U - \frac{1}{4}\right], \tag{5c}$$

$$S = \sqrt{3}a\left[\frac{1}{3U} + \frac{1}{8}\right], \tag{5d}$$

Me – O distances:

$$b = \sqrt{2}\left(\frac{a}{4}\right), \tag{6a}$$

$$c = \sqrt{11}\left(\frac{a}{8}\right), \tag{6b}$$

$$d = \sqrt{3}\left(\frac{a}{4}\right), \tag{6c}$$

$$e = \sqrt{3}\left(\frac{3a}{8}\right), \tag{6d}$$

$$f = \sqrt{6}\left(\frac{a}{4}\right), \tag{6f}$$

3.3. Bond angles

$$\theta_1 = \cos^{-1}\left[\frac{p^2 + q^2 - c^2}{2pq}\right], \tag{7a}$$

$$\theta_2 = \cos^{-1}\left[\frac{p^2 + r^2 - e^2}{2\,pr}\right], \tag{7b}$$

$$\theta_3 = \cos^{-1}\left[\frac{2p^2 - b^2}{2p^2}\right], \tag{7c}$$

$$\theta_4 = \cos^{-1}\left[\frac{p^2 + s^2 - f^2}{2ps}\right], \tag{7d}$$

$$\theta_5 = \cos^{-1}\left[\frac{r^2 + s^2 - f^2}{2rs}\right] \tag{7f}$$

3.4. Magnetic studies

The saturation magnetization (M_s) was evaluated from the extrapolation of $1/H$ verses M plots. The experimental magnetic moment (n_e) was calculated using the given relation [27].

$$n_e = \frac{M \cdot M_s}{N_A \cdot \mu_B} \tag{8}$$

where M = molar mass of ferrite sample, M_s = saturated magnetization, N_A = Avogadro's number and μ_B = Bohr magneton.

In order to understand the magnetic behaviour in a comprehensive way, the experimental magnetic moment is used to calculate canting angles in the following way.

$$n_e = n_B \cdot \cos\alpha_{Y-K} - n_A \tag{9}$$

where n_B and n_A magnetic moments at B– and A– sites.

3.5. Temperature dependence magnetization studies

The dependence of magnetic field on the magnetic behaviour with respect to temperature (M-T) is useful to understand the role of magnetocrystalline anisotropy. In the present study, M-T graphs are recorded in the applied field of 100 Oe. In zero field cooling (ZFC), the magnetization becomes maximum at a particular temperature where the magnetic moments don't relax and completely blocked is known as blocking temperature (T_B). At blocking temperature (T_B), magnetocrystalline energy (KV) and thermal agitation energy (k_BT_B) are balanced together and is given by [28].

$$K \cdot V_p = 25k_B \cdot T_B \tag{10}$$

where K = magnetic anisotropy constant, V_P = volume of the nanoparticle exhibiting superparamagnetism, k_B = Boltzmann's constant and T_B = blocking temperature.

In present study, the values V_p are calculated using the average particle sizes estimated from FE-SEM micrographs. The values of V_P are used in the above relation to estimate K.

3.6. Mössbauer studies

The Mössbauer spectra have been recorded at room temperature for $Mn_xZn_{1-x}Fe_2O_4$ (x = 0.5, 0.6, 0.7). The values of isomer shift are determined relative to α-Fe metal foil. The spectra were analyzed by fitting with a WINNORMOS site fit program to evaluate the Mössbauer parameters. Generally, magnetocrystalline anisotropy will determine the magnetic behaviour of a ferrite sample. The magnetocrystalline energy (E_A) for a system of nanoparticles is given by [29].

$$E_A = (k_B \cdot T_B) \cdot \log\frac{\tau}{\tau_o} \tag{11}$$

where τ = superparamagnetic relaxation time, τ_o = relaxation time constant, k_B = Boltzmann's constant and T_B = blocking temperature.

The dependence of magnetocystalline energy (KV) on the thermal energy (k_BT) of a ferrite system will influence its magnetic behaviour. For an assembly of superparamagnetic nanoparticles, above blocking temperature (T_B) thermal energy overcomes the magnetocrystalline anisotropy and then during the measurement the relaxation time (τ) is greater than the characteristic time (τ_o). Therefore, during the measurement the hyperfine interaction averages to zero and the sextet collapses into a quadruple doublet. Below the blocking temperature (T_B), the magnetocrystalline anisotropy overcomes the thermal energy and the system is spontaneously magnetized along the easy axis of magnetization and then during the measurement the relaxation time (τ) is less than the characteristic time (τ_o). Therefore, the hyperfine interaction at the Mössbauer nucleus is not zero and depending upon the strength of the hyperfine interaction the relative (either weakly resolved or fully resolved) sextet can be observed in the Mössbauer spectrum.

4. Discussion

4.1. Inter-ionic bond lengths and bond angles

The X-ray diffractogram of $Mn_{0.5}Zn_{0.5}Fe_2O_4$ prepared by sol-gel auto combustion method is once again displayed in Fig. 1 for our convenience. The detailed description about structural parameters like

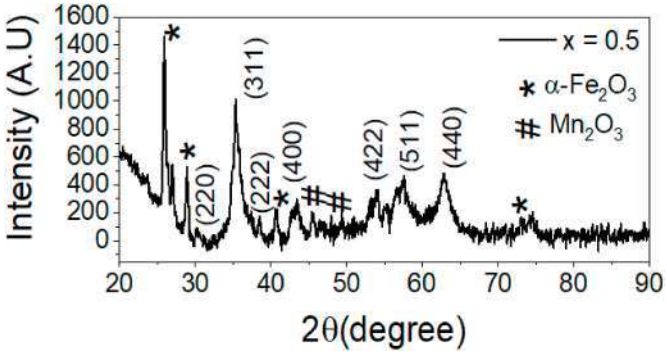

Fig. 1. XRD patterns of $Mn_{0.5}Zn_{0.5}Fe_2O_4$ nanoferrite system [19].

experimental lattice parameter (a), X-ray density (ρ_x), crystallite size (D) and lattice strain (η) can be found in our previous publication [19]. The cation distributions and bond angles are summarized in Table 1. The theoretical lattice parameter calculated using estimated cation distributions is denoted as a'. The theoretical lattice parameter (a'), oxygen positional parameter (U) and bond lengths (R_A & R_B) are listed in Table 2 along with the reported values of experimental lattice parameter (a) and impurity to ferrite phase ratios. The values of experimental lattice parameter (a) are cope up with the theoretical lattice parameter (a') which are calculated using the cation distributions estimated from X-ray peak intensities. It indicates that the present cation distributions in the ferrite systems are reliable. It can be observed that the values of R_A is increasing and again decreasing while R_B is monotonically increasing with the substitution of Mn^{2+}. The value of U is similar to the varying trend of R_A. The tetrahedral and octahedral site sizes increase in the ratio of 0.375 with the incorporation of metal ions into their usual sites in the spinel structure. This value is normally referred as ideal oxygen positional parameter. Any deviation from this ideal value is attributed to the cation redistribution in the spinel structure. The bond angles θ_1, θ_2 and θ_5 seem to be increasing whereas θ_3 and θ_4 seems to be decreasing with the increase in Mn^{2+} ion concentration. Moreover, for the composition $x = 0.6$, the values of θ_1, θ_2 and θ_5 are low while θ_3 and θ_4 are high when compared to the compositions $x = 0.5$ and 0.7. From this analysis, we can expect A–B superexchage interaction is weak and B–B superexchange interaction is strong in the composition $x = 0.6$. These changes in the bond angles are providing useful informations to understand the magnetic behaviour of present ferrite systems under investigation.

4.2. Magnetic studies

The hysteresis loops recorded for $Mn_xZn_{1-x}Fe_2O_4$ ($x = 0.5$, 0.6, 0.7) nanoferrite systems are presented in Fig. 2. The experimental values of saturation magnetization (M_s), coercive field (H_c), remnant magnetization (M_r) and magnetic moment (n_e) are tabulated in Table 3. The variation of M_s and H_c against to the Mn^{2+} ion substitution is shown in Fig. 3. It can be observed that the value of M_s is increasing with the substitution of Mn^{2+}. Shobana et al. [30] observed a decrease in M_s with increase in Mn^{2+} ion concentration and reported this is due to decrease in crystallite size. In the present study, the variations of crystallite sizes (from XRD) and particle sizes (from FE- SEM) (see Table 3) are not supporting the increasing trend of M_s. As reported in our previous publication [19], it can be observed the formation of ferrite is increasing and secondary phases are appeared to be decreasing with the substitution of Mn^{2+} (see Table 2). The decrease of secondary phase is ascribed to the thermodynamical stability of Mn^{2+} in the spinel structure. Therefore, the dilution of secondary phase with the substitution of Mn^{2+} in turn increase the M_s. In order to understand the magnetic behaviour of present ferrite systems more precisely, consider the bond angles given in Table .1. It was observed that the bond angles θ_1, θ_2 and θ_5 seem to be increasing whereas θ_3 and θ_4 are decreasing. It is well known that the increase of θ_1, θ_2 and θ_5 is related to the increase of A-B superexchange interaction and decrease of θ_3 and θ_4 is related to the decrease of B–B superexchange interaction [31]. Basing on this consideration we can support the increasing trend of M_s. However, to provide comprehensive analysis on the magnetic behaviour of present ferrite systems, the magnetic moment of ferrite systems are calculated from the cation distributions (from Table 1) and are listed in Table 3 as n_t. It can be observed that n_t is increasing and again decreasing with the substitution

Table 2
Theoretical lattice parameter (a'), tetrahedral & octahedral bond lengths (R_A & R_B), oxygen positional parameter (U) and reported values experimental lattice parameter as well as ratios of impurity phase.

Comp. (x)	*a (Å)	a' (Å)	R_A (Å)	R_B (Å)	U	*$\frac{I_{\alpha-Fe_2O_3}}{I_{Ferrite}}$	*$\frac{I_{Mn_2O_3}}{I_{Ferrite}}$
0.5	8.355	8.356	1.974	1.994	0.386	1.46	0.19
0.6	8.373	8.375	1.983	1.996	0.387	1.26	0.27
0.7	8.356	8.357	1.956	2.001	0.385	0.86	0.17

* The values of experimental lattice parameter and ratios of impurity phases are taken from our previous reports for the purpose of comparison and interpretation, Ref [19].

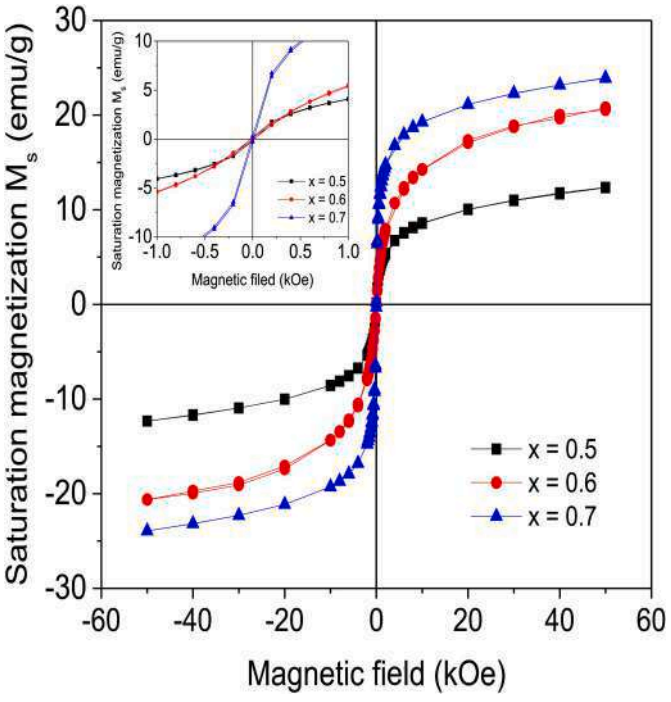

Fig. 2. Hysteresis loops of $Mn_xZn_{1-x}Fe_2O_4$($x = 0.5$, 0.6, 0.7) nanoferrites.

Table 3
Saturation magnetization (M_s), remnant magnetization (M_r), coercivity (H_c) and magnetic moments (n_e andn_t).

Comp (x)	M_s (emu/g) ±1	M_r (emu/g) ±0.01	H_c (Oe) ±1	n_e	n_t	*$<D_{XRD}>$ (nm) ±0.1	*$<D_{FE\text{-}SEM}>$ (nm) ±0.1
0.5	12	0.17	20	0.52	5.05	5.4	8.9
0.6	21	0.09	13	0.93	5.70	4.5	7.8
0.7	25	0.28	9	1.04	4.78	5.5	6.4

* The average crystallite size $< D_{XRD} >$ and particle size $< D_{FE\text{-}SEM} >$ are taken from our previous reports for the purpose of comparison and interpretation, Ref [19,33].

Table 1
Cation distribution and bond angles of $Mn_xZn_{1-x}Fe_2O_4$ ($x = 0.5$, 0.6, 0.7) nanoferrites.

Comp. (x)	Cation distribution	θ_1	θ_2	θ_3	θ_4	θ_5
0.5	$(Mn_{0.305}Zn_{0.255}Fe_{0.44})$ $[Mn_{0.195}Zn_{0.245}Fe_{1.56}]O_4$	121.54	137.69	95.14	126.18	70.14
0.6	$(Mn_{0.315}Zn_{0.275}Fe_{0.41})$ $[Mn_{0.275}Zn_{0.125}Fe_{1.59}]O_4$	121.19	137.25	95.61	126.21	69.69
0.7	$(Mn_{0.330}Zn_{0.148}Fe_{0.522})$ $[Mn_{0.370}Zn_{0.152}Fe_{1.478}]O_4$	121.89	137.94	94.67	126.09	70.59

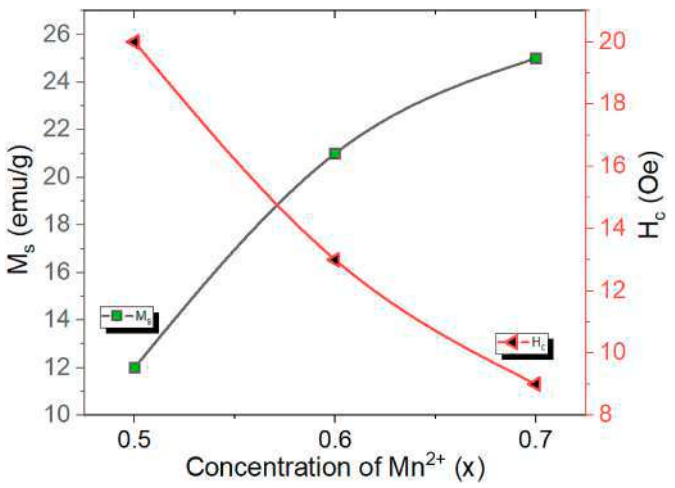

Fig. 3. Variation of M_s and H_c against to the Mn^{2+} ion concentration.

of Mn^{2+} ions. But the varying trend of M_s and n_e in the present studies is a contrast to the varying trend of n_t. In the present study, the estimated cation distributions are successfully supporting the structural studies. However, these cation distributions are not supporting the magnetic behaviour. Therefore, the increase of M_s can be speculated due to the increase of the magnetocrystalline anisotropy (K). Devi and Soibam [32] reported a value of M_s (50.025 emu/g) and is higher than the present value of M_s (24.85 emu/g). Their reported values are higher than our present values of M_s. This can be explained basing on core-shell morphology of nanoparticles. The unsaturated magnetization even at 60 kOe represents that the ferrite nanoparticles are single domain state exhibiting superparamagnetic behaviour. Moreover the low values of H_c and M_r are also representing that the ferrite nanoparticles are in superparamagnetic state. As the size of the particle is reduced to nano dimensions, then the magnetic spins that are residing on the surface of nanoparticle increases. The coordination between the spins on the surface of nanoparticle is uneven. This region is known as a shell. The magnetic spins inside the particle is ferromagnetic ordering is known as the core. When magnetic field is applied the magnetic spins in the core are aligned in the direction of field gives rise to magnetization. But the spins on the shell does not align in the field direction even at higher values. This leads to decrease in saturation magnetization to give low values of M_s than their counter bulk parts. It can be observed that H_c is decreasing with the substitution of Mn^{2+} ions. From the values of particle sizes, we can expect that the ferrite nanoparticles are in single domain state. For the nanoparticles in single domain state, the H_c decreases as the size of the nanoparticle decreases following the given relation [14].

$$H_c = g - \frac{h}{D^{3/2}} \tag{12}$$

where g and h = constants, D = particle size.

In the view of average particle sizes ($<D_{FE\text{-}SEM}>$), the variation of particle sizes (see Table 3) is not supporting the increase of H_c. So, the above relation may not be adoptable to incorporate the results. For the particles in the single state showing the superparamagnetic behaviour, the variation of H_c is also related as [14].

$$H_c \propto \frac{K^4 D^6}{M_s A^3} \tag{13}$$

where K = magnetocrystalline anisotropy, D = particle size, A = exchange energy constant and M_s = saturation magnetization. Since we speculated the increase of K may be the probable factor for the increase of M_s, so increase of K does not support the decrease of H_c. The variation of either $<D_{FE\text{-}SEM}>$ or $<D_{XRD}>$ is not supporting the variation of H_c. Therefore, the decrease of H_c can be expected due to increase of M_s with the substitution of Mn^{2+}.

4.3. Temperature dependence magnetic studies

The M–T graphs for $Mn_xZn_{1-x}Fe_2O_4$ ($x = 0.5, 0.6, 0.7$) nano ferrites are shown in Fig. 4. The values of blocking temperature (T_B), magnetocrystalline anisotropy (K) and volume of nanoparticles (V_p) are tabulated in Table 4. A plot T_B, K, $<D_{XRD}>$ and $<D_{FE\text{-}SEM}>$ is displayed in Fig. 5. It can be observed that T_B decreases and increases with the substitution of Mn^{2+} ions. Depending upon the values of K we can expect the variation of T_B is in the order T_B ($x = 0.7$)>T_B ($x = 0.6$)>T_B ($x = 0.5$) as well as based on the values of V_p, we can expect this order as T_B ($x = 0.5$)>T_B ($x = 0.6$)>T_B ($x = 0.7$). But the variation of T_B in the present study is quite different from these expectations. An interesting dependence of parameters was observed between $<D_{XRD}>$ and T_B. Based on the relation $25KV_p = k_BT_B$, the variation of $<D_{XRD}>$ (see this order D_{XRD} ($x = 0.7$)>D_{XRD} ($x = 0.5$)>D_{XRD} ($x = 0.6$)) is supporting the variation of T_B (T_B ($x = 0.7$)>T_B ($x = 0.6$)>T_B ($x = 0.5$)). In this study, we calculated the values of K only considering the volume of particle size estimated from FE-SEM. The major contribution for magnetocrystalline anisotropy can also be speculated due to surface anisotropy, dipolar interactions in nanocrystalline materials, shape anisotropy, etc. In the present study, M_s is increasing while H_c is decreasing with the substitution of Mn^{2+} ions. It can be observed from FC-ZFC curves that the magnetization M at 10 K is unevenly varying in the order $M_{(10K)}$ ($x = 0.7$)>$M_{(10K)}$ ($x = 0.5$)>$M_{(10K)}$ ($x = 0.6$). This indicates that the cation distribution estimated at room temperature is different from the cation distribution to be estimated at temperature 10 K, resulting to the changes in the magnetocrystalline anisotropy. During the FC the magnetization reaches the maximum for the three compositions at low temperature and became flattened. This type of nature reveals the extreme strong magnetic interactions among the ferrite nanoparticles, supporting the observation made from

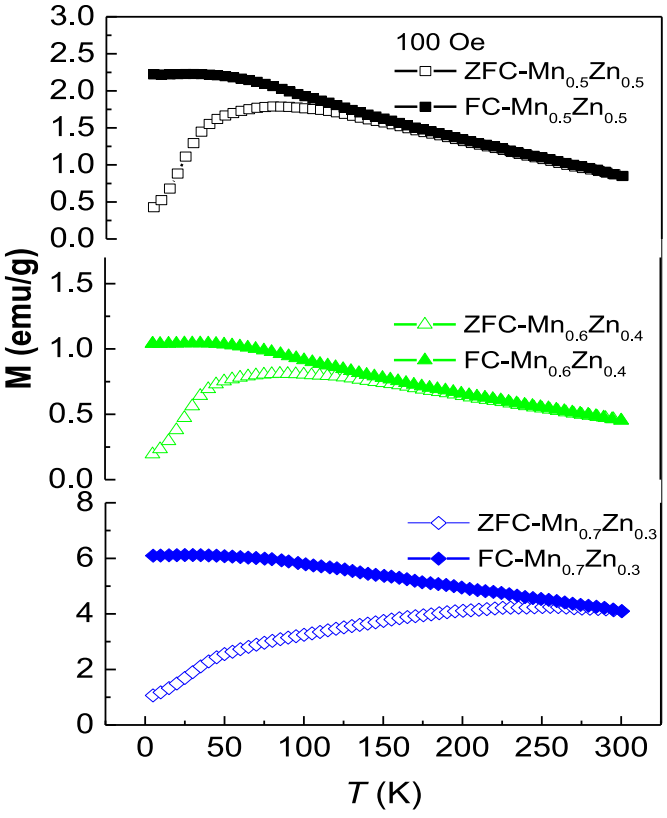

Fig. 4. FC–ZFC curves of $Mn_xZn_{1-x}Fe_2O_4$($x = 0.5, 0.6, 0.7$) nanoferrites in the presence of field 100 Oe.

Table 4
Blocking temperature (T_B), magnetocrystalline anisotropy (K) and volume of particle (V_p).

Comp. (x)	T_B (°K)	$K \times 10^5$ (erg/cm^3)	V_p (cm^3)
0.5	74	6.919	368.93
0.6	65	9.029	248.35
0.7	251	63.121	137.19

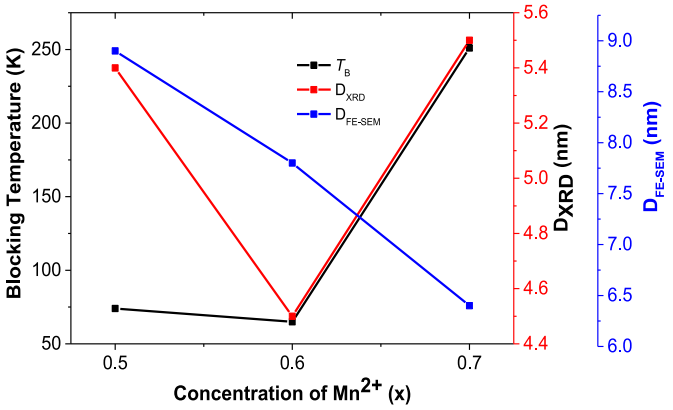

Fig. 5. Variation of T_B, $<D_{XRD}>$ and $<D_{FE\text{-}SEM}>$ against to the Mn^{2+} ion concentration.

microstructural studies. Similar studies have been reported in the literature [34–36]. From our previous reports [17], it can be observed that the secondary phases present ferrite samples are showing predominant influence on the microscopic magnetic behaviour. Finally, the magnetic behaviour depends on the magnetocrystalline anisotropy, super exchange interactions and magnetic dipolar interactions.

4.4. Mössbauer studies

Mössbauer spectra of $Mn_xZn_{1-x}Fe_2O_4$ ($x = 0.5, 0.6, 0.7$) nanoferrites are shown in Fig. 6. Mössbauer spectroscopic parameters like isomer shift (δ), quadruple splitting (Δ), line width (Γ), hyperfine fields (H_{hf})and area under spectral lines (A%) are tabulated in Table 5. The isomer shift of A–site is less than that of B–site due to the higher covalence of Fe^{3+}–O^{2-}complexes at A–site when compared to the B–site and many reports are available in the literature [37,38]. The values of δ was observed to be less than 0.5 mm/s, indicating the presence of Fe^{3+} in high spin state, ruling out the existence of Fe^{2+} ions, as isomer shift values for Fe^{2+} ions is greater than 0.5 mm/s (in between 0.9 and 1.1 mm/s) [39]. The quadruple splitting (Δ) is a representative of cubic symmetry around the Mössbauer nucleus. The values of Δ in the present study are found to be in the range of 0.022–0.127 mm/s. This is a long range and variation of Δ is attributed to random distribution of Mn^{2+} and Zn^{2+} around Fe^{3+} ions in the spinel structure. This is supporting the cation redistribution in the series of ferrite samples understudy. When the particle size is reduced to nano dimensions due to the finite size effects, surface energy of nanoparticles is not enough to regulate the normal occupancy of cations in the spinel structure. The samples with composition $x = 0.5$ and $x = 0.6$ are exhibiting paramagnetic doublets while the composition $x = 0.7$ possessed the paramagnetic doublet along with weakly resolved sextets. This indicates that the ferrite nanoparticles in the samples of composition $x = 0.5$ and $x = 0.6$ are in superparamagnetic state whereas the sample with composition $x = 0.7$ has the ferrite nanoparticle sizes simultaneously showing superparamagnetism and hyper fine interaction. We observed the similar behaviour in Ni–Zn and Mn–Ni ferrite systems prepared in our laboratory using co-precipitation method [29,40]. It can be seen that each composition has two central doublets. In the present study, core-shell

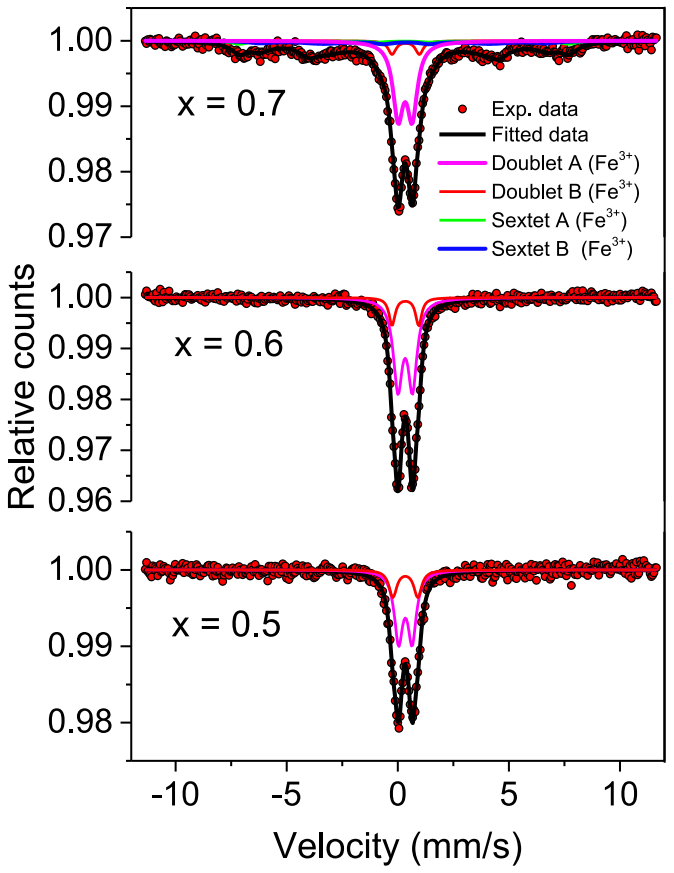

Fig. 6. Room temperature Mössbauer spectra of $Mn_xZn_{1-x}Fe_2O_4$($x = 0.5, 0.6, 0.7$) nanoferrites.

Table 5
Values of Hyperfine field (H_{hf}), isomer shift (δ), quadruple splitting (Δ), line width (Γ) and areas in percentage of tetrahedral and octahedral sites of Fe^{3+}ions.

Comp. (x)	Iron Sites	H_{hf} (T)	Δ mm/s	δ mm/s	Γ mm/s	A (%)
0.5	Doublet 1	–	0.615	0.241	0.453	75.4
	Doublet 2	–	1.120	0.230	0.413	24.6
0.6	Doublet 1	–	0.660	0.234	0.484	87.6
	Doublet 2	–	1.196	0.242	0.321	12.4
0.7	Doublet 1	–	1.226	0.257	0.401	3.5
	Doublet 2	–	0.638	0.236	0.549	35.3
	Sextet 1	45.96	0.022	0.177	0.894	18.8
	Sextet 2	39.97	0.127	0.229	1.067	42.4

model might be suitable to explain magnetic behaviour of ferrite systems under Mössbauer analysis. The paramagnetic doublets with higher value quadruple splitting $\Delta = 1.120(x = 0.5)$, $1.196(x = 0.6)$ and 1.226 ($x = 0.7$) are assigned to core and the paramagnetic doublets with the values of quadruple splitting $\Delta = 0.615$ ($x = 0.5$), $0.660(x = 0.6)$ and 0.638 ($x = 0.7$) are assigned shell region of nanoparticles. From VSM studies, it can be observed that M_s is increasing with the substitution of Mn^{2+} ions and this is expected due to increase in K. From Table 5, it can be observed that the relative area under Mössbauer spectral lines assigned to the shell region is decreasing with the substitution of Mn^{2+} ions. This is attributed to the decrease of core-shell interactions and increase of M_s resulting to the increase of K. From M–T studies we can see that K is increasing with the increasing in Mn^{2+} ion concentration. The increase of K is a probable factor to increase in M_s with the substitution of Mn^{2+}. Moreover, as K is with the substitution of Mn^{2+}, it can be anticipated that the distribution of particles showing hyperfine interaction are increasing which can be clearly evident from the nature of the present Mössbauer spectra.

5. Conclusion

The incorporation of metal ions resulted in expansion of tetrahedral and octahedral sites and therefore, the increase and decrease of tetrahedral bond length (R_A) are a result of cation redistribution. The varying trend of bond angles seemed to support the increase of M_s, but small deviations for the composition $x = 0.6$. More precisely, the increase of the ferrite phase with the substitution of Mn^{2+} is the probability factor for the increase of M_s as well as K also is the another factor. The decrease of coercivity (H_c) with the substitution of Mn^{2+} is found to be dependent of D^6 law. The variation of crystallite size ($<D_{XRD}>$) and particle size ($<D_{FE\text{-}SEM}>$) is not supporting the increase of blocking temperature (T_B). The flat nature of FC curves at lower temperature indicates the spin-glassy interactions of nanoparticles. This is a supportive to adopt core-shell model for the paramagnetic doublets present in the Mössbauer spectra. The core-shell morphology of present ferrite nanoparticles supports the present trend of M_s. The factors like ferrite phase and K are the probable factors for the typical magnetic behaviour of present ferrite systems. The present ferrite nanoparticles are adequately suitable for sensor and bio-medical applications.

Credit author statement

All the authors contributed equally.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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AN ENDEAVOR TO OUTSTRIP PATRIARCHY: MANJU KAPOOR'S '*DIFFICULT DAUGHTERS*'

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Abstract
The women characters in Manju Kapur's novels seem to be left without the freedom to act and they remain solely in the field of hallucination, mere fantasy to be dreamt and loved. Manju Kapur in *Difficult Daughters* projects the image of the rebellious, but stoic women ultimately breaking the customary confines in the backdrop of conformist narrative thread. Manju Kapur in her works presents women who try to establish their own self. In *Difficult Daughters*, Virmati, in her pursuit of identity, who is also the focal character of the novel, revolts against convention? The very name of the Manju Kapur is one of the best known celebrated post-independence writers exploring sociological and psychological sensitive issues. Kapur tries to explore the insight or human psyche of her protagonist Virmati, torn between desire for love and duty towards family. Thus, the conflict of internal and external experiences, pressures and expectation produce worries. The novel "*Difficult Daughters*'' is a connotation to a point that a woman, who tries to search for self, is recognized as a difficult daughter in the family and the society. A woman is "new" if her basic concerns are deeper than purely seeking equality with men, asserting her own persona and insisting upon her own rights as a woman. The woman today has her own quest for self-discovery and self-fulfillment. A woman is trying now to be her own gravitational force, beyond the pull of patriarchy. Manju Kapur's novel *Difficult Daughters* is a story of a daughter's journey back into her mother's aching past. It spans the genres of literature and history and falters in both. *Difficult Daughters* is a story of three generations of women: Ida, the narrator, who is a divorcee. Virmati, her mother, who marries an already married professor for love, and Kasturi, her grandmother, who come to terms with a difficult daughter, Virmati. This was not a fictional family, but the story of a real, middle class home with fathers, mothers and brothers and sisters that one had seen and lived with.

Key Words: family, generation, identity, marriage, men, patriarchy, society, values, women

Introduction

Patriarchy is an institutionalized social system in which men dominate over others, but can also refer to dominance over women specifically; it can also extend to a variety of manifestations in which men have social privileges over others to cause exploitation or

oppression, such as through male dominance of moral authority and control of property. - Sylvia Welby

Manju Kapur is an outstanding women novelist in the field of Indian literature. In all her writings she deals with the silent suffering of Indian middle class women either as a victim of tradition or patriarchy or searching for identity. She usually sketches her thoughts and ideas, around women issues and voices out through her female protagonist.

Kapur's perception of women liberation is deeply stretched with social and cultural matters. Her novels play a significant role to insist the self-development of every woman for the furtherence of Indian society. She is the author of six novels. Her first novel *Difficult Daughters* wins the commonwealth prize in the year 1999. Other works of Manju Kapoor are *A Married Women* (2002), *Home* (2006), *The Immigrant (* 2008), *Custody* (2011) and *Brothers (2016). Difficult Daughters* is the story of a woman tattered amid family duty, the aspiration for education, and illegal love. Virmati, a young woman born in Amritsar into a stern and didactic family, falls in love with a neighbour, the Professor--a man who is already married.

That the Professor ultimately marries Virmati, installs her in his home and helps her towards further studies in Lahore. It is a small consolation to her offended family or even to Virmati, who ascertains that the scuffle for her own liberty has created irretrievable lines of partition and pain around her.

Difficult Daughters is about the chronicle of three generations of women and how intergenerational trauma affects them. It also picturises the quest of women for identity in a patriarchal system. The family compromises of Lala Diwan Chand who has two sons Suraj Prakash and Chander Prakash. Suraj is married to Kasturi and Chander Prakash is married to Lajwanti. Essentially the story is of three generations- Kasturi, the mother of Virmati, Virmati (the main protagonist), and Ida, the daughter of Virmati. Ida who belongs to the third generation is the protagonist of the book. Virmati is the 'difficult daughter' in the prosperous merchant family of Lala Diwan Chand. Ruby Mihoutra comments in her article "Existential Images of Women in Manju Kapur's Difficult Daughters" that 'While in the generation of Kasturi, woman's role was confined to childbearing and kitchen work, the generation of Virmati …..breaks away from the tradition bound limits of Indian women' (Milhoutra. P.164. 2005). Fortunately she is saved and Indumati is married off to Inderjeet in the name of the family name and honour. Virmati then is sent to Lahore for further studies. As Jaideep Rishi points out in his essay: "Mother–Daughter Relationship in Manju Kapur's Difficult Daughters: "Kasturi unknowingly becomes the voice of patriarchy. She holds those values as ideals which patriarchy has taught her to be so and when her daughter rebels against such values she takes it to be a rebellion against her own self." She deems the patriarchal postulations about the superiority of male in the family as well social system. Kasturi's believes that marriage is her destiny. After her graduation, her education continued at home.

Lalaji, the grandfather of Virmati the great supporter of patriarchal system, has a jewelry shop and a mill and he hoped that his sons would continue his work. "Lala Diwan Chand was vehemently opposed to any kind of division in the family…….his property that he refused to divide. He had worked all his life to make it grow, and he was not about to halve and quarter it now" (DD,25). Even after the division of the family into two units Lalaji had given clear instructions that his sister would be looked after 'with the dignity and respect that was her due' (DD, 28). His sister was clearly thankful for it was her brother who had given

her a home after she was widowed at the age of fourteen. Indeed the joint family structure can be a blessing for the old who are looked after and get companionship, the children who have the support and help of both the elders and the youngsters and the ill and sick are looked after. Following the customs he postpones Virmati's marriage to Inderjeet due to sudden deaths in the family. Rather than sit at home, she joins college. There she is inextricably drawn to the Professor who woes her on the pretext that his wife is not his companion and he yearns for an invigorating scholarly partner. Virmati is caught between familial and romantic love. Her family is opposes this match because Harish is already married.

Virmati takes the step to commit suicide to avoid marriage with Inderjeet. Kasturi considers this as an insult to the reputation of the family. She cannot understand why her daughter does not want to get married and have a family. She believes "A woman's shaan is in her home." In this context Dr. Ruby Milhoutra opines: "Her mother tried to ensure her future happiness by the impeccable nature of her daughter's qualifications.

She was going to please her in- laws… "(DD,57). Virmati revolts against deep rooted family traditions and marries the professor. She prepares herself to stay with the professor's family, which compromises of his first wife, mother-in-law, sister-in-law and children. Although Virmati succeeds in marrying the Professor, it proves to be a disaster. She has to live as a second wife and under the hostile gaze of Ganga, her husband's first wife without identity. Only her mother-in-law accepts her to some extent and that too at the behest of her son. During her conjugal life Virmati feels that it would have been better if she had not married Harish. "I should never have married you" (DD, 212).

After some time she suffers a miscarriage. Sometimes Virmati blames herself to be responsible for the destruction of Ganga's life. Dr. Arpita Ghosh comments: An "intensive education" fails to teach Virmati that resistance to patriarchy to forge an identity and ensure independence is not equivalent to trespassing into another woman's domain. Marrying a man of her choice was not an issue for her family but marrying a family-man who was already married with children was objectionable. She failed to realize the gravity of the situation.

Virmati blooms into a type of 'new woman' as Irish writer Sarah Grand (1854–1943) used the term that refers to independent women seeking radical change. She displays her strength of mind in overcoming her dejection. She is "strong to bear the pain, silently, without anyone knowing" (DD, 91). She is still stressed to institute her Self and to gain belligerence. She embodies the modern woman has frayed between craving for Self and her vulnerability. After her first sex encounter with Harish, she tries to justify by saying that there was no point in foolishly denying it on the basis of an "outmoded morality" (DD, 114). The novel also explores the problems of women in a male dominated society. Born out of typical Indian family, Virmati is caught between tradition and modernity. It results only in self-alienation and she becomes a symbol of female imagination. Responding to the pressures and the family structure at the Professor's house, she struggles to get the Professor's love and attention. Though she considers herself as a new woman She is amazed at her former roommate Swarna Lata's efforts to participate and help to bring about alteration in her life and be a part of the change in patriarchal system. With Swarna Lata's help she underwent abortion in the hands of a proper doctor at Mohini Dutta's guest room before marriage. Swarna asks Virmati to come and demonstrate against the Hindu code bill "Men don't want family wealth to be divided among women. Say their sisters get dowry, that's their share, and the family structure will be threatened, because sisters and wives will be seen as rivals,

instead of dependents who have to be nurtured and protected. As a result women will lose their moral position in society! Imagine!"(DD, 232).

Ida, Virmati's daughter and the narrator, understands their family structure. She thinks that 'when I grow up I should be very careful to tailor my needs to what I knew I could get. That is my female inheritance. That is what she tried to give me. Adjust, compromise, adapt, assertion, though difficult to establish, is easy to remember' (DD, 236). Manju Kapoor remarks: "conflict between mother and daughter is inevitable and I suppose I was a difficult daughter. The conflict carries on through generation because mothers want their daughters to be safe. We want them to make the right choices- right in the sense that they are socially acceptable. My mother wanted me to be happily married; I want my daughters to have good jobs."

Ida becomes the typical daughter of a 'difficult daughter' Virmati. She could not grow an understanding with her mother during her lifetime and after Virmati's death this realization engulfs her with guilt. Ida sets on a journey into her mother's past in search of a woman she could know and understand She rebels against Virmati, rejects her own womanhood and follows her own whims, even though she experiences a strong bond with her mother, "without her I am lost, I look for ways to connect" (DD 3). The story of Virmati is basically a story of Manju Kapur's own mother. She acclaimed that the heroine of her writing is her mother. In an interview with Jo Stimpson she asserts: that 'I based my first novel on her. I admire her fighting spirit, her generosity, her capacity to endure. She irritated me when she was alive, but now I see these things more clearly. I think of her every day'.

The act of setting out a quest and then to write out the mother's life keeps Ida connected with her mother. It would be appropriate to say that Ida longs for her mother even after her death. Thus through the voice of Ida the writer narrates the gripping tale of three women, Virmati, Shakuntala and Swarna, set in the pre-independence era who choose to not conform to society's standards, and to rise above expected domestic ambitions; they "thought to be something other than a wife."

Kritika Agarwal comments in the book review: "They think for themselves, prioritise their life before others, go for higher studies, participate in the Satyagraha movement, and choose not to ever get married. From the Hindu code bill, dowry, to abortion, and property rights, Virmati, Shakuntala and Swarna lay the foundation of the rights that women today enjoy. Set around the time of partition, Kapur traces the life of her mother in undivided Punjab. This novel mirrors the society's obsession with its conservative ideas of women, superstitions, male child, and the family's sacred duty of marriage."

Manju kapoor brilliantly portrays the desire, struggle, and effort to emerge out of patriarchal system of the society by Virmati. The novel sings the struggles of women who despite facing their personal battles, significantly contribute to India's independence; but is equally relatable today's society and the woes that still fight to outgrow.

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Cloud Assembling Engineering In light of Block chain Innovation

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Abstract:

IT infrastructure has started to be used more effectively in the manufacturing sector. Iot, digital actual frameworks, big data, and cloud producing were a part of the capacities that complete the contemplations. Various difficulties in current business have been addressed on the grounds that as far as possible. Cloud producing is one of these innovations that has emerged because of the pay-more only as costs raised. Assembling assets now be leased and shared on an overall scale on account of this innovation. They may have issues starting from focal construction and the need for a dependable outsider. We feel that cloud-producing frameworks controlled by BC can defeat some recently referenced issues and kill the need for are liable outsider with these characteristics. Block chain (BC) is a decentralized and distributed technology. This examination utilizes a few nearby applications done on brilliant agreements dependent on BC to carry out correspondence and arrangements across the space isolating the asset provider. The created application is known as the decentralized cloud-producing application (DCMApp). DCMApp is a cross breed network that utilizes Ethereumasa public BC organization It is clashing with a completely open BC affiliation .Gathering plans have turned into even more straight forward, and secure considering the way that to DCMApp's crossbreed structure. Without the prerequisite for any server framework. With the Ethereum organization, controlling arrangements are basically incomprehensible.

I. Introduction:

Cloud fabricating applications/design structure is formed by previous encounters, alternate points of view of industry and scholastic climate and arrangements proposed to various necessities. Regardless of the distinctions in cloud fabricating applications, which have normal highlights. An Application ISP Centralized Server Application cloud fabricating application is brought together for whatever reason it is ready for. There are numerous issues that cloud fabricating stages have as a result of their focal design. To execute cloud fabricating stages, the framework should be planned and introduced. Establishment and support of such frameworks are expensive. This expense should be met here and there from clients. Clients should pay this expense to get administrations from the application. Since cloud producing stages are applications running over the web, it is beyond the real of possibilities to expect to profit from the elements of the application by downloading the application to the PC once. Not with standing, in a dApp, clients are not associated with in middle. They can run the capacities presented by the application from any point. In case the capacities presented by cloud fabricating frameworks are given to clients inside a BC upheld dApp, clients can utilize these capacities openly with an application that is free of an essential issue and can move esteem between the gatherings. In synopsis, BC-upheld decentralized cloud fabricating applications will dispense with the requirement for a focal server, and clients won't be charged for the activity and upkeep of the focal application. an ordinary application needs a focal server, though decentralized applications

needn't bother with any focal server. In unified application, admittance to the application happens from a solitary actual point. The application might become blocked off because of harm to the actual server, assaulting the application, or ending the application. This will intrude on the cycle and cause genuine misfortunes. Disappointment in cloud fabricating frameworks brings about material misfortunes for both the specialist co-op and the getting party. To offer continuous support, the stage should have a top-notch network association and the actual machines on which the application is running should be without bother and secure. In BC upheld dApps, the coherence of administration won't rely upon a solitary point. If there should be an occurrence of an issue in any hub of the BC organization, the congruity of the help can be effectively accomplished by associating with another hub. The BC-upheld decentralized cloud producing application can be run persistently from any point and give the usefulness required between the gatherings. A similar application serves numerous clients in cloud producing frameworks. Inside the application, client information is put awayina typical data set. Theclient signingintotheapplication, withtheapproval givento him/her, makes exchanges on the screens identified with him in the application. A client validation framework is utilized to permit theclient to sign into theapplication. These frameworks are generally founded on client name and secret word data. There are likewise applications that utilization 2FA strategy (two element confirmation) to expand the security of the login framework. Not with standing, these safety efforts are intended to shield clients from outside dangers as it were. It doesn't offer any safety efforts against the dangers of the individuals who oversee or foster the application. Any individual who approaches the applications information base is a possible danger to the framework. In the BC-upheld cloud producing application, the client stores the information that are confirmed by a hugecrowd in the BC organization. By running theapplication on its own PC, the client can do every one of the activities by utilizing the mysterious key of his wallet without sharing the client's name and secret phrase data with a far-off server.

Cloud based plan fabricating(CBDM) refers to an assistance situated arranged item advancement model in which administration clients are empowered to design them, select, and use altered item acknowledgment assets and administrations going from PC supported designing programming to reconfigurable assembling frameworks. A continuous discussion on CBDM in the examination local area rotates around a few angles like definitions, key attributes, figuring structures, correspondence and cooperation processes, publicly supporting cycles, data and correspondence framework, programming models, information stockpiling, and new plans of action relating to CBDM. One inquiry, specifically, has regularly been raised: is cloud-based plan and assembling really another world view, or is it simply old wine in new jugs? To respond to this inquiry, we examine and look at the current definitions for CBDM, distinguish the fundamental attributes of CBDM, characterize a precise prerequisites agenda that an admired CBDM framework ought to fulfill, and contrast CBDM with other significant however more customary community-oriented plan and conveyed fabricating frameworks like web-and specialist-based plan and assembling frameworks.

II. Literature survey:

A Cyber-Physical Systems architecture for Industry 4.0-based manufacturing systems (Jay Lee, Behrad Bagheri , Hung-An Kao). Late advances in assembling industry has cleared way for a systematical sending of Cyber-Physical Systems (CPS), inside which data according to all connected points of view is firmly observed and synchronized between the actual manufacturing plant floor and the digital computational space. In addition, by using progressed data investigation, arranged machines will actually want to perform more effectively, 10 cooperatively and versatilely. Such pattern is changing assembling industry to the future, in particular Industry 4.0. At this early advancement stage, there is a critical requirement for anun mistakable meaning

of CPS. Inthispaper, a brought together 5-level engineering is proposed as a rule for execution of CPS Cloud Computing for Cloud Manufacturing: Benefits and Limitations(Peng Wang, Robert X. Gao1, Zhaoyan Fan).
Distributed computing, as another worldview for amassing figuring assets and conveying administrations over the Internet, is of considerable interest to both scholarly world and the business. In this paper, the fundamental qualities of distributed computing are summed up, in view of its application to the assembling business. Scientific models for example, scientific progression process (AHP) technique for choosing fitting cloud administrations are investigated, as for computational cost and organization correspondence that present a bottle neck for powerful usage of this new foundation. The survey presented in this paper plans to help scholastic analysts and manufacturing ventures in getting an outline of the state-of the-information on distributed computing while investigating this arising stage for administration Cloud-Based Design and Manufacturing: Status and Promise (Dazhong Wu, David W. Rosen and Dirk Schaefer) The data innovation industry has benefited impressively from distributed computing, which permits associations to shed a portion of their costly data innovation framework and movements processing expenses for more manageable functional costs. Considering these advantages, we propose a new paradigm for item plan and assembling, alluded to as cloud-based plan and producing (CBDM). This part presents a definition and vision for CBDM, verbalizes the distinctions and likenesses among CBDM and customary standards like web- and specialist based advances, features the essentials of CBDM, and presents a model framework, create dat Georgia Tech, called the Plan and Manufacturing Cloud(DMCloud). At long last, we close this part with a diagram of future exploration headings. Two Bitcoins at the Price of One? Double- Spending Attacks on Fast Payments in Bit coin(Ghassan O.Karame, Elli And roulaki, Srdjan Capkun). Bitcoin is a decentralized installment framework that is in light of Proof-of-Work. Bitcoin is presently acquiring notoriety as an advanced money; a few organizations are beginning to acknowledge Bitcoin exchanges. An example instanceof thedeveloping utilization of Bitcoin was as of latedetailed in the media; here, Bitcoins were utilized as a type of quick installment in a neighborhood drive-through joint. In this paper, we investigate the security of utilizing Bitcoin for quick installments, where the time between the trading of money and merchandise is short (i.e., in the request for few moments).

III. RELATED WORK

BLOCKCHAIN TECHNOLOGY

BC technology can be seen of as a disseminated information base that holds a rundown of information records, or as an overall record, keeping all cycles shared and run between members [25]. Unlike the classical database and ledger, it is decentralized and distributed. A paper, ‘‘Bitcoin: A Peer-To-Peer Electronic Cash System’’, was published in 2008 by a person / group under the name Satoshi Nakamoto [26]. This article presents electronic cash that permits direct internet based installments starting with one party then onto the next without a delegate [27]. After a few months, in 2009, Bitcoin application was implemented [25]. Similar applications were developed using the basic features of Bitcoin cryptocurrency and the applications were labeled with the term cryptocurrency. Bitcoin, one of the most used cryptocurrencies, achieved a great success in 2019 with a capital market of approximately 223 billion dollars [30]. BC technology, which forms the basis of Bitcoin, became popular with the success of Bitcoin. Bitcoin has increased people’s interest in BC technology, but it has also made people skeptical about this technology. Many economists have criticized Bitcoin. Many experts have advocated Bitcoin as the best investment tool as a global unit. Bitcoin is based on classic BC technology. Classical BC technology suffers from data synchronization, double spending problems in distributed systems. The data synchronization problem has been attempted to be solved by applying the consensus model. The double spending problem is solved by a decentralized payment system rely on proof of work (PoW).

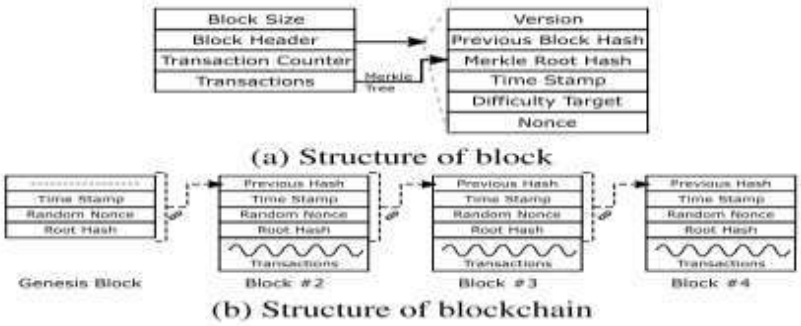

Fig 1. Structure of Block chain

[22], [23]. This solution was first applied by Satoshi Nakamota in the Bitcoin white paper [24]. With the improvements made, the BC technology currently used is slightly different from conventional BC technology. The BC technology used in this article is a completely distributed and decentralized system with P2P communication VOLUME 8, 2020 2165 B. Kaynak et al.: Cloud Manufacturing Architecture Based on Public Blockchain Technology FIGURE 1. Structure of blockchain. based on consensus and PoW models [33]. The general architecture of BC technology used in the study is shown in figure 1. As shown in Figure 1a, each block has block size, block header, transaction counter and transaction fields. The block header field has 6 fields in itself: version, previous block hash, merkle root hash, time stamp, difficulty target and nonce. Version field allows you to monitor the software protocol. The previous block hash field holds the hash value of the previous block. If there is any change in the transaction field, the value in the hash field changes. This area ensures data security. The Merkle root field holds the hash value of the merkle tree in BC. Approved transactions are included in the merkle tree, and if there is any change in the transaction, the root hash value of the local tree associated with the transaction changes, and all blocks created after the modified block are lost. The Difficulty target field indicates the degree of difficulty of the PoW algorithm. The nonce value is a random value used for the proof of work algorithm. As shown in Figure 1b, the BC network starts with the genesis block. The genesis block has different areas than the other blocks and represents the starting block of the chain. The Genesis block is created by the person who started the chain. This block contains the basic structure and rules of the BC.

BLOCKCHAIN SUPPORTED DAPP

The data of recently popular applications such as Whatsapp, Twitter, etc. are stored on servers that are responsible for a single organization / person. Such applications are centralized applications. Centralized applications have limitations such as security, the need for reliable third-party, less transparency, single point failure. Because of these limitations, it may be to avoid a central structure in all or some of the applications. Decentralized applications (dApp) are distributed applications running on a peer-to-peer (P2P) network. Application data is not maintained on a single server, and multiple nodes on the network have copies of the data. Torrent applications are examples of dApps running on P2P networks. Large files are shared with the torrent application. There are many websites that provide access to these files and search engines. Thus, the loss of a node and the loss of access to the node do not prevent accessibility to the system. As the number of people sharing files increases, the file downloads faster. When the sharing stops, the network breaks and the previous steps and the shared file disappear. DApps built on the basic architecture of BC technology have some different features from traditional dApps: • The application should use a crypto token. • The application must be open source. • Application data must be stored on a decentralized BC. • Consensus algorithms (PoW based algorithm, Proof of Stake, Delegated Proof of Stake algorithm) should be used. • With SCs, local functions in dApp have become functions that run on the BC network. So, It is guaranteed that the functions cannot be changed.

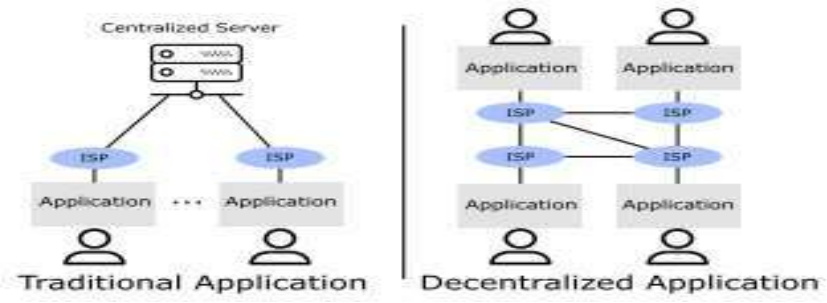

Fig 2. Centralized Vs Decentralized

Clients work demands. In this recreation, a sumof 939 work demands are created, which around prompts a normal number of 10 for the 100 clients. The reason for the reproduction is to acquire bits of knowledge on the exhibition and assessment of suppliers. For effortlessness,weexpectthatfor this situation study, clients don't depend on disseminated figuring for getting answers for their administration demands. As shows the recreation flowchart, with nitty gritty depiction in the accompanying segments.

IV. CONCLUSION:

Asset proprietors and assets looking for buyers can promptly trade assets across stages on account of cloud assembling's qualities. Customers, on the opposite side, are worried about the stages unwavering quality. ABC-based application is given in this review empowers clients to make arrangements among themselves without the prerequisite for an outsider. There view is remarkable in that the arrangements are made conceivable by means of the Ethereal organization, which is a public BC network that additionally upholds SC. Then again, the application is in here with in a half and half structure. On account of the mixture structure, clients just compensation for arrangements that should be gotten. If the modification were to be run totally in a public design, all information would need to be kept on the public organization, which would be very costly. A side from that, the way that an individual or gathering control s an organization is a private organization makes extreme worries for clients. Also, for private organizations, a server foundation should be given by an outsider. A half and half development were picked consequently. Clients will actually want to make creation concurrences with each other and make installments straightforwardly to another utilizing this methodology.

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Rainfall activity over Vijayawada region during 2019

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N. Umakanth, N. Ranga Babu, B. T. P. Madhav, et al.

Rainfall Activity over Vijayawada Region During 2019

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Abstract. Heavy rainfall events occurred in recent years lead to severe human loss and damages to crops and property. We have examined the rainfall, maximum temperature, minimum temperature, total perceptible water (TPW), total totals index and k index parameters over Vijayawada region (16.5°N 80.64°E) using IMD dataset and ERA5 reanalysis dataset. High TPW values in monsoon season indicate the moisture availability need for severe rainfall. In post-monsoon season, some extreme events are recorded in October. The TTI and KI values also indicated higher values when there is a severe rainfall.

Keywords: Heavy, Rainfall, Maximum, Minimum.

INTRODUCTION

In northern hemisphere, the heavy rainfall occurring events have been escalating in recent years [1]. The statistical indices which assess the extreme events have shown 30% increase in heavy rainfall over USA during 1950-2009 [2]. The rainfall events intensity has been increasing over Europe. The trends have shown sudden spikes which support the rainfall increase in Europe [3, 4]. A research work by Goswami [5] stated that the extreme rainfall occurrences have shown drastic increase over central India during the time period 1951–2000. A significant increase in natural hazards will be seen in future due to rainfall over central India. Another work by Ghosh et al. [6] demonstrated that extreme events vary spatially. When we do not consider long term datasets increases nor has decrease in rainfall been seen over Indian region. However, Vittal et al. [7] stated that the occurrences of extreme events over India showed contrasting results when we compare before 1950 and after 1950. These contrasting results are due to the rapid urbanization globally.

In recent research works, many researchers pointed that apart from global warming; rapid urbanization also influenced the occurrence of heavy precipitation events [8-11]. In the recent years, the urban areas across India have been expanded beyond the imagination in order to meet the financial tempo globally [12]. As there is an increase in economic growth, opportunities increase widely in urban area. This plays huge role in increasing the population in urban areas [13]. Any measures regarding these extreme events have to be implemented first in the urban areas [14]. Although the extreme events are increasing widely, it cannot be portrayed on single station due to data handling issues [15–22]. Therefore, we examined the trend of daily rainfall, temperature heavy and few parameters over Vijayawada region.

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DATA AND METHODLOGY

This work has been performed completely on the Vijayawada city at 16.5°N and 80.64°E. We have collected very high spatial resolution 0.25° daily gridded rainfall data from INDIA METEOROLOGICAL DEPARTMENT (IMD) [23]. We have collected very high spatial resolution 1.0° daily gridded temperature data from IMD [24]. ERA5 reanalysis dataset is also collected from the website https://climate.copernicus.eu/climate-reanalysis [25].

The pressure level ERA5 dataset are utilized in estimating the below parameters given below:

(i) K-Index (KI)

The K Index is the subtraction of temperature and dew point temperature at different pressure levels of the atmosphere as shown below [26].

$$KI = (Temp850 - Temp500) + Tempd850 - (Temp700 - Tempd700) \quad \text{---------- (1)}$$

Where Temp represents temperature; Tempd represents dew point temperature.

(ii) Total Totals Index (TTI)

The total totals index is obtained by differencing the temperature and dew point temperatures at 850 and 500 hpa pressure levels. The threshold values of TTI values are shown below [27].

$$\text{Cross Totals, } CT = Tempd850 - Temp500$$

$$\text{Vertical Totals, } VT = Temp850 - Temp500$$

$$\text{Total Totals Index, } TTI = CT + VT = Temp850 + Tempd850 - 2Temp500 \quad \text{------- (2)}$$

Where Temp denotes temperature, Tempd denotes dew point temperature.

RESULTS AND DISCUSSION

From FIGURE 1&2, the rainfall, maximum temperature and minimum temperature parameters have been monitored over Vijayawada region of Andhra Pradesh state for 2019. Very less rainfall has been seen over January and February months. On 28th January there was an extreme rainfall event i.e. 26 mm of rainfall was recorded. Apart from that event there were no events. The temperatures were very less. The minimum temperatures lie between 14 to 23°C whereas, the maximum temperatures values lies between 29 to 32°C. In pre-monsoon season, there were two extreme rainfall events i.e. on 10th March and 30th May. The minimum temperatures lie between 25 to 30°C whereas, the maximum temperatures values lies between 36 and 42°C. In monsoon season, there were 27 extreme rainfall events. The rainfall activity was higher in July and September moths. The extreme event was recorded on 14th July. The minimum temperatures lie between 24 and 28°C whereas, the maximum temperatures values lies between 32 and 40°C. In post-monsoon season, there were six extreme rainfall events. The rainfall activity was higher in October month. No extreme rainfall events in November and December. On 23rd October, 44 mm of rainfall was seen. The minimum temperatures lie between 20 to 24°C whereas, the maximum temperatures values lies between 28 to 33°C.

In FIGURE 3, the rainfall and total perceptible water (TPW) parameters have been monitored over Vijayawada region of Andhra Pradesh state for, 2019. In winter season, very less TPW has been seen over January and February months. On 28th January when there is an extreme rainfall event the TPW value is 40 mm. Apart from that event there was no events. The TPW values were very less ranging between 23 to 35 mm. In pre-monsoon season, the TPW values were ranging between 35 and 57 mm. In monsoon season, the TPW values were higher. The TPW values ranges between 54 and 67 mm. In post-monsoon season, the TPW values were higher mainly in October. The TPW values were very low in November and December months.

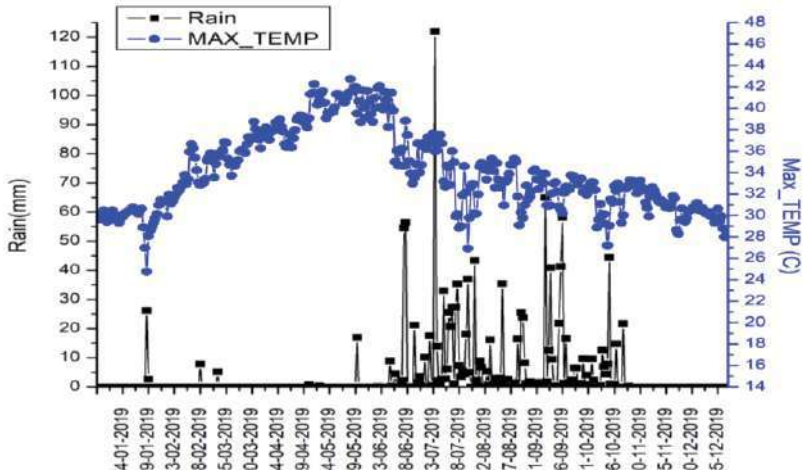

FIGURE 1. Time series plot of rainfall and maximum temperature for Vijayawada region during 2019.

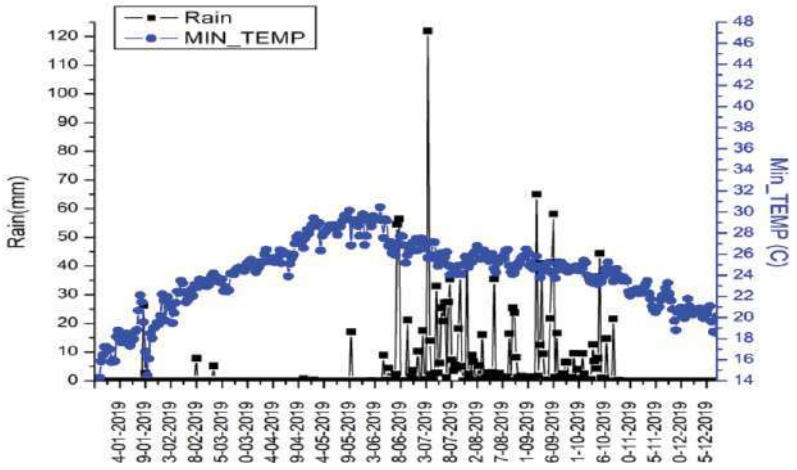

FIGURE 2. Time series plot of rainfall and minimum temperature for Vijayawada region during 2019.

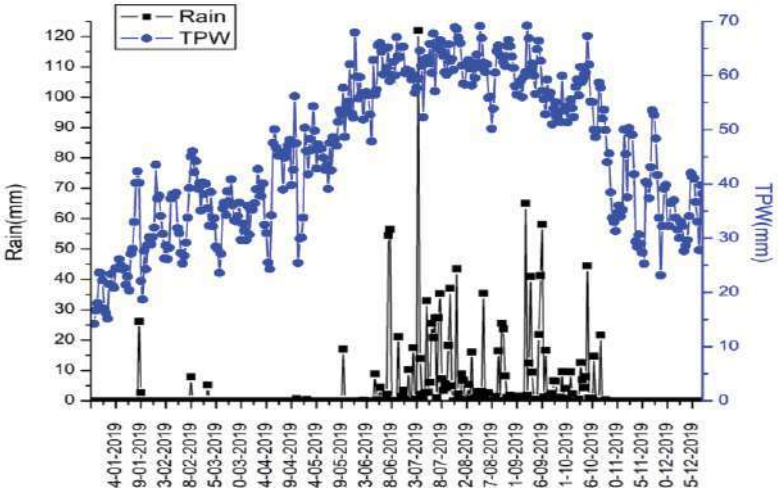

FIGURE 3. Time series plot of rainfall and total precipitable water for Vijayawada region during 2019.

In FIGURE 4, the rainfall and K-Index (KI) parameters have been monitored over Vijayawada region of Andhra Pradesh state for 2019. In winter season, very less KI has been seen over January and February months. On 28th January when there is an extreme rainfall event the KI value is 32 C. Apart from that event there was no events. The TPW values were very less ranging between 3 and 22 mm. In pre-monsoon season, the KI values were ranging between 20 and 36 C. In monsoon season, the KI values were higher. The KI values ranges between 22 and 42 C. In post-monsoon season, the KI values were higher mainly in October. The KI values were very low in November and December months. In FIGURE 5, the rainfall and Total Totals Index (TTI) parameters have been monitored over Vijayawada region of Andhra Pradesh state for 2019. In winter season, very less TTI has been seen over January and February months. On 28th January when there is an extreme rainfall event the TTI value is 42 K. Apart from that event there were no events. The TTI values were very less ranging between 23 and 42 K. In pre-monsoon season, the TTI values were ranging between 40 and 52 K. In monsoon season, the TTI values were higher. The TTI values ranges between 36 and 50 K. In post-monsoon season, the TTI values were higher mainly in October. The TTI values were very low in November and December months.

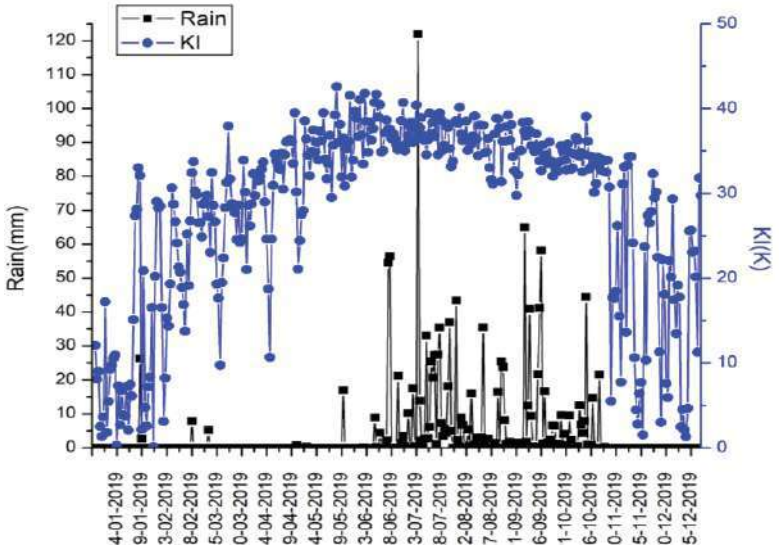

FIGURE 4. Time series plot of rainfall and K-Index (KI) for Vijayawada region during 2019.

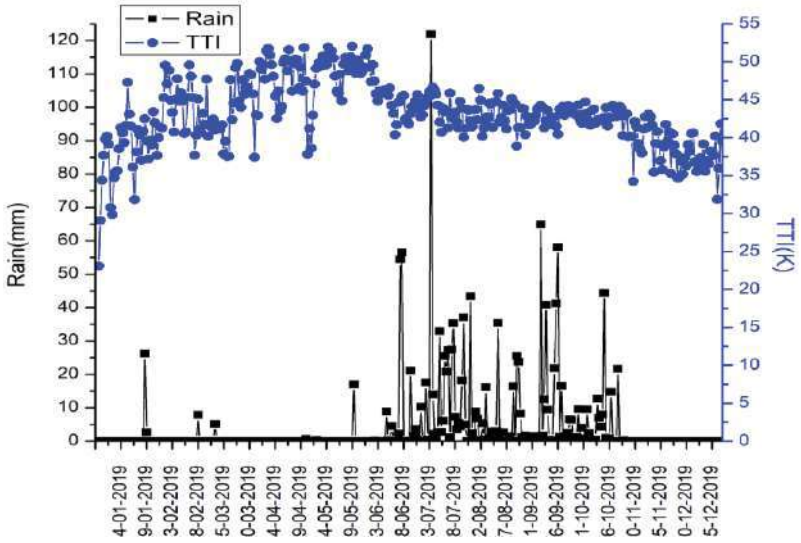

FIGURE 5. Time series plot of rainfall and Total Totals Index (TTI) for Vijayawada region during 2019.

CONCLUSION

Our work brings out the heavy rainfall events over Vijayawada region during 2019. In winter season, a extreme event on 28th January recorded 26 mm of rainfall. In pre-monsoon season, there were two extreme rainfall events whereas 27 events in monsoon season. Finally in post-monsoon season, 6 events were recorded. The pre-monsoon temperatures were higher ranging between 36 and 42°C. High amount of total perceptible water (TPW) is seen in monsoon season. The TTI and KI values also indicated higher values when there is a severe rainfall. The TTI values were higher in pre-monsoon but heavy rainfall events were more in monsoon. This indicates that TTI is indicating a chance for rainfall based on temperature fluctuations. For extreme rainfall, moisture is more important than temperature.

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Analysis of rainfall activity over Allahabad region during 2019

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Analysis of Rainfall Activity over Allahabad Region during 2019

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Abstract. In recent years**,** India has experienced many horrible heavy rainfall events which lead to huge loss to humans, crops and property. We have examined the rainfall, maximum temperature, minimum temperature, total perceptible water (TPW), total totals index and k index parameters over Allahabad region (25.43°N 81.84°E) using IMD dataset and ERA5 reanalysis dataset. High temperatures were seen during pre-monsoon season. Almost 45^{o}C has been observed. The heavy rainfall in monsoon season indicates that moisture availability plays more roles in these events than temperature variation indices. Total perceptible water parameters help in projecting the accumulated moisture at the region.

Keywords: Temperature, Rainfall, k index, Extreme.

INTRODUCTION

An abnormal rise in extreme rainfall events has been noticed in the recent decades [1-5]. The changes in rainfall are mainly affected by relative humidity and wind patterns. The Clausius–Clapeyron relation is very helpful in determining the variations in precipitation [6, 7]. These events are mainly based on temperature, season and geographical position [8]. The daily rainfall occurrences are mainly influenced by the surface temperature which warms the air [9]. The previous atmospheric conditions used for assessing these events fail in predicting the occurrence of event [10, 11]. Satellite predictions and climate model predictions indicate that global warming is mainly responsible for the extreme rainfall events [12]. The rise in rainfall events has been observed in recent years over USA and Europe [13]. Coumou and Rahmstorf [14] stated that the global temperature rise helps the air to carry more moisture required for the occurrence of rainfall events. Goswami et al. [15] stated that the extreme rainfall occurrences have shown drastic increase over central India during the time period 1951–2000. A significant increase in natural hazards will be seen in future due to rainfall over Central India. Ghosh et al. [16] demonstrated that extreme events vary spatially. When we do not consider long term datasets increases nor has decrease in rainfall been seen over Indian region. A research by Chaturvedi [17] indicated that no perfect pattern of increase in extreme rainfall over India. These heavy rainfall events lead to the occurrence of natural disasters like landslides and flash floods. They also impose adverse affect on crops. This impacts the financial status of nation. However, the forecasting of these events is tough due to technical and real time data issues. The exact prediction of

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these events in future will help in saving human lives and a lot of property loss. The correct prediction of these events also helps in taking the mitigation measures prior to the occurrence [18-25].

DATA AND METHODLOGY

This work has been performed completely on the Allahabad city, at 25.43°N and 81.84°E. We have collected very high spatial resolution 0.25° daily gridded rainfall data from INDIA METEOROLOGICAL DEPARTMENT (IMD) [26]. We have collected very high spatial resolution 1.0° daily gridded temperature data from IMD [27]. ERA5 reanalysis dataset is also collected from the website https://climate.copernicus.eu/climate-reanalysis [28].

The pressure level ERA5 dataset is utilized in estimating the below parameters given below:

(i) K-Index (KI)

The K Index is the subtraction of temperature and dew point temperature at different pressure levels of the atmosphere as shown below [29].

$$KI = (Temp850 - Temp500) + Tempd850 - (Temp700 - Tempd700) \quad \text{---------- (1)}$$

Where Temp represents temperature; Tempd represents dew point temperature.

(ii) Total Totals Index (TTI)

The total totals index is obtained by differencing the temperature and dew point temperatures at 850 and 500 hpa pressure levels. The threshold values of TTI values are shown below [30].

$$\text{Cross Totals, } CT = Tempd850 - Temp500$$

$$\text{Vertical Totals, } VT = Temp850 - Temp500$$

$$\text{Total Totals Index, } TTI = CT + VT = Temp850 + Tempd850 - 2Temp500 \quad \text{------- (2)}$$

Where Temp denotes temperature, Tempd denotes dew point temperature.

RESULTS AND DISCUSSION

From FIGURE 1&2, the rainfall, maximum temperature and minimum temperature parameters have been monitored over Allahabad region of Uttar Pradesh state for 2019. Very less rainfall has been seen over January and February months. On 26th January there was a normal rainfall event i.e. 4 mm of rainfall was recorded. Apart from that event there were no events. The temperatures were very less. The minimum temperatures lie between 5 and 15°C whereas, the maximum temperatures values lies between 23 and 29°C. In pre-monsoon season, there were no extreme rainfall events. The minimum temperatures lie between 13 and 27°C whereas, the maximum temperatures values lies between 24 and 45°C. In monsoon season, there were 35 extreme rainfall events. The rainfall activity was higher in August and September moths. The extreme event was recorded on 28th September. The minimum temperatures lie between 22 and 28°C whereas, the maximum temperatures values lies between 29 and 42^{o}C. In post-monsoon season, there were five extreme rainfall events. The rainfall activity was very less. No extreme rainfall events in November and December. On 3rd October, 60.9 mm of rainfall was observed. The minimum temperatures lie between 3 and 20°C whereas, the maximum temperatures values lies between 15 and 30°C.

In FIGURE 3, the rainfall and total perceptible water (TPW) parameters have been monitored over Allahabad region of Uttar Pradesh state for 2019. In winter season, very less TPW has been seen over January and February months. On 26th January when there is a normal rainfall event the TPW value is 22 mm. Apart from that event there was no events. The TPW values were very less ranging between 9 and 22 mm. In pre-monsoon season, the TPW values were ranging between 12 and 35 mm. In monsoon season, the TPW values were higher. The TPW values ranges between 35 and 70 mm. In post-monsoon season, the TPW values were higher mainly in October. The TPW values were very low in November and December months (14 -41 mm).

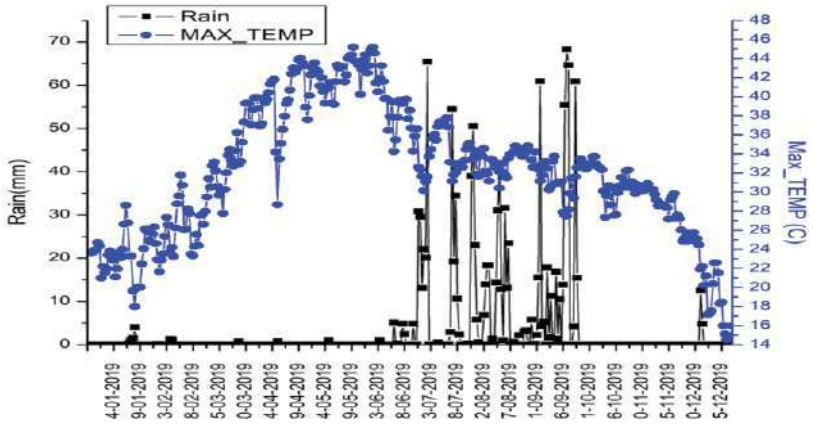

FIGURE 1. Time series plot of rainfall and maximum temperature for Allahabad region during 2019.

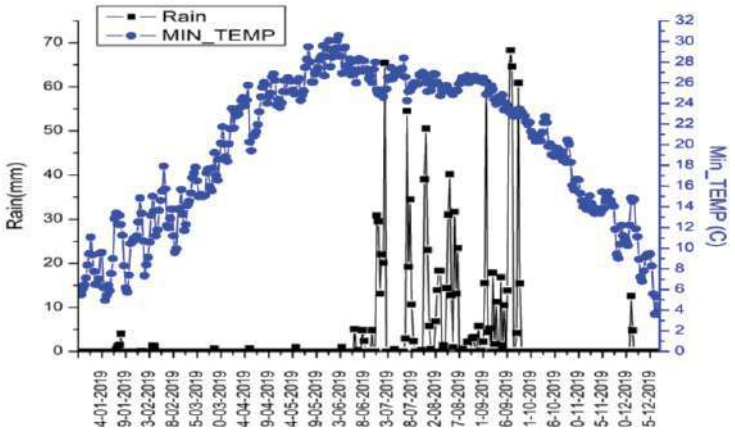

FIGURE 2. Time series plot of rainfall and minimum temperature for Allahabad region during 2019.

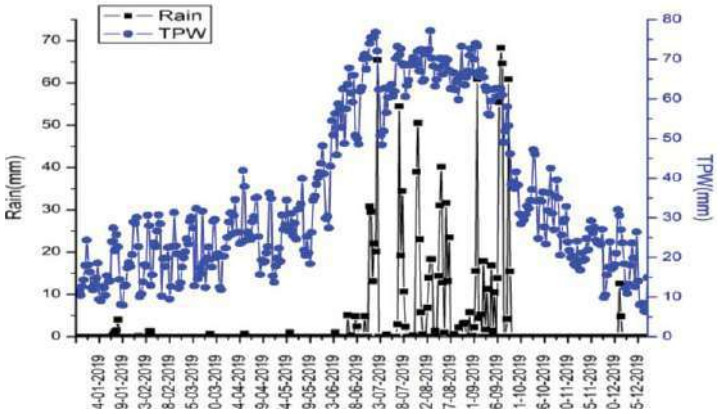

FIGURE 3. Time series plot of rainfall and total perceptible water for Allahabad region during 2019.

In FIGURE 4, the rainfall and K-Index (KI) parameters have been monitored over Allahabad region of Uttar Pradesh state for, 2019. In winter season, very less KI has been seen over January and February months. On 26th January when there is a normal rainfall event the KI value is 20 C. Apart from that event there were no events. The KI values were very less ranging between 3 and 30 K. In pre-monsoon season, the KI values were ranging between 10 and 36 C. In Monsoon season, the KI values were higher. The KI values ranges between 20 and 42 K. In post-

monsoon season, the KI values were higher mainly in October. The KI values were very low in November and December months.

In FIGURE 5, the rainfall and Total Totals Index (TTI) parameters have been monitored over Allahabad region of Uttar Pradesh state for 2019. In winter season, very less TTI has been seen over January and February months. On 26th January when there is n normal rainfall event the TTI value is 47 K. Apart from that event there were no events. The TTI values were very less ranging between 10 and 30 K. In pre-monsoon season, the TTI values were ranging between 36 and 52 K. In monsoon season, the TTI values were higher. The TTI values ranges between 39 and 45 K. In post-monsoon season, the TTI values were higher mainly in October. The TTI values were very low in November and December months.

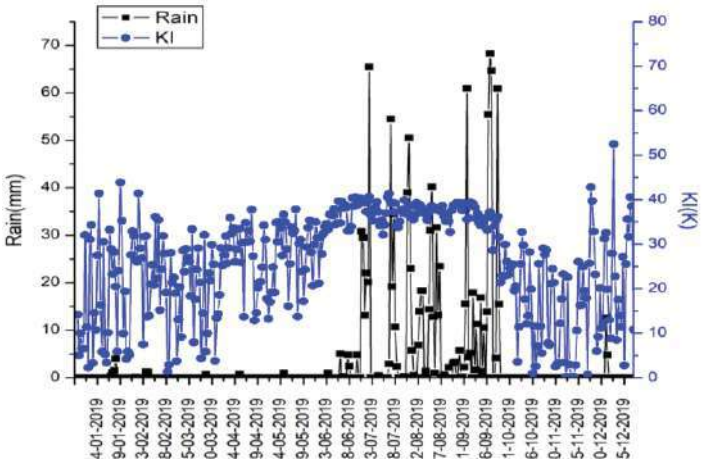

FIGURE 4. Time series plot of rainfall and K-Index (KI) for Allahabad region during 2019.

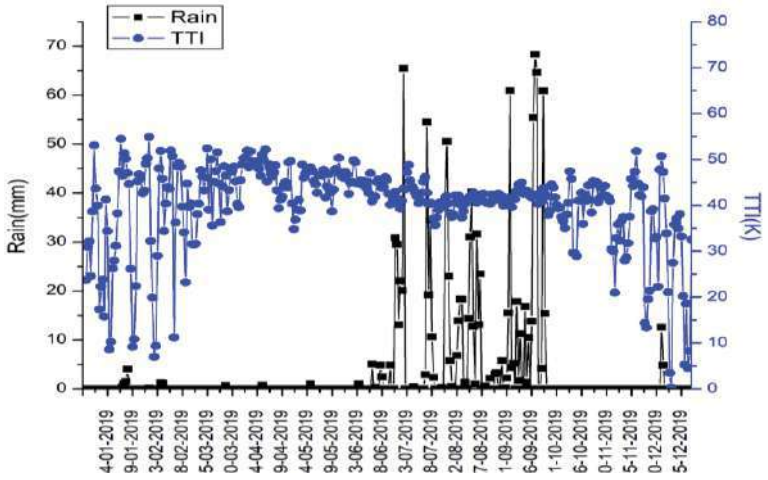

FIGURE 5. Time series plot of rainfall and Total Totals Index (TTI) for Allahabad region during 2019.

CONCLUSIONS

This research exposes the heavy rainfall events over Allahabad region during 2019. In winter season, a normal event was seen. In pre-monsoon season, there were no extreme rainfall events whereas 35 events in monsoon season. Finally in post-monsoon season, 5 events were recorded. The pre-monsoon temperatures were higher ranging between 36 and 45°C. High amount of total perceptible water (TPW) is seen in monsoon season. The KI and TTI values also indicated higher values when there is a severe rainfall. The KI values were higher in pre-

monsoon but heavy rainfall events were more in monsoon. This indicates that KI is indicating a chance for rainfall based on temperature fluctuations. For extreme rainfall, humidity based moisture is more important than temperature.

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DNA barcoding and molecular systematics in genus *Coelogyne* Lindl. (*Orchidaceae*)

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Abstract

Subtribe *Coelogyninae* (*Epidendroideae*, *Orchidaceae*) comprises 16 genera, including *Coelogyne* Lindl. The aim of this study was to reveal the sectional relationships, and relations to the genus *Pholidota*, using DNA bar coding. This technique helps the identification of species even if only a small piece of material is available. In the present study, the DNA barcode was based on *rbcL*, the Rubisco large sub unitfrom the chloroplast genome. The inter-specific divergence values and species discrimination rates were calculated by Kimura 2 parameter (K2P) using MEGA 4.0. The *rbcL* with average interspecific divergence values yielded 72.72% species resolution, and could distinguish all the species of *Coelogyne* and *Pholidota* investigated. Invariably the genus *Pholidota* shows a close affinity with *Coelogyne*. This supports the inclusion of the genus *Pholidota* in the subtribe *Coelogyninae* of tribe *Coelogyneae*.

Keywords: DNA barcodes, *Coelogyne, Pholidota, rbcL*, sectional delineation

INTRODUCTION

The *Orchidaceae* is one of the largest families of flowering plants, comprising 763 genera and 28,000 species. With 1256 species across 155 genera, orchids in India represent the third largest flowering plant family (Singh et al., 2012). The genus *Coelogyne* Lindl. comprises c. 550 species, with over 200 species distributed throughout South-East Asia, Sumatra and Himalayas (Butzin, 1992). Most *Coelogyne* species are epiphytes, some are lithophytes, and there are a few terrestrial plants (Comber, 1990). In India, most orchid habitats are dwindling due to habitat destruction and fragmentation, landscape development, river valley projects and other infrastructural developments. Except few a recent publications (Parab and Krishnan, 2008; Khasim and Ramesh, 2010; Chaudhary et al., 2012; Ramudu et al., 2012), there has been little research directed at the molecular characterization of Indian orchids. In view of above background, this study explored the molecular characterization of some Indian orchids belonging to subfamily *Epidendroideae*.

MATERIALS AND METHODS

For molecular studies, plant materials (one population each) belonging to the tribe *Coelogninae*, sub-tribe *Coelogneae*, sub-family *Epidendroideae* were collected from geographical locations in Andhra Pradesh, Tamil Nadu and Kerala in Southern India. Some species were also procured from Tropical Botanical Garden and Research Institute (TBGRI), Pallode (Kerala) and National Orchidarium, Yercaud (Tamil Nadu).

Isolation of genomic DNA

The genomic DNA was isolated using the CTAB (N-acetyl-N,N,N tri methyl-ammonium bromide) technique (Doyle and Doyle, 1987). Leaf tissue of 100 mg was ground into powder and 600 mL of cold extraction buffer (3% CTAB, 1.42 M NaCl, 20 mM EDTA, 100 mM Tris-HCl, pH 8.0, 2% polyvinylepyrrolidone, 5 mM ascorbic acid) added. The tissue was further homogenized for 3 min. The entire homogenization process was performed in liquid nitrogen.

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Homogenized samples were treated at 65°C for 15 min and then extracted once with chloroform-isoamyl alcohol (24:1, v/v) to obtain a clear supernatant. This supernatant was centrifuged at 12,000 rpm for 5 min. This supernatant containing plant genomic DNA was transferred to a fresh test-tube to which was added a one-fifth volume of 5% CTAB solution in 0.7 M NaCl, and extracted again with chloroform-isoamyl alcohol. The DNA was precipitated from the supernatant by the addition of two volumes of cold absolute ethanol. After incubation at -80°C for 15 min, the DNA was centrifuged at 12,000 rpm for 20 min at 4°C. After rinsing the DNA pellet in cold 70% ethanol, the DNA was dried under vacuum and resuspended in 100 µL of distilled water. DNA concentration was determined by absorbance at 260 nm and the quality assessed by using 1% agarose gel electrophoresis.

Amplification of *rbcL* and sequencing

The universal primers were downloaded from Kew website (http://www.kew.org/barcoding) and aligned with the chloroplast genome of some orchids (Table 1).

Table 1. Primers used for amplification of *rbcL* DNA barcodes.

S. no.	Locus	Primer name	Primers sequence
1.	*rbcL*	*rbcL_F*	5' ATGTCACCACAAACAGAGACTAAAGC 3'
		rbcL_R	5' GAAACGGTCTCTCCAACGCAT 3'

Some changes toward with 5' end served as new primers for the amplification of the *rbcL* locus. These new primers were obtained from Helini Biomolecules, Chennai, Tamil Nadu. The PCR reaction mixture contained 1 unit of *Pfu* DNA polymerase, 2 µL each of 10× PCR buffer with $MgSO_4$, 2 mM of each of the dNTPs, 10 µM forward and reverse primers, and 20-30 ng of template DNA. The final volume was made to 20 µL with autoclaved MQ. For amplification of *rbcL* locus from the chloroplast genome the thermal cycle was: one cycle of 5 min at 94°C; 35 cycles of 30 s each at 94°C, 40 s at 50°C, 1 min at 72°C with a final extension of 7 min at 72°C. The PCR products were electrophoresed in 1% TAE agarose gels, containing 5 mg mL^{-1} EtBr and visualized on a UV trans-illuminator. The PCR products were cleaned by Exo-SAP method: the samples that yielded single band of amplicon were cleaned using a mixture of two enzymes (exonuclease and shrimp alkaline phosphatase). The enzymes treatment degrades the unused primers left after the amplification and removes the phosphate group from all the left over dNTPs in the reaction mixture. The removal of these two components is necessary as these can cause hindrance in Sanger's di-deoxy sequencing reaction. A total of 2 µL of enzyme mixture, consisting of 0.5 µL of exonuclease I, 1 µL of shrimp alkaline phosphatase and 0.5 µL of MQ was used to clean 8 µL of PCR product, making the reaction mixture a total of 10 µL. The mixture was then incubated at 37°C for 15 min, 85°C for 15 min, and finally held at 4°C in a thermal cycler. The cleaned-up PCR products were stored at -20°C. The purified PCR product was subjected to bi-directional Sanger's di-deoxy sequencing (Sanger et al., 1977) using BigDye terminator v3.1 cycle sequencing kit on an ABI Prism 3700 DNA Analyzer (Applied Biosystems Inc., USA).

Analysis of DNA sequence data

The chromatograms obtained from the sequencer were base-called, using Phred. The sequences with a Phred score of >20 were taken for further analysis. The forward and reverse sequences were trimmed and assembled, using Sequencher (Gene Codes Corporation, Ann Arbor, Michigan, USA). Each sequencher project file (.spf) consisted of all the sequences of a single species and its consensus sequence was taken as the representative sequence for that particular species. The sequences were submitted to GenBank, NCBI and accession numbers were obtained for each sequence.

RESULTS

Interspecific K2P distances and species discrimination for *rbcL* at the tribe and sub-tribe level

The interspecific variation and species discrimination rates among the congeneric species were also calculated individually for the *rbcL* locus for the *Coelogyneae* tribe. The species discrimination rates were calculated using both genetic distance and phylogenetic tree methods. For the later, neighbor joining (NJ) trees with thousand bootstrap replicates were constructed for the tested *rbcL* locus.

The tribe *Coelogyneae* is divided into subtribes *Thuniinae* and *Coelogyninae* (Dressler, 1993) and two genera (*Coelogyne* and *Pholidota*) of sub-tribe *Coelogyninae* were studied here (Figure 1). The interspecific K2P distance matrix of 11 *rbcL* sequences obtained from 11 species of *Coelogyninae* analyzed averaged 0.005 (range from 0 to 0.017). Out of the 11 species analyzed, seven species had zero distance and the maximum interspecific K2P distance (0.017) was recorded between *Coelogyne trinervis* and *C. flaccida*.

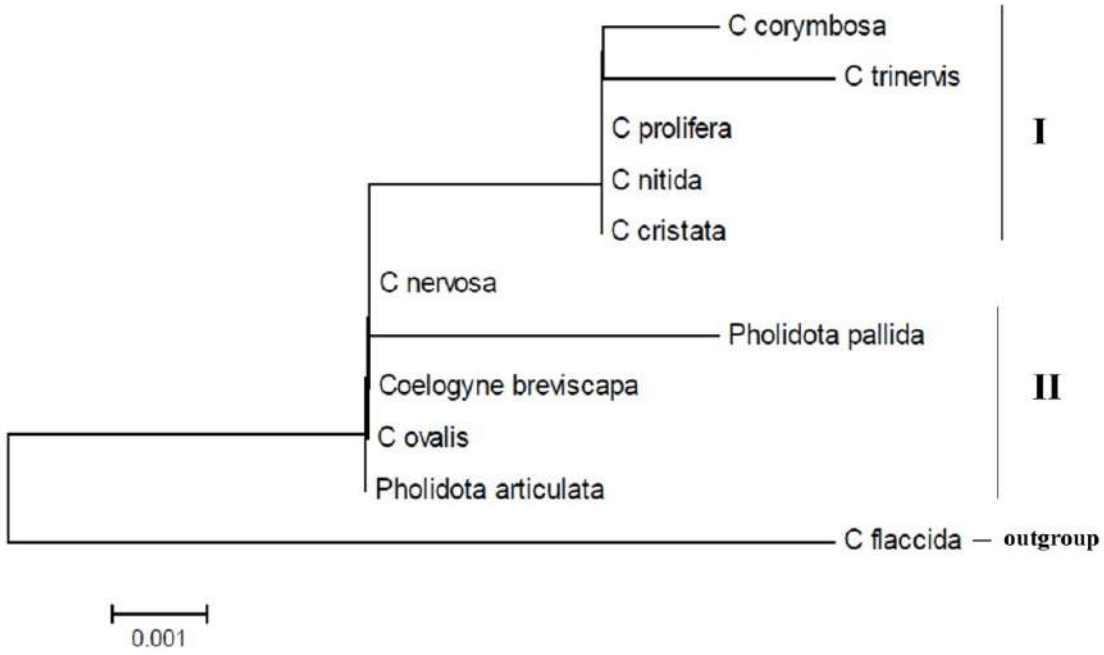

Figure 1. Dendrogram of *Coelogyneae* based on phylogenetic tree building method.

Species resolution

1. Distance based method.

The *rbcL* distance matrix of 11 species belonging to subtribe *Coelogyninae* could not be resolved because of their zero distances with other species. Consequently, seven species remained unresolved (Figure 1). Therefore, species resolution based on *rbcL* sequences was only 36.36%. The species with zero distance estimates are *C. breviscapa, C. cristata, C. nervosa, C. nitida, C. ovalis, C. prolifera* and *Pholidota articulata*.

2. Phylogenetic tree building method.

The analyses of aligned *rbcL* sequences simplified from 11 species revealed that there are 144 variable sites, parsimony informative 17, singleton sites 29, 861conserved sites out of total 1,127 nucleotide sites. The neighbor joining tree with thousand boot straps replicates revealed three different clusters comprising seven unresolved species; thus enabling species resolution of 72%. The species clusters formed are shown in Figure 1. These clusters are as

follows: i) *C. corymbosa, C. trinervis, C. nitida, C. prolifera* and *C. cristata*; ii) *C. nervosa, C. breviscapa, C. ovalis*, *P. articulata, P. pallida.* Outgroup *C. flaccida.*

3. BLAST method.

The *rbcL* locus of 11 investigated species on BLAST analysis afforded 60.15% species resolution. Species discrimination rate based on this method was 51.29%.

Interspecific K2P distances and species discrimination rates for *rbcL* at the generic level

The interspecific variations and species discrimination rates among the congeneric species were also calculated individually for *rbcL* locus for three genera, based on genetic distance and phylogenetic tree methods. For the latter, the Neighbor joining trees with thousand bootstrap replicates were constructed for tested *rbcL* locus.

***Coelogyne* Lindl.**

In *Coelogyne*, nine species were evaluated (all species in Table 2, plus *Coelogyne stricta*). The average interspecific K2P distance for *rbcL* was 0.007. The *rbcL* locus had resulted in three species pairs with distances estimate zero (Table 2). The species discrimination rate for *rbcL* was 44.44. Out of the 1108 nucleotides site of *rbcL* sequences, 276 were variable sites, 11 parsimony informative and 27 singleton. The *rbcL* locus showed species clusters with different number of unresolved species (Figure 1). Thus resulting species resolution is 44.44%. The *rbcL* sequences showed 11 parsimony sites and 276 variable sites.

Table 2. Sectional delineation in the genus *Coelogyne* Lindl. Arranged according to Subedi et al. (2011).

Section	Taxa
Ocellatae Pfitzer & Kraenzlin	1. *C. corymbosa* 2. *C. nitida*
Cristatae Pfitz.ex. Pfitz. & Kranzl.	1. *C. cristata* 2. *C. nervosa*
Elatae Pfitz.ex. Pfitz. & Kranzl.	*C. stricta*
Fuliginosae Pfitz. & Kranzl.	*C. ovalis*
Lentignosae Pfitz.ex. Pfitz. & Kranzl.	*C. breviscapa*
Proliferae sens. str. Lindl.	*C. prolifera*
Flaccidae Lindl.	1. *C. flaccida* 2. *C. trinervis*

***Pholidota* Lindl**

In the genus *Pholidota*, two species, viz., *P. pallida* and *P. articulata* were studied. Average interspecific distance of *rbcL* locus is 0.002; and the *rbcL* sequences of two species are variable enough to distinguish two species (Figure 1).

DISCUSSION

The *rbcL*, a plastid gene, has proven to be an important tool to address phylogenetic relationships at various taxonomic levels (Cameron et al., 1999). This gene is located in the large single copy region of the chloroplast genome and encodes the large subunit of ribulose 1,5-biphosphate corboxylase/oxygenase (RUBISCO) (Soltis and Soltis, 1998). It has been sequenced in about 5000 plant species (Sanderson, 2003). Studies on *Cypripediodeae* by Alberts et al. (1994) and *Dendrobiinae* indicate that the amount of sequence divergence exhibited by *rbcL* is sufficient and suitable to address the phylogenetic relationships at generic and species level within the family *Orchidaceae*. *rbcL* was used here for DNA barcoding based on various recommendations and published methods (Chase et al., 2005; Kress et al., 2005; Consortium for the Barcode of Life (CBOL, http://www.barcoding.si.edu/protocols.html);

BOLD (Barcode of life Datasystems); Ratnasingham and Hebert, 2007).

Species discrimination

Genetic distance, the phylogenetic tree method and BLAST analysis methods have been used to resolve species. The perfect barcoding gap assigns an unknown individual to its respective species correctly (Meyer and Paulay, 2005). In the phylogenetic tree method with cluster analysis, the phylogenetic tree is constructed using sequences of the *rbcL* locus based on percent species resolution (Lahaye et al., 2008). The NJ tree based analysis for species discrimination also provides a convenient method of viewing the data as the unresolved species clusters can be identified easily. It also helps in identifying synonymous species. Thus, *Pholidota pallida* and *P. imbricata* used to be considered as two different species, but are now accepted as synonyms in the Kew Checklist (Govarts et al., 2010).

The *rbcL* locus from chloroplast genome exhibited very low and varied species discriminatory powers (50-75%) using all the three methods. The present study is based on previous investigations of *rbcL* locus for reconstructing phylogenies only at family and subfamily level as it has limited application at the species level (Cameron et al., 1999; Singh et al., 2012; Parveen et al., 2012). Moreover, among congeneric species, the resolving power was very low.

Interspecific variability and phylogenetic relationships

From all dendrograms constructed based on K2P distances and bootstrap percentage, it is evident that *Coelogyne flaccida* belongs to *Coelogyne* section *Flaccidae* seems to be a outgroup (Figure 1). However, another species, viz., *C. trinervis* belonging to same section shows separately in cluster II of the dendrogram (Figure 1). These results indicate that *Flaccidae* appears to be polyphyletic. However, Gravendeel and Vogel (2000) observed that *Flaccidae* is monophyletic but with low bootstrap support. From the dendrogram (Figure 1), it is evident that *C. ovalis* belonging to section *Fuliginosae* shows close affinity with *Proliferae* and *Crystatae*. The present molecular data indicate that the genus *Coelogyne* is polyphyletic. These results are in accordance with studies of Gravendeel et al. (2001). The dendrogram (Figure 1) shows that the *Coelogyne* section *Ocellatae* under which *C. corymbosa* and *C. nitida* placed, forms single cluster (bootstrap percentage of 50 and 24, respectively) with other species of *Crystatae* and *Lentiginosae*. The present molecular results and dendrogram (Figure 1) indicate that there is a clear species discrimination between *C. nitida* and *C. corymbosa.* The term *Ocellatae* refers to the eye-shaped spots present on the lip of the flower; this section is characterized by having relatively few flowers, glabrous ovary and pedicel, and lateral lobes of the lip with distinct color patches (Subedi et al., 2011). However, Subedi et al. (2011) also stated that it is monophyletic based on DNA sequence data. Whereas *Coelogyne* section *Crystatae* under which *C. cristata* and *C. nervosa* were placed, seems to be polyphyletic (Figure 1). However, further study is needed to ascertain whether the entire *Coelogyne* genus is polyphyletic.

The present study also showed that there is a clear species discrimination of *Pholidota articulata* and *P. pallida*, but with a low bootstrap percentage of 24 and 15, respectively. Invariably the genus *Pholidota* shows close affinity with *Coelogyne*. This supports the inclusion of genus *Pholidota* in the subtribe *Coelogyninae* of tribe *Coelogyneae*. Present anatomical evidences also support the placing of *Pholidota* in the subtribe *Coelogyninae*.

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Genetic diversity of an endemic medicinal orchid, *Coelogyne nervosa*, from southern India using morphological and molecular markers

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Abstract

Genetic diversity of *Coelogyne nervosa* A. Rich. was investigated using SDS-PAGE, RAPD markers and morphological characters. *C. nervosa* grows as an epiphyte as well as a lithophyte in the Eastern and Western Ghats of India. Leaf samples collected from these two reference sites were taken for RAPD and protein profile analysis. Vegetative parts such as leaves, pseudobulbs and roots were fixed in FAA. Free-hand and microtome sections were cut and stained with safranin-fastgreen. The objective of this study is to assess the genetic diversity of endemic orchid *C. nervosa* distributed in southern India. The six populations collected from these two geographical regions exhibited significant variation in their morphological and molecular characters. The stomata are tetracytic and hypostomatic in distribution. The maximum thickness of cuticle and midrib region in leaf and, extensive lignification in exodermis and endodermis of root were recorded in populations located in the Western Ghats as compared to those of the Eastern Ghats. It is interpreted to be associated with the greater need to conserve water. RAPD and protein profile data showed the inter population diversity between these two reference sites. This can be attributed to the different ecological and climatic conditions prevailing in the Eastern and Western Ghats of India.

Keywords: SDS-PAGE, RAPD analysis, anatomical features, *C. nervosa*

INTRODUCTION

The endemic orchid, *Coelogyne nervosa* R. Rich. belongs to the *Coelogyninae* of tribe *Coelogyneae*, in the family *Orchidaceae* (Dressler, 1993). The genus *Coelogyne* is distributed in Australasia, tropical Asia, including India, and China. The Western Ghats and Eastern Ghats of India are rich in orchid flora. These habitats face destruction, and illegal collection of plants has jeopardised the size and frequency of orchid natural populations. There is a need to evolve conservation strategies for this group of angiosperms before the path to extinction is irreversible. As part of a strategy for a long-term conservation, the maintenance of genetic diversity within and among the populations is very important (Avila-Díaz and Oyama, 2007).

Recently genetic polymorphism in many plants has been documented by using various molecular markers including isozymes. For example, the analysis of isozymes and RFLP (restriction fragment length polymorphism) revealed relatively little polymorphism in *Scutellaria* (*Lamiaceae*) (Hosokawa et al., 2000). The disadvantages of complex procedures and expensive costs strongly restrict the application of AFLP (amplified fragment length polymorphism) and SSR (Zha et al., 2009). By contrast RAPD (random amplified polymorphic DNA), amplified by arbitrary primers could be very useful and low cost genetic markers (Williams et al., 1990; Hosokawa et al., 2000). Besse et al. (2004) studied the genetic diversity in cultivated vanilla orchid using RAPD markers. In orchid species studied, genetic diversity

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has varied from very low to very high. Widespread species generally having higher levels of variation than endemic species with a narrow geographical range, and usually larger populations have more diversity (Gustafsson, 2000; Avila-Díaz and Oyama, 2007). RAPD is a powerful tool to estimate the range of genetic variability and the findings are a valuable consideration when evolving conservation strategies of particular species. The objective of present study was to assess the genetic diversity of *C. nervosa* by using morphological and molecular markers, such as RAPD and SDS-PAGE (sodium dodecyl sulphate polyacrylamide gel electrophoresis) protein profiles.

MATERIALS AND METHODS

Study area

Two major reference sites, i.e., the Western and Eastern Ghats of India were selected for the present study (Figure 1). Populations 2, 4, 5, 6 were situated in the Western Ghats whereas remaining two (P-1 and P-3) were located in the Eastern Ghats (Table 1).

Table 1. *Coelogyne nervosa* populations from the Eastern and Western Ghats of India.

Population site	Host or substrate	Altitude (m a.s.l.)
1. Yercaud (Eastern Ghats)	*Lithophyte*	1500
2. Dodabetta (Western Ghats)	*Terminalia alata*	2623
3. Palani Hills (Eastern Ghats)	*Proteum serratum*	2195
4. Kodaikanal (Western Ghats)	*Pterocarpus marsupium*	2010
5. Waynad (Western Ghats)	*Lithophyte*	1500
6. Munnar (Western Ghats)	*Lithophyte*	1400

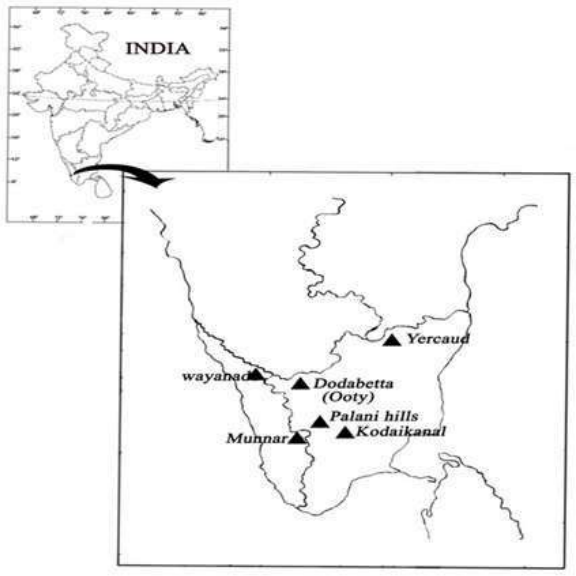

Figure 1. Study area map showing sampling sites in India.

The Western Ghats harbour a rich variety of plant life in its scrub jungles, moist and dry deciduous forests, tropical wet evergreen forests, montane grasslands and sholas. The Western Ghats are known for the luxuriant growth of orchids. The Eastern Ghats comprises a disconnected of hills extending along northeastern-southwestern direction in the east coast. This ranges starts from Tamil Nadu in south and extends up to Orissa through Andhra Pradesh in the north. The vegetation is dry deciduous.

Anatomical studies

In total, six populations (P_1 to P_6) were selected from two major geographical areas. Vegetative parts (leaves, pseudobulbs and roots) were collected from these six populations growing on different host trees and substrate (Table 1). The materials were fixed in formaline-acetic-alcohol and the standard procedure of dehydration and embedding followed (Berlyn and Miksche, 1976; Khasim, 2002). Microtome and free-hand sections were cut at a thickness of 10-15 µm and stained with safranin-fastgreen.

Molecular studies

Leaf material collected from the six populations was used for the molecular studies.

1. SDS-PAGE.

Fresh leaves of 2 g were crushed in extraction buffer containing 1.4 M NaCl, 20 mM EDTA (ethylene diamine tetracetic acid) 100 mM Tris-HCl (pH 8.0), 2% CTAB (N-cetytl-N, N, N trimethyl ammonium bromide) and 0.2% marcaptoethanol with mortar and pestle. The preparation was subjected to SDS-PAGE (Shi and Jackowski 1998). The protein banding pattern was observed. The protein molecular weight markers ranging from 14 to 116 kD were used for comparison.

2. RAPD analysis.

A modified CTAB technique (Doyle and Doyle, 1987) was used for the extraction of genomic DNA and PCR amplification. Only six primers were used in this study (Table 2). PCR was performed in a reaction volume of 25 µL containing 50 mM KCl, 10 mM Tris HCl (pH 9.0), 0.1% triton X-100, 1.5 mM $MgCl_2$, 100 µM each of dNTPs, 25 P mole primer, 100 ng genomic DNA and 1 unit of Taq DNA polymerase.

Table 2. Primer sequencing, amplified bands, polymorphic bands and percentage polymorphism in RAPD analysis of six populations of *C. nervosa*.

S. no.	Primer	Primer sequence 5'-3'	Amplified bands (n)	Polymorphic bands (n)	Polymorphism (%)
1	Primer 1	5'GGTGCGGGAA 3'	5	4	80
2	Primer 2	5'CCCGTCAGCA 3'	5	4	86.6
3	Primer 3	5'GTTTCGCTCC 3'	4	3	75
4	Primer 4	5'AAGAGCCCGT 3'	5	4	80
5	Primer 5	5'GTAGACCCGT 3'	4	2	50
6	Primer 6	5'AACGCGCAAC 3'	7	5	71.4
Total			**31**	**22**	

Amplified products were resolved electrophoretically on 1.5% agarose gel run at 100 V, and visualized by staining with ethidium bromide. RAPD bands were scored as present or absent for each DNA sample and analysed according to the definition of genetic similarity of Nei and Li (1979), i.e., Sij = 2a/(2a+b+c), where SiJ is the similarity coefficient between two individuals (i and J), 'a' is number of bands in both i and J, 'b' is number of bands present in *i* and absent in *J* and 'c' is the number of bands present in *J* and absent in *J*. The matrix of similarity was clustered using UPGMA algorithm and a dendrogram constructed.

RESULTS AND DISCUSSION

Morphological and anatomical studies

In *C. nervosa*, leaf was coriaceous and pseudobulb showed nerve like lines on its surface.

1. Leaf.

Epidermal cells in the leaf were relatively larger on the abaxial surface, and were rectangular to polygonal in shape. Stomata were (Figure 2a) confined to the abaxial surface

(hypostomatic distribution). The leaves are similarly hypostomatic in most orchid species (Avadhani et al., 1982). Rasmussen opined that hypostomaty is more frequent in mesophytic orchids and amphistomaty dominates in those of dry and humid habitats. Tetracytic stomata were observed in all six populations (Figure 2a). The length and width of guard cells are given in Table 3. The maximum and minimum length of guard cells was 35 µm in both P_2 and P_3, and 31.2 µm and P_4, respectively.

Table 3. Morphological characters of *Coelogyne nervosa*.

Morphological characters	P_1	P_2	P_3	P_4	P_5	P_6
Leaf						
Thickness of cuticle (µm)	3, 3-4	4, 4-5	3, 4	3, 4	4, 5-6	4, 6
Thickness of midrib region (µm)	253	298	273	272	307	328
Thickness of laminar region (µm)	203	231	220	205	251	234
Midrib vascular bundle length (µm)	172	190	189	192	182	162
Midrib vascular bundle width (µm)	149	139	132	132	129	143
Guard cell length (µm)	32.3	35	35	31.2	32	32
Guard cell width (µm)	26.2	30	29	24	26	27
Size of the stomatal pore (µm)	14.5	22.2	19.1	20	18	18.2
No. of phloem cap layers (µm)	5	5	5-6	6-7	6	5
No. of xylem cap layers (µm)	3-4	4	4	4	5	4
Pseudobulb						
Thickness of cuticle (µm)	28	32	35	29.2	32	35
No. of xylem cap layers (µm)	3-4	3	4	3	4	3-4
No. of phloem cap layers (µm)	5	5	5	4	6-7	5-6
Root						
No. of velamen layers (µm)	3-4	4-5	2-4	3-4	4-5	4
Vascular bundle size (µm)	529	512	412	382	332	402
Lignification of exodermis (µm)	20.2	15.2	16.3	15.1	17.2	19.1
Lignification of endodermis (µm)	29	26.9	25	28	26.7	30.2

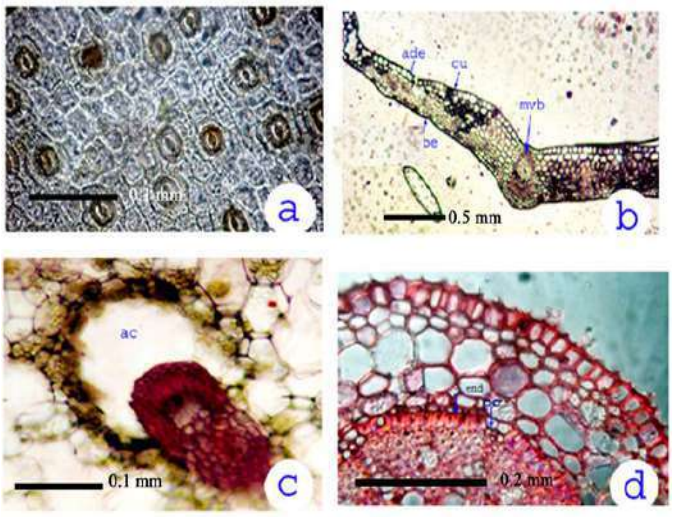

Figure 2. Anatomical features of *C. nervosa*. (a) Stomata from abaxial epidermis of leaf; (b) Transection of leaf showing midrib vascular bundle; (c) Pseudobulb transaction showing air cavity towards the phloem cap; (d) Root transaction showing 'O' shaped thickened endodermal cells. Key: ade – adaxial epidermis, cu – cuticle, mvb –midrib vascular bundle, ac – air cavity, end – endodermis, pc – passage cell.

In transection, the leaf was V-shaped at the midrib and flattened at the laminar region (Figure 2b). A thick cuticle was developed on both surfaces; however, it was more thickened in P-5 and P-6 (both lithophytes from the Western Ghats) compared to other populations. Mesophyll was homogeneous. Highest number of fibre cap layers (6-7) was observed in populations of Western Ghats (Table 3).

2. Pseudobulb.

In transection, the pseudobulb was circular in outline. The highest cuticular thickening was observed in P3 (Eastern Ghats), and also in P-5 and P-6 (both from the Western Ghats). Ground tissue consists of large and small parenchymatous cells with abundant mucilage. Large and small vascular bundles were distributed in ground tissue. Air cavities were conspicuous towards phloem cap in all six populations (Figure 2c). Such air cavities were also reported in *Otochilus alba* (Mohana Rao and Khasim, 1987). The presence of air cavities in some members of *Coelogyninae* enables them to be light in weight (Kaushik, 1983).

3. Root.

In all populations of *C. nervosa* velamentous roots were observed. An exodermis, which lies just below the velamen, possessed U-shaped thick-walled cells and also thin-walled passage cells (Figure 2d). Endodermis was highly lignified with squarish, uniformly thickened cells; it was interrupted by a cluster of passage cells lying opposite to passage cells (Figure 2d). Maximum lignification in the endodermal cells was observed in lithophytic populations when compared to epiphytes.

Though the Western Ghats are congenial for the luxuriant growth of orchids, the lithophytic populations had a xerophytic nature. It was also evident from the anatomical data that the host tree plays an important role in supplying nutrients. In this context, Khasim and Ramesh (2010) also opined that the degree of supply of nutrients varied from one host tree to other. P-2 from the Western Ghats showed maximum cuticle thickening and a higher number of velamen layers. This can be attributed to the host tree, *Terminalia alata* on which P-2 grows, as the nutrient supply would be little. Accordingly, it has undergone structural adaptations so as to conserve the nutrients and utilise them judiciously.

Molecular diversity

1. SDS-PAGE protein profile.

In *C. nervosa*, the SDS-PAGE protein profile showed multiple bands of varied molecular weight ranging from 14 to 116 kD in six populations (Figure 3). Out of 83 bands, an average of 13 bands per population were observed. There were 36 polymorphic bands observed in all populations. The protein band thickness and staining intensity showed variation among six populations. The SDS-PAGE protein profile (Table 4) also showed that higher molecular weight proteins were present in P-2 (110.86 kD) and the lowest in P-4 and P-6, all from the Western Ghats (14 kD).

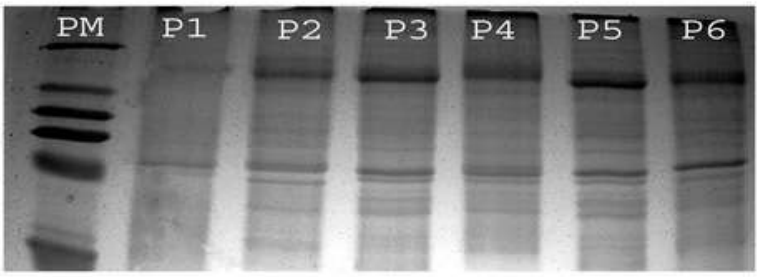

Figure 3. SDS-PAGE protein banding pattern in six populations of *C. nervosa*. Key: PM – Protein marker, P1 – Yercaud, P2 – Dodabetta, P3 – Palni, P4 – Kodaikanal, P5 – Wayanad, and P6 – Munnar.

Table 4. Protein banding pattern and molecular weight in six populations of *C. nervosa* based on SDS-PAGE.

Protein bands	1	2	3	4	5	6	7	8	9
Population 1	81.4	29.3	28.5	27.9	25.2	18.1			
Population 2	110.8	106.3	86.9	76.4	62.3	42.2	36.4	32.0	29.1
Population 3	109.6	105.9	98.2	91.8	80.6	72.9	41.9	35.4	33.3
Population 4	110.6	103.5	95.5	76.8	62.8	50.1	43.2	34.7	30.7
Population 5	105.9	104.3	94.0	88.0	78.9	70.9	70.7	57.7	48.3
Population 6	102.3	97.0	91.8	72.9	59.3	47.0	41.2	36.3	31.9
Protein bands	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
Population 1									
Population 2	27.5	23.0	16.0						
Population 3	31.3	28.5	27.5	24.9	22.9	16.8			
Population 4	29.1	28.6	27.5	25.8	22.6	17.1	14		
Population 5	40.6	34.5	30.8	28.6	27.5	25.5	25.8	24.5	22.5
Population 6	29.0	27.8	26.6	24.7	23.8	16.5	14.8		

2. RAPD banding pattern.

The RAPD amplification profile showed variability among six populations of *C. nervosa* (Figure 4). There were 6 primers chosen to generate 31 RAPD fragments, of which 22 bands were polymorphic for all populations (Figure 4). Primer 2 was found to produce the highest percentage of polymorphism. The percentage of polymorphism ranged from 50 to 86.6%. These data showed that there is a considerable degree of genetic diversity at the interspecific level. The Nei's genetic similarity matrix of all populations is presented in Table 5. The highest value for the similarity coefficient (0.926) was found between P-5 and P-1, while the lowest (0.838) was between P-4 and P-3. In order to analyse the relationship among populations studied, the UPGMA-based dendrogram was constructed using paired matrix values (Figure 5). From the dendrogram, it is evident that P-1 (Eastern Ghats), P-5 (Western Ghats), and P-4 (Western Ghats) form one cluster and the remaining P-2 (Western Ghats), P-6 (Western Ghats) and P-3 (Eastern Ghats) another cluster. This suggests that not only geographical conditions but also habitat (epiphyte, lithophyte) play vital roles in the survival of species in the forests.

Table 5. Nei's genetic similarity matrix of populations of *C. nervosa* based on RAPD analysis.

Populations	P1	P2	P3	P4	P5	P6
P_1	-					
P_2	0.924	-				
P_3	0.901	0.876	-			
P_4	0.905	0.870	0.838	-		
P_5	0.926	0.868	0.879	0.865	-	
P_6	0.910	0.909	0.875	0.862	0.842	-

The present study showed that there is much genetic diversity among the populations of the same reference site. Besides, there has been a considerable variation found in samples collected from two distinct geographical locations. The gene flow was limited due to the great distance between these two geographical sites. The isolation by distance as well as climatic conditions brought about considerable variation (molecular/genetic and morphological) (Raymond and Rousset, 1995). However, the wide range of molecular weight of protein bands of SDS-PAGE indicate that *C. nervosa* across the Western Ghats has not separated into very distinct sub-populations. Therefore, any threat to the genetic diversity in this species in near future seems limited.

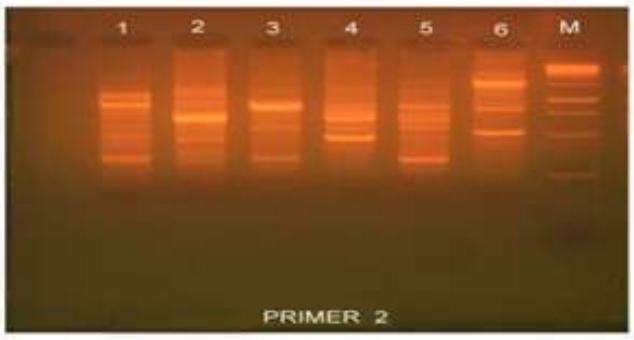

Figure 4. RAPD amplification profiles of *C. nervosa*. Key: P1 – Yercaud, P2 – Dodabetta, P3 – Palni, P4 – Kodaikanal, P5 – Wayanad, and P6 – Munnar.

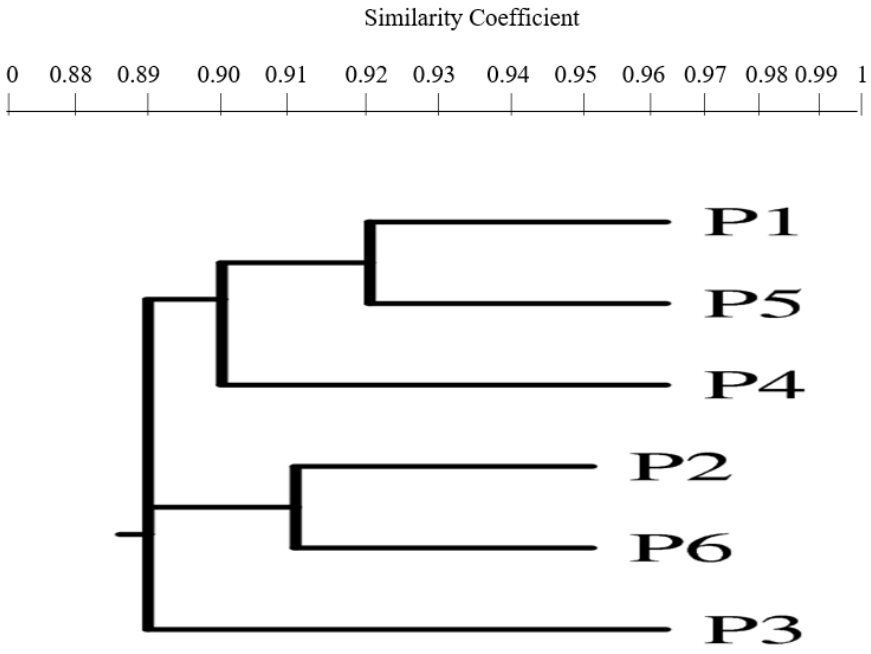

Figure 5. UPGMA dendrogram of *C. nervosa* based on RAPD analysis.

According to Misra's opinion (1995) orchids are highly habitat-specific and they therefore, suffer very much due to the destruction of their delicate habitats. Basumatary et al. (2008) opined that the epiphytic orchids form a variety of associations in the ecosystem and the knowledge on their community dynamics has much significance in formulating effective conservation measures. Therefore, apart from molecular analysis, the studies on community dynamics and interaction with the host tree are equally important before finalizing orchid conservation strategies (Khasim and Ramesh, 2010).

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In vitro multiplication of some selected banana cultivars (*Musa* spp.) from India and their genetic fidelity using ISSR markers

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Abstract

In the present study, in vitro multiplication of three banana cultivars, viz., 'Grand Naine', 'Monthan' and 'Red banana', and genetic fidelity of regenerated plantlets have been taken up. The objective of this investigation is to carry out micropropagation of banana on commercial scale using cost-effective cytokinin (BAP) and auxin (IAA) in order to supply quality saplings to farmers at affordable price. Commercial standard medium and MS basal medium supplemented with BAP at different concentrations and IAA at fixed concentration have been used for micropropagation. To study the genetic fidelity of in vitro-derived plants, ISSR markers were used. In micropropagation experiments, shoot proliferation in commercial standard medium was found to be better in 1.0 and 5 mg L^{-1} BAP for 'Grand Naine'; whereby, production schedule could be determined accurately with quality shoots. However, for 'Monthan', 5 and 10 mg L^{-1} BAP, and for 'Red banana' 10 and 5 mg L^{-1} BAP along with 0.2 mg L^{-1} IAA for each, appeared to be more suitable for obtaining productive shoots. Genetic fidelity studies showed that 'Monthan' is 'true-to-type' as it shows high MIC value of 64.51%; whereas, 'Grand Naine' with 48.41%. In case of 'Red banana', a lot of variation was reported with PIC value of 78.43. This genetic variation could be due to somaclonal variation that had occurred in the micropropagated plants.

Keywords: banana cultivars, in vitro multiplication, ISSR markers, genetic fidelity

INTRODUCTION

Banana is the most popular fresh fruit all over the world and its name comes from the Arabic word 'banan', which means finger. Almost all banana cultivars are derived from *Musa acuminata* and *Musa balbisiana*. India is now the second largest producer of fruits and vegetables in the world and banana stands in the second place for export. Banana is grown in more than 150 countries, producing 105 million tons of fruit per year. The global production of banana is around 102028.17 thousand t of which India contributes 29.19%. Banana (*Musa* spp.) is an important fruit crop in India. Main banana growing states in India are Tamil Nadu, Maharashtra, Gujarat, Andhra Pradesh, and Karnataka.

Banana is a very popular fruit due to its low price and high nutritive value. Its high vitamin B6 content helps fight infection and is essential for the synthesis of 'heme', the iron containing pigment of haemoglobin. The fruit is also rich in carbohydrates and potassium, and is a great source of fibre too.

It is also a good source of phosphorus, calcium, and magnesium. The fruit is easy to digest, free from fat and cholesterol. It helps in reducing the risk of heart diseases when consumed regularly and is recommended for patients suffering from high blood pressure, arthritis, ulcer, gastroenteritis, and kidney disorders.

At present, India is the largest producer of banana in the world with about 30% of total global production. However, the export market share is a meager 1%. With increased productivity/unit area, export capabilities can be improved. This is possible by substituting

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the conventional suckers with virus indexed, tissue cultured plants and adopting scientific methods of cultivation. Most popular varieties cultivated in Andhra Pradesh (India) are 'Dwarf Cavendish', 'Robusta', 'Amritpani', 'Thellachakrakeli', 'Karpoora Poovan', 'Chakrakeli', 'Monthan', and 'Yenagu Bontha'. Farmers are cultivating local cultivars which are low yielding and the productivity of banana is quite low, i.e., 35 t ha^{-1} as against 65 t ha^{-1} in Maharashtra (Karuna and Kameswara, 2016). Micropropagation of selected banana cultivars, such as 'Grand Naine' (G9), 'Monthan', and 'Red banana' on commercial scale using economical cytokinin (BAP) and an effective auxin (IAA) has been taken up in this study in order to supply them to farmers at affordable prices.

MATERIALS AND METHODS

The presently studied cultivars viz., 'Grand Naine' and 'Red banana' were collected from Bangalore; whereas, 'Monthan' from Mysore, southern India. Sword suckers were taken as explants and the plants were regenerated. Suckers were washed with sterilized distilled water, then sterilized in 70% ethyl alcohol and finally surface sterilized in 0.4% mercuric chloride for 15 min, followed by several washes in sterilized distilled water.

Media preparation and sterilization

MS basal medium (Murashige and Skoog, 1962) was used for the culture of selected banana cultivars. The basal medium with different concentrations of BAP with a fixed concentration of IAA at 0.2 mg L^{-1} was used for optimizing an in vitro production protocol for the different selected banana cultivars. The media composition consisting of different concentrations of BAP used along with a fixed concentration of IAA as the auxin at 0.2 mg L^{-1} in MS basal medium used in the present study were designated as M1, M2, M3, M4 and M5 (Table 1). One L of each of the media was dispensed into 25 glass jars and all the media were prepared at one stretch. MS basal medium without growth regulators was taken as control.

Table 1. Different growth regulators used in MS basal medium for optimizing in vitro protocol for selected banana cultivars.

Media code	Growth regulators used	Concentration (mg L^{-1})
M1	BAP+IAA	10+0.2
M2	BAP+IAA	5+0.2
M3	BAP+IAA	3+0.2
M4	BAP+IAA	2+0.2
M5	BAP+IAA	1+0.2

The final volume of medium was made up to 1 L. Each of the glass jars was dispensed with 40 mL of this medium and capped tightly. The jars were sealed with autoclavable polypropylene wrap and autoclaved for 45 min at a temperature of 121°C and a pressure of 15 psi in a horizontal autoclave. The process of media preparation was repeated again every two or three weeks for subsequent culture transfers until the final data recording was completed. The glass jars were incubated at room temperature for a week in a separate clean room to check the sterility of the medium and then used for explants/culture inoculation purpose.

The shoot tip explants measuring 2 to 4 cm were aseptically dissected in the laminar air-flow cabinet and these were inoculated on initiation medium (M1 to M5) (Table 1; Figure 1). Several explants of each of the cultivar were inoculated in initiation and multiplication medium.

Once the cultures attained their full growth by four weeks, they were ready for subculturing and re-transfer to multiplication medium. After repeated multiplication thrice the slightly elongated shoots were transferred to MS basal medium consisting of 1% activated charcoal for further elongation of shoot and rooting to obtain the complete regenerated plantlet.

Figure 1. One explant each of the selected banana cultivars inoculated in each tissue culture jar.

Clonal genetic fidelity of in vitro-derived plants

In vitro grown plants (10 plantlets) from 6th and 8th cycle of 'Grand Naine', 'Monthan', and 'Red banana' were selected for genetic fidelity studies. The leaves of each of the banana cultivars (in vitro derived, as well as mother plant) were taken for isolation of DNA.

Isolation of DNA and selection of primers

Plant genomic DNA was isolated by using CTAB technique (Doyle and Doyle, 1987; Hasan and Khasim, 2018). About 20 ISSR primers were screened for each cultivar to select some best primers. Finally, six selected primers were used to assess the clonal fidelity of the regenerated plantlets in comparison with their respective mother plants (Table 2). The samples of 10 numbers of in vitro generated plants for testing were taken based on the scale of sampling mentioned by NCS-TCP (National certification system for Tissue culture raised plants, Department of Biotechnology, Government of India).

Table 2. Nucleotide sequence of ISSR primers employed for genetic fidelity testing.

ISSR	Primer nucleotide sequence	Annealing temperature (°C)
807	5'-AGA GAG AGA GAG AGA GT-3'	51
808	5'-AGA GAG AGA GAG AGA GC-3'	53
810	5'-GAG AGA GAG AGA GAG AT-3'	51
830	5'-TGT GTG TGT GTG TGT GG-3'	54
834	5'-AGA GAG AGA GAG AGA GYT-3'	52
840	5'-GAG AGA GAG AGA GAG AYT-3'	56

Y = C/T.

PCR and data analysis

ISSR amplification was carried out in 25 µL reaction mixture which consists of 2.5 µL of 10× PCR buffer, 0.2 mM dNTPs, 2 mM $MgCl_2$, 0.6 µM of primer, 0.625 units of *Taq* DNA polymerase (3 U μL^{-1}) and 1 µL of DNA sample (40-100 ng). Amplified products were analysed on a 1.5% agarose gel stained with ethidium bromide in 1×Tris-acetate EDTA buffer and documented under gel documentation system. DNA ladder 1 Kb (Promega) was used for estimating the amplicon sizes.

The amplified products were scored in terms of a binary code as present (1) or absent (0), each of which was treated as a unit character regardless of its intensity. All amplifications were repeated twice and only reproducible bands were scored.

RESULTS AND DISCUSSION

Micropropagation of selected banana cultivars

All the explants inoculated in the initiation medium started responding within 15 days under the given conditions. The outer leaf sheaths of the explants changed from off-white to

green, and started to unfold externally. At the base of explants of 'Grand Naine' and 'Red banana', blackening was noticed due to phenolic exudation. However, there was no blackening observed in 'Monthan', clearly indicating that phenolic exudation was found to be controlled and is not a serious problem. The shoot tip explants of different banana cultivars exhibited varied response in terms of both encouraging growth signs. The categories of general growth response at this stage were assessed with its ability to unfold the leaf sheath and turn into green colour. It was excellent (+++) when the response was in 2 to 3 weeks of incubation; very good (++) when the response was in 3 to 4 weeks of incubation; good (+) when the response was in 4 weeks of incubation; poor (-) when there was no response at all.

In vitro multiplication of initiated explants in different media

During the first four weeks in culture, the external leaf sheaths of each explant, which was initially white, later turned green. Some elongation of the explant could be observed in all the three banana cultivars. Most of the axenic explants established in culture, formed fresh multiple shoots by 4 to 8 weeks of longitudinally splitting and re-inoculating into the respective medium. However, each of the explant in each of the cultivar, showed a vast variation in their ability to proliferate in different media.

The multiplication ratio

1. 'Grand Naine'.

Good multiplication of proliferating clusters i.e., 3.5 and 2.76 was obtained in M5 and M2 media, respectively, by three successive transfers (Table 3). Poor multiplication ratio was seen in M4 medium.

Table 3. Multi ratio of banana cultivar 'Grand Naine' in different initiation medium in three successive growth cycle, each cycle of four weeks.

Media code	I multi cycle	II multi cycle	III multi cycle	Ave. no. of elongated shoots jar^{-1} (mean ± SE)	Culture quality
M1	2.2	2.0	1.5	1.90±0.20	+
M2	3.0	2.8	2.5	2.76±0.14	+++
M3	2.5	2.2	2.0	2.23±0.14	+++
M4	2.0	1.8	1.5	1.76±0.14	++
M5	3.5	3.5	3.5	3.50±0.0	+++

+++ Excellent; ++ Very good; + Good.

2. 'Monthan'.

The explants of 'Monthan' in M2 to M1 showed an impressive proliferating pattern (Figure 2) with good multiplication ratio in four weeks of culturing (Table 4). Elongation of shoots and leaf expansion were hardly found in these series of media in four weeks of culturing. Most of the cultures were very compact with the emerging shoot tips covered with a blackened scale. None of the clump was more than 2 cm in height.

Figure 2. Response of banana cultivars in MS basal medium containing 1% activated charcoal.

Table 4. Multiplication ratio of banana cultivar 'Monthan' in different initiation media in three. successive growth cycles each cycle of four weeks.

Media code	I multi cycle	II multi cycle	III multi cycle	Ave. no. of elongated shoots jar^{-1} (mean ± SE)	Culture quality
M1	3.7	3.8	3.6	3.70±0.05	+++
M2	3.9	3.9	3.9	3.90±0.0	+++
M3	2.5	2.4	2.3	2.40±0.05	++
M4	2.5	2.5	2.3	2.43±0.06	++
M5	1.8	1.7	1.5	1.66±0.08	+

+++ Excellent; ++ Very good; + Good.

3. 'Red banana'.

All the explants in M1 and M2 media showed considerably good response in four weeks of culturing (Table 5). In M3 medium, a mix of shoot elongation and proliferation was found. Somehow, quality of cultures was not good in M4. Instead, all the shoot initials exhibited shoot elongation and produced sturdy plantlets in M5. In all stages of growth, the red shade was found on the stems once the cultures were incubated in 12 h light.

Table 5. Multi ratio of banana cultivar, 'Red banana' in different initiation media in three successive growth cycles, each cycle of four weeks.

Media code	I multi cycle	II multi cycle	III multi cycle	Ave. no. of elongated shoots jar^{-1} (mean ± SE)	Culture quality
M1	3.2	3.5	3.4	3.66±0.08	+++
M2	2.8	2.7	2.5	2.66±0.08	++
M3	1.8	1.9	2.0	1.90±0.05	++
M4	1.5	1.3	1.2	1.33±0.08	+
M5	1.5	1.3	1.2	1.33±0.08	++

+++ Excellent; ++ Very good; + Good.

A model for in vitro micropropagation protocol for banana

It can be clearly understood that M1 and/or M2 was good for multiplication and bulking the proliferating clusters over a period of several cycles at least up to six multiplication cycles. The shoot elongation was the best in M5 medium for all the three cultivars. However, for 'Monthan' and 'Red banana', it was essential to culture the shooting clusters for two successive cycles. The shoots could be completely rooted ready to be sent to greenhouse for acclimatization after 3 to 4 weeks of culture in MS basal medium containing 1% activated charcoal.

Consistent shoot proliferation of commercial standard was the best in 5 and 3 mg L^{-1} BAP for 'Grand Naine'; whereas, the production scheduling could be formatted accurately, with quality shoots. However, for 'Monthan' 10 mg, 2 mg L^{-1} BAP and for 'Red banana' 10 mg and 3 mg L^{-1} BAP along with 0.2 mg L^{-1} IAA for each, respectively, appeared to be more suitable for obtaining productive shoots for a viable commercial production scheduling.

For all the three cultivars investigated, shoot elongation was the best in 1 mg L^{-1} BAP; where, the shoot initials elongated and the leaves expanded with 5-8 roots. However, for 'Monthan' and 'Red banana', one cycle of 4 weeks in lesser BAP was not enough for shoot elongation. A second cycle of another 4 weeks was essential to obtain elongated shoots which could then be transferred to medium containing activated charcoal for development of complete plants.

The ex-agar plants of all three cultivars of banana performed very well in poly-tunnel during the process of primary acclimatization. Later plants were transformed to primary nursery. The net pot plants started to grow very rapidly after shifting them from poly-tunnel to the greenhouse under higher light intensity. Then, the plants were transferred to natural

soil conditions.

Plant cells growing in vitro are considered to be under some degree of stress and may be predisposed to direct infection. Thus, microbial contaminants are the major challenges in plant's in vitro propagation during the different stages of culture processes (Helaly et al., 2014).

Roles of IAA (auxin) and BAP (cytokinin) in tissue culture media

The multiplication ratio of banana cultivars is strongly supported by BAP and IAA (Reddy et al., 2014; Sahoo et al., 2015; Karule et al., 2016) as also obtained in the present study substantiating that the combination of BAP and IAA is an influential cytokinin-auxin combination for in vitro micropropagation of banana. Adenine-based cytokinins are used in several *Musa* spp. for in vitro propagation (Gubbuk and Pekmezci, 2004). N6-benzylaminopurine (BAP) is the most commonly preferred cytokinin (Vuylsteke, 1989).

Increased levels of cytokinin inhibit apical dominance and promote lateral shoot proliferation. This principle holds good for banana cultivars; which, higher multi ratio was obtained at 5 or 10 mg L^{-1} of BAP along with 0.2 mg L^{-1} of IAA and lesser multiplication ratio, but elongation of shoots at 1 mg L^{-1} BAP. However, the multiplying cultures of 'Grand Naine' were not good in medium with 10 mg L^{-1} BAP over a period of 3 to 4 multiplication cycles. Yellowing of leaves occurred after three to four subcultures. Higher concentrations of BAP and kinetin beyond optimum levels were also reported to cause necrosis and reduction in shoot formation during in vitro multiplication of 'Nendran' (Rabbani et al., 1996). Reddy et al. (2014) also stressed the importance of BAP at low concentration about 2 mg L^{-1} that had given the best induction of 'Grand Naine' plantlets.

Sahoo et al. (2015) reported that MS medium supplemented with 2 mg L^{-1} BAP and 1 mg L^{-1} IBA was found to be ideal for the early shoot elongation after 30 days of inoculation in 'Grand Naine'. Further, Lalrinsanga et al. (2013) revealed the highest multiple shoot induction in MS medium fortified with 5 mg L^{-1} BAP with 2.17 shoots; while, MS with 1 mg L^{-1} NAA + 0.2 mg L^{-1} BAP with longest regenerated shoots after 45 days of incubation.

Micropropagation of banana through initiation of shoot tip explants has been reported by a number of researchers in MS basal medium supplemented with 5 to 20 mg L^{-1} BAP (Noor Aziah and Khalid, 2002; Venkatachalam et al., 2006). Some researchers have reported that a combination of BAP and an auxin were enhanced proliferation and shoot length during the tissue culture of banana (Ngomou et al., 2014). TDZ, a phenyl urea based cytokinin is frequently used along with BAP and IAA (Hamide and Mustafa, 2004).

The earlier study on 'Robusta' also demonstrated that BAP along with an auxin is most suited for commercial in vitro micropropagation. In the present study, among the various BAP concentrations (1 to 10 mg L^{-1}) along with a fixed concentration of IAA (0.2 mg L^{-1}), for the tested cultivars, it was found that maximum shoot multiplication was obtained in 5 mg L^{-1} BAP for 'Grand Naine'. However, maximum shoot multiplication for 'Monthan' and 'Red banana' was obtained in 10 mg L^{-1} BAP.

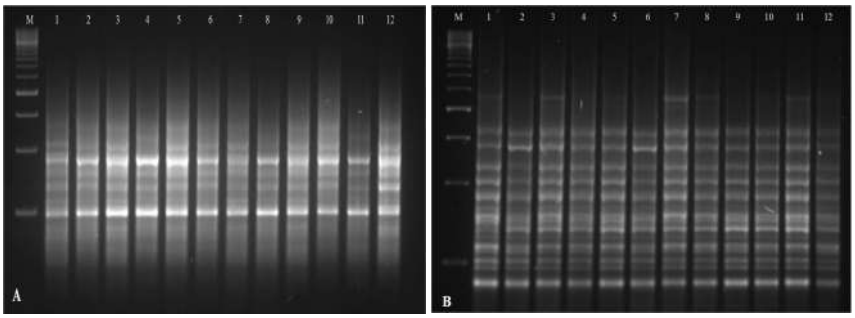
Clonal genetic fidelity

The result of DNA amplification showed that the three cultivars viz., 'Grand Naine', 'Monthan' (Figure 3) and 'Red banana' and their respective parents produced a wide array of strong and weak bands. However, only distinct, reproducible well-resolved fragments were scored. The total number of bands for each of the primer in three different cultivars studied is given in the table (Table 6).

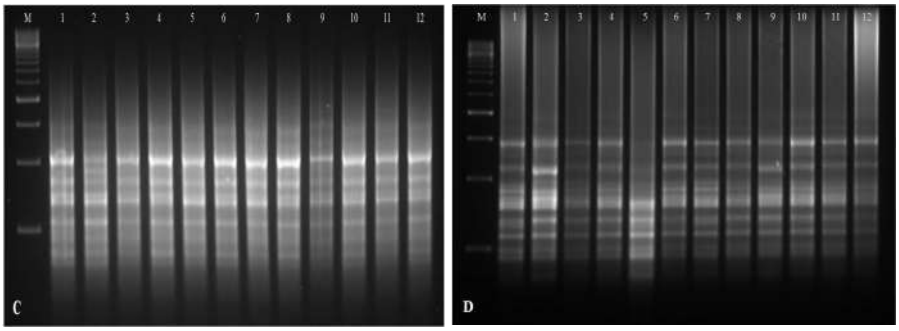
When compared the in vitro-derived plants with that of mother plants, 'Grand Naine' showed 48.14% of monomorphic and 51.85% of polymorphic bands; 'Monthan' 64.51 and 35.48%, respectively, and regenerated plants of this are almost true-to-type. However, in 'Red banana' polymorphic percentage is very high, i.e., 78.43% (Table 7) and showing significant variation.

Table 6. Banding pattern of ISSR primers obtained for different banana cultivars along with the bands for mother plant (MP) tissue.

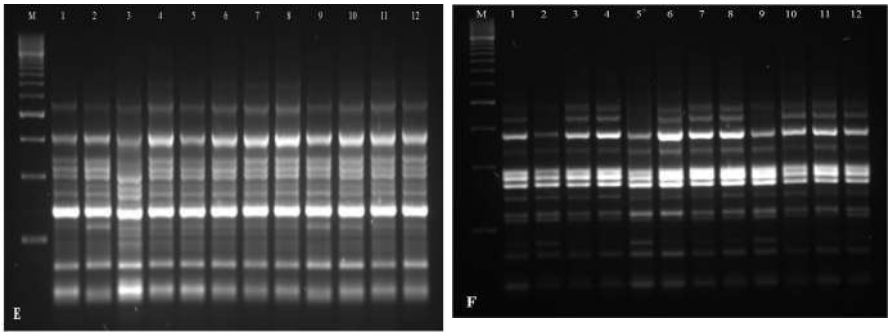
ISSR primers	Total no. of bands obtained for each of the marker					
	G9	G9 MP	Monthan	Monthan MP	Red banana	Red banana MP
807	106	20	57	10	109	22
808	55	12	138	24	122	20
810	71	12	69	12	78	16
830	50	10	73	14	70	12
834	69	12	131	24	92	24
840	85	16	150	26	60	14
Total	**436**	**82**	**618**	**110**	**531**	**108**



A. Lane 1 & 7-mother plant, Lane 2 to 6, 8 to 12 – Monthan samples**; B.** Lane 1 & 7-mother plant, Lane 2 to 6, 8 to 12 – Monthan samples



C. Lane 1 & 12-mother plant, Lane 2 to 11 – Monthan samples**; D.** Lane 1 & 7-mother plant, Lane 2 to 6 & 8 to 12 – Monthan sample



E. Lane 1 & 12-mother plant, Lane 2 to 11 – Monthan samples**; F.** Lane 1 & 12-mother plant, Lane 2 to 11 – Monthan samples

Figure 3. ISSR banding pattern of PCR amplified products of in vitro plants (10 samples) for banana cultivar 'Monthan' ISSR primers used - (A) UBC 807, (B) UBC 808, (C) UBC 810, (D) UBC 830, (E) UBC 834, and (F) UBC 840.

Table 7. ISSR percentage of MIC and PIC values of in vitro derived plants and mother plants by using ISSR marker.

No.	Cultivars	Percentage of monomorphic with mother plant & in vitro plants (%)	Percentage of polymorphic with mother plant & in vitro plants (%)
1	Grand Naine	48.14	51.85
2	Monthan	64.51	35.48
3	Red Banana	21.56	78.43

As per the MIC (monomorphic information content) values 'Monthan' showed true-to-type regenerated plants as it showed 64.5% MIC (Table 7); whereas, 'Grand Naine' had 48.14% MIC value. That means it had nearly 50% of similarity with mother plants. But 'Red banana' showed the highest MIC value 21.56%. All the variations might be due to the somaclonal variation that has been occurred in the micropropagated plants. Shei Sheidai dai et al. (2008) also explained that the presence of specific gene/loci in the parental plants could be lost in the regenerated plants due to somaclonal variations. This must be the possible reason for 'Red banana' that showed the highest variation in regenerated plants compared to mother plant.

The results of the present study clearly showed that in vitro multiplication of 'Monthan' can be carried out up to 8th cycle without any matter of concern of getting off-types. Whereas in case of 'Grand Naine' and 'Red banana', there is a need for further careful observations and stricter protocols to be followed in order to reduce the in vitro stress factors that could have caused genetic variations, manifested in the form of varying banding pattern.

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Structural and photoluminescence characteristics of PbO-M_2O_3(M_2O_3 = Al_2O_3, Sb_2O_3 and Bi_2O_3)-WO_3-B_2O_3: Sm_2O_3 glasses suitable for orange-red lasers

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ABSTRACT

PbO-M_2O_3(M_2O_3 = Al_2O_3, Sb_2O_3 and Bi_2O_3)-WO_3-B_2O_3:Sm_2O_3 glasses are synthesized by the traditional method of melt-quenching. The glassy nature and glass structural units are characterized by XRD and FTIR studies respectively. The Judd–Ofelt theory is successfully applied to absorption and emission transitions of Sm^{3+} ions. The values of Ω_λ have shown the following order: $\Omega_4 > \Omega_6 > \Omega_2$, which confirm the covalent nature of Sm-O bonds and excellence rigidity of the samples. Radiative properties of Sm^{3+} ions viz., branching ratio (β_r), experimental life time (τ_{exp}), quantum efficiency (η) and rate of non-radiative decay (W_{NR}) are evaluated. The glass containing Bi_2O_3 shows good deed of host environment around Sm^{3+} ions for possible orange-red laser transition $^4G_{5/2} \rightarrow {}^6H_{7/2}$ ($\approx$ 600 nm).

1. Introduction

Among various oxide glasses, particularly B_2O_3 glasses have become as good hosts for rare earth ions because of their high thermal stability, low melting temperature, wide range of transparency in UV–visible-NIR regions [1,2]. Samarium (Sm^{3+}) ions- doped borate glasses have gained better attention over other rare earth ions as they are suitable for specific applications because of their customary visible luminescence [1,3]. Particularly, the red-orange emission band ($^4G_{5/2} \rightarrow {}^6H_{7/2}$) of samarium ion in the vicinity of host glass network exhibit prominent intensity of luminescence with higher stimulated emission cross section (σ_E) and larger quantum efficiency (η). These are the necessary characteristics of optical materials suitable for emerging technological applications as optical memories, solid state lasers, color emissive display panels etc [1,3,4].

However, samarium ions- doped borate glasses have high phonon losses by means of non-radiative transitions. Although, the phonon losses can be minimized by the amalgamation of transition metal oxide (like WO_3) and/or heavy metal oxides (like PbO, Al_2O_3,

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Table 1
The chemical composition of the prepared glass samples.

Glass sample	Composition (mol%)
WAl	35 PbO–3 Al_2O_3–2 WO_3 – 60 B_2O_3
WSb	35 PbO–3 Sb_2O_3–2 WO_3 – 60 B_2O_3
WBi	35 PbO–3 Bi_2O_3–2 WO_3 – 60 B_2O_3
SmAl	35 PbO–2 Al_2O_3–2 WO_3 – 60 B_2O_3: 1 Sm_2O_3
SmSb	35 PbO–2 Sb_2O_3–2 WO_3 – 60 B_2O_3: 1 Sm_2O_3
SmBi	35 PbO–2 Bi_2O_3–2 WO_3 – 60 B_2O_3: 1 Sm_2O_3

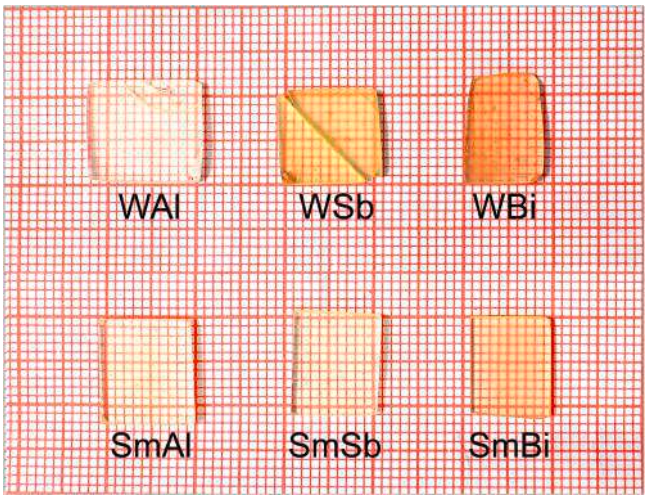


Fig. 1. Synthesized glass samples by melt-quenching method.

Table 2
Physical parameters of the PbO-M_2O_3(M_2O_3 = Al_2O_3, Sb_2O_3 and Bi_2O_3)-WO_3-B_2O_3: Sm_2O_3 glasses (±margin of error).

Physical parameter	WAl	WSb	WBi	SmAl	SmSb	SmBi
Density d (g/cm^3) (±0.0001)	3.134	5.671	5.044	4.7322	4.9101	5.0671
Average molecular weight (M)	122.91	128.59	133.83	125.38	129.17	132.66
Ion concentration N_i (10^{20} ions/cm^3) (±0.005)	–	–	–	0.2273	0.2289	0.2300
Interionic distance r_i (A°) (±0.005)	–	–	–	16.385	16.346	16.320
Polaron radius r_p (A°) (±0.005)	–	–	–	6.6020	6.5864	6.5758
Field strength (10^{15} cm^{-2}) (±0.005)	–	–	–	6.8826	6.9154	6.9376
Electronic polarizability α_e (10^{-22} ions/cm^3) (±0.005)	–	–	–	4.3640	4.3695	4.2962
Refractive index n_d (±0.0001)	1.7836	1.7862	1.7789	1.7657	1.7782	1.7857
Dielectric constant (ε')	3.1812	3.1905	3.1644	3.1311	3.1619	3.1776
Reflection loss (R_L)	0.0428	0.0431	0.0424	0.0416	0.0424	0.0413
Molar reflectivity (R_m) (cm^{-3}) (±0.005)	16.5108	9.5690	11.1194	11.0043	11.0183	10.8336

Sb_2O_3 and Bi_2O_3) in the composition of glass; and consequently, the quantum efficiency (η) of radiative transitions will be improved significantly. More ever, the refractive index, rigidity and mechanical strength of the glasses can be improved [1,4,5].

Thus, we can expect that the incorporation of small concentration of Al_2O_3, Sb_2O_3 and Bi_2O_3 to the PbO-WO_3-B_2O_3 glass composition would increase the glass forming ability by structural modifications around the samarium ions for better laser emission transitions in the visible band.

2. Methodology

2.1. Synthesis of glass samples

Confining to synthesis of good quality of high transparent glasses, a precise composition of PbO-M_2O_3(M_2O_3 = Al_2O_3, Sb_2O_3 and Bi_2O_3)-WO_3-B_2O_3: Sm_2O_3 is chosen; and six samples are synthesized by melt-quenching [1,2]. Table 1 shows the chemical composition of the fabricated glasses. The compositions of the samples have been heated in alumina crucibles at 1000 °C in a muffle furnace for 30 min; and the acquired glass samples are annealed at 350 °C at the rate of cooling 1 °C/min. The photographs of all the synthesized samples are shown in Fig. 1, which confirms their good optical transparency.

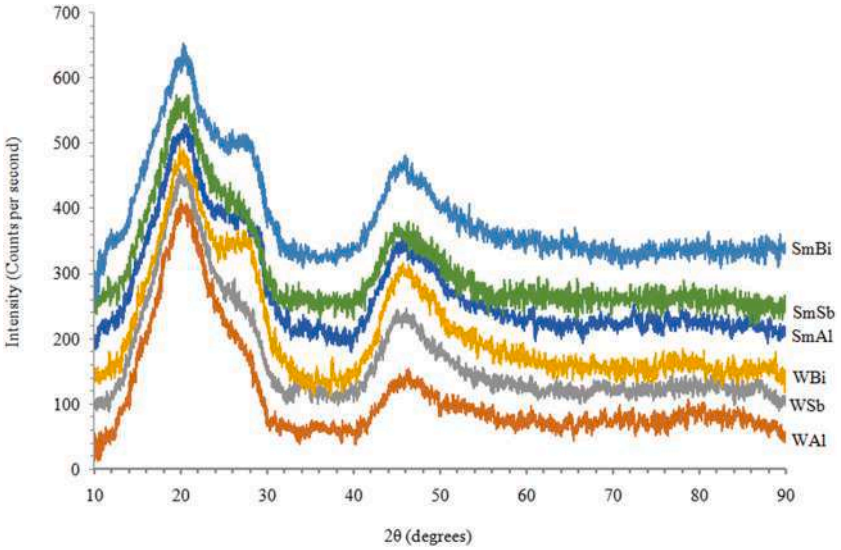


Fig. 2. XRD pattern of the prepared glass samples.

2.2. Characterization tools

The density (d) of the samples is estimated by Archimedes' principle with o-xylene as floating liquid. The refractive index (n_d) is determined by using Abbe's refractometer. The XRD spectra are recorded by using JEOL 8530 X-ray diffractometer. Scanning electron microscopy (SEM) and Energy dispersive spectral (EDS) profiles have been recorded by using JCM-6000PLUS microscope. The FTIR spectra are recorded by Perkin-Elmer Paragon 500 FTIR spectrometer. The absorption spectra are captured on JASCO V-570 photometer. The excitation, photoluminescence and decay mechanisms are recorded by Hitachi F-3010 spectrometer. All the readings are recorded at room temperature.

2.3. J-O theory

The spectral intensities of Sm^{3+} ions have been computed by adapting the Judd-Ofelt theory [6,7]. Using the J–O parameters (Ω_λ), radiative characteristics of Sm^{3+} ions such as radiative rate of spontaneous emission (A), damping rate of spontaneous emission by all the transitions (A_T), fluorescent state branching ratio (β_r), radiative life time(τ_{rad}) of the excited state and stimulated emission cross-section (σ_E) have been studied.

2.4. Decay mechanism

The life time (τ_{exp}) of $^4G_{5/2}$ state is obtained at $\lambda_{excitation} = 402$ nm by the fluorescence decay curves. The intensity of fluorescence is defined as bi-exponential function [8]. And, hence the experimental life time (τ_{exp}) of Sm^{3+} ions is determined [8]. Thus, Quantum efficiency (η) and rate of non-radiative decay (W_{NR}) are calculated in terms of experimental and theoretical life times (τ_{exp} and τ_{rad}) [9,10].

3. Results

3.1. Physical parameters

The physical parameters of the samples are determined and are listed in Table 2. One can notice that these glasses have shown higher values of density (d), electronic polarizability (α_e) and refractive index (n_d) because of the presence of various heavy metal ions viz., Al^{3+}, Sb^{3+} and Bi^{3+} ions in the glass composition. Thus these glasses may act as good candidates of dielectrics with higher nonlinear optical susceptibility [11].

3.2. XRD, SEM and EDS studies

Fig. 2 shows the XRD spectra of the glass samples. The spectra have exhibited multiple broad bumps around ($2\theta = 26^\circ$ and 45°) without any prominent sharp peaks, which show the absence of long-range periodicity [11,12]. Thus, we confirm the amorphous state of the samples. Fig. 3(a) and (b) shows the SEM and EDS profiles of the SmBi sample. The SEM and EDS studies have evidently confirmed that all the elements are presented in the original homogeneous composition without any contamination.

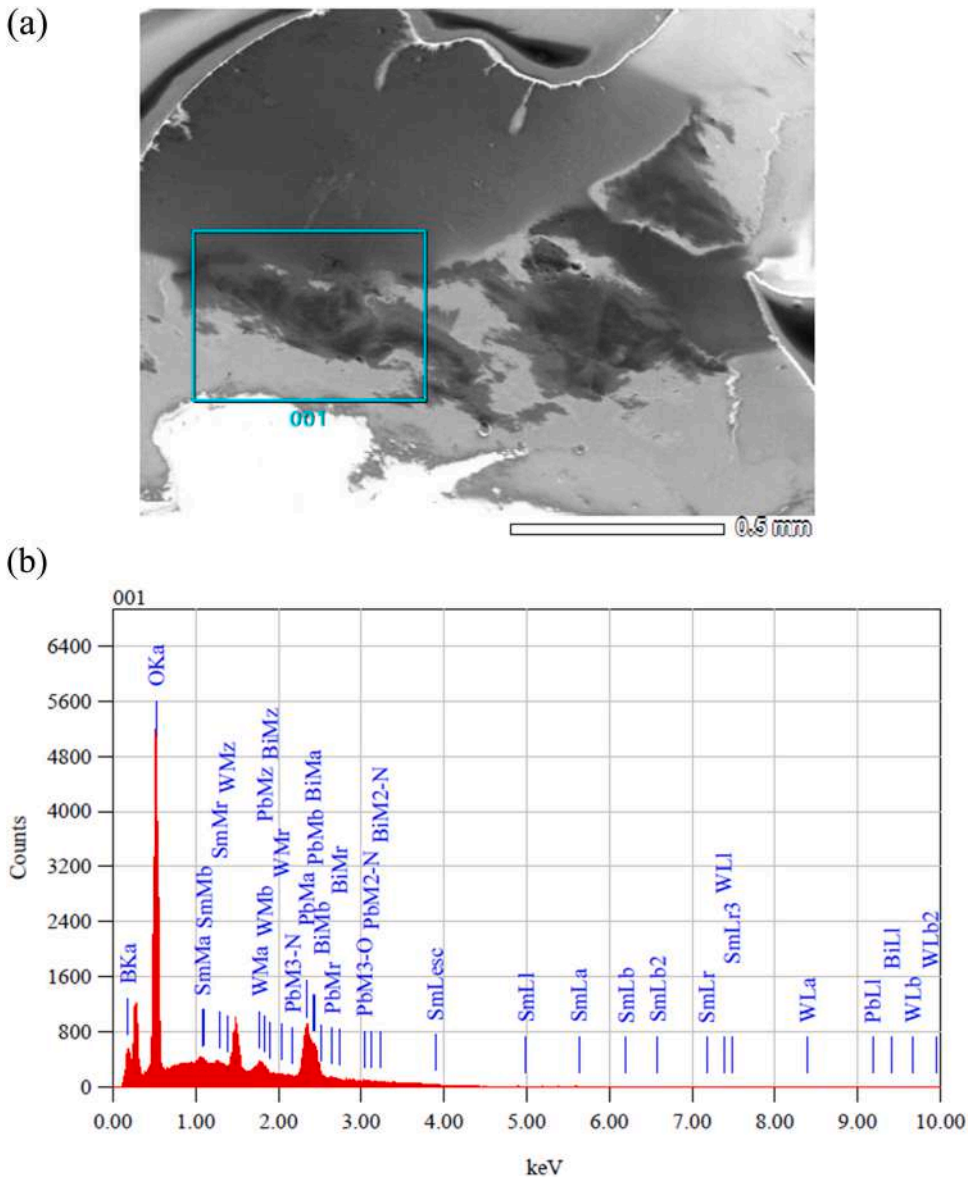


Fig. 3. (a) Scanning electron microscopic (SEM) image of SmBi sample, (b) Energy dispersive spectrum (EDS) of SmBi sample.

3.3. FTIR spectra

Fig. 4 shows the Fourier transform infrared spectra of the glasses. Assignment of various band positions is listed in Table 3. The spectra have exhibited well-known bands at metacenters 1330 cm^{-1} and 1080 cm^{-1} attributed to the stretching relaxations of B-O bonds of BO_3 units and BO_4 units respectively. Also, the spectra have displayed the bands at 910 cm^{-1} attributed to ν_1 vibrations of WO_4 units [13]. Another clear band positioned at 720 cm^{-1}, which is a characteristic note of bending vibrational frequencies of Pb-O bonds or O–B–O bonds [14]. In this region ($\approx$ 685 cm^{-1}) we may expect vibrations of [AlO_6] units, [SbO_6] units and [BiO_6] units too in the present glass samples containing Al_2O_3, Sb_2O_3 and Bi_2O_3 respectively [4,5,14]. Finally, stretching vibrational frequencies of Pb-O bond in PbO_4 units may be depicted at $\approx$ 420 cm^{-1} [15]. This band may be combined with the doubly degenerate bending (ν_4) vibrations of WO_4 units [13].

3.4. Optical absorption spectra

The optical absorption spectra (Fig. 5(a) and (b)) of Sm^{3+}- doped glasses have exhibited the significant bands [1,3,4,15]: $^6P_{3/2}\rightarrow{}^6H_{5/2}$; $^4G_{9/2}\rightarrow{}^6H_{5/2}$; $^4I_{13/2,\ 11/2,\ 9/2}\rightarrow{}^6H_{5/2}$; $^4M_{15/2}\rightarrow{}^6H_{5/2}$; $^4G_{5/2}\rightarrow{}^6H_{5/2}$; $^6F_{11/2,\ 9/2,\ 7/2,\ 5/2,\ 3/2,\ 1/2}\rightarrow{}^6H_{5/2}$ and $^6H_{15/2}\rightarrow{}^6H_{5/2}$. Thus, the theoretical and experimental oscillator strengths (f_{cal} and f_{exp}) of Sm^{3+} ions have been computed by using J-O theory; and the values are presented in Table 4. To investigate nature of the glasses, we have estimated the J-O parameters (Ω_λ) and presented in Table 5.

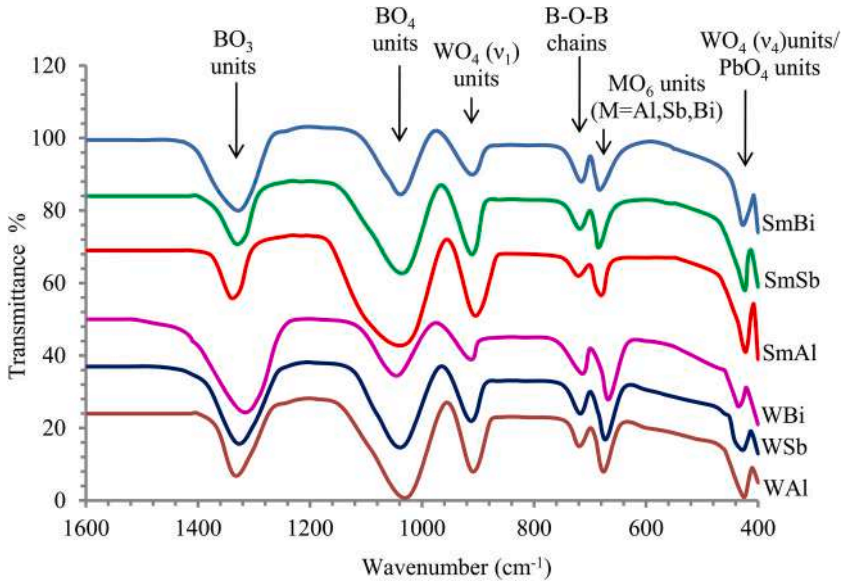


Fig. 4. FTIR spectra of the PbO-M_2O_3(M_2O_3 = Al_2O_3, Sb_2O_3 and Bi_2O_3)-WO_3-B_2O_3: Sm_2O_3 glasses.

Table 3
Pertinent data on FTIR spectra (band positions in cm^{-1}) of the glasses.

Glass sample	BO_3 units	BO_4 units	WO_4 (ν_1) units	B-O-B linkages	MO_6 units (M = Al,Sb,Bi)	WO_4 (ν_4)/PbO_4 units
WAl	1335	1025	910	720	675	424
WSb	1332	1033	914	718	671	426
WBi	1326	1038	915	714	666	429
SmAl	1342	1022	906	722	677	422
SmSb	1334	1030	912	719	683	423
SmBi	1329	1032	913	716	680	427

3.5. Florescence spectroscopy

The excitation spectra of Sm^{3+} ions (for $\lambda_{emission}$ = 600 nm) are shown in Fig. 6. From these spectra, the excitation wavelength is chosen as $\lambda_{excitation}$ = 402 nm by corresponding to the prominent $^6P_{3/2} \rightarrow {}^6H_{5/2}$ transition for the three samples [10]. The photo-luminescence spectra of the Sm^{3+} ions doped glasses are shown in Fig. 7. The spectra have showcased the four well-known bands [1,3, 10,15]: $^4G_{5/2} \rightarrow {}^6H_{11/2,\ 9/2,\ 7/2,\ 5/2}$. Among all the transitions, the highest intensity is shown by $^4G_{5/2} \rightarrow {}^6H_{7/2}$ transition (orange emission), whereas the lowest intensity is observed for $^4G_{5/2} \rightarrow {}^6H_{11/2}$ transition (red emission). Additionally, two hypersensitive transitions $^4G_{5/2} \rightarrow {}^6H_{5/2,\ 9/2}$ have exhibited yellow and reddish-orange emission respectively [10,16]. From the emission spectra, various radiative parameters are determined and furnished in Table 6. The energy level diagram of Sm^{3+} ions in the SmBi host glass system is shown in Fig. 8.

3.6. Decay curves

The bi-exponential decay curves (Fig. 9) of the transition $^4G_{5/2} \rightarrow {}^6H_{7/2}$ ($\approx$ 600 nm) of Sm^{3+} ions have been plotted for the excitation wavelength 402 nm (by Inokuti-Hirayama model) [17]. Then, the values of τ_R, τ_{exp} and η of Sm^{3+} ions for the three glasses are obtained and presented in Table 7.

4. Discussion

4.1. Glass structure

The FTIR spectra (see Fig. 4) are used to understand the glass structure. When Sm_2O_3 is added to the composition, the intensity of B-O bond of tetrahedral BO_4 units is observed to increase at the expense of BO_3 units. Obviously, the strength of the Sm^{3+} ions- doped glasses could be more than that of their pure samples [18]. The gradual conversion of $BO_3 \rightarrow BO_4$ units has been observed as a function of the modifier oxide in the order ($Al_2O_3 > Sb_2O_3 > Bi_2O_3$). More ever, the intensities of BO_4, WO_4 and PbO_4 units are the highest for Al_2O_3- added glasses. Because, the atomic radius of aluminum ions (0.68 A°) is less than that of antimony ions (0.90 A°) and bismuth

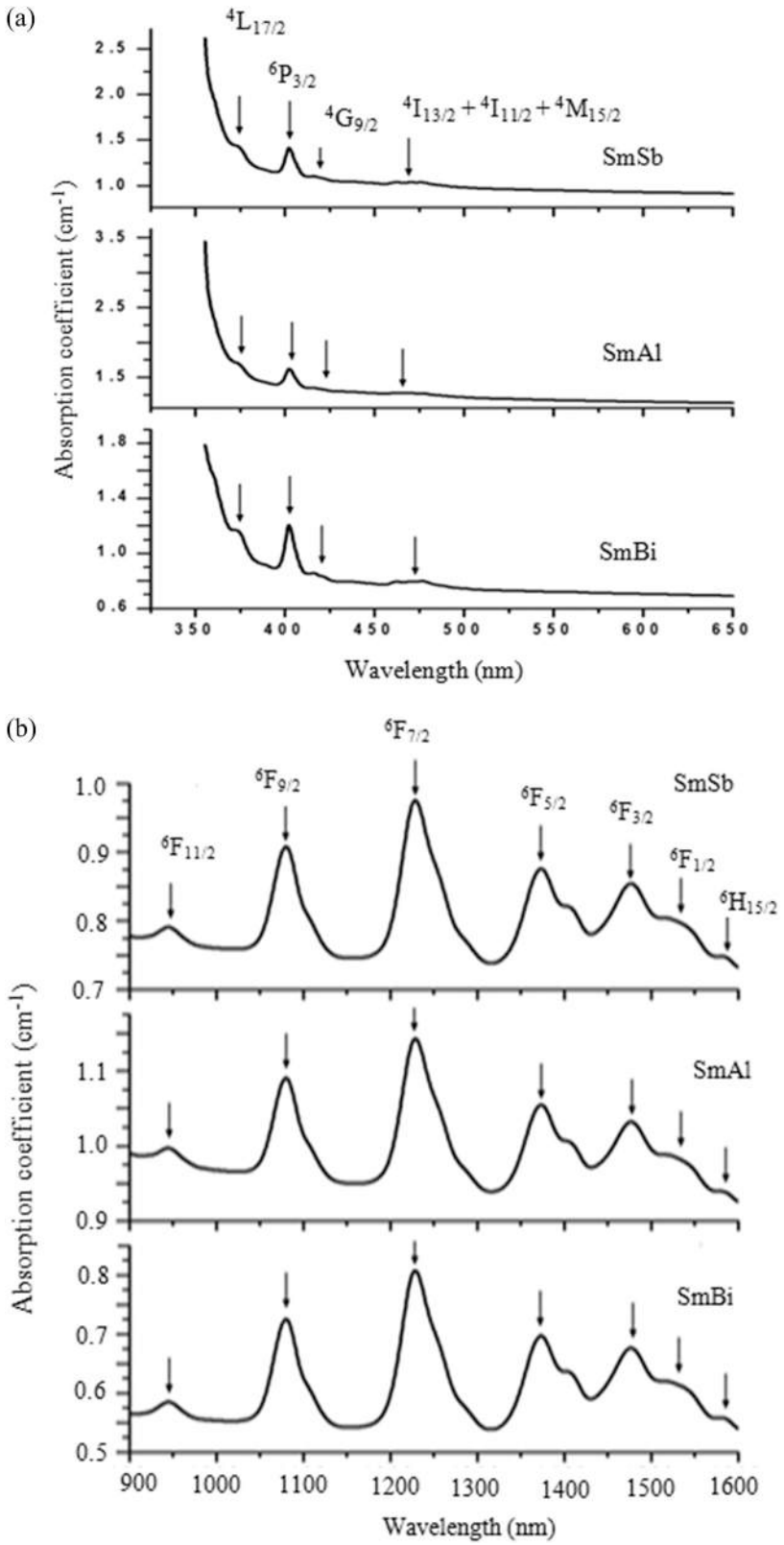


Fig. 5. (a) Optical absorption spectra of the PbO-M_2O_3(M_2O_3 = Al_2O_3, Sb_2O_3 and Bi_2O_3)-WO_3-B_2O_3: Sm_2O_3 glasses (UV-Visible region), (b) Optical absorption spectra of the PbO-M_2O_3(MO = Al_2O_3, Sb_2O_3 and Bi_2O_3)-WO_3-B_2O_3: Sm_2O_3 glasses (NIR region).

Table 4
Theoretical and experimental oscillator strength of Sm^{3+} ions in the glasses.

Transition $^6H_{5/2}$	SmAl		SmSb		SmBi	
	f_{exp} (x10^{-6})	f_{cal} (x10^{-6})	f_{exp} (x10^{-6})	f_{cal} (x10^{-6})	f_{exp} (x10^{-6})	f_{cal} (x10^{-6})
$^6P_{3/2}$	4.3862	3.4632	4.326	3.428	3.2862	2.5268
$^4G_{9/2}$	0.1342	0.0768	–	–	–	–
$^4I1_{3/2} + {}^4I_{11/2} + {}^4M_{15/2}$	1.936	1.328	0.98	0.84	1.2678	0.8672
$^4I_{9/2}$	–	–	1.47	1.58	–	–
$^4G_{5/2}$	–	–	1.39	1.37	–	–
$^6F_{11/2}$	0.527	0.826	–	–	0.1587	0.3216
$^6F_{9/2}$	4.243	4.427	2.93	2.87	2.0816	1.9832
$^6F_{7/2}$	6.052	5.864	–	–	2.3568	2.3242
$^6F_{5/2}$	1.816	1.836	–	–	1.1986	1.4276
$^6F_{3/2}$	0.7682	0.7846	4.32	4.58	0.6842	0.6214
$^6F_{1/2}$	0.0831	0.0352	2.48	2.16	0.0634	0.0483
$^6H_{15/2}$	0.4361	0.0186	–	–	0.3672	0.0186
rms deviation	±0.3802		±0.3950		±0.3253	

Table 5
J–O intensity parameters of Sm^{3+} ions in the glasses.

Glass sample	$\Omega_2 \times 10^{-20}$ (cm^2)	$\Omega_4 \times 10^{-20}$ (cm^2)	$\Omega_6 \times 10^{-20}$ (cm^2)	Spectroscopic ratio (Ω_2/Ω_6)
SmAl	2.893	5.553	4.614	1.204
SmSb	2.342	5.015	4.446	1.128
SmBi	2.028	4.629	4.178	1.108

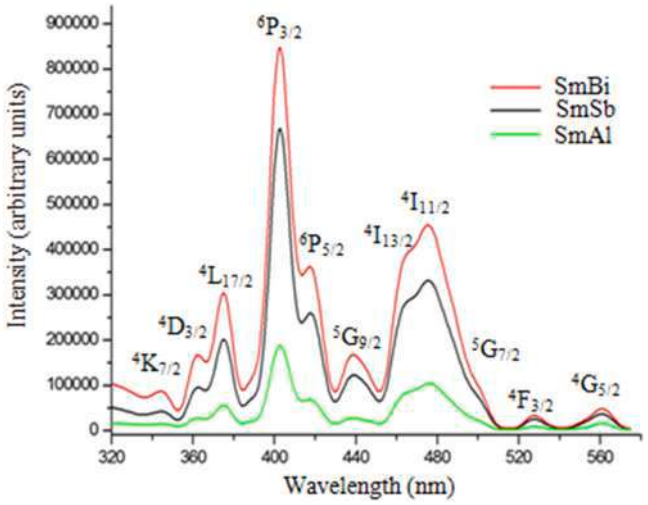


Fig. 6. Excitation spectra of Sm^{3+} ions- doped glass samples (for $\lambda_{emission} = 600$ nm).

ions (1.17A°) [19]. The smaller radius of the modifier ion gives a smaller mean distance between B–O–B, W-O-W, B-O-W, B-O-Pb intermolecular chains etc. Consequently, the degree of polymerization of the glass system would be less for WAl and SmAl glass samples, where as it may be high for WBi and SmBi samples. Thus, there is significant effect of the modifier oxides on structural properties of these glasses [20].

4.2. Judd-Ofelt parameters of Sm^{3+} ions

The trend of the obtained values of Ω_λ (in Table 5) shows that the prepared glasses are covalent in nature. High values of Ω_4 and Ω_6 shows the greater rigidity of all the glass samples. The spectroscopic quality factor is observed as $\Omega_4/\Omega_6 > 1$; which validate that the Sm^{3+} ions in present glasses can serve as potential laser active elements [21].

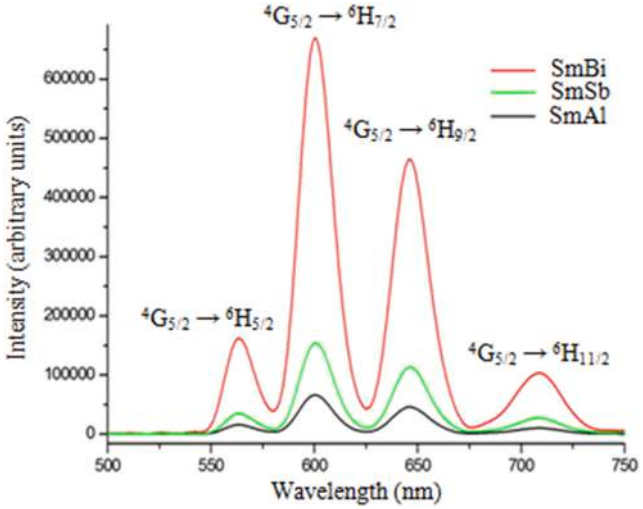


Fig. 7. Photoluminescence spectra of Sm^{3+} ions-doped glass samples (for $\lambda_{excitation}$ = 402 nm).

Table 6
Radiative properties viz., wavelength of emission band (λ), radiative rate of spontaneous emission (A), branching ratio (β_r) and high stimulated emission cross section (σ_E) of Sm^{3+} ions in the glasses.

Transition from $^4G_{5/2}$	λ (nm)	A (s^{-1})	β_r (%)	σ_E x 10^{-22} (cm^2)
SmAl				
$^6H_{11/2}$	709	94	13.00	10.14
$^6H_{9/2}$	645	252	34.81	15.49
$^6H_{7/2}$	598	358	49.37	13.91
$^6H_{5/2}$	561	20	2.82	0.783
SmSb				
$^6H_{11/2}$	709	89	13.24	5.22
$^6H_{9/2}$	647	223	33.33	9.66
$^6H_{7/2}$	599	340	50.69	9.17
$^6H_{5/2}$	562	18	2.75	5.50
SmBi				
$^6H_{11/2}$	710	83	13.34	3.25
$^6H_{9/2}$	648	204	32.71	5.34
$^6H_{7/2}$	600	319	51.24	4.10
$^6H_{5/2}$	564	17	2.71	3.38

4.3. Radiative properties

4.3.1. Branching ratio and stimulated emission cross section

The transition $^4G_{5/2} \rightarrow {}^6H_{7/2}$ of Sm^{3+} ions shows the branching ratio $\beta_r \geq 50\%$, which confirms the highest possibility of emission of laser light in orange color [1,16]. Further, the values of β_r of the transition $^4G_{5/2} \rightarrow {}^6H_{7/2}$ of Sm^{3+} ions are observed to be highest for SmBi glass. And, the glass sample SmBi has shown the lowest value of the stimulated emission cross section (σ_E) and highest radiative life time (τ_{rad}), because of the sustainable broad width of emission by all the transitions [22].

4.3.2. Life time and quantum efficiency

The decay curves are bi-exponential as shown in Fig. 9 because of two factors: (i) non-radiative decay mechanisms by phonon losses; and (ii) cross-relaxation processes by the electric dipole–dipole interactions between the pairs of samarium ions (one Sm^{3+} ion at excited state and other Sm^{3+} ion at ground state). Mainly, two cross-relaxation (CR) channels (as shown in Fig. 8) may be presented in the SmBi glasses as follows [23]:

CR 1: $(^4G_{5/2}, {}^6H_{5/2}) \rightarrow ({}^6F_{5/2}, {}^6F_{11/2})$

CR 2: $(^4G_{5/2}, {}^6H_{5/2}) \rightarrow ({}^6F_{7/2}, {}^6F_{9/2})$

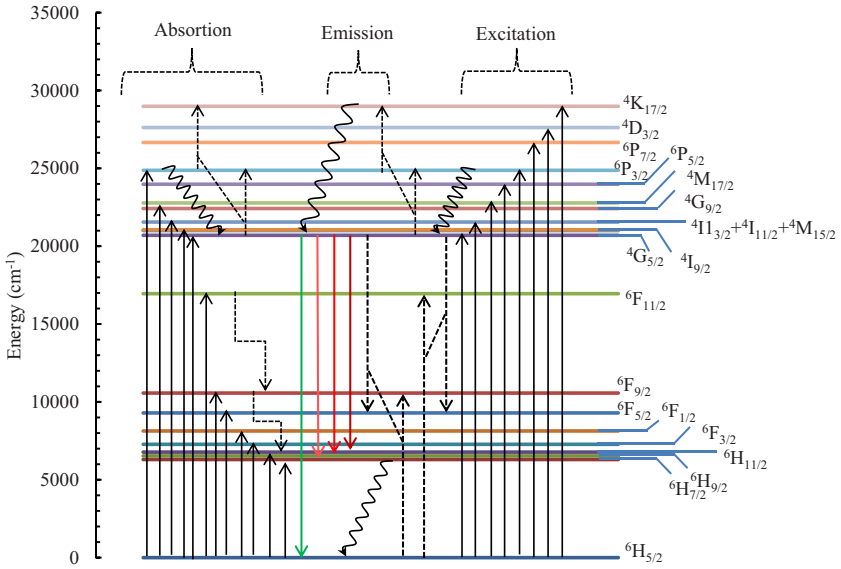


Fig. 8. Energy level diagram of Sm^{3+} ions- in SmBi glass system.

The values of radiative life time (both τ_R and τ_{exp}) and quantum efficiency (η) of Sm^{3+} ions are observed to be the lowest for the sample SmAl. It is well known fact that shorter life time (both τ_{rad} and τ_{exp}) and lower quantum efficiency (η) of the any rare earth ion leads to greater non-radiative losses (W_{NR}) [24]. Thus, non-radiative losses or multi-phonon relaxations are more for SmAl glass sample. Fig. 10 clearly shows that the emission intensities are augmented with the modifier Bi_2O_3 due to low phonon losses and reduced non-radiative transitions [25]. Therefore, the quantum efficiency is observed in the order: SmBi > SmSb > SmAl.

4.3.3. CIE chromaticity graph

The (x, y) color coordinates of the Sm^{3+}ions- doped glasses are shown in Fig. 11. The coordinates of chromaticity assessed for these glasses are presented in Table 7. We can see that these co-ordinates have been placed in the orange-red segment of the CIE 1931 chart. Thus, these results confirm that Sm^{3+} ions- doped PbO-M_2O_3-WO_3-B_2O_3 glasses will act as promising fluorescent materials for various applications like warm light sources [10], ultra-efficient LEDs [18], solid state visible lasers [26] and other photonic devices [27].

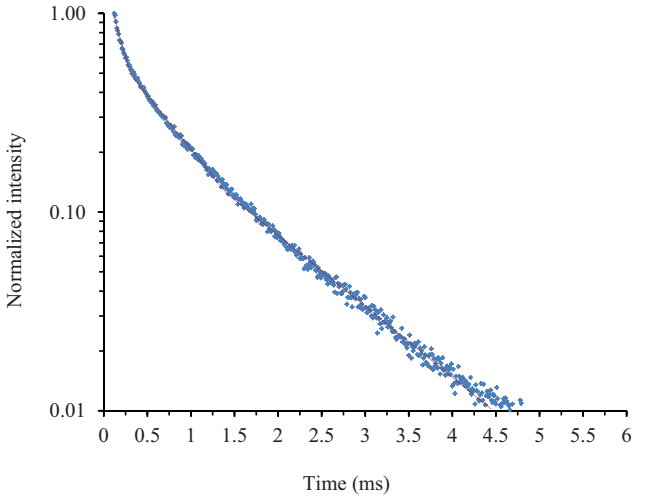
5. Conclusions

The structural and photoluminescence characteristics of the PbO-M_2O_3(M_2O_3 = Al_2O_3, Sb_2O_3 and Bi_2O_3)-WO_3-B_2O_3:Sm_2O_3 glasses have been studied. The key findings are as follows:

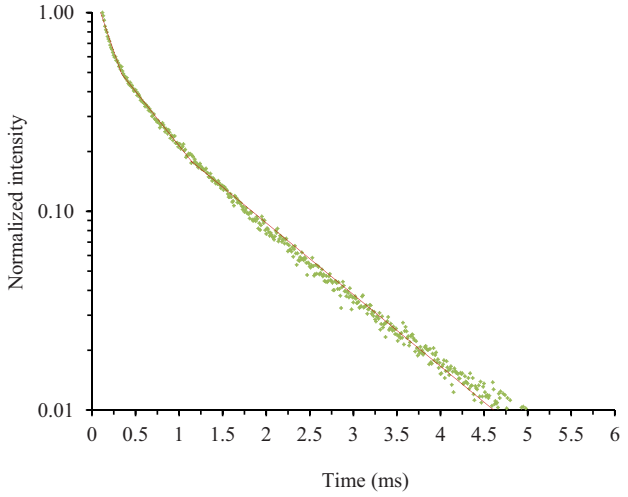
i) The Physical parameters, XRD, SEM and FTIR spectral studies have confirmed the structural modification of the glasses a function of modifier oxides Al_2O_3, Sb_2O_3 and Bi_2O_3.
ii) The tendency of the Ω_λ values shows that the prepared glasses are covalent in nature. The spectroscopic quality factor $\Omega_4/\Omega_6 > 1$; which indicates that the Sm^{3+} ions in present glasses can act as potential laser sources.
iii) The photoluminescence spectra have exhibited the laser transition $^4G_{5/2} \rightarrow {}^6H_{7/2}$ of Sm^{3+} ions in these glasses in orange color (≈ 600 nm). The quantum efficiency is observed in the order: SmBi > SmSb > SmAl.
iv) It has been observed that chromaticity coordinates of emission wavelengths fall in the orange-red region of the CIE color chart. Thus, these glasses may find potential scientific and technological applications in the fields of solid state lasers and photonics.

Declaration of Competing Interest

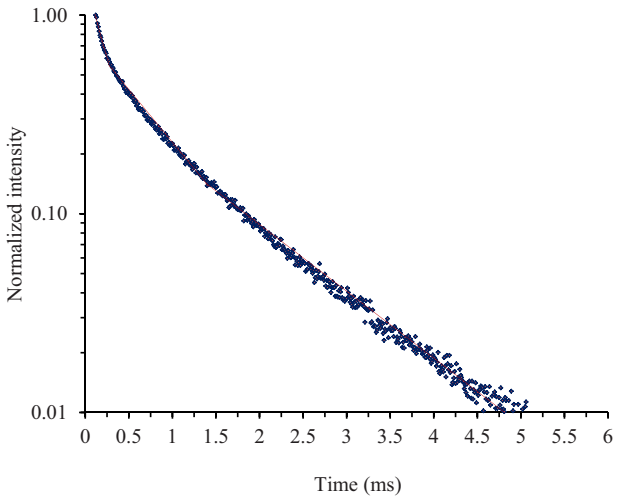
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.



(a) SmAl



(b) SmSb



(c) SmBi

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Fig. 9. Decay curves of Sm^{3+} ions for $\lambda_{excitation} = 402$ nm and $\lambda_{emission} = 600$ nm (fitted by Inokuti-Hirayama model).

Table 7
Radiative properties viz., damping rate of spontaneous emission by all transitions (A_T), radiative life time (τ_{rad}), experimental life time (τ_{exp}), quantum efficiency (η), rate of non-radiative decay (W_{NR}) and color coordinates of Sm^{3+} ions in the glasses.

Glass sample	A_T (s^{-1})	τ_{rad} (ms)	τ_{exp} (ms)	η (%)	(W_{NR}) s^{-1}	Color coordinates	
						X	Y
SmAl	725	1.380	0.694	50.31	716	0.556	0.427
SmSb	670	1.493	0.768	51.45	632	0.532	0.395
SmBi	623	1.606	0.862	53.68	537	0.573	0.402

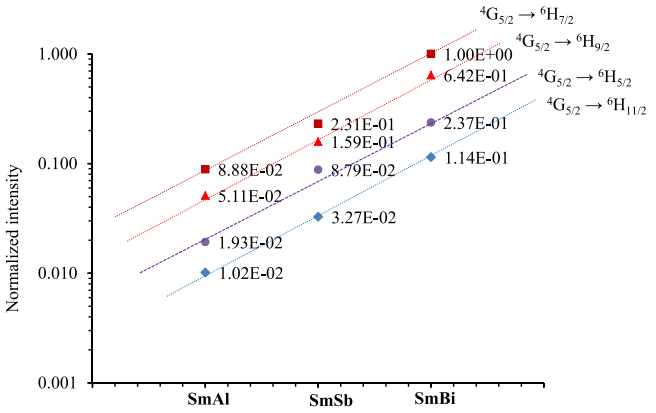

Fig. 10. Comparative intensity of emission bands of Sm^{3+} ions excited at 402 nm (SmBi > SmSb > SmAl).

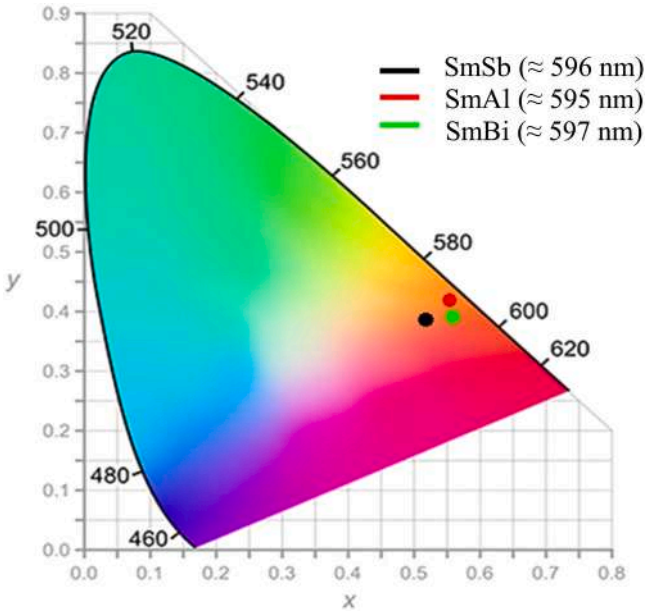

Fig. 11. The color space chromaticity diagram for emission scheme of the Sm^{3+}ions- doped glasses.

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Mechanism of Action of Essential Oils and their Major Components

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Abstract

The essential oil of lemongrass, palm rosa and eucalyptus were found to be good antimicrobial agents. To a large extent the results suggest their potential use as chemotherapeutic agents, food preserving agents, and disinfectants. However before considering these compounds as chemotherapeutic agents against human/animal diseases, it is important to study their cytotoxic and mutagenic effects. Studies were then carried to investigate the probable mechanism by which these compounds act against Gram negative (E. coli) and Gram-positive (Staphylococcus aureus) bacteria. The leakage of potassium ions from the cell suspension of bacteria and change in absorption maxima in presence of the test compounds was monitored. The results indicate that, in presence of crude essential oils the leakage of bacterial cellular material was higher than that showed in presence of the individual major components of essential oils, which is due their ability to disrupt the permeability barrier of microbial membrane structures, although the presence of additional mechanisms or targets cannot be ruled out.

Key words: *Plant essential oils, anti bacterial, anti fungal, potassium leakage, absorption maxima*

INTRODUCTION

Laboratories across the world have found literally thousands of phytochemicals, which have inhibitory effects on all types of microorganisms in vitro. It is well known that various types of secondary metabolites produced by plants are responsible for the biological activities of these phytochemicals [1]. Though much is known about the chemistry and the antimicrobial activity of several phytochemicals, very few reports are available on the possible mechanism of action. The bioactive compounds isolated from plants are substances whose chemical structures are widely different, with only rare exceptions, from those of the antibiotics derived from bacteria, actinomycetes, fungi, etc. [2, 3]. Most of the studies on phytochemicals and their antimicrobial activity are not followed by investigations on the mechanism of action of these compounds. This is regrettable, because the antimicrobial agents isolated from higher plants may act as regulators of intermediary metabolism by activating or blocking an enzyme reaction, removing or neutralizing an inhibitor influencing nutrient uptake from the medium, acting as a depressor of or otherwise affecting enzyme synthesis at nuclear or ribosomal level, changing membrane structures or substituting a limiting factor in intermediary metabolism [4, 5]. In this context it is desirable that the possible mode of action of these phytochemicals is studied. However, of late we could see an increase in the number of reports studying some aspects of mode of action of phytochemicals.

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Most of the plant secondary metabolites with antibacterial activity are lipophilic compounds. Lipophilic compounds were reported to act predominantly by dissipating the pH gradient across the cytoplasmic membrane. Food preservatives such as lactic acid, benzoic acids and some other lipophilic drugs were shown to act on the cytoplasmic membrane by disturbing hydrophobic interaction between the lipids and proteins [6, 7]. The cytoplasmic membranes of bacteria provide a barrier to the passage of small ions such as H^+, K^+, Na^+ and allow cells to control the entry and exit of different compounds. This permeability barrier role of cell membranes is integral to many cellular functions, including the maintenance of the energy status of the cell, other membrane coupled energy-transducing processes, solute transport, regulation of metabolism and control of turgor pressure [8, 9, 10]. Hence any compound which damages the cytoplasmic membrane of bacteria, will have bactericidal activity. For phenols and phenolic compounds an injury of membrane functions has been proposed as a mechanism of action [11, 12]. The observation by two different groups [13, 14] that the toxicity of phenols correlates well with hydrophobicity indicates cell membrane, which is rich in lipid content, as the possible main target of phenolic antimicrobial agents. Independent research reports by Hugo & Bloomfield and Lee et al., [15, 16, 17] have shown that fentichlor which is toxic to bacteria shows an increase in permeability of cytoplasmic membrane to protons with a consequent dissipation of the proton motive force (PMF) and an uncoupling of oxidative phosphorylation. There is a close relationship between the leakage of UV_{260} absorbing material by bacteria and the bactericidal activity of fentichlor. In general, the mode of action by which essential oils, which are lipophilic in nature, act seems to involve the cytoplasmic membrane of bacteria [18]. However, enzymes and DNA of the bacteria have also been mentioned as possible targets [19].

Knobloch et al., [20.] have studied more than 40 terpenoids isolated from essential oils and investigated their possible influence on the reaction mechanisms of primary energy metabolism, in particular NADH and succinate dehydrogenase activities, membrane-bound respiratory electron flow and oxidative phosphorylation [21, 22]. All reactions investigated were inhibited by terpenoids, with thymol and carvacrol being the most effective. Studies by Ultee et al., Cristani et al., and Nazzaro et al., [23, 24, 25] with liposome model systems confirmed that cyclic terpenes accumulate in the membrane, affecting membrane integrity and resulting in the dissipation of proton motive force which is in accordance with the earlier report by Sikkema et al., [26].

Studies by Cox et al., and Nazzaro et al., [25, 27] on tea tree oil, which is found to contain cyclic monoterpenes, showed that the antibacterial activity of this oil is related to its ability to disrupt the permeability barrier of cell membrane structures of bacteria, and the accompanying loss of chemi-osmotic control.

Some of the studies showed that Gram-negative bacteria are less sensitive to lipophilic compounds than Gram-positive bacteria [28]. Studies by Sikkema et al., [26] indicated that the higher tolerance of Gram-negative bacteria to lipophilic compounds is related to the resistance shown by the outer membrane of Gram- negative bacteria to these molecules.

In light of the above knowledge, it was proposed to investigate the effect of these compounds on cell permeability of Gram-negative (E. coli) and Gram-positive (S. aureus) bacteria. We have selected the essential oils lemon grass; palm rosa, eucalyptus and major components citral, geraniol and citronellal for the present studies. Studies were undertaken to monitor the response of E. coli and Staphylococcus aureus against these compounds in terms of permeability control i.e., leakage of potassium ions and UV_{260} and UV_{280} absorbing substances from the cell suspension in presence of the test compounds.

MATERIALS

Chemicals (analytical grade) were purchased from M/s Qualigens and Hi Media laboratories India Ltd.

Test compounds*: Three essential oils have been used in the present investigation viz- lemongrass oil, palm rosa oil, eucalyptus oil. These were obtained by steam distillation at CIMAP, Boduppal, Hyderabad. The major components used in the present investigation viz. Citral, Citronellal and Geraniol were obtained from M/s Sigma Chemicals.*

METHODS

Potassium Efflux

Preparation of Bacterial cell suspension: E. coli and S. aureus were grown overnight in nutrient broth at 37°C with shaking at 120 rpm. Bacterial cells were then harvested and washed once with 10mM EDTA and then twice with distilled water by centrifuging each time at 6000 rpm for 15 minutes at 4°C, and resuspended such that the absorbance of the final suspension was 2.0 at A_{450}.

The cell suspension was incubated for half an hour at room temperature and the potassium concentration in the cell free supernatant, obtained after centrifuging the cell suspension at 6000 rpm for 15 minutes, was determined by flame photometry. This served as control.

Effect of Essential Oils and Major Components on Potassium Efflux

The cell suspension was prepared as described above. After incubation for half an hour at room temperature, the test compounds viz. lemon grass, palmrosa, eucalyptus and major components citral, geraniol and citronellal were added at MBC values to the cell suspension separately. At regular intervals of 15 minutes, aliquots of the samples were drawn and extra cellular potassium ion levels were measured as described above.

Leakage of UV_{260} And UV_{280} Absorbing Material

The method of Heipieper et al., [29] was followed to determine the leakage of UV_{260} absorbing material. The cell suspension was prepared as described above and the absorbance of the cell free supernatant was determined at 260nm using Spectronic UV-spectrophotometer. This served as control for the leakage studies of UV_{260} absorbing material.

The absorbance at 280nm was also measured and this served as control for the leakage studies of UV_{280} absorbing material.

Effect of Essential Oils and Major Components on Leakage of UV_{260} and UV_{280} Absorbing Material

The procedure followed was similar to the measurement of potassium ion efflux. Samples treated with test compounds viz. lemon grass, palm rosa, eucalyptus and major components citral, geraniol and citronellal at MBC values were drawn at regular intervals and the A_{260} and A_{280} of the supernatant obtained after centrifugation was measured.

RESULTS

The effect of essential oils of lemongrass, palmrosa, eucalyptus and major components citral, geraniol and citronellal on the membrane permeability of E. coli and S. aureus cells was studied at their minimum bactericidal concentration in terms of leakage of potassium ions, and UV 260 and UV280 absorbing material.

Efflux of Potassium Ions

Escherichia coli and S. aureus cells were treated with the test compounds at minimum bactericidal concentration and the extracellular potassium ion concentration was measured both in the presence and absence of test compounds at regular time intervals. In the absence of test compounds, the extracellular concentration of K^+ ions of E. coli and S. aureus cell suspension was 1.1 ppm and 1.4 ppm respectively.

The E. coli cell suspension when treated with test compounds viz. lemon grass oil, palm rosa oil, eucalyptus oil, citral, geraniol and citronellal at their minimum bactericidal concentration showed an increase in the extracellular concentration of potassium ions compared to the control which implicates increased cell permeability in the presence of test compounds. The results are presented in Figure 1a and 1b.

In the presence of lemon grass oil, leakage of potassium ions from E. coli cell suspension could be seen after 30 minutes of incubation. In the presence of palm rosa oil and eucalyptus oil, the leakage could be seen after 45 minutes of incubation (Figure 1a). citral, the major component of lemon grass oil induced leakage of K^+ ions from the E. coli cell suspension after 30 minutes. Citronellal and geraniol induced leakage of K^+ ions after 60 minutes, and the leakage of K^+ ions in presence of these two compounds is low compared to the other test compounds (Figure 1b).

Among the test compounds, lemongrass oil displayed high activity in terms of inducing K^+ ion leakage from E. coli cell suspension. In the presence of lemongrass oil efflux of potassium ions from E. coli cell suspension could be observed from 30 minutes of incubation and after 120 minutes of incubation the concentration of extracellular K^+ ions was 6.6ppm. In the presence of palm rosa oil and eucalyptus oil, after 120 minutes of incubation the extra cellular K^+ ions was 4.8ppm and 5.4ppm respectively (Figure 1a). Similarly in presence of citral, geraniol and citronellal, after 120 minutes of incubation the extra cellular K^+ ion concentration was 5.8ppm, 2.8ppm and 3.6 ppm respectively (Figure 1b).

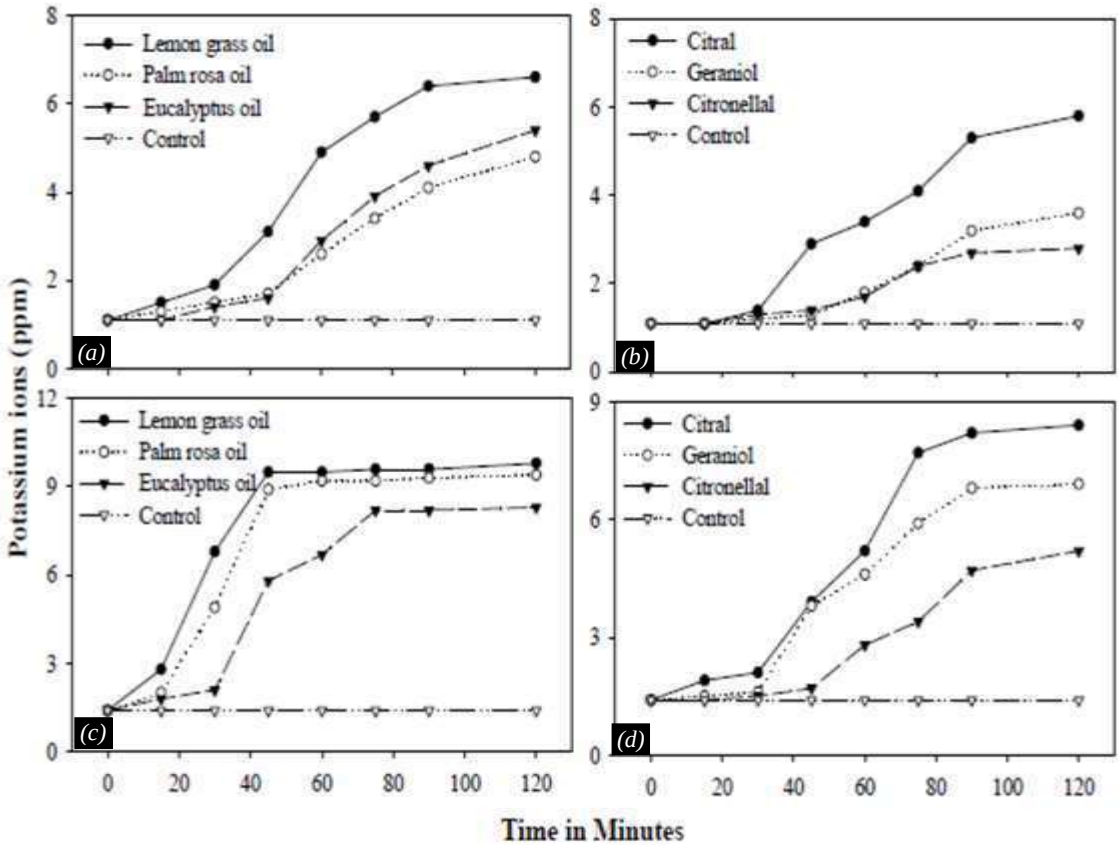

Figure 1. *Efflux of potassium ions from the cell suspension of E. coli and S.aureus treated with essential oils and their major compounds, (a, b) Efflux of potassium ions from the cell suspension of E. coli treated with lemon grass, palm rosa, eucalyptus oils and citral, geraniol, citronellal, respectively, (c, d) Efflux of potassium ions from the cell suspension of S. aureus treated with lemon grass, palm rosa, eucalyptus oils and citral, geraniol, citronellal, respectively.*

In case of S. aureus cell suspension, all the test compounds induced a high leakage of potassium ions. The leakage was induced after 15 minutes of incubation in presence of lemon grass oil and palm rosa oil, and after 30 minutes of incubation in presence of Eucalyptus oil (Figure 1c). In the presence of citral an increase in leakage could be seen from 30 minutes. In the presence of geraniol also an increase in leakage could be observed from 30 minutes of incubation, while in presence of citronellal an increase in leakage could be observed after 45 minutes of incubation (Figure 1d). In the presence of lemongrass oil the extracellular K^+ concentration after 120 minutes of incubation was 9.8ppm. In the presence of palm rosa oil and eucalyptus oil, the extracellular K^+ concentration after 120 minutes of incubation was 9.4ppm and 8.3ppm respectively (Figure 1c). Similarly in the presence of citral, geraniol and citronellal, the extracellular K^+ concentration after 120 minutes of incubation was 8.4 ppm, 6.9 ppm and 5.2 ppm, respectively (Figure 1 d).

Leakage of UV_{260} and UV_{280} – Absorbing material

The leakage of UV_{260} absorbing material (mainly nucleic acid material) was monitored in presence of test compounds against E. coli and S. aureus. In the absence of test compounds the OD of UV_{260} absorbing material was between 0.012 to 0.064 for E. coli cell suspension, and 0.013 to 0.053 for S. aureus cell suspension.

The cell suspension of E. coli in the presence of test compounds showed an increase in absorbance of extra cellular UV_{260} material giving an indication of membrane leakage. Lemongrass oil was the most effective compound in terms of inducing leakage of UV_{260} absorbing material. In the presence of lemon grass oil there was an increase in the UV_{260} absorbing material from 30 minutes of incubation, and a maximum absorbance of 2.24 was recorded after 90 minutes of incubation. Palm rosa oil induced leakage of UV_{260} absorbing material from 45 minutes of incubation, and a maximum absorbance of 1.26 was recorded after 120 minutes of incubation. Similarly eucalyptus oil induced leakage of UV_{260} absorbing material after 45 minutes of incubation reaching a maximum of 0.74 OD after 120 minutes (Figure 2a). Among major components citral showed highest activity. In the presence of citral leakage of UV_{260} material started after 30 minutes of incubation, with a maximum absorbance of 1.38 recorded after 120 minutes of incubation. Geraniol induced leakage of UV_{260} absorbing material after 60 minutes with a maximum of 0.56 OD recorded after 120 minutes. In the presence of citronellal leakage of UV_{260} absorbing material was slow and steady. The leakage of UV_{260} absorbing material in presence of citronellal reached a maximum of 0.289 OD after 120 minutes. Thus, citronellal induced very low leakage of UV_{260} material from the cell suspension of E. coli (Figure 2b).

The cell suspension of S. aureus was very sensitive to all the test compounds in terms of leakage of UV_{260} absorbing material. In the presence of lemon grass oil the leakage of UV_{260} absorbing material started immediately and a maximum absorbance of 3.0 was recorded after 30 minutes of incubation indicating the quick action of lemon grass oil (Fig 2c). Palm rosa oil induced the leakage of UV_{260} absorbing material after 15 minutes of incubation and a maximum absorbance of 2.18 was recorded after 60 minutes of incubation. Eucalyptus oil induced the leakage from 15 minutes of incubation with a maximum absorbance of 0.882 recorded after 60 minutes of incubation. The results show that these three essential oils are very quick in their action against S. aureus (Figure 2c). In presence of the major component citral, leakage of UV_{260} absorbing material could be seen immediately and a maximum absorbance of 3.0 was recorded after 45 minutes of incubation. In presence of geraniol an increase in the leakage could be observed from 45 minutes reaching a maximum absorbance of 1.61 after 120 minutes. In presence of citronellal, increase in the leakage could be observed from 45 minutes with a maximum absorbance of 0.84 recorded after 120 minutes of incubation (Figure 2d).

The leakage of UV_{280} absorbing material (mainly proteins) was also monitored. In the absence of test compounds the OD of UV_{280} material was between 0.015-0.034 for coli cell suspension, and 0.019-0.049 for S. aureus cell suspension. Control cultures that were not treated with test compounds,

showed negligible increase in the UV_{280} absorbing material, whereas the treated cultures showed a rapid increase in presence of certain compounds.

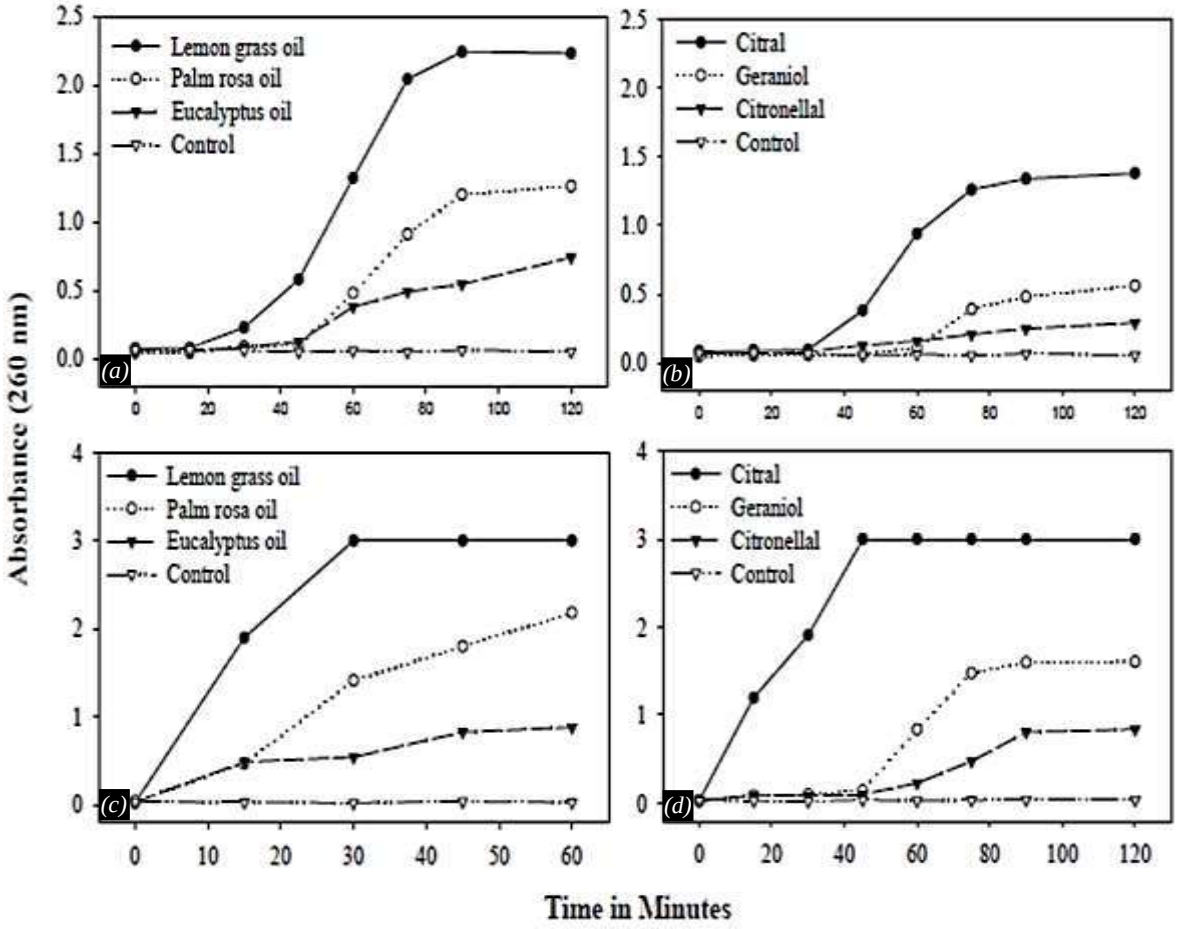

Figure 2. *Leakage of UV_{260} absorbing material from the cell suspension of E. coli and S. aureus treated with essential oils and their major compounds, (a, b) Leakage of UV_{260} absorbing material from the cell suspension of E. coli treated with lemon grass, palm rosa, eucalyptus oils and citral, geraniol, citronellal, respectively, (c, d) Leakage of UV_{260} absorbing material from the cell suspension of S. aureus treated with lemon grass, palm rosa, eucalyptus oils and citral, geraniol, citronellal, respectively*

In the presence of lemon grass oil, the cell suspension of E. coli showed leakage of UV_{280} material from 15 minutes. The leakage was very rapid with absorbance of UV_{280} absorbing material increasing from 0.184 at 30 minutes to 1.04 at 45 minutes (Figure 3a). The maximum OD of UV_{280} absorbing material in presence of lemon grass was 2.04 after 90 minutes of incubation. The leakage in presence of palm rosa could be observed from 30 minutes reaching a maximum absorbance of 0.98 after 120 minutes of incubation. In presence of eucalyptus oil the leakage was slow and steady. The leakage of UV_{280} absorbing material in presence of eucalyptus could be observed from 30 minutes of incubation and a maximum absorbance of 0.361 was recorded after 90 minutes of incubation (Fig 3a). The major component citral induced leakage of UV_{280} absorbing material from 45 minutes, and the leakage was rapid with a maximum absorbance of 0.79 recorded after 120 minutes of incubation. Geraniol and citronellal induced leakage of UV_{280} absorbing material from 30 minutes of incubation with a maximum absorbance of 0.54 and 0.220 respectively, recorded after 120 minutes of incubation (Figure 3b).

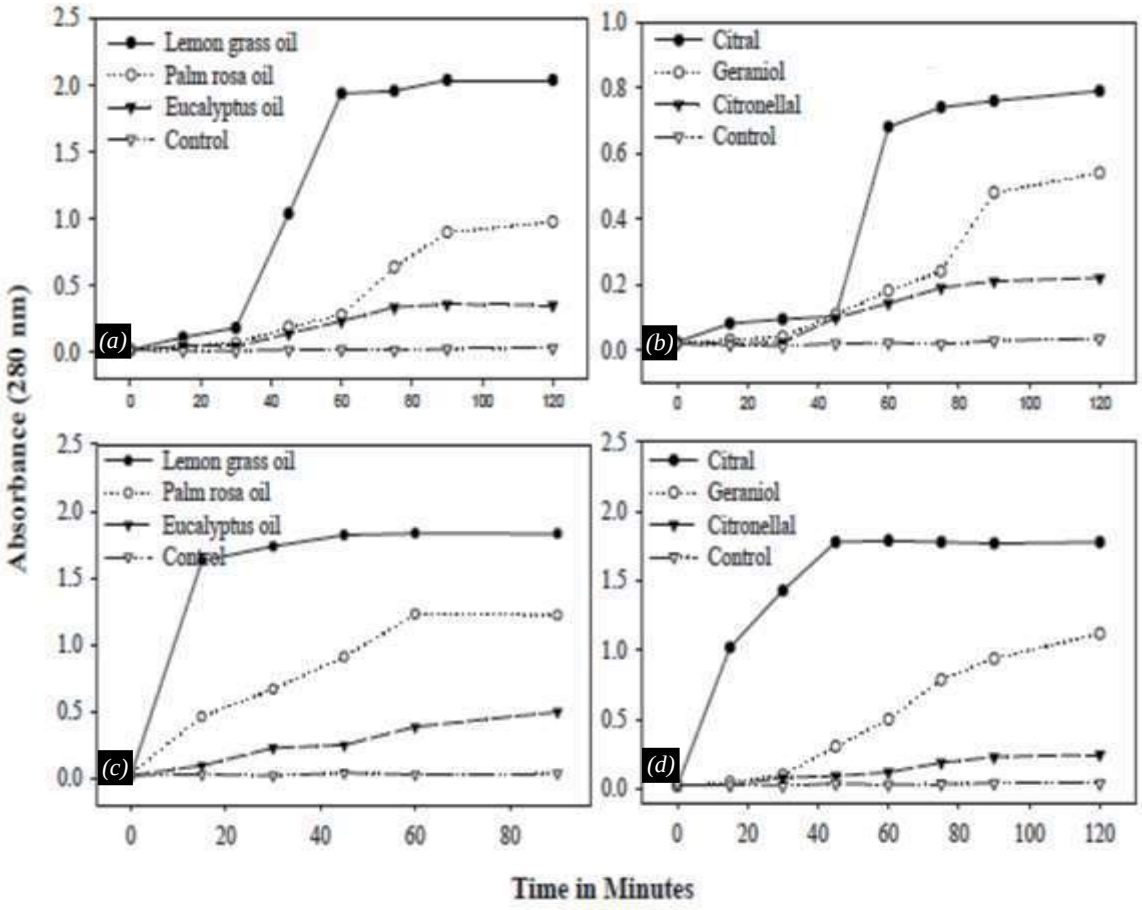

Figure 3. *Leakage of UV_{280} absorbing material from the cell suspension of E. coli and S. aureus treated with essential oils and their major compounds, (a, b) Leakage of UV_{280} absorbing material from the cell suspension of E. coli treated with lemon grass, palm rosa, eucalyptus oils and citral, geraniol, citronellal, respectively, (c, d) Leakage of UV_{280} absorbing material from the cell suspension of S. aureus treated with lemon grass, palm rosa, eucalyptus oils and citral, geraniol, citronellal, respectively.*

The cell suspension of S. aureus was very sensitive to the test compounds in terms of leakage of UV_{280} absorbing material. In the presence of lemon grass oil the leakage was very quick. The leakage commenced immediately in presence of lemon grass oil with a maximum absorbance of 1.834 recorded after 60 minutes of incubation. In the presence of Palm rosa oil also the leakage of UV_{280} could be observed immediately with a maximum absorbance of 1.234 recorded after 60 minutes of incubation. In presence of eucalyptus oil the leakage of UV_{280} material could be observed from 30 minutes with a maximum absorbance of 0.496 recorded after 90 minutes of incubation (Fig 3c). The major component citral induced leakage of UV_{280} material immediately from the cell suspension of S. aureus with a maximum absorbance of 1.79 recorded after 60 minutes of incubation. Geraniol induced leakage from 30 minutes of incubation with a maximum of absorbance 1.12 recorded after 120 minutes of incubation. Citronellal induced a very low leakage of UV_{280} material. The leakage in presence of citronellal was very slow with a maximum absorbance of 0.248 recorded after 120 minutes of incubation (Figure 3d).

DISCUSSION

Our present investigation has shown that the essential oils lemon grass, palm rosa and eucalyptus, and their major components citral, geraniol and citronellal show high antimicrobial activity compared to the other compounds tested [30]. It would be ideal if the possible mechanism of action of these compounds is also known as this will have implications for its spectrum of activity, selective toxicity, development of resistance etc. Hence it was aimed to study the probable mechanism by which these compounds act on bacteria. For our studies we have selected one Gram-negative bacterium E. coli, and one Gram-positive bacterium S. aureus.

It is well known that the correct and precise functioning of the cytoplasmic membrane, which is rich in hydrophobic lipid molecules, is indispensable to the cell. Therefore, any compound that disrupts or compromises the cytoplasmic membrane will have a lethal effect on cells. It is already known that interactions with the hydrophobic structures of bacteria play a key role in the antimicrobial actions of hydrocarbons [25, 26, 31]. Numerous investigations by various authors have pointed out that the ability of various essential oils to act as anti bacterial agent's stems from their high lipophilic character [18]. Studies by Cox et al., and Lopez-Romero et al., [27, 32] have shown that exposing E. coli and S. aureus at MBC concentrations of tea tree oils leads to increased permeability of bacterial cytoplasmic membranes, which was indicated by potassium ion leakage. Carson et al., [33] have also shown that tea tree oil induces leakage of UV_{260} absorbing material indicating a gross and irreversible damage of cytoplasmic membrane in S. aureus. Many other studies have implicated membrane damage by essential oils as principal contributor to their antibacterial ability [34, 35].

Loss of cytoplasmic material, leakage of cellular potassium ions from the cell suspension were taken as indicators for gross and irreversible damage to cytoplasmic membrane. It was speculated that the essential oils and their major components in the present study, owing to their extremely lipophilic character may disrupt the membrane integrity resulting in the leakage of intracellular components into the extra cellular medium. Until now different studies have shown that an efflux of potassium ions is a first indication of membrane damage in bacteria [26, 36, 37]. Potassium ion is the major cytoplasmic cation of growing bacterial cells involved in several key functions. It plays a role in the activation of cytolpasmic enzymes, maintenance of turgor pressure and possibly regulation of cytoplasmic pH. Hence the effect of these compounds on leakage of K^+ ions and UV_{260} and UV_{280} absorbing material into extra cellular medium was studied. The data on effects of lemon grass oil, palm rosa oil, eucalyptus oil and their major components on the leakage of potassium ions and UV_{260} and UV_{280} absorbing material showed that these oils and their components did affect the membrane integrity of E. coli and S. aureus.

The test compounds at minimum bactericidal concentration caused an increased efflux of K^+ ions into the extra cellular medium of the cells. In terms of absolute value of K^+ ion leakage, lemon grass oil caused the highest efflux of K^+ ions from E. coli cell suspension compared to the other two essential oils. It could be observed that lemon grass oil, which showed highest antibacterial activity of the tested essential oils [30] was the most effective in causing K^+ ion efflux.

One interesting observation is that in presence of crude essential oils the leakage of bacterial cellular materials was higher than that showed in presence of the individual major components of essential oils. The results of minimum bactericidal concentration, time course of lethal action of these compounds also suggested the same. This strengthens the hypothesis that components other than major component within the oil also affect the susceptibility of microorganisms. The results also point that the essential oils are more active against S. aureus, than E. coli. The reason for this could be due to the higher levels of tolerance shown by the outer membrane of Gram-negative bacteria to lipophilic compounds, as indicated [26] and very recently by [38]. However before coming to any conclusion about the preferential activity of essential oils are to be studied.

When we compare the time course lethal action of essential oils, eucalyptus oil and palm rosa oil was similar to certain extent, while there is much difference observed in case of inducing leakage of cellular material in presence of these oils. Given the heterogeneous composition of these essential oils, it seems unlikely that there is only one mechanism of action or that only one component is responsible for the antimicrobial action. So, it is possible that in addition to membrane damaging effects, there could be additional targets for these essential oils to act and this could be the reason for the small differences observed when we compare various results of our study. There were few reports showing [20,39] that terpenoids derived from essential oils act on primary energy metabolism, in particular NADH and succinate dehydrogenase activities, membrane bound respiratory electron flow and oxidative phosphorylation. Hence, studies on these additional targets might provide clear answer to the differences seen in the results.

However, the loss of UV_{260} and UV_{280} absorbing material and increased K+ ion efflux from the cell suspensions of bacteria in presence of all the test compounds suggest that cytoplasmic membrane of bacteria is compromised and damaged irreversibly by treatment with these compounds. The other observation of presence of correlation between the time course of lethal action and damage to cell membrane in majority of the cases suggest that membrane damage is one of the likely causes of cell death. Further, work on the effect of these compounds on microbial energy transduction, electron microscopy studies will give additional information about the mechanism of action.

CONCLUSION

In conclusion, our observations confirm that the antimicrobial activity of essential oils result from their ability to disrupt the permeability barrier of microbial membrane structures, although the presence of additional mechanisms or targets cannot be ruled out. The mode of action may be same against E. coli and S. aureus, and could be similar to that of other broad- spectrum membrane active disinfectants and preservatives, such as phenol derivatives, chlorhexidine, para benzoic acid derivatives and tea tree oil [27,40].

Declaration

Funding Source: *No funding is available for the present study.*

Conflict of Interest: *None of the authors (MNP, VK, SKP and JNLL) expressed conflict of interest.*

Ethical Approval: *This article does not contain any studies with human participants or animals performed by any of the authors.*

Data Availability Statement: *The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.*

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ISSUES AND CONCERNS OF CORPORATE GOVERNANCE IN INDIA

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Abstract

Corporate Governance conceptually is highly associated with ethical business. It is "A set of systems, processes and principles which ensure that a company is governed in the best interest of all stakeholders." It provides a commitment of transparency and observance of ethics in code of conduct and discharge of duties by all the persons involved in the affairs of the company. They are the ones who agree to take responsibility towards the shareholders. Corporate Governance is a broad term is today's business environment. Good Corporate Governance became a key word to handle accounting scandals and to mitigate growing concern about the quality of financial statements. In short Corporate Governance is about promoting corporate fairness, transparency and accountability. Corporate Governance is now an issue and important factor that can be used as tool to maximize the wealth of shareholders of a corporate. Corporate Governance aims are the Vision, Values and Visibility. In this paper we will study the concept, origin, principles, Issues and Concerns of Corporate Governance from the viewpoint of India.

Keywords: Corporate, Governance, Stakeholders, Ethical and Responsibility

Introduction: Corporate Governance is the system of rules, practices, and processes by which a firm is directed and controlled. Corporate Governance essentially involves balancing the interests of a company's many stakeholders, such as shareholders, senior management executives, customers, suppliers, financiers, the government, and the community. Corporate Governance involves a set of relationships between a company's management, its board, its shareholders and other stakeholders. Corporate Governance also provides the structure through which the objectives of the company are set, and the means of attaining those objectives and monitoring performance are determined.

Evolution of Corporate Governance

There have been several major corporate governance initiatives launched in India since the mid-1990s. The first was by the **Confederation of Indian Industry** (CII), India's largest industry and business

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association, which came up with the first voluntary code of corporate governance in 1998. The second was by the **SEBI**, now enshrined as Clause 49 of the listing agreement. The third was the **Naresh Chandra Committee**, which submitted its report in 2002. The fourth was again by SEBI — the **Narayana Murthy Committee**, which also submitted its report in 2002. Based on some of the recommendation of this committee, SEBI revised Clause 49 of the listing agreement in August 2003. Subsequently, SEBI withdrew the revised Clause 49 in December 2003, and currently, the original Clause 49 is in force.

Meaning and Definition of Corporate Governance

Corporate Governance in a set of acceptable, transparent, ethical, responsible, reasonable actions clerihew through the value system having large societal acceptability so as to achieve the vision of an organization. Corporate Governance in also concerned with developing and maintaining a balance between economic and social goals of an organization while also justifiable fulfilling the individual goals of the promoters and key investors.

The Corporate Governance framework should promote transparent and fair markets, and the efficient allocation of resources. Effective corporate governance requires a sound legal, regulatory and institutional framework that market participants can rely on when they establish their private contractual relations. In shorter terms `Corporate Governance' in related to how well, cohesively and with transparent efficiency the board of an organization leads it towards its goals. `Corporate Governance' has a relationship with the values and moral of a company and its directors. Corporate governance acts as a bridge between shareholders, stakeholders, and board of directors. It should be able to restore the trust and confidence of management and the company to the shareholders in the company.

The main function of Corporate Governance is to make agreements that describe the privileges and tasks of shareholders and the organization. In case of disagreements because of conflict of interest, it is the responsibility of corporate governance to bring everyone together. An important role of corporate Governance in to create vendors and benchmarks for the conduct of its directors and to ensure that they abide by their duties, responsibilities and obligations in the best of the interest of their shareholders, stakeholders, employees and society at large.

Significance of Corporate Governance: A corporation is a congregation of various stakeholders, namely customers, employees, investors, vendor partners, government and society. In this changed

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scenario an Indian corporation, as also a corporation elsewhere should be fair and transparent to its stakeholders in all its transactions. This has become imperative in today's globalized business world where corporations need to access global pools of capital, need to attract and retain the best human capital from various parts of the world, need to partner with vendors on mega collaborations and need to live in harmony with the community. Unless a corporation embraces and demonstrates ethical conduct, it will not be able to succeed. Corporations need to recognize that their growth requires the cooperation of all the stakeholders; and such cooperation is enhanced by the corporations adhering to the best Corporate Governance practices

Principles of Corporate Governance

- **Recognition to each shareholder**: Small shareholders in an organization have little impact on the stock price and so their interests generally disregarded and more importance is given to interests of majority shareholders and the executive in board. But principle of corporate governance says that organizations must value the rights of each shareholder and make sure that all shareholders are allowed to participate.
- **Duties toward other stakeholders**: Every organizations should be aware of that they have legal, social, and market driven obligations to non-shareholder stakeholders i.e. employees, creditors, suppliers, customers etc. And fulfilling of these obligations is must for the good corporate governance.
- **Board Size**. In determining appropriate board size, directors should consider the nature, size and complexity of the company as well as its stage of development. Larger boards often bring the benefit of a broader mix of skills, backgrounds and experience, while smaller boards may be more cohesive and may be able to address issues and challenges more quickly
- **Board Meetings**. The board of directors, with the assistance of the nominating/corporate governance committee, should consider the frequency and length of board meetings. Longer meetings may permit directors to explore key issues in depth, whereas shorter, more frequent meetings may help directors stay current on emerging corporate trends and business and regulatory developments.
- **Effective role of the Board**: The board needs to be of adequate size and have appropriate levels of independence. It also needs relevant skills to review and challenge management performance.

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All board members must be on the same page and share a similar vision for the future of the company.

- **Remuneration to the Board of Directors**: The board of directors' overall remuneration and benefits shall be decided by the annual general shareholders´ meeting. Considerations related to the company's size and complexity, the members' expertise and the amount of time committed as well as the possibility of recruiting suitable members shall be considered when evaluating the level of board fees.
- **Ethical behavior**: Every organization should develop such a code of conduct for their directors, executives and other members that promote ethical and responsible decision making.
- **Transparency**: Organizations should clarify the roles and responsibilities of board and management to provide stakeholders with a level of accountability. All the relevance matters concerning the organization should be made available to concerning party timely.

Review of Literature

- **Yoshikawa and Rasheed** (2009) The Governance codes become a source of normative institutional pressure for convergence within a country.
- **Rujitha** (2012) studied Regulatory issues in Corporate Governance and found that the loop holes in the provisions have to be removed. Companies should not be left to escape by taking advantage of the limitations of the clause 49 of the listing agreement.
- **Unadkat** (2017) found that India has witnessed several enactments which have contributed significantly in strengthening governance norms and in increasing accountability by way of disclosures. Interestingly, these changes have been inspired by the Anglo-Saxon or Anglo-US model of corporate governance.
- **Arora and Bodhanwala** (2018) Corporate Governance aims at facilitating effective monitoring and efficient control of business. Its essence lies in fairness and transparency in operations and enhanced disclosures for protecting interest of different stakeholders.

Objectives of the Study

- ✓ To understand the concept and origin of Corporate Governance.
- ✓ To Study the principles of Corporate Governance.
- ✓ To Study the Issues & Concerns on the way to Corporate Governance.

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Research Methodology

This paper is a descriptive in nature. The study is based on secondary data. The secondary data was collected through internet and print sources. Internet source is the main source of data for the data collection. Also, secondary data was collected from books, magazines, journals, Newspapers, past researches and various websites.

Issues & Concerns in Corporate Governance

- **The gap between the interest of management and isolated shareholders**: The constitutions of many companies stress and underline the business is to be managed "by or under the direction of" the board. In such a practice, the responsibility for managing the business is delegated by the board to the CEO, who in turn delegates the responsibility to other senior executives. Thus the board occupies a key position between the shareholder and the company's management.

- **They represent shareholder's interest by monitoring managers, approving strategies and policies and disciplining poorly performing managers**. A family owned controlled and managed business with intergenerational time horizons and material, direct shareholding may present far lower governance risks to long term investors than a listed company controlled by a foreign multinational where management have little incentive to grow the value of the local subsidiary.

- **Selection procedure and term of Board:** Selection procedure adopted in Indian corporations is biggest challenge for good corporate governance. Law requires a healthy mix of executive and non-executive directors, independent directors and woman director. Most companies' in India tend to only comply on paper; board appointments are still by way of "word of mouth" or fellow board member recommendations. It is common for friends and family of promoters and management to be appointed as board members. Life-term board members can pose many problems to business say fixed beliefs, power gaining etc. so no business prefers to appoint board members for life-term.

- **Performance Evaluation of Directors**: SEBI, India's capital markets regulator, has released a 'Guidance Note on Board Evaluation' in January 2017. Which cover all major aspects of Board Evaluation including the Subject & Process of Evaluation, Feedback to the persons being evaluated, Action Plan based on the results of the evaluation process, Disclosure to stakeholders,

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Frequency & Responsibility of Board Evaluation. But for achieving the desired objectives from performance evaluation, they need to make the evaluation result public and these disclosures may put the corporate in big trouble.

- **Missing Independence of Directors**: Independent directors' appointment was supposed to be the biggest corporate governance reform by Kumar Mangalam committee on corporate governance in 1999. However in reality independent directors have hardly been able to make the desired impact. Till now the appointment of directors in most of companies is made at the discretion of promoters, so it is still questionable. For providing the true success it is necessary to limit the promoter's powers in matters relating to independent directors.

- **Removal of Independent Directors**: Under law, an independent director can be easily removed by promoters or majority shareholders. When an independent director doesn't take the side with promoter's decisions, they are removed from their position by promoters. So to save their post directors have to work for the interest of promoters. To resolve this issue SEBl's International Advisory Board had proposed an increase in transparency for the appointment and removal of directors

- **Liability toward Stakeholders**: Indian company act 2013 mandates that directors owe duties not only towards the company and shareholders but also towards the other stakeholders and for the protection of environment. But generally board tries to limit and escape from these kinds of accountability. For this it may be a good idea to require the entire board to be present at general meetings to give stakeholders an opportunity to pose questions to board.

- **Protection of minority shareholders**: The protection of minority shareholders affected more by national legislation rather than the behavior of individual companies much of global corporate governance focus on boards and their committees, independent directors and managing CEO succession. In the Indian corporate, boards are not as empowered as in several western economies and since the board is subordinate to the shareholders, the will of majority shareholders prevails. Therefore there is a conflict in India between the majority and minority shareholders. The minority shareholders themselves have today a distribution that varies significantly from the past. Only the supervision boards and the institutes of ethics will be main safeguard of interest of minority shareholders.

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- **Founder/Promoter's extensive Role**: In India, instead of separate entity of businesses, promoters or founders continuously influence the business decisions Family owned Indian companies suffer an inherent inhibition to let go of control. They affect the decisions by influencing the board and management. This is done because they had the significant portion of company's share. So to remove this issue it will be good idea to amplify the shareholder base and reduce the shareholding of founders.

- **Transparency and Data Protection**: Corporate Governance is based on the principle of transparency but it cannot be defined what information is to be disclosed or not. In today's cut throat environment of competition it can be very dangerous if wrong information be disclosed. In digitalization Privacy and data protection is a central governance issue. For this the board must be capable of handling data and to ensure the protection of such data from potential misuse. And by looking at the importance of data and the potential cost if data be misused, we can say that organization must invest a reasonable amount of resources to protect the data.

- **Business Structure and internal conflicts**: Business structures also put hindrance on the way to governance as they require many layers of management, executives and other officers. This makes it very difficult for the company leaders to receive accurate, important data from the lower levels and to command orders to lower level of the company as the data may be distorted at any point of chain. Board of executives can make much good decisions and policies. But if the internal relationship in organization say between board and managers is not good then the implementation of decisions and policies also get affected.

- **Corporate Governance is about ethical conduct in business**. Ethics is concerned with the code of values and principles that enables a person to choose between right and wrong. Further, ethical dilemmas arise from conflicting interests of the parties involved.

- **Environment of Mistrust**: In recent years, many scams, frauds, misappropriation of public money and corrupt practices have taken place and because of the doubtful practices of key executives and board members, confidence of investors and society has diminished. It is happening in the stock market, banks, financial institutions, companies and government offices. This has made the business environment distrustful.

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Conclusion

In this paper, we have studied the Concept, Origin, Significance and Principles of Corporate Governance to a corporation or organization. In the process of Corporate Governance organizations may have to face some problems in short run, but in long run it will be advantageous and investors would be promoted to act like owners rather than just traders. We also discussed the issues and concerns in the way of corporate governance in India. Directors are appointed by the promoters and then they influence the decisions of directors for their personal interest. These issues can be tackled by regulating the size, selection criteria and procedure of independent directors. And also regulating the role of promoters or founders in corporate, so that directors can take their decisions without any biases. This will create competition for having best governance practice. After studying this paper we can conclude that although India has achieved a significant Corporate Governance regulation but being a developing country, has a long way to go on the path of Corporate Governance.

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Medicinal Plants: Biodiversity, Sustainable Utilization and Conservation

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Editors

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Preface

Plant wealth has been a source of medicine since the inception of human civilization. In spite of tremendous development in the field of synthetic drugs and antibiotics during the twenty-first century, plants play a vital role in modern as well as traditional medicine across the globe. According to WHO, 3.5 billion people have been depending on traditional medicine because of its safety features and effective curing of diseases. In recent past, there was a paradigm shift towards the herbal medicine for the reason that the pronounced adverse effects of many synthetic drugs and chemicals. Over 35,000 plants have been used in various human cultures around the world, while about 20,000 plants are marked for medicines and cosmetics. India is at the forefront in using and exporting herbal drugs because it enjoys the unique position with diverse flora spread over the entire Indian subcontinent. Ayurveda, Unani, and Homeopathic systems of medicine rely heavily on medicinal plants only. There is a need to have sustainable scientific cultivation and extensive phytochemical research. Similarly, there is a need to protect the wild genes of medicinal plants as these are at the verge of extinction due to unsustainable exploitation. No doubt, there is a lot of scope for organized sectors such as phytochemical, pharmaceutical, and herbal drug industries in India. Therefore, proper documentation of this group of plants is need of the hour.

The edited volume on *Medicinal Plants: Biodiversity, Sustainable Utilization and Conservation* is an outcome of Proceedings of International Symposium on 'Biodiversity of Medicinal Plants & Orchids: Emerging Trends and Challenges' held during 9–11 February, 2018 at Acharya Nagarjuna University, Guntur, India, convened by Dr. S.M. Khasim and sponsored by UGC, DST, and CSIR. It aims to report on the state of the art of scientific investigations that have been going on during the last half century on medicinal plants. All papers contained in the book are peer reviewed. Further, the manuscripts were reviewed by editors and editorial board of International Symposium, and those papers judged suitable for publication following the authors' consideration of reviewer suggestions appeared in this edited book.

In view of the importance of medicinal plants globally for their large-scale cultivation and emerging value for the human health, we felt the necessity of this first comprehensive compilation by International experts. To inculcate the basic knowledge and recent trends among the researchers and teachers, the broad spectrum of medicinal plants and their sustainable utilization have been dealt in the book.

The book contains four parts, (1) Biodiversity and Conservation, (2) Ethnobotany and Ethnomedicine, (3) Bioactive compounds from plants and microbes, and (4) Biotechnology. All four parts contain 49 papers authored by eminent scientists/professors of India as well as abroad.

In Part I, biodiversity and application of sea weeds as a resource of medicine for humanity have been discussed; there are two sea weed biodiversity hotspots in India viz., Gulf of Kutch (Gujarat) and Gulf of Mannar (Tamil Nadu) representing species richness with high endemism. Another chapter in this part deals about biodiversity of mangroves in Eastern Ghats of India, particularly Gautami–Godavari estuary of Andhra Pradesh. A comprehensive data on biodiversity of medicinal plants in Eastern Ghats of Andhra Pradesh has been documented in another chapter. Some new species from Eastern Ghats of Andhra Pradesh were well documented in this chapter. In Part II, structural design and establishment of database application system for Miao medicinal plants in Guizhou Province (China) have been explained. The Miao ethnomedicine is regarded as one of the most famous traditional medicines in China. Promoting a complementary in situ and ex situ conservation strategies for medicinal plants of the Qiandongnan Miao and Dong autonomous prefecture is highly recommended. In another chapter, Shui communities of Guizhou, Southwest China, their traditional knowledge of herbal medicinal plants was discussed. Dr. Tapan Mukherjee (former Scientist CSIR-NISCAIR, New Delhi, India) stressed the importance of documentation and protection of traditional knowledge in India and abroad. In Part III, established immunoassays for the determination of Ginsenosides were discussed, and they must be useful for quality control of various ginseng medicines. In another chapter in this part, elicitation of flavonoids in *Kalanchoe pinnata* by *Agrobacterium rhizogenes*-mediated transformation has been documented; various elicitation strategies were discussed with respect to the enhancement of bioactive compounds in *K. pinnata* leaves in this paper. GC-MS profile of secondary metabolites from *Cassia occidentalis* and *Coldenia procumbens* were dealt by some workers. Other topics on veterinary medicinal plants of Andaman and Nicobar Islands and herbal medicine market in China were elaborated in this part. In Part IV, molecular and cytogenetical approaches for genetic diversity analysis of wild and cultivated *Curcuma* from North-East India have been discussed. The high proportion of polymorphism observed in the gene pool of *Curcuma* would be significant in identification of novel genotypes for breeding programs and, their subsequent introduction and usage for therapeutic purposes. In another paper, standard protocols for micropropagation of banana cultivars from India and testing of their genetic fidelity using DNA markers were discussed. DNA barcodes of *Phyllanthus amarus* and physicochemical quality of potable water in the agency areas of India were well documented by some workers.

This book serves as a reference book for the researchers, teachers, and students of Biotechnology, Botany, Microbiology, Biochemistry, and Pharmaceutical sciences. It will be of equal interest to pharmaceutical industry, medical scientists particularly Ayurveda and Allopathy, agricultural scientists, and policy makers.

We would like to express our sense of gratitude to all the contributors from India and abroad for accepting our invitation to contribute chapters and for not only

sharing their knowledge, but also for admirably integrating their expertise in composing the chapters of various aspects. We greatly acknowledge Dr. Hiroyuki Tanaka (Fukuoka, Japan), Dr. Tomofumi Miyamoto (Fukuoka, Japan), Prof. V.S. Raju (Warangal, India), Prof. T. Pullaiah (Anantapur, India), Prof. M. Venkaiah (Visakhapatnam, India), Dr. N. Sanjappa (Former Director General, Botanical Survey of India, Howrah, India), Prof. P.B. Kavikishore (Hyderabad, India), and Dr. Tapan Mukherjee (NISCAIR, New Delhi), for sparing their valuable time for bringing the final shape of this edited book.

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Part IV Biotechnology

45 Development of Standard Protocols for In Vitro Regeneration of Some Selected Banana Cultivars (*Musa* spp.) from India

Saifuldeen Ahmed Hasan, S. M. Khasim, and J. Ramudu

Abstract

Banana is a very popular fruit due to its high nutritive value. It helps in reducing the risk of heart diseases and is also recommended for patients suffering from high blood pressure, arthritis, ulcers, and gastrointestinal and kidney disorders. Micropropagation of selected banana cultivars such as Grand Naine (G9), Monthan, and Red Banana on commercial scale using economical cytokinins (BAP) and an effective auxin (IAA) has been taken up in this study in order to supply them to farmers on an affordable price. Consistent shoot proliferation of commercial standard was best in 5 and 3 mg/L BAP for Grand Naine where the production schedule could be formatted accurately with quality shoots. However, for Monthan 10 and 2 mg/L BAP and for Red Banana 10 and 3 mg/L BAP along with 0.2 mg/L IAA for each, respectively, appeared to be more suitable for obtaining productive shoots for a viable commercial production scheduling.

Keywords

Banana cultivars · *Musa* spp. · In vitro regeneration · Auxins and cytokinins

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45.1 Introduction

Banana is the most popular fresh fruit all over the world, and its name comes from the Arabic word "banan," which means finger. Almost all banana cultivars are derived from *Musa acuminata* and *Musa balbisiana*. India is now the second largest producer of fruits and vegetables in the world, and banana stands in second place for exports (Anonymous 2016). Banana is grown in more than 150 countries, producing 105 million tonnes of fruit per year. The global production of banana is around 102,028.17 thousand tons of which India contributes 29.19%. Banana (*Musa* spp.) is an important fruit crop in India. Main banana-growing states in India are Tamil Nadu, Maharashtra, Gujarat, Andhra Pradesh, and Karnataka.

Banana is a very popular fruit due to its low price and high nutritive value. Its high vitamin B6 content helps fight infection and is essential for the synthesis of "heme," the iron-containing pigment of hemoglobin. The fruit is also rich in carbohydrates and potassium and a great source of fiber too. It is also a good source of phosphorus, calcium, and magnesium. The fruit is easy to digest and free from fat and cholesterol. It helps in reducing the risk of heart diseases when consumed regularly and is recommended for patients suffering from high blood pressure, arthritis, ulcer, gastroenteritis, and kidney disorders.

At present, India is the largest producer of banana in the world with about 30% of total global production. However, the export market share is a meager of 1%. With increased productivity/unit area, the export capabilities can be improved. This is possible by substituting the conventional suckers with virus-indexed and tissue-cultured plants and adopting the scientific methods of cultivation. In Andhra Pradesh, major banana-producing districts are East Godavari, West Godavari, Anantapura, Cuddapah, and Vizianagaram. Most popular varieties cultivated are Dwarf Cavendish, Robusta, Rasthali, Amritpant, Thella Chakkarakeli, Karpoora Poovan, Chakrakeli, Monthan, and Yenagu Bontha. The farmers are cultivating local cultivars which are low yielding, and the productivity of banana is quite low, i.e., 35 tonnes per hectare as against 65 tonnes per hectare in Maharashtra (Karuna and Rao 2016). Micropropagation of selected banana cultivars, such as Grand Naine (G9), Monthan, and Red Banana, on commercial scale using economical cytokinin (BAP) and an effective auxin (IAA) has been taken up in this study in order to supply them to farmers on affordable prices.

45.2 Media Preparation and Sterilization

MS basal medium (Murashige and Skoog 1962) was used for the culture of selected banana cultivars. The basal medium with different concentrations of BAP with a fixed concentration of IAA at 0.2 mg/L was used for optimizing an in vitro production protocol for the different selected banana cultivars. The media compositions consisting of different concentrations of BAP along with fixed concentration of IAA as the auxin at 0.2 mg/L in MS basal medium (Table 45.1) used in the present study

Table 45.1 Different growth regulators used in MS basal medium for optimizing in vitro protocol for selected banana cultivars

Media code	Growth regulators used	Concentration (mg/L)
M1	BAP + IAA	10 + 0.2
M2	BAP + IAA	5 + 0.2
M3	BAP + IAA	3 + 0.2
M4	BAP + IAA	2 + 0.2
M5	BAP + IAA	1 + 0.2

were designated as M1, M2, M3, M4, and M5. One liter of each of the media was dispensed in to 25 glass jars, and all the media was prepared at one stretch.

The final volume of medium was made up to 1 L. Each of the glass jar was dispensed with 40 mL of this medium and capped tightly. The jars were sealed with autoclavable polypropylene wrap and autoclaved for 45 min at a temperature of 121 °C and a pressure of 15 psi in a horizontal autoclave. The process of media preparation was repeated again every 2 or 3 weeks for subsequent culture transfers until the final data recording was completed. The glass jars were incubated at room temperature for a week in a separate clean room to check the sterility of the medium and then used for explant/culture inoculation purpose.

45.3 Micropropagation of Selected Banana Cultivars

45.3.1 Initiation of Explants

All the explants inoculated in the initiation medium started responding within 15 days under the given conditions. The outer leaf sheaths of the explants changed from off-white to green and started to unfold externally. At the base of explants of Grand Naine and Red Banana, blackening was noticed due to phenolic exudation. However, there was no blackening observed in Monthan cultivar, clearly indicating that phenolic exudation was found to be controlled and is not a serious problem (Tables 45.2, 45.3, and 45.4). The shoot tip explants of different banana cultivars exhibited varied response in terms of both encouraging growth signs. The categories of general growth response at this stage were assessed with its ability to unfold the leaf sheath and turn into green color. It was excellent (+++) when the response was in 2–3 weeks of incubation, very good (++) when the response was in 3–4 weeks of incubation, good (+) when the response was in 4 weeks of incubation, and poor (−) when there was no response at all.

In vitro propagation of banana preferably uses sword sucker as explant source. Despite pretreatment in antibiotic solution and surface sterilization in mercuric chloride, some bacterial contamination was observed in all the three banana cultivars. None of the explants had any fungal contamination. This study demonstrates reduction in microbial contamination, saving up to 85% of the explants in aseptic culture establishment supporting the subsequent micropropagation of the banana suckers.

Table 45.2 Response of Grand Naine to different initiation media

Media code	No. of explants initiated	No. of explants survived free of contamination	% Survival	General growth response	Distinct remarks
M1	5	4	80	++	Slight phenolic exudation
M2	5	5	100	+++	Slight phenolic exudation
M3	5	4	80	++	Slight phenolic exudation
M4	5	4	80	++	Slight phenolic exudation
M5	5	5	100	+++	Slight phenolic exudation
Total/ ave.	25	22	88		

+++ Excellent; ++ very good; + good; − poor

Table 45.3 Response of Monthan to different initiation media

Media code	No. of explants initiated	No. of explants survived free of contamination	% Survival	General growth response	Distinct remarks
M1	5	5	100	+++	No phenolic exudation
M2	5	4	80	++	No phenolic exudation
M3	5	4	80	++	No phenolic exudation
M4	5	5	100	+++	No phenolic exudation
M5	5	3	60	+	No phenolic exudation
Total/ ave.	25	21	84		

+++ Excellent; ++ very good; + good; − poor

In the present study, the overall bacterial contamination in Grand Naine was only about 12%, while that in Monthan was 16%, and in Red Banana, it was 20% based on survival rate. It is worth noting that the suckers of Grand Naine and Monthan were robust, and many outer leaf sheaths were eliminated at every stage of pretreatment and surface sterilization. However, the suckers of Red Banana when received from the field were smaller, and only a few outer leaf sheaths were removed during

Table 45.4 Response of Red Banana to different initiation media

Media code	No. of explants initiated	No. of explants survived free of contamination	% Survival	General growth response	Distinct remarks
M1	5	4	80	++	Slight phenolic exudation
M2	5	3	60	+	Slight phenolic exudation
M3	5	4	80	++	Slight phenolic exudation
M4	5	4	80	++	Slight phenolic exudation
M5	5	5	100	+++	Slight phenolic exudation
Total/ ave.	25	20	80		

+++ Excellent; ++ very good; + good; – poor

the entire process. The excessive bacterial contamination in Red Banana (20%) can be attributed to this aspect, and it cautions on choosing well-grown robust suckers for improved culture establishment.

45.3.2 In Vitro Multiplication of Initiated Explants in Different Media

During the first 4 weeks in culture, the external leaf sheaths of each explant, which were initially white, later turned green. Some elongation of the explant could be observed in all the three banana cultivars. Browning or blackening at the base of the explants occurred but only externally, which could be removed during the process of subculturing. Most of the axenic explants established in culture formed fresh multiple shoots by 4–8 weeks of longitudinally splitting and re-inoculating into the respective medium. However, each of the explant in each of the cultivar showed a vast variation in their ability to proliferate in different media.

45.3.2.1 The Multiplication Ratio

The establishment of substantial multiplication of the explant approximately took about 120–150 days depending on the cultivar. Eventually, until that period of 120–150 days, the individual explant/cluster was periodically transferred to fresh medium every 3 or 4 weeks in order to provide fresh nutrients and also prevent the ill effects of phenolics and tissue blackening. The base of the explants in the multiplication

stage expanded horizontally forming a compact base from which cluster of shoots usually 6–15 in number appeared from the basal mass. Each proliferating cluster is subdivided into smaller clusters of minimum three microshoots in each culture cycle, and the number of such smaller clusters thus produced is known as multiplication ratio throughout the present study. Usually each cluster consisting of three or four small proliferating shoot initials is the requirement as per the commercial standard.

45.3.2.2 Grand Naine

All the explants in M1 to M5 series of media showed considerably good response (Fig. 45.1) in 4 weeks of culturing. However, mixed type of multiplication and shoot elongation was seen in different combinations (Table 45.5). Good multiplication of proliferating clusters was obtained in M2 and M3 medium by three successive transfers in the respective medium. Poor multiplication ratio was seen in M1 medium. The shoots had poor quality often with yellow leaves.

The clumps were neither multiplied vigorously nor elongated sufficiently. Even in M5, horizontal proliferations of shoots were not observed. Instead, all the shoot initials exhibited shoot elongation, and sturdy plantlets were obtained.

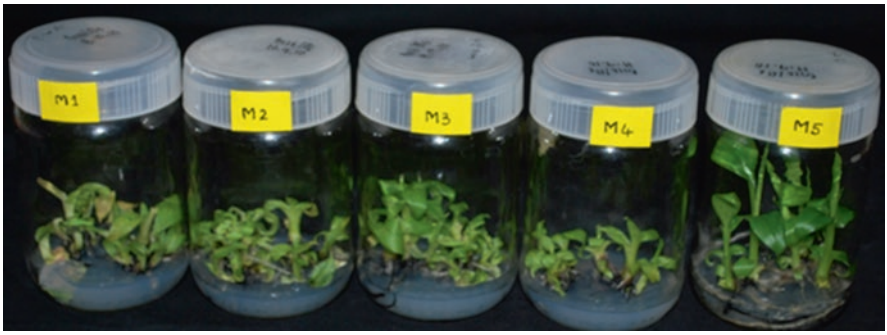

Fig. 45.1 Response of Grand Naine banana cultivar in M1 to M5 multiplication media after three subcultures

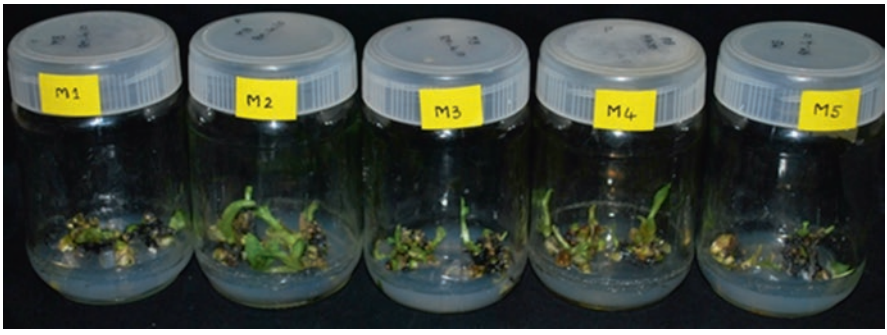

Fig. 45.2 Response of Monthan in M1 to M5 multiplication media after three subcultures

45.3.2.3 Monthan

The explants of Monthan in M1 to M5 also showed an impressive proliferating pattern (Fig. 45.2) with good multiplication ratio in 4 weeks of culturing (Table 45.6). However, the cultures did not appear to be remarkable as obtained for that of Grand Naine in M1 to M5 series of media. Elongation of shoots and leaf expansion were hardly found in these series of media in 4 weeks of culturing. Most of the cultures were very compact with the emerging shoot tips covered with a blackened scale. None of the clump was more than 2 cm in height.

45.3.2.4 Red Banana

All the explants in M1 to M5 series of media showed considerably good response (Fig. 45.3) in 4 weeks of culturing (Table 45.7). Good multiplication of proliferating clusters was obtained in M1 and M2 medium by three successive transfers in the respective medium. In M3 medium, a mix of shoot elongation and proliferation was found. Somehow, the quality of cultures was not good in M4. Instead, all the shoot initials exhibited shoot elongation and produced sturdy plantlets in M5. In all stages of growth, the red shade was found on the stems once the cultures were incubated in 12-h light.

Table 45.5 Multi-ratio of banana cultivar Grand Naine in different initiation media in three successive growth cycles, each cycle of 4 weeks

Media code	I multi-cycle	II multi-cycle	III multi-cycle	Ave. no. of elongated shoots/jar	Culture quality
M1	2.2	2.0	1.5	0	+
M2	3.0	2.8	2.5	0	+++
M3	2.5	2.2	2.0	3	+++
M4	2.0	1.8	1.5	3	++
M5	3.5	3.5	3.5	5	+++

+++ Excellent; ++ very good; + good; – poor

Table 45.6 Multi-ratio of banana cultivar Monthan in different initiation media in three successive growth cycles each cycle of 4 weeks

Media code	I multi-cycle	II multi-cycle	III multi-cycle	Ave. no. of elongated shoots/jar	Culture quality
M1	3.7	3.8	3.6	0	+++
M2	3.9	3.9	3.9	0	+++
M3	2.5	2.4	2.3	2	++
M4	2.5	2.5	2.3	3	++
M5	1.8	1.7	1.5	4	+

+++ Excellent; ++ very good; + good; – poor

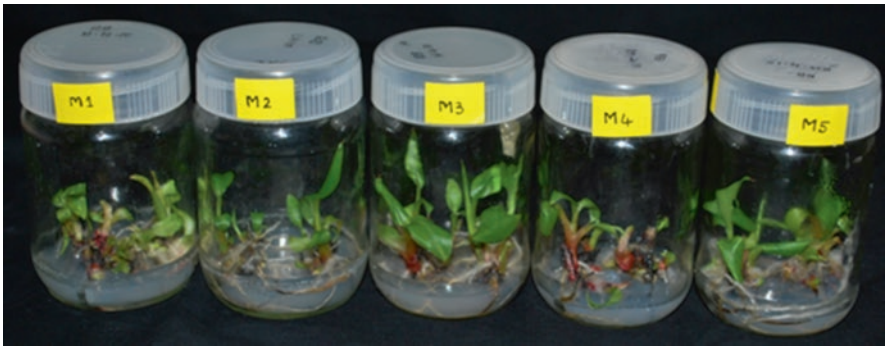

Fig. 45.3 Response of Red Banana in M1 to M5 multiplication media after three subcultures

45.3.3 Response of Banana Shoots in Medium Containing Activated Charcoal

Shooting and rooting stage is a pre-final in vitro growth stage required for banana cultivars. The microshoots produced during multiplication stage will generally carry the effect of cytokinin and hence will not possess sturdy stems although they may or may not have the roots. It is very important to have sturdy stems and sufficient roots prior to transferring them to rooting medium. In order to obtain good quality plantlets, the multiplying cultures will pass through a shooting and rooting stage where individual microshoots are allowed to elongate and mature in the absence of cytokinins. This was achieved by transferring separate single plants extracted from small clusters of 2–3 slightly elongated shoots into MS basal medium with 1% activated charcoal (AC) without any plant growth regulators. The elongated shoots of banana in AC medium further elongated and grew up into sturdy plantlets by 3–4 weeks with an average shoot length of about 6 cm (Table 45.8).

45.3.4 A Model for In Vitro Micropropagation Protocol for Banana

It can be clearly understood from the flow charts (Figs. 45.4, 45.5, and 45.6) that M1 and/or M2 was good for multiplication and bulking of the proliferating clusters over a period of several cycles at least up to six multiplication cycles. The shoot elongation was best in M5 medium for all the three cultivars. However, for Monthan and Red Banana, it was essential to culture the shooting clusters for two successive cycles. The shoots could be completely rooted ready to be sent to greenhouse for acclimatization after 3–4 weeks of culture in MS basal medium containing 1% activated charcoal. The entire process of initiation up to getting the rooted plants in vitro for each of the cultivar carried out in the present study is shown as flow charts (Figs. 45.4–45.6).

Table 45.7 Multi-ratio of banana cultivar Red Banana in different initiation media in three successive growth cycles each cycle of 4 weeks

Media code	I multi-cycle	II multi-cycle	III multi-cycle	Ave. no. of elongated shoots/jar	Culture quality
M1	3.2	3.5	3.4	0	+++
M2	2.8	2.7	2.5	0	++
M3	1.8	1.9	2.0	3	++
M4	1.5	1.3	1.2	3	+
M5	1.5	1.3	1.2	5	++

+++ Excellent; ++ very good; + good; – poor

Table 45.8 Effect of charcoal medium on general growth parameters and quality of shoots on different banana cultivars after 4 weeks of growth

Banana cultivars	Leaf color	Growth vigor	No. of leaves/ plant	Shoot length (cm)	Stem girth (mm)	Root quality
Grand Naine	Dark green	+++	5	5–6	6–8	A lot of black elongated roots
Monthan	Pale green	++	3	4–5	4–5	Black roots
Red Banana	Reddish green	+++	4	8	6–8	A lot of white roots, with root hairs

+++ Excellent; ++ very good; + good; – poor

Consistent shoot proliferation of commercial standard was best in 5 and 3 mg/L BAP for Grand Naine where the production scheduling could be formatted accurately, with quality shoots. However, for Monthan 10 and 2 mg/L BAP and for Red Banana 10 and 3 mg/L BAP along with 0.2 mg/L IAA for each, respectively, appeared to be more suitable for obtaining productive shoots for a viable commercial production scheduling.

For all the three cultivars investigated, shoot elongation was best in 1 mg/L BAP wherein the shoot initials elongated and the leaves expanded with profuse rooting. However, for Monthan and Red Banana, one cycle of 4 weeks in lesser BAP was not enough for shoot elongation. A second cycle of another 4 weeks was essential to obtain elongated shoots which could then be transferred to medium containing activated charcoal for development of complete plants.

The ex-agar plants of all three cultivars of banana performed very well in poly-tunnel during the process of primary acclimatization. Later plants were transformed to primary nursery. The net pot plants started to grow very rapidly after shifting them from poly-tunnel to the greenhouse under higher light intensity. Then, the plants are transferred to natural soil conditions.

Plant cells growing in vitro are considered to be under some degree of stress and may be predisposed to direct infection, even by microbes, which are not normally pathogenic to them. Thus, microbial contaminants are the major challenges in

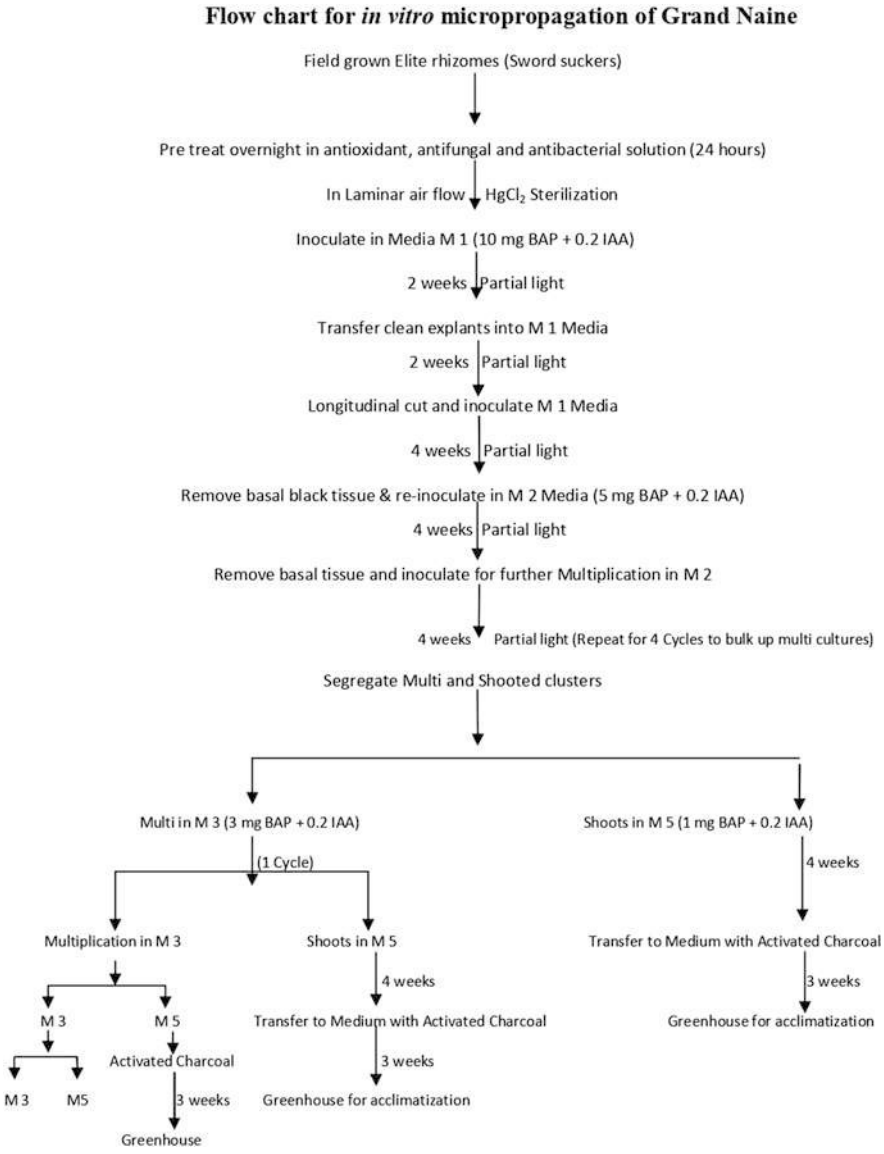

Fig. 45.4 Flow chart for in vitro micropropagation of Grand Naine

plant's in vitro propagation during the different stages of culture processes (Helaly et al. 2014), especially during the initiation stage; to minimize the microbial contamination and to promote healthy growth, explants were treated with 70% absolute alcohol for 6 min, 0.1% mercuric chloride for 10 min and 0.2% for 10 min, 1% sodium hypochlorite for 15 min, 0.1% cefotaxime for 5 min, and 0.05% gentamycin for 5 min (Shashikumar et al. 2015).

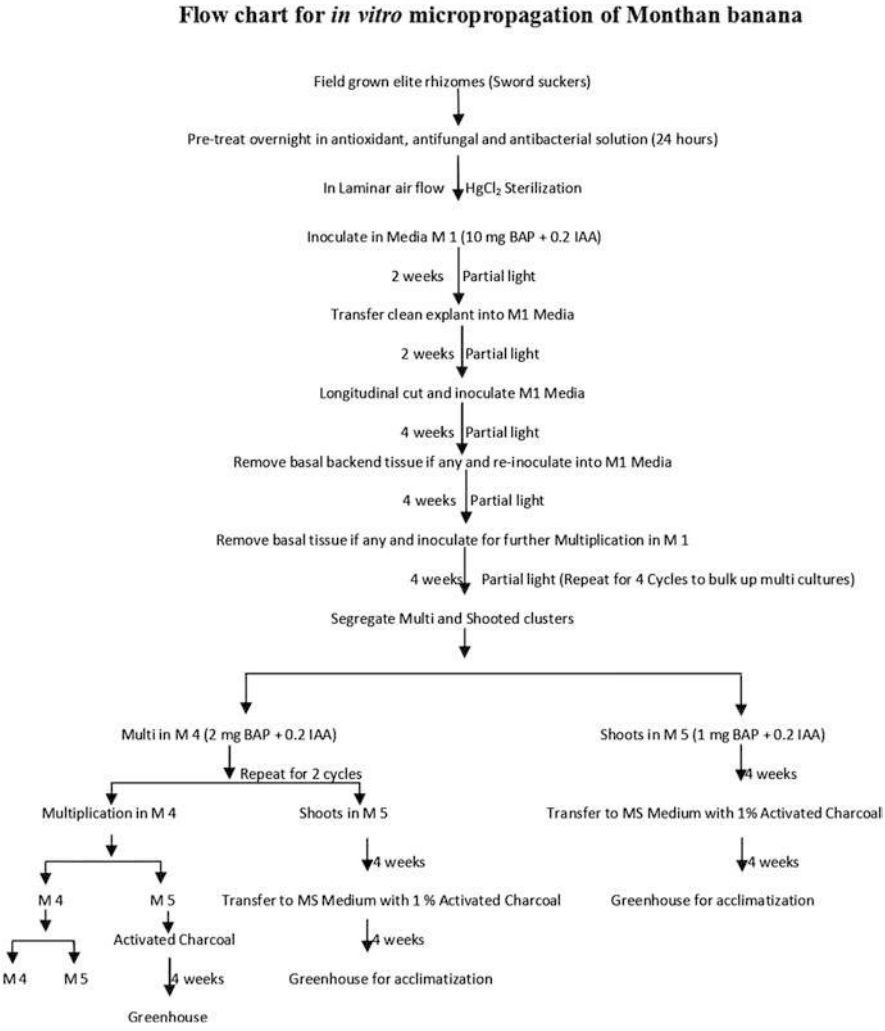

Fig. 45.5 Flow chart for in vitro micropropagation of Monthan banana

45.3.5 Importance of Bud Splitting Technique

In the present study, the axenic explants were longitudinally split to promote rapid multiplication. In this study, bud splitting technique is applied; a remarkable multiplication of shoots is achieved in about 120 days in Grand Naine cultivar and about 150 days in Monthan and Red Banana. The rate of multiplication was more than threefold in all the three cultivars in the present study. The concept of injury to shoot tip buds to promote axillary proliferation by breaking the apical dominance was reported by Woolley and Wareing (1972). Bud splitting is not a common practice in banana tissue culture. But splitting of the shoot tip in banana was reported to

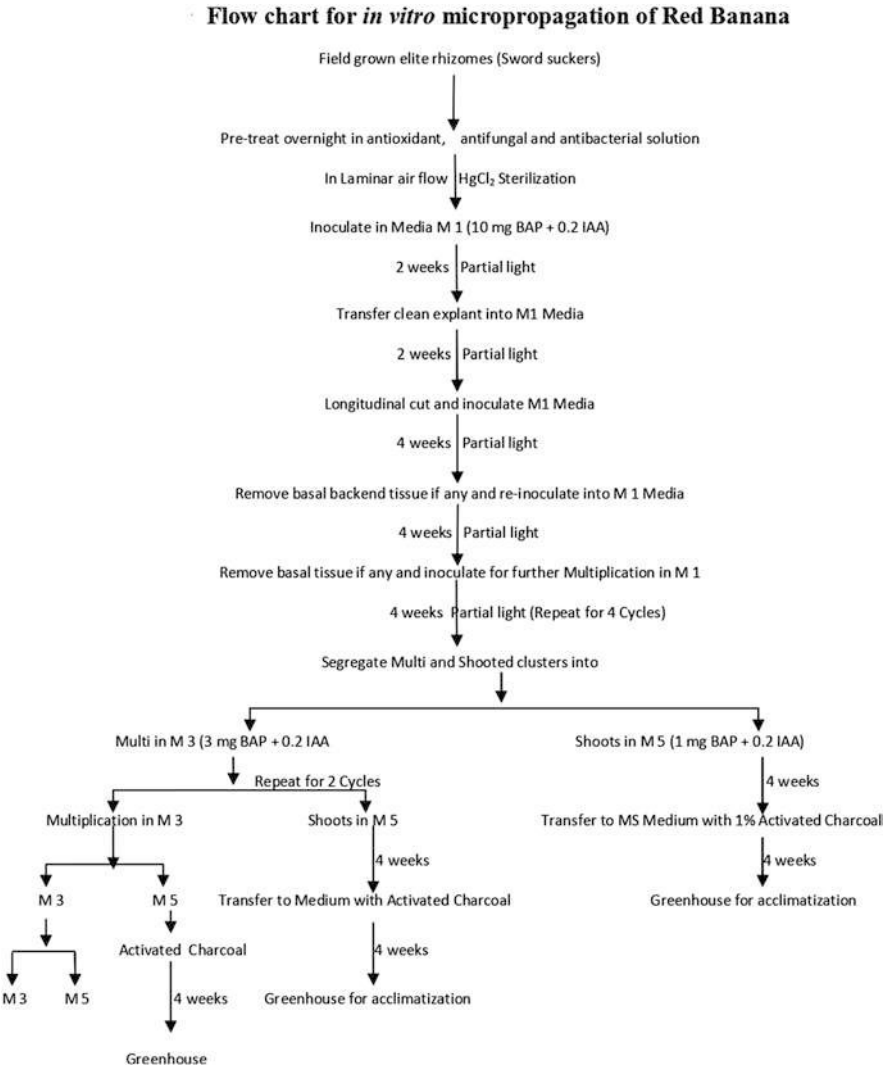

Fig. 45.6 Flow chart for in vitro micropropagation of Red Banana

enormously increase the growth rate and shoot proliferation (Swamy et al. 1983). In the application of bud splitting technique, the meristematic tissues in juvenile state are very competent that are found in young suckers. Generally longitudinal bud splitting promotes the rapid multiplication (Hussein 2012). These auxiliary meristems are commonly the source of formation of buds in nature especially when the apical shoots are damaged or injured (Burrows 1989). In another study, Mateille and Foncelle (1988) noticed that longitudinal cuts of buds induced a threefold increase in multiplication in *Musa* cv. Poyo from the Ivory Coast. The multiplication rate was also found to depend on the origin of suckers, whereby lateral buds doubled in

size within 3 weeks, while the apical buds reached three times their size. In general, different cultivars show variation in the degree of their shoot bud proliferation. This is because the multiple budding appears to be linked to genome configuration of a given cultivar (Khatri et al. 1997) and apical dominance which is under control of plant growth regulators. Thus, appropriate concentration of plant growth regulators and bud splitting technique in effort to break dormancy and induce multiple buds development are necessary to ensure production of large number of shoots in vitro, which is a commercial requirement in banana cultivars.

45.3.6 Role of Activated Charcoal (AC) and Antioxidants in Tissue Culture Media

In the present study, phenolic exudation in the initiated explants was found in Grand Naine and Red Banana cultivars in the first few cycles. It was interesting to note that no phenolic exudation was found in Monthan cultivar. Gradually, the phenolic exudation was found to be controlled even in Grand Naine and Red Banana too. Generally, banana and plantain explants are susceptible to tissue blackening with excessive exudation of phenolics, which hinders the normal growth of tissue in vitro. A wide variety of chemicals and antioxidants have been used to prevent the phenolic exudation (Safwat et al. 2015). Antioxidant, i.e., ascorbic acid, has been successfully used by Strosse et al. (2004) and Abdelwahd et al. (2008) to inhibit phenolics and to reduce the oxidative blackening in various plant species.

The use of activated charcoal in micropropagation was first reported by Fridborg et al. (1978), and it was suggested that charcoal was responsible for absorption and desorption which controlled the release of nutrients in the production of synthetic seeds (Ganapathi et al. 1992). Activated charcoal has a very fine network of pores with large inner surface area on which many substances can be adsorbed (Pan and Van-Staden 1998). Safwat et al. (2015) reported in their studies the effect of some antioxidants on blackening and growth of in vitro culture of banana. They were of opinion that the cultivated charcoal could be acted as antioxidant and used to minimize the blackening process of in vitro derived plantlets.

Activated charcoal has some effect on the morphogenesis by the irreversible adsorption of inhibitory compounds in the culture medium, thus substantially decreasing the toxic metabolites, phenolic exudation, and accumulation of brown exudates (Thomas 2008). In the present study, soaking the explants in pretreatment solution consists of citric acid and ascorbic acid at 0.1 g/L for 24 h, and removal of outer leaf sheaths at every stage coupled with frequent washes at every stage during process of initiation has effectively controlled harmful effects of phenolics. Antioxidant growth regulators are considered as one of the most important factors in the development of a standard tissue culture protocol (Dayarani and Dhanarajan 2013), and the present study endorses previous reports. In this present study, it could be seen that all plants in activated charcoal media had sturdy with glossy leaves, which can be attributed to the adsorption capability of charcoal eliminating the remnant effects of cytokinins and auxins.

45.3.7 Role of IAA (Auxin) and BAP (Cytokinin) in Tissue Culture Media

The multiplication rate of adventitious buds under the influence of cytokinin is one of the determining factors deciding on the efficiency of the micropropagation system. It is evident from the present study that the multiplication of the explants inoculated in MS basal medium containing BAP and IAA resulted in axillary shoot proliferation and shoot elongation. Axillary shoot proliferation and shoot elongation required for commercial purpose were successfully achieved for banana cultivars in this investigation. In the methodology adopted here, elongation of the terminal shoot is suppressed by excising the shoot tip, thereby promoting the proliferation of the axillary shoots. The suppression of terminal shoot allows multiplication of a large number of microshoots. The process of axillary proliferation in banana is further augmented by the use of BAP and IAA.

The multiplication ratio of banana cultivars is strongly supported by BAP and IAA (Dagnew et al. 2012; Ahmed et al. 2014; Reddy et al. 2014; Sahoo et al. 2015; Karule et al. 2016) as also obtained in the present study substantiating that the combination of BAP and IAA is an influential cytokinin-auxin combination for in vitro micropropagation of banana. Adenine-based cytokinins are used in several *Musa* spp. for in vitro propagation (Gubbuk and Pekmezci 2004). N6-benzylaminopurine (BAP) is the most commonly preferred cytokinin (Vuylsteke 1989).

Increased levels of cytokinin inhibit apical dominance and promote lateral shoot proliferation. This principle holds good for banana cultivars wherein higher multiratio was obtained at 5 or 10 mg/L of BAP along with 0.2 mg/L of IAA and lesser multiplication ratio with elongation of shoots was obtained at 1 mg/L BAP. However, the multiplying cultures of Grand Naine were not good in medium with 10 mg/L BAP over a period of 3–4 multiplication cycles. Yellowing of leaves occurred after three to four subcultures. Higher concentrations of BAP and kinetin beyond optimum levels were also reported to cause necrosis and reduction in shoot formation during in vitro multiplication of Nendran (Rabbani et al. 1996). Reddy et al. (2014) also stressed the importance of BAP at low concentration about 2 mg/L that had given the best induction of Grand Naine plantlets.

After the initial shoot multiplication in M1 as booster multiplication medium for a few cycles, the normal multiplication for bulking was carried out in M2 for Grand Naine, while M1 continued to be used for Monthan and Red Banana. However, an intermediate shoot elongation medium was adopted for each of the cultivar, i.e., M3 for Grand Naine and Red Banana (both belong to AAA genome) and M4 for Monthan in which a combination of dwarf multiplying clumps and also clumps with elongated shoots was obtained. The dwarf clumps were excised on top and re-inoculated for further multiplication, while the elongated clumps were transferred to M5 without cutting the top for further elongation. Shoot elongation was observed in the lowest concentration tried in M5 medium (1 mg/L BAP + 0.2 mg/L IAA). These results clearly establish the fact that optimal growth and morphogenesis of tissues may vary among the cultivars of same genus based on their nutritional and hormonal requirements.

Sahoo et al. (2015) reported that MS medium supplemented with 2 mg/L BAP and 1 mg/L IBA was found to be ideal for the early shoot elongation after 30 days of inoculation in Grand Naine cultivar. Further, Lalrinsanga et al. (2013) revealed highest multiple shoot induction in MS medium fortified with 5 mg/L BAP with 2.17 shoots while MS with 1 mg/L NAA + 0.2 mg/L BAP with longest regenerated shoots after 45 days of incubation.

Micropropagation of banana through initiation of shoot tip explants has been reported by a number of researchers in MS basal medium supplemented with 5–20 mg/L BAP (Priyono 2001; Noor-Aziah and Khalid 2002; Venkatachalam et al. 2006). Some researchers have reported that a combination of BAP and an auxin has enhanced proliferation and shoot length during the tissue culture of banana (Ngomuo et al. 2014). TDZ, a phenyl urea-based cytokinin, is frequently used along with BAP and IAA (Gubbuk and Pekmezci 2004).

In the previous study with banana cultivar Robusta (Saifuldeen 2015), it was demonstrated that BAP along with an auxin is most suited for commercial in vitro micropropagation. In the present study, among the various BAP concentrations (1–10 mg/L) along with a fixed concentration of IAA (0.2 mg/L), for the tested cultivars, it was found that maximum shoot multiplication was obtained in 5 mg/L BAP for Grand Naine. However, maximum shoot multiplication for Monthan and Red Banana was obtained in 10 mg/L BAP.

45.3.8 Acclimatization of In Vitro Derived Plants

Micropropagated plants are cultured within in vitro environments, with a high relative humidity and low light intensity, taking nutrients and energy from the culture medium. These plants do not possess protective mechanisms against desiccation. Impaired stomatal function (Marín et al. 1988) and reduced epicuticular waxes (Gaspar and Coumans 1987; Sutter 1988) have been noted in these plants. In addition, their photosynthetic competence is reported to be reduced (Preece and Sutter 1991). During acclimatization, physiological and structural changes allow micropropagated plants to adapt to the new environment conditions, mainly to low relative humidity and high light intensity. As a result, plants become autotrophic and develop as normal plants.

Pospisilova et al. (1999) have opined that abnormalities occurring in morphology, anatomy, and physiology of in vitro derived plantlets could be repaired after transfer to ex vitro conditions and further stated that many plant species need gradual changes in environmental conditions to avoid desiccation losses and photoinhibition. By manipulating the in vitro environment, leaves that have greater tolerance to water stress and are photosynthetically competent could be developed as part of the acclimatization process in preparing plantlets for transferring out of culture (Seelye et al. 2003).

45.4 Conclusions

The present work has contributed to the development of suitable set of media for different stages of in vitro production protocol for Grand Naine, Monthan, and Red Banana cultivars. It is concluded from the results that each of the cultivars had specificity in the concentration with plant growth regulator requirement at different stages of growth for obtaining optimum results.

Further, under ideal conditions as per the in vitro production flow chart, each established explant is expected to generate about 10,000 plants in 12 months' time with 6–8 multiplication cycles. The entire laboratory process starting from selection of elite sword suckers followed by initiation to obtaining the ex-agar rooted plants has to be carefully planned and judiciously executed. It is essential to give extreme importance to dissections of multiplying cultures to obtain productive cultures clumps and the final product.

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Foreword

The family Orchidaceae is the largest family of flowering plants next only to Asteraceae. A great majority of orchids are epiphytic herbs. Although orchids are familiar to the general public due to their ornamental value, they also represent one of the most interesting groups of plants in a number of areas – ecology, morphology, physiology and embryology. Flowers of no other groups of plants show such diversity as orchids; apart from their variations in size, shape and colour, many of them show amazing resemblance to various animals including humans. Also, the flowers of orchids remain fresh for several weeks both on the plants and as cut inflorescences. As it is comparatively easy to raise interspecific and even intergeneric hybrids in orchids, a large number of hybrids, showing unparalleled diversity of flowers, are available in the market. Because of these desirable ornamental features, there is a great demand for orchid plants as well as cut flowers. Unfortunately, though India has great diversity of orchids, our floriculture industry of orchids has remained poor when compared to many Southeast Asian countries such as Singapore, Philippines and Thailand. As orchids are generally slow-growing, their multiplication through conventional means is very time-consuming. Orchids were the first to be commercially propagated using the technique of micropropagation. Now, most of the standard nurseries dealing with orchids use this technology routinely.

Pollination biology of orchids is fascinating and highly variable. Extensive studies have been carried out on pollination of orchids since the time of Darwin. Some species exhibit typical entomophily and a good number of them have also evolved autogamous self-pollination. However, a large number of species exhibit highly specialized pollination system – each orchid species is pollinated by just one specific pollinator. Many of them have evolved deceptive pollination syndrome; they attract animal pollinators by falsely exhibiting the presence of rewards, but do not provide any rewards. Sexual deception is the extreme form of pollination deception evolved to attract species-specific male pollinators. In all the species of *Ophrys*, for example, the flower not only resembles the female of the pollinating insect but also secretes species-specific pheromone to attract male pollinator. The male insect attracted by these features lands on the flower and tries to mate; this is termed as pseudo-copulation, during which it brings about pollination. Seeds of orchids are very small, almost microscopic, and are produced in large numbers. In some species the number of seeds per fruit is reported to be over a million!

In recent decades, overexploitation, human-induced environmental changes, biological invasions and climate change are creating havoc to the sustainability of our biodiversity. These changes have initiated the 'sixth mass species extinction crisis' in which a large proportion of species would become extinct in a geologically short time. As orchids require special habitat, they are highly vulnerable to these changes and require urgent conservation efforts using all available means. Apart from their horticultural importance, in recent years bioactive compounds from plant sources including orchids are gaining importance throughout the world. This again leads to overexploitation of wild species. Many of the overexploited orchid species have already been included in Appendices I and II of CITES. One of the basic requirements for effective conservation is the availability of data on the biology of the species. There is an urgent need to generate such data on most of the wild species of orchids.

Because of their ornamental and other biological importance, orchids have been favorite materials for research for centuries. Extensive studies are being carried out on both fundamental and applied areas of orchids. However, most of the recent literature is scattered in a large number of research papers and reviews. Although there are several books on horticultural aspects of orchids, there are no recent books bringing together the exciting details on other areas of orchid biology in recent years. The present book on *The Orchid Biology: Recent Trends and Challenges* edited by Professor S. M. Khasim and his coeditors from India, Mexico and Thailand is most welcome. This book is the outcome of the proceedings of an international symposium on 'Biodiversity of Medicinal Plants and Orchids: Emerging Trends and Challenges' held during 9–11 February 2018 at Acharya Nagarjuna University, Guntur, India, and also some invited chapters by experts. All the chapters have been grouped under five relevant sections: Cryopreservation and Biotechnology, Orchid Biodiversity and Conservation, Anatomy and Physiology, Pollination Biology and Orchid Chemicals and Bioactive Compounds. I understand that all the chapters have been peer-reviewed before sending to the press. I congratulate the editors for undertaking this compilation. I am confident that various chapters would provide a critical account of the past, present and future trends in diverse areas of orchid biology. Studies on the biology of orchids of India are very limited in spite of the vast diversity it has. Apart from providing consolidated data on various areas of orchid biology, I hope the book would encourage young researchers to take up studies on orchids, particularly of Indian biodiversity hotspots such as North-East Himalaya and the Western Ghats.

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Preface

The Orchidaceae constitutes one of the largest families of flowering plants comprising about 28,484 species. It contributes about 40 percent of monocotyledons. In India, it represents the second largest flowering plant family with 1,141 species in 166 genera and contributes about 10% Indian flora. Orchids comprise a unique group of plants, and their flowers are the most enchanting and exquisite creation of nature. Phylogenetically and taxonomically, the Orchidaceae has been considered as a highly evolved family amongst angiosperms. Orchids show the incredible range of diversity in shape, size and colour of flowers. Orchids with the most attractive and bewitchingly beautiful flowers have commercial importance in floriculture market around the globe. Millions of cut flowers of *Cymbidium*, *Dendrobium*, *Cattleya*, *Paphiopedilum*, *Phalaenopsis*, *Vanda*, etc. besides pot plants of orchids are sold in Western countries, and thus, orchid cut flower industry has now become a multimillion-dollar business in Europe, USA and Southeast Asia.

Besides ornamental value, orchids have got immense pharmaceutical potential. Root tubers of *Habenaria edgeworthii* form an important composition of 'Astavarga' group of drugs in Ayurvedic medicines. It is an established fact that tubers of some terrestrial orchids have been used for treatment of diarrhoea, dysentery, intestinal disorders, cough, cold and tuberculosis. Some orchids, particularly belonging to the genera such as *Aerides*, *Arachnis*, *Cattleya*, *Cymbidium*, *Dendrobium*, *Epidendrum*, *Oncidium*, *Paphiopedilum*, *Phalaenopsis*, *Renanthera*, *Vanda*, etc., have been extensively used to produce the internationally acclaimed hybrids. The Indian orchids are paradoxically victims of their own beauty and popularity. As a result, their natural populations have been declining rapidly because of unbridled commercial exploitation in India and abroad as well. Further this situation has led the orchids to the verge of extinction, e.g. *Renanthera imschootiana*, *Diplomeris hirsuta*, *Paphiopedilum fairrieanum* (already extinct), *Cypripedium elegans*, *Taeniophyllum andamanicum*, etc.

An edited book titled *The Orchid Biology: Recent Trends and Challenges* is the outcome of the proceedings of an international symposium on 'Biodiversity of Medicinal Plants and Orchids: Emerging Trends and Challenges' held during 9–11 February 2018 at Acharya Nagarjuna University, Guntur, India. Besides that, we also invited eminent orchid experts across the globe to contribute to this book, so as to enable us to report on state of the art of scientific investigations that have been going on for the last several decades on orchid biology. All papers contained in this

book were peer-reviewed by international experts. Further, the manuscripts were reviewed by editors and those papers that were judged as suitable for publication following the authors' considerations of reviewer suggestions appeared in this edited book.

In view of the importance of orchids globally for their large-scale production and exploitation for the human health and wealth, we felt that the comprehensive compilation by international experts is the need of the hour.

The present book contains five sections: (I) Cryopreservation and Biotechnology, (II) Orchid Biodiversity and Conservation, (III) Anatomy and Physiology, (IV) Pollination Biology and (V) Orchid Chemicals and Bioactive Compounds. All five sections contain 28 papers authored by eminent orchid experts/professors across the globe. This book serves as a reference book for researchers, teachers, orchid enthusiasts, orchid growers and students of biotechnology, botany, pharmaceutical sciences and ethnomedicine. It would be of equal interest to horticultural industry especially orchid industry, agricultural scientists and policy makers.

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Orchid Seed Ultrastructure: Ecological and Taxonomic Implications with Reference to Epidendroideae (Orchidaceae)

14

J. Ramudu, S. M. Khasim, and G. Ramesh

Abstract

Ultrastructure of orchid seed belonging to subfamily Epidendroideae (Orchidaceae) has been discussed. Orchid seeds are tiny and microscopic and produced lakhs in a single pod. In *Cycnoches ventricosum* it contains four million seeds. Since orchid seeds are non-endospermic, very few seeds germinate successfully and give rise to mature plant. The present paper deals with quantitative data related to the length and width of seed and embryo, percentage of airspace and number of testa cells. It is evident from the present study that *Cymbidium* spp. showed higher values of seed volume/embryo volume compared to the vandoid genera whereby higher percentage of airspace had been recorded in cymbidiums. Hence *Cymbidium* deeds are more buoyant and widely distributed throughout Indo-Malayan region. Based on seed morphometry, *Pholidota* is closely allied to *Coelogyne.* Similarly *Oberonia* and *Malaxis* show close affinity with *Vanda*.

Keywords

Orchid seed · SEM studies · Epidendroideae · Functional adaptations · Taxonomic implications

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14.1 Introduction

The Orchidaceae is one of the largest families of flowering plants. It comprises about 28,484 species all over the world (Govaerts et al. 2017). In India, with 1350 species, it represents the second largest flowering plant family and contributes about 10% of Indian flora (Kumar and Manilal 1994; Jalal and Jayathi 2012). Orchid seeds are light in weight and the tiniest among the seeds produced by flowering plants, and these are non-endospermic and vary considerably in their size, shape, morphology and colour, and minute details and morphological characters have got tremendous ecological significance. In majority of orchid species, seed size shows variation from 300 to 800 μm (Molvray and Kores 1995). Orchid seeds exhibit a wide range of diversity in their sizes (ranging from 0.1 mm in *Oberonia* to 6 mm in *Epidendrum*) and shapes but also complexity of light weight seed coat architecture and hierarchical surface sculpturing (Barthlott 2014). The taxonomic significance of the seed characteristics was first reported by Clifford and Smith (1969). Besides, serving as taxonomic markers, the morphological characters of seeds can be used to deduce phylogenetic relationship (Barthlott 1976) and to identify their involvement in hybrid genotypes (Arditti et al. 1979). The present paper deals with an overview on seed ultrastructure with reference to subfamily Epidendroideae (Orchidaceae).

Orchid seeds show the remarkable diversity not only in their sizes (from 0.1 mm in *Oberonia* to 6 mm in *Epidendrum*) and shapes but also in the complexity of light weight seed architecture and surface sculpturing (Barthlott et al. 2014). Verma et al. (2014) studied the seed physical characteristics of 32 Western Himalayan orchids (threatened) using light and scanning electron microscopy; they stressed the importance of seed characteristics in elucidating the taxonomic and phylogenetic interrelationships. Seed morphology has got importance in delineation of species within the genus and also delineation of subgeneric groups (Matthews and Levins 1986; Ness 1989; Vij et al. 1992; Larry 1995; Augustine et al. 2001). Molvray and Kores (1995) also reported that the orchid seed varies in shape from filiform to fusiform, clavate to ellipsoidal and oftenly prominently winged. Barthlott and Ziegler (1981) worked elaborately on the seed coat structure of orchids. In their study, they have recognized 20 different seed types by taking varying seed characteristics.

The taxonomic importance of seed characteristics was first pointed out by Clifford and Smith (1969); later Dressler (1981) proposed several classifications based on conventional micromorphological characters. Matthews and Levins (1986) and Larry (1995) opined that seed micromorphology serves as a source of systematic characters to circumscribe subgeneric groups or hypothesize relationship among species within the genus.

Based on SEM studies of orchid seeds, Barthlott (1976) concluded that the morphological characters can be used to deduce phylogenetic relationships. Arditti (1979) also opined that seed volume in orchids reflects their size of seeds. Arditti et al. (1980) revealed that L/W (length/width) ratio of seed provides some very important information on the relative degree of truncation of orchid seeds. Arditti et al. (1980) and Augustine et al. (2001) found that seed testa cells show reticulation; if reticulation is present, the pattern may be varied from genus to genus.

Barthlott and Ziegler (1981) worked on the shape of the orchid seed. They had recognized 20 different seed types based on shape, testa cells length, sculpturing pattern of testa cells, presence of intercellular gaps and beading. An extensive review on orchid seed and their taxonomic significance was given by Barthlott (1976), Tohda (1986), Chase and Pippen (1988, 1990), Kurzweil and Weber (1991), Petersson (1991) and Arditti and Ghani (2000).

Vij et al. (1992) had done substantial work on orchid seeds and opined that the seed protected with the thickening and sculpturing pattern had varied from habitat to habitat. They also observed that the thickenings are thin in terrestrial orchids whereas thick in epiphytic orchids.

Kurzweil (1993) had done pioneering work on seed micromorphology using SEM in South African Orchidaceae and classified them into two seed types: (1) *Satyrium* type in which orchid seed testa cells have straight or slightly undulate and thickened anticlinal cell walls and (2) *Disa uniflora* type in which seed coat consists of convex cells with undulate anticlinal walls.

Molvray and Kores (1995) observed that seeds are covered by hard coat made up of testa cells and embryo loosely arranged and papery in texture. Augustine et al. (2001) also made SEM studies on *Bulbophyllum* seed micromorphology.

Swamy et al. (2004) studied the seed micromorphometry of orchids of Karnataka using SEM and found that presence of twisted ropelike testa cells in both *Aerides maculosa* and *Xenikophyton smeeanum* shows close affinity in these species. Sharma et al. (2004) studied the seed morphometry of *Paphiopedilum* spp. using SEM and reported spindle-shaped seeds in this genus. Swamy et al. (2004) also studied based on SEM of orchid seeds of Western Ghats of Karnataka and concluded that maximum relative degree of truncation is found in *Coelogyne breviscapa* and minimum in *Eria dalzelli.* They also studied SEM studies of orchid seed of Western Ghats and found that embryo volume can change during its development from zygote to seedlings; the seeds with higher percent of airspace get dispersed over wide geographical areas.

Gamarra et al. (2007) have been done pioneering work on seed micromorphology of genus *Neottinae* (Orchidaceae) and observed that all seeds are fusiform in shape with transverse ridges on the inner periclinal walls, which is the characteristic feature of genus *Neottinae.*

Gamarra et al. (2007, 2008, 2012) extensively studied the seed micromorphology in subtribe Orchidinae and found that sculpturing pattern of testa cells plays a significant role in identification of orchid species. Aytasakcin et al. (2009) studied seed morphometry of Turkish orchids. They were of opinion that some of the seed characters such as seed length, testa cell reticulation, seed volume/embryo volume and airspace are useful for taxonomy of Orchidaceae.

Verma et al. (2014) studied the seed micromorphometry of threatened orchids of Western Himalaya based on SEM and observed different seed shapes such as fusiform, spathulate, ovoid and filiform shaped seeds. They were of opinion that the embryo was tiny and most of the seed occupied with 79% airspace is found in Himalayan epiphytic orchids. The highest seed volume/embryo volume ratio is found in terrestrial orchids.

Chaudhary et al. (2014) studied the comparative seed micromorphology in *Dendrobium* (Orchidaceae) and concluded that species from temperate region have larger seed volumes and seed volume/embryo volume ratio than species from subtropical or tropical regions. They also reported maximum airspace in tropical and subtropical dendrobiums compared to temperate species. Brzosko et al. (2017) studied the seed dispersal in some terrestrial orchids in Biebrza National Park, North East Poland.

14.2 Seed Micromorphology

14.2.1 General Seed Micromorphological Features and Functional Adaptations in the Orchidaceae

Orchid seeds are unique in their tiny nature and produced in large numbers, ranging from 1300 to 10,00,000 per capsule (Garg et al. 1992) and even in a single pod of *Cycnoches ventricosum* that contains four million seeds (Arditti and Ghani 2000). Due to lack of endosperm, all seeds will not germinate, out of which very few seeds germinate whenever the fungal infection and other specific requirement is available (desired habitat and substrate); even in seedling stage itself, most of individuals will die. There is a heavy mortality at any stage of seedling development; very few seedlings successfully grow and give rise to mature plant. In order to cope with this problem, orchids have been developed some adaptive features such as occurrence of lakhs of seeds in a pod, minute seeds, extremely light in weight, etc. The seeds differ from those of the most other angiosperms and resemble the so-called dust seeds of other plants (Molvaray and Chase 1999; Arditti and Ghani 2000). The dustlike seeds (Figs. 14.1 and 14.2), a significant character exhibited by orchids, are well suited for long-distance dispersal by wind.

Seed Shape The SEM studies revealed that the seeds of all studied taxa are very minute and these varied in their shape and size (Figs. 14.1 and 14.2). Seed shape varies from quadrilateral in *Malaxis densiflora* and fusiform in *Cymbidium aloifolium* to elongated in *Coelogyne nervosa* and shorter with bulged central part in *C. nitida* (Fig. 14.1a–d). In the case of *Calanthe triplicata*, they are filamentous, whereas in *Vanda tessellata*, ropelike appearance is seen (Fig. 14.2a, b). Spindle shape has been reported in *Oberonia* and *Pholidota* (Fig. 14.2c, d). The seed shape of studied taxa varies from fusiform or short to elongated and narrowly ellipsoid, spathulate and spindle shaped. Similar observations have been reported in several other orchid taxa by many workers (Kurzweil 1995; Swamy et al. 2004; Chaudhary et al. 2014; Verma et al. 2014). Barthlott and Ziegler (1981) reported 20 different types of seed based on shape, testa cells, length and sculpturing of cells and presence of intercellular gaps and beading. Arditti et al. (1979, 1980) and Verma et al. (2012) had advocated the evolutionary significance of seed shape. Fusiform seeds that are observed in all the subfamilies appear to be basic form in orchids from which all other seed shapes evolved. Vij et al. (1992) reported that fusiform seed is found in primitive orchids and ovoid, elliptical, filamentous, cylindrical seeds are found in advanced epidendroid orchids. Molvray and Chase

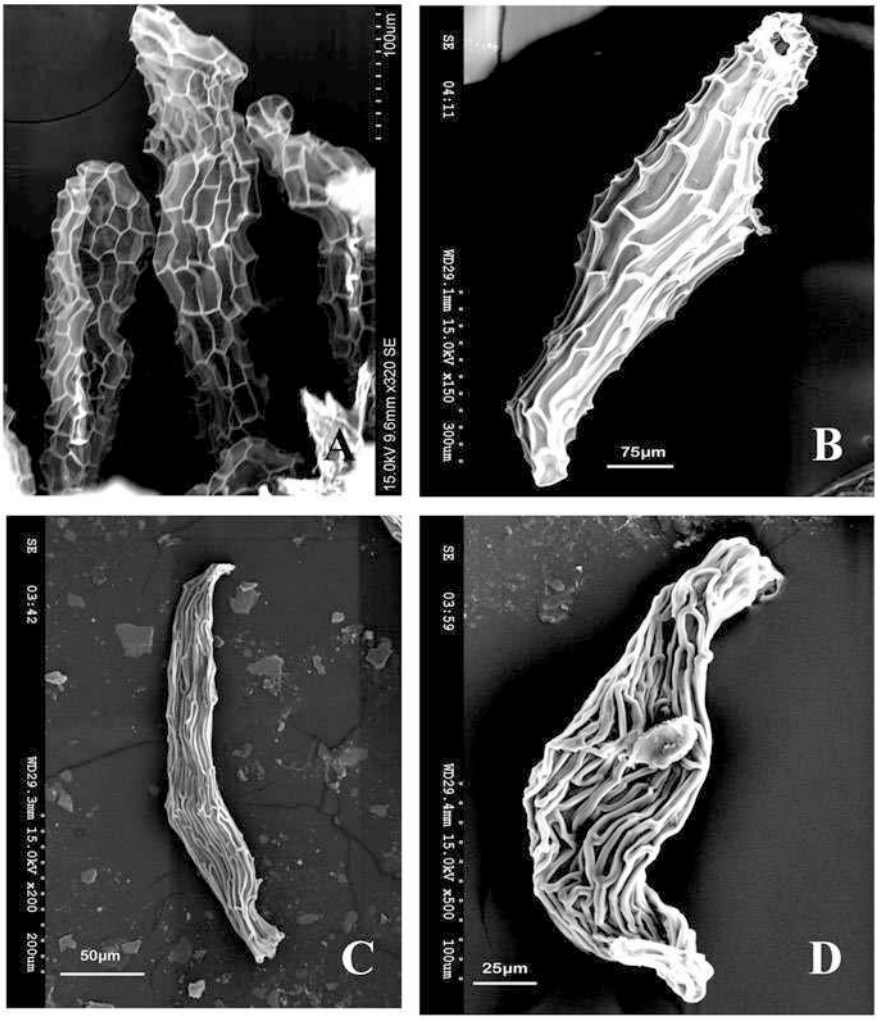

Fig. 14.1 SEM photographs of orchid seed. (**a**) *Malaxis densiflora*, transparent seed, quadrilateral-shaped seed with blunt ends; (**b**). *Cymbidium aloifolium*, fusiform-shaped seed; (**c**) *Coelogyne nervosa*, elongated seed; (**d**) *C. nitida*, shorter seed with a bulged central part having ellipsoid embryo

(1999) reported that seeds of fusiform, oblong or filiform shape are very common in Epidendroideae, whereas fusiform and ovoid seeds are typical of Orchidoideae.

Seed Size (Length of Seed) and Colour Orchid seed ranges from 100 µm (*Oberonia similis*) to 6000 µm (*Epidendrum secundum*). According to Barthlott et al. (2014), seed size has been classified into five categories.

Very small	100–200 μm
Small	200–500 μm
Medium	500–900 μm
Large	900–2000 μm
Very large	2000–6000 μm

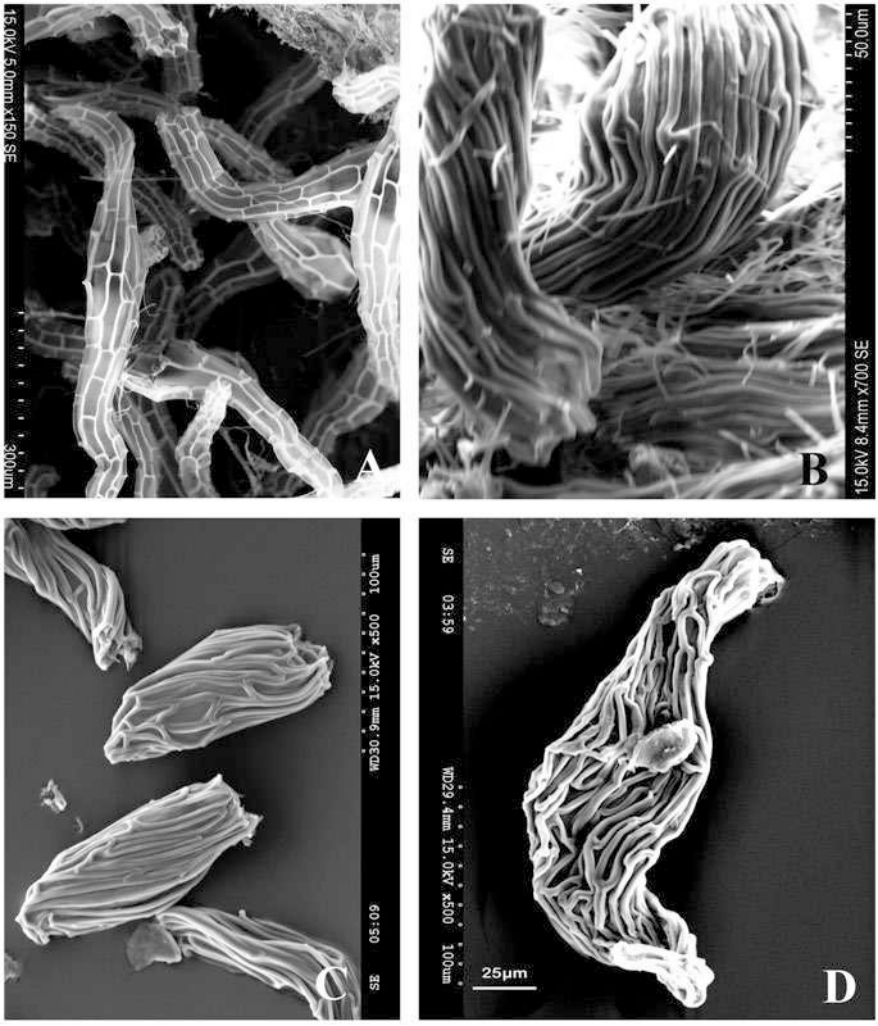

Fig. 14.2 SEM photographs of orchid seed. (**a**) *Calanthe triplicata*, filamentous shaped seed; (**b**) *Vanda tessellata*, ropelike appearance of seed; (**c**) *Oberonia arnottiana*, spindle-shaped seeds; (**d**) *Pholidota pallida*, spindle-shaped seeds with slight curvature

In most of the cases, orchid seed is whitish, brownish or dark brown and also beige, yellow, reddish, orange, greenish, yellowish brown or black. The colour is

determined by the testa especially embryo which may be intense yellow, orange or red orange colour or greenish due to presence of chloroplast pigment.

L/W Ratio According to Augustine et al. (2001), length/width ratio has some significance in expressing the relative degree of truncation of seeds in orchids. In present study, L/W ratio has been observed in 18 species belonging to 6 genera (Table 14.1). Maximum L/W ratio was observed in *Calanthe triplicata* (9.55), witnessed with highly truncated seeds, when compared to other investigated taxa. Verma et al. (2012) observed the highest L/W ratio in *Arundina graminifolia* but the lowest in *Aerides multiflora*. In the present study, maximum L/W ratio was recorded in *Calanthe triplicata*; medium L/W ratio in *C. breviscapa* (5.80) followed by *C. ovalis* (5.80*)*, *V. testacea* (4.87), *P. pallida* (4.64) and *C. nervosa* (4.53); and lowest L/W ratio recorded in *A. praemorsa* (2.67). Therefore, the genus possessing highly truncated seeds is *Calanthe* and genera possessing the medium truncated seeds are *Coelogyne, Vanda testacea* and *Pholidota* and the lowest truncated seeds are *Cymbidium*, *Oberonia* and *Acampe*.

Seed Volume The seed volume in orchids is a reflection of the size of the seeds (Arditti et al. 1979). In the present work, maximum seed volume was recorded in *Cymbidium giganteum* (16.674×10^{-3}) followed by *C. bicolor* ($11.880\,\text{mm} \times 10^{-3}$). Lowest seed volume was recorded in *Acampe praemorsa.* Almost all vandoid species studied here had lowest seed volume when compared to other taxa

Testa Cells The seed coat consists of testa cells which are transparent at maturity. Testa cells are varied in their shape and size (Fig. 14.3a, b). The thickenings in the testa cells are variously developed along the transverse and longitudinal walls. In *Malaxis densiflora* and *Calanthe triplicata*, testa cells showed transverse cell wall thickenings (Fig. 14.4a, b), whereas epiphytic species of *Cymbidium* showed very prominent longitudinally oriented cell wall thickenings (Fig. 14.4c, d). Similarly, an epiphytic orchid, viz. *Pholidota pallida*, also recorded prominent cell wall thickenings. Similar observations were recorded by Vij et al. (1992) that testa cell wall thickenings are more prominent in epiphytes but less prominent in terrestrial taxa

According to Vij et al. (1992), prominent development of cell wall thickenings in testa has got adaptive significance, provides rigidity to the seed coat and protects the embryo, and also hygroscopic nature of seed provides metabolic activities during germination.

Presence of chalazal pore in seeds of *Luisia* (Fig. 14.3c) and *Vanda* spp. possibly serves as an entry point for the fungal endophytes required for germination (Garg et al. 1992). Such chalazal openings were also reported in South African Orchidoideae (Kurzweil 1993). Each seed comprises an undifferentiated embryo enclosed within a transparent integument or seed coat. It is difficult to study its

Table 14.1 Seed characters and quantitative data

Sl. no.	Taxa	Time of fruiting	Colour	Length (mm)	Width (mm)	L/W	Seed volume $mm^3 \times 10^{-3}$	Average length of testa cells (μm)	Average width of testa cells (μm)	Average no. of testa cells
1	*Malaxis densiflora* (A.Rich) O.Kutze	Mar–Jun	White	0.3289 ± 0.0497	0.0985 ± 000983	3.33	0.0008355 0.355 $mm^3 \times 10^{-3}$	37.81	13.69	11.62
2	*Oberonia arnottiana*	Sept–Oct	Yellow	0.27398 ± 0.004986	0.09012 ± 0.004733	3.03	0.0005805 0.5605 $mm^3 \times 10^{-3}$	105.03	17.88	3.62
3	*O. ensiformis*	Sep–Oct	Light yellow	0.2657 ± 0.00546	0.08009 ± 0.00434	3.31	0.000443 0.443 $mm^3 \times 10^{-3}$	107.5	20.91	3.79
4	*Cymbidium aloifolium*	Jan–Feb	Yellow	0.8838 ± 0.1174	0.2216 ± 0.0209	3.98	0.011360 (11.36×10^{-3})	142.90	50.19	7.85
5	*C. bicolor*	Nov–Dec	Yellow	0.9568 ± 0.05001	0.2406 ± 0.0233	3.97	0.011449 (14.49×10^{-3})	148.92	53.28	8.10
6	*C. eburneum*	Nov–Dec	Yellow	0.83769 ± 0.2406	0.2462 ± 0.0770	3.40	0.01328 (13.28×10^{-3})	149.15	53.20	8.5
7	*C. giganteum*	Nov–Dec	Light yellow	0.9967 ± 0.08896	0.2529 ± 0.0377	3.94	0016674 (16.674×10^{-3})	158.21	54.15	9.5
8	*Calanthe bimuricata*	Apr–May	White	0.9474 ± 0.1701	0.0992 ± 0.0227	9.55	0.002440 2.440 $mm^3 \times 10^{-3}$	140.54	31.18	9.87
9	*Coelogyne breviscapa*	Sept–Oct	Light yellow	0.54718 ± 0.05372	0.09426 ± 0.015517	5.80	0.001271 (1.271×10)	161.29	35.38	11.21
10	*C. nervosa*	Sept–Oct	Yellow	0.54435 ± 0.5057	0.1199 ± 0.1553	4.53	0.002045 ($2.045\ mm^3 \times 10^{-3}$)	152.32	62.1	9.3

11	*C. nitida*	Sept–Oct	Pale yellow	0.2930 ± 0.0575	0.0727 ± 0.0164	4.03	0.00040534 (0.4053 $mm^3 \times 10^{-3}$)	72.71	14.16	2.20
12	*C. ovalis*	Oct–Dec	Light yellow	0.3261 ± 0.05885	0.0553 ± 0.014599	5.89	0.00026102 (0.2610 $mm^3 \times 10^{-3}$)	71.42	15.28	4.10
13	*Pholidota pallida*	Nov–Dec	Pale yellow	0.45017 ± 0.1116	0.096843 ± 0.02635	4.64	0.0011043 (1.1043 $mm^3 \times 10^{-3}$)	142.29	27.42	7.20
14	*Acampe praemorsa*	Mar–Jun	Light brown	0.1847 ± 0.06906	0.06906 ± 0.00345	2.67	0.0002306 (0.2306 $mm^3 \times 10^{-3}$)	68.56	11.19	3.66
15	*A. rigida*	Mar–Jun	Light brown	0.2402 ± 0.003910	0.0633± 0.00452	3.79	0.0002520 (0.2520 $mm^3 \times 10^{-3}$)	79.22	13.24	5.42
16	*Luisia zeylanica*	Jun–Jul	Yellow	0.2545 ± 001553	0.07445 ± 0.003838	3.39	0.00037045 (0.37045 $mm^3 \times 10^{-3}$)	84.52	12.29	3.1
17	*Vanda testacea*	Mar–Apr	Light yellow	0.2185 ± 0.0344	007232 ± 0.0004432	4.87	0.00029855 (0.2985 $mm^3 \times 10^{-3}$)	47.82	13.91	4.42
18	*V. tessellata*	Apr–May	Yellow	0.1892 ± 0.021051	0.06829 ± 0.000453	2.77	0.0002308 (0.2308 $mm^3 \times 10^{-3}$)	69.50	11.06	4.81

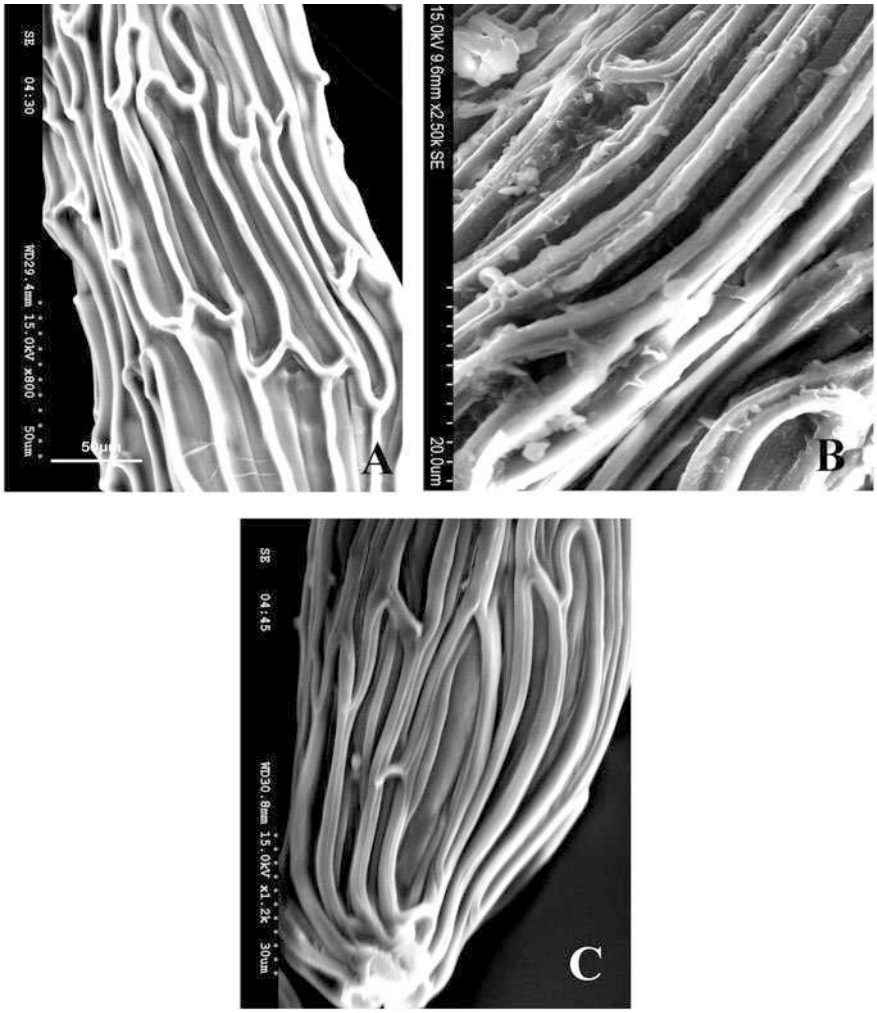

Fig. 14.3 SEM photographs of orchid seed. (**a**) *Pholidota pallida*, seed testa cells, rectangular and longitudinally oriented; (**b**). *Acampe rigida*, testa cells elongated with longitudinally oriented cell wall thickenings and blister like dots; (**c**). *Luisia zeylanica*, seed possesses opening at chalazal end

structural details with the help of optical microscope; such details can be obtained only with the help of scanning electron microscope (Arditti et al. 1979, 1980).

Embryo Characters Just like seeds, embryos are still more tiny in the Orchidaceae (Arditti and Ghani 2000). In all investigated taxa embryo, colour varies from yellow to pale yellow and light brown to white. According to Patrick et al. (1980), orchid embryos tend to be uniform in size within a genus, whereas the dimensions of testa are more variable. Orchid embryos are generally spherical or oval in shape

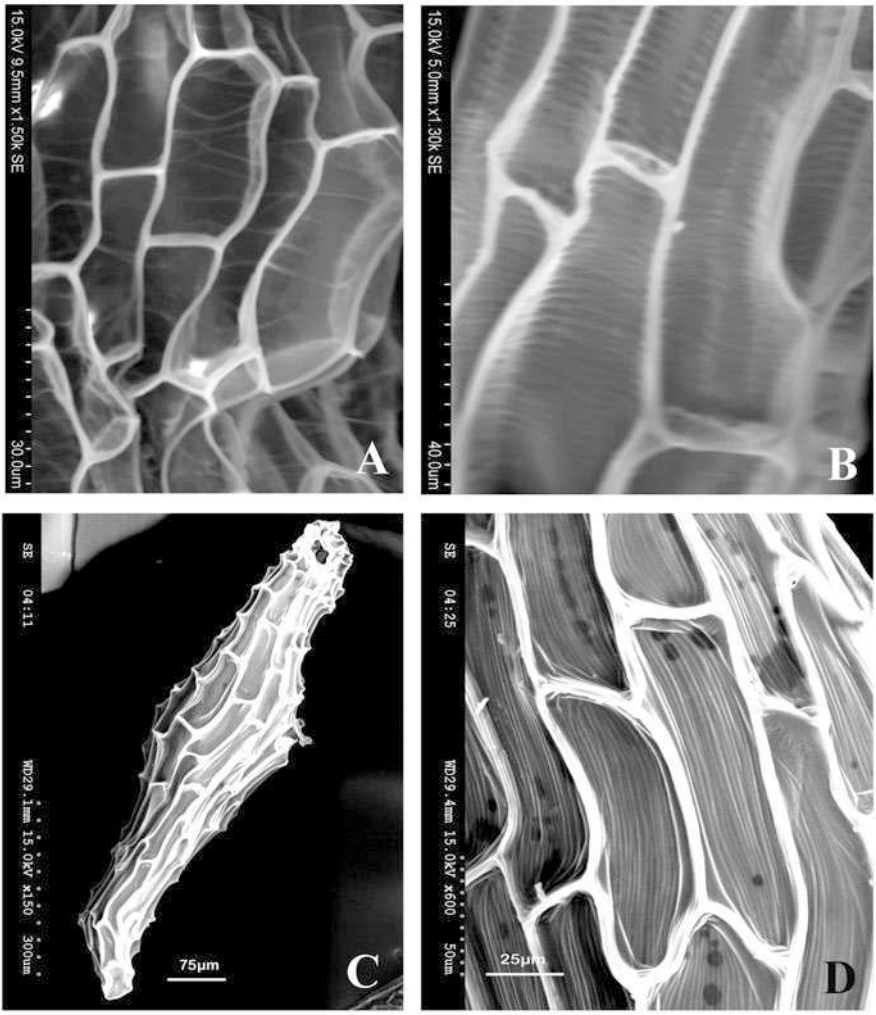

Fig. 14.4 SEM photographs of orchid seed. (**a**) *Malaxis densiflora*, seed testa cells showing fine transverse cell wall thickenings; (**b**) *Calanthe triplicata*, seed testa cells longitudinally oriented with cell wall thickenings; (**c**) *Cymbidium aloifolium*, seed testa cells with longitudinally oriented cell wall thickenings; (**d**) *Cymbidium giganteum*, seed testa cells with longitudinally oriented cell wall thickenings

In the presently studied taxa, the volume of embryo changes during the development of the seed. Young seeds have small undifferentiated embryos, whereas the mature seeds from the dehisced capsules have large volume of embryos. Embryo volume directly reflects the percentage of airspace inside the seed (Verma et al. 2014), and therefore, it has an important role in seed dispersal and species distribution. Arditti (1992) and Yan et al. (2002) reported that the tiny nature of embryos makes them exceedingly air filled, therefore helping them to float across longer distances in air for a wider dispersal (Table 14.2).

Table 14.2 Embryo characters and quantitative data

S. no.	Taxa	Colour	Length (mm)	Width (mm)	L/W	Embryo volume $mm^3 \times 10^{-3}$	Seed volume to embryo volume	Airspace (%)
1	*Malaxis densiflora*	White	0.1621 ± 0.002952	0.0628 ± 0.02501	2.58	0.0003339 (0.3339 $mm^3 \times 10^{-3}$)	2.50	60.50
2	*Oberonia arnottiana*	Yellow	0.09975 ± 0.00769	0.00937	1.20	0.00003505 (0.03505 $mm^3 \times 10^{-3}$)	1.65	39.62
3	*O. ensiformis*	Light yellow	0.09689 ± 0.01744	0.07394 ± 0.007629	1.31	0.0002756 (0.2756 $mm^3 \times 10^{-3}$)	1.60	38.19
4	*Cymbidium aloifolium*	Light yellow	0.3010 ± 0.0125	0.1108 ± 0.008485	2.716	0.001930 (1.930 $mm^3 \times 10^{-3}$)	5.886	83.00
5	*C. bicolor*	Pale yellow	0.1919± 0.0542	0.1317 ± 0.0350	1.45	0.001738 (1.738 $mm^3 \times 10^{-3}$)	8.34	88.04
6	*C. eburneum*	Yellow	0.3251 ± 0.011507	0.1119 ± 0.006677	2.905	0.002125 (2.125 $mm^3 \times 10^{-3}$)	6.249	83.99
7	*C. giganteum*	Light yellow	0.1981 ± 0.09015	0.1304 ± 0.003645	1.5191	0.001759 (1.759 $mm^3 \times 10^{-3}$)	6.479	89.47
8	*Calanthe bimuricata*	White	0.1413 ± 0.0591	0.07513 ± 0.0251	1.88	0.00041661 (0.4166 $mm^3 \times 10^{-3}$)	5.85	82.92
9	*Coelogyne brevisapa*	Yellow	0.2495 ± 0.04370	0.06437 ± 0.0755	3.816	0.000538 (0.538 $mm^3 \times 10^{-3}$)	2.362	57.67
10	*C. nervosa*	Pale yellow	0.2019 ± 0.03780	0.1032 ± 0.02336	1.95	0.0011232 (0.1232 $mm^3 \times 10^{-3}$)	1.82	47.07
11	*C. nitida*	Light yellow	0.1524 ± 0.01125	0.03912 ± 0.00168	3.89	0.00012183 0.1218 $mm^3 \times 10^{-3}$	3.327	69.94
12	*C. ovalis*	Pale yellow	0.2015 ± 0.000628	0.03125 ± 0.001663	6.44	0.00010286 0.1028 $mm^3 \times 10^{-3}$	2.53	60.59
13	*Pholidota pallida*	Pale yellow	0.2912 ± 0.01217	0.06293 ± 0.004003	4.62	0.0006017 (0.617 $mm^3 \times 10^{-3}$)	1.835	45.51

S. no.	Taxa	Colour	Length (mm)	Width (mm)	L/W	Embryo volume $mm^3 \times 10^{-3}$	Seed volume to embryo volume	Airspace (%)
14	*Acampe praemorsa*	Light brown	0.1073 ± 0.00295	0.0515 ± 0.00654	2.08	0.0001486 (0.1486 $mm^3 \times 10^{-3}$)	1.55	35.53
15	*A. rigida*	Light brown	0.1703 ± 0.02150	0.04215 ± 0.002150	4.09	0.0001579 (0.1579 $mm^3 \times 10^{-3}$)	1.59	37.34
16	*Luisia zeylanica*	Yellow	0.1212 ± 0.01217	0.05333 ± 0.00321	2.27	0.0001791 (0.179 $mm^3 \times 10^{-3}$)	2.06	51.63
17	*Vanda testaceae*	Light yellow	0.1250 ± 0.0150	0.0452 ± 0.00264	2.76	0.0001334 (0.1334 $mm^3 \times 10^{-3}$)	2.23	55.31
18	*V. tessellata*	Yellow	0.1452 ± 0.001829	0.0340 ± 0.01252	4.26	0.000087734 (008773 $mm^3 \times 10^{-3}$)	2.63	62.00

Seed Volume/Embryo Volume with Reference to Species Distribution Fahn and Werker (1972) classified the wind dispersed seeds (anemochores) into flyers (meteoranemochores) and rollers (chamaechores). The orchid seeds belong to flyers because of their tiny and light weight in nature adapted for wind dispersal. The orchid seed contains a more tiny embryo with air-filled space causing buoyancy

Seed morphological characters such as shape, size, weight, airspace play a key role in distribution of orchid species (Arditti and Ghani 2000, Murren and Ellison 1998). Kiyohara et al. (2012) and Shimizu et al. (2012) found different seed velocities based on their weight and proportion of airspace. Seeds with large airspace drop more slowly and therefore will have lower settling velocity. Further seed shape (relationship between seed length and width) is also connected with dispersal distances of seeds (Brzosko et al. 2017). Arditti and Ghani (2000) and Eriksson and Kainulainen (2011) stated that elongated seeds can disperse faraway distances. However Eriksson and Kainulainen (2011) reported that low-weight seeds could travel long distances irrespective of their shape. The other morphological character is size of the fruiting plants that reflects the distance that seed dispersed (Alexandersson and Agren 2000). Seed dispersal distance is strongly dependent on plant height (Thomson et al. 2011). Brzosko (2017) observed that the plants having shortest shoot had recorded the seed dispersal of short distance. Plants with tallest shoots had seed dispersal of farthest distances. Generally the wind dispersal was taller than the other plants in the wild habitat (Willson and Traveset 2000).

From Table 14.1, it is evident that *Cymbidium* spp. showed higher values of Sv/Ev ratio when compared to vandoid genera such as *Acampe*, *Luisia* and *Vanda*; as a result percentage of airspace is high in *Cymbidium*. Hence, *Cymbidium* seeds are more buoyant and widely distributed throughout Indo-Malayan region. Within the *Cymbidium* genus, *C. giganteum* shows the highest airspace percentage whereby it is distributed in Western Ghats and also Kumaon to Khasi Hills including Sikkim Himalaya.

Similarly among coelogynes *C. nitida* showed higher percentage of airspace. Hence, it is widely distributed in Sikkim and Khasi Hills of North East Himalaya (Bose and Bhattacharjee 1980). However, in the present study, *C. nitida* and also *Cymbidium giganteum* collected from botanical gardens, not from wild, need further study to confirm their distribution pattern in India.

In the case of tribe Vandeae, *Vanda tessellata* shows higher percent of airspace; hence it is widely distributed in Western and Eastern Ghats and also in Western Himalayan region of India. Among *Acampe* genus, *A. rigida* with 37.34% of airspace is an old world orchid restricted to peninsular region, whereas *A. praemorsa* with comparatively lower percentage (35.53%) of airspace is distributed in Southern India and nearby Sri Lanka.

As already mentioned that there is no generalised pattern of orchid distribution in India and elsewhere, Vij et al. (1998) also opined that some orchids exhibit high habitat specificity (narrow preferences towards exposure and shade, moisture, soil pH, mineral nutrients, etc.) and it is more pronounced in the mycoheterotrophs. Those species which have more buoyant seeds can successfully disperse long

distances but unable to establish themselves in the absence of biotic (mycorrhizal) and abiotic factors. As Zotz and Heitz (2001) pointed out, a more integrative approach to study the epiphytic biology is needed including physiological investigations, substrate instability, dispersal limitation and competition (intra- and interspecific level).

14.3 Taxonomic and Phylogenetic Implications

In all presently investigated taxa, testa cells are transparent and variously thickened. Their shape, size and wall thickenings have got taxonomic significance in species identification (Healey et al. 1980). Vij et al. (1992) classified the three categories of seeds based on length of testa cells; these are long (>200 μm), intermediate (>100–200 μm) and short (up to 100 μm). Based on their classification, the presently investigated taxa are grouped into following categories:

(i) ***Intermediate seeds:*** *Oberonia arnottiana*, *O. ensiformis*, *Cymbidium aloifolium*, *C. bicolor*, *C. eburneum*, *C. giganteum*, *Calanthe triplicata*, *Coelogyne breviscapa*, *C. nervosa* and *Pholidota pallida*.
(ii) ***Shorter seeds:*** Rest of all studied taxa have shorter seeds, such as *Malaxis densiflora*, *Coelogyne nitida*, *C. ovalis*, *Acampe praemorsa*, *A. regida*, *Luisia zeylanica*, *Vanda testaceae* and *V. tessellata.* Category of species having long seeds was not reported from this study.

Among intermediate category, *Coelogyne breviscapa* possesses the longest testa cells (161.29 μm) whereas *Malaxis densiflora* with the shortest testa cells (37.81; Table. 14.1). In general, the shorter testa cells were observed in the tribe Vandeae when compared to other tribes studied here. Similar observations were recorded by Swamy et al. (2004) in *Aerides maculosa* and *Vanda parviflora* of the tribe Vandeae (Table 14.3).

The micromorphological features of seed and embryo (quantitative data, Tables 14.1 and 14.2) from various species are taken and subject to hierarchical cluster analysis using Euclidean distance to determine the distance among various species (Table 14.4). The dendrogram (Fig. 14.5) based on quantitative seed micromorphological characters of various species belonging to Epidendroideae revealed the following clusters:

Cluster I:	*Coelogyne breviscapa*, *C. nervosa*, *Pholidota pallida*
II:	*C. giganteum*, *C. bicolor*, *C. eburneum*, *C. aloifolium*, *Calanthe triplicata*
III:	*Luisia zeylanica*, *Acampe praemorsa*, *A. rigida*
IV:	*Coelogyne nitida*, *C. ovalis*, *Vanda tessellata*
V:	*Oberonia arnottiana*, *O. ensiformis*, *Malaxis densiflora*, *Vanda testaceae*

It was already mentioned in the previous section that *Pholidota* is closely allied to *Coelogyne*. The dendrogram (Fig. 14.5) also showed that *Pholidota pallida* is

Table 14.3 Diagnostic quantitative characters of seed and embryo taken for dendrogram construction of subfamily Epidendroideae

	Seed/embryo characters					
Species	L/W of seed	Average length of testa cells	Average no. of testa cells	Embryo volume	Vs/Ve	Airspace percentage
Malaxis densiflora	3.33	37.81	11.62	0.0003339	2.50	60.50
Oberonia arnottiana	3.03	105.03	3.62	0.0003505	1.65	39.62
O. ensiformis	3.31	107.5	3.79	0.0002756	1.60	38.29
Cymbidium aloifolium	3.98	142.9	7.85	0.001930	5.88	83.00
C. bicolor	3.97	148.92	8.10	0.001738	8.34	88.04
C. eburneum	3.40	149.15	8.5	0.002125	6.24	83.99
C. giganteum	3.94	158.21	9.5	0.001759	6.42	89.47
Calanthe triplicata	9.55	140.54	9.87	0.00041661	5.85	82.92
Coelogyne breviscapa	5.80	161.29	11.21	0.000538	2.34	57.67
C. nervosa	4.53	152.32	9.3	0.0011232	1.82	47.07
C. nitida	4.03	72.71	2.20	0.00012183	3.32	69.94
C. ovalis	5.89	71.42	4.10	0.00010286	2.53	60.59
Pholidota pallida	4.64	142.29	7.20	0.0006017	1.83	45.51
Acampe praemorsa	2.67	68.56	3.66	0.0001486	1.55	35.53
A. rigida	3.79	79.22	5.42	0.0001579	1.59	37.34
Luisia zeylanica	3.39	84.52	3.66	0.0001791	2.06	51.63
Vanda testaceae	4.87	47.82	4.87	0.0001334	2.23	55.31
V. tessellata	2.77	69.50	4.81	0.000087734	2.63	62.00

Table 14.4 Distance matrix (Euclidean distance) based on quantitative seed features in the subfamily Epidendroideae

Name of the taxa	*Malaxis densiflora*	*Oberonia arnottiana*	*O. ensiformis*	*Cymbidium aloifolium*	*C. bicolor*	*C. eburneum*	*C. giganteum*	*Calanthe triplicata*	*Coelogyne breviscapa*
Malaxis densiflora	–								
Oberonia arnottiana	70.847	–							
O. ensiformis	73.567	2.825	–						
Cymbidium aloifolium	107.593	57.902	57.336	–					
C. bicolor	114.677	65.852	65.231	8.231	–				
C. eburneum	113.895	62.931	62.185	6.398	4.621	–			
C. giganteum	123.918	73.289	72.437	16.711	9.695	10.651	–		
Calanthe triplicata	105.400	56.878	56.369	8.378	11.701	10.730	19.674	–	
Coelogyne breviscapa	123.538	59.638	57.713	31.732	33.532	29.489	32.307	33.110	–
C. nervosa	14.084	48.233	46.020	37.398	41.646	37.344	43.058	38.285	14.084
C. nitida	89.901	44.381	47.09	71.664	78.712	78.033	88.060	69.750	89.901
C. ovalis	90.198	39.731	42.505	75.103	82.542	81.418	91.731	73.034	90.198
Pholidota pallida	22.947	37.927	35.720	37.724	43.548	39.376	47.040	38.078	22.947
Acampe praemorsa	95.689	36.701	39.04	88.419	96.346	94,282	104.910	86.783	95.689
A. rigida	47.861	25.984	28.347	78.513	86.495	84.248	94.852	76.869	84.776
Luisia zeylanica	48.209	23.774	26.576	66.519	74.381	72.561	83.159	64.870	77.414
Vanda testaceae	13.234	59.367	62.092	99.146	106.49	105.46	115.72	97.053	113.67
V. tessellata	32.453	42.020	44.817	76.484	83.848	82.793	93.069	74.607	92.165

(continued)

Table 14.4 (continued)

Name of the taxa	*C. nervosa*	*C. nitida*	*C. ovalis*	*Pholidota pallida*	*Acampe Praemorsa*	*A. rigida*	*Luisia zeylanica*	*Vanda testaceae*	*V. tessellata*
Malaxis densiflora									
Oberonia arnottiana									
O. ensiformis									
Cymbidium aloifolium									
C. bicolor									
C. eburneum									
C. giganteum									
Calanthe triplicata									
Coelogyne breviscapa									
C. nervosa	–								
C. nitida	83.149	–							
C. ovalis	82.201	9.838	–						
Pholidota pallida	10.366	73.931	72.537	–					
Acampe praemorsa	84.760	34.762	25.450	74.513	–				
A. rigida	73.851	33.445	24.667	63.628	11.012	–			
Luisia zeylanica	68.197	21.883	16.080	58.215	22.687	15.355	–		
Vanda testaceae	104.91	29.027	24.219	95.007	28.778	36.204	36.934	–	
V. tessellata	84.927	9.068	3.990	74.700	26.534	26.553	18.308	22.789	–

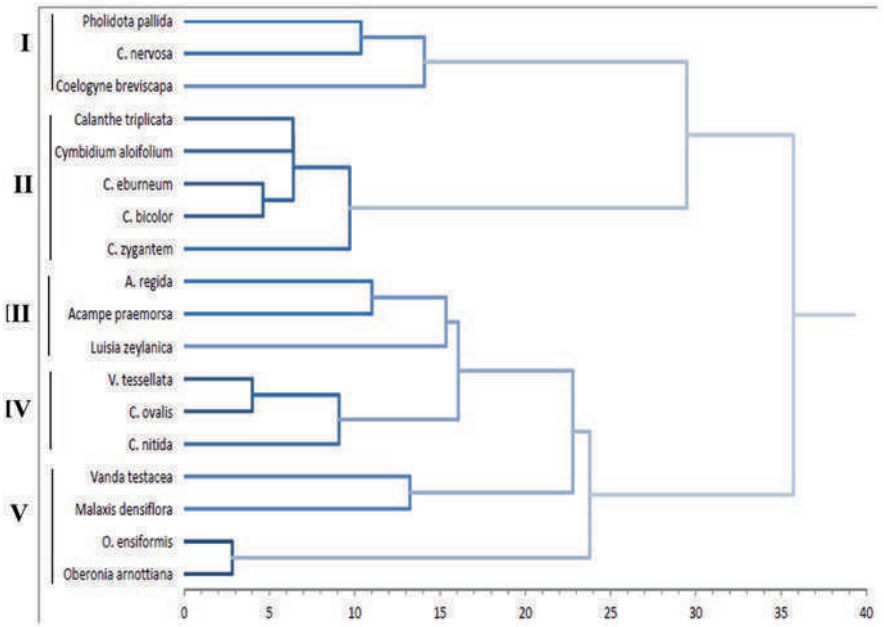

Fig. 14.5 Dendrogram of all 18 studied taxa belonging to subfamily Epidendroideae based on quantitative characters of seed and embryo

clustered with *Coelogyne breviscapa* and *C. nervosa* (cluster 5); this supports the inclusion of *Pholidota* in the subtribe Coelogyninae of the tribe Coelogyneae by Dressler (1993).

From the dendrogram (Fig. 14.5), it is also evident that there is clear species differentiation among *Cymbidium* which form a clear cluster, i.e. cluster 4; it does not share any character with other genera. Dendrogram also showed that the *Oberonia* and *Malaxis* had close affinity of *Oberonia* and *Malaxis* with that of *Vanda testaceae*. Similarly *Vanda testaceae* and *V. tessellata* are spread into cluster 1 and cluster 2 along with *Oberonia* and *Coelogyne*, respectively.

The average number of testa cells in the longest axis of seeds in *Malaxis densiflora* (11.62) is higher followed by *Coelogyne breviscapa* and lowest in *C. nitida* (2.20). From Table 14.3, it is evident that the tribe Vandeae shows lesser number of testa cells when compared to other tribes of Epidendroideae such as Malaxideae, Cymbidieae, Arethuseae and Coelogyneae.

Regarding L/W value of embryo, it is highest in *Coelogyne ovalis* (6.44) followed by *Pholidota pallida* whereas lowest in *Oberonia arnottiana* (1.20). Among all tribes studied here, Coelogyneae and Vandeae show higher L/W values than the other tribes.

Calevo et al. (2017) also opined that the morphological traits such as seed size, embryo size, cell wall thickenings, etc. are potential markers to resolve the taxonomic disputes and assess the phylogenetic relationship of various orchid taxa.

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15 Structural Adaptations of *Bulbophyllum* and *Dendrobium* (Orchidaceae) to the Epiphytic Habitat and Their Phylogenetic Implications

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Abstract

The morpho-anatomical studies in *Bulbophyllum* and *Dendrobium* (Orchidaceae) with special reference to ecological adaptation and phylogenetic implications have been carried out. The vegetative parts were collected from different parts of North-East Himalaya and Western and Eastern Ghats of India. All were epiphytes belonging to tribe Dendrobieae. These plant parts were fixed in FAA (Formaline-Acetic acid-Alcohol) and usual methods of microtomy had followed. Stomata were confined to abaxial surface in all the investigated taxa. The presence of stomatal ledges and substomatal chambers is helpful in reducing leaf transpiration and evaporation of water. Absorbing trichomes were recorded only in Sikkim collections of *D. anceps* whereas they were absent in Darjeeling collections. In case of *D. herbaceum* and *D. moschatum*, these were present only in Kerala collections and absent in Karnataka collections. Single- or multi-layered velamen has been reported in both genera. It was observed that tilosomes were always associated with single layered velamen roots whereas completely absent in multilayered velamen taxa. Based on anatomical data, sectional delineation and phylogenetic interrelationships have been discussed.

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Bulbophyllum · *Dendrobium* · Anatomical adaptations · Habitat tolerance · Phylogenetic implications

15.1 Introduction

The Orchidaceae constitutes one of the largest families of flowering plants comprising about 28,484 species (Govaerts et al. 2017). It contributes about 40% of the monocotyledons (Rasmussen 1985). In India, it represents the second largest flowering plant family with 1350 species (Jalal and Jayathi 2012) and contributes about 10% of Indian flora (Jain 1980; Kumar and Manilal 1994). A majority of orchid habitats in India are dwindling in state due to many anthropogenic activities. The present paper deals with some of the insights in anatomy related to ecological adaptability and phylogenetic interrelationship of genera *Bulbophyllum* and *Dendrobium*.

Both *Bulbophyllum* and *Dendrobium* belong to the tribe Dendrobieae Endl. and sub tribes Bulbophyllinae Schltr. and Dendrobiinae Lindl. respectively (Dressler 1993). In India, the tribe Dendrobieae is represented by about 189 species, distributed in Western and Eastern Ghats and, Eastern and Western Himalayas. Most of the taxa are primarily epiphytic, although some are lithophytic or terrestrial. In general, the great diversity of orchids and their different habitats have been made possible by structural, ecological and physiological adaptations (Mehra and Vij 1974; Khasim and Mohana Rao 1986; Mohana Rao and Khasim 1987a, b; Pridgeon 1986; Arditti 1992; Stern and Morris 1992). Vegetative structures such as roots, stems and leaves are specialized in water and nutrient absorption (Benzing et al. 1983; Moreira and Isaias 2008). Physiologically, the Crassulacean Acid Metabolism (CAM) helps in water economy by closure of stomata during the day (Luittge 2004) and, photosynthesis in roots is equally important in the maintenance of oxygen supply (Dycus and Knudson 1957; Moreira et al. 2009).

However, the vegetative anatomy of this highly evolutionary important family is completely neglected or has received little attention. From the ecological point of view Sanford (1974) did some work on African orchids, Kaushik (1983) on some Himalayan orchids and Metusala et al. (2017) on *Dendrobium* of Indonesia. During the last two decades few important monographs on orchid biology and systematics have appeared (Dressler 1993; Vermeulen 1993; Pridgeon et al. 1999, 2001, 2003, 2005; Ramesh et al. 2017). By critical reading of the available literature, it is evident that the authors had studied the anatomy with respect to systematics; but they did not explain the ecological adaptation of orchids. From the ecological point of view Sanford (1974) did some work on African orchids and Kaushik (1983) on some Himalayan orchids. As such, there has been no single paper on anatomy of orchids in relation to ecological adaptability for the last 20 years. In view of this the present anatomical investigation has been undertaken in the *Bulbophyllum* and *Dendrobium* species, the largest genera in the family Orchidaceae, so as to throw light on their

ecological adaptability and also ascertain the tribal, subtribal and sectional delineation, and phylogenetic relationships.

Plant materials were collected from Arunachal Pradesh, Darjeeling, Sikkim, Himalayas, Karnataka and Kerala at various altitudes over a period of 3 years (Table 15.1, Fig. 15.1). Plants were identified with the help of standard floras (Hooker 1894, 1895; King and Pantling 1898; Brühl 1926; Bose and Bhattacharjee 1980; Abraham and Vatsala 1981; Hegde 1984; Dressler 1993; Manilal and Kumar 2004; Mabberley 2008); these were confirmed by comparing them with the authentic herbarium specimens stocked at the Botanical Survey of India, Coimbatore,

Table 15.1 [a]Details of collections of orchid plant materials

S.No.	Species	Place, altitude and date of collection	Host tree	Accession No.
	Family: **ORCHIDACEAE**			
1	Subfamily: **EPIDENDROIDEAE**			
	Tribe: **DENDROBIEAE LINDL.**			
	Subtribe: **BULBOPHYLLINAE**			
	BULBOPHYLLUM Thouars			
	Bulbophyllum affine Lindl.	(i) Tipi (Arunachal Pradesh), 1500 m; May, 2011	*(i) Castonopsis indica*	(i) **RO1** (Arunachal Pradesh)
		(ii) Araria (Darjeeling), 1650 m; April, 2011	*(ii) Azadirachta indica*	(ii) **RO2** (Darjeeling)
2	*B. bisetum* Lindl.	(i) Jalalgarh (Darjeeling), 2250 m; February, 2011	(i) *Azadirachta indica*	***(i)*** *RO3*
3	*B. careyanum* W.J. (Hook.) Spreng.	(i) Packyong (Sikkim), 2500 m; February , 2011	(i) *Saurauia nepalensis*	(i) **RO4**
4	*B. cauliflorum* Hk. f.	(i) Jalalgarh (Darjeeling), 2250 m; February, 2011	(i) *Mangifera indica*	(i) **RO5**
5	*B. cornutum* (Lindl.) Rchb.f.	(i) Araria (Darjeeling), 1650m; February, 2011	(i) *Alnus nepalensis*	(i) **RO6**
6	*B. crassipes* J.D. Hook. f.	(i) Qasba (Darjeeling), 1250 m; February, 2011	(i) *Schima wallichii*	(i) **R07**
7	*B. fischerii* Seidenf.	(i) Jalalgarh (Darjeeling), 2250 m;	(i) *Mangifera indica*	(i) **R08**
		(ii) Lingtam (Sikkim), 2680 m; February, 2011	(ii) *Meliosma dillenifolia*	(ii) **R09**
8	*B. khasyanum* Griff.	(i) Taplejorg (Darjeeling), 1650 m; February, 2011	(i) *Schima wallichii*	(i) **R10**

(continued)

Table 15.1 (continued)

S.No.	Species	Place, altitude and date of collection	Host tree	Accession No.
9	*B. protractum* Hook. f.	(i) Ramda (Arunachal Pradesh), 1650 m, May 2011.	(i) *Elaeocarpus floribundus*	(i) **R11**
10	*B. scabratum* Rchb. f.	(i) Saddlepoint (Arunachal Pradesh, 2000 m; May 2011	(i) *Bischofia jaramica*	(i) **R12**
11	*B. stenobulbon* Par et Rchb. f.	(i) Qasba (Darjeeling), 1250 m; February, 2011	(i) *Alnus nepalensis*	(i) **R13**
12	*B. tremulum* Wight.	(i) Lingtam (Sikkim), 2680 m; February, 2011	(i) *Castanopsis indica*	(i) **R14**
13	*B. umbellatum* Lindl.	(i) Packyong (Sikkim), 2500 m; February, 2011	(i) *Alnus nepalensis*	(i) **R15** (Sikkim)
		(ii) Araria (Darjeeling), 1650 m; February, 2011	(ii) *Alnus nepalensis*	(ii) **R16** (Darjeeling)
	SUBTRIBE*: DENDROBIINAE*			
1	***DENDROBIUM* Swartz**	(i) Araria (Darjeeling), 1850 m; February, 2011	(i) *Alnus nepalensis*	(i) **R17 (**Darjeeling)
	Dendrobium anceps Sw.	(ii) Packyong (Sikkim), 2500 m; February, 2011	(ii) *Persea oderatissima*	(ii) **R18** (Sikkim)
2	*D. bicameratum* Lindl.	(i) Phidim (Darjeeling), 2000 m; February, 2011	(i) *Mangifera indica*	(i) **R19**
3	*D. densiflorum* Lindl.	(i) Phidim (Darjeeli ng), 2000 m; February, 2011	(i) *Schima wallichii*	(i) **R20**
4	*D. haemoglossum* Thw.	(i) Qasba (Darjeeling), 1785 m; February, 2011	(i) *Schma wallichii*	(i) **R21**
5	*D. herbaceum* Lindl.	(i) Karuman code (Kerala), 985 m; January, 2011	(i) *Mangifera indica*	(i) **R22** (Kerala)
		(ii) Khanapur (Karnataka), 850 m; June, 2011	(ii) *Terminalia elliptica*	(ii) **R23** (Karnataka)
6	*D. heyneanum* Lindl.	(i) Karuman code (Kerala), 985 m; June, 2011	(i) *Phoenix sylvestris*	(i) **R24**
7	*D. jenkinsii* Wall. ex. Lindl.	(i) Jalalgarh (Darjeeling), 1750 m; February, 2011	(i) *Azadirachta indica*	(i) **R25**

(continued)

Table 15.1 (continued)

S.No.	Species	Place, altitude and date of collection	Host tree	Accession No.
8	*D. microbulbon* A. Rich.	(i) Palavara (Kerala), 950 m; January, 2011	(i) *Terminalia bellirica*	(i) **R26** (Kerala)
		(ii) Halsi (Karnataka), 850 m; June, 2011	(ii) *Syzygium cumini*	(ii) **R27** (Karnataka)
9	*D. moschatum* (Buch.-Ham.) Sw.	(i) Karuman code (Kerala), 925 m; January, 2011	(i) *Mangifera india*	(i) **R28** (Kerala)
		(ii) Hanbur (Karnataka), 875 m; June, 2011	(ii) *Phoenix sylvestris*	(ii) **R29** (Karnataka)
10	*D. nobile* Lindl.	(i) Araria (Darjeeling), 2210 m; February, 2011	(i) *Alnus nepalensis*	(i) **R30**
11	*D. nutantiflorum* Hawkes & Heller	(i) Peringammala (Kerala), 950 m; June, 2011	(i) *Madhuca latifolia*	(i) **R31**
12	*D. pendulum* Roxb.	(i) Rongli (Sikkim), 1950 m; February, 2011	(i) *Albizia gamblei*	(i) **R32**

[a]Arranged according to Dressler (1993)

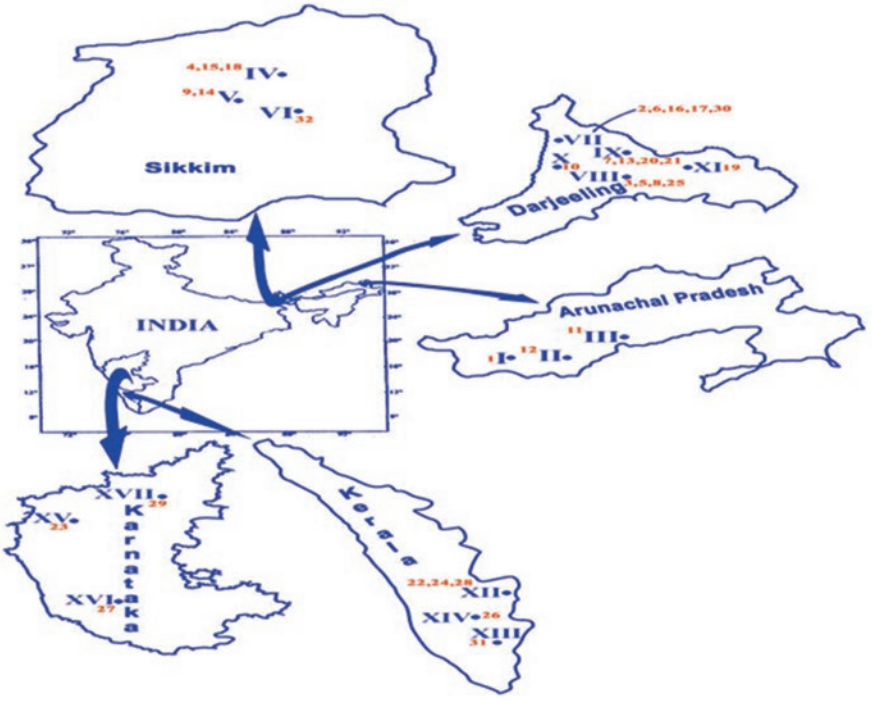

Fig. 15.1 India map and places of collection of orchid plant material

India. Voucher specimens were deposited in the Department of Botany and Microbiology, Acharya Nagarjuna University, India.

Vegetative organs such as leaves, stems, pseudobulbs and roots were fixed in FAA (5 cc formalin + 5 cc acetic acid + 90 cc 70% ethanol) for 24 h and then they were transferred to 70% alcohol and stored in it for laboratory studies. Free-hand cross sections of all vegetative organs were made at standardized levels (Metcalfe 1963; Cutter 1978). Cross section of mature leaves was done in a region equidistant from the base and apex of lamina. Stems were sectioned at median internodes, and mature roots at half way between the apex and junction with the rhizome. Sections were stained with safranin and fast green. For leaf epidermal peelings, small bits of leaves were put in 10% potassium hydroxide solution and then boiled until the epidermis was loosened from the mesophyll and veins. These peelings were mounted in 50% glycerine.

15.2 General Anatomy of *Bulbophyllum* and *Dendrobium*

The genera *Bulbophyllum* and *Dendrobium* are sympodial orchids, in which growth of the stem is arrested at certain stage and shoots are produced laterally from the base.

15.2.1 Leaf

Leaf anatomical features of *Bulbophyllum* and *Dendrobium* were given in the Tables 15.2 and 15.3. In a majority of the taxa studied here, the leaf is thick and fleshy. A fully developed leaf consists of a tubular leaf sheath and a lamina, often separated by an abscission layer, which involves in shedding and consequently helps in reducing the transpiring surfaces under stress conditions (Goh and Kluge 1989). In cross section, the leaf is generally V-shaped at the midrib and flattened at the laminar region.

Epidermal cells possess smooth and thin walls in almost all investigated taxa belonging to tribe Dendrobieae. According to Solereder and Meyer (1930) smooth cell walls are present in advanced epiphytic orchids whereas sinuous walls in primitive terrestrial ones.

In most of the presently studied taxa, the size of the adaxial epidermal cells is comparatively larger than abaxial ones. In some cases, these cells are two or three times larger in their size than the abaxial epidermal cells (Fig. 15.2a–d). Khasim (1996) reported adaxial epidermal cells that are three times larger than abaxial ones in *Paphiopedilum fairrieanum*. Mohana Rao and Khasim (1987b) reported bulliform cells on adaxial surface in *Anthogonium gracile* of Thuniinae. Bulliform cells are also reported in presently investigated taxon viz., *D. moschatum*.

Stomata The stomata are hypostomatic in distribution, restricted to abaxial surface of leaf. Similarly hypostomatic distribution is found in other groups of Orchidaceae (Möbius 1887; Singh 1981; Williams 1979; Avadhani et al. 1982). Interestingly Vij et al. (1991) observed the hypostomatic leaves in mesophytic orchids. Rasmussen

Table 15.2 **Leaf:** anatomical features in *Bulbophyllum* (in μm)

Anat. feat.	Access. no.															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. Absorbing trichome	–	–	+	–	+	+	+	–	–	–	–	+	+	–	–	+
2. Cuticle thickness	0.008	0.015	0.011	0.009	0.007	0.004	0.012	0.009	0.008	0.005	0.005	0.019	0.008	0.008	0.007	0.005
3. Stomatal width (two gua rd cells including pore)	0.023	0.018	0.021	0.019	0.016	0.024	0.028	0.024	0.020	0.011	0.021	0.022	0.015	0.018	0.017	0.014
4. Stomatal length (only guard cell)	0.017	0.012	0.019	0.015	0.019	0.011	0.021	0.011	0.015	0.008	0.012	0.011	0.018	0.019	0.015	0.012
5. Midrib vb. Size	0.089	0.075	0.062	0.084	0.058	0078	0071	0.075	0.069	0.08	0.086	0.083	0.077	0.054	0.095	0.038
6. Laminar vb. size	0.041	0.047	0.052	0.051	0.044	0.049	0.057	0.064	0.058	0.061	0.047	0.058	0.062	0.042	0.050	0.032
7. Water storage cell	0.064	0.051	0.059	0.062	0.054	0.064	0.057	0.060	0.069	0.068	0.066	0.054	0.056	0.058	0.061	0.051
8. Substomatal chamber size	0.023	0.021	0.021	0.021	0.020	0.019	0.025	0.018	0.021	0.026	0.020	0.029	0.027	0.022	0.024	0.024
9. No.of ph. cap layers	3	4	3	3	2	3	2	3	2	3	5	3	5	4	2	2
10. No.of xy. cap layers	2	4	1	2	A	A	1	2	1	2	2	1	4	2	2	1

[1. *Bulbophyllum affine* (Arunachal Pradesh), 2. *B. affine* (Darjeeling), 3. *B. bisetum,* 4. *B. careyanum,* 5. *B. cauliflorum,* 6. *B. cornutum,* 7. *B. crassipes,* 8. *B. fischerii* (Darjeeling), 9. *B. fischerii* (Sikkim), 10. *B. khasyanum,* 11. *B. protractum,* 12. B. scabratum, 13. B. stenobulbon, 14. B. tremulum, 15. B. umbellatum (Sikkim).16. B. umbellatum (Darjeeling)]

ph. = phloem, xy. = xylem, vb. = vascular bundles; + = present, – = absent

Table 15.3 **Leaf:** anatomical features in *Dendrobium* (in μm)

Anat. feat.	Access. No.															
	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
1. Absorbing trichome	–	+	–	–	–	+	–	+	+	–	+	+	–	–	–	+
2. Cuticle thickness	0.010	0.007	0.009	0.006	0.004	0.008	0.006	0.011	0.006	0.009	0.004	0.004	0.008	0.007	0.005	0.012
3. Stomatal width (two guard cells including pore)	0.020	0.025	0.028	0.019	0.029	0.015	0.014	0.022	0.019	0.021	0.015	0.022	0.017	0.025	0.028	0.011
4. Stomatal length (only guard cell)	0.015	0.018	0.010	0.012	0.012	0.014	0.017	0.012	0.019	0.022	0.015	0.012	0.018	0.011	0.015	0.025
5. Mid vb. Size	0.082	0.070	0.078	0.071	0.057	0.068	0.083	0.079	0.088	0.081	0.078	0.076	0.081	0.085	0.073	0.084
6. Laminar vb. size	0.051	0.055	0.047	0.060	0.041	0.048	0.055	0.063	0.059	0.044	0.057	0.048	0.041	0.062	0.054	0.059
7. Water storage cell	0.065	0.062	0.067	0.064	0.068	0.060	0.067	0.057	0.066	0.026	0.059	0.068	0.061	0.069	0.053	0.057
8. Substomatal chamber size	0.026	0.027	0.021	0.029	0.025	0.023	0.029	0.028	0.026	0.021	0.025	0.020	0028	0.022	0.022	0.024
9. No. of ph. Cap layers	2	2	3	3	3	3	2	3	2	2	2	2	3	2	3	2
10. No. of xy. Cap layers	1	–	2	2	1	2	1	2	2	–	1	1	-	+	1	1

[17. *Dendrobium anceps* (Darjeeling), 18. *D. anceps* (Skkim), 19. *D. bicameratum*, 20. *D. densiflorum*, 21. *D. haemoglossum*, 22. *D. herbaceum* (Kerala), 23. *D. herbaceum* (Karnataka), 24. *D. heyneanum*, 25. *D. jenkinsii*, 26. *D. microbulbon* (Kerala), 27. *D. microbulbon* (Karnataka), 28. *D. moschatum* (Kerala), 29. *D. moschatum* (Karnataka), 30. *D. nobile*, 31. *D. nutantiflorum*, 32. D. pendulum

ph. = phloem, xy. = xylem, vb. = vascular bundles; + = present, – = absent

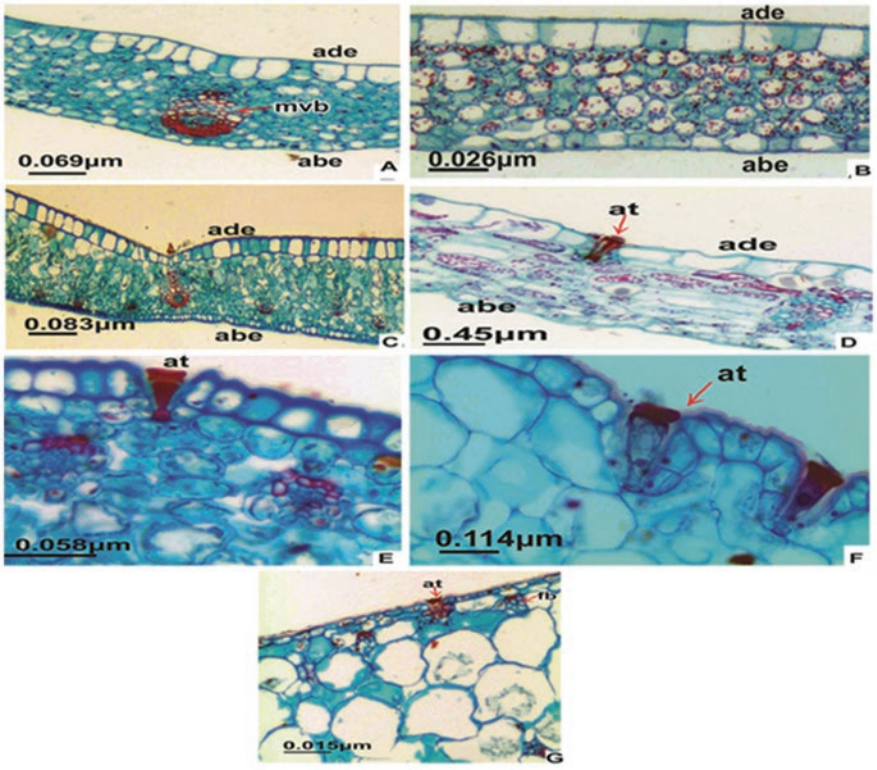

Fig. 15.2 **a**–**g**. Leaf
(**a**) *Bulbophyllum fischerii.* Leaf cross section showing larger adaxial epidermal cells and midrib vascular bundle
(**b**) *B. khasyanum.* Leaf cross section showing adaxial and abaxial epidermis
(**c**) *B. herbaceum.* Leaf cross section showing larger adaxial epidermal cells and midrib vascular bundle
(**d**) *B. umbullatum* (Sikkim collection). Leaf cross section showing absorbing trichome towards adaxial epidermis
(**e**) *B. herbacetum*. Leaf cross section indicating elongated 3-celled absorbing trichome on adaxial epidermis
(**f**) *B. umbellatum* (Darjeeling collection). Leaf cross section showing absorbing trichomes towards adaxial epidermis
(**g**) *Dendrobium anceps.* Leaf cross section indicating the absorbing trichome and fibre bundles towards adaxial epidermis

(1987) opined that hypostomaty is more frequent in mesophytic orchids whereas amphistomaty dominates in those of dry and humid habitats. Parkhurst (1978) observed that thick leaves tend to be amphistomatous, thus producing a secondary dependence of stomatal distribution on the environment. The thick leaves, generally associated with crassulacean acid metabolism have been considered an additional feature promoting amphistomaty in orchids (Rasmussen 1987). During the unfavourable period leathery leaves get folded and, in *R. retusa* two sides of lamina come so close to each other that there is no chance of transpiration from the adaxial

side of leaf (Kaushik 1983). With few exceptions, cyclocytic stomata with 5–6 subsidiary cells have been observed in presently investigated taxa. Epiphytes generally have smaller stomata than terrestrials. In the presently investigated taxa, the width of guard cells (including pore) varies among *Bulbophyllum* species (minimum of 0.011 μm to maximum 0.028 μm) and also *Dendrobium* (from 0.011 to 0.029 μm). Guard cells with prominent cuticular ledges (stomatal ledges) were observed on the leaf surface view of presently investigated taxon *B. affine* and *B. careyanum*. In *D. nobile* also, cuticular projections were observed around the stomatal apparatus; this type of projections has not been reported so far in any other orchid.

Absorbing trichomes The trichomes known to be absorbing in function, are 2 or 3-celled structures with dome-shaped apical cell and basal stalk cell (Fig. 15.2e–g). Kaushik (1983) preferred to call them as 'Handle cells'. The presence of absorbing trichomes is a regular feature in the members of Epidendroideae except tribe Vandeae (Khasim 1986). However, in the present investigation, these were observed in some species such as *Bulbophyllum bisetum, B. scabratum, B. stenobulbon*, *B. umbellatum* and also in *Dendrobium anceps, D. densiflorum, D. herbaceum, D. heyneanum* and *D. jenkinsii*. Pridgeon (1981) also studied the absorbing trichomes in Pleurothallidinae. He stated that the movement of water-soluble stain in these trichomes indicates an absorbing function similar to that of absorbing process of some bromeliad trichomes (Schimper 1888, quoted in Tomlinson 1969; Benzing et al. 1976).

Hypodermis In the presently investigated taxa, hypodermis is almost absent. However, fibre bundles at hypodermal position have appeared in *D. anceps* (Fig. 15.2g). Isaiah (1993) also reported fibre bundles in *Agrostophyllum khasianum, B. bhotanense* and *Epidendrum xanthum*. Mohana Rao and Khasim (1987b) observed these fibre bundles in *Agrostophyllum callosum, Cymbidium grandiflorum, C. lowianum, C. marstersii, C. traceyanum* and *Epidendrum radicans*. They also stated that fibre bundles provide mechanical strength to the plant body.

Mohana Rao and Khasim (1987b, c) reported multispiral thickenings in hypodermal cells in *B. dyerianum, Phaius maculatus, Pholidota imbricata* and *Otochilus alba.* Isaiah (1993) also observed hypodermal cells with multispiral thickenings in *B. bhotanense, B. gymnopus* and *D. jenkinsii*.

Mesophyll In all the investigated taxa, mesophyll is homogeneous, not differentiated into palisade and spongy parenchyma. Mesophyll tissue is tightly packed in some cases, which favours the fixation of carbon through C_4 pathway. Various tracheoidal elements including water storage cells with cellulosic thickenings and without thickenings were observed in the presently studied taxa. Olatunji and Nengim (1980), who coined the term 'tracheoidal elements', opined that certain specialized elements which possess annular, spiral or pitted secondary wall thicken-

ings, resemble the tracheids of vascular system. Pridgeon (1986) referred to these tracheoidal elements as 'spirally thickened idioblasts'.

In general, vascular bundles are arranged in a single series in all the presently investigated taxa. In all vascular bundles of leaf, phloem is situated towards abaxial side, and xylem towards adaxial side. The phloem and xylem ends possess some amount of sclerenchyma (sclerotic sheath). Tracheids with helical thickenings and vessel-like tracheids are abundant in leaves and also other parts of the plant body. Vessel-like tracheids were also reported by Ayensu and Williams (1972) in *Palumbina* and *Odontoglossum,* and also by Kaushik (1983) in several Himalayan orchids.

15.2.2 Pseudobulb/Stem

Anatomical features of Pseudobulb/stem were given in Tables 15.4 and 15.5. The stem shows morphological variation. In some species of *Dendrobium* and other orchids, the upper portion of the stem is fleshy whereas lower portion is thick and hard. Pseudobulbs are present in epiphytic orchids. Both fleshy stem and pseudobulb are concerned with storage of water. Pseudobulbs are consistent with sympodial growth, that leads to the shortening of shoots and thus to a compact habit reducing the transpiring surface; at the same time, sympodial habit promotes water storage and accumulation of starch materials (Benzing 1989a, b, c; Goh and Kluge 1989).

Fleshy stem and pseudobulb show anatomical similarities such as cuticle on the epidermis, and barrel-shaped or squarish and turgid epidermal cells; cortex and ground tissue with large polygonal to oval-shaped cells, function in storage of water (Fig. 15.3a, b). However, pseudobulb differs from stem in certain features. In pseudobulb, distinct cortex is absent; directly ground tissue in which numerous vascular bundles are scattered, appeared immediately below the epidermis (Fig. 15.3a, b). In case of stem, in the presently investigated taxa viz., *B. bisetum, B. cauliflorum* and *D. nobile*, a distinct cortex is present; this cortex is demarcated from the ground tissue by a ring of 3–4 layered sclerenchyma. Such type of demarcation was also reported by Morris et al. (1996) in some members of the subtribe Dendrobiinae.

Some of the cortical cells in the ground tissue region are showing pitted wall thickenings in most of the dendrobiums, such as *D. anceos, D. microbulbon, d. densiflorum* and *D. haemoglossum* (Fig. 15.3c–f). In some cases, cortical cells with multispiral cellulosic thickenings are involved in water storage (Fig. 15.3g). Vascular bundles showed well developed phloem cap made up of sclerenchymatous tissue. Large and small, numerous, collateral vascular bundles are scattered in the ground tissue region. In general, small vascular bundles are scattered at the peripheral region and large vascular bundles located in the centre.

Table 15.4 Stem/pseudobulb: anatomical features in *Bulbophyllum* (in μm)

	Access. no.															
Anat. feat.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. Cuticle thickness	0.006	0.004	0.005	0.006	0002	0.003	0.004	0.005	0.004	0.003	0.003	0.003	0.007	0.004	0.002	0.003
2. Water storage cells	0.021	0.024	0.020	0.021	0.026	0.021	0.024	0.018	0.021	0.019	0.017	0.025	0.038	0.022	0.024	0.025
3. Size of vb.	0.041	0.046	0.049	0.095	0.051	0.048	0.032	0.038	0.065	0.041	0.051	0.040	0.049	0.041	0.065	0.059
4. No. of ph. cap layers	3	3	3	2	1	3	2	2	3	3	3	3	2	2	2	2
5. No. of xy cap layers	2	2	1	–	–	–	–	1	2	–	1	1	–	–	1	–
6. Length of tracheid/vessel member	0.029	0.031	0.041	0.021	0.019	0.025	0.019	0.029	0.028	0.022	0.029	0.041	0.019	0.021	0.018	0.029
7. Length of xy. Fibre	0.035	0.045	0.019	0.025	0.042	0.037	0.029	0.039	0.027	0.035	0.039	0.019	0.032	0.022	0.041	0.035
8. Length of ph. Fibre	0.044	0.031	0.038	0.041	0.032	0.038	0.015	0.037	0.028	0.039	0.037	0.038	0.041	0.045	0.033	0.040

[1. *Bulbophyllum affine* (Arunachal Pradesh), 2. *B. affine* (Darjeeling), 3. *B. bisetum,* 4. *B. careyanum,* 5. *B. cauliflorum,* 6. *B. cornutum,* 7. *B. crassipes,* 8. *B. fischerii* (Darjeeling), 9. *B. fischerii* (Sikkim), 10. *B. khasyanum,* 11. *B. protractum,* 12. *B. scabratum,* 13. *B. stenobulbon,* 14. *B. tremulum,* 15. *B. umbellatum* (Sikkim), 16. *B. umbellatum* (Darjeeling)]

ph. = phloem, xy = xylem, vb. = vascular bundles; + = present, – = absent

Table 15.5 Stem/pseudobulb: anatomical features in *Dendrobium* (in μm)

	Access. No.															
Anat. feat.	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
1. Cuticle thickness	0.006	0.005	0.005	0.003	0.006	0.003	0.004	0.003	0.005	0.006	0.003	0.004	0.006	0.004	0.005	0.006
2. Water storage cells	0.021	0.026	0.030	0.021	0.025	0.019	0.020	0.024	0.027	0.016	0.014	0.021	0.024	–	0.021	0.028
3. Size of vb.	0.041	0.047	0.040	0.031	0.044	0.051	0.031	0.042	0.044	0.049	0.040	0.045	0.039	0.047	0.047	0.042
4. No. of ph. cap layers	2	3	4	3	4	3	3	3	3	3	4	4	3	1	2	2
5. No. of xy. cap layers	–	1	2	–	1	2	3	–	1	1	2	2	1	–	1	–
6. Length of tracheid/vessel member	0.021	0.025	0.031	0.025	0.018	0.021	0.018	0.021	0.018	0.022	0.027	0.019	0.024	0.026	0.025	0.020
7. Length of xylem fibre	0.031	0.042	0.030	0.034	0.031	0.039	0.031	0.041	0.036	0.029	0.038	0.01	0.034	0.038	0.032	0.029
8. Length of phloem fibre	0.034	0.031	0.040	0.031	0.044	0.032	0.039	0.024	0.041	0.039	0.031	0.042	0.041	0.037	0.033	0.039

[17. *Dendrobium anceps* (Darjeeling), 18. *D. anceps* (Skkim), 19. *D. bicameratum*, 20. *D. densiflorum*, 21. *D. haemoglossum*, 22. *D. herbaceum* (Kerala), 23. *D. herbaceum* (Karnataka), 24. *D. heyneanum*, 25. *D. jenkinsii*, 26. *D. microbulbon* (Kerala), 27. *D. microbulbon* (Karnataka), 28. *D. moschatum* (Kerala), 29. *D. moschatum* (Karnataka), 30. *D. nobile*, 31. *D. nutantiflorum*, 32. *D. pendulum*]
ph. = phloem, xy. = xylem, vb. = vascular bundles; + = present, – = absent]

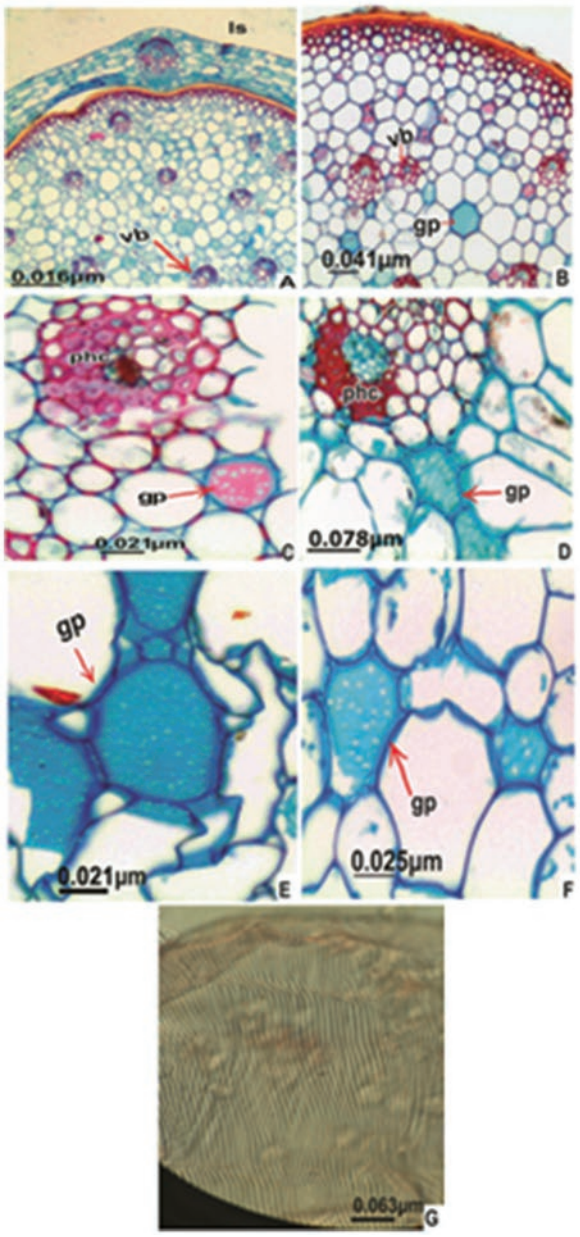

Fig. 15.3 (**a**–**g**). Pseudobulb/stem (**a**) *B. khasyanum*. Pseudobulb cross section indicating leaf sheath and scattered vascular bundles in the ground tissue (**b**) *B. tremulum.* Pseudobulb cross section showing ground tissue and vascular bundles (**c**) *D. anceps*. Fleshy stem cross section indicating the phloem cap and pitted wall thickenings in the ground tissue cell (**d**) *D. microbulbon* Pseudobulb cross section indicating vascular bundle with well-developed phloem cap and cells with pitted thickenings in ground tissue region (**e**) *D. densiflorum* Part of cross section of fleshy stem indicating pitted cell wall thickenings in ground tissue region (**f**) *D. haemoglossum* Stem cross section indicating pitted thickenings in cells of ground tissue (**g**) *D. bicameratum* Water storage cell with multispiral thickenings from stem maceration

15.2.3 Root

In general velamen roots are present in all epiphytic taxa of *Bulbophyllum* and *Dendrobium* (Tables 15.6 and 15.7) and occasionally in terrestrials. The epidermis

Table 15.6 Root: anatomical features in *Bulbophyllum* (in μm)

	Access. No.															
Anat. feat.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. No. of velamen layers	1	2–3	1	1	1	1	6	7	–	5	8	5–7	5–7	1	6–8	4–6
2. Fibrous mats/tilosomes	+	–	+	–	+	+	–	–	–	–	–	+	–	+	–	–
3.Exodermis cell lignification	0.021	0.028	0.031	0.019	0.015	0.021	0025	0.024	0.023	0.020	0.022	0.031	0.026	0.021	0.025	0.030
4.Passage cell size	0.004	0.007	0.003	0.004	0.006	0.003	0.002	0.003	0.005	0.004	0.006	0.003	0.008	0.007	0.004	0.005
5.Endoermis cell lignifiction	0.008	0006	0.010	0.009	0.004	0.007	0.011	0.008	0.005	0.015	0009	0.010	0.006	0.008	0.011	0.013
6.Vascular cylinder diameter	0.051	0.051	0.047	0.053	0.072	0.061	0.052	0.054	0.050	0049	0.044	0.047	0.051	0.049	0.057	0.041
7. No. of protoxylem poles	8	10	13	9	9	8	16	10	12	12	8	13	8	6	10	9

[1. *Bulbophyllum affine* (Arunachal Pradesh), 2. *B. affine* (Darjeeling), 3. *B. bisetum,* 4. *B. careyanum,* 5. *B. cauliflorum,* 6. *B. cornutum,* 7. *B. crassipes,* 8. *B. fischerii* (Darjeeling), 9. *B. fischerii* (Sikkim), 10. *B. khasyanum,* 11. *B. protractum,* 12. *B. scabratum,* 13. *B. stenobulbon,* 14. *B. tremulum,* 15. *B. umbellatum* (Sikkim), 16. *B. umbellatum* (Darjeeling)]

Table 15.7 Root: anatomical features in *Dendrobium* (in μm)

	Access. No.															
Anat. feat.	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
1. No. of velamen layers	9	3–4	6	5–7	1	1	1	1	1	1	2–5	3–6	5–8	1	6	3–5
2. Fibrous mats/tilosomes	–	–	–	9	+	+	+	+	+	+	–	–	–	+	–	–
3. Exodermis cell lignification	0.029	0.028	0.027	0.019	0.030	0.026	0.027	0.021	0.027	0.021	0.028	0023	0.029	0.026	0.029	0.015
4. Passage cell size	0.004	0.006	0.004	0.004	0.005	0.003	0.005	0.004	0.006	0.007	0.004	0.003	0.007	0.005	0.002	0.003
5. Endoermis cell lignifiction	0.012	0.005	0.007	0.010	0.011	0008	0.012	0.011	0.007	0.013	0.009	0.014	0.016	0.010	0.009	0.009
6. Vasclar cylinder diameter	0.0 39	0.047	0.054	0.050	0.055	0.048	0.047	0.045	0.037	0.051	0.058	0.053	0.043	0.046	0.060	0.049
7. No. of protoxylem poles	8	10	12	10	8	10	11	10	11	8	10	10	8	9	10	9

[17. *Dendrobium anceps* (Darjeeling), 18. *D. anceps* (Skkim), 19. *D. bicameratum*, 20. *D. densiflorum*, 21. *D. haemoglossum*, 22. *D. herbaceum* (Kerala), 23. *D. herbaceum* (Karnataka), 24. *D. heyneanum*, 25. *D. jenkinsii*, 26. *D. microbulbon* (Kerala), 27. *D. microbulbon* (Karnataka), 28. *D. moschatum* (Kerala), 29. *D. moschatum* (Karnataka), 30. *D. nobile*, 31. *D. nutantiflorum*, 32. *D. pendulum* + = present, – = absent

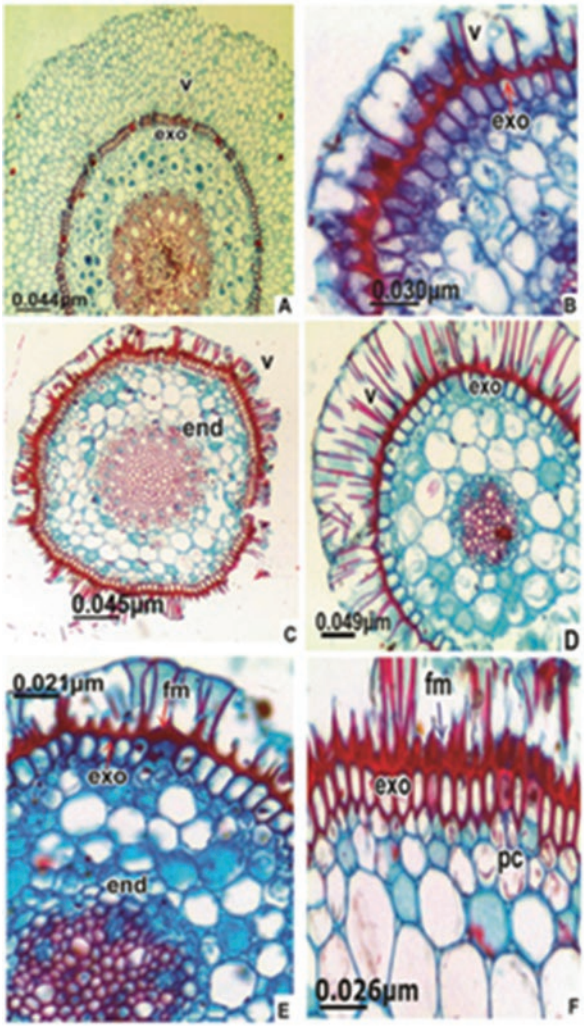

Fig. 15.4 (**a**–**f**) Root (**a**) *B. protractum*. Gross structure of root in cross-section showing multilayered velamen, exodermis and vascular cylinder (**b**) *D. haemoglossum* Root cross section showing single-layered velamen and exodermis with highly thickened inner tangential walls (**c**) *D. heyneanum* Gross structure of root in cross section showing single-layered velamen and vascular cylinder (**d**) *B. tremulum.* Water storage cell from root maceration (**e**) *B. cornutum.* Root transection showing velamen-exodermis complex with fibrous mats (tilosome) and endodermis (**f**) *D. nobile.* Root cross section indicating, fibrous mats, exodermis and also cortical cells possessing pitted thickenings

of mature root is multiseriate with velamen tissue (Fig. 15.4a). In epiphytic taxa, an extensive root system is developed to collect humus from the surrounding area. These roots are classified into two types: (1) substrata roots which penetrate the soil and absorb water and nutrients, and (2) aerial roots that are totally exposed to air and invariably they have multilayered velamen for water absorption, conservation and to provide mechanical strength to the plant body (Dycus and Knudson 1957; Morriset 1964; Benzing 1986, 1989a, b). Roots with single layered velamen (Fig. 15.4b–e) were recorded in almost all the presently studied taxa of *Bulbophyllum* and *Dendrobium*. In young roots, the cells in the outermost velamen layer are smaller than the inner ones; this layer is known as ‘epivelamen’ which is ruptured in mature

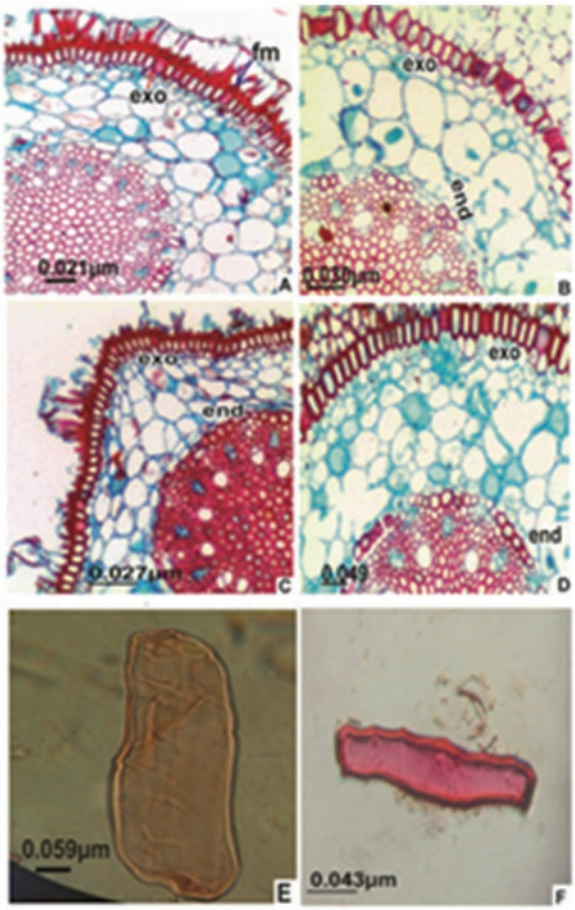

Fig. 15.5 (**a**–**f**) Root (**a**) *D. heyneanum.* Part of cross section of root showing velamen with thickened inner tangential walls, cortex and interrupted endodermis (**b**) *B. umbellatum.* Root cross section indicating exodermis and endodermis (**c**) *D. jenkinsii.* Root cross section showing peeled-off velamen with thickened inner tangential walls, exodermis, cortex, endodermis and vascular cylinder (**d**) *D. pendulum* Part of root cross section showing slightly elongated thick-walled exodermis, cortex and interrupted endodermis (**e**) *B. tremulum*. Water storage cell from root maceration (**f**) *D. moschatum.* Vessel-like tracheid from root maceration

roots. Wide bands of thickenings are found in velamen roots of some presently studied taxa.

Fibrous mats also known as tilosomes (Pridgeon et al. 1983) or fibrous bodies/fibrous mats (Benzing et al. 1983) in more specialized form are observed in some taxa of present investigation, such as *B. affine, B. bisetum, B. careyanum, B. cauliflorum, B. cornutum, B. tremulum, D. haemoglossum, D. herbaceum, D. heyneanum, D. jenkinsii, D. microbulbon and D. nobile* (Fig. 15.4f). Tilosomes also appeared in other Indian species such as *B. leopardianum, D. rotundatum* and *Otochilus alba* (Khasim 1986; Mohana Rao and Khasim 1987a). Tilosomes were observed only in epiphytes whereas absent in terrestrial taxa (Pridgeon et al. 1983; Khasim 1986; present work).

The exodermis, a single layer of cells, is situated in-between the velamen and cortex (Fig. 15.5a–d); in fact, it is the outermost layer of the cortex (Janczewski 1885; Leitgeb 1864; Engard 1944; Shushan 1959). It differs from the velamen by its greater degree of vacuolation and its elongation parallel to the long axis of the root.

At maturity, most exodermal cells do not contain protoplast and they are thick-walled, although a few remain living, thin-walled and these cells are known as "passage cells" (Fig. 15.5b). It is believed that water and solutes pass into the cortex through these cells (Dycus and Knudson 1957).

Cortex is situated in between exodermis and endodermis. It comprises thin-walled cells with cellulosic nature (Fig. 15.5e) and some may be chlorenchymatous. Some cortical cells may give an illusory appearance of vessel-like elements but the thickenings are cellulosic in nature (Fig. 15.5f). Occurrence of endotrophic mycorrhiza in the velamen and cortex is a regular feature in the family Orchidaceae.

Endodermis is uniseriate in all the investigated taxa. It is made up of thick-walled protective cells and interrupted at protoxylem poles by thin-walled passage cells. However, multiseriate endodermis was also reported in *Paphiopedilum venustum, Phragmipedium caudatum* and *P. achroederos* (Rosso 1966). Endodermal cells possess 'O' shaped thickenings (uniform lignification) in all the presently studied taxa.

Vascular cylinder comprises pericycle, phloem, xylem and pith. Phloem strands alternate with xylem strands. Vessel elements are not found in all the presently investigated taxa. However, these were reported in the roots of *Dendrobium peirardii* (Singh 1986). Vessel types and their occurrence constitute an important aspect for estimating evolutionary sequence and degree of advancement in monocotyledons (Dahlgren and Rasmussen 1983). Dahlgren and Clifford (1982) reported vessels in some orchid roots. The presence of vessels in roots is considered to be more advanced than the rhizome, stem and leaf (Cheadle and Kosakai 1980). Since all the investigated taxa are epiphytes, vessels are absent but recorded very long tracheids and vessel-like tracheids in their vegetative parts.

15.3 Anatomy in Relation to Ecological Adaptability

Some of the anatomical features of ecological interest are given below in the Table 15.8. In *Bulbophyllum* leaves are fleshy and differ in their size and form; in some cases leaf is deciduous at flowering. Leaves in *Dendrobium* are commonly distichous, conduplicate and articulate, but they range from terete and coriaceous, to laterally flattened and fleshy (Morris et al. 1996). Leaves may be persistent or deciduous. Persistent leaves are succulent ones and they commonly store water (Holttum 1960), whereas deciduous leaves remained present during wetter season of the year.

Stomatal ledges are prominent on the guard cells in *B. affine, B. careyanum, D. jenkinsii, D. moschatum* and *D. nobile*. These stomatal ledges are helpful in reducing the rate of transpiration from leaf surface and increases resistance to water loss (Yukawa et al. 1991, 1992; Ramesh et al. 2017). The presence of substomatal chambers in all taxa is an added advantage for epiphytic orchids in reducing leaf transpiration and evaporation of water.

In general, adaxial epidermal cells are larger in their size than abaxial epidermal cells. In some cases, e.g. *B. fischerii, B. khasyanum, B. pendulum, B. protractum, B. scabratum, B. stenobulbon, B. umbellatum, D. haemoglossum, D. herbaceum, D.*

Table 15.8 Morphological and anatomical features of ecological interest

Taxa	Habitat	External features	Ade cells size; stomata, distribution; ssc	Absorbing trichomes	Water storage cells and other trachaeoidal elements; fb, vlt	No. of velamen layers	Fibrous mats/ tilosomes
Bulbophyllum affine	E	Thick leaves, fleshy pseudobulbs	**ade** cells comparatively larger; with 2 or 4 subsidiary cells, prominent stomatal ledges, **h**; well-developed **ssc**	–	Simple water storage cells with abundant mucilage; **fb** absent; **vlt** abundant	Single layered	+
B. bisetum	E	Thick leaves, fleshy pseudobulb	**ade** cells comparatively larger; with 4 subsidiary cells, **h**; **ssc** present	+	Simple water storage cells with mucilages; **fb** absent	Single-layered	+
B. careyanum	E	Fleshy pseudobulb, leathery leaves	**ade** cells comp aratively larger; with 2 subsidiary cells (paracytic), stomatal ledges present **h**; well-developed **ssc**	–	Special type of water storage cells with cellulosic thickenings, other cells rich with mucilage; **fb** absent. **vlt** numerous	Single-layered	+
B. cauliflorium	E	Long-sheathed rhizome, fleshy pseudobulbs	**ade** cells comparatively larger; with 2 or 4 subsidiary cells, **h**; small **ssc**	+	Simple water storage cells rich with mucilage; **fb** absent; **vlt** numerous	Single-layered	+
B. cornutum	E	Thick leaves, fleshy pseudobulb	**ade** cells are comparatively larger; with 4–6 subsidiary cells (mostly cyclocytic, **h**; **ssc** present	–	Simple water storage cells rich with mucilage; **fb** absent; **vlt** numerous	Single-layered	+
B. crassipes	E	Leathery leaves, fleshy pseudobulb	**ade** cells are comparatively larger; with 4 subsidiary cells (tetracytic), **h**; **ssc** present	–	Simple water storage cells rich with mucilage; *fb* absent; **vlt** numerous.	6-layered	–

B. fischerii	E	Fleshy pseudobulb	**ade** *c*ells two times larger; with 4–5 subsidiary cells, **h; ssc** very small	–	Special type of water storage cells with multi- spiral cellulosic thickenings, simple water storage cells rich with mucilage; **fb** absent; **vlt** present	7–8 layered	–
B. khasyanum	E	Leaves coriaceous, fleshy pseudobulbs	**ade** cells two times larger; with 5 subsidiary cells, **h**; **ssc** present	–	Simple water storage cells rich with mucilage; **fb** absent	5- layered	–
B. protractrum	E	Fleshy pseudobulb	**ade** cells two times larger; with 5 subsidiary cells, **h**; **ssc** present	–	Simple water storage cells rich with mucilage; **fb** absent; **vlt** present	8- layered	–
B. scabratum	E	Fleshy pseudobulb	**ade** cells two times larger; with 4 subsidiary cells, **h; ssc** present.	+	Simple water storage cells with mucilage	Single-layered	+
B. stenobulbon	E	Fleshy leaves, cylindrical pseudobulbs	**ade** *c*ells 2–3 times larger; with 2–3 subsidiary cells, **h; ssc** present	+	Simple water storage cells rich with mucilage; cells with pitted thickenings **fb** absent; **vlt** present	5- layered	–
B. tremulum	E	Fleshy pseudobulb	**ade** cells comparatively larger; with 4–5 subsidiary cells, **h; ssc** absent	–	Simple water storage cells rich with mucilage, cells with pitted thickenings; **fb** absent; vessel-like tracheids	Single-layered	+

(continued)

Table 15.8 (continued)

Taxa	Habitat	External features	Ade cells size; stomata, distribution; ssc	Absorbing trichomes	Water storage cells and other trachaeoidal elements; fb, vlt	No. of velamen layers	Fibrous mats/ tilosomes
B. umbellatum	E	Ovoid pseudobulb, fleshy	**ade** cells 2–3 times larger, with 4–5 subsidiary cells or anomocytic in some cases. **h; ssc** very small	+	Special type of water storage cells with multispiral cellulosic thickenings, simple water storage cells with mucilage; **vlt** present	4–6 layered	–
Dendrobium anceps	E	Stem stout, leaves leathery	**ade** cells comparatively larger; with 4–5 subsidiary cells, **h**; no. **ssc**	+	Special water storage cells with cellulosic thickenings, cells with pitted thickenings; **fb** present, **vlt** numerous	5–9 layered	–
D. bicameratum	E	Stem fleshy	**ade** cells comparatively larger; with 4–5 subsidiary cell, **h; ssc** present	–	Special type of water storage cells with multispiral cellulosic thickenings, **fb** absent; **vlt** present.	6- layered	–
D. densiflorum	E	Thick leaves; fleshy stems	**ade** cells comparatively larger; with 45 subsidiary cells, **h**; very small, **ssc** present	–	Simple water storage cells rich with mucilage, some cells with pitted thickenings; **fb** absent; **vlt** present.	5–6 layered	–
D. haemoglossum	E	Fleshy stem	**ade** cells two times larger; with 5 subsidiary cells, **h**; smaller size, **ssc** present	–	Simple water storage cells rich with mucilage and also abundant starch **fb** absent; **vlt** numerous	Single layered	+
D. herbaceum	E	Fleshy stem	**ade** *c*ells, 2–3 times larger; with 4–5 subsidiary cells, **h**; small **ssc**	+	Simple water storage cells with abundant mucilage; **fb** absent; **vlt** numerous	Single layered	+

D. heyneanum	E	Leathery leaves, fleshy stems	**ade** cells two times larger; with 4–6 subsidiary cells, **h**;	+	Simple water storage cells rich with mucilage, cells with pitted thickenings; **fb** absent.	Single layered	+
D. jenkinsii	E	Leathery leaves, fleshy pseudobulb	**ade** cells comparatively larger; with 4 subsidiary cells, prominent stomatal ledges, **h; ssc** present	+	Simple water storage cells with abundant mucilage; **fb** absent; **vlt** numerous.	Single layered	+
D. microbulbon	E	Thick leaves, fleshy pseudobulb	**ade** cells 2–3 times larger; with 4 subsidiary cells, **h; ssc** present	+, some times absent	Simple water storage cells with abundant mucilage, cells with pitted thickenings; **vlt** present	1–4 layered	+ (inconspicuous)
D. moschatum	E	Leaves leathery, fleshy stems	**ade** cells 2 or 3 times larger; with 5 subsidiary cells, prominent stomatal ledges, **h; ssc** not seen	+	Simple water storage cells with abundant mucilages, cells with pitted thickenings**;** **fb** absent; **vlt** numerous	6–8 layered	–
D. nobile	E	Fleshy pseudobulb like stem	**ade** cells 2 times larger; with 4 subsidiary cells, **h; ssc** present	–	Simple water storage cells with abundant mucilage, **vlt** present	Single-layered	+
D. nutantiflorum	E	Thick stems	**ade** cells 2–3 times larger; with 5 subsidiary cells, **h**; **ssc** present	–	Simple water storage cells rich with mucilage; **fb** absent, **vlt** numerous	6- layered	–
D. pendulum	E	Thick leaves, fleshy pseu- dobulbs	**ade** cells comparatively larger; with 4–5 subsidiary cells, **h; ssc** present	+	Simple water storage cells rich with mucilage, presence of bulliform ells, **fb** absent; **vlt** present	3–5 layered	–

ade = adaxial**, e** = epiphyte, **h** = hypostomatic, **fb** = fibre bundle, **ssc** = substomatal chamber, **vlt** = vessel-like tracheid; **+** = present, **–** = absent

heyniyanum, D. microbulbon, D. moschatum, D. nutantiflorum, D. nobile and *D. pendulum*, adaxial epidermal cells are 2–3 times larger in their size than abaxial ones. Such type of larger adaxial epidermal cells were also reported in *D. cumulatum, D. falconeri, D. gibsonii and D. parishii* (Isaiah 1993). All these large epidermal cells are thin-walled, mucilagenous, hyaline and function in storage of water.

Absorbing trichomes are present in both *Bulbophyllum* and *Dendrobium* species. These may be 3-celled in *B. bisetum, B. umbellatum, D. anceps*, *D. herbaceum, D. jenkinsii* and *D. pendulum;* whereas 2-celled in *B. cauliflorum, B. stenobulbon, B. heyneanum, D. microbulbon* and *D. moschatum*. Systematic occurrence of absorbing trichomes were recorded in Pleurothallidinae (Pridgeon 1981). These are sunken and glandular with a basal cell. Apical cells of trichomes on adaxial leaf surface generally rupture and a brown opaque residue covers the exposed portion of the basal cell (stalk cell). Pridgeon (1981) observed that amylacetate-alcohol dissolves the residue, rendering the stalk cell's lateral walls clearly visible. Inward movement of eosin or safranin stain from the apical cell through the stalk cell and into hypodermal cells indicates an absorptive function.

As such distinct hypodermis is absent; however, fibre bundles are observed in *D. anceps* at hypodermal position. Fibre bundles provide mechanical strength to the plant body along with special type of water storage cells with multispiral cellulosic thickenings.

Pseudobulbs are uninodal or polynodal organs. They show a great range of variation in their size and shape. Pseudobulbs are conical-ovoid in *B. bisetum* and *B. fischerii*, ellipsoidal in *B. crassipes*, cylindrical in *B. protractum, B. umbellatum* and *B. stenobulbon* and sub-globose in *B. tremulum*. In the case of *D. jenkinsii*, pseudobulbs are bottle-shaped; mostly in presently studied taxa of *Dendrobium*, stems are fleshy and erect or pendulous without bulbous nature. Thick cuticle and sinuous walls of epidermal cells are helpful in reducing the transpiration and also harden the tissue (Yukawa and Uehara 1996).

In some cases, these larger cells are modified into special type of water storage cells with multispiral cellulosic thickenings observed in *B. careyanum, B. fischerii, B. umbellatum, D. anceps* and *D. bicameratum*. All these larger parenchymatous cells with or without thickenings serve as water storage cells and comprise the succulent tissue of the organ (Wilder 1985; Koller and Rost 1988a, b; Stern and Morris 1992).

As it was opined by Moreira et al. (2013) that the well-developed velamen roots, distinct exodermis and endodermis, and specialized thick-walled cortical cells are the characteristic features of epiphytic orchids. This tissue is supposed to act as a sponge, absorbing the moisture from the atmosphere. In fact, the velamen stores water which is utilized by plant during dry conditions. Pridgeon (1987) reviewed the functional aspects of velamen. Tangential walls of cells in the innermost velamen layer are much thickened and form fibrous mats, also known as fibrous bodies or tilosomes (Benzing et al. 1982; Pridgeon et al. 1983). It was observed in the presently investigated taxa that the well-developed fibrous mats (tilosomes) are always associated with single-layered velamen (Table 15.8). This type of situation was also reported from *D. rotundatum* (Khasim and Mohana Rao 1984). Dycus and Knudson

(1957) called these fibrous bodies as "layers of even mats" situated on velamen cell walls immediately above the passage cells in several epiphytic taxa. The fibrous bodies in *Sobralia macrantha* (Benzing et al. 1982) and *D. rotundatum* (Khasim and Mohana Rao 1984) have been described as hygroscopic device designated to facilitate the condensation of atmospheric moisture prior to its absorption through underlying passage cells, and as a possible aid to the acquisition of atmospheric ammonia (Haberlandt 1914). The fibrous mats/tilosomes promote water economy in orchids. The labyrinthine structure of tilosome lengthens the pathway, the water vapour must traverse to breach the exodermis-velamen barrier during transpiration (Khasim and Mohana Rao 1984). This notion parallels to the plug hypothesis of (Leitgeb 1856). If the fibrillar components of tilosome alternately shrink and swell upon desiccation and hydration, its mass would function as a one way valve and not as a plug (Benzing et al. 1982).

Velamen may be single-layered or multi-layered. In single-layered velamen roots, exodermis is well developed with long thick-walled and short thin-walled passage cells. This single-layered velamen is peeled-off in mature roots. As a result, the entire interior part of the root is exposed out and there is a possibility of losing water from the root very easily. So as to prevent the water loss, tilosomes were conspicuously seen just above the exodermis in these single-layered velamen roots. Tilosomes are completely absent from the multi-layered velamen roots of *Bulbophyllum* and *Dendrobium* species. From this discussion, it can be presumed that the single-layered velamen roots have undergone anatomical adaptations so as to protect the root from desiccation and transpiration of water from interior parts of root.

The diversification of velamen characters is also exemplified by the type of habitat and host tree on which *Bulbophyllum* and *Dendrobium* species are growing continuously (Ramesh et al. 2017). When compared to Darjeeling collections, plants collected from Arunachal Pradesh habitat show some xeric characters; with respect to velamen, it is well-developed in Arunachal collections of *B. umbellatum* at altitude 1500 m when compared to Darjeeling at 1650 m elevation. However, velamen is well developed in *D. anceps* plants collected from Darjeeling (9-layered) than that of Sikkim collections at 2500 m elevation (3–4 layered velamen). Besides, tilosomes are observed in *B. affine* of Arunachal collections whereas absent in Darjeeling accessions. Isaiah (1993) reported 1–2 layered velamen with tilosomes in *B. protractum*, whereas in the presently studied collection at 1650 m elevation from Arunachal Pradesh velamen is multilayered without tilosomes. This report further indicates that Arunachal Pradesh habitat shows more xeric elements than that of Darjeeling where luxuriant growth of orchids is found. Similarly Karnataka region at 850 m altitude shows some xeric condition when compared to Kerala at 950 m elevation, both come under Western Ghats of India. This is evident from the well developed velamen roots of *D. microbulbon* and *D. moschatum* collected from Karnataka region (Tables 15.6 and 15.7). Moreover, more number of water storage cells with abundant mucilage is observed in *D. microbulbon* collected from Karnataka than that of Kerala collections. From the above discussion, it is evident

that those plants growing in lower elevation are showing more xeric conditions than those plants of higher elevation.

Exodermis in root possesses long, thick-walled and broad thin-walled passage cells. The thick-walled cells prevent water escaping from the conducting tissues in the interior of roots; thin-walled passage cells allow the water-soluble nutrients to pass through from outside into the conducting tissues. Water-soluble nutrients are checked by tilosomes and these pass through the passage cells into the interior of roots. Just like velamen, the exodermal thickenings aid in the reduction of water loss by root transpiration (Benzing et al. 1983), turning into an important apoplastic barrier (Hose et al. 2001; Ma and Peterson 2003).

Endodermis is interrupted by thin-walled passage cells at protoxylem poles. In all the investigated taxa, endodermal cells are uniformly lignified ('O' shaped thickenings). In both samples of *D. anceps* collected from Darjeeling and Sikkim have showed poor lignification of endodermal cells. But Isaiah (1993) reported high lignification in endodermis of some species, also collected from Sikkim. This can be attributed that not only the habitat conditions but also the supply of nutrients by host plant plays a vital role for the survival of epiphytic orchids.

Vascular cylinder in the root consists of pericycle, phloem, xylem and pith. In *Bulbophyllum* the number of protoxylem poles is 6–16 whereas in *Dendrobium*, it is 8–12 (Tables 15.6 and 15.7). On the basis of the number of protoxylem poles Rosso (1966) classified orchids belonging to Cypripedioideae into two groups: (I) protoxylem points 8 or less and (ii) protoxylem points 9 or more.

In orchid root, vascular cylinder is polyarch in nature. In most of the investigated taxa, fibre sheath was observed around xylem and phloem. Vessels were not found in majority of taxa, instead vessel-like tracheids were abundant in almost all the taxa. However, vessels were reported from the roots of *Dendrobium pierardii* (Singh 1986), *D. amplum* and *D. thyrsiflorum* (Isaiah 1993). Carlquist and Schneider (2006) reported vessels in other members of Epidendroideae. Cheadle (1942) found vessels with scalariform perforation plates and also with simple perforation plates in some orchid taxa; he opined that the vessels do not occur in the shoot system of typically bulbose or cormose plants, but occur most commonly in roots, less commonly in leaves, and an intermediate way in aerial roots. This can be interpreted as an adaptation for rapid uptake of water during brief periods of water availability (Carlquist and Schneider 2006). Kaushik (1983) also opined that vessels must have been eliminated due to development of other water storage mechanisms in the plant body; in fact, epiphytes, which are cut-off from the ground perhaps, have no need of possessing vessels.

Mycorrhizal association is found in the roots of presently investigated taxa. It was also observed in rhizomes of *Zeuxine gracilis* (Muthu Kumar et al. 2011). In fact orchid seed germinates only after being infected by fungal mycelium. No other members of angiospermous family, except Orchidaceae, have maximum exploitation of endotrophic fungus for their nutritional requirements. Withner (1974) postulated that orchid-fungus association in various types of soils as indicative of deficient soil nutrient supply rather than that of a particular host-fungus specificity. Rayer (1927) opined that the possession of mycorrhiza is infrequently beneficial to

vascular plants. Phytoalexins undoubtedly play an important role in this respect (Arditti 1979).

Having no direct root contact to the soil, epiphytes lack access to the most important nutrient source of ground-rooted plants. Sources for epiphytic orchids are atmospheric inputs (rain, dust and intercepted mist), nutrients released from ground-rooted host plants through leaching or decomposition and to a lesser extent, remains of animals as well as mineral and organic matter (Benzing 1990). Awasti et al. (1995) reported that stemflow leachates are the main source of ammonium-N and nitrate-N for uptake by orchids of Sikkim Himalaya. Nutrient scavenging in epiphytes is assisted by unusual morphological structures such as velamen roots with tilosomes, extensive development of roots, absorbing trichomes etc. However, some ecologists pointed out that though nutrients are scarce, this may not be of much importance, but the prime limiting factor is water (Zotz and Heitz 2001). So as to store and conserve the water, orchid has undergone various morphological adaptations such as presence of pseudobulbs, succulent/leathery leaves, presence of water storage cells with multispiral cellulosic thickenings.

From the entire discussion of this chapter, it is evident that there is no generalized pattern of growing of epiphytic orchids; not only the geographical conditions and type of habitat, but also the host-tree on which orchid grows, is playing vital role in survivability of epiphytic orchids (Ramesh et al. 2017) However, this needs further study to confirm. Those orchids that are getting poor supply of nutrients from host plant, undergo anatomical adaptations so as to survive under extreme environmental conditions (Khasim and Ramesh 2010; Ramudu et al. 2012). Sikkim-Himalaya is known to be congenial for orchid growth as it has sufficient rainfall and, warm and humid conditions prevailing throughout the year. However, leaves and roots of *B. fischerii* collected from Sikkim itself showed much larger size of leaf adaxial epidermal cells and well-developed, 12-layered velamen roots. This could be attributed to the host-tree, *Meliosma dillenifolia*, on which it is growing (Table 15.1); leaves and roots have undergone structural adaptations so as to conserve the nutrient supply appropriately (Khasim and Ramesh 2010). As Zotz and Heitz (2001) pointed out, a more integrative approach to study the epiphytic biology is needed including physiological investigations, substrate instability, dispersal limitation and competition (intra and inter specific level).

15.3.1 Tribal and Subtribal Delineation in Dendrobieae of Subfamily Epidendroideae

Lindley (1830–1840), Bentham and Hooker (1883), Rolfe (1909), Mansfeld (1937a, b), Hatch (1954), Dressler and Dodson (1960), and Melchior (1964) treated the Epidendroideae as one of the major tribes in family Orchidaceae; whereas Vermeulen (1966), Garay (1972), Thorne (1976), Dressler (1981), Rasmussen (1985) and Dressler (1986, 1993) regarded the Epidendroideae as a subfamily of Orchidaceae.

The subfamily Epidendroideae resembles the other members of Orchidaceae in possessing both terrestrial and epiphytic habits, larger adaxial epidermal cells and

homogeneous mesophyll. However, Epidendroideae deviate markedly in having hypodermis in leaf; stomata of cyclocytic, diacytic and in some cases paracytic type, heterogeneous mesophyll; well-developed sclerenchymatous sheath around vascular bundles; and velamen roots with uniformly thickened or U-shaped thickenings in exodermal and endodermal cells. With respect to embryology, they show similarities with members of Orchidaceae in possessing zygomorphic flowers, column, rostellum, unilocular ovary, parietal placentation, capsular fruit and numerous tiny transparent non-endospermic seeds. But Epidendroideae deviate from other members of Orchidaceae in possessing well-developed suspensor and thick cell-walled seed coat.

With respect to chemistry, Epidendroideae show affinity with members of Orchidoideae in having flavone C-glycosides, anthocyanins, phenanthrenes and coumarins, but they deviate significantly in other chemical constituents like alkaloids, 9, 10-dihydrophenanthropyrans and pyrones, steroides, triterpenoids and bibenzyls (Veerraju 1990). Due to these significant differences in anatomy, embryology and chemical constituents, Epidendroideae deserves the status of subfamily.

The members of tribe Dendrobieae are characterized by the presence of naked pollinia without caudicles or any other appendages, a prominent column foot and *Dendrobium* seed type. *Bulbophyllum* belonging to subtribe Bulbophyllinae is quite distinct from subtribe Dendrobiinae in its habit (pseudobulbs of a single internode and basal inflorescence), absence of silica bodies, presence of leaf hypodermis and more number of protoxylem poles upto 16 in roots (Table 15.9). However, both genera *Bulbophyllum* and *Dendrobium* share several common anatomical features such as presence of prominent stomatal ledges, substomatal chambers, well-developed phloem cap, water storage cells with multispiral cellulosic thickenings and single-layered velamen roots (Table 15.10). These resemblances strongly

Table 15.9 Dissimilar anatomical features (quantitatively) of *Bulbophyllum* and *Dendrobium*

Anatomical features	*Bulbophyllum*	*Dendrobium*	References
Leaf Cuticle thickness in leaf	0.005–0.019	0.004–0.012 µm	Present study
Length of guard cells	0.008–0.021	0.010–0.025 µm	Present study
Hypodermis	Present; however, absent in most of the taxa of present study	Absent	Isaiah (1993); present study
Laminar vascular bundles	0.032–0.062 µm	0.041–0.063 µm	Present study
Root Exodermis lignification	0.015–0.31 µm	0.015–0.029 µm	Present study
Endodermis lignification	0.003–0.013 µm	0.005–0.016 µm	Present study
Vascular cylinder diameter	0.041–0.061 µm	0.037–0.060 µm	Present study
No. of protoxylem poles	6–16	8–12	Kaushik 1983; present study

Table 15.10 Common anatomical features shared by *Bulbophyllum* and *Dendrobium*

Anatomical features	*Bulbophyllum*	*Dendrobium*	References
Stomata	With 2–6 subsidiary cells (cyclocytic)	With 4–6 subsidiary cells (cyclocytic)	Mohana Rao and Khasim (1987c), present study
Stomatal ledges	Prominent	Prominent	Mohana Rao and Khasim (1987c), present study
Substomatal chambers	Present	Present	Mohana Rao and Khasim (1987c), Isaiah 1993 present study
Absorbing trichomes	Present	Present	Mohana Rao and Khasim (1987c), present study
Fibre bundles in leaf	Present in few cases	Present in few cases	Mohana Rao and Khasim (1987c), present study
Mesophyll	Homogeneous, in few cases differentiated	Homogeneous	Mohana Rao and Khasim (1987c), present study
Phloem cap in leaf and stem	Well developed	Well developed	Mohana Rao and Khasim (1987c), present study
Special water storage cells	Present, columnar or oval shaped	Present, club or oval shaped	Mohana Rao and Khasim (1987c), present study
Single-layered velamen	Present in some cases with fibrous mats (tilosomes)	Present, in some cases with fibrous mats (tilosomes)	Isaiah (1993), present study

support the view of Dressler (1993) that both subtribes Bulbophyllinae and Dendrobiinae are sister groups of tribe Dendrobieae.

15.4 Interrelationships and Phylogenetic Implications

Morris et al. (1996) stated that the comparative anatomy and systematics are common strategies to understand the relationships among *Dendrobium* and also *Bulbophyllum* spp. Nor Hazlina et al. (2013) opined that morphological characters are very important in distinguishing various species and also interspecific hybrid progenies. They also stated that the data on relationships among the species and hybrids are useful to select the parents for hybrid production.

In *Bulbophyllum* sections (Table 15.11), viz., *Desmosanthus* is characterized by larger adaxial epidermal cells (two to three times larger in their size); similarly in the section *Cirrhopetalum* species such as *B. scabratum* and *B. umbellatum* larger adaxial epidermal cells are found; further phloem cap is well developed in the section *Cirrhopetalum*. Both single- and multi-layered velamen is observed in the

Table 15.11 [a]Sectional delineation in *Bulbophyllum* Thou. and *Dendrobium* Sw

Section	Species
BULBOPHYLLUM Thouars	
Sestochilos (Breda) Benth. Hk.f.	(i) *Bulbophyllum affine* Lindl.
Desmosanthes (Bl.) J.J.Sm.	(i) *B. cauliflorum* Hk. f.
	(ii) *B. protractum* Hk.f.
	(iii) *B. stenobulbon* Par. et Rchb. f.
Racemosae Benth. ex Hk. f.	(i) *B. bisetum* Lindl.
	(ii) *B. careyanum* (Hook.) Sprngl.
	(iii) *B. crassipes* Hk.f.
Cirrhopetalum Lindl.	(i) *B. cornutum* (Lindl.) Rchb.f. (*Cirrhopetalum cornutum* Lindl.)
	(ii) *B. fischerii* Seidenf. (*Cirrhopetalum gamblei* Hk.f. *C. thomsonii* Hk.f.)
	(iii) *B. scarbratum* Rchb. f. (*C. caespitosum* Lindl.)
	(iv) *B. umbellatum* Lindl. [=*B.maculosum* (Lindl.) Rchb. f.]
Globiceps Schltr.	(i) *B. khasyanum* Griff.
Pleiophyllus J.J. Sm.	(i) *B. tremulum* Wt.
DENDROBIUM Swartz	
Aporum Bl.	(i) *D. anceps* Sw.
Breviflores Hk.f.	(i) *D. bicameratum* Lindl.
Dendrobium Sw.	(i) *D. nobile* Lindl.
	(ii) *D. pendulum* Roxb. (*D. crassinode* Benson & Rchb.f.)
Densiflora Finet	(i) *D. densiflorum* Lindl.
	(ii) *D. jenkinsii* Wall. ex Lindl.
Grastidium Bl.	(i) *D. haemoglossum* Thw.
Formosae (Benth. et. Hk.f.) Hk.f.	(i) *D. nutantiflorum* Hawk. et Helr. (D. *jerdonianum* Wt.)
Herbacea Krzl.	(i) *D. herbaceum* Lindl.
Holochrysa Lindl.	(i) *D. moschatum* (Buch.-Ham.) Sw.
Stachyobium Lindl.	(i) *D. heyneanum* Lindl.
	(ii) *D. microbulbon* A.Rich.

[a]According to Garay et al. (1994), Wood (2006)

section *Cirrhopetalum.* Single-layered velamen is also found in other studied taxa belonging to *Sestochilus* and *Pleiophyllus*. Mohana Rao and Khasim (1987c) reported the same type of velamen roots in *B. andersonii*. Further well-developed multi-layered velamen is also recorded in sections *Desmosanthes, Racemosae* and *Globiceps*. This anatomical data clearly indicates that the section *Cirrhopetalum* is a unique one, from which other groups have originated. This strengthens the Schlechter's (1912) opinion that *Cirrhopetalum* species are true bulbophyllums. The assemblage of some vegetative characters in the section *Cirrhopetalum* and appearance of these characters in other sections of *Bulbophyllum*, justify that *Cirrhopetalum* must have existed prior to the origin of other *bulbophyllums*. Molecular data also support this assumption (Ramesh et al. 2017). However, some more studies are needed to ascertain this statement.

In case of genus *Dendrobium*, both single and multi-layered velamen was recorded in the sections *Dendrobium* and *Densiflora*. The section *Stachyobium* in the present study had sheerly showed single-layered with well-developed tilosomes and also with larger adaxial epidermal cells in leaf; whereas in the sections of *Aporum, Breviflores, Formosae* and *Holochrysa*, well-developed multi-layered velamen was recorded.

The presently investigated taxon *D. anceps* of *Dendrobium* section *Aporum*, is characterized by the presence of 3-celled absorbing trichomes, suberized epidermal cells and fibre bundles at subepidermal region in leaf. Similar anatomical features were recorded in *D. aloifolium* also belongs to the section *Aporum* by Solereder and Meyer (1930) and Morris et al. (1996).

Leaf anatomy of *D. anceps*, only species representing the section *Aporum* in the present investigation, is similar to that of species of the section *Rhizobium* in possessing three-celled absorbing trichomes, suberized epidermal cells and fibre bundles (Carlsward et al. 1997). This anatomical data supports the view of Stern et al. (1994) that the section *Aporum* is a sister group of the section *Rhizobium*. Based on cladistic analysis with leaf anatomical features, Carlsward et al. (1997) demonstrated that both these groups are monophyletic.

The morphological characters (quantitative data; Tables 15.12 and 15.13) from various species of *Bulbophyllum* and *Dendrobium* are taken and subjected to Hierarchical cluster analysis using Euclidean distance to determine the distance among various species (Tables 15.14 and 15.15).

Bulbophyllum In *Bulbophyllum*, a range of 1.00–11.87 Euclidean distance values are observed (Table 15.14). *B. crassipes* has highest (11.87) and *B. careyanum* lowest (1.00) values. The dendrogram based on anatomical features of *Bulbophyllum* (Fig. 15.6) revealed 3 clusters as follows:

Cluster I –	*B. umbellatum, B. scabratum, B. fischerii, B. khasyanum, B. stenobulbon, B. protractum.*
Cluster-II –	*B. cauliflorum, B. careyanum, B. cornutum, B. affine, B. tremulum, B. bisetum.*
Cluster-III –	*B. crassipes*

From the dendrogram (Fig. 15.6), it is evident that *B. umbellatum* is closely related to *B. scabratum*; in the same way *B. stenobulbon* has close affinity with *B. protractum*; similarly *B. cauliflorum* with *B. careyanum*.

It is also noted from the dendrogram (Fig. 15.6) that the section *Cirrhopetalum* species, such as *B. cornutum, B. fischerii, B. scabratum* and *B. umbellatum*, are scattered among two clusters. This indicates that all species of the section *Cirrhopetalum* are in one way or other related to other sections of *Bulbophyllum*. In other words, other *Bulbophyllum* species show some affinity with this section. This supports the view that all other *Bulbophyllum* species might have derived from the section

Table 15.12 Diagnostic anatomical features (quantitatively) in *Bulbophyllum* used for dendrogram construction

Taxa	Size of adaxial epidermal cells in leaf (μm)	No. of subsidi- ary cells in stoma	No. of phloem cap layers in leaf	No. of velamen layers in root	No. of proto- xylem poles in root
Bulbophyllum affine	0.025	2–4	2–4	1	8–10
B. bisetum	0.023	4	3	1	13
B. careyanum	0.026	4–5	3	1	9
B. cauliforum	0.028	4–5	2	1	9
B. cornutum	0.025	4–6	3	1	8
B. crassipes	0.029	4	2	5–7	16
B. fischerii	0.029	4–5	2–3	7–8	10–12
B. khasyanum	0.005	5	2	5	12
B. protractum	0.024	5	5	8	8
B. scabratum	0.037	4	3	5–7	10
B. stenobulbon	0.025	4–5	6	5–7	8
B. tremulum	0.024	4–5	4	1	6
B. umbellatum	0.031	4	2	6–8	10

Table 15.13 Diagnostic anatomical features (quantitatively) in *Dendrobium* used for dendrogram construction

Taxa	Size of adaxial epidermal cells in leaf (μm)	No. of subsidi- ary cells in stoma	No. of phloem cap layers in leaf	No. of velamen layers in root	No. of proto- xylem arches in root
Dendrobium anceps	0.019	4–5	2	7–9	8–10
D. bicameratum	0.021	2	3	6	12
D. densiflorum	0.025	4	3	5–7	8
D. haemoglosum	0.031	5	3	1	8
D. herbaceum	0.027	4–5	2–3	1	10–11
D. heyneanum	0.032	4–6	3	1	10
D. jenkinsii	0.002	4–5	2	1	11
D. microbulbon	0.024	5	2	2–5	8–10
D. moschatum	0.026	4	2–3	5–8	8–10
D. nobile	0.029	4	2	1	9
D. nutantiflorum	0.003	4	3	6	7
D. pendulum	0.028	4–5	2	3–5	9

Table 15.14 Distance Matrix (Euclidean Distance) based on anatomical features in *Bulbophyllum*

Taxa	*B. affine*	*B. bisetum*	*B. careyanum*	*B. cauliflorum*	*B. cornutum*	*B. cressipes*	*B. fischeri*	*B. Khasyanum*	*B. protractum*	*B. scabratum*	*B. stenobulbon*	*B. tremulum*	*B. umbellatum*
Bulbophyllum affine	–												
B. bisetum	3.162	–											
B. careyanum	1.732	4.123	–										
B. cauliflorum	2.449	4.243	1.000	–									
B. cornutum	3.000	5.385	1.414	1.732	–								
B. crassipes	8.718	6.782	9.327	9.274	10.247	–							
B. fischerii	7.416	7.141	7.616	7.681	8.124	4.359	–						
B. khasyanum	5.000	4.359	5.099	5.000	5.831	4.583	3.162	–					
B. protractum	7.416	8.888	7.348	7.681	7.348	8.660	4.472	5.831	–				
B. scabratum	6.083	6.708	6.164	6.245	6.633	6.083	2.450	3.162	3.162	–			
B. stenobulbon	6.708	8.426	6.782	7.280	6.782	9.000	5.099	6.000	1.414	3.742	–		
B. tremulum	4.123	7.141	3.162	3.606	2.449	11.874	9.274	7.483	7.348	7.348	6.633	–	
B. umbellatum	7.280	7.681	7.211	7.141	7.616	6.083	2.449	3.742	3.742	1.414	4.690	8.367	–

Table 15.15 Distance Matrix (Euclidean Distance) based on anatomical features in *Dendrobium*

Taxa	*D. anceps*	*D. bicameratum*	*D. densiflorum*	*D. haemoglosum*	*D. herbaceum*	*D. heyneanum*	*D. jenkinsii*	*D. microbulbon*	*D. moschatum*	*D. nobile*	*D. nutantifium*	*D. pendulum*
Dendrobium anceps	–											
D. bicameratum	4.796	–										
D. densiflorum	3.162	4.583	–									
D. haemoglosum	8.307	7.071	6.083	–								
D. herbaceum	8.124	5.916	6.782	3.000	–							
D. heyneanum	8.124	6.708	6.633	2.236	1.414	–						
D. jenkinsii	8.063	6.000	6.856	3.162	1.000	1.732	–					
D. microbulbon	4.000	3.873	3.162	4.583	4.243	4.243	4.123	–				
D. moschatum	1.732	3.464	2.236	7.348	7.141	7.280	7.211	3.317	–			
D. nobile	8.124	6.245	6.164	1.732	2.449	2.449	2.236	4.243	7.141	–		
D. nutantiflorum	4.472	5.385	1.414	5.196	6.481	6.164	6.557	3.464	3.606	5.477	–	
D. pendulum	4.123	4.472	2.646	4.243	4.583	4.359	4.472	1.000	3.464	4.123	2.646	–

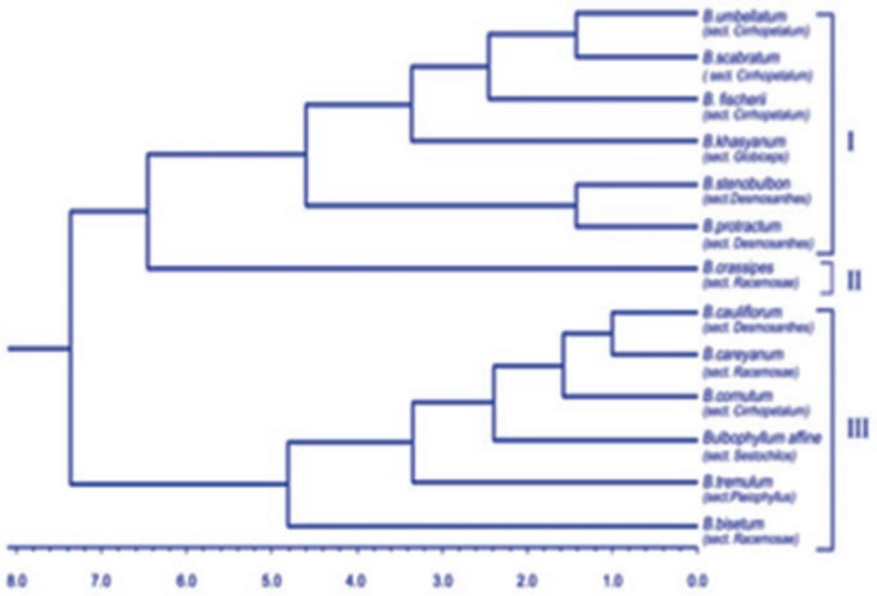

Fig. 15.6 Dendrogram showing dissimilarity among *Bulbophyllum* based on anatomical features

Cirrhopetalum which is considered to be the ancestral one to others. However, this needs further study. *B. crassipes* showing 6.5 dissimilarity value, does not form cluster with any other group of species.

Dendrobium

In *Dendrobium*, a range of 1.00–8.30 Euclidean Distance values are observed (Table 15.15); *D. haemoglossum* has the highest (8.30) whereas *D. jenkinsii* lowest (1.00) Euclidean distance values. The dendrogram (Fig. 15.7) reveals the following clusters.

Cluster-I – *D. nutantiflorum, D. densiflorum, D. pendulum, D. microbulbon, D. moschatum, D. anceps, D. bicameratum.*

Cluster-II – *D. nobile, D. haemoglossum, D. jenkinsii, D. herbaceum, D. heyneanum.*

The *Dendrobium* section *Formosae*, to which *D. nutantiflorum* (=*D. jerdonianum* Wt.) belongs (Table 15.11), was thoroughly analysed by Sathapattayanom (2008); according to him, the two morphologically aberrant species, such as *D. nutantiflorum* and *D. trigonopus*, remain unplaced. But from this study, preliminarily dendrogram shows that *D. nutantiflorum* has close affinity with *D. densiflorum* (section *Densiflora*) based on quantitative anatomical features.

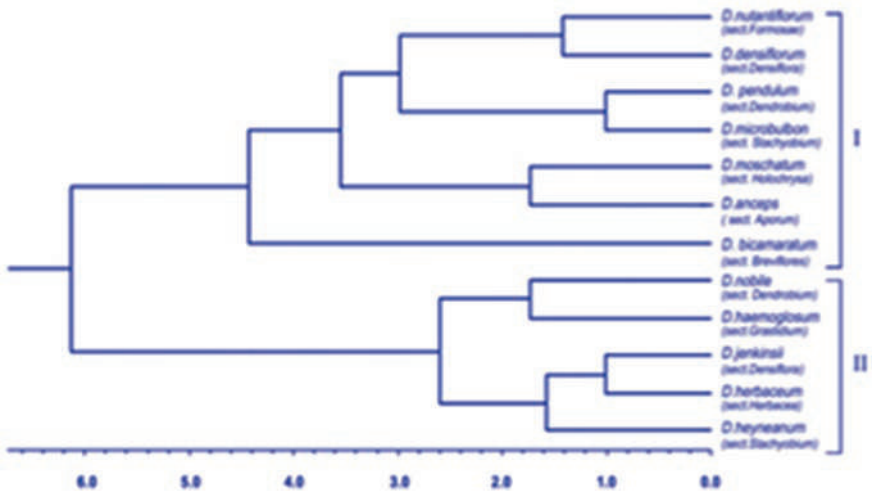

Fig. 15.7 Dendrogram showing dissimilarity among *Dendrobium* based on anatomical features

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Anatomical Studies in Some Indian Coelogyneae (Orchidaceae)

17

J. Ramudu and S. M. Khasim

Abstract

Root anatomical studies in some Indian Coelogyneae species, such as *Coelogyne breviscapa*, *C. corymbosa*, *C. flaccida*, *C. nervosa*, *C. nitida*, *C. ovalis*, *C. prolifera*, *C. stricta* and *Pholidota pallida*, have been taken up. Multiseriate velamen, parenchymatous cortex and defined endodermis were recorded in all studied species. The root anatomical characters could be considered as adaptation to epiphytism and their survival and sustainability in their respective habitats. The geographical conditions and type of habitat, also the host tree on which orchid grows, are playing a vital role in the survivability of epiphytic orchids. Those orchids that are getting poor supply of nutrients by host plant undergo adaptations so as to survive under extreme environmental conditions.

Keywords

Root anatomy · *Coelogyne* · *Pholidota* · Epiphytism · Ecological adaptability

17.1 Introduction

The Orchidaceae is one of the largest families of flowering plants comprising about 28,484 species worldwide (Govaerts et al. 2017). In India, with 1350 species, it represents the second largest flowering plant family and contributes about 10% of Indian flora (Kumar and Manilal 1994; Jalal and Jayathi 2012).

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In South India, there are about 250 species spreading to 70 genera reported (Abraham and Vatsala 1981). The vegetative anatomy of this highly evolutionary important family is neither completely taken up nor received attention. During the last two decades, few important monographs on orchid biology and systematics have appeared (Dressler 1993; Vermeulen 1993; Pridgeon et al. 1999, 2001, 2003, 2005). By critical reading of the available literature, it is evident that authors have studied the anatomy in relation to systematics; but they did not explain the ecological adaptation of orchids. From the ecological point of view, Sanford (1974) did some work on African orchids, Kaushik (1983) on some Himalayan orchids and Metusala (2017) on *Dendrobium* of Indonesia. As such, there has been no single paper on anatomy of orchids in relation to ecological adaptability for the last 20 years.

The orchid plant materials were collected from different parts of Southern India. Orchid species taken for anatomical studies were listed in Table 17.1. Roots of various species were collected and fixed in FAA (formalin 0.5 parts, acetic acid 0.5 parts, 70% ethanol 9 parts); later these were preserved in 70% ethanol before processing. Free-hand cross sections of roots were made at standardized levels (Cutter 1978). Roots of the plant were dehydrated in alcohol and xylene series, infiltrated and embedded in paraffin wax (melting point 60–62 °C) and sectioned with a rotary

Table 17.1 List of species taken for anatomical studies[a]

S.No.	Species	Place of collection and elevation	Habitat and host tree	Voucher No.
I.	Subfamily **Epidendroideae**			
	Tribe **Coelogyneae**			
	Subtribe **Coelogyninae**			
1	*Coelogyne breviscapa* Lindl.	Shevaroy Hills, Yercaud (TN), 2000 m.	Epi and*Alnus nepalensis*	ANUH 1001
2	*C. corymbosa* Lindl.	Doddabetta, Ooty (TN), 2200 m.	Epi and*Mangifera indica*	ANUH 1002
3	*C. flaccida* Lindl.	Doddabetta, Ooty (TN), 2200 m.	Epi and *Castanopsis indica*	ANUH 1003
4	*C. nervosa* A. rich.	Doddabetta, Ooty (TN), 1800 m.	Epi and *Schima wallichii*	ANUH 1004
5	*C. nitida* Lindl.	National Orchidarium,Yercaud (TN)	___	ANUH 1005
6	*C. ovalis* Lindl.	Doddabetta, Ooty (TN), 2200 m.	Epi and *Terminalia bellirica*	ANUH 1006
7	*C. prolifera* Lindl.	Doddabetta, Ooty (TN), 2100 m.	Epi and *Terminalia alata*	ANUH 1007
8	*C. stricta* (D.Don) Schltr.	National Orchidarium,Yercaud (TN)	___	ANUH 1008
9	*Pholidota pallida* Lindl.	Shevaroy Hills, Yercaud (TN), 1800 m.	Epi and*Mangifera indica*	ANUH 1009

[a]Arranged according to Dressler (1993)
Epi Epiphyte, *TN* Tamil Nadu

microtome at a thickness of 15–20 μm. Double staining was done by safranin-fast green combination, and sections were mounted in DPX mount (Vijayaraghavan and Shukla 1990; Khasim 2002). Optical photomicroscope (Motic 2.0, 5 megapixels) was used to take anatomy photographs.

17.2 Root Anatomy of Indian Coelogyneae

The present investigation roots of all studied taxa were circular in outline (Fig. 17.1a). The velamen was formed by dead tissue; velamen cells are polygonal to oval shaped (Fig. 17.1a). Numbers of velamen layers are highest in *C. stricta*, i.e. 7 (Table. 17.2). In *C. flaccida* epivelamen is distinct (Fig. 17.1b). However in C. corymbosa velamen was bistratified in roots of characteristic wall thickenings (Fig. 17.1c).

Outer layer of cortex consists of long, thick-walled passage cells which do not necessarily alternate each other (Fig. 17.1c). Exodermal size is equal in all studied taxa. However the presence of secondary thickenings in these cell walls plays an important role in water storage function and mechanical support. Exodermal cell lignifications are highest in *P. pallida* compared to other taxa (Fig. 17.2a, b).

The highest cortical layers are found in *C. breviscapa* (Fig. 17.2c, d), *C. flaccid* and *C. nervosa* (Fig. 17.2e). Cortices consist of small and large oval-shaped cells with intercellular spaces. Some cortical cells are with pitted thickenings (Fig. 17.1e). In *C. stricta* some layers of cortical cells are hyaline and mucilaginous and have water storage function (Fig 17.1e).

The endodermis cell walls were thickened. However in *C. stricta*, the highest endodermal cell lignifications were observed (Fig. 17.1d). The vascular cylinder, phloem and xylem, strands alternate each other. In *P. pallida* xylem comprises tracheids with helical thickenings (Fig. 17.2a, b). Vessel members and vessel-like tracheids were abundantly observed in root macerations of *C. ovalis*. Pith is parenchymatous (Figs. 17.1f and 17.2f).

17.3 Anatomy in Relation to Ecological Adaptability

All the presently investigated taxa possess velamen roots. The epidermis of mature root is multiseriate with velamen tissue. According to Dycus and Knudson (1957) and Benzing (1986, 1989a, 1989b), epiphytic roots are of two types: (1) substrata roots which penetrate the soil and absorb water and nutrients and (2) aerial roots that are totally exposed to air and provide mechanical strength to plant body. Epivelamen which differs from inner velamen layers was also reported by Khasim (1986) in *Cymbidium grandiflorum*, *C. mastersii* and *Oberonia wightiana*,but it is peeled off at maturity.

Velamen is dead tissue filled with air in the dry condition, giving the root the characteristic grey colour. This tissue is supposed to act as a sponge, absorbing the moisture from the atmosphere. In fact, the velamen stores water which is utilized by

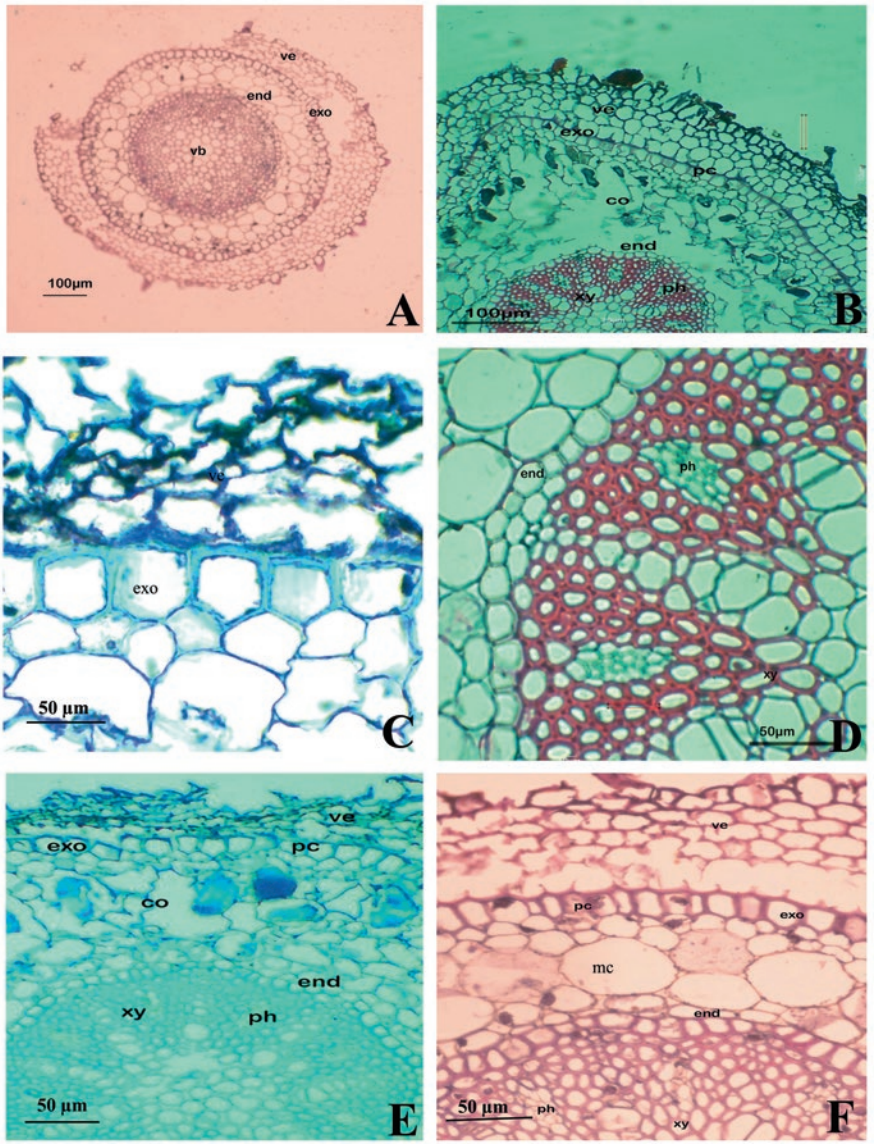

Fig. 17.1 (**a**–**f**). Anatomical studies of *Coelogyne nervosa*, *C. flaccid*, *C.corymbosa*, *C. stricta* and *C.ovalis*roots. (**a**). Root cross section showing gross structure with cortex and vascular cylinder of *C. nervosa*; (**b**). Root cross section showing velamen, exodermis, cortex and endodermis in *C.flaccida*; (**c**). Root cross section showing epivelamen and exodermis and cortex of *C. corymbosa*; (**d**). Root cross section showing endodermis of *C. stricta*; (**e**). Root cross section showing cortex of *C.* stricta; (**f**). Root cross section showing velamen, cortex and endodermis of *C. ovalis* (***co*** Cortex, ***end*** Endodermis, ***exo*** Exodermis, ***mc*** Mucilage cavity, ***pc*** Passage cell, ***ph*** Phloem, ***pt*** Pith, ***vb*** Vascular bundle, ***ve*** Velamen, ***xy*** Xylem)

Table 17.2 Root: anatomical features in Epidendroideae (in μm)

Species / Anatomical feature	*Coelogyne breviscapa*	*C. corymbosa*	*C. flaccida*	*C. nervosa*	*C. nitida*	*C. ovalis*	*C. prolifera*	*C. stricta*	*Pholidota pallida*
1. No. of velamen layers	5	4	5	6	5	4	4	7	6
2. Exodermis size	32.12	33.32	34.01	30.08	34.04	31.87	29.85	31.90	33.46
3. Exodermis cell lignification	2.1	2.0	2.1	2.2	1.9	1.8	2.1	2.0	2.3
4. Cortex no. of layers	7	6	7	7	5	6	6	6	6
5. Endodermis thickness	18.81	23.41	21.59	18.48	16.61	21.21	14.53	21.21	14.53
6. Endodermal cell lignification	12.11	13.22	12.91	11.11	13.00	15.99	9.08	16.44	9.20
7.Vascular cylinder diameter	316.02	499.9	452.05	5808	486.6	730.16	328.32	395.5	664.17
8. No. of protoxylem poles	10	9	8	9	10	19	8	9	14
9. Vessel member	+	+	+	+	+	+	+	+	−

(+), Present; (−), Absent

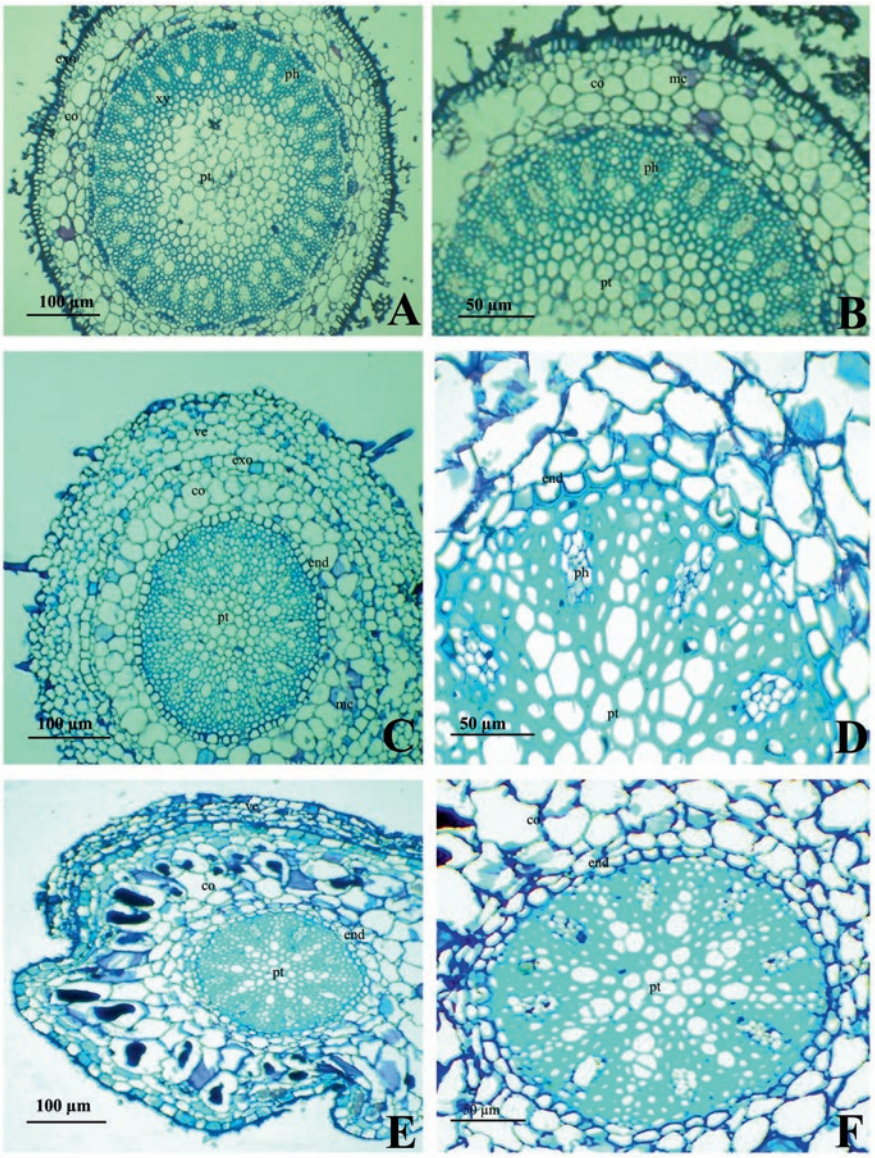

Fig. 17.2 (**a–f**). Anatomical studies of *Pholidota pallida*, *C. breviscapa*, *C. nitida* and *C. flaccida* roots. (**a**). Root cross section showing gross structure with cortex and vascular cylinder of *P. pallida*; (**b**). Root cross section showing exodermis, cortex and endodermis of *P. pallida*. (**c**). Root cross section showing epivelamen and exodermis and cortex of *C. breviscapa*. (**d**). Root cross section showing cortex and endodermis of *C. breviscapa*. (**e**). Root cross section showing cortex of *C. nitida*. (**f**). Root cross section showing velamen, cortex and endodermis of *C. flaccida* (*co* Cortex, ***end*** Endodermis, ***exo*** Exodermis, ***mc*** Mucilage cavity, ***pc*** Passage cell, ***ph*** Phloem, ***pt*** Pith, *vb* Vascular bundle, ***ve*** Velamen, ***xy*** Xylem)

plant during dry conditions. Velamen also protects the inner cortex and other tissues against UV damage (Zotz and Winkler 2013; Chomicki 2015).

The diversification of velamen character is also exemplified by type of habitat and host tree on which both *Coelogyne* and *Pholidota* species are growing continuously. However, velamen is well developed in taxa collected from Yercaud (Tamil Nadu) at an elevation of 1500 m with six to seven layers than that of Doddabetta (Ooty, 2200 m) plant collections. The present investigation further indicates that the Yercaud plant collections, viz. *C. breviscapa* and *P. pallida*, growing in Eastern Ghats show more xeric elements than that of Ooty and Doddabetta that come under Western Ghats where luxuriant growth of orchids is found.

In some orchids, flavonoids are accumulated in the aerial photosynthetic roots. These flavonoids are helpful in protecting the velamen root against the UV radiation damage and also providing long-lasting nature to the aerial roots even after their death (Chomicki et al. 2015), whereas in terrestrial orchid root, flavonoids are completely absent.

Fibrous mats are completely absent from the presently studied taxa except *Pholidota pallida*. Fibrous mats (tilosomes) are reported in the tribes Polystacheae and Dendrobieae and other subtribes of Epidendroideae (Pridgeon 1983; Khasim 1986; Mohana Rao and Khasim 1987a, c; Ramesh 2014).

According to Shushan (1959), the outermost layer of cortex, close to the velamen, is differentiated as an exodermis. Exodermis consists of two types of cells: (1) larger cells, along with root axis, with thickened walls without protoplast, and isodiametric and (2) shorter cells with thin walls, known as passage cells with a dense cytoplasm and prominent nucleus. The longer lignified cells of exodermis protect the root cortex against dehydration, while shorter cells, with thin walls, drive nutrients from velamen to root cortex (Dycus and Knudson 1957; Oliveira and Sajo (1999). In almost all presently investigated taxa, exodermis comprises 'O'-shaped thickenings.

Benzing et al. (1983) reported that just like velamen, the exodermal thickenings aid in the reduction of water loss by root transpiration. Thickenings of exodermal cell wall are also reported maximum in *P. pallida* collected from Yercaud (Eastern Ghats of Tamil Nadu). Cortical cells with pitted thickenings are found in *C. ovalis*. These were also reported in *C. cristata* (Mohana Rao and Khasim 1987b) and *Eria bicolor* (Isaiah et al. 1990). Bur and Barthlott (1991) described these cells as pseudovelamen cells. These cells provide mechanical strength to the plant body. Moreira et al. (2013) opined that the well-developed velamen, distinct exodermis and specialized thick-walled cortical cells are the characteristic features of epiphytic orchids.

Cortex is situated in between exodermis and endodermis. It consists of thin-walled oval- to circular-shaped cells of various sizes. The cortical layers close to the exodermis and endodermis are smaller than those of the central region. Cortex comprises thin-walled chlorenchymatous cells. Occurrence of endotrophic mycorrhiza in the velamen and cortex is a regular feature in the family Orchidaceae (Leitgeb 1864).

Exodermis in root possesses long, thick-walled and broad thin-walled passage cells. The thick-walled cells prevent water escaping from the conducting tissues in the interior of roots; thin-walled passage cells allow the water-soluble nutrients to pass through from outside into the conducting tissue.

Endodermis is interrupted by thin-walled passage cells at protoxylem poles. In all investigated taxa, endodermal cells are uniformly lignified ('O'-shaped thickenings). Species such as *C. nervosa*, *C. prolifera* and *Pholidota pallida* collected from Ooty, Doddabetta (both under Western Ghats) and Yercaud (Eastern Ghats), respectively, showed high lignification in exodermal cells. This must be attributed that not only habitat conditions (including altitude) but also the host tree supplying nutrients play a vital role for the survival of epiphytic taxa. Ramesh (2014) also made similar observations in *Dendrobium anceps* collected from Darjeeling and Sikkim Himalaya. However, this needs further study on interpopulation diversity and its ecology.

In vascular cylinder of roots, the maximum number of protoxylem poles (protoxylem points) was observed in *C. ovalis* followed by *Pholidota pallida*. Based on number of protoxylem poles, Rosso (1966) classified orchids belonging to Cypripedioideae into two groups: (I) protoxylem points 8 or less and (ii) protoxylem points 9 or more.

Vascular cylinder is polyarch in orchid root. Xylem strands alternate with phloem. In most of the investigated taxa, fibre sheath is present around xylem and phloem. Sclerenchymatous pith in some of taxa is merging with vascular sclerenchyma. Vessel members are present in all investigated *Coelogyne* whereas absent in *Pholidota*. Carlquist and Schneider (2006) also reported vessels in other members of Epidendroideae. Cheadle (1942) reported vessels with scalariform perforation plates and also with simple perforation plates. However, in most of the cases, vessels were not reported, but vessel-like tracheids are abundant. In this context, Kaushik (1983) opined that vessels must have eliminated due to development of other water storage mechanisms in the plant body; in fact, epiphytes, which are cutoff from the ground perhaps, have no need of possessing vessels.

According to Cheadle and Kosakai (1980), the presence of vessels in roots is considered to be more advanced than the rhizome, stem and leaf. Since most of the investigated taxa are epiphytes, vessels rarely appeared but very long tracheids and vessel-like tracheids are abundant in their vegetative parts.

From the entire discussion of this section, it is evident that there is no generalised pattern of growth of epiphytic orchids; not only the geographical conditions and type of habitat but also the host-tree on which orchid grows is playing a vital role in survivability of epiphytic orchids. Those orchids that are getting poor supply of nutrients by host plant undergo adaptations so as to survive under extreme environmental conditions (Khasim and Ramesh 2010; Ramudu et al. 2012).

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The Mother-Daughter Dyad in Alice Munro's "The Peace of Utrecht"

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Abstract : *The mother-daughter relationship appears not just peripherally but centrally in a relatively large number of Canadian novels and short stories. In many cultures—the evocation of women's experience condemns a writer to marginalization but in the case of Canada the portrayal of mother-daughter relationship in fiction seemed to strike a responsive chord in Canadian cultural context. The centrality of the mother-daughter relationship in Alice Munro's fiction is noticeable right from her 1968 debut short story collection Dance of the Happy Shades. The frequency by which the dyad of an ill mother and a (more or less) caregiving daughter reoccurs throughout her oeuvre makes it appear as if her treatment of the theme bordered on the obsessive. "The Peace of Utrecht" is a first-person narrative, told through the voice and consciousness of an adult woman, by name Helen. The story describes her brief return to the hometown and family which she left behind several years ago, to move ahead and find her destiny in the bigger world, in a less restrained environment. The story is narrated in two parts, set in the present tense of Helen's visit, although in each part there are complex shifts back and forth in time as the story proceeds inexorably toward its final revelation. The two parts correspond to two sets of problematic relationships: the unresolved mother-daughter relationship and the bond between the siblings. Munro creates a duplicitous world where everyday reality is overlaid by memory and fabricated stories about the past, so at every turn the sisters confront their doubled selves as adults and as the adolescents they were ten years ago.*

KEY WORDS: *Canadian fiction -- mother-daughter relationship -- cultural past -- female identity -- psychological journey – autobiographical -- degenerative illness -- caregiver – desertion -- traumatic experience – ambivalence – reconciliation -- guilt – individuality-- autonomy*

The Mother-Daughter Dyad in Alice Munro's "The Peace of Utrecht"

The problem, the only problem, is my mother. And she is the one of course that I am trying to get; it is to reach her that this whole journey has been undertaken. With what purpose? To mark her off, to describe, to illumine, to celebrate, to get *rid* of, her; and it did not work, for she looms too close, just as she always did. She is heavy as always, she weighs everything down, and yet she is distinct, her edges melt and flow. Which means she has stuck as close as ever and refused to fall away, and I could go on and on, applying what skills I have, using what tricks I know, and it would always be the same.

Alice Munro, "The Ottawa Valley"

For much of modern Canadian fiction, the mother has been an obsessive figure, as for the narrator of Alice Munro's story. This is true for both English and French Canada, thus establishing something of a Canadian rarity—a truly national phenomena. It is not surprising to learn that many of these writers who have placed the mother at the centre of their work have been women. What is surprising is the fact that these writers, writing about women's experience from an obviously female point of view, have been able to evoke recognition not only in the Canadian readers of both genders but also assume a place of vital importance in their national literature.

The major English-Canadian women writers Margaret Atwood, Margaret Laurence and Alice Munro typically focus on creating female characters in their fiction. In fact a large number of Canadian short stories and novels offer female perspectives on Canadian culture. The various women's movements of the 1960s and 70s encouraged and empowered women to make their voices heard and this led to the emergence of a distinctive Canadian literature. As the Canadian women writers participated in developing a literary tradition, rather than opposing an already established one, they hence occupy an enviable position in the creation of their country's fictional landscape. With a significant space for themselves, they treat women's psychological struggles with intense seriousness and, using a minimum of political rhetoric, try to give these struggles a past and a future.

Consequently, mother-daughter relationship appears not just peripherally but centrally in a relatively large number of Canadian novels and short stories. In many cultures—the evocation of women's experience condemns a writer to marginalization but in the case of Canada the portrayal of mother-daughter relationship in fiction seemed to strike a responsive chord in Canadian cultural context. In the works of some Canadian writers like Jane Rule and Margaret Gibson only isolated and somewhat idiosyncratic aspects of the relationship predominate but in that of Atwood, Laurence and Munro, the struggle between mothers and daughters emerges dynamically through different stages of its development. These latter writers identify the pervasive influence of the mother—as so many men writers have recognized for themselves that of the father—they tend to represent it through the daughter's steadily emerging discovery of her own female identity. Interestingly, this self-discovery very frequently dramatizes the necessity of coming to terms with the past. The underlying principle that makes Canadian fiction particularly significant in studies of female development is, the seriousness with which it treats women's quests and its emphasis on a past that, for women, is bound with the mother. In the works epitomizing mother-daughter relationship, the mother figure is often strongly connected with the past. The mother is, of course, the natural embodiment of a woman's personal past, but in many ways, she is identified with a broader cultural past as well.

The psychological journey that appears in a great deal of the Canadian fiction reveals the ambivalence that characterizes the daughter's feelings

about her mother. In her pursuit to achieve autonomy, anger and affection compete with each other so that she suffers from her desired separation, often felt to be a desertion of the mother, while at the same time she resents her childlike dependence. Indeed, whether the mother appears to be malevolent or benign, her representation epitomizes the anger, guilt and affection of the daughter as she tries to accept her own femininity. Such acceptance often necessitates the painful recognition of the transference of power involved in taking the mother's place. As the daughter grows stronger, the mother weakens; the power seems to have been stolen and the triumphant daughter can despair of her own victory. Correspondingly where the mother remains all powerful, the daughter agonizes over her own impotence. Though the struggle is ambivalent the longing for freedom propels action. And the resultant journey illustrates an apparently necessary movement from negation (the effort to cut off from the past), to recognition (an awareness of the conflict between subjection and autonomy) and concludes with reconciliation (the achieved inclusion of the past in the present).

The centrality of the mother-daughter relationship in Alice Munro's fiction is noticeable right from her 1968 debut short story collection *Dance of the Happy Shades*. The frequency by which the dyad of an ill mother and a (more or less) caregiving daughter reoccurs throughout her oeuvre makes it appear as if her treatment of the theme bordered on the obsessive. Magdalene Redekop, in her extensive study dedicated to the image of mother in Munro's fiction suggests that, "the writing daughter's conscious failure to understand and represent the mother remains at the heart of Munro's aesthetic" (1992: 209). However, Munro does not simply re-write the same story again and again, but rather attempts to negotiate new meanings and to "exorcise" past traumas. The preponderance of mother-daughter nexus in Munro's fiction has a significant biographical antecedent. In 1943, when Munro was twelve years old, her mother, Anne Laidlaw, began exhibiting troubling symptoms that would lead to the diagnosis of Parkinson's disease. Being the eldest daughter, with her nearest sister five years behind, Munro was her mother's primary caregiver, responsible for the domestic chores that her mother could no longer manage, duties that often kept Munro home from school. The impact of this period on Munro's fiction has been considerable and persistent since "the onset of Anne's Parkinson's disease came just as Munro had reached puberty and was realising her vocation as a writer" (2011: 73). The time Munro spent managing the household provided the opportunity to think her thoughts the labour of care produced the simultaneous resentment and empowerment reflected in many of her stories depicting caregiving. Thus the incorporation of painful autobiographical elements makes the image of the mother more prominent in her fiction.

> The whole mother-daughter relationship interests me a great deal. It probably obsesses me...because I had a very intense relationship with my own mother. She became ill when I was quite young. The incurable illness of a parent makes a relationship ('s)...stresses more evident... and so her illness and death and the whole tension

> between us...was very important... This is just something I keep going back to over and over again (1983:103-4).

The present article deals with the narrative treatment of an obsessive theme in Alice Munro's fiction: the mother-daughter relationship. There are two problematic sets of interrelations: the first one refers to the young daughter's inability to cope with her mother's neurodegenerative disease, the second one concerns the adult daughter, especially her revisiting of traumatic childhood experiences. Drawing on recent developments in psychoanalysis and development psychology this article analyses the way Munro presents family relationships, offering deep insights into her characters' psychological development and discussing the reasons of the adult daughter's apparent failure to come to terms with her past. In this article, I focus primarily on "The Peace of Utrecht" from *Dance of the Happy Shades* which Munro described as her "first real story".

Munro's mother, Anne Laidlaw died of Parkinson's disease in 1959 after several years of illness. Followed by this incident, in the same year "The Peace of Utrecht" was written. "The Peace of Utrecht" was published in the 1960 spring issue of the *Tamarack Review*. In an interview with Metcalf, Munro confesses the fact that "The Peace of Utrecht" was her "first really painful autobiographical story" and also "the first time I wrote a story that tore me up" (1972:58). There are several correspondences between the short story and Munro's personal experiences: her own mother's degenerative illness, her departure from her hometown in Ontario and her return.

"The Peace of Utrecht" is a first-person narrative, told through the voice and consciousness of an adult woman, by name Helen. The story describes her brief return to the hometown and family which she left behind several years ago, to move ahead and find her destiny in the bigger world, in a less restrained environment. In the first draft of the story about the two sisters and their problematic relationship Munro decided to let Ruth, a neighbour and girlhood friend of Helen, tell the story instead of one of the sisters. This attempt was later abandoned for Helen's narrative. Ruth' story presents the town's point of view which would have been more objective than Helen's, but Munro doesn't want objectivity. She wishes to revisit her own past in a fictional way and make sense of a traumatic experience.

The story is narrated in two parts, graphically marked by the use of the roman numerals, which is extremely rare in Munro's fiction. Both parts are set in the present tense of Helen's visit, although in each part there are complex shifts back and forth in time as the story proceeds inexorably toward its final revelation. In the view of E. D. Blodgett, the reader "must follow the narrator, surrendering to the narrative unfolding, perceiving that discovery is a relative process, each insight modified by a new one" (1988:23). The two parts correspond to two sets of problematic relationships: the unresolved mother-daughter relationship and the bond between the siblings. Munro creates a duplicitous world where everyday reality is overlaid by memory and fabricated stories about the past, so at every turn the sisters confront their doubled selves as adults and as the adolescents they were ten years ago. This story is Munro's first

narrative representation of what Luce Irigaray calls as "a highly explosive nucleus of emotions", mother-daughter relationship which she refigures in different versions throughout her writing career.

In this story, Helen is both the girl who was once at home and the woman who is watching that girl. This peculiar doubleness makes it possible to explore the issue of identity without collapsing inward into the claustrophobic centre of the story. Two sisters who grew up together sharing more or less the same childhood experience meet after a long period of time. Helen, narrator and protagonist, shortly after her mother's death, comes back to her hometown with her two children for a three-week visit with her older, unmarried sister Maddy, who still continues to live in the family home. During their childhood both Helen and Maddy had to take care of their mother who was suffering from a degenerative disease, probably Parkinson's. Helen to her part left her mother in declining health to get married and lead a free life while Maddy stayed behind and continued to be her mother's caregiver. Munro sets the tone from the beginning, anticipating the atmosphere of repressed guilt, shame, distressing emotions, secret thoughts, hypocrisy and helplessness that dominate the entire story. The relationship between the two sisters is problematic as it conceals a latent conflict and an apparently impossible sincere reconciliation. The opening lines of the story reveal Helen's disappointment and estrangement from her sister: "I've been at home now for three weeks and it has not been a success. Maddy and I, though we speak cheerfully of our enjoyment of so long and intimate a visit, will be relieved when it is over. Silence disturbs us" (1968: 190).

Helen begins by opening wounds and asserting her separate pain. She embarks, through memory, on a voyage of self-discovery, moving steadily toward a searing recognition. She realises that she has deserted both her sister—far more importantly—her diseased mother to their respective fates. By examining herself, Helen is gauging her own responsibility, and her own interdependence and independence. Helen's narration is evidence to Munro's reliance on the evocative effects of memory. The story's narrative order is not chronological as it begins with Helen's acknowledgement of the rift between herself and her sister. Instead, its movement depends upon a dialectic relationship between Helen's memory and her perception of her present situation, and is directed by her looming awareness of her dead mother. The mother acts like a wedge between the sisters, a function emphasized by Helen's nostalgic reminiscence of the fullness of their childhood relationship, juxtaposed against the emptiness of their present situation. Helen speaks of their shared childhood in "the dim world of continuing disaster, of home" but does not explain this disaster. With Maddy's warning to Helen, "No exorcising here", we understand that it was truly disastrous. Even here Helen doesn't specify who or what must be exorcised. Instead, she describes the memories of their childhood that she and Maddy share with Fred Powell, whom she guesses to be Maddy's lover. With these stories, the sisters try to safely wrap their unhappy childhood memories "in a kind of mental cellophane" to entertain their listener. (193).

In order to analyse the mother-daughter relation we need to examine the way the maternal illness affected the daughter's childhood and adult experiences. Both Helen and Maddy had different responses to maternal illness as they strived to deal with their new role, that of their mother's caregiver. We can observe here two different discourses imposed by the pressures of cultural norms: that of mothers as primary 'attachment figures' who are supposed to look after their children, protect and provide 'good enough mothering' (1953:90), and that of the daughters as primary caregivers who are expected to look after aged and sick parents. The two discourses are similar to a certain extent since they mutually stem from cultural stereotypes about motherhood and daughterhood. Mothers are supposed to sacrifice their individuality and subjectivity in order to be recognised as good mothers. The same condition implies for caregiving daughters who are trapped in a similar binary between the positions of good and bad daughter. Accepting the caregiving position makes her a good daughter, opting for a non-caregiving position turns her into a bad daughter.

Almost till the end of the story, Maddy remained tied to her mother by means of an invisible umbilical cord. According to psychologists this kind of relationship is always dangerous. Nancy Chodorow in her work *Reproduction of Mothering* argues that in the case of daughters there is less social pressure to differentiate from their mothers than on sons, who are expected to differentiate from their fathers. A mother-daughter relationship is characterized by less clearly defined boundaries than the father-son relationship. Consequently, greater effort is required of adolescent girls to achieve separateness and autonomy. Discussing Chodorow's theory about mother-daughter dyad, Christina Wieland states that "incomplete separation is also the source of women's ambivalence towards their mothers; because of the way the daughter feels strongly connected to her mother, yet also strives to be independent from her" (2002:107). Girls have a tendency to take up their mother's pain and depression and also experience intense guilt if they try to assert their individuality and autonomy. Maddy seems to be caught in this mother-child dyad from which she is unable to escape even after her mother's death. On the other hand, Helen who ran away and tried to escape the pressure also continues to feel guilty for having done so.

In the second section of Part I, Helen explains that her mother died the preceding winter after a prolonged illness but that she did not return home for her funeral. Upon entering Maddy's empty house with her two children, Helen sees herself in a mirror that reflects a face quite different from the smooth-faced young girl who left that house for marriage and motherhood. She remembers the panic and disorder that lay behind that deceptively smooth surface. Now, she realises the significance of the altered surface as that of a tensed, watchful young mother who watches not only her children but also herself. She feels as if she is trapped in a haunted space, overpowered by once familiar rooms and objects. Her own carefully constructed adult identity begins to wither as she senses herself becoming all at once not only a mother and sister but also a daughter who stands still in the hall instinctively awaiting the sound of

her mother's "ruined voice" calling out to her—a desperate cry for help. This is followed by a brief flashback which explores a double humiliation: the terrible humiliation of the flesh, caused by the mother's strange, deteriorating disease and the adolescent humiliation of her two daughters, caused by their inevitable condition to cope with their sick mother. In the words of Helen:

> While she (mother) demanded our love in every way she knew, without shame or sense, as a child will. And how could we have loved her, I say desperately to myself, the resources of love we had were not enough, the demand on us was too great (199).

In this story we witness a reversal of roles which has long-term effects on the girls' personalities as from now on they are the ones who have to do the mothering. Until the moment Helen left home, the sisters used to form a partnership "reversing the power relationship between generations" (2004: 22). The daughters were forced to adopt the position of caregiver for which they were not psychologically ready. Both Helen and Maddy took care of their mother during the first few years of her illness. However, at one point, Helen realised that the only way to assert her individuality is to assume the non-caregiving position. Hence, she went away to college, got married and started her own family. Superficially, it seems as if Helen finally attained her independence as an individual, but she is still a prisoner of the binary good daughter-bad daughter. She continues to feel guilty because she deserted her sick mother, placed the burden on her sister's shoulders and, ultimately, became a bad daughter. Nevertheless, she gained the necessary psychological distance which allowed her to move past denial towards self-assertion. For instance, Helen declares that she had had a number of distressing experiences in childhood and that these experiences affected her adult personality: "Is it possible that children growing up as we did lose the ability to believe in—to be at home in—any ordinary and peaceful reality?" (191). Moreover, Helen appears to be able to refer back to her past without being resentful; speaking about her dead mother, she says: "I no longer feel that when they say the words 'your mother' they deal a knowing, cunning blow at my pride. I used to feel that; at those words I felt my whole identity, that pretentious adolescent construction, come crumbling down" (194).

On the contrary, Maddy, partly because she did not distance herself from her mother, does not have the courage to address her childhood pain and suffering: "No exorcising here, says Maddy in her thin, bright voice" (191). Maddy uses 'suppression', a psychological process, in order to accept the reality of her life. She strongly wants to forget the time when she was forced to become her mother's "mother" and provide love, nurturing, warmth, security, when in fact it was she who needed all these things in that phase of life. More so, she was compelled to face stronger pressure because "society encourages [women] to carry [their] mothers with [them] in every breath, every decision, every success, and every failure" (2004: 11). It is evident that Maddy could not and did not escape this pressure. Toward the end of the story she realises the burden of these expectations which made her a prisoner in her own house. She is not in a position to leave

the house and lead a new life even after the object of her dependency (her mother) is gone.

Helen's haunting memories introduce a sequence of prison metaphors describing her mother's life. In "Working for a Living," Munro speaks of her mother as "walled in by increasing paralysis..." (37). Here in this story, Helen describes her mother as physically walled in by her "house of stone" and psychologically isolated by her daughters' grimly self-protective roles as 'prison guards' declining her the loving pity for which she yearned and yelled. Being deprived of any emotion, their caregiving used to be pragmatic and efficient but entirely insensitive: "We grew cunning, unfailing in cold solicitude; we took away from her our anger and impatience and disgust, took all emotion away from our dealings with her, as you might take away meat from a prisoner to weaken him, till he died" (199). This harrowing admission has been arrived at slowly, quietly, lethally. When the mother gradually loses her ability to speak, the daughters start acting as her interpreters. The theatricality of this new role humiliated the sisters almost to death or, to put it in Carrington's words they felt as if performing "a vulgar circus act" (1989: 22).They start treating their mother as an inanimate object or as a baby who lacks physical autonomy and the ability to express itself. Helen and Maddy are ashamed both of their mother and of their position of caregivers which is clearly reflected in the way they present their mother's appearance:

> Our Gothic mother, with the cold appalling mask of the Shaking Palsy laid across her features, shuffling, weeping, devouring attention wherever she can get it, eyes dead and burning, fixed inward on herself; this is not all (200).

In order to be recognised as good daughters, they took care of their mother though this situation filled them with shame. Outside the house, they were publicly embarrassed by their mother's symptoms whereas inside, they could not bear the sound of her voice: "the cry for help—undisguised, oh, shamefully undisguised and raw and supplicating" (198). Maddy appears to be the ultimate victim of this dramatic situation, the good daughter who sacrificed herself for her mother by accepting the caregiving position no longer assumed by Helen. The problem is that Maddy's choice is not determined by her selflessness, but rather by her inability to claim her own independence. Maddy suffers from close identification with her mother resulting into, as Nancy Chodorow suggested, a lack of distinctiveness which makes it almost impossible for her to build a reliable autonomous self.

The Part II of the story juxtaposes a situation parallel to that of Helen and her sister. Helen announces that she has been visiting her two old spinster aunts, also sisters, and fears if she and Maddy will resemble their aunts when they grow old, trapped similarly in the web of sisterhood. Helen in one of her visits senses a mild disagreement between the sisters for the first time ever. Aunt Annie despite Aunt Lou's objection shows Helen her dead mother's carefully cleaned and mended clothes and offers some of them to her. When Helen refuses to accept "those brocades and flowered silks," (205) in which her mother disguised her dying body, she realises that she is going against family values: "Things must be used, everything

must be used up, saved and mended and made into something else and used again; clothes were to be worn" (206). Perhaps the clothes served as the introduction to a conversation that Aunt Annie intended to start. She reveals Helen about her mother's frantic, dying escape from the hospital where Maddy had forcibly admitted her under the pretext of a check-up, as she no longer wanted to take care of her. Like a prisoner, wearing only a robe and slippers, she tried to run away in a snowstorm. After she was caught and returned to the hospital, a board was nailed across her bed to prevent further escape. Unable to communicate with the nurses and get out of bed, she led a life worse than imprisonment. In Carrington's opinion, "she was quite literally nailed into her coffin of silence and motionlessness before her death" (1989:188). Surprisingly enough, Helen does not seem to be harrowed by this unexpected discovery.

In the last section of Part II Helen attempts exorcism for Maddy. She urges her sister not to feel guilty for hospitalizing their mother but to leave the past and move ahead in life: "Take your life, Maddy. Take it" (210). Just before accepting this advice Maddy accidentally drops and breaks a pink glass bowl. It is at this point she tears "the mental cellophane" that prevented her from expressing her emotions and from asserting her true self. It is more likely that the bowl symbolizes her true self, the Maddy she had been hiding behind her role of "surrogate mother" (2009:23). For the first time she takes responsibility for the choice she made: "I couldn't go on . . . I wanted my life" (210). Maddy confirms the fact that, unlike Helen, she cannot accept the guilt she feels for refusing the caretaking position and for putting the mother in the hospital. The last line of the story which follows this incident strikes directly to the heart of not only the sister's situation but also her character: "But why can't I, Helen? Why can't I?" (210). The journey from knowledge of the mother to self knowledge has resulted in this helpless, anguished cry that has no answer. Munro's ending leaves the readers staggered by Maddy's overwhelming self knowledge that she cannot 'begin'. This is an example of failed 'exorcism'. The true poignancy of the story arises from the inability of Maddy to find freedom and take full control of her life. Her personality prohibits her from turning her back on what is tried and true, what is predictable. For Maddy, as for Helen, exorcising the guilty past is not so simple. Maddy took over her mother's pain and depression, and experienced intense guilt when she tried to assert her individuality and autonomy. She continues to remain tied to her mother even after her death. Thus, Maddy is caught in a mother-daughter dyad from which she is incapable to escape.

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GENDER ISSUES IN ALICE MUNRO'S "BOYS AND GIRLS"

Smt. J. Kalpana, Lecturer In English V.S.R. Government Degree & P.G. College Andhra Pradesh

Abstract:

Alice Munro, the master of the contemporary short story, has often written about the seemingly unbridgeable gap that separates men and women. In the short story "Boys and Girls," this gap is examined in the small world of a farm. Because the narrator is female, she is expected to behave in a subdued and frivolous way, to be devoted to domestic chores, and to ally with her mother against "male" pursuits such as farming, shooting, and heroism. Initially, she identifies more readily with her father than with her mother, noting that her father's work seems important and interesting while her mother's is depressing. The daughter is proud that her father appreciates her hard work, but she is ambivalent about the violence and callousness that is necessary to please him. Her gender forces a whole complex of behaviours on her; she is scorned by her mother and grandmother for not being enough of a girl and is ridiculed by the men in the family for being too much of a girl.

"Boys and Girls" is a short story by Alice Munro, the master of the contemporary short story, the Canadian winner of the Nobel Prize in Literature . It was originally published in 1964 and later included in Alice Munro's debut collection *Dance of the Happy Shades (1968).* The CBC produced a television adaptation of "Boys and Girls" in 1983. It won an Academy Award in 1984 for Best Short Subject. Among many others, a significant element in Munro's narratives of growing up is her exposure of the constructedness of rigid concepts of femininity and masculinity. The young female characters in her growing-up cycles *Lives of Girls and Women* (1971) and *Who Do You Think You Are?* (1978) and also in several other individual stories are deeply confused by the dictates of gender-scripts.

Alice Munro has often written about the seemingly unbridgeable gap that separates men and women. In "Boys and Girls," Munro records the humiliated and anguished psychology of a young girl conditioned by the society from her early formative stage. It highlights the invisible forces which shape children, in this case, the narrator and her brother Laird, into gendered adults. As Marlene Goldman states, "one such 'invisible' mechanism central to the production of gendered adults, involves the division and control of space". Here in this story, spatial divisions and control of space are emphasized by a female narrator still young enough to remark upon details which the adults ignore. Because the narrator is female, she is expected to behave in a subdued and frivolous way, to be devoted to domestic chores, and to ally with her mother against "male" pursuits such as farming, shooting, and heroism. Initially, she identifies more readily with her father than with her mother, noting that her father's work seems important and interesting while her mother's is depressing.Throughout the story the narrator is torn between the "girl" life with her mother inside the house, assisting in the kitchen, and the "boy" life with her father outside the house, helping out with the farm. The story explores the gender bias prevalent in the society and also the protagonist's feelings toward, and struggle to find, an identity of her own.

The action of this story takes place entirely on the fox farm. In the opening passage of admirably clear and restrained description Munro creates the feeling of the place and details the daily tasks the girl performs as she helps her father, keeping the pens supplied with water and spreading grass over them to prevent the foxes' pelts from being darkened by sunlight. The narrator's mother disliked the whole pelting operation which includes the killing, skinning and preparation of furs. The

narrator, however, seems to find her father's work comforting and to her "the smell of blood and animal fat" is "reassuringly seasonal, like the smell of oranges and pine needles" (112). She prefers her father's outdoor activities to her mother's domestic sphere and chores: "It seemed to me that work in the house was endless, dreary and peculiarly depressing; work done out of doors and in my father's service, was ritualistically important" (117). Indeed, the whole story centers on an inside/outside dichotomy linked to gender spaces. The young girl identifies with the male world and feels at ease with it; she even considers herself more suitable for it than her younger brother Laird, whom she regards as a sissy for a major part of the story: "Laird came too, with his little cream and green gardening can, filled too full and knocking against his legs and slopping water on his canvas shoes. I had the real watering can, my father's, though I could carry it three-quarters full" (114). The curiously detached centre of all this activity is formed by the foxes which, despite generations of captivity, have not ceased to be wild animals, hostile and intractable: "Naming them did not make pets out of them, or anything like it" (115).

The challenges to the narrator's connection to the father and her right to occupy the male 'outside' space are launched from within the household itself. The female family members begin to coerce the narrator. Efforts to restrict her behaviour occur at every level of existence. Statements by her visiting grandmother affirm the fact that the female gender role is an utterly restrictive one at the time when the story is set. Her grandmother tells her, "girls don't slam doors like that"; "girls keep their knees together when they sit down"; and when she asks a question, she is told "that none of girls' business" (119).

The stories which the narrator tells herself before she falls asleep at night, suggest something about her desires and wishes. They allow the narrator to imagine herself in alternative versions of her own life. In these stories, she casts herself into the role of heroic subject, as male savior, she rescues people from a bombed building, shoots rabid wolves and rides "a fine horse spiritedly down the main streets" (113). These stories of rescue and heroism seem, at least in terms of their content, quite clearly to place the narrator precisely where she would like to be: in the male-centered world of work which she associates with her father.

The symbolic act of letting Flora free also constitutes the protagonist's first rebellion against her father, who adheres to the authoritative gender patterning. It can be considered as a silent outcry against her own domestication. It is also interesting to note that she finds herself wanting to *tell* her mother about this incident. But she ends up remaining silent, still in the hope that her brother, who saw the incident, might not, mention anything to her father and her loyalty to her father's world might remain intact.

The climax occurs at the end of the story with Flora being captured and Laird telling the family at dinner that his sister was responsible for letting Flora escape. Laird's telling is itself an act which challenges the completeness of any opposition between father's world and mother's world, between boys and girls. For the first time in the story he sides with his father against her. When the narrator begins to cry, her father, having overcome his immediate consternation about his daughter's apparently ill-advised act, reacts in a manner that is even more threatening to his daughter than either fury or reproach. He simultaneously absolves and dismisses her by saying that she is "only a girl" (127).

"Boys and Girls," written at the beginning of the "second wave" of feminist involvement with literature in North America, renders gender relations in a rather programmatic manner: the systematic, highly symbolic opposition between interior/female and outer/male space, the almost stereotypical characterization of the father and the mother, the seemingly logical mirroring of the girl's ongoing socialization process in her different dreams, the divergent character of the male and female horses, the clear-cut socializing influences imposed on the girl both by family members and by the closed rural society. The story illustrates the restrictive, de-individualizing forces of an essentialist gender concept

during the adolescent phase of development. Simultaneously,it also points out positively valued, liberating opportunities for women to rebel against dominant male codes of behaviour.

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Editorial...

Writing in English literature is a global phenomenon. It represents ideologies and cultures of the particular region. Different forms of literature like drama, poetry, novel, non-fiction, short story etc. are used to express one's impressions and experiences about the socio-politico-religio-cultural and economic happenings of the regions. The World War II brings vital changes in the outlook of authors in the world. Nietzsche's declaration of death of God and the appearance of writers like Edward Said, Michele Foucault, Homi Bhabha, and Derrida bring changes in the exact function of literature in moulding the human life. Due to Globalization and liberalization, society moves to the post-industrial phase. Migration and immigration become common features of postmodern society. These movements give birth to issues like race, ethnicity, gender, crisis for identity, cultural conflict, dislocation, isolation and many others. Thus multiculturalism becomes the key note of new literatures written in English. The colonial legacy, immigrants and migrated authors attempt to define Britishness in literature and the result is postethnicity in English literature. The writers like Salman Rushdie, Hanif Kureishi, Andrea Levy and many others attempted to redefine and revaluate the singular authority of text and plead for the plurality of themes. There is another form of literature growing consciously in the country like India. This literature is called as Fourth World Literature or the literature of protest. The marginalized sections of society attempt to protest against upper caste ideologies in Dalit Literature. All these issues are reflected in the present issue of Literary Endeavour.

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WOMEN PARTICIPATION IN POLITICS IN INDIA

Smt Suneetha. Y, Lecturer in English V.S.R. Government Degree & P.G. College Andhra Pradesh

Abstract:

Political participation is not only relates to right to vote but also relates to participation in decision-making process, political consciousness, campaigns to create political awareness about political issues and promote political reforms. It is a fundamental prerequisite for gender equality and real democracy. It helps in women's direct involvement in public decision-making and it is a source of ensuring better accountability for women. To fight with gender inequality in politics the Indian government has given reservations for seats in local governments. Apart from all these from the local to the global level, women's leadership and political participation is restricted. women are seen as mere voters even after proving their abilities as leaders. There are many challenges to women's participation in politics. In India women are sexually abused. Child marriages, domestic violence, low literacy rates, discrimination had lowered Indian women's economic opportunities. Unlike men there are fewer opportunities for women to get involved in organizations to gain leadership skills.

Key Words: *Political participation-right to vote-decision making-political issue political reforms- real democracy-reservations-challenges- barriers to overcome women empowerment.*

Introduction

In any political system, right from the developed to the developing countries, presence of women is very low compared to men. In many countries women had to wage long battles to get right to vote. Today the percentage of women as voters has increased considerably, but their political participation is not equal to men and therefore women are unable to get an equal share in organization that require decision making. Women have not been regarded as significant part of the political arena.From the local to the global level, women's leadership and political participation are restricted. Women are underrepresented as voters, as well as in leading positions, whether in elected office, the civil service, the private sector or academia. This occurs despite their proven abilities as leaders and agents of change, and their right to participate equally in democratic governance.

Role of Women in Nation Building

Many women around the world have proved themselves as dynamic, vibrant, sincere and perfect in many fields. They are efficient and perseverant enough to face all odds and challenges and obstacles. Today's educated modern women are successful both as home makers as well as professionals, academicians, bureaucrats and Politicians.Women such as Indira Gandhi, Margaret Thatcher , Sirimao Bandarunaike , Vijayalaksmi Pandit, , Kiran Bedi have proved themselves in shaping their own destiny as well as country's destiny. While India was struggling to liberate from the clutches of the British Raj, the women folk came forward to shoulder their responsibility. Rani Lakshmi Bai is an epitome of bravery and courage. A number of Freedom fighters such as Sarojini Naidu, Sucheta Kripalini, Durgabai Deshmukh etc., display their courage and determination in the struggle for Independence. Mary Kom, Sania Mirza, Saina Nehwal, P.V.Sindhu, Sumitra Mahajan have made their presence strongly felt in their respective fields. Arundhati Battacharya, Indra Nooyi, Kiran Mazumdar, Shika sharma, Chanda Kochhar are some of the women who are contributing in their unique way in economic journey of India as CEOs of various organizations and Financial Institutions.

Political Parties

India has a multi party system with the seven registered parties at the nation level out of which three are the largest parties. They are Indian National Congress (INC), Bharatiya Janatha Party (BJP) and Communist Party of India (CPI) and these parties created Women's wings. The INC wing is All India Mahila Congress, the BJP wing is the BJP Mahila Morcha and the CPI wing is the National Federation of Indian Women. The INC has increased women participation by instituting a 33% quota for women. In June 2009, the INC nominated a woman to become a first Speaker of Loksabha and also supported the election of Smt Pratibha Patil, India's first female President. The BJP has encouraged representation of women by inducting 7 women as Ministers in Union Cabinet.

Women Representation among Elected Representatives

As per the data from ECI, out of the total 4896 MPs/MLAs across the country, only 418 or 9% are women. Among MPs, Lok Sabha has 59 (11% of 543 MPs) and Rajya Sabha has 10% or 23 (10% of 233 MPs) women MPs. Among State assemblies, West Bengal 34 (out of 294 MLAs), Bihar 34 (ou of 243 MLAs) and Andhra Pradesh 34 (out of 294 MLAs) have the maximum no of women MLAs followed by Uttar Pradesh with 32 women out of 403 MLAs and Rajasthan with 28 women out of 20 MLAs. In terms of percentage, among state assemblies, the highest percentage of Women MLAs is from Bihar with 14% (34 out of 243 MLAs) followed by Rajasthan with 14% (28 out of 200 MLAs) women votes and West Bengal with 12% (34 out of 294 MLAs).

Women Poltical Participation in India Low, Need More

The Economic Times conducted a survey on women's poltical participation and reveals that Women's political participation in India is low inspite of their 49% share of population.The survey fo 2017 stated that factors such as domestic, responsibilities prevailing cultural attitudes, role of women in society and lack of support from their families were some of the main obstacles that prevented women from entering politics. The survey said that there are countries like Rwanda which has 60 percent women representation in 2017 but countries like India, Japan, Egypt etc have less than 15% representation of women. Quoting an Inter- parliamentary Union (IPU) and UN Women report- Women in politics the survey said Loksabha had 64 (11.8 percent of 542 MPs) and Rajya Sabha 27 (percent of 245 MPs) and out of 4118 MLA's only 9 percent were women. But however there has been substantial representation of women at local government levels. Women Sarpanch accounted fo 43% of total Gram Panchayats across the country exhibiting their active leaderships.Stressing on the importance of more women participation in the survey said , "Recognising the significance of roles o women in decision making process in the society is critical to strengthen women's agencies for building a progressive society with equality of opportunities among all citizens.

Women's Vote in 2014

The gender-wise patterns of vote in the 2014 elections can be seen at two levels. At the first level, it is about the increased turn out among women voters in these elections. At the back drop of a significant increase in the overall voter turnout (from 58 to 66 percent) at the all India level, there is a remarkable closing of the gender gap between men and women voters (men at 67 and women at 66 percent at the all India level). In quite a few states women have outnumbered men voters. If this is not so new a phenomenon for states in the North East like Manipur, Meghalaya and Sikkim; it is definitely happening for the first time in states like Bihar, Rajasthan, Punjab, Odisha, Tamil Nadu, Uttarkhand etc.Lastly, it must be noted that the parties with women leaders (AIADMK,TMC and BSP) have all gained more support among women voters in these elections and it may point to another possible space for arrival of a women's constituency in the future .Women turnout during India's 2014 parliamentary general elections was 65.63%, compared to 67.09% turnout for men.[12] In 16 out of 29 states of India, more women voted than men.[12] A total of 260.6 million women exercised their right to vote in AprilMay 2014 elections for India's parliament.

Rise of Women Ministers in Union Cabinet

Seven women Ministers were inducted in the Union Cabinet after formation NDA Government. They were

- Smt Sushma Swaraj Cabinet Minister for External Affairs
- Smt Smriti Zubin Irani - Cabinet Minister for Textile Ministry
- Dr Nazma A Heptulla - Cabinet Minister for Minority Affairs
- Smt Maneka Sanjay Gandhi- Cabinet Minister for Women and Child Development
- Smt Harsimrat Kaur Badal- Cabinet Minister for Food Processing Industries

The number is higher in terms of previous Governments. The portfolios held by these ministers show how the modern woman have successfully balanced their personal as well as professional commitments.

The level and forms of women's participation in politics is largely shaped by cultural and societal barriers in the form of violence, discrimination and illiteracy. In the larger society, violence and the threat of violence affects many women's ability to participate actively in many forms of social and political relationship, to speak in public, to be recognized as dignified beings whose worth is equal to that of others. In India sexually abused. Child marriage and domestic violence and low literacy rates had lowered Indian womens economic opportunities and contributed to sexual violence in India. Although the Constitution of India removed gender inequalities among caste and gender, discrimination continues to be a widespread barrier to women's political participation. Women also lack leadership experience why many Indian due to the fact they are burdened with household duties. There is little public space for them as men have dominated the political arena for many years in India. Literacy among Indian women is 53.7% which is much lower than literacy among men reported at 75.3%. Illiteracy limits the ability of women to understand the political system and issues. Problems with exploitation, such as women being left off of voters lists, have been reported as illiteracy limits the ability of women to ensure their political rights are exercised.

Overcominng Obstacles of Participation

To combat with gender inequality in politics the Government of India has given reservations for seats in local governments. The Indian government directed the state and local governments to promote equality by giving equal pay, free legal aid, maternity relief, right to work and education and improving the standard of living.

Conclusion

Although there are many institutions that promote women's civic engagement and political participation, obstacles to women's political participation and leadership persist. Women's lesser economic resources compared with men's, their greater care giving responsibilities, their more limited access to important supports that would help them to run for office, and succeed as office holders, and the greater scrutiny that women candidates seem to face from the public and the media all restrict women's political participation and leadership in states across the nation. Progress in advancing women's political status continues to move at a glacial pace. But a ray of hope of can be seen in the 2014 General Elections as they were unique and unsurpassed in many ways. They not only showed greater rate of women voting but also their representations in Cabinet. This went on to prove the rising status of the women.

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Source: Inter Parliamentary Union, April, 2014
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Synthesis and Characterization of Nickel Oxide Nanoparticles Synthesized via Chemical Precipitation Method

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Abstract:
The Nickel oxide Nanoparticles were synthesized from Nickel Nitrate Hexahydrate aqueous solution under the chemical method at 90°C. The average crystallite size was calculated from De-Bye Scherrer's equation. FESEM, EDX, XRD were used to characterize the structural features of the product. FTIR spectra confirmed the adsorption of the Nickel oxide nanoparticles. In addition, UV-visible absorption spectra were employed to estimate the band gap energy of the Nickel oxide nanoparticles. This method may be suitable for large scale production of Nickel oxide nanoparticles for practical applications. The effect of Nickel oxide nanoparticles is screened in vitro for antimicrobial activity by Disc diffusion method. The bacterial organisms used in this study are E.coli, Bascillus Subtilis and also fungi Aspergillus Niger. The observed inhibition zones for these nanoparticles are in the range of 6mm for E.coli and 7mm for Bascillus Subtilis and 7mm for fungi Aspergillus Niger. The cytotoxicity activities of Nickel oxide nanoparticles screened by MTT assay. We have screened for one type of cancer cell-line i.e MCF-7(Breast Cancer). Nickel oxide nanoparticles obtained IC_{50} values in the range of 32.59ug/ml for MCF-7 cell line.

Keywords: *Nickel Oxide Nanoparticles, SEM, EDX, XRD,FTIR,UV-Vis, Disc diffusion method, Cytotoxicity.*

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I. Introduction:

Nanoparticles are being synthesized globallyowing to various exciting and unique propertieswhich facilitates its ther exploitations in completely unrelated fields such as nanomedicine, photocatalysis etc.The main use of inorganic oxides for nanoparticles are theur stability, robustness, and long shelf life.

Producing nanoparticles involves breaking up a bulk maternal into atoms or ions and then allowing those atoms or ions to condense into nanoparticles. Ni nanoparticles are having applications as catalyst, conducting and magnetic materials.Ni nanoparticles in particular being cheap, need mild reaction conditions for high yields of products in short reaction times as compared to the traditional raney-Nickel.NiO is an anti-ferromagnetic Oxide semiconductor with P-type conductivity due to its wide band gap energy range from 3.6-4.0evIt also having applications as smart windows, spin valves, giant magneto resistance(GMR) sensor, solar cell etc.

II. Materials:

All of chemicals used in experiment are of analytical grade and used as purchased without any purification. Nickel nitrate hexahydrate Ni $(No_3)_2{\cdot}6H_2O$), of 98% purity is used. De-ionized water used as a solvents. Sodium hydroxide (NaOH) is used to adjust the pH.

Synthesis of nickel oxide Nanoparticles:

Nickel nitrate hexahydrate $Ni(No_3)_2{\cdot}6H_2O$ and sodium hydroxide NaOH were each dissolved separately in deionized water to form the liquid media of the desired concentrations of 0.05M (4.575g/500mL)and 0.1M (2g/500mL) for sample A and B respectively the ratio of the concentrations was 1:1 ($Ni(No_3)_2{\cdot}6H_2O$: NaOH). The nickel nitrate hexahydrate was slowly added drop- wise to NaOH solutions under vigorous stirring at room temperature, forming transparent white solutions, then inserted into an electrical oven at 90°C for 2 hours. These solutions were reacted to produce nickel oxide precipitates. Following the precipitation, the solution was centrifuged at 3000 rpm for 30 minutes. The supernatant was then removed, and the precipitation which contains nickel oxide was obtained. Finally, cnickel oxide was grinded with mortar to be shaped into powder

Theoretical Velocities of Binary Mixtures of Di Methyl Malonate with Branched Alkanols at Different Temperatures

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Abstract

Ultrasonic velocities and densities of the binary liquid mixtures of di methyl malonate with branched alkanols like 2– methyl-1-propanol(2M1P), 2- propanol (2P), and 2- butanol (2B) have been measured in temperatures range 303.15 K to 318.15 K with an interval of 5K over the entire composition range of mole fractions. Using Nomoto's relation (U_N), impedance relation (U_I), ideal mixing relation (U_{id}), Junjie's relation (U_J), Rao's specific velocity relation (U_R) and Kudriavtsev relation (U_K), the theoretical values of ultrasonic velocity were evaluated. The computed estimations of ratio in velocity (U^2/U^2imx) from measured estimations of ultrasonic velocity (U) are graphically shown.From the values of experimental and theoretical velocities the molecular interaction parameter (α) has been evaluated and discussed its variation with the composition mixture has been conferred in terms of molecular interactions. The validity of the theories were checked by calculating standard deviation and chi square test.

Key Words: *Di Methyl Malonate, Ultrasonic velociets, Theoretical Velocities, Relative percenta ge of error, Chi-square test.*

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Design, Synthesis, and Biological Evaluation of Novel 2-(4-Arylsubstituted-1*H*-1,2,3-triazol-1-yl)-*N*-{4-[2-(thiazol-2-yl)benzo[*d*]thiazol-6-yl]phenyl}acetamide Derivatives as Potent Anticancer Agents

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Abstract—A novel series of benzothiazole bearing 1,2,3-triazole derivatives **11a**–**11j** are synthesized, and their structures are confirmed by ^{1}H and ^{13}C NMR, and mass spectral data. The synthesized compounds are tested for their anticancer activity towards human cancer cell lines including MCF-7 (breast), A549 (lung), Colo-205 (colon), and A2780 (ovarian) and demonstrate moderate to high activity. Among those, compounds **11b**, **11c**, **11d**, **11e, 11f,** and **11g** are characterized by more potent activity than the standard drug.

Keywords: NSC-710305, tazobactum, benzothiazole, 1,2,3-triazoles, anticancer activity

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INTRODUCTION

Various benzothiazole derivatives are highly recognized for their biological and pharmacological activities including anticancer [1], antiinflammtory [2], antitubercular [3], analgesic [4], antidiabetic [5], fungicidal [6], antiviral [7], and antimicrobial [8]. For example, benzothiazole containing compound NSC-710305 (Fig. 1a) demonstrated significant anticancer properties and has undergone to phase-1 clinical trial [9]. Numerous biological properties of 1,2,3-triazoles are well known and presented elsewhere. The 1,2,3-triazole unit containing antibiotic drug tazobactum (**2**) inhibited the bacterial β-lactamases [10].

Cu(I)-Catalyzed "click-chemistry" reaction is presented as one of efficient methods of synthesis of 1,2,3-trizoles via 1,3-dipolar cycloaddition of azide intermediates to alkynes [11–14].

In view of the above, we have designed and synthesized different substituted 2-(4-aryl-1*H*-1,2,3-triazol-1-yl)-*N*-{4-[2-(thiazol-2-yl)benzo[*d*]thiazol-6-yl]phenyl}acetamide derivatives **11a**–**11j** and tested their anticancer activity.

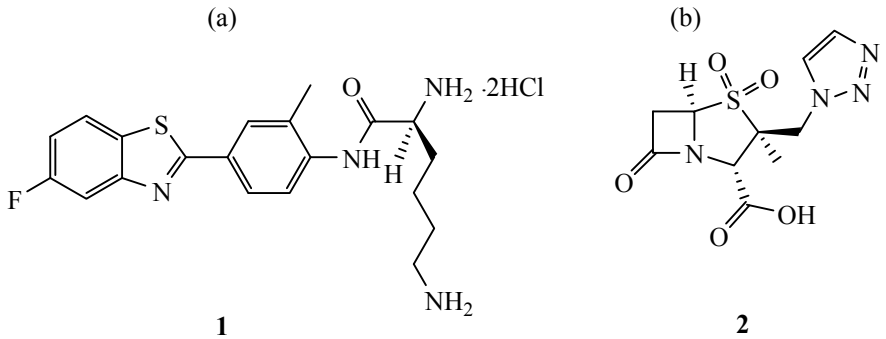
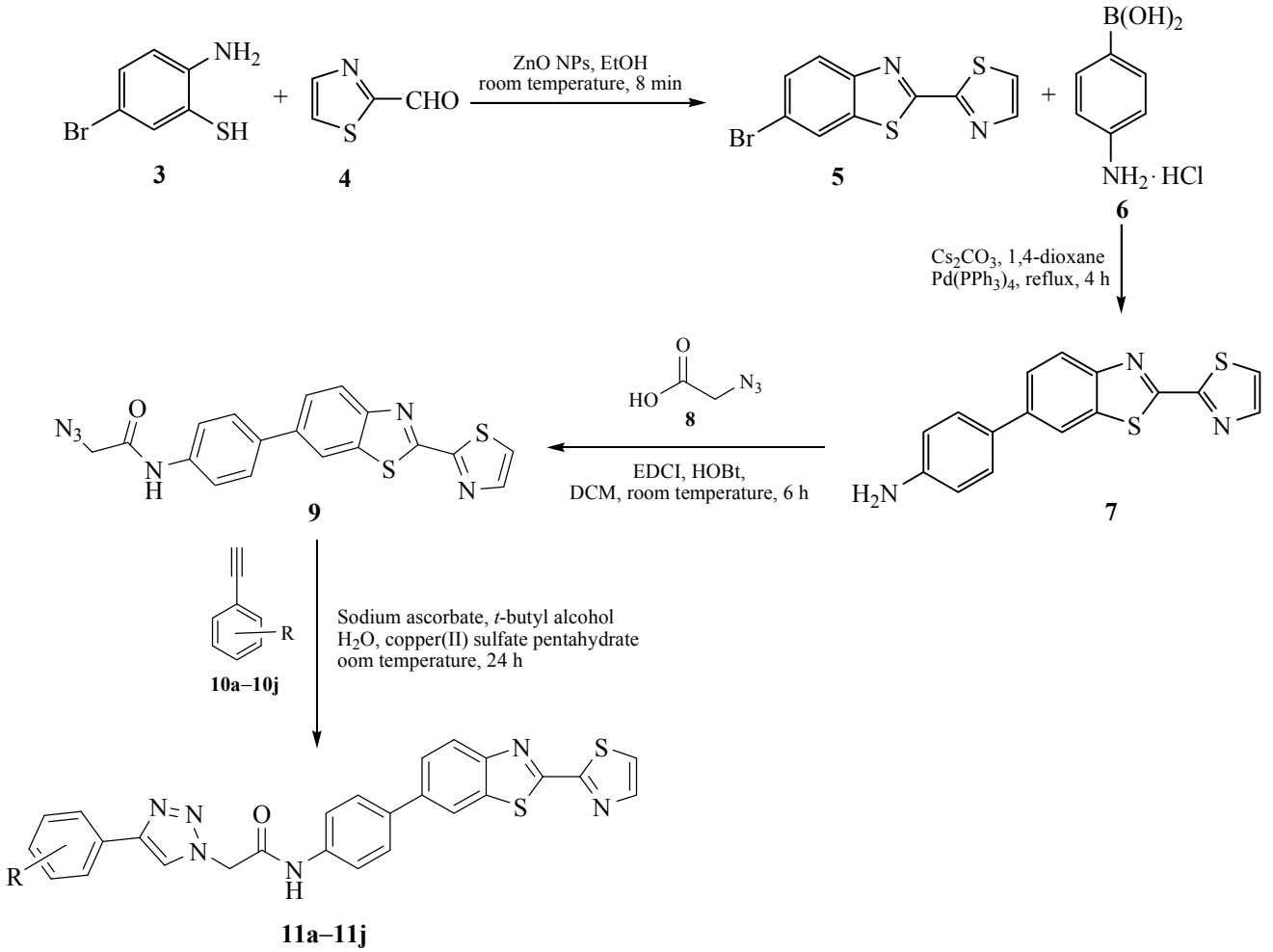


Fig. 1. Structures of (a) NSC-710305 and (b) Tazobactum.

Scheme 1. Synthesis of 2-(4-aryl substituted-1*H*-1,2,3-triazol-1-yl)-*N*-{4-[2-(thiazol-2-yl)benzo[*d*]thiazol-6-yl]phenyl}-acetamide derivatives **11a**–**11j**.



R = H (**10a, 11a**), 3,4,5-trimethoxy (**10b, 11b**), 3,5-dimethoxy (**10c, 11c**), 4-methoxy (**10d, 11d**), 4-nitro (**10e, 11e**), 3,5-dinitro (**10f, 11f**), 4-chloro (**10g, 11g**), 4-bromo (**10h, 11h**), 2,4,6-trimethyl, (**10i, 11i**), 3,5-dimethyl (**10j, 11j**).

RESULTS AND DISCUSSION

As outlined in Scheme 1, the reaction of 2-amino-5-bromobenzenethiol (**3**) with thiazole-2-carbaldehyde (**4**) in presence of ZnO-NPs led to 6-bromo-2-(thiazol-2-yl)benzo[*d*]thiazole (**5**). The intermediate **5** was introduced in the Suzuki-coupling reaction with 4-aminophenylboronic acid hydrochloride (**6**) in presence of Cs_2CO_3 and $Pd(PPh_3)_4$ catalyst. The process led to the substituted benzothiazole **7**, which was coupled with 2-azidoacetic acid (**8**) in presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI) and hydroxybenzotriazole (HOBt) with formation of triazoamide **9**. The following cycloaddition of azide **9** to substituted arylalkynes **10a**–**10j** catalysed by $Cu_2SO_4 \cdot 5H_2O$ in presence of sodium ascorbate, afforded the corresponding target compounds **11a**–**11j.**

In vitro cytotoxicity. The derivatives **11a**–**11j** were tested for their anticancer activity towards four human cancer cell lines, including MCF-7 (breast), A549 (lung), Colo-205 (colon), and A2780 (ovarian), by using MTT assay (see the table). Etoposide was used as the standard drug. All synthesized compounds **11a**–**11j** displayed moderate to high activity with IC_{50} values ranging from 0.10±0.039 to 20.5±6.23 μM against the standard drug (0.13±0.017 to 3.08±0.135 μM). According to the structure-activity relationship (SAR), the compound **11b** with 3,4,5-trimethoxy substitution on the phenyl ring demonstrated the most potent

In vitro cytotoxicity activity of target compounds **11a**–**11j**

Compounds	IC_{50}[a], μM			
	MCF-7	A549	Colo-205	A2780
11a	4.78±2.330	6.12±3.010	12.20±4.340	Not active
11b	0.10±0.039	0.15±0.037	0.18±0.034	1.22±0.620
11c	1.37±0.660	1.42±0.690	0.98±0.073	1.65±0.800
11d	2.89±1.550	1.99±0.820	1.73±0.760	2.08±1.920
11e	0.12±0.036	0.19±0.040	1.83±0.740	0.54±0.044
11f	1.29±0.770	2.00±1.480	2.10±1.580	Not active
11g	1.63±0.110	1.88±0.780	Not active	1.60±0.450
11h	13.20±5.780	9.15±4.550	8.12±4.510	3.44±1.230
11i	14.02±5.110	5.87±2.340	Not active	18.30±6.110
11j	20.50±6.230	Not active	3.99±2.090	Not active
Etoposide	2.11±0.024	3.08±0.135	0.13±0.017	1.31±0.270

[a] Each data is presents as mean ±S.D. values from the experiments performed in triplicates.

anticancer activity against all three cell lines. The compound **11c** containing 3,5-dimethoxyphenyl attached to triazole ring was characterized by lower activity than compound **11b**. Compound **11d** with 4-methoxy substituent exhibited lower activity than compounds **11b** and **11c.** Replacement of the 4-methoxy group by the electron-withdrawing 4-nitro group resulted in increased activity of compound **11e**, whereas incorporation of two electron-withdrawing groups (3,5-dinitro in **11f**) resulted in a remarkable decline in activity. The 4-chloro substituent in compound **11g** enhanced its activity.

EXPERIMENTAL

All chemicals and reagents were obtained from Aldrich (Sigma–Aldrich, St. Louis, MO, USA), Lancaster (Alfa Aesar, Johnson Matthey Company, Ward Hill, MA, USA) and used without further purification. Reactions were monitored by TLC, performed on silica gel glass plates containing 60 F-254 and visualized under UV light or by iodine indicator. ^{1}H and ^{13}C NMR spectra were measured on a Bruker, Bruker UXNMR/XWIN-NMR (400 MHz) spectrometer using DMSO-d_6 as a solvent and TMS as the internal standard. ESI spectra were measured on a Micro mass, Quattro LC using ESI+ software with capillary voltage 3.98 kV and ESI mode positive ion trap detector. Melting points were determined on an electrothermal melting point apparatus, and are uncorrected.

6-Bromo-2-(thiazol-2-yl)benzo[*d*]thiazole (5). A mixture of 2-amino-5-bromobenzenethiol (**3**) (10 g 48.9 mmol) with thiazole-2-carbaldehyde (**4**) (4.3 mL, 48.9 mmol) and ZnO NPs (362 mg, 4.44 mmol) in absolute ethanol (40 mL) was stirred at room temperature for 8 min. After completion of the reaction (TLC), the solvent was evaporated in vacuum and the crude solid product was purified by column chromatography (ethyl acetate–hexane, 1 : 1) to give pure compound **5**. Yield 93%. ^{1}H NMR spectrum, δ, ppm: 7.49 d (1H, J = 8.09 Hz), 7.68 d (1H, J = 8.10 Hz), 7.75 d (1H, J = 8.09 Hz), 7.84 d (1H, J = 8.10 Hz), 7.93 s (1H). MS (FAB): 298 $[M]^+$.

4-(2-(Thiazol-2-yl)benzo[*d*]thiazol-6-yl)benzenamine (7). To a mixture of compound **5** (13 g, 43.7 mmol) with 4-aminophenylboronic acid hydrochloride (**6**) (10.7 g, 61.8 mmol) dissolved in 1,4-dioxane (70 mL), were added $Pd(PPh_3)_4$ catalyst (504 mg, 0.437 mmol) and an aqueous solution of Cs_2CO_3 (10 mL, 28.4 g, 87.4 mmol) upon stirring. The reaction mixture was refluxed upon stirring for 4 h, then cooled down, and the solvent was evaporated under vacuum. Diethyl ether (100 mL) was added, and the organic solution was washed with brine (3× 30 mL), dried over with Na_2SO_4 and evaporated to dryness. The crude product was recrystallized from

ethyl acetate to obtained pure compound **7**. Yield 84%. ^{1}H NMR spectrum, δ, ppm: 4.89 s (2H), 7.16 d (2H, J = 7.19 Hz), 7.50 d (1H, J = 8.10 Hz), 7.60 d (2H, J = 7.19 Hz), 7.66 d (1H, J = 8.11 Hz), 7.73 d (1H, J = 8.10 Hz), 7.75 d (1H, J = 8.11 Hz), 7.80 s (1H). MS (ESI): 311 $[M + H]^+$.

2-Azido-*N*-{4-[2-(thiazol-2-yl)benzo[*d*]thiazol-6-yl]phenyl}acetamide (9). To the solution of compound **7** (10 g, 32.2 mmol) in 25 mL of anhydrous dichloromethane, were added 2-azidoacetic acid (**8**) (2.4 mL, 32.2 mmol), EDCI (7.4 g, 48.3 mmol) and HOBt (400 mg, 0.0322 mmol). The reaction mixture was stirred at room temperature for 6 h, then washed with saturated solution of $NaHCO_3$, extracted with CH_2Cl_2, and dried over anhydrous Na_2SO_4. The crude product was purified by column chromatography (ethyl acetate–hexane (6:4)) to afford the pure compound **9**. Yield 89%. ^{1}H NMR spectrum, δ, ppm: 5.37 s (2H), 7.52 d (1H, J = 8.12 Hz), 7.57 d (2H, J = 7.25 Hz), 7.67 d (1H, J = 8.13 Hz), 7.73–7.80 (m, 3H), 7.82 s (1H), 7.86 d (1H, J = 8.12 Hz), 9.86 s (1H). MS (ESI): 394 $[M + H]^+$.

Synthesis of compounds 11a–11j. Azide **9** (300 mg, 7.6 mmol) and an appropriate ethynylbenzene **10a–10j** (7.6 mmol) were dissolved in a 1:1 mixture of water with *t*-butyl alcohol (15 mL). Sodium ascorbate (225 mg, 15 mol%, 1.14 mmol) was added followed by copper (II) sulfate pentahydrate (95 mg, 5 mol %, 0.38 mmol). The mixture was stirred vigorously in darkness for 24 h. After completion of reaction *tert*-butyl alcohol was evaporated in vacuum, and the aqueous phase was extracted with ethyl acetate (3× 30 mL). The combined organic phases were washed with water and dried over Na_2SO_4. The crude product was purified by column chromatography (ethyl acetate–hexane, 1 : 1) to obtain the corresponding pure compound **11a–11j**.

2-(4-Phenyl-1*H*-1,2,3-triazol-1-yl)-*N*-{4-[2-(thiazol-2-yl)benzo[*d*]thiazol-6-yl]phenyl}acetamide (11a). Yield 56%, mp 249–251°C. ^{1}H NMR spectrum, δ, ppm: 5.60 s (2H), 7.51 d (1H, J = 8.12 Hz), 7.56 d (2H, J = 7.24 Hz), 7.61 d (2H, J = 7.24 Hz), 7.64–7.68 (m, 3H), 7.72–7.79 (m, 4H), 7.81 s (1H), 7.84 d (1H, J = 8.12 Hz), 8.23 s (1H), 10.09 s (1H). ^{13}C NMR spectrum, δ, ppm: 55.3, 114.7, 116.3, 120.4, 124.5, 125.3, 126.4, 127.2, 128.3, 129.5, 131.5, 132.8, 134.6, 143.4, 143.7, 147.5, 148.5, 149.6, 152.8, 157.4, 162.8. MS (ESI): 496 $[M + H]^+$.

2-[4-(3,4,5-Trimethoxyphenyl)-1*H*-1,2,3-triazol-1-yl]-*N*-{4-[2-(thiazol-2-yl)benzo[*d*]thiazol-6-yl]phenyl}-acetamide (11b). Yield 49.7%, mp 256–258°C. ^{1}H NMR spectrum, δ, ppm: 3.86 s (3H), 3.90 s (6H), 5.61 s (2H), 7.32 s (2H), 7.52 d (1H, J = 8.09 Hz), 7.55 d (2H, J = 7.23 Hz), 7.63 d (2H, J = 7.23 Hz), 7.67 d (1H, J = 8.14 Hz), 7.72 d (1H, J = 8.14 Hz), 7.78 s (1H), 7.82 d (1H, J = 8.09 Hz), 8.24 s (1H), 10.10 s (1H). ^{13}C NMR spectrum, δ, ppm: 55.4, 57.4, 61.9, 110.8, 114.5, 116.4, 117.8, 120.5, 124.3, 125.6, 126.7, 127.3, 131.2, 134.7, 143.5, 144.4, 145.6, 147.6, 148.5, 149.4, 152.7, 155.7, 157.5, 162.7. MS (ESI): 586 $[M + H]^+$.

2-{4-(3,5-Dimethoxyphenyl)-1*H*-1,2,3-triazol-1-yl]-*N*-{4-[2-(thiazol-2-yl)benzo[*d*]thiazol-6-yl]phenyl}-acetamide (11c). Yield 59%, mp 253–255°C. ^{1}H NMR spectrum, δ, ppm: 3.91 s (6H), 5.60 s (2H), 7.10 s (1H), 7.34 s (2H), 7.51 d (1H, J = 8.08 Hz), 7.54 d (2H, J = 7.24 Hz), 7.63 d (2H, J = 7.24 Hz), 7.66 d (1H, J = 8.13 Hz), 7.72 d (1H, J = 8.12 Hz), 7.79 s (1H), 7.82 d (1H, J = 8.08 Hz), 8.23 s (1H), 10.08 s (1H). ^{13}C NMR spectrum, δ, ppm: 55.3, 57.4, 99.5, 110.5, 114.7, 116.4, 117.5, 120.5, 124.6, 125.3, 126.7, 131.2, 132.7, 134.5, 143.4, 145.2, 147.5, 148.3, 149.4, 154.3, 157.3, 160.4, 162.9. MS (ESI): 556 $[M + H]^+$.

2-[4-(4-Methoxyphenyl)-1*H*-1,2,3-triazol-1-yl]-*N*-{4-[2-(thiazol-2-yl)benzo[*d*]thiazol-6-yl]phenyl}-acetamide (11d). Yield 59%, 240–242°C. ^{1}H NMR spectrum, δ, ppm: 3.88 s (3H), 5.61 s (2H), 7.13 d (2H, J = 7.20 Hz), 7.52 d (1H, J = 8.10 Hz), 7.55 d (2H, J = 7.22 Hz), 7.59–7.67 (m, 5H), 7.70 d (1H, J = 8.13 Hz), 7.79 s (1H), 7.83 d (1H, J = 8.10 Hz), 8.24 s (1H), 10.09 s (1H). ^{13}C NMR spectrum, δ, ppm: 55.6, 57.6, 114.7, 115.3, 116.4, 117.6, 120.6, 124.3, 125.6, 125.9, 126.3, 127.5, 131.8, 134.6, 143.2, 143.6, 147.6, 148.5, 149.3, 154.2, 156.8, 157.6, 162.8. MS (ESI): 526 $[M + H]^+$.

2-[4-(4-Nitrophenyl)-1*H*-1,2,3-triazol-1-yl]-*N*-{4-[2-(thiazol-2-yl)benzo[*d*]thiazol-6-yl]phenyl}-acetamide (11e). Yield 65%, mp 259–261°C. ^{1}H NMR spectrum, δ, ppm: 5.67 s (2H), 7.51 d (1H, J = 8.10 Hz), 7.54 d (2H, J = 7.26 Hz), 7.59 d (2H, J = 7.26 Hz), 7.61 d (2H, J = 7.28 Hz), 7.65 d (1H, J = 8.13 Hz), 7.69 d (1H, J = 8.12 Hz), 7.80 s (1H), 7.83 d (1H, J = 8.10 Hz), 8.27 s (1H), 8.32 d (2H, J = 7.28 Hz), 10.14 s (1H). ^{13}C NMR spectrum, δ, ppm: 55.7, 114.5, 116.5, 117.6, 120.6, 124.5, 125.3, 126.4, 127.5, 130.6, 131.5, 134.5, 135.6, 143.5, 144.5, 147.4, 148.5, 149.7, 150.3, 154.3, 157.8, 162.9. MS (ESI): 541 $[M + H]^+$.

2-[4-(3,5-Dinitrophenyl)-1*H*-1,2,3-triazol-1-yl]-*N*-{4-[2-(thiazol-2-yl)benzo[*d*]thiazol-6-yl]phenyl}-acetamide (11f). Yield 63%, mp 264-266°C. ^{1}H NMR spectrum, δ, ppm: 5.69 s (2H), 7.51 d (1H, J =

8.11 Hz), 7.54 d (2H, *J* = 7.27 Hz), 7.60 d (2H, *J* = 7.27 Hz), 7.65 d (1H, *J* = 8.14 Hz), 7.68 d (1H, *J* = 8.12 Hz), 7.81 s (1H), 7.83 s (1H), 8.28 s (1H), 8.36 s (2H), 8.40 s (1H), 10.15 s (1H). ^{13}C NMR spectrum, δ, ppm: 55.7, 114.7, 116.5, 117.4, 118.5, 120.6, 124.5, 125.4, 126.5, 131.4, 133.5, 134.7, 135.5, 143.5, 147.4, 148.2, 149.5, 150.3, 151.7, 154.7, 157.8, 162.9. MS (ESI): 586 $[M + H]^+$.

2-[4-(4-Chlorophenyl)-1*H*-1,2,3-triazol-1-yl]-*N*-{4-[2-(thiazol-2-yl)benzo[*d*]thiazol-6-yl]phenyl}-acetamide (11g). Yield 61%, mp 260–262°C. ^{1}H NMR spectrum, δ, ppm: 5.62 s (2H), 7.50 d (1H, *J* = 8.11 Hz), 7.55 d (2H, *J* = 7.26 Hz), 7.58 d (2H, *J* = 7.28 Hz), 7.62 d (2H, *J* = 7.26 Hz), 7.67 d (1H, *J* = 8.14 Hz), 7.71 d (1H, *J* = 8.10 Hz), 7.77 d (2H, *J* = 7.28 Hz), 7.82 s (1H), 7.84 d (1H, *J* = 8.11 Hz), 8.25 s (1H), 10.11 s (1H). ^{13}C NMR spectrum, δ, ppm: 55.6, 114.5, 116.5, 117.3, 120.7, 124.5, 125.4, 126.5, 128.6, 130.6, 131.5, 132.5, 133.4, 134.7, 143.2, 144.7, 148.3, 149.3, 149.8, 154.6, 157.9, 162.9. MS (ESI): 530 $[M + H]^+$.

2-[4-(4-Bromophenyl)-1*H*-1,2,3-triazol-1-yl]-*N*-{4-[2-(thiazol-2-yl)benzo[*d*]thiazol-6-yl]phenyl}-acetamide (11h). Yield 66%, mp 266–268°C. ^{1}H NMR spectrum, δ, ppm: 5.64 s (2H), 7.48 d (2H, *J* = 7.29 Hz), 7.53 d (1H, *J* = 8.10 Hz), 7.56 d (2H, *J* = 7.25 Hz), 7.59 d (2H, *J* = 7.29 Hz), 7.63 d (2H, *J* = 7.25 Hz), 7.67 d (1H, *J* = 8.09 Hz), 7.72 d (1H, *J* = 8.07 Hz), 7.81 s (1H), 7.84 d (1H, *J* = 8.10 Hz), 8.27 s (1H), 10.10 s (1H). ^{13}C NMR spectrum, δ, ppm: 55.7, 114.5, 116.4, 117.3, 120.5, 122.4, 124.6, 125.3, 126.5 128.6, 130.5, 131.2, 132.6, 134.4, 143.5, 144.3, 147.6, 148.4, 149.5, 154.6, 157.9, 162.8. MS (ESI): 575 $[M + H]^+$.

2-(4-Mesityl-1*H*-1,2,3-triazol-1-yl)-*N*-{4-[2-(thiazol-2-yl)benzo[*d*]thiazol-6-yl]phenyl}acetamide (11i). Yield 52%, mp 247–249°C. ^{1}H NMR spectrum, δ, ppm: 2.38 s (3H), 2.41 s (6H), 5.49 s (2H), 7.16 s (2H), 7.50 d (1H, *J* = 8.09 Hz), 7.55 d (2H, *J* = 7.25 Hz), 7.60 d (2H, *J* = 7.25 Hz), 7.66 d (1H, *J* = 8.13 Hz), 7.70 d (1H, *J* = 8.12 Hz), 7.79 s (1H), 7.83 d (1H, *J* = 8.09 Hz), 8.22 s (1H), 10.09 s (1H). ^{13}C NMR spectrum, δ, ppm: 23.5, 24.8, 55.6, 114.5, 116.4, 117.3, 120.5, 124.5, 125.6, 126.3, 129.6, 130.2, 131.3, 134.5, 136.4, 138.6, 143.2, 144.5, 147.6, 148.6, 149.7, 154.6, 157.8, 162.7. MS (ESI): 538 $[M + H]^+$.

2-[4-(3,5-Dimethylphenyl)-1*H*-1,2,3-triazol-1-yl]-*N*-{4-[2-(thiazol-2-yl)benzo[*d*]thiazol-6-yl]phenyl}-acetamide (11j). Yield 54%, mp 262–264°C. ^{1}H NMR spectrum, δ, ppm: 2.37 s (6H), 5.46 s (2H), 7.10 s (2H), 7.17 s (1H), 7.50 d (1H, *J* = 8.08 Hz), 7.53 d (2H, *J* = 7.23 Hz), 7.57 d (2H, *J* = 7.23 Hz), 7.63 d (1H, *J* = 8.11 Hz), 7.69 d (1H, *J* = 8.10 Hz), 7.78 s (1H), 7.83 d (1H, *J* = 8.08 Hz), 8.23 s (1H), 10.09 s (1H). ^{13}C NMR spectrum, δ, ppm: 23.7, 55.7, 114.7, 116.4, 117.5, 120.6, 124.5, 125.4, 126.5, 129.6, 131.3, 132.6, 134.5, 135.8, 140.5, 143.3, 146.8, 147.4, 148.4, 149.7, 154.6, 157.8, 162.6. MS (ESI): 524 $[M + H]^+$.

MTT assay. Individual wells of a 96-well tissue culture micro titre plate were inoculated with 100 μL of complete medium containing 1×10^4 cells. The plates were incubated at 37°C in a humidified 5% CO_2 incubator for 18 h prior to the experiment. After medium removal, 100 μL of fresh medium containing the test compounds and etoposide (Eto) at different concentrations (0.5, 1, and 2 μM) were added to each well and incubated at 37°C for 24 h. Then the medium was discarded and replaced with 10 μL MTT dye. Plates were incubated at 37°C for 2 h. The resulting formazan crystals were solubilized in 100 μL extraction buffer. The optical density was recorded at 570 nm with a micro plate reader (Multi-mode Varioskan Instrument-Themo Scientific). The percentage of DMSO in the medium never exceeded 0.25%.

CONCLUSIONS

Ten novel 2-(4-arylsubstituted-1*H*-1,2,3-triazol-1-yl)-*N*-{4-[2-(thiazol-2-yl)benzo[*d*]thiazol-6-yl]phenyl}-acetamides **11a**–**11j** are synthesized. Their structures are confirmed by ^{1}H and ^{13}C NMR and mass spectral data. The products are tested for their anticancer activity towards human cancer cell lines including MCF-7 (breast), A549 (lung), Colo-205 (colon), and A2780 (ovarian). The synthesized compounds **11b, 11c, 11d, 11e**, **11f**, and **11g** demonstrate activity higher than the standard drug.

CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

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Green Synthesis Of ZnO nanoparticles using Milletia Pinnata Leaf extract and their Characterization

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Abstract: ***During the last decade, arrays of exploratory experiments conducted to gauge the solid impact of nanotechnology have comparatively proved its effeciency. Global emphasis is given to the importance of biological synthesis. Biomolecules present in plant extracts can be used to reduce metal ions to nanoparticles in a single-step green synthesis process. This biogenic reduction of metal-ion to base metal is quite rapid,readily conducted at room temperature and pressure and easily scaled up. The involved reducingg agents include the various water soluble metabolites (eg.,alkaloids,phenolic compounds,terpenoids) and co-synthesis. ZincOxide(ZnO) Nanoparticles have the particular focus of plant based synthesis. Extracts of a diverse range of milletia Pinnata have been successfully used in making ZnO nanoparticles. The formation and stability of the reduction ZnO nanoparticles in the colloidal solution were monitored by UV-Vis spectrophotometer analysis. The mean particle diameter of ZnO nanoparticles was calculated from XRD pattern. FT-IR spectra of the leaf extract after the development of nanoparticles are determined to allow identification of possible functional groups, responsible or the conversion of metal ions to metal nanoparticles. In this paper we reported the synthesis of Milletia pinnata mediated ZnO nanoparticles and their characterization.***

KEYWORDS: Green synthesis, nanoparticles, leaf extract, Milletia pinnata, characterization.

INTRODUCTION:

Over the past decade the green nanoscience and nanotechnology is a sprouting interdisciplinary field of research, interpressing material science, bio nanoscience and technology. Remarkable advances are made in the field of biotechnology and nanotechnology to harness the benefit of lifesciences(1), health care and industrial biotechnology(2,3). Nanoparticles may provide solutions to technological and environmental challenges in the area of solar energy conversion, Catalysis, medicine and water treatment(4-7). Thus there has been increasing interest in the development of clean synthetic procedure" Green Chemistry" for nanoproducts targeted as potential applications in the fields of catalysis in chemical reactions, drug delivery in medical, bio-labelling, microelectronic, informationstorage and optoelectronic devices(8-13). Nanocrystalline ZnO nanoparticles have been found tremendous applications in the fields of high sensitivity biomolecular detection, diagnostics, antimicrobials, therapeutics, catalysis and micro-electronics(13). Group of researchers develop ZnO nanoparticles being extensively synthesized using various plant leaf extract such as camellia sinensis, magnolia kobus and diopyros kaki leaf, geranium leaf, acalypha india leaf, coriandrum sativum, sorbus aaucuparia leaf, gliricidia sepium, rose leaf, cinnamomum camphora, aloevera and neem(8-19).

For environmental concerns , there is a need to develop benign nanoparticles using non toxic chemicals in the synthesis, protocols are to avoid adverse effect in medicinal applications. At present several groups of researchers concentrated on biomimetic approaches such as plant or plant extracts, microorganisms and yeast to synthesize the metal nanoparticles called as "green chemical or phyto chemical" approaches (7). The Milletia pinnata is a traditional mediciine in the treatment and prevention of several kinds of ailments in many countries such as for treatment of piles, skin diseases and wounds .In the present paper the synthesis and characterization of ZnO nanoparticles from Milletia pinnata leaf extract is discussed.

MATERIALS AND METHOD:

1. Preparation Of *MILLETIA PINNATA* Leaf Extract

Initially Indian Beech tree leaves (Milletia pinnata) leaves are cleaned several times using distilled water to remove the dust particles and residual moisture. 20g of thoroughly washed finely cut milletia pinnata leaves in 500ml Erlenmeyer flask along with 100ml of distilled water and then boiling the mixture at 70^0C until it turns into brownish colour. Further, the extract was filtered with whatman no:1 filter paper and extracted solution used for further process.

2. Synthesis Of ZnO Nanoparticles

0.2 grams of Zinc Nitrate is taken and dissolved in 20 ml distilled water And 5 ml of indian Beech tree Leaves solution is added to the zinc nitrate solution. Both solutions are mixed using magnetic Stirrer at room temp for 30 minutes And the solution is Centrifuged at 300 rpm. The sample is placed in micro oven at 80^0c for duration of 20 hours. The precipitate is washed for several times using distilled water and it is filtered using nano filter paper. Finally Zinc oxide nano powder is obtained and used for further process.

RESULTS AND DISCUSSIONS:

X-ray Diffraction Analysis

X-ray diffraction is an analytical technique generally used for phase identification of a crystalline material and can provide information on a unit cell dimensions as well. X-ray diffraction is now a common technique for studying crystal structures and atomic spacing. Although single crystal X-ray crystallographic investigation is the most precise source of information regarding the structure of a complex, the difficulty of obtaining crystalline complexes renders this method unsuitable for that study. However, a variety of other spectroscopic techniques could be used with good effect for characterizing the metal complexes in X-ray powder diffraction. The, X-ray powder diffraction (XRD) measurements of three complexes are performed. The diffractogram obtained complexes has been given in figures and the observed diffraction data, with the help of the data obtained from the powder XRD, the particle size calculations are performed using Scherrer equation.

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PERFORMANCE EVALUATION OF THE KRISHNA DISTRICT CO-OPERATIVE CENTRAL BANK LTD., KRISHNA DISTRICT OF ANDHRA PRADESH

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Abstract

Co-operative banks are often created by persons belonging to the same local or professional community or sharing a common interest. Cooperative banks generally provide their members with a wide range of banking and financial services (loans, deposits, banking accounts etc.). Co-operative banks differ from stockholder banks by their organization, their goals, their values and their governance. Present paper attempts to Performance Evaluation of The Krishna District Cooperative Central Bank Ltd., through selective indicators, it analyses the Deposits, Credits, and C/D Ratios.

Keywords: Deposits, Capital, Borrowings, Credit, Investment.

Introduction

The Krishna District Co-operative Central Bank Ltd., (Krishna DCCB) started functioning with Machilipatnam as Head Quarters and regional office at vijayawada. Krishna DCCB is the biggest DCCB in the State of A.P. not only in terms of no. of Branches (55 Brs.), no. of PACS (425 PACS after restructuring). (As on 31.03.2018)

Objective of the Study

- To evaluate the growth of Capital, Reserves and Borrowings of the Krishna DCCB Ltd.
- To analyse the Deposits, Credits and C/D Ratios of the Krishna DCCB Ltd.
- To examine the growth of investment by The Krishna DCCB Ltd.
- To understand the profitability position in The Krishna DCCB Ltd.

Methodology of the Study

The study is mainly based on secondary data, which has been gathered from annual reports of The Krishna DCCB Ltd., mainly from the last eight years reports. The secondary data is also collected from NABARD, RBI bulletins, Government of India reports and online sources like apcob.org, krishnadccb.com etc.

Sample of the Study

The present study is mainly based on secondary sources drawn from National Federation of State Cooperative Banks Ltd (NFSCOB) reports, and other web sites, papers, books and journals relating to Co-operative banking sector.

Data in Tools

Data were collected for period of ten years from 2008-09 to 2017-18. For analysis of

the data, various statistical tools (Mean, S.D, C.V, Trend analysis) has been used to arrive at conclusion in a scientific way

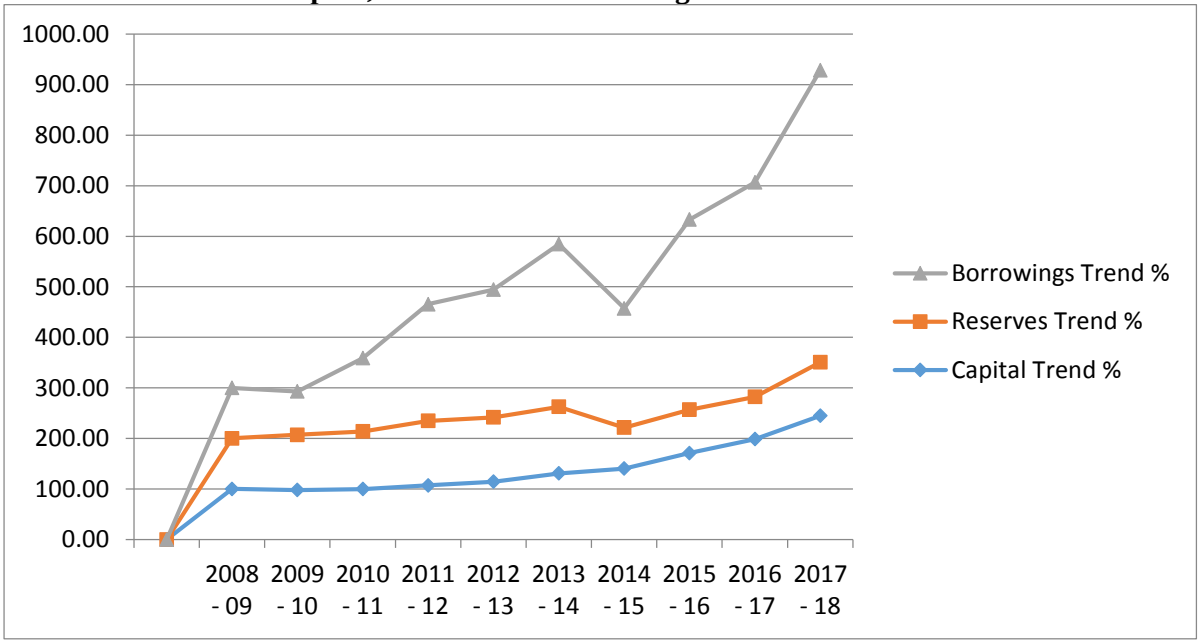
Analysis of the Study

Trend of Capital, Reserves and Borrowings of the Krishna DCCB LTD. *(Rs. In lakhs)*

Year	Capital		Reserves		Borrowings	
	Amount	Trend %	Amount	Trend %	Amount	Trend %
2008 - 09	5684	100	12886	100	26211	100
2009 - 10	5565	97.91	14058	109.10	22517	85.91
2010 - 11	5667	99.70	14714	114.19	37981	144.90
2011 - 12	6089	107.13	16454	127.69	60536	230.96
2012 - 13	6491	114.20	16405	127.31	66336	253.08
2013 - 14	7429	130.70	16986	131.82	84411	322.04
2014 - 15	7964	140.11	10480	81.33	61870	236.05
2015 - 16	9716	170.94	11072	85.92	98676	376.47
2016 - 17	11292	198.66	10777	83.63	111247	424.43
2017 - 18	13918	244.86	13609	105.61	151524	578.09
Mean	7981.50	140.42	13744.10	106.66	72130.90	275.19
S.D	2824.37	49.69	2434.15	18.89	40412.43	154.18
C.V	35.39	35.39	17.71	17.71	56.03	56.03

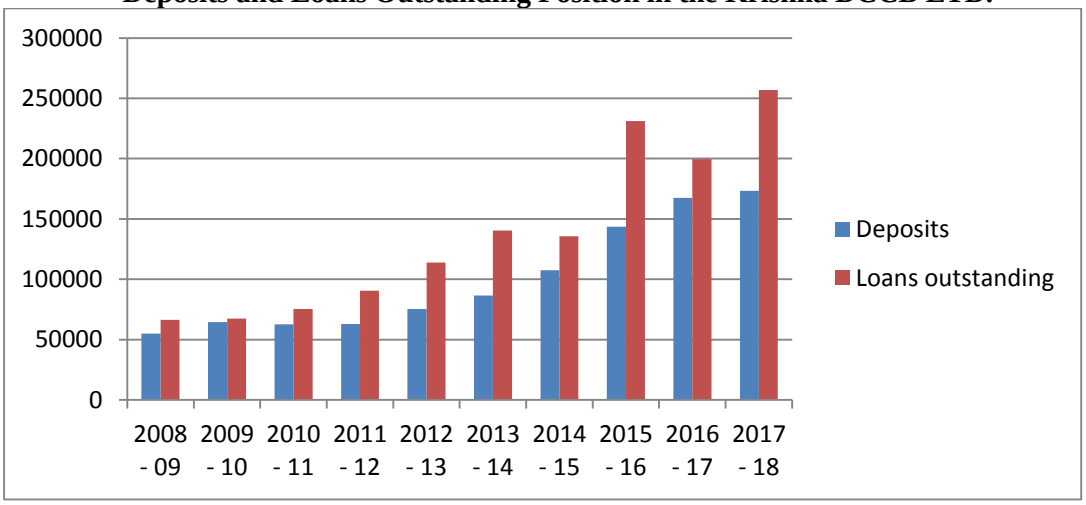
Source: www.krishnadccb.com

Trend of Capital, Reserves and Borrowings of the Krishna DCCB LTD.

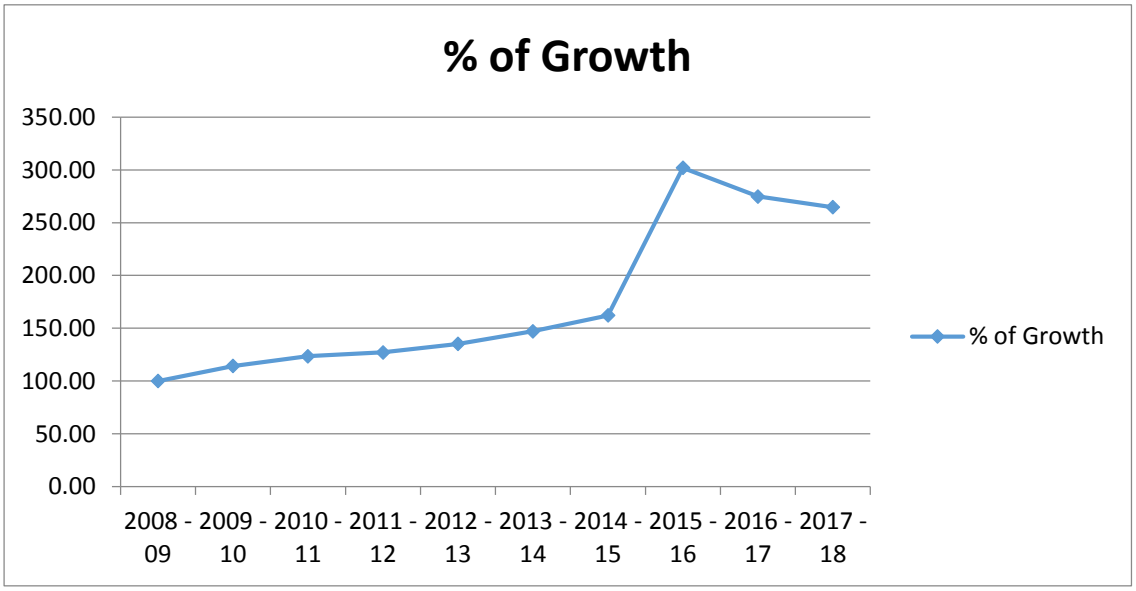


The above table 1analysed the funds of The Krishna DCCB Ltd. and their trend in terms of Capital, Reserves and Borrowings. The amount of capital is 5,684 lakhs in 2008-09, it has been gradually increased (except in the year 2009-10) and reached 13,918 lakhs in 2017-18, with a percentage of 144.86 (244.86 -100). In case of reserves, they are 12,886 lakhs in 2008-09 where as in 2017-18 it was recorded 13,609 lakhs with 5.61% (105.61-100) growth trend. The borrowings of The Krishna DCCB Ltd. during the year 2008-09 are 26,211 lakhs, it has been fluctuating and finally the borrowings are recorded 1,51,524 lakhs in the year 2017-18 with 478.09 % (578.09-100) growth. The average growth of Capital, Reserves and Borrowings of The Krishna DCCB Ltd. is 140.42%, 106.66% and 275.19% respectively. The S.D of capital is 49.69%, Reserves are 18.89%, and borrowings are 154.18%. When compare to the Standard deviation of capital, reserves and borrowings, Reserves have more consistent than Capital and borrowings.

Deposits and Loans Outstanding Position in the Krishna DCCB LTD.



The above table 2 has been depicted that during the year 2008-09 the Deposits are registered Rs.54, 972 lakhs it has been increased to Rs.1, 73,214 lakhs in 2017-18. The mobilization of deposits has been increased gradually during the study period, and the average deposits mobilized by bank is 99,902.70. Whereas Loans Outstanding of Krishna DCCBs is just Rs.66,343 lakhs in 2008-09, it has been increased to 4 times with an amount of 2,56,783 lakhs in the year 2017-08. The average credit issued by the banks is Rs.1,37,707.60 lakhs. The Credit Deposit Ratio of The Krishna DCCB Ltd. from 2007-08 to 2017-18 is shown a fluctuating trend. The highest C/D Ratio of the banks estimated with 162.29% in 2013-14 and the lowest C/D Ratio 104.22% is recorded in the year 2009-10. The average C/D ratio during the study period 135.70%, with a variance of 14.81%. The S.D is 20.10 which is less it indicates high degree of uniformity of observations as well as homogeneity of the series.

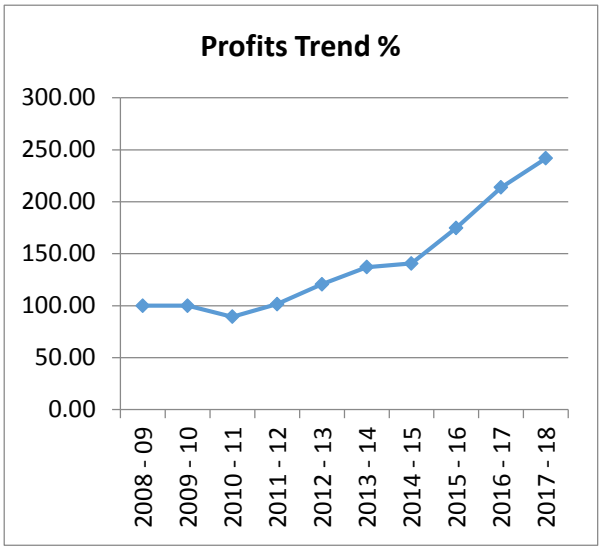


Growth of Investments by the Krishna DCCB LTD.

The table-3 depicted growth pattern of investment by The Krishna DCCB Ltd., the investment in 2008-09 is Rs. 32,614 lakhs and it has been increased gradually year by year and recorded Rs. 86,317 lakhs in the year 2017-18 with a growth rate 164.66 (264.66-100). The average investment of the bank is 57,095.40 lakhs during the study period. The Standard Deviation is 24,529.80 lakhs and the C.V is 42.96 which is less, it indicates high degree of

uniformity of observations as well as homogeneity of the series.

Trends of Profits in the Krishna DCCB LTD.



The table-4 revealed the growth pattern of profits in The Krishna DCCB Ltd., the profit in 2008-09 is Rs. 582 lakhs and it has suddenly increased to Rs. 1407 lakhs in 2017-18, with a growth rate 141.75 (241.75-100), The average profit of the bank is Rs. 826.10 lakhs during the study period. The Standard Deviation is 304.22 lakhs and the C.V is 36.83 which is higher it indicates low degree of homogeneity as well as heterogeneity of the series.

Suggestions and Recommendations

The Krishna DCCB Ltd. should also provide loans such as education loans, vehicle loans on par with commercial banks.

- The Bank should start deposit schemes such as kiddy banks, Weekly saving schemes, daily saving schemes, Women Deposit schemes, Student deposit schemes, Marriage deposit schemes, Pension deposit schemes, Reinvestment deposit scheme
- The Bank should try to upgrade technology. It should adopt the modern methods of banking like internet banking, credit cards, ATM, etc.

Conclusion

The DCCBs have been showing maximum growth in investment. It is suggested that government should formulate specific policies and they should be implemented for the upliftment of the Krishna District Central Cooperative Banks. DCCBs should try to upgrade technology and should formulate customer friendly policies to face competition with commercial banks.Finally profits of the Krishna DCCB Ltd., has been increased almost three and half times during the study period.

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AN EMPIRICAL STUDY OF DEMONETIZATION IMPACT ON RURAL PUBLIC

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ABSTRACT

Demonetization technically is a liquidity shock, a sudden stop in terms of currency availability. The term demonetization is not new to the Indian economy. The highest Bank notes ₹1,000 and ₹10,000 demonetized in 1946 and ₹1,000, ₹5,000, and ₹10,000 were demonetized in 1978. Recently on 8 November 2016, India's Prime Minister Narendra Modi announced the Government of India's decision to cancel the legal tender character of ₹500 and ₹1,000 banknotes with effect from 9 November 2016. He also announced the issuance of new ₹500 and ₹2,000 banknotes in exchange for the old banknotes up to 30 December 2016. Demonetization affects the economy through the liquidity side. Its effect will be a telling one because nearly 86% of currency value in circulation was withdrawn without replacing bulk of it. Demonetization may have had considerable negative effect in the first few days but the situation now has improved and it will have a positive impact on the economy. The demonetization of the highest denomination currency notes is part of several measures undertaken by the government to address tax evasion, bogus currency and funding of illegal activities by encouraging cashless transactions. It would help combat black money and reduce corruption, while having a positive result on the economic front. Demonetization has significant and immediate impact on the state of the Indian economy. The present study relates to Krishna District of Andhra Pradesh. The main object of this research paper is to measure the Impact of Demonetization on Rural Public. The researcher has collected data through primary sources and secondary sources as well.

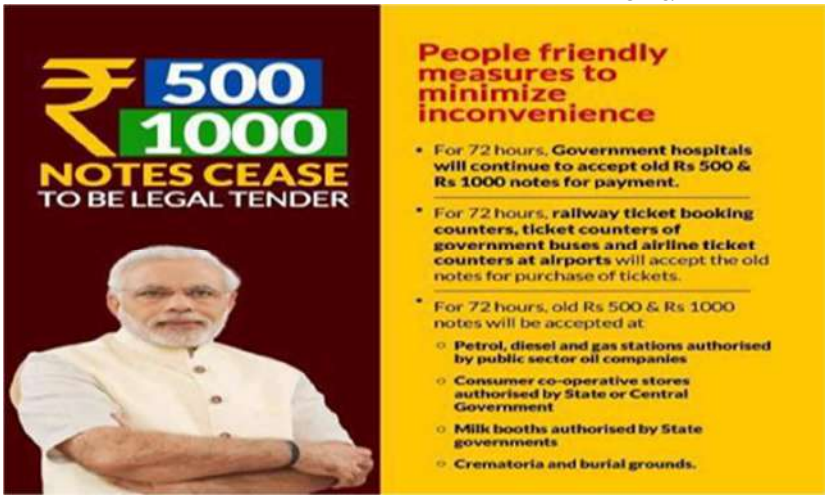
KEYWORDS

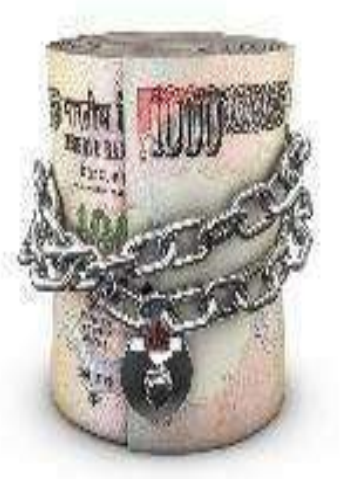
demonetization, black money, corruption, tax evasion and cashless transactions.

INTRODUCTION

The government has implemented a major change in the economic environment by demonetizing the high value currency notes – of Rs 500 and Rs 1000 denomination. These ceased to be legal tender from the midnight of 8th of November 2016. People have been given up to December 30, 2016 to exchange the notes held by them. The proposal by the government involves the elimination of these existing notes from circulation and a gradual replacement with a new set of notes. In the short term, it is intended that the cash in circulation would be substantially squeezed since there are limits placed on the amount that individuals can withdraw. In the months to come, this squeeze may be relaxed somewhat. The reasons offered for demonetization are two-fold: one, to control counterfeit notes that could be contributing to terrorism, in other words a national security concern and second, to undermine or eliminate the 'Black Economy'. 86% of India's currency was nullified in a great demonetization effort that aimed to clean out the black market's cash supply and counterfeit notes which completely disrupted the social, political, and economic spheres of the world's second largest emerging market.

FIG. 1 & 2





CAUSES FOR DEMONETIZATION

The common public and bankers are undoubtedly in hassles in the present scenario. Now the question arises that why was demonetization required to be done at this point of time. Here are certain points to clarify on the need of demonetization of currency:

- To Introduce New Currency in to the country.
- To develop a good banking system
- The Fake currency in the economy.
- To Block the inflow of fake currency notes and their use for criminal activities including terrorist activities.
- To Eliminate Black Money, Currency Storage, Corruption and Tax Evasion etc.,
- Destabilizing election campaigns being done through black money.
- Destroying hoardings of public money by few influential people.

HISTORY OF DEMONETIZATION IN INDIA?

This is not the first time when Indian currency is demonetized in India. The first instance was in 1946 and the second in 1978 when an ordinance was circulated to phase out notes with denomination of Rs. 1,000, Rs. 5,000 and Rs. 10,000. The highest denomination note ever printed by the Reserve Bank of India was the Rs. 10,000 note in 1938 and again in 1954. But these notes were demonetized in January 1946 and again in January 1978. Higher denomination banknotes of Rs. 1,000, Rs. 5,000 and Rs. 10,000 were reintroduced in 1954 and all of them were demonetized in January 1978. The Rs. 1,000 note made a comeback in November 2000. Rs 500 note came into circulation in October 1987. The move was then justified as attempt to contain the volume of banknotes in circulation due to inflation. However, this is the first time that Rs. 2,000 currency note is being introduced.

THE POSITIVE IMPACT OF DEMONETIZATION

- ✓ What happens, when people go to bank and deposit their money? Deposits in banks will increase and this eventually makes the interest rates come down.
- ✓ Curb on black money, corruption, terrorism etc.
- ✓ Boost to cashless economy with people adopting the habits of using Debit cards, Credit cards, Net banking, Mobile banking and Wallets etc.
- ✓ Political related activities, real estate business, etc., will be fair and transparent.
- ✓ A trap on tax evaders will bring good revenues to Government.

THE NEGATIVE IMPACT OF DEMONETIZATION

- Withdrawal of currency in circulation, in the short-run, might actually drive up interest rates.
- Very Short-term liquidity squeeze could be severe and hence economic activity could suffer.
- There is short-term impact on economic activity which reduces government revenues and widens deficit.
- Cost of printing the new currency would create another problem.
- Immediate confusion and public disorder.

CATEGORIES EFFECTED BY DEMONETIZATION

The following are various categories affected by Demonetization,

- Agriculture sector
- Real Estate and Construction
- Automobiles and Auto Ancillary
- Mobile phones and pc sellers
- Juices/fruit drinks sale
- Durable goods sellers
- Cigarettes sellers
- Petty vendors
- Hair saloons
- Kirana Shops
- Employees
- Women
- Students

OBJECTIVES OF THE STUDY

To study and analyses the demonetization post- impact on rural public in Krishna District.

- ➢ To know the problems and challenges faced by the rural public due to demonetization.
- ➢ To know the effect of demonetization on banking transactions.
- ➢ To know the interest of the public to move for cashless transactions instead of using paper currency.

METHODOLOGY

The research paper is analytical in nature. The research is mainly based on primary data as well as secondary data. The sample size is 120 from various rural households in Krishna District of Andhra Pradesh. Questionnaire was canvased on selected respondents using Convenience Sampling Method.

FINDINGS OF THE STUDY

- ❖ 90% of the respondents told that the new currency note i.e., Rs. 2000 is very inconvenient to them.
- ❖ Out of the total respondents, about 75% were effected by demonetization
- ❖ Out of the total respondents, about 95% are having bank accounts and 87.5% have ATM cards. Of them only 52.5% availing ATMs for their payments.
- ❖ Of the total sample, only 15% of the respondents are using e-banking for their payments and 65% of the respondents are showing interest to use e-banking for their future payments.
- ❖ 50% of the respondents are dissatisfied with the continuation of cash withdrawal limits from their bank accounts.
- ❖ Respondents had faced tough situation to get lower denomination currency for Rs. 500 and 2000 notes particularly the rural mass and illiterate labour.
- ❖ Liberalizing the limits on cash withdrawals and steady availability of cash at Banks & ATMs slowly relieving the rural people from the negative impact of demonetization at micro level.

PICTURE 3

RECOMMENDATIONS & SUGGESTIONS

- ✓ Immediate action is needed for the installation of POS machines at all retail outlets, like Kiraana Shops, Medical stores, Oil bunks, PDS dealers ect.,
- ✓ Awareness programs on cashless transactions to be continued.
- ✓ Awareness programs on the use of Debit cards, Credit cards, Smart cards, e-Banking, Mobile Banking, Paytms/ wallets etc. in rural areas to be undertaken.
- ✓ Monitoring the progress of cashless transactions
- ✓ Encouraging the public to go for cashless transactions by offering monetary incentives, like discounts.
- ✓ Implementation of Aadhaar enabled e-Payment system
- ✓ Elimination of service charges on cashless transactions.

PICTURE 4

CONCLUSION

The study observes that the demonetization effect on public initially was painful but has led the sellers and buyers to adopt cashless means such as paytm, debit & credit cards use, online banking to buy goods. By switching over to cashless means, economy will reap its fruits in due course of time. Of course Indian consumers particularly the rural and illiterates need to learn the means of going to cashless transactions. By adopting the cashless means, certainly there will be a definite check on black money and the tax evaders.

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