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Morphological characterization, propagation strategy and identification of Boucerosia frerei accessions for their hanging basket exploitation- an attractive succulent with star-shaped flowers from the Northern Western Ghats of India

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Abstract

Boucerosia frerei (G. D. Rowley) Mieve & Liede (formerly *Frerea indica*; family-Apocynaceae) is one of the little known, endemic and critically endangered succulent herbs with pretty flowers distributed sparsely in the Northern Western Ghats of India. Naturally, the plant shows phenotypic variation in numerous qualitative and quantitative characters especially stem, leaves and flowers. Consequently, this is challenging and stimulating taxa that merit considerable further attention of taxonomists, horticulturalist, gardeners, plant enthusiast etc. The present investigation describes the first report of collection, phenotypic characterization, commercially practicable propagation strategy development and identification of the accession for their usage in hanging baskets for *B. frerei.* The results highlighted some of the important and interesting features of this plant. A total of 32 morphological traits (9 qualitative and 23 quantitative) were noted for five accessions collected from Kate's Point, Mahabaleshwar (KP), Meruling, Satara (MS), Sajjangad, Satara (SS), Purandar, Pune (PP) and YCIS, Satara (YCIS) and evaluated using ANOVA, PCA and cluster analysis. Ten morphological traits *viz*. plant height, stem color, number of nodes, number of roots, number of leaves, number of flowers, size, shape, architecture and seed weight contributed to the variation between the accessions. For succulents to be used for hanging baskets, plant height, number of leaves and floral and floral attributes (number, color, size, shape and architecture) are among the most important selection traits. Morphological characterization identified the Meruling, Satara (MS) as the potential accession for hanging baskets. A combination of soil, coconut coir and vermiculite (3:1:1) escalated the phenotypic traits in the aerial parts in hanging basket cultivation. Among the propagation techniques employed, vegetative propagation served superior over tissue-culture technique for large-scale propagation for its conservation and availing the stock material to floriculture industry.

1. Introduction

Ornamental plants illustrate natural living prettiness that contributes immensely to the well-being of mankind and constitutes a principal profit-making group in the horticulture industry (Barrera Necha and Bautista-Banos 2016). Unequivocally, wild ornamental plants contributed significantly towards the transformation of horticultural industry since last decade. Now a day, diverse promising ornamental plants from the natural origin are being extensively used in professional landscaping, home gardening as well as cut flowers (Heywood 2003; Malhotra and Ram 2017). Due to such rising needs, numerous wild flowering plants have been domesticated as potential ornamentals. However, identification of elite clones having ornamental attributes in wild plants is a challenging task. For overcoming such issues, in recent time the scientists across the globe have concentrated on strengthening of quality attributes for floral industry (Noman et al. 2017; Parisi et al. 2016).

The reputation of succulents as ornate plant has been raised due to the unique geometric shapes, coloring patterns, flower architecture together with its high level moisture retaining ability (Cabahug et al. 2018). The genus *Boucerosia* Wight & Arn (Apocynaceae) is distributed with seven species in the Indian Subcontinent. *Boucerosia frerei*, described as *Frerea indica* by Nicol A. Dalzell in 1865 is now included under the genus *Boucerosia* (Meve and Liede 2002). The succulent grows naturally in the Northern Western Ghats of India is endemic to the narrow pockets in the state of Maharashtra (Fig. 1). According to ICN this plant species is among the world's 12 most endangered species and was included in Appendix II of the Convention of International Trade in endangered species of wild fauna and flora (CITES) (Mishra and Arya 2010). *B. frerei* is a perennial herb bearing fleshy and succulent stem with persistent leaves which grows naturally in remote places especially on rock crevices and exposed hill slopes (Fig. 2A-B). Though *B. frerei* has limited occurrence, it gained attention from botanical enthusiast, taxonomists, horticulturalist and gardeners due to its diverse ornamental attributes especially good-looking flowers, succulent stem, leaves and follicles (Fig. 2B-D). The plant has extraordinary ability to hold out against drought by detaching the leaves and further lowering transpiration area by developing the thick succulent knots on stem with silver grey outer surface for sunlight deflection (Fig. 2E). Naturally occurring variations within individuals as well as populations enforced the *B. frerei* in horticultural implementation.

However, the species is facing hurdles for survival in nature due to its indiscriminate collection as stem and leaf vegetable (Jadhav et al. 2015), destruction of its natural habitat, complex pollinating mechanism, lack of specific pollinators and inadequate propagation approaches.

Despite the curious flowers, succulent nature and existence of morphological variances, the efforts to collect and characterize these variations for horticultural implications have not yet been done for *B. frerei*. It is a pretty succulent with star shaped flowers and could make a good indoor plant especially used as potting plant and as hanging basket. To the authors' knowledge, no selections have been identified for this purpose. To amplify the diversity and horticultural use in the nursery trade, the collection, characterization and evaluation of *B. frerei* materials is very much essential. Hence, this study is concentrated on (1) characterization of five accessions collected from the state of Maharashtra, *viz*. Kate's Point (Mahabaleshwar), Meruling (Satara), Sajjangad (Satara), Purandar (Pune) and YCIS (Satara), (2) identification of accessions suitable for hanging basket use, (3), development of effective propagation strategy and (4) optimization of hanging basket requirements.

2. Materials And Methods

2.1. Collection of plant material

The five accessions of *B. frerei* were sampled over a 360 km^2 range between latitudes 17°65' to 18°27' N and 73°90' to 74°01' E from the Northern Western Ghats region of India. Altitude varied from 703 to 1376 metre above sea level. At random, we sampled 10 individuals from each five accessions (Table 1; Fig. 2F).

2.2. Establishment of stock plantlets in garden conditions

The study was performed from July, 2018 to November, 2018 at Yashavantrao Chavan Institute of Science (YCIS), Satara, India (lat: 17.685066, long: − 74.010591, elevation: 691 m above sea level). Stem cuttings (with 6–8 nodes) from four natural localities from known provenance in the Northern Western Ghats as well as only *in situ* source from YCIS, Satara with no available records of its origin were studied (Table 1). Stock plants of these accessions were established by rooting cuttings with IAA (100 ppm) treatment for 12 hrs. The treated cuttings were inserted vertically in 15 cm plastic pots filled with coconut coir. The stem cuttings were allowed for rooting in a mist chamber for 30 days where misting was done after every 60 minutes. The rooted plantlets were transferred vertically in large size pots (20 cm) filled with garden soil, coconut coir and leaf manure (1:2:1) and maintained in green house for 30 days. Then the plants were exposed under full sunlight and watered twice a day for a min through sprinkler irrigation for next 15 days. Each pot received approximately 200 ml of water daily. Plants were held for another month under the same outdoor conditions.

2.3. Selection of morphological traits and their assessment

The morphological characterization was carried out in the month of September, 2018. Observations on 32 selected morphological traits comprising 9 qualitative and 23 quantitative traits were scored for each plant of each accession. Few amongst the quantitative morphological traits *viz*. plant height, number of branches, leaves, flowers, pod/follicle, seed, length and diameter of follicles were considered from the descriptor developed for closely allied *Caralluma edulis* (Singh et al. 2016), however in this study several new traits have been considered for creating a distinguished and complete descriptor for *B. frerei*. Ten plants were randomly selected from each accession and studied for various morphological traits as mentioned in Table 2 and Table 3. Qualitative traits recorded for each accession were leaf shape, apex, stalk, stem shape, colour, flower colour, architecture, shape and hairs on corolla edges (Table 3). Distribution of hairs on corona edges of the five accessions was categorized as sparse, medium or dense. Fifth edition of Royal Horticultural Society colour chart (2007) was considered for determination of flower and stem color.

Aside from qualitative characters, twenty-three quantitative characters were also measured for each five accessions. Average plant height, number of roots, branches, nodes, leaves, flowers and follicles were measured for 10 plants from each accession. Stem diameter and length of internodes were obtained individually measuring all the internodes of 10 mature stems of each plant from each accession. Average leaf length, width, petiole diameter and length were measured from 10 mature leaves of each plant in each accession. Average flower bud size, flower diameter, pedicel length was recorded by randomly selecting 10 bud/flowers from each accessions. The average number of flower buds and average number of days for flower opening for each plant in each accession were calculated. Average follicle length, diameter, number of seeds per follicle, average seed length, width and seed weight per 10 seeds were also calculated.

2.4. PCA, bi-plot and cluster dendrogram

Analysis of variance (ANOVA) of quantitative data was carried out in Statistix 10 software. Dennett's multiple comparison test was used to separate accession means. To establish the relationships and resemblance among five accessions and to decide the interrelation between morphological characters, a principal component analysis (PCA) was performed using the 9 qualitative and 23 quantitative characters. The qualitative data were transformed by assigning ordinal numbers for all characters. Data were analysed using Past software (version 4.10). Contributions of the morphological characters in the components were also calculated. For qualitative traits, principal component loadings greater than 0.04 or less than − 0.04 were accepted as significant, while PCA loadings greater than 0.1 were accepted as significant for quantitative traits. Hierarchical clustering was done using Euclidean distance.

2.5. Optimization of propagation strategy

Conventional (stem cuttings) and non-conventional (*in vitro* micropropagation) means were employed for propagation *B. frerei*. The basal cut ends of stem cuttings (8–10 cm) were treated with numerous concentrations of indole-3-aceic acid (IAA), α-naphthalene acetic acid (NAA) and inole-3-butyric acid (IBA) solution (IAA: 50–200 ppm; NAA: 100–400 ppm; IBA: 50–250 ppm) by dipping the stem cuttings (1 cm) for 12 h. After 12 h, the excess auxin was washed with running water. The treated stem cuttings were tagged separately and planted in plastic pots filled with coconut coir and watered periodically for 30 days. The plantlets with healthy root-shoot system were then transferred to soil, coconut coir and vermiculite (1:1:1) and placed in the greenhouse under shade. The greenhouse temperature was ranged between 30–33 ^{o}C during day and 21-24^{o}C during night with 60–80% relative humidity. The plantlets were irrigated periodically by taking safeguard against water-logging.

For *in vitro* micropropagation, healthy and young nodal segments excised from outdoor grown plants were cleaned under flowing tap water for 10 min. The explants were then soaked in 5% v/v labogent for 5 min, subsequently washed with distilled water. After repeated wash the nodal segments were pre-treated in Bavistin (0.5% w/v) for 5 min. Stem pieces were disinfected by immersing in 0.1% $HgCl_2$ (w/v) for 7 min. The sterilized segments were rinsed thrice with sterilized distilled water and single node explant was transferred to Murashige and Skoog (1962) medium fortified with different plant growth regulators (PGRs). Diverse concentrations of PGRs (BAP, KN, and IAA) either singular supplementation or in combination were mixed in the medium for shoot production. Approximately 2–3 cm long apical microshoots with 2–3 pairs of leaves were segregated from *in vitro* grown shoots and cultured on rooting medium. All the cultures were settled at 25 ± 2°C under 16-hours light/8-hour dark photoperiod with light (2000 lux) provided with white fluorescent tubes (Philips, India, Ltd.). After removal from culture vessel, the roots of *in vitro* derived plantlets were cleared for traces of medium to circumvent the pathogenic impurity during hardening. The plantlets were then moved to portable plastic pots containing potting composition of sterile soil, sand and coconut coir (1:1:1).

2.6. Optimization of planting substrate for hanging basket

The plantlets (15–17 cm) with well-grown root and shoot system obtained through conventional propagation (stem cutting) technique were used for hanging basket experiments. Different planting substrates were tested to evaluate the

growth and development of *B. frerei* plantlets in the hanging basket. Hanging baskets (20 x 10 x 10) filled with soil, brick pieces, coconut coir and vermiculite either alone or in combination was tested as planting substrate for *B. frerei* in hanging basket. The plantlets were allowed to grow for 60 days in hanging basket and the growth analysis was done for aerials parts.

2.7. Statistical analysis

Assessment of quantitative morphological traits was based on triplicate experiments with 10 replicates and data obtained were presented in the form of mean ± SE for each parameter. The vegetative propagation experiment consisted of 13 treatments (including control), each repeated three times in a randomized block design with 10 stem cuttings per treatment. The data were subjected to one-way ANOVA (analysis of variance) for calculation of significant differences. Plant tissue culture experiments were also set in completely randomized design. Data presented in the table are treatments of 20 replicates (culture tubes) and all experiments were repeated twice. Data were analysed using analysis of variance (ANOVA) and means were compared using Dunnett Multiple Comparison Test at the 0.01 and 0.05 level of significance, using software GraphPad Instat (GraphPad Software Inc., USA).

3. Results And Discussion

3.1.1. Nomenclature

Boucerosia frerei (G.D.Rowley) Meve & Liede in Pl. Syst. Evol. 234: 200 2002;. *Caralluma frerei* G.D.Rowley Natl. Cact. Succ. J. 13: 78. 1958; *Frerea indica* Dalz. in J. Linn. Soc. Lond. 8: 10, t. 3. 1865; Hook. f. Fl. Brit. India 4: 76. 1883; Cooke, Fl. Pres. Bombay 2: 243. 1958 (Repr.). Sant. and Irani in Univ. Bombay Bot. Mem. no. 4: 44. 1962; Jagtap et Singh in Fasc. Fl. India 24: 243. 1999; Jagtap and Das in Singh et al. Fl. Maharashtra St. Dicot. 2: 361. 2001; *Desmidorchis dalzellii* M.R.Almeida in Fl. Maharashtra 3A: 241. 2001; *Ceropegia frerei* (G.D.Rowley) Bruyns in S. African J. Bot. 112: 414. 2017.

3.1.2. Vernacular Names

Mankudi, Makadshingi, Shindal -Makudi

3.1.3. Flowering and fruiting

August - December

3.1.4. Ecology and distribution

It grows between 850-1400 m altitude on rock crevices and exposed hill slopes facing south-east or north-west directions forming patches along with *Euphorbia neriifolia* as its closest associate (Tetali et al. 1997). In natural habitats, the species is represented by very few populations with limited number of mature individuals. A typical habitat of *B. frerei* consists of steep slopes, preferably the crest region with a thin layer of soil between lateritic rocks (Fig. 2A-D). Except fire, the habitat is free from human disturbance and in general is unapproachable. The distribution of the species is restricted only to the Northern Western Ghats and that too the state of Maharashtra in India. The distribution range is limited to six localities in four districts of the state *viz*. Pune, Satara, Ahmednagar and Raigad. The species is basically a high-altitude plant with characteristic distribution in nature (Mishra and Arya 2010).

3.1.5. Taxonomic description

A succulent, perennial, trailing or pendulous herb. 9-15 cm long. Stem glabrous, branched, branches thick, pale green with scars of fallen leaves, slightly swollen nodes, shiny internodes, fleshy, 0.4-2 cm long and 4-6 mm across, watery sap.

Leaves opposite, fleshy, decussate, exstipulate, subsessile, 3-5 x 1- 2.5 cm, elliptic-oblong, obtuse, shining, glabrous, decurrent on petioles, petioles terete, 4-10 mm long with flap-like structure. Flowers extra-axillary, solitary or in pairs, 1-1.5 x 0.5 mm, ovate-deltoid, bracteate; pedicels ca. 5 mm long, glabrous, stout, curved, terete, arising from between petioles. Calyx 5 lobed, ca. 3 x 1.5 mm, valvate, glabrous, lobes deltoid, divided 2/3 way down, apex acute, fringed with fine deep purple hairs on the edges. Corolla fleshy, rotate, ca. 2-2.5 cm across, 5-lobed; lobes valvate, divided less than ½ way down, deltoid, acute, fringed with fine deep purple hairs on edges, yellowish green on the outer side, purple with pale yellow irregularly shaped spots on the inner surface. Staminal corona, outer corona 1-2 mm long, 5-lobed, broad, slightly divided at the apex, bowl shaped; inner ca. 1.5. mm long, 5-lobed, arising from the inner side of outer corona, ca 1.5 mm long, linear, apex acute, incurved, resting on the anther. Stamens five, ca 1.5 mm long, red anthers with short staminal columnrt, pollinia 5, pollinia 1 in each cell, yellow, pollen masses solitary in each anther cell, waxy with pellucid layer attached by light-brown caudicles wide to a dark-brown corpuscle. Gynostegium *ca* 3 mm long. Style apex flat, pearl white. Follicles paired or single, 6.3-8.2 cm long, terete, smooth, linear-lanceolate, curved at apex. Seeds many, ca 8 x 3.5 mm; oblong, flat, dark brown with light brown margin, coma silky white, ca 1.3 mm long (Kamble 2008; Mishra and Arya 2010). Natural occurrence, morphological attributes and associates of *B. frerei* is presented are presented in figure 2A-F.

3.1.6. Threats and conservation status

Illegitimate collection of leaf and stem as vegetable is the major threats identified (Mamgain et al. 1996; Jadhav et al. 2015). Most of the populations of *B. frerei* are on the verge of vanishing due to destruction and shrinking of their natural habitats. Fire and landslides also serve among major causes of habitat destruction. Additionally, complex pollinating mechanism is identified as one of the hurdle for its survival in natural existence. Infestation by caterpillars of the plain and stripped tiger and *Aphis* species also limited the size of the natural populations (Tetali et al. 1997). The plant is also exploited for its pretty, elegant, and star shaped flowers considered among top-ten most attractive flowers. The plant detaches the leaves and diminishing the stem into thick succulent knots with silver grey upper surface throughout drought period giving it a structure of silvery worms and vultures at times destroy its branches (Tetali et al. 1997). To some extent, vegetative propagation strategy has been employed for multiplication of *B. frerei* (Mamgain et al. 1996; Mishra and Arya 2010), however the data obtained were very scanty to design the conservation policy and domestication strategies.

3.2. Morphological appraisal

Naturally, the plants show variation across several morphological traits including plant height, length of internodes, number of nodes, leaf number, leaf shape, leaf length, flower colour and architecture. In general, confirmation and diversity of flowering plants as well as its association with close taxa can be assessed through phenotypic traits or by molecular basis (Kshirsagar et al. 2017; Guan et al. 2020). Diversity across leaf (size and shape) and flower (color and architecture) are the major potential ornamental traits found in *B. frerei* (Fig. 3A-B). Literature survey revealed that, insufficient information is available on the descriptors for morphological characterization of succulent plants especially asclepiads. Only few succulents have been characterized by considering a small number of morphological traits, *viz*. *Caralluma edulis* (Singh et al. 2016) and *Opuntia* species (Amani et al. 2019). In the present investigation, a comprehensive descriptor has been developed for morphological characterization (both qualitative and quantitate attributes) of *B. frerei* (Table 2 & 3).

3.2.1. Qualitative traits

Leaf shape, apex, stalk, stem shape, color, flower color, architecture, shape, hairs on corolla edges (purple coloured) were used to differentiate between five accessions (Table 2). Leaf shape is one of the major attribute contributes to the ornamental value of succulents. The Meruling, Satara accession had broadly oblong leaves, while all other accessions

possessed elliptic leaf shapes (Fig. 2A). The variety of leaf shapes observed in all accessions co-ordinated with the species descriptions by various authors (Kamble 2008; Mishra and Arya 2010). The MS and YCIS accessions possessed obtuse leaf apex while KP, SS, and PP were having acute to subacute leaf apex. KP, PP, and YCIS accessions had petiolate leaves while subsessile leaves were reported for MS and SS accessions. Slightly quadrangular stem was observed in SS and PP while other accessions were with cylindrical stem. MS and SS accessions possessed green coloured stem, while KP, PP and YCIS accessions had slightly pale green coloured stem. In all five accessions, either red or deep red coloured flowers were observed. Deep red color was reported for MS and YCIS while other accessions possessed red coloured flowers. Diverse flower architecture and colouring patterns made *B. frerei* flowers more attractive (Fig. 2B). Distribution of yellow coloured spots or patches on corolla lobe is the major floral attribute in *B. frerei* (Fig. 2B). Irregular network of yellow coloured patches was observed for the accessions collected from MS and YCIS; however single yellow spot on each corolla lobe were reported for KP accession. Ring connection between the yellow spots was reported for SS accession, while corolla lobe with full of yellow patches was reported for PP accession. In general, either ovate or deltoid flower shape was reported in all five accessions of *B. frerei*. Occurrence of purple coloured hairs on the corolla edges makes *B. frerei* flowers more attractive, however great variation has been reported for its distribution especially treated them as sparse, medium and dense. MS and YCIS accessions showed densely distributed hairs on the corolla lobes while KP and PP accessions possessed moderate distribution of hairs and SS was the only accession which had very sparsely distributed hairs on corolla lobes. The diversity among the flower color and architecture made this more attractive, however the divergence in flower traits were not reported previously.

3.2.2. Quantitative traits

Differences were found among accessions for 23 quantitative traits including plant height, number of roots per plant, number of branches per plant, stem diameter, number of nodes per plant, length of internodes, number leaves per plant, leaf length, width, petiole diameter, length, flower bud size, the period required for flower induction, number flowers per plant, flower diameter, pedicel length, number of follicles/plant, follicle length, width, number seeds/follicle, seed length, seed width and weight/10 seeds (Table 3). No significant differences between the accessions were observed for vegetative characters such as branches per plant (3.20±0.20 to 4.80±0.20), stem diameter (2.46±0.04 to 3.76±0.07 cm), length of internodes (1.05±0.06 to 01.45±0.06 cm), leaf length (4.14±5.40 to 5.40±0.04 cm), leaf width (1.88±0.07 to 2.66±0.08 cm), petiole diameter (5.00±0.32 to 6.40±0.24 mm) and petiole length (04.14±0.04 to 9.61±0.07 mm). Furthermore, no significant differences were observed with respect to reproductive traits such as flower bud size (1.96±0.05 to 3.06±0.07 mm), days to flowering (8.00± 0.31 to11.80± 0.37), pedicel length (1.60±0.06 to 2.12±0.06 cm), number of follicles per plant (1.60±0.25 to 3.60±0.24), follicle length (7.36±0.28 to 8.24±0.10 cm), follicle width (1.48±0.13 to 1.80±0.11 cm), number of seeds per follicle (7.40±0.51 to 12.20±0.73), seed length (7.32±0.09 to 8.02±0.09 mm) and seed width (2.56±0.15 to 3.28±0.10 mm). Significant differences in length plant height, number of roots per plant, number of nodes per plant, number of leaves per plant number of flowers per plant, flower diameter and seed weight were observed among the five different accessions (Table 3). Plant height is one of the key traits for ornamental plants grown in indoor conditions especially in hanging basket. In the present study, the average plant height among the five accessions was significantly higher in MS accession (39.38±1.02 cm). Moreover, the average numbers of roots were also recorded maximum for MS accession (11.72±0.55 cm). Differences observed in leaf and flowers, when compared to leaf and flowers of each accession, supported the qualitative observations on leaf and flowers (Table 3&4). The MS accession exhibited the highly significant values for utmost quantitative traits followed by YCIS, PP, KP and SS accessions. Of the quantitative characters recorded, length of internodes, leaf length, petiole length, seed length and length of follicles were within the range of published literature (Kamble 2008; Mishra and Arya 2010), whereas, slight higher value was recorded for petiole length.

3.3. PCA, Bi-plot and Cluster analysis for identification of elite plantlets for hanging basket

Among nine qualitative traits, significance differences were observed in three traits including plant stem colour, flower architecture and flower shape. Plant height, number of roots, number of leaves, number of flowers, flower diameter, and seed weight are among the quantitative traits showed significance difference (Table 4). PCA indicated retention of both PC1 and PC2. These two principal components explained 48.59% of the total variation among the five accessions (Table 4). PC1 had an eigenvalue of 28.99 and accounted for 32.27% of the variation. This component was significantly associated with the plant height, number of nodes, leaf number, flower number, flower architecture, flower diameter, and seed weight (Table 4). PC2 had an eigenvalue of 4.54, contributing 16.32% of the variation and had plant height, number of roots; stem colour, flower shape and seed weight as the main contributing factors (Table 4). The biplot (Fig. 4) clustered the five accessions in relation to the ten main morphological characters contributing to the principal components A close association was observed between MS and YCIS accessions based on green coloured stem and the higher average values for plant height, number of roots, number of leaves, flower size, number of nodes, number of seeds, number of flowers and seed weight (Fig. 4). However, flower shape and architecture were negatively correlated with aforementioned morphological traits. These characters separated the accessions into two groups: (1) accessions possessing ovate shaped flowers and flower architecture other than irregular network of pale yellow spots and (2) accessions possessing green coloured stem and the higher average values for plant height, number of roots, number of leaves, flower size, number of nodes, number of seeds, number of flowers and seed weight (Fig. 4). KP and PP accessions were located on the axis of deltoid shaped flowers while SS was located on the opposite side with flower architecture other than irregular network of pale yellow spots.

The dendrogram (Fig. 5) assembled the five accessions in two major clusters. The first cluster contained the MS, Satara and was grouped separately due to stem colour and significant contribution of plant height, number of roots, number of leaves, flower size, number of nodes, number of seeds, number of flowers and seed weight. The YC, SS, PP and KP accessions were grouped into a second cluster due to characters such as elliptic leaves, acute to subacute leaf apex, pale green coloured and slightly quadrangular to cylindrical stem, ovate leaves and sparse to medium distribution of hairs on corolla edges. Moreover, these accessions showed resemblance in quantitative characters such as stem diameter, length of internodes, leaf length, width, petiole diameter, length, flower bud size, days to flowering, pedicel length, number of follicles, follicle length, width, seed length and width. Qualitative and quantitative data, together with principal components analysis, indicated Meruling, Satara as potential accession for its use in hanging baskets. Due to shorter internodes, this accession might respond well during propagation and exhibit a compact form and high number of lateral branches. These plant characters are important criteria in potted flowering plants (Antesco and Baldos 2020).

3.4. Propagation strategy

Profit-oriented production of ornamental herbaceous perennial plants primarily depends on seed, vegetative (stem) cuttings, or tissue culture (Cabahug et al. 2018; Chavan et al. 2018; Lewis et al. 2020). In the current study, vegetative propagation through stem cuttings and tissue culture technique has been employed.

3.4.1. Vegetative propagation

Plant propagation by vegetative means make certain the preservation of genetic integrity of a specific genotype over many production succession (Lewis et al. 2020). Moreover, vegetative propagation also offers planters a shorter production cycle and more unvaried crop growth compared to seed production (Ecker and Barzilay 1993). Much of what is familiar about propagation of *B. frerei* is built on studies of Mamgain et al (1996); while add-on studies have uncertain or deficient procedures and are inadequate for mass-propagation and conservation perspectives (Tetali et al. 1997; Chakraverty 1999). In the present study, thorough investigation has been carried out using different types and concentrations of auxins during vegetative growth (both root and shoot development) using stem cuttings. Various growth parameters, *viz*. percent of stem cuttings responded to the treatment, average root and shoot number as well as

their lengths were recorded for each treatment and are presented in table 5. The stem cuttings with 8-10 pairs of leaves (Fig. 6A) were treated with various concentrations of IAA, NAA and IBA. Stem cuttings without auxin treatment showed poor response during root-shoot production (Table 5; Fig. 6B). Among the different auxins tested, IAA (100 ppm) showed optimal response during root-shoot production from stem cuttings of *B. frerei*. This concentration resulted with emergence of roots and shoot in cent percent of cuttings. A maximum response to all studied growth parameters, *viz*. number of roots (14.15±0.64), length of roots (5.36±0.17), number of shoots (5.30±0.26) and length of shoots (17.35±0.66) were recorded after one month of propagation period (Table 5, Fig. 6C-D). Subsequently, the plantlets grew well after transferred to soil beds as well as plastic bags (Fig. 6E-F). The same auxin type and concentration also supported the shoot elongation which is very much essential for introduction of this succulent into hanging basket. NAA (150 ppm) also stimulated the root-shoot induction in 90% of stem cuttings. Similarly, propagation through stem cutting was successfully employed in numerous succulent's including *Hoya imperialis* (Don et al. 2021). On the contrary, numerous genera including lantern flowers from same plant family failed to propagate through stem cuttings (Chavan et al. 2018).

3.4.2. *In vitro* propagation

The usage of biotechnology especially plant tissue culture technique for the expansive propagation and preservation of numerous plant groups have been well documented for wild ornamental plants (Raut et al. 2006; Chavan et al. 2018; Kshirsagar et al. 2021). Of the only studies that have been published on micropropagation protocols for *B. frerei*, development of optimum media composition to initiate shoots and roots (Desai et al. 2003). In the current work, the *in vitro* cultures were established using young nodal bud segments of *B. frerei* using stock plants from the accessions of Meruling, Satara (Fig. 7A). MS medium without growth regulators induced up to 20% of shoot induction from nodal explants; however emergence and multiplication new shoots were largely dependent on incorporation of different types, concentration and combinations of auxins and cytokinins (Table 6). The nodal explants responded (45–95%) to both the cytokinin (BAP, KN) as well as combinations among them or with IAA (Table 6). Inside 30 days of culture period, highest number of multiple shoots (5.9+0.6 per explant) was achieved on 3.0 mg/l of BAP combined with 1.5 mg/l of IAA (Fig. 7B-D). This number seems to be the higher compared to previous reports on the same plant species (Desai et al, 2003). The shoots grown on BAP and IAA containing media were more vigorous, than those developed in PGR supplementation. A synergistic effect of BAP along with various auxins on bud proliferation and multiple shoot formation has been reported for several plant species including *Hemidesmus indicus* (Sreekumar et al. 2000), *Huernia hystrix* (Amoo et al. 2009), *Ceropegia panchganiensis* (Chavan et al. 2013), and *Ceropegia mohanramii* (Adsul et al. 2019), another members of Apocynaceae. A combination of BAP and KN exhibited symptoms of hyperhydricity and reduced shoot elongation. It was noticed that, Kinetin (1.0-4.0 mg/l) induced shoots produced callus at the base of shoots in all of its four tested combinations at variable frequencies. On contrary, singular supplementation of BAP supported shoot multiplication in medicinally important succulent plant, *Caralluma diffusa* (Karthik et al. 2013). In the present study, *in vitro* rooting experiments were conducted for regenerated shoots using varied concentrations and mixtures of auxins, however *in vitro* derived shoots showed very poor response towards root induction (Fig. 7E). These results are in agreement with earlier report on *in vitro* rooting of *B. frerei* (Desai et al. 2013). On the other hand, during present study *in vitro* derived shoots showed substantial numbers of roots under *ex vitro* rooting environment following treatment with IAA (100 ppm). The plantlets rooted *ex vitro* were grown robustly in greenhouse (Fig. 7F). Similarly, *ex vitro* rooting approach have been successfully employed in *Ceropegia bulbosa* (Phulwaria et al. 2013).

In the present investigation, vegetative propagation of *B. frerei* was achieved through stem cutting and micropropagation technique where stem cutting was found more feasible in propagating *B. frerei*. Recent studies on propagation of two succulent plants, *Hoya imperialis* and *H. coronaria* confirmed the viability of stem cutting over micropropagation approach (Don et al. 2021).

3.5. Hanging basket conditions

The plantlets (15 cm in length) of *B. frerei* (Meruling, Satara accession) with completely developed roots and shoots were vertically placed in hanging baskets filled with diverse types and concentrations of planting substrates (Table 7). It was noticed that, the type and concentration of planting substrate significantly altered the growth responses of aerial part (shoot length, number of nodes, number of leaf pairs, and numbers of flowers). A singular use of planting substrate showed poor response towards all the studied parameters, while mixtures of 2 or more planting substrates triggered the growth in hanging basket condition. A combination of soil, coconut coir and vermiculite (3:1:1) showed maximum response towards the growth parameters of aerial parts (Table 7). After 15 days of transfer, the stem was started bending downwards. The plantlets were watered periodically were grew well, showed quickness towards vegetative growth. Plant attained an average height of 56.6±2.1 cm after 60 days in the hanging basket (Fig. 8A-B). Flowering was observed after 60 days in the hanging basket (Fig. 8C). As the species is a seasonal herb which completes its life cycle during four months of monsoon season (June-September) and a month followed by monsoon (October). When it grows naturally, the plants started shedding of its leaves, loses water from the stem which further reduces the size and forms silver-grey coating which covers stem. However, the present study revealed that the plants can withstand and grew well during next 3 months (November-January), when proper care is taken and irrigated periodically. It was also noticed that, plants produced fewer flowers during November-February; however the pretty succulent stem and leaves gave decent beauty to it in hanging basket plant in home/office gardens. After February, the plants from hanging basket start shedding their leaves, stem forms outer covering with reduced stem size and remains in this situation till next monsoon.

4. Conclusions

Morphological diversity analysis of *B. frerei* revealed that enough morphological variation existed between five accessions which provide great scope for selection of accessions for its use in hanging basket. Stem color, plant height, number of roots, number of nodes, number of leaves, number of flowers flower diameter are among the identified traits which possesses significantly while differentiating the accessions. Cluster analysis of the five accessions revealed two distinct groupings based on shared characters. Identification of these important characters and relationships between accessions provide relevant information that revealed the Meruling Satara as potential accession for hanging basket use. Among the propagation techniques employed, vegetative propagation using stem cuttings was found more promising compared to micropropagation. Moreover, results suggested that, treatment of 100 ppm IAA to stem cuttings serve better for production of stock plants which will fulfil the horticulture industry needs. This study provides benchmark information for further evaluation and exploitation of this potential horticulture crop.

5. Future Perspectives

B. frerei is a useful plant resource that could be supportive to upraise the floriculture industry. Aside from its ornamental usefulness, the succulent is edible and possesses nutritional desirability. In all probably it holds crucial secondary metabolites as it is being used by traditional people in many ailments. Exploitation of newly evolved techniques of extraction, purification and identification of novel metabolites could be used for its biochemical verification which might be very helpful in understanding complete profile of the plant. In consequence, collecting more germplasm from diversified domains (field/homestead/natural) across altitudinal and distributional ranges and examining them for nutritive potential and biomolecules should also gain paramount attentiveness. The striking feature of this succulent as they can resist extreme conditions and as potted plants for indoor areas as they are in need of little watering. To promote the maintainable use of *B. frerei*, we propose intensifying the knowledge on farming and cultivation exercise for enhanced seed production, growing cultivated lines, and ensuring preservation of this undomesticated genetic resource as a vegetable as well as ‘future plant of hanging basket’.

Declarations

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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Tables

Table 1 Details of collection site and distribution range of *B. frerei*.

S. No.	Collection no.	Locality	Abbreviation	Latitude (N)	Longitude (E)	Altitude (m)
1	MMB-FI-1	Kate's Point, Mahabaleshwar	KP	$17^0 94'$	$73^0 71'$	1280
2	MMB-FI-2	Meruling, Satara	MS	$17^0 77'$	$73^0 90'$	1005
3	MMB-FI-3	Sajjangad, Satara	SS	$17^0 65'$	$73^0 90'$	1019
4	MMB-FI-4	Purandar Hills, Pune	PP	$18^0 27'$	$73^0 96'$	1381
5	MMB-FI-5	YCIS, Satara	YC	$17^0 68'$	$74^0 01'$	703

Table 2

Qualitative morphological assessed in various accessions of *B. frerei*.

S. No.	Descriptor	Abbreviation	Accessions (Locality name)				
			Kate's Point, Mahabaleshwar	Meruling, Satara	Sajjangad, Satara	Purandar, Pune	YCIS, Satara
1.	Leaf shape	LFS	Elliptic	Oblong	Elliptic	Elliptic	Elliptic
2.	Leaf apex	LFA	Acute	Obtuse	Subacute	Acute	Obtuse
3.	Leaf stalk	LFST	Petiolate	Sub-sessile	Sub-sessile	Petiolate	Petiolate
4.	Stem shape	STS	Cylindrical	Cylindrical	Slightly quadrangular	Slightly quadrangular	Cylindrical
5.	Stem color	STC	Pale green	Green	Green	Pale green	Green
6.	Flower color	FLC	Deep red	Deep Red	Red	Red	Deep Red
7.	Flower architecture	FLA	Single yellow spot on each corolla lobe	Irregular network of pale yellow spots	Ring connection throughout spots	Corolla with full of yellow patches	Irregular network of pale yellow spots
8.	Flower shape	FLS	Ovate	Deltoid	Deltoid	Ovate	Deltoid
9.	Hairs on corolla edges (purple colored)	HCE	Medium	Dense	Sparse	Medium	Dense

Quantitative morphological attributes of *B. frerei*.

Table 3

Quantitative morphological attributes of *B. frerei*.

S. No.	Descriptor	Abbreviation	Accessions (Locality name)				
			Meruling, Satara	Kate's Point, Mahabaleshwar	Sajjangad, Satara	Purandar, Pune	YCIS, Satara
1	Plant height (cm)	PH	39.38±1.02	22.94±0.66	15.76±0.47	21.48±0.30	30.86±1.25
2	No. of roots/plant	NR	10.72±0.55	08.00±0.31	06.24±0.30	07.00±0.15	08.72±0.23
3	No. of branches/plant	NB	04.80±0.20	03.20±0.20	03.60±0.24	04.00±0.32	04.40±0.24
4	Stem diameter (cm)	SD	03.76±0.07	02.46±0.04	02.70±0.04	03.32±0.12	03.10±0.03
5	No. of nodes/plant	NN	26.20±1.02	16.20±1.13	11.60±0.68	16.20±1.39	23.80±1.06
6	Length of internodes (cm)	LI	01.05±0.06	01.17±0.07	01.45±0.06	01.22±0.07	1.08±0.08
7	No. of leaves/plant	NL	51.07±1.74	30.60±0.87	21.00±1.41	31.80±1.85	44.20±2.42
8	Leaf length (cm)	LL	05.04±0.04	04.14±0.08	03.46±0.06	04.26±0.10	04.62±0.06
9	Leaf width (cm)	LW	02.66±0.08	02.32±0.10	02.34±0.07	01.88±0.07	02.32±0.09
10	Petiole diameter (mm)	PD	06.40±0.24	05.00±0.32	05.80±0.20	05.00±0.45	05.80±0.37
11	Petiole length (mm)	PL	09.61±0.07	04.14±0.04	04.28±0.06	05.54±0.07	05.60±0.06
12	Flower bud size (mm)	FB	03.06±0.07	02.08±0.04	01.96±0.05	02.54±0.10	02.38±0.11
13	Days to flowerings	DF	08.00±0.31	11.20±0.37	11.80±0.37	09.80±0.66	10.20±0.73
14	No. of flowers/plant	NF	15.20±0.80	04.80±0.58	10.00±0.71	07.60±0.40	10.20±0.80
15	Flower diameter (mm)	FD	14.40±0.09	06.20±0.05	08.40±0.07	07.40±0.04	10.20±0.06
16	Pedicel length (cm)	PDL	02.12±0.06	02.02±0.08	01.84±0.05	01.60±0.06	02.04±0.13
17	No. of follicles/plant	NF	03.60±0.24	01.60±0.25	02.20±0.50	02.40±0.24	02.80±0.37
18	Follicle length (cm)	FL	08.24±0.10	07.36±0.28	07.50±0.17	07.26±0.07	07.62±0.24
19	Follicle width (cm)	FW	01.80±0.11	01.48±0.13	01.62±0.17	01.74±0.06	01.70±0.17
20	No. of seeds/follicle	NS	12.20±0.73	07.40±0.51	10.20±0.86	10.80±0.58	11.20±1.00

21	Seed length (mm)	SL	08.02±0.09	07.46±0.23	07.32±0.09	07.52±0.08	07.90±0.12
22	Seed width (mm)	SD	03.28±0.10	02.56±0.15	02.62±0.10	02.72±0.14	03.02±0.21
23	Seed weight-10 seeds (mg)	SW	66.40±1.32	40.80±1.82	54.80±1.56	49.40±2.16	64.60±1.75

Table 4

Principal component analysis of 32 morphological characters recorded in five accessions of *B. frerei*.

	PC1	PC2
Eigenvalue	28.99	4.54
% variance	32.27	16.32
Qualitative morphological characters		
Leaf shape	-0.018886	-0.02554
Leaf apex	-0.027424	-0.017613
Leaf stalk	-0.014623	0.0051971
Stem shape	-0.012745	-0.026178
Stem color	-0.018899	**0.043862**
Flower color	-0.012745	-0.026178
Flower architecture	**-0.044388**	-0.0084951
Flower shape	-0.018899	**-0.043862**
Hairs on corolla edges (purple colored)	-0.027424	-0.017613
Quantitative morphological characters		
Plant height (cm)	**0.34459**	**0.71434**
No. of roots/plant	0.066158	**0.12983**
No. of branches/plant	0.026858	-0.014181
Stem diameter (cm)	0.018971	0.0050003
No. of nodes/plant	**0.30977**	-0.019815
Length of internodes (cm)	0.00069025	0.035154
No. of leaves/plant	**0.71099**	0.095477
Leaf length (cm)	0.023467	0.0043326
Leaf width (cm)	0.010068	0.006063
Petiole diameter (mm)	0.026451	-0.019765
Petiole length (cm)	0.010339	0.012468
Flower bud size (mm)	0.016401	0.019215
Days to flowerings	-0.055394	-0.067888
No. of flowers/plant	**0.17359**	-0.090241
Flower diameter (mm)	**0.15241**	-0.0073812
Pedicel length (cm)	0.0063039	0.0053987
No. of follicles/plant	0.033559	-0.010113
Follicle length (cm)	0.017983	0.0076634
Follicle width (cm)	0.004182	-0.0034513

No. of seeds/follicle	0.066125	-0.094897
Seed length (mm)	0.012545	0.0018667
Seed width (mm)	0.013952	-0.00051171
Seed weight-10 seeds (mg)	**0.45261**	**-0.65751**

Bold values indicate characters significantly contributing to the principal components

Table 5 Efficiency of auxins on *ex vitro* rooting and subsequent shoots development in *B. frerei*.

S. No.	Type of auxin	Conc. (ppm)	% response	No. of roots ± SE	Length of roots (cm) ± SE	No. of shoots ± SE	Length of shoots (cm) ± SE
1	Control	-	60	1.20±0.33	0.71±0.18	0.90±0.27	8.40±2.41
2	IAA	50	80	8.65±1.05	3.94±0.47	3.25±0.40	11.61±1.44
3		100	100	14.15±0.64	5.36±0.17	5.30±0.26	17.35±0.66
4		150	85	10.00±1.46	4.13±0.57	3.55±0.48	13.41±1.87
5		200	75	8.05±1.12	3.23±0.40	2.85±0.39	12.18±1.66
6	IBA	100	70	7.25±1.14	2.89±0.41	2.90±0.46	11.01±1.70
7		200	65	6.55±1.13	2.67±0.41	2.75±0.49	10.26±1.77
8		300	75	7.90±1.10	3.14±0.39	3.10±0.44	11.88±1.63
9		400	60	2.05±0.41	1.02±0.19	1.35±0.30	9.15±1.92
10	NAA	50	80	7.70±0.93	3.65±0.46	3.10±0.40	12.00±1.46
11		100	80	7.90±0.95	3.66±0.43	3.20±0.39	11.61±1.45
12		150	90	8.85±0.73	4.33±0.36	4.15±0.38	14.11±1.19
13		200	75	7.50±1.03	2.80±0.36	2.70±0.39	11.32±1.55

Table 6

Efficiency of plant growth regulators on *in vitro* shoot multiplication in *B. frerei*.

S. No	PGRs	Conc. (mg/l)	Response (%)	No. of shoots ± SE	Shoot length (cm) ± SE
1	PGR free	0.0	20	0.5+0.1	0.8+0.2
2	BAP	1.0	65	$1.4+0.9^{ns}$	$3.3\pm0.8^{*}$
3		2.0	70	$3.1+1.5^{*}$	$4.0\pm1.2^{**}$
4		3.0	90	$5.3+1.7^{**}$	$4.5+0.2^{**}$
5		4.0	90	$2.8+1.8^{*}$	$4.3\pm1.0^{**}$
6	KN	1.0	75	$2.0+1.3^{*}$	$3.1+0.9^{*}$
7		2.0	70	$1.7+1.3^{ns}$	$2.8+1.1^{*}$
8		3.0	60	$1.8+1.1^{*}$	$3.0+0.5^{*}$
9		4.0	45	$1.1+0.9^{ns}$	$2.5+0.2^{*}$
10	BAP+KN	3.0±0.5	60	$1.1+0.9^{ns}$	$2.6+2.2^{*}$
11		3.0±1.0	85	$1.4+0.9^{ns}$	$2.2+3.1^{*}$
12		3.0±1.5	85	$3.8+1.8^{*}$	$2.7+3.3^{*}$
13		3.0±2.0	80	$2.6+1.7^{*}$	$1.4+1.5^{ns}$
14	BAP+IAA	3.0±0.5	70	$2.1+0.8^{*}$	$3.8+1.2^{**}$
15		3.0±1.0	85	$3.8+1.1^{*}$	$4.1+0.9^{**}$
16		3.0±1.5	95	$5.9+0.6^{**}$	$5.2+0.6^{**}$
17		3.0±2.0	90	$3.1+0.4^{*}$	$4.2+1.0^{**}$

Values are significant at ns – non significant, *- significant at **- significant at level when performed with one way ANOVA using Dunnett Multiple Comparison Test.

Table 7: Planting substrate requirement for *B. frerei* in hanging basket.

S. No.	Planting substrate (per 100 gm)				Parameters	
	Soil (g)	Brick pieces (g)	Coconut coir (g)	Vermiculite (g)	Length of aerial shoot in hanging basket (cm) ± SE	Observations
1	100	-	-	-	30.7±1.2	Stunted growth
2	50	50	-	-	37.3±0.8	Vegetative growth
3	50	-	50	-	36.0±1.7	Vegetative growth
4	50	-	-	50	45.5±1.5	Vegetative growth
5	25	25	25	25	47.1±2.0	Flowering
6	40	20	20	20	51.2±1.1	Flowering
7	60	-	20	20	56.6±2.1	Flowering
8	40	10	25	25	48.4±0.9	Flowering

Figures

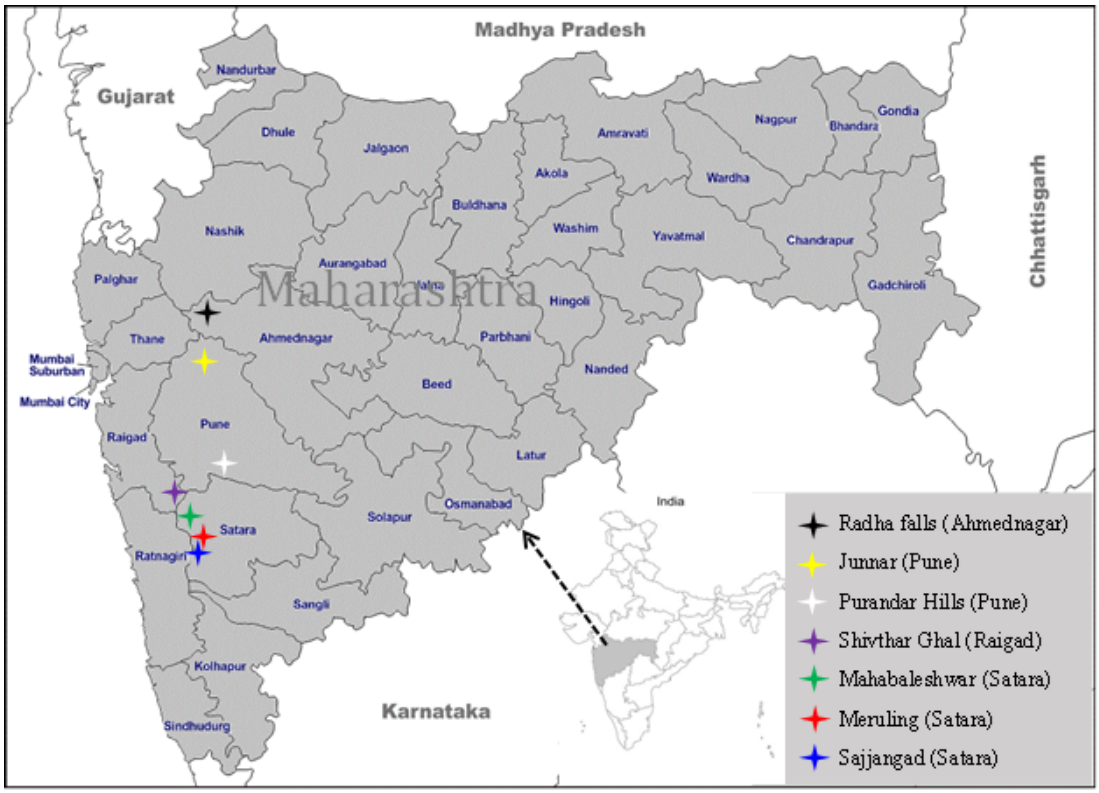


Figure 1

Map of Maharashtra State, India showing natural localities of *B. frerei*.

Figure 2

Boucerosia frerei (G. D. Rowley) Mieve & Liede. A-B: Natural occurrence and association, C: Flowering twig, D: Follicle, E: Shedding of leaves and forming silver grey outer surface of stem at the end of monsoon, F: Potted plants after collecting from different localities.

Figure 3

Variation among leaf and flower attributes of *B. frerei*. A: Leaf, B: Flower color and ornamentation.

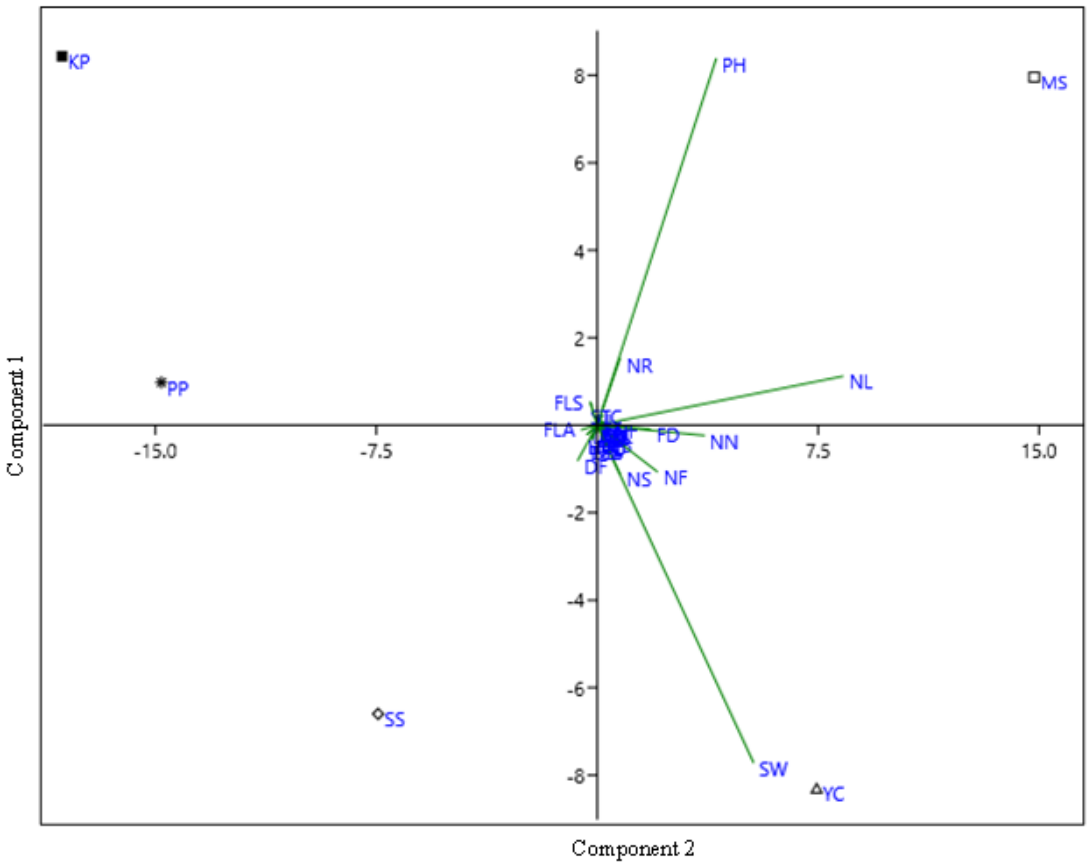


Figure 4

Bi-plot showing five *B. frerei* accessions (KP = Kate's Point, Mahabaleshwar; MS = Meruling, Satara; SS = Sajjangad, Satara; PP = Purandar, Pune; YC = YCIS, Satara) and 32 qualitative and quantitative morphological characters.

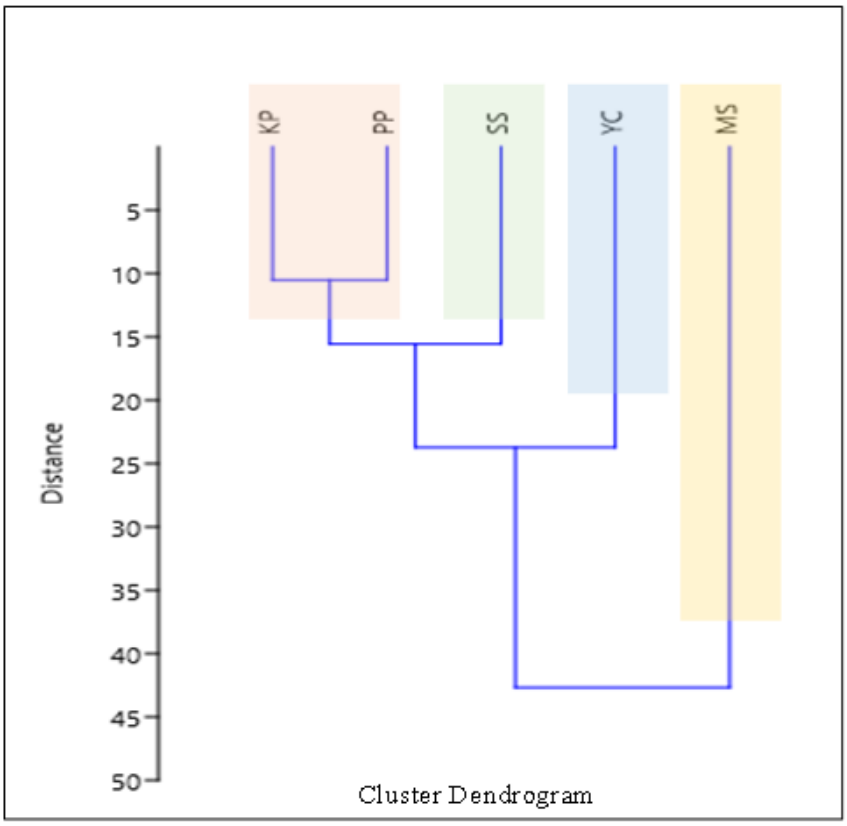


Figure 5

Cluster dendrogram of five *B. frerei* accessions generated from 32 qualitative and quantitative morphological characters.

Figure 6

Vegetative propagation of *B. frerei*. A: Stem cuttings with 8-10 pairs of leaves, B: Root induction (control), C-D: Root induction in IAA (100 ppm) treated cuttings, E: Cultivation of plantlets in open field, F: Cultivation of plantlets in bags.

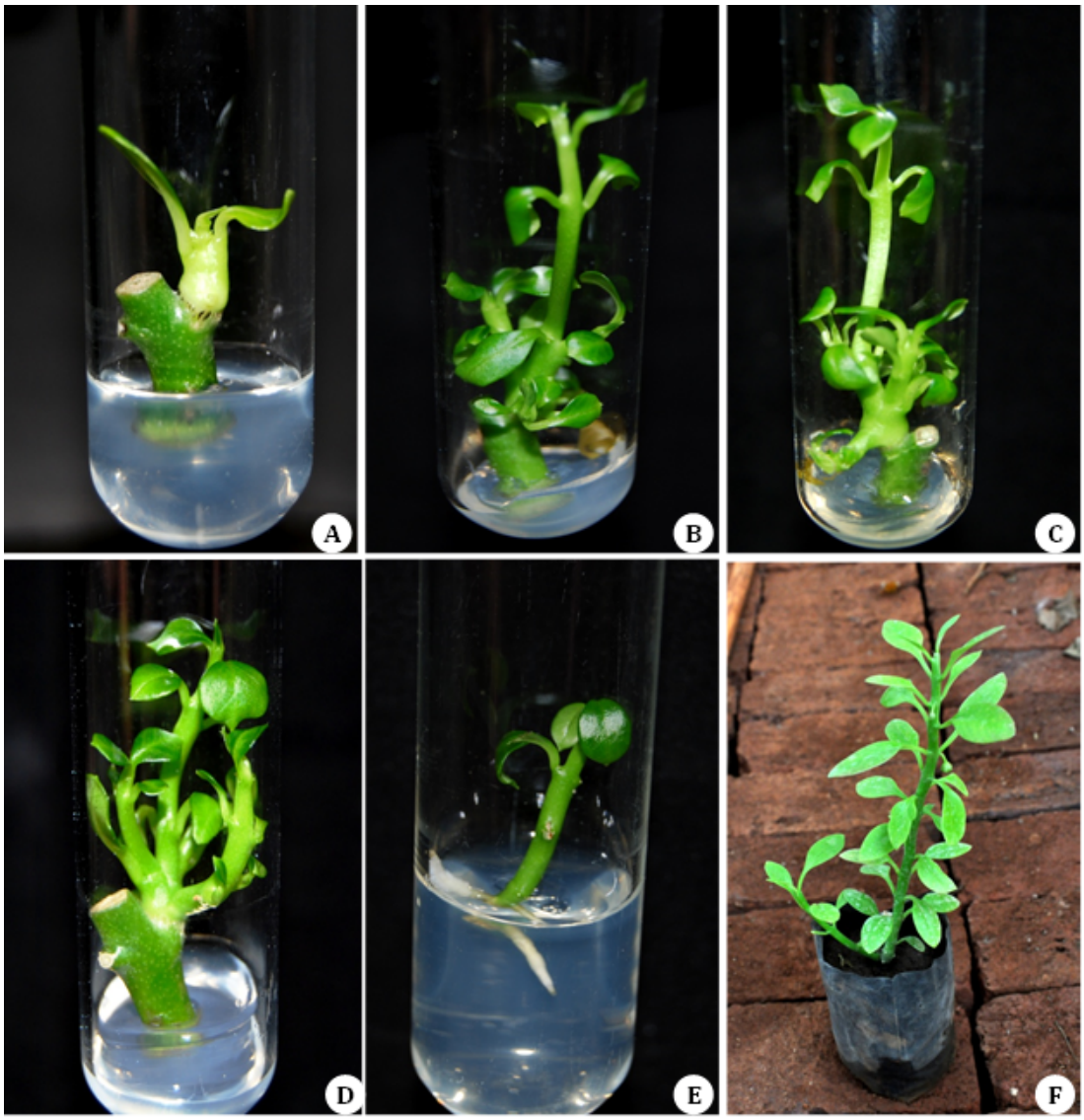


Figure 7

Micropropagation of *B. frerei*. A: Shoot initiation from nodal buds (MS+BAP 1.0 mg/l), B-D: Shoot multiplication (MS+BAP 3.0 mg/l), D: *In vitro* rooting (MS+IAA 1.5 mg/l), E: Hardened plant.



Figure 8

Differential views of hanging basket of *B. frerei*.