resulting seeds. Another study on other species also showed that nitrogen concentration of seeds did increase in response to maternal nutrient status[3].

In this study, correlation analysis of two nutrient treatments showed that the number of pods per plant was significantly negatively correlated with the mean number of seeds per pod. This result indicates that there was a trade-off between pod size and the number of seeds rather than between seed size/mass and the number of seeds. This result disagrees with that reported by Wulff[22], who found no correlation between mean seed mass per plant and total number of seeds produced per plant. The numbers of pods and seeds are affected by environmental conditions during flowering, seed set[23] and genetic factors[24].

The present results imply that reproductive characters were significantly affected by nutrient treatments. Seed mass is much less influenced by soil nutrient conditions than the number of pods and number of seeds per pod. Both germination percentage and germination rates are affected by seed mass. The nitrogen concentration showed a significant increase in the seed in response to an increase in the parental nutrient status. In addition, poor growth of *V. grandiflora* seedlings under low nutrient conditions indicates that this species is not tolerant of infertile soils.


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# Variation studies in surface micromorphology on seed coat and endosperm of *Ensete superbum* (Roxb.) Cheesman: a conservation concern species of India

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***Ensete superbum* (Roxb.) Cheesman is an endemic wild banana species of Western Ghats, northeastern hills of India and northern Thailand. The white powdery endosperms of the seeds are widely used to treat**

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**various human disorders. The present study examined the exomorphic characteristics of *E. superbum* seed coat and starch crystals of endosperm using scanning electron microscopy to highlight and document the surface ornamentation pattern of *E. superbum* grown at different locations. Interestingly, the size of seeds decreased with the increase in latitude from 8°N to 22°N and unveiled the phenotypic variation in genetic diversity of *E. superbum* in India. Information on macro and micromorphological variations of seed coat and endosperm of *E. superbum* has been reported for the first time in India.**

**Keywords:** Cliff banana, *Ensete superbum*, endosperms, medicinal plant, micromorphology.

THE genus *Ensete* comprises nine species spread across tropical and subtropical regions of Africa, Asia and Madagascar. Among these, *Ensete superbum* and *Ensete glaucum* are found in India[1]. The former, commonly known as cliff banana, is distributed on rocky slopes (Figure 1) and crevices across evergreen and deciduous forests of Western Ghats, Aravalli Range and northeastern sub-Himalayan tracts of India.

*E. superbum* is a monocarpic, non-stoloniferous herbaceous plant. It does not produce suckers like other members of Musaceae. Seeds are the only known mode of natural multiplication[2]. Seeds are hard and dark brown in colour, sub-globose and 0.4–0.8 cm in length, 0.5–1.0 cm in breadth and 0.6–1.3 cm in diameter. Powdery endosperms of seeds are widely used by ethnic communities as a remedy for treating debility[3], diabetes[4], kidney stone[5] and leucorrhoea[6]. Pharmacognostical features of *E. superbum* seeds and endosperm are well documented[7,8]. The white powdery endosperm of *E. superbum* seeds are rich in starch grains, calcium oxalate and parenchyma cells[7]. Documentation and conservation of genetic diversity is a primary objective in the management of rare and threatened species[9]. *E. superbum* is a rare medicinal plant distributed from tropical rain forest of Western Ghats, northeastern sub-Himalayan tracts and dry deciduous forests of central and western India[1]. The wide distribution of *E. superbum* under diverse climatic conditions may result in the variation of seed and plant morphology. Documentation of the effect of latitudinal variation on seed size, exomorphic characteristics of seed coat and endosperm using scanning electron microscopy (SEM) are lacking regarding this species. Therefore, this study examined the exomorphic characteristics of *E. superbum* seed coat and starch crystals of endosperm using SEM to document the variations in ornamentation pattern.

**Figure 1.** *Ensete superbum* growing under natural condition.

The fresh seeds of *E. superbum* were collected in the month of February–June 2012–2013, from five locations of southwestern part of India, i.e. Ponmudi, Kerala (L1), Jog, Karnataka (L2), Veer, Maharashtra (L3), Saputara, Gujarat (L4) and Pavagadh, Gujarat (L5). Coordinates of the collection sites were geographically recorded using a hand held global positioning system (GPS). The coordinates of the collection area are presented in Table 1. The samples were identified and authenticated by comparison with the botanical description mentioned in Floras[10] and voucher specimens (No. 174/12101801–174/12101805) were deposited at the Pharmacognosy Laboratory of SDM Centre for Research in Ayurveda and Allied Sciences, Udupi, for future references.

The seeds collected from various locations were examined for comparative size variation using a digital caliper (Mitutoyo Digimatic caliper – CD-6″ CSX). The seed length and breadth were measured for 25 seeds, randomly selected from the geo-tagged samples of five locations (L1–L5).

Seed samples were then examined under SEM to document the seed coat and surface ornamentation pattern of starch crystals in endosperm. Each sample were mounted on stubs using double-sided conductive tape coated with 8 nm of gold/palladium and examined in a ZEISS EVO 18 SEM at 10.00 kV with 16 mm working distance. The images of the seed coat surface were obtained at 1000× and 2000×. On the other hand endosperm starch crystals' samples were captured at magnifications of 200×, 500× and 1000× at the Department of Electron Microscopy, National Institute for Interdisciplinary Science and Technology, Thiruvananthapuram, Kerala, India.

The seeds of *E. superbum* are quite hard, black to light brown in colour, variable in size and shape from nearly spherical to flattened and irregular, with a smooth surface. The seeds are dark brown, sub-globose but angled

**Table 1.** Coordinates of the study area

| Site code | Sites | Latitude | Longitude |
|---|---|---|---|
| L1 | Ponmudi, Kerala | 8°39′26.16″N | 77°4′58.23″E |
| L2 | Jog falls, Karnataka | 14°13′35.56″N | 74°48′52.57″E |
| L3 | Veer, Maharashtra | 18°04′36.56″N | 73°21′46.07″E |
| L4 | Saputara, Gujarat | 20°33′59.01″N | 73°44′56.89″E |
| L5 | Pavagadh, Gujarat | 22°27′49.05″N | 73°30′58.41″E |


**Figure 2.** Variation in seeds size of *E. superbum* grown under different latitudes (L1–L5).

**Table 2.** Comparative macroscopic measurements of *E. superbum* seeds from the five locations

| Location | Seed length (mm) mean ± SEM | Seed breadth (mm) mean ± SEM |
|---|---|---|
| Ponmudi, Kerala (L1) | 10.0 ± 0.3 | 8.3 ± 0.5 |
| Jog falls, Karnataka (L2) | 8.4 ± 0.4 | 7.2 ± 0.2 |
| Veer, Maharashtra (L3) | 5.7 ± 0.3 | 5.3 ± 0.3 |
| Saputara, Gujarat (L4) | 4.6 ± 0.3 | 4.6 ± 0.3 |
| Pavagadh, Gujarat (L5) | 4.2 ± 0.3 | 4.8 ± 0.3 |

by pressure, varied in size depending on the habitat. The seeds shape and structure are similar in all the locations studied, however, seed size varied from location to location. Longitudinally cut seed shows a micropyle, embryo embedded by endosperm, a white coloured powder. The lower end contains a cavity called chalazal mass. The present study resulted in documenting the natural variations of seed size in *E. superbum*. According to morphometric measurement of collected seeds from these locations, the size of seeds in terms of length and breadth, varied to a maximum of 10.0 and 8.3 respectively, in Ponmudi, Kerala (L1) and the lowest was recorded at 4.2 and 4.8 respectively in Pavagadh, Gujarat (L5). The comparative macroscopic measurements of seeds from the five locations are shown in Figure 2 and Table 2.

The SEM studies were conducted to examine the exomorphic seed coat ornamentation of the five different locations (L1–L5). In Ponmudi, Kerala (L1), the seed surface patterns were non-identical and showed tuberculate ornamentation pattern (Figure 3 *a* and *b*). The centre of testa cells had depressions and did not have sharp lines. The depressions were also located in all the cell centres. In Jog Falls, Karnataka (L2), the seed coat surface patterns were tuberculate epidermal cells of non-identical and irregular shapes. Few were granular in appearances (Figure 3 *c* and *d*). These tuberculate elevations had irregular structures and arrangement and were defined by soft textured and undulate hills. Cell shapes of semi circle to elongated tuberculate surface ornamentation patterns were observed.

The seed coat surface patterns in Veer, Maharashtra (L3) were tuberculate epidermal cells of non-identical and irregular shapes (Figure 3 *e* and *f*). Cell shapes of semi circle to elongated surface ornamentation patterns were observed. In L4 (Saputara, Gujarat), there were tuberculate epidermal cells having semi circle or semi oval shape. Some cell boundaries had irregular or uneven areas (Figure 3 *g* and *h*). In L5 (Pavagadh, Gujarat), the testa surface ornamentation pattern was non-identical in shape. Few cells showed reticulate with tuberculate pattern. Some epidermal projections had ribbed structures (Figure 3 *i* and *j*).

In addition to the seed coat surface analysis, the micromorphological characters of the endosperm starch crystal surface of *E. superbum* grown under different habitats were also examined by SEM. Accordingly, in Ponmudi, Kerala (L1), examination of powdery endosperm showed plenty of starch grains varying from small to medium sized crystals measuring from 92.30 to 130.50 μm in length and 36 to 90 μm in breadth. Endosperm starch grains were found to be compound granules with highly polygonal and irregular shape. Corners of each crystal appeared as blunt. The surface ornamentations of starch crystals were verrucate-tuberculate with numerous perforations and formed by a continuous membranous layer clustered with globular cells of different sizes (Figure 4 *a–c*).

In Jog falls, Karnataka (L2), endosperm starch grains size varied from small to medium sized crystals measuring 80.46–106 μm in length and 32.2–43.3 μm in breadth. A trend similar to L1 was observed in shape and surface ornamentation pattern of starch crystals in (L2) (Figure 4 *d–f*). In Veer, Maharashtra (L3), the starch grains size varied from small to medium sized crystals measuring 70.24–103.50 μm in length and 38.20–45.40 μm in breadth (Figure 4 *g–i*). L3 resembled L1 and L2 in terms of similar shape and surface ornamentation pattern.

In Saputara, Gujarat (L4), grains size of starch crystals varied from 90.34 to 180.70 μm in length and 53.10 to 84.02 μm in breadth. Surface ornamentation of the crystals exhibited more angular shape at each corner than the other sites (Figure 4 *j–l*). And in Pavagadh, Gujarat (L5), crystal grain size varied from small to medium measuring from 108.24 to 124.20 μm in length and 58.70 to 60.10 μm in breadth. Ornamentation pattern of the crystals surface were observed to be similar in appearance with other locations studied. Locations L4 and L5 exhibited similarity in individual crystal angularity (Figure 4 *m–o*).

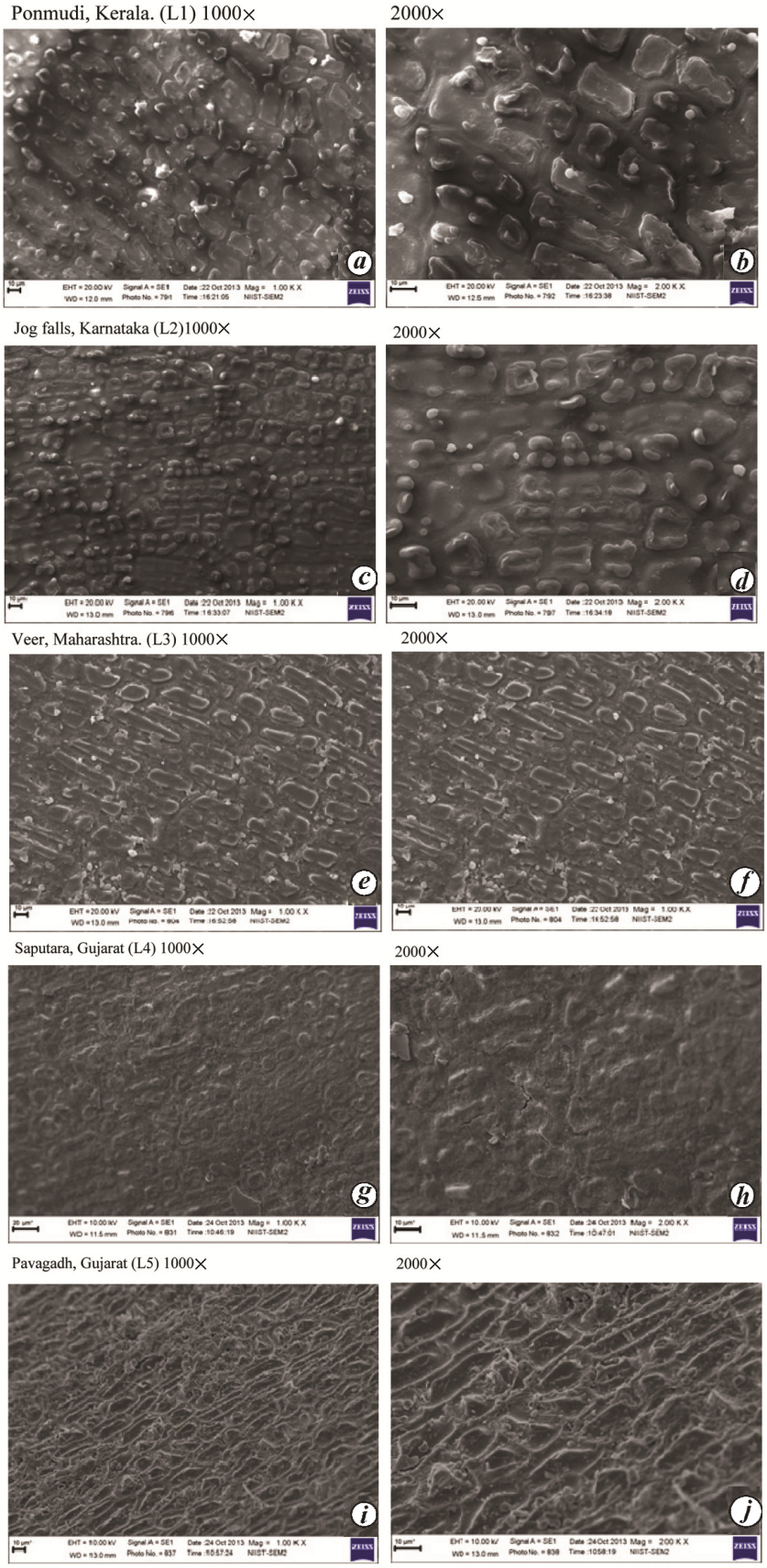


**Figure 3.** SEM analysis of seed coat surface of *E. superbum* under 1000× and 2000× magnifications at different locations (L1–L5).

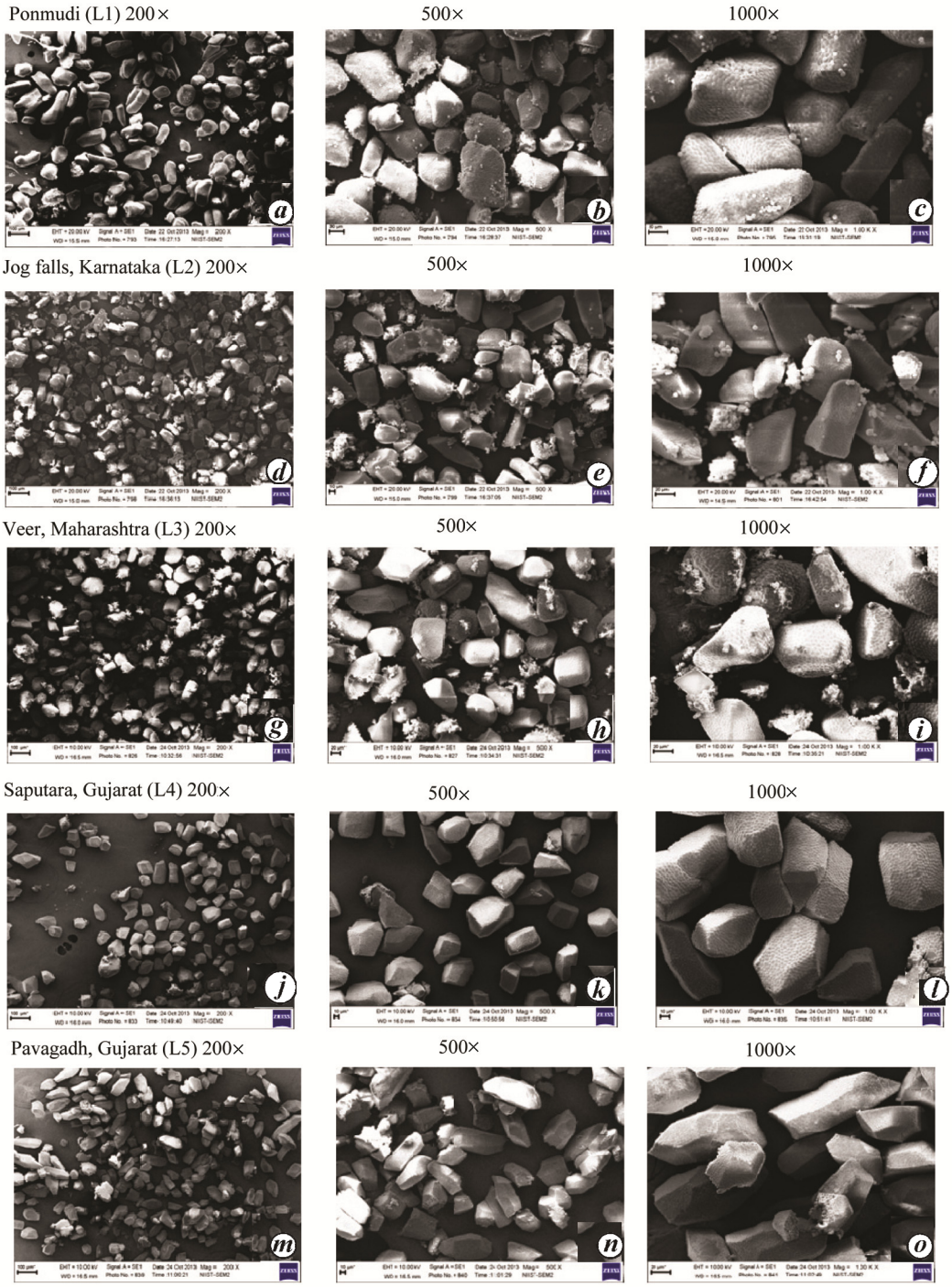


**Figure 4.** SEM analysis of endosperm starch crystals of *E. superbum* at different locations under 200, 500 and 1000× magnifications (L1–L5).

The seeds of *E. superbum* are dark brown, sub-globose but angled by pressure with a harder seed coat. The wild musaceae has fleshy fruits which are dispersed by bats and brown palm civet[11,12]. Seed coat consists of exotesta and mesotesta made from sclerenchyma cells, which offer hardiness to the coat. The seed varies irregularly in shape and colour[1]. Information on the seed size variation within the species is scarce in case of *E. superbum*. Promising variations were recorded for varied natural populations in terms of seed length and breadth. Interestingly, the size of seeds decreases with the increase in latitude from 8°N to 22°N. Ponmudi, Kerala (L1) lies at 8°N and situated in the southern part of Western Ghats which receives more frequent rainfall than the other parts of the Western Ghats. Likewise, Pavagadh, Gujarat (L5) at 22°N lat. receives the lowest rainfall among the studied locations

and thus recorded the least seed size. Seed size, usually expressed as seed weight showing variations within species is often associated with persisting environmental factors, availability of soil moisture and nutrients[13,14]. Hence the different maternal growing climates might have influenced variability in the seed sizes.

Exomorphic seed coat surface examination under SEM revealed considerable variation in the diversity of seed coat's micromorphology. Five different types of tuberculate pattern were observed. All the regions showed minor or major type of projection in seed coat with identical or non-identical patterns. According to Graven[15], seed coats of the Musaceae taxa are almost similar in their main structure. The hard seed coat in Musaceae[16] offers an efficient protection for seeds during maturation, dispersal and protects the embryo and endosperm from potential mechanical injuries.

The endosperm starch crystal appears with two distinct groups. The samples L1, L2 and L3 exhibited similar pattern with blunted corners; whereas L4 and L5 exhibited angular corners on starch crystals. Functionally, endosperm acts as a nurse tissue to support the growth of the embryo in the developing seed. The endosperm of *E. superbum* is formed of parenchyma cells with prismatic crystals of calcium oxalate and plenty of starch grains[7]. Compound starch granules of *E. superbum* are developed from multigranules, as similar to the starch granules observed in Poaceae members[17]. The present study revealed that the starch grains of *E. superbum* were compound polygonal and irregular in shape. The surface ornamentation of starch grains in *E. superbum* seeds were similar to those observed in the other members of Musaceae taxa[15].

In India, *E. superbum* is being neglected because of its scanty and scattered distribution. Information on the genetic variation in these species is not yet been reported. Hence, the present study on the comparative macro- and microscopic variability of seeds grown in the natural conditions from different regions, unveiled the variation in genetic diversity of *E. superbum* in India. The observation on various parameters of seeds showed considerable level of variability with respect to different locations. The latitudinal influence on variation of seed size and microscopic characters of seed coat and endosperm reported in the present study is first of its kind on *E. superbum*.


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