

Anatomical and Micromorphological Features of Desiccation Tolerant Forked Fern *Dicranopteris linearis* (Burm. F.) Underw.

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Abstract Ferns are widely distributed in the tropics and commonly occur in areas of water scarcity such as road open places, road side along the cutting and as evergreen thickets indicating that the species are tolerant to desiccation. The rhizome, petiole and leaf anatomy of the species was examined in terms of anatomical and ultra-structural characteristics of the forked fern that facilitate the establishment of the species in water-scarce environments. The presence of sclerenchyma cells, well developed xylem vessels in rhizome and heavily lignified cells in the cortex of the petiole point towards the study nature of the plant parts. The frond anatomy revealed uniseriate upper and lower epidermis with thick cuticle, lignified bundle

sheath cells surrounding vascular bundle. Endodermis uniseriate with casparian strips. SEM analysis of the abaxial and adaxial surface of frond reveal specific features which confer adaptation to desiccation such as restriction of stomata to lower epidermis, thick epicuticular wax deposits and velvety hairy indumentums. Micromorphological results of desiccated fronds revealed the tolerance of the fern against the induced abiotic stress.

Keywords Forked fern, *Dicranopteris linearis*, Desiccation tolerance, Lignified cells, Epicuticular wax.

Introduction

The morphological and anatomical features of plant species is used as an indicator of the environmental conditions in which they survive. Desiccation tolerant plant species and their physiological and morphological adaptations has been a subject of research. Ferns represent one of the oldest vascular groups of plants and the second most diverse group of vascular species followed to angiosperms. They show a wide variety of adaptive strategies against drought or desiccation [1]. To unravel fern's success to survive under extreme conditions, studies have been focused on their eco-physiological potential towards stress tolerance in terms of their sporophytic and the gametophytic generations. Proctor and Pence [2] recorded about 64 species of ferns exhibiting desicca-

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tion tolerance and approximately less than 1% of ferns tolerate extreme drought.

In the present study morphological and anatomical features of the drought tolerant forked fern was conducted in order to identify their tolerant features. This was followed by SEM analysis of their adaxial and abaxial frond surface in order to provide an insight of micromorphological characters of the fern. *Dicranopteris linearis* (Burm. F.) Underw. commonly called as forking fern is distributed all over Western Ghats and low lands. It is an evergreen perennial fern. Dixit [3] has listed two species of *Dicranopteris* in India viz. *D. curanii* Copel. and *D. linearis* (Burm. F.) Underwood. Manickam and Irudayaraj [4] reported the presence of almost 12 varieties of *D. linearis* of which var. *montana*, *sebastiana*, *brevis* and *tenuis* has been found in South India growing on fully exposed clearings or roadsides.

Innate or induced biochemical, physiological and morphological mechanisms are seen in plants that survive in water scarcity areas [5, 6]. Several structural characteristics may contribute to survival in such conditions such as reduction of leaf surface, increased thickness of epidermal cell walls, extreme cuticularization, high trichome density and sunken stomata located on the abaxial surface [7, 8]. In addition to these features, the presence of water storage tissues, high sclerenchyma tissue density and mucilage storage result in improved mechanical conditions and/or reduced tissue damage [8].

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Materials and Methods

Plant material

Dicranopteris linearis (Burm.F.) Underw. commonly known as forking fern belongs to Gleicheniaceae and is widespread in tropical and subtropical regions of the world. It grows horizontally at ground level with stalked compound fronds. It is found extensively

growing along the road cuttings in shaded or open areas where water availability is scarce. The sporophyte of the fern reaches up to 3 m tall, with characteristic dichotomously divided leaves and rhizome is several meter long creeping, brown and covered with septate, branched hairs.

Desiccation treatment protocol

Fresh *D. linearis* was fully hydrated and equilibrated in a controlled environment chamber for 48 h at 20°C and a radiant flux intensity 75 $\mu\text{M}/\text{m}^2/\text{s}$. The samples were desiccated in a desiccator over polyethylene glycol (PEG) in a controlled environment chamber using the same light and temperature regimes as described above for 96 h.

Anatomical studies

Fully expanded leaves were harvested and fixed in 70% FAA [9]. Cross sections of the thizome, the petiole and median region of the pinnae were made. Sections were cleared using commercial sodium hypochloride (50%), neutralized and stained with safranin, subsequently mounted in glycerin (50%) and viewed through light microscope. Lignin was localized by phloroglucinol-HCl test [9].

Scanning electron microscopy

Leaf surface micro morphology was analyzed by scanning electron microscope as per the method of Wakui et al. [10] using ZEISS EVO18, Varian Vacuum Technologies, Ion 25 Scanning Electron Microscope.

Results and Discussion

Morphological and anatomical traits of the fern

The terrestrial fern *Dicranopteris linearis* possess typical characteristics of plants growing in tropical drought conditions. The creeping rhizome which helps the plant to form dense extensive thicket is covered with septate, branched hairs with erect petiole



Fig.1. C S of petiole showing lignified cells stained with phloroglucinol-HCl

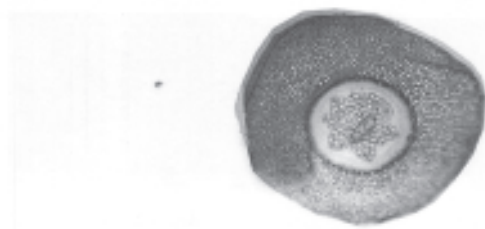


Fig.2. Cross section of rhizome showing lignified cells stained with phloroglucinol-HCl

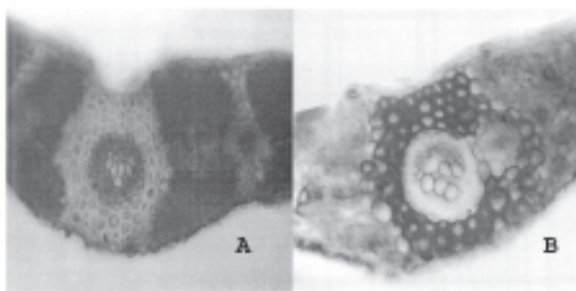


Fig.3a & b. C S of leaf blade showing localized lignin in the cells.

Fig. 1. CS of petiole showing lignified cells stained with phloroglucinol-HCl. **Fig. 2** Cross section of rhizome showing lignified cells stained with phloroglucinol-HCl. **Fig. 3a & b.** CS of leaf blade showing localized lignin in the cells.

arising at regular intervals. The surface of the petiole is glabrous and it is woody in nature. Localization of lignin in the cross section of the petiole, showed highly lignified cells in the cortex (Fig. 1). To analyze the lignin accumulation in the cells lignin staining dye was used. Those cells contain lignin will be stained deep red color; mean while, non-lignin cells remain colorless. Phloroglucinol-HCl-lignin reaction

(Wiesner reaction) revealed intense red color whereas, the parenchyma tissues remained colorless. Thus, the sturdy nature of the petiole is due to the high lignification. The CS of the rhizome also revealed the presence of sclerenchyma cells and lignified xylem vessels (Fig. 2). Further sectional views reveal the well developed vascular system for their efficient water transporting system. Tao et al. [11] compared stone

cell distribution among *Pyrus* species using lignin localization technique. Similarly, Leroux et al. [12] analyzed lignin deposition in *Equisetum ramosissimum* using Wiesner reaction. Sano et al. [13] also employed the similar technique in rose prickles.

The characteristic dichotomously divided fronds have large lamina bearing deeply pinnate blades. The fronds are hard, thick, leathery and glabrous in nature. In the cross section through the leaf blade (Fig. 3a) shows distinct uniseriate upper and lower epidermis. Thick waxy cuticle lining is found on both the epidermis. The dorsiventral mesophyll seems to be tightly packed and made up of chlorenchyma cells. The vascular bundle is concentric i.e.; xylem surrounded by phloem and has also a thickened endodermis with casparian strips. Lignin staining reveals the presence of 3–4 layers of lignified cells surrounding the vascular bundle (Bundle sheath) (Fig. 3b). The presence of compactly arranged mesophyll cells and lignified cells suggests that the fern sporophytes has specific adaptation to drought conditions.

Mazumdar [14] reported the presence of phytolith among ferns diagnosed 168 species of ferns all along the different parts of the plant body. Similarly, the presence of phytoliths has also been noticed in the fronds of *D. linearis*. Biological occurrence of silicon as phytolith in plants has been correlated with mechanical support, reduction of water loss, protection from herbivores, prevention of entry of pathogens, protection from metal toxicity, root elongation, increased capture of light for photosynthesis and also cooling of leaves [15]. Currie and Perry [16] found that silicon protects the plants from various biotic and abiotic stresses via inducing the chitinases, peroxidases, polyphenol oxidases enzyme and also flavonoid phytoalexins pathway related to stress resistance, but the detailed mechanism has yet to be fully elucidated.

Eventhough the presence of silica bodies/ phytolith was not visible through light microscopy, the cells of the epidermis especially at the region of vascular bundle seems to be extremely thickened (Fig. 3a) resembling stegmata of two desiccation tolerant fern species *Anemiatomentosa* var. *anthriscifolia* and *Anemivillosa* (Schizaeaceae) [17]. Stegmata are struc-

tures formed inside the cells by the incrustation of silica on the cell walls. According to Prychid et al. [18] the presence of stegmata in organs helps to maintain rigidity and provides resistance against pests and pathogens. When present in the leaf epidermis, they reduce transpiration, thus improving water-use efficiency. The presence of stegmata in the epidermis has been associated with xeromorphic characteristics in orchids [19].

Morphological and micromorphological features of desiccated (96 h) and fresh fronds of *Dicranopteris linearis* were compared to evaluate the changes happening in the frond as a result of desiccation and thereby understanding its structural features of tolerance. The SEM (Scanning Electron Microscopic) studies of the micromorphological features of fresh and desiccated fronds showed unique features indicative of the fern's desiccation tolerance capacity. Cuticular ridging and stomatal distribution showed wide variation between the epidermis of the frond. Stomata were absent on the dorsal surface. The upper surface of the fresh and desiccated fronds showed electron dense areas. This nature increased with desiccation. The surface view of the upper epidermis from hydrated and dry frond is shown in Figure 4a and 4b, respectively. Cuticular ridges on the upper surface of the desiccated frond appeared more prominent with deep furrowed pattern. It appears that the upper epidermal cell wall has undergone folding during desiccation, resulting in a more wrinkled appearance of the adaxial frond surface, when compared to control. Epicuticular wax crystal deposits were apparent on adaxial surface. The wrinkled pattern was more visible on the desiccated frond (Fig. 4b) indicating more deposition on cuticular wax with desiccation, and thereby desiccation tolerance. According to Cameron et al. [20] cuticular wax deposition and their composition affects drought tolerance of tree tobacco (*Nicotiana glauca*) i.e.; total leaf cuticular wax load increasing with higher dehydration stress periods. Increased cuticular wax deposition was noticed in *Arabidopsis* plants under drought stress and interestingly, the gene regulating the transcription factor MYB94 showed over expression [21]. Reduction in the biosynthesis of β -diketone, failure of development of glaucousness, and reduction of cuticle permeability in common wheat, mutant for the gene determining wax pro-

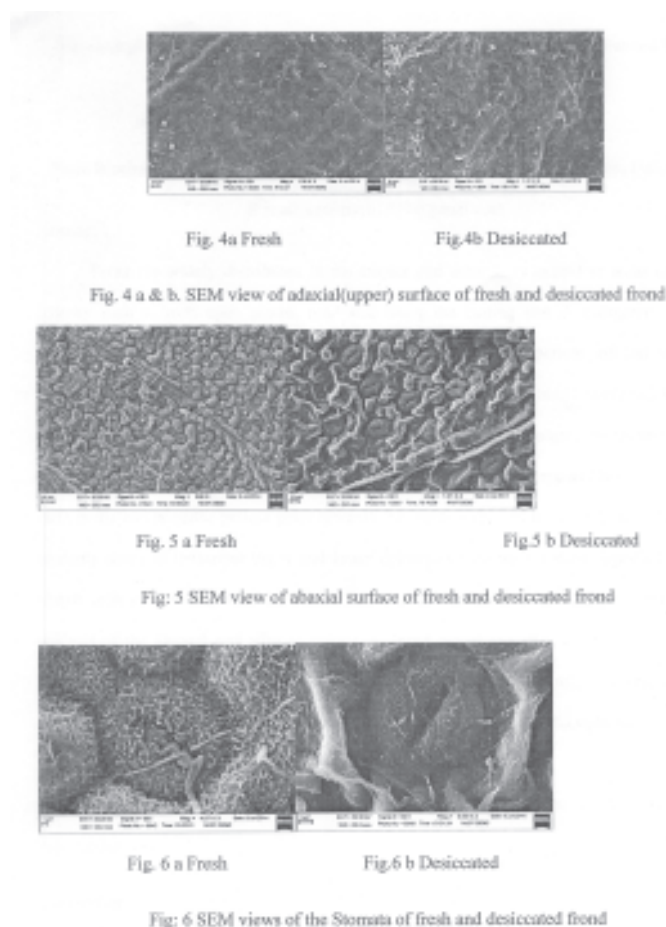


Fig. 4a & b. SEM view of adaxial (upper) surface of fresh and desiccated frond. **Fig. 5.** SEM view of abaxial surface of fresh and desiccated frond. **Fig. 6.** SEM views of the stomata of fresh and desiccated frond.

duction has been reported by Zhang et al. [22] reiterating the importance of cuticular wax in drought and heat tolerance by the crop. Thus the increase of wax content is associated with enhanced drought tolerance in many plants.

The abaxial surfaces of the frond have large number of stomata. Stomata were restricted to the lower surface only. The increased stomatal number and its restriction towards lower surface were common among ferns growing in the xeric environment. The distribu-

tion pattern is also irregular i.e.; the stomata were located at close proximity to each other. The guard cells were with their long axis lying almost parallel to the veins. Stomatal type as described by Mehra and Soni [23] is filicineanpolocytic. However, Sen and Bhunia [24] redefined it aspolocytic and mesoperigenousanomocytic stomata. Stomata were found to be seated in small pits surrounded by thick rims formed by the bulged subsidiary cells around it. The number of subsidiary cells was limited with a large semilunar conspicuous bulging. The stomatal opening with the

large guard cells is seems to be covered up by this single semilunar subsidiary cells, while small bulged spherical cells were also visible which were shared by the adjacent stoma (Fig. 5a).

The bulged subsidiary cell in the desiccated frond shows shrinkage, while the stomata remain unaffected even after desiccation as seen in Figure 5b. The lower leaf surface showed characteristic velvety hairy indumentums covering the surface of both subsidiary and guard cells (Fig. 6a). These hairy indumentums are papillate glandular structures secreting waxy compounds helping indirectly to tolerance against desiccation. The disappearance of the papillate hairs in the desiccated frond was clear in the Figure 6b. Comparison of the magnified view of the stomatal opening of the fresh and desiccated frond (Fig. 6) shows that the large guard cells remains intact without any structural distortion even after desiccation. Thus, the fern exhibit characteristics that seems to be adaptive to water stress, such as glaucous nature, epidermis covered by a thick waxy cuticle, lignified cells surrounding the vascular bundle, presence of sunken stomata and papillate hairy indumentums. These leaf characteristics help the fern to protect the photosynthetic tissues against damage from excessive water loss, extreme temperatures, and UV light.

Conclusion

The forked fern profusely grows along the dry habitats. The morphological, anatomical and micromorphological features showed an array of special features that confer its capability to establish and grow in water scarce areas. The occurrence of large amount of sclereified cells play a key role in the leaf texture towards tolerance. This was further validated by Wiesner test for lignin. Further the cell wall architecture as revealed by SEM corroborates with the desiccation tolerant potential of the fern. Future studies on the physiological and biochemical properties will give more insight into its extant of desiccation tolerance capacity.

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