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Testa characterization of indigenous and widely adapted exotic species of *Cupressus* L. growing in Central (Kumaon) Himalaya

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Micromorphological and anatomical features of seed coat of two well adapted exotic species of *Cupressus*, viz., *C. glabra* Sudw. and *C. arizonica* Greene and one indigenous species, i.e. *C. torulosa* D. Don growing at an altitude of 1500-2700m in Kumaon Himalaya were studied under scanning electron microscope to evaluate taxonomically valuable characters of the testa. Significant differences in the shape of hilum, spermoderm pattern and anatomy of seed coat were observed. The three species exhibited three different types of hilum region, i.e. slightly curved and cylindrical (*C. glabra*), sunken and obovate (*C. arizonica*), and large, raised and widely obovate (*C. torulosa*). Basic spermoderm pattern in the three species was scalariform with some distinction in secondary structure. In transections of the seed coat, shape, size and arrangement of cells in both the exotic species were more or less similar while in *C.torulosa*, these were completely different. The study underlines the potential of testal morphology as a taxonomic feature in the genus.

Key-words—Ultra-structure, Seed coat, *Cupressus* spp., Exotic, Indigenous.

INTRODUCTION

CUPRESSUS L., commonly known as cypress (Eckenwalder, 1993) belongs to the family Cupressaceae Rich. ex. Bartling 1830. It has about 28 species distributed in the Old World from the Mediterranean region to China, and in the New World from the western United States to Mexico, Guatemala and Costa Rica (Wolf, 1948; Little, 2006). The genus has been extensively investigated and revised for its taxonomy and embryology (Dallimore, 1931; Wolf, 1948; Dallimore & Jackson, 1966; Florin, 1967; Eckenwalder, 1976; Little, 1970, 2004, 2006; Dogra, 1980, 1984; Silba, 1981, 1994, 1998; Watson & Eckenwalder, 1993; Rehfeldt, 1997; FU *et al.* 1999; Farjon, 2001, 2005; Little *et al.*, 2004). However, no comprehensive studies on ultra-structure of seed coat of indigenous and exotic *Cupressus* species have ever been made. Scanned seed coat characters have proved universally useful in clearing systematic confusions, establishing evolutionary relationships, segregating hybrids and in palaeobotanical studies (Lersten, 1979; Gopinathan & Babu, 1985; Rejdali, 1990). *Cupressus* has some confusion regarding its two very closely related species namely *C. glabra* and *C. arizonica*, the only species equally well adapted to Central Himalayan region. There is a necessity of identifying additional parameters for distinguishing and delimiting these species. Hence, present investigations were carried out to elucidate in detail micromorphological and microanatomical characteristics of testa of the two widely adapted and high yielding exotic species, viz., *C. glabra* and *C. arizonica* and one indigenous *C. torulosa*.

MATERIAL AND METHOD

Mature seeds of the indigenous *Cupressus* species, i.e. *C. torulosa* D. Don. and the two widely adapted exotic species i.e. *C. arizonica* Greene and *C. glabra* Sudw. were randomly collected from trees growing in Central (Kumaon) Himalaya at 1500-2700m altitude. Ten healthy seeds of comparable shape and size of each species were selected from the bulk seeds extracted from twenty cones and collected randomly from ten trees of each species. Seeds were cleaned in 90% ethanol, air-dried and mounted on aluminium stubs with glue and coated with palladium in a sputter coater. For uniformity, the middle part of the seed and the surface adjacent to the hilum were scanned. For anatomical observations, seeds were sectioned longitudinally through the mid plane with a sharp razor blade, coated and mounted in the same way as above. Each sample was scanned under JSM-35C scanning electron microscope at an accelerating voltage of 15 KV.

TAXONOMIC DESCRIPTION

Cupressus arizonica Greene 1882 popularly known as 'Arizona Cypress' or 'Rough Barked Arizona Pine' occurs wild in the mountains of Arizona, New Mexico, Texas, California and Mexico (Vidakovic, 1991; Lanner, 1999). In India it is planted in the wild in northwest central Himalaya. Seeds are medium to dark brown in colour and 3.5 to 4mm long.

C. glabra Sudworth 1910 also known as 'Smooth Barked Arizona Cypress' is confined to central western Arizona at 975-2652 m altitude. It is widely cultivated in U.K. and usually

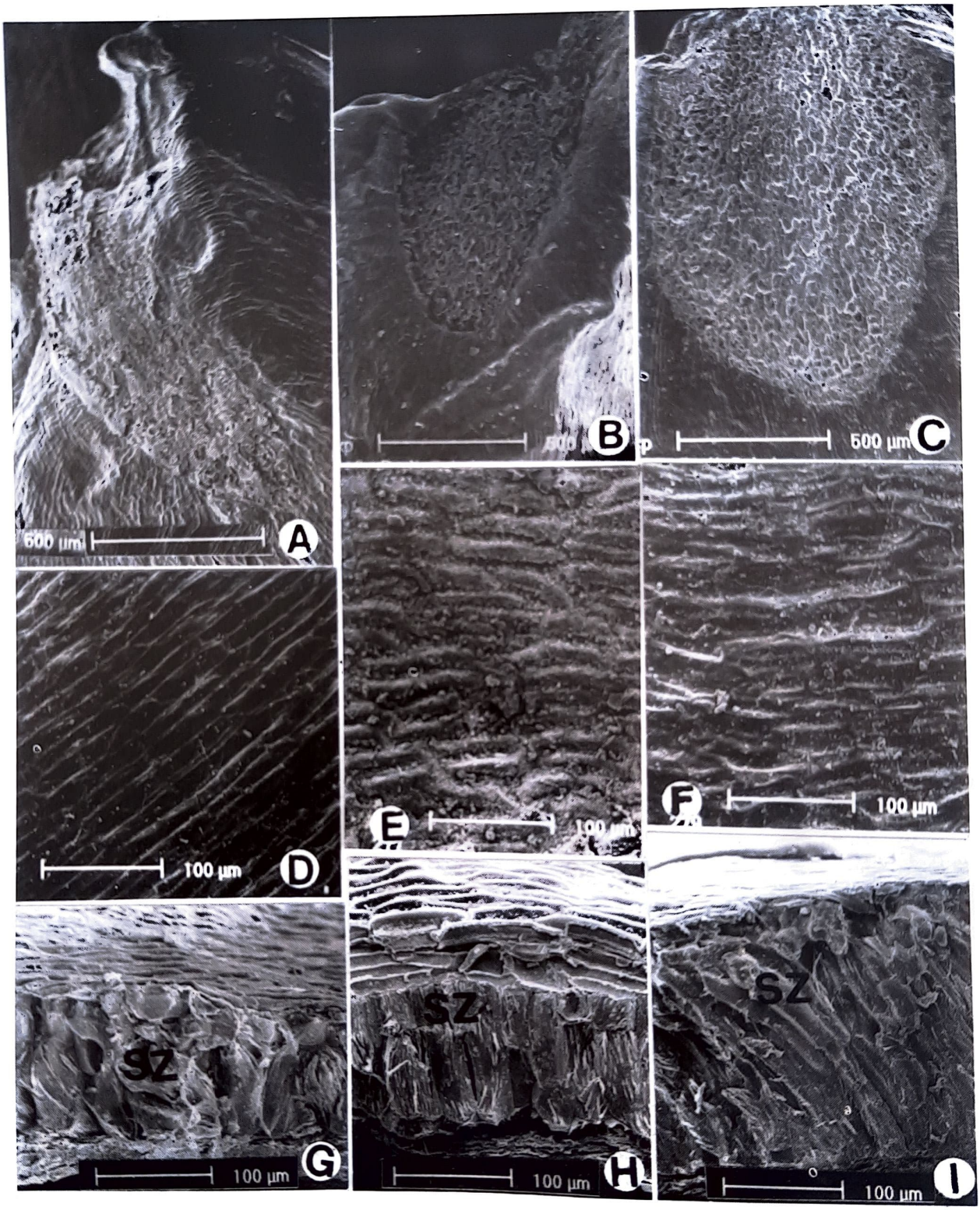

PLATE-1

Figures A-I :

A. *Cupressus glabra*–A part of seed showing hilum. B. *C. arizonica*–A part of seed showing hilum. C. *C. torulosa*–A part of seed showing hilum. D. *C. glabra*–A part of seed showing diagonally arranged rows of spermoderm cells. E. *C.arizonica*–Seed coat showing spermoderm pattern. F. *C.torulosa*–Seed coat showing spermoderm pattern. G. *C.glabra*–T.S. of seed coat. H. *C. arizonica*–T. S. of seed coat. I. *C.torulosa.*–T.S. of seed coat. SZ= sclerenchymatous Zone.

considered as a variety of *C. arizonica*. It differs from the latter in having smoother, reddish to greyish bark exfoliating in thin flakes and strips during its lifetime, glandular and glaucous foliage, large cones and glaucous seeds. Many trees grown as *C. arizonica* in Britain were later referred to *C. glabra* (Dallimore & Jackson, 1966). However, on account of the wide distribution some taxonomists have concluded *C. arizonica* as the basic or parental species of a group of species including *C. glabra*, despite its distinguishing characteristic features (Wolf, 1948; Little, 1970). Silba (1981) and Farjon (1998) revised *C. glabra* as a variety of *C. arizonica* and stated the possibility of its being a hybrid between typical *C. arizonica* and *C. bakeri*. According to the data based on amplified polymorphic DNAs, Bartel *et al.*, (2003) suggested that these taxa might be better recognized at the specific level rather than varietal level. In India, *C. glabra* is widely planted in Central Himalaya in Kumaon and Garhwal regions. It is a highly fertile exotic species as compared to *C. arizonica* (Sahai, 1990). Seeds are light pale brown, oblong, larger than *C. arizonica*, *i.e.*, about 4-5mm long.

C. torulosa D. Don 1824 known, as 'Bhutan Cypress' (Silba, 1986) or 'Himalayan Cypress' (Vidakovik, 1991), is indigenous to the outer ranges of Western Himalaya up to Nanga Parbat-base camp trial in Pakistan. It also occurs in China, Vietnam (Vidakovic, 1991) and Argentina (Malizia *et al.*, 2000). It can be recognized by slender whip-like divisions of the flattened branching systems and by its uniform leaves. Seeds are triangular, reddish to blackish brown and 2.5- 3mm long.

OBSERVATIONS

A uniform plane of seed was used for each seed sample for the observation of following characteristics.

Micromorphology–The three *Cupressus* species display three different types of hilum region i.e. slightly curved and cylindrical with deposition in *C. glabra*, sunken and obovate with reticulate surface in *C. arizonica* and raised and widely obovate with thin reticulate surface in *C. torulosa* (Pl 1, Figs A, B, C). Basic spermoderm pattern in all the three species is scalariform (having small fairly regular cross bands) with some variations in secondary structures. In *C. glabra*, cells are prominent, evenly distributed in the diagonally arranged rows and attached with each other by transverse walls. In *C. arizonica*, each cell is somewhat cylindrical, arranged in irregular and discontinuous horizontal rows and separated by its cell boundary but in *C. torulosa* cells vary in shape and are arranged irregularly in the discontinuous rows (Pl. 1, Figs D, E, F).

Microanatomy: In transections of seed coat, shape, size and arrangement of cells in both the exotic species are more or less similar. Multi-layered outer surface has been noticed in both the exotic species. Cells of outer surface are lignified and compact in *C. glabra* (Pl. 1, Figs G, H)) whereas, in *C. torulosa*, they are indistinct due to heavy deposition (Pl. 1, Fig. I). Inwards from the outer surface, a broad zone of sclerenchyma cells is present. The cells of this zone are irregularly arranged in a bundle in both the exotic species but are thin, papery and discontinuous in *C. arizonica* whereas, in *C. glabra* they are thick and compact (Pl. 1, Figs G, H). The sclerenchymatous zone in *C. torulosa* is broader than the other two species and consists of very thin, irregular, vague and indistinct cells. The cells of innermost layer are slightly lignified and flattened (Pl. 1, Fig. I).

DISCUSSION

In the present study the finer characteristics of seed coat have been investigated with SEM techniques. SEM allows separation of species that apparently look very similar (Brisson & Peterson, 1976). Though in transections, spermoderm pattern and hilar region of seeds of three species of *Cupressus* showed some similarities with each other, seeds of all the three taxa have their own hilum, seed coat pattern and anatomical characteristics. Similarly, recent analysis using morphological, chemical and multiple lines of molecular/genetic data found sufficient distinct characters in the different populations of this taxa, which enables to identify them as discrete species (Little, 2004, 2006). In transections, stony layer of exotic species is quite different in *C. torulosa*. Although, seed characters are genetically constant, some ecological factors may have operated on the structure of seed coat that help in establishing a link to their native environment as observed during the study of exotic pines (Sahai, 1994). For a precise conclusion in this direction a much broader sample needs to be studied. Since present investigations were made on the first generation of *C. arizonica* and *C. glabra* introduced in Central Himalaya, results thus obtained are meaningful in distinguishing the species, correlating the exotic and indigenous species and searching for clues related to their native habitat. The present study supports statements of Heywood (1971), Whiffin and Tom (1972), Gutterman and Heydeker (1973) and Barthlott (1981) that scanning electron microscopy of seed surface is useful for interpretation of different aspects of the seed, and can be used in distinguishing closely related taxa, in highlighting the interrelationship of indigenous, widely growing and well adapting exotic species of the genus *Cupressus*.

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