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#### Research Article

# Seed and seedling morphology of two near threatened Indian species: *Cryptomeria japonica* and *Cupressus cashmeriana* (Cupressaceae *sensu lato*)

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**Abstract**: Seed and Seedling morphology of two species of Cupressaceae – *Cupressus cashmeriana* Royle ex Carriéreand *Cryptomeria japonica* (Thunb. Ex L.f.) D. Don have been studied using light microscope (LM) and scanning electron microscope (SEM). Both the species are categorized as Near Threatened (NT) in IUCN Red List Category (2013). For characterization of seed, different parameters like shape, size, base, apex, surface were considered. For the study of seedlings, the parameters like germination pattern, root, hypocotyl, number of paracotyledons, number and nature of first leaf and subsequent leaves have been characterized. The seed and seedling characters as studied in this investigation are important as taxonomic markers for identification purpose.

**Keywords:** Seedling morphology, identification, *Cupressus cashmeriana* Royle ex Carriére and *Cryptomeria japonica* (Thunb. Ex L.f.) D. Don.

#### Introduction

Cupressus cashmeriana Royle ex Carriére and Cryptomeria japonica (Thunb. Ex L.f.) D. Don belong to family Cupressaceae sensu lato. Cupressus cashmeriana Royle ex Carriére is an evergreen tree, native to the eastern Himalaya in Bhutan and Arunachal Pradesh in India. It is categorised as Neat Threatened species in The IUCN Red List of Threatened Species 2013 (Zhang and Christian, 2013, http://dx.doi.org/10.2305/IUCN.UK.2013-1.RLTS.T32311A2813777.en). It grows moderately high altitudes ranging from 1250 to 2670 mts elevation. It possesses horizontal main branches and pendulous side branches, with closely appressed, scale-like, opposite decussately arranged leaves (Page, 1998). The male cones are borne terminally, whereas the female cones are axillary on the drooping branches. The male cone is composed of opposite decussately arranged microsporophylls on the cone axis and the female cone is composed of 4 pairs of ovuliferous – bract scale pairs. Both the male and female cones are globose and greenish vellow in colour becoming dark brown when matured. Several reports of medicinal properties of this plant have been made. In vitro antiinflammatory, antioxidant and antimicrobial activities of essential oils from the leaves and twig parts of this plant have been reported (Yc, S., et al., 2015). Phytochemical studies have reported the presence of a flavanol diglycoside in the leaf extracts of Cupressus cashmeriana (Khabir et al., 1987). Several other investigations have been carried out in morphology (Maerki, 2014), cytology (Hair, 1968),

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Assistant Professor, Department of Botany, Surendranath College, 24/2, Mahatma Gandhi Road, Kolkata- 700009., West Bengal, India. E-mail: sonyyrr@gmail.com phylogenetic studies (Damon et al, 2006), molecular studies (Rushforth, 2003), anatomy (Romàn-Jordàn et al., 2016) and palynology (Danti et al., 2010) for Cupressus cashmeriana.

Cryptomeria japonica (Thunb. Ex L.f.) D. Don, formerly belonging to family Taxodiaceae, now belongs to Cupressaceaes. It is regarded as a monotypic genus, endemic to Japan, have been introduced to other countries like India, It is also categorised as Near Threatened (NT) species in The IUCN Red List of Threatened Species 2013 (Thomas, Katsuki and Farjon, 2013, http://dx.doi.org/10.2305/IUCN.UK.2013-

1.RLTS.T39149A2886821.en), which grows at elevations ranging from 600 mts to 1200 mts. It is a large branched evergreen tree, with fibrous, brown bark that peels off in longitudinal strips, with awlshaped spirally arranged leaves. They are monoecious with male and female cones usually borne on different branchlets, sometimes on the same branchlet. Male cones form clusters of more than 10 in number. Each male cone bears 15-25 spirally arranged microsprophylls. Female cones are globular, about 20 mm in diameter, pale brown in colour, with 20 - 30 spirally arranged wedge-shaped scales; the bract and ovuliferous scales are fused except at the tip, that ends up in a recurved process. This tree is economically and medicinally very important. Its timber is extremely fragrant, weather and insect resistant, durable and therefore used extensively for furniture and building. This tree



exudes a resin containing cryptopimaric acid and phenolic acid. The bark also yields 6% tannin. The chemical composition of Cryptomeria japonica essential oil (CJE) has revealed excellent antifungal activity (Cheng et al., 2005) and antibacterial activities against acne producing Propionibacterium acnes and Staphylococcus epidermidis (Yoon et al, 2009). Mosquito larvicidal activities have been reported in case of extracts from red heartwood-type (Cheng et al., 2008) and the black heartwood-type Cryptomeria japonica (Gu et al., 2009). Other than the investigations on the medicinal and economical properties, reports have been made on morphology (Hashimoto, 1990), anatomy (Abe et al., 2003, Tadooka et al., 2005, Kitin et al., 2009), embryology (Lawson, 1904), palynology (Panzani et al., 1986, Uehara et al., 2000, Suárez-Cervera et al., 2003), DNA variation (Kado et al., 2003), AFLP and CAPS linkage maps (Nikaido et al., 2000), tissue culture techniques (Ogita et al., 1999), etc.

But seedling morphological studies are not emphasized for these two taxa. The systematic values of seedling of flowering plants have been stressed by different authors abroad (Duke, 1969; Burger, 1972; de Vogel, 1980). Paria and his group reported seedling structures as a key characteristic for systematic as well as phylogenetic studies of angiosperms (1986, 1996, 1999, 2002 and 2014). Studies on seedlings of gymnosperms have been carried out by Lubbock (1892), Hill and Fraine (1914), Philipson and Molloy (1990) and Donald and Joseph (1991). Since seedling morphological characters are genetically stable, they can play an important role in identifying the taxa. The medicinally and economically useful threatened plants may be identified and thereby, conserved at the juvenile stage by using the knowledge of seedling identification. In an attempt towards this, the seedling morphology of two Near Threatened (IUCN, 2013) species of Cupressaceae - Cupressus cashmeriana Royle ex Carriére and Cryptomeria japonica (Thunb. Ex L.f.) D.Don - is taken up in the present study.

#### **Material and Methods**

In the present investigation, seedlings of Cupressus cashmeriana Royle ex Carriére and Cryptomeria japonica (Thunb. Ex L.f.) D.Don were collected from natural habitats of various areas of Darjeeling district, West Bengal and Pelling, Sikkim. The seedlings of various stages were studied by using light microscope (LM) and scanning electron microscope (SEM). Seeds were also taken from the seed cones collected from identified adult plants and grown in the nursery of temperate climate. The different developmental stages of raised seedlings were considered. Both the raised seedlings from the nursery and those collected from natural habitats were studied and compared. Similar data on seedling morphology were noted in both the cases.

At least ten to fifteen specimens of different growth forms were studied taken from different habitats and the nursery, which revealed the uniform features of seedlings. The specimens showing various stages were documented in the form of herbarium sheets, which have been deposited in the Calcutta University Herbarium (CUH). The description of seeds were prepared in consultation with Martin (1946), Martin and Barkley (1961) and Corner (1976). The description of seeds were prepared in consultation with Bojňanský and Fargašová (2007). The morphology of seedlings was described following the terminology as proposed by Burger (1972), Hickey (1973), Vogel (1980), and Radford (1986). For method of description of seedlings, Lubbock (1892), Vogel (1980), Mukhopadhyay et al. (2013) and Paria (2014) were followed. The seedling of both the taxa were placed on glass slide and observed under binocular stereomicroscope (Wild M3). The morphological characters of seeds were noted using different magnifications and documented by taking photographs using Nikon coolpix S7000 digital camera. The scanning electron micrographs were taken at the SEM laboratory, Centre for Research in Nanoscience and Nanotechnology (CRNN), University of Calcutta, Kolkata, West Bengal. Paracotyledons and first leaves were previously desiccated and cleaned with absolute alcohol. The cleaned and dried samples were directly mounted on the adhesive tapes, placed on the stabs and subsequently were palladium gold coated. Surface patterns were studied under different magnifications using CARL ZEISS EVO 18 Special Edition scanning electron microscope. Photographs were taken by selecting the appropriate fields of the specimens.

#### **Results**

Cryptomeria japonica Thunb. Ex L.f.) D.Don Seed Description: Seed shape irregular, ellipsoid, trigonous, dorsal side convex, ventral side flattened, with wings. Dimension 5-8 x 2-3.2 mm. Apex emarginate. Base attenuated. Surface smooth with raised triradiate ridge on dorsal surface. Colour lustrous dark brown, membranous wings pale. (Fig 1A).

Seedling Description: Seedlings epigeal. phanerocotylar. Tap root tapering downwards, 1.4 -6.6 cm, flexuous; unbranched at early stages with 3-7 thin, pointed lateral roots, flexuous; light vellowish in colour. Hypocotyl shortly elongating, 1.8 - 2.5 cm, erect, straight, terete, herbaceous to semiwoody, pale green, glabrous, nonpubescent. Paracotyledons three, equal, whorled, ascending, fleshy, glabrous, persistent up 45 leaves stage, exstipulate, dark glaucous green, sub-sessile; blade linear  $(0.9 - 1.2 \text{ cm} \times 0.2 \text{ cm})$ , apex acute, base subcuneate, margin entire; presence of a single prominent midrib. First leaves simple, cauline, spirally and closely arranged on the main stem, three per node, smaller than the paracotyledons, exstipulate, deep glossy green on upper surface, paler underneath, glabrous, sessile; blade linear to awlshaped, triangular in section, flattened or slightly concave above, convex beneath,  $(0.6-2.5~\rm cm~x~0.1-0.2~cm)$ , apex acute, base sub-cuneate, margin entire, presence of a single prominent midrib. Subsequent leaves simple, triangular to awl shaped, all other characters same as first leaves except larger in size,  $0.7-3.2~\rm cm~x~0.2-0.3~cm$ . (Fig. 1B, C, 3A, 4)

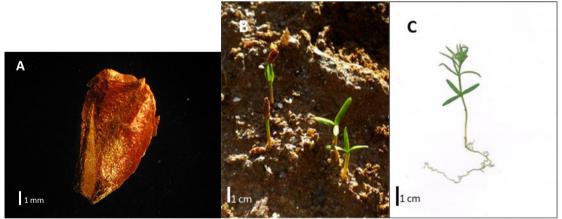
**SEM Characters**: *Paracotyledons*— waxy deposition on both upper and lower surfaces, with unicellular unbranched hairs (8  $\mu$ m - 9  $\mu$ m) on upper surface (Figs 6 and 7). *First leaf* — amphistomatic, stomata present on the upper surface, are rectangular, dimension 20  $\mu$ m — 28  $\mu$ m × 10  $\mu$ m — 22  $\mu$ m, stomata that are present on the lower surface are arranged in vertical rows, rectangular, dimension 22  $\mu$ m — 30  $\mu$ m × 10  $\mu$ m — 25  $\mu$ m, often stomata openings have waxy occlusions; few unbranched uniseriate epidermal hairs (14  $\mu$ m — 18  $\mu$ m) with acute tips and swollen bases present on upper surface with thick epicuticular waxy deposition in form of crystalloids and small tubules. (Figs. 8 and 9).

#### Cupressus cashmeriana Royle ex Carriére

**Seed Description**: *Seed shape* irregular, ellipsoidal, compressed lateral, with indistinct wing-like margin. *Dimension* 4-7 x 3-4 mm. *Apex* truncate. *Base* flattened. *Surface* Smooth ventral surface, with slightly raised median ridge on dorsal surface. Colour lustrous brown, membranous wings pale. (Fig 2 A)

Seedling Description: Seedlings epigeal, phanerocotylar. Tap root tapering downwards, 2.3 -3.9 cm, subflexuous, unbranched at early stages with 1-2 very thin, lateral roots, flexuous; green in colour. Hypocotyl shortly elongating, 2.0 - 2.4 cm, erect, straight, terete, herbaceous, dark green at upper halfbecoming paler at lower half, glabrous, nonpubescent. Paracotyledons two, equal, opposite, ascending, fleshy, glabrous, persistent up to 72 leaf stage, exstipulate, dark glaucous green, subsessile; blade linear  $(0.8 - 1.1 \times 0.1 \text{ cm})$ , apex acute, base round, margin entire; presence of a single prominent midrib. First leaves simple, two per node, opposite decussately arranged with respect to paracotyledons, smaller than paracotyledons, exstipulate, deep green on upper surface, paler underneath, glabrous, sessile; blade narrowly linear  $(0.6 - 0.8 \times 0.1 \text{cm})$ , apex acute, base decurrent, margin entire; presence of a single prominent midrib. Subsequent leaves simple, four, from each node, whorled, scale-like, with a slight bluish tint, other characters same as first leaves except size 0.6  $-1.0 \text{ cm} \times 0.1 \text{ cm}$  [branching starts at 3<sup>rd</sup> and 6<sup>th</sup> nodel. (Fig. 2B, C, 3B, 5).

**SEM Characters**: *Paracotyledons*— heavy waxy deposition on both upper and lower surface, with unbranched hairs on both surfaces (Fig 10A, B). *First leaf* — hypostomatic, stomata on the lower surface are arranged in vertical rows, rectangular, dimension 15  $\mu$ m — 22  $\mu$ m × 5  $\mu$ m — 9  $\mu$ m, often stomata stomatal openings have waxy occlusions; numerous unbranched uniseriate hairs (30  $\mu$ m — 34  $\mu$ m) with acute tips and swollen bases present on upper as well as lower surface with thick epicuticular waxy deposition in form of small round tubules. (Fig 11 and 12).



**Figure 1.** *Cryptomeria japonica* Thunb. Ex L.f.) D. Don: **A -** Seed; **B -** Seedlings (7 days old); **C -** Scanned photograph of seedling (28 days old)

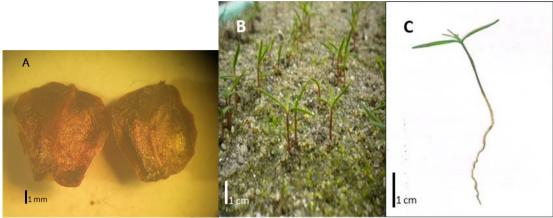


Figure 2. CupressuscashmerianaRoyle ex Carriére: A - Seed; B - Seedlings (10 days old); C – Scanned photograph of seedling (15 days old)



Figure 3. Developmental stages of seedlings: A - Cryptomeria japonica Thunb. Ex L.f.) D.Don **B** – Cupressus cashmeriana Royle ex Carriére

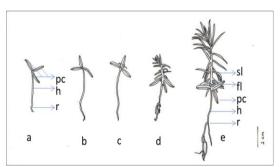


Figure 4. Cryptomeria japonica Thunb. Ex L.f.) D.Don (fl- first leaves, h- hypocotyl, pc- paracotyledons, rroot, sl- subsequent leaves)

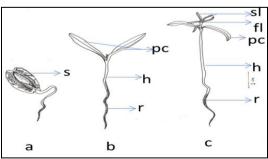
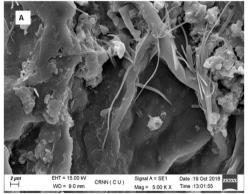


Figure 5. Cupressus cashmeriana Royle ex Carriére (flfirst leaves, h- hypocotyl, pc- paracotyledons, rroot, s- seed, sl- subsequent leaves)



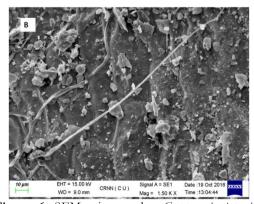


Figure 6. SEM micrographs: Cryptomeria japonica. A, B: Paracotyledon upper surface with waxy deposition & very few hair.

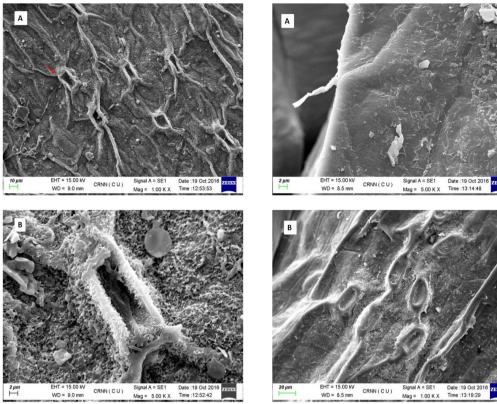


Figure 7. SEM micrographs: Cryptomeria japonica. A, B: Paracotyledon showing stomata undersurface, marked by arrow.

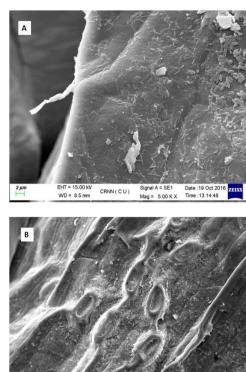


Figure 9. SEM micrographs: Cryptomeria japonica. A, B: Lower surface of first leaf showing hair with waxy deposits and stomata.

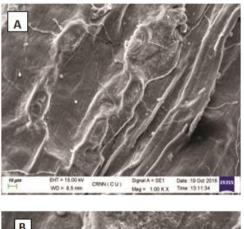
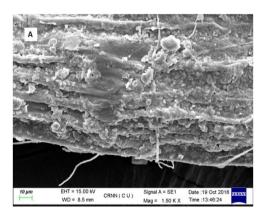




Figure 8. SEM micrographs: Cryptomeria japonica. A, B: Upper surface of first leaf showing waxy deposition in some stomata, along with hair.



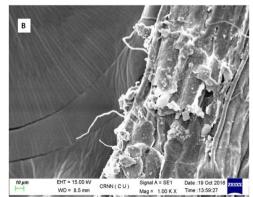
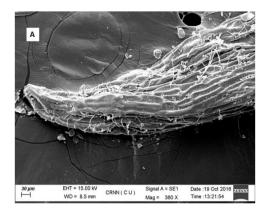


Figure 10. SEM micrographs: Cupressus cashmeriana. A, B: Paracotyledon with heavy waxy deposition and hairs on upper & lower surface.



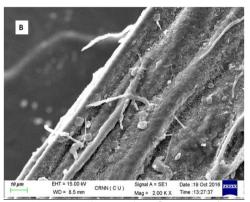
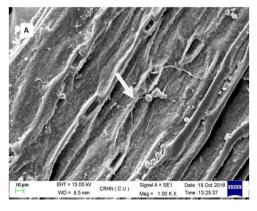


Figure 11. SEM micrographs: Cupressus cashmeriana. A, B: Upper surface of first leaf with many hairs and waxy deposits.



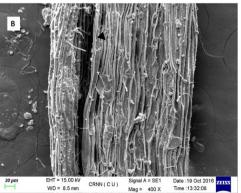


Figure 12. SEM micrographs: Cupressus cashmeriana. A, B: Under surface of first leaf with hairs, waxy deposits and stomata (marked by arrow).

Table 1. Comparative account of characters studied for two taxa:

Characters		Cryptomeria japonica	Cupressus cashmeriana
Paracotyledon	Number	Three	Two
	Phyllotaxy	Whorled	Opposite
	Number	Three per node	Two per node
First leaves	Phyllotaxy	Spirally arranged	Opposite decussate wrtparacotyledons
	Shape	Linear to awl-shaped	Narrowly linear
	Base	Subcuneate	Decurrent
	Apex	Acute	Acute
Subsequent Leaves	Shape	Triangular to awl-shaped	Scale-like

#### **Discussion**

The study of the two taxa revealed some important characteristics of seed and seedling morphology which are potential for serving as taxonomic markers for identification. Both the taxa belonging to Cupressaceae sensu lato are characterized by phanerocotylar, epigeal pattern of germination. Since these two taxa are identified as Near Threatened (NT) by IUCN Red List, their identification at an early stage of seedling will assist to protect this plant in nature in the midst of unfavourable environmental and biotic factors. From the table 1, it is observed that the seedling characters, such as, the number of paracotyledons is remarkably differentiating for the two taxa. Cryptomeria japonica is characterised having three, linear, paracotyledons with whorled phyllotaxy two, linear, oppositely arranged paracotyledons is observed in Cupressus cashmeriana. Three first leaves in whorled phyllotaxy in Cryptomeria japonica is in constrast with the presence of two opposite decussately arranged first leaves in Cupressus cashmeriana. These characters would be remarkable markers for taxonomic identification of the two economically important species. The identification at the seedling stage would be an aid in protecting the two Near Threatened species of Cupressaceae sensu lato from further depletion from natural habitats due to over exploitation.

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