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An insight on the reproductive aspects in *Tricyrtis hirta* (Thunb.) Hook.: An ornamental toad lily from the Himalayas

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Abstract
In the present investigation, some aspects of embryo and helobial endosperm in *Tricyrtis hirta* (Thunb.) Hook. (Toad Lily) of the family Liliaceae collected from Laitkseh, Nongstoin, Meghalaya was studied. *Tricyrtis hirta* is often regarded as an ornamental plant and because of which it is exploited by indiscriminate collection and destruction of habitat. The flowers of *Tricyrtis hirta* bloomed during the month of July to August and were showy, bisexual and actinomorphic. The sections of the capsules showed a small ovule which was anatropous and bitegmic. Embryo sac observed was of the Normal or Polygonum type. Endosperm development was observed to be of helobial type. But as development takes place and when mature, the chalazal nuclear endosperm also becomes cellular. Endosperm haustoria was also seen at the chalazal portion. From the phase contrast view of the photograph, a six celled pro-embryo was seen which confirmed a chenopodiad type of embryogeny in *Tricyrtis hirta*. These findings on the reproductive aspect of *Tricyrtis* could help in the development of regeneration protocol and in conservation of this species.

Keywords: toad lily, capsules, embryo, helobial endosperm, haustoria

Introduction

India is a vast country with rich diversity of biotic resources due to varied physiographic and climatic regime and encompasses a wide spectrum of habitats. India is one of the 12 mega diversity countries in the world having two hotspots *viz*., the Western Ghats and the Eastern Himalayas, based on species rarity and endemism [1].

According to an estimate based on satellite images the northeastern region has 1,63,799 km^2 of the forest, which is about 25% of the total forest cover in the country. Eastern Himalayas has 82% forest cover which is one of the global biodiversity hotspot and is also among the 200 globally important eco-regions [2]. Jain and Sastry [3] reported that the knowledge of threatened species is very meager. Haridasan and Rao [4] have reported 54 rare and threatened species in tropical forest of Meghalaya, out of which 15 plants are trees.

North-East India is indeed a ‘store house of biodiversity’ because of its rich spectrum of species of flora and fauna. The Northeastern region comprises the eight states of India *viz*., Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Tripura and Sikkim. However, this plant wealth is eroding at a fast pace due to habitat loss, fragmentation, over exploitation of plants of economic importance, invasion of exotics, Jhum cultivation, encroachment in forest areas for the developmental activities and settled agriculture, pollution and climate change. According to Singh and Khurana [5], about 25% of the higher plant species is expected to disappear in the next few decades and another 25% may be lost by the end of the 21st century.

The process leading to flower, fruit and seed formation remain poorly known particularly in the rare, endemic and threatened plants of North East India. Plants need to develop all parts of the reproductive structures and need to repeat that each time they re-initiate sexual reproduction. Understanding the reproductive biology of species is essential for conservation efforts [6, 7], particularly of rare, endangered and threatened (RET) species where there are only a very few population.

Tricyrtis

The name *Tricyrtis* comes from the Greek words "tri" (three) and "kyrtos" (swelling, arched, bulging or humped) which refers to the 3 sack-like nectaries at the base of the tepals. The most common explanation for the name toad lily is that the flowers and leaves are spotted like toads. In addition, the flowers have warty, sack-like (saccate) bumps at the base of the flowers that are "toadish" to some. The bumps are actually nectaries. A variation on the common name is the hairy toad lily, referring to their hirsute nature. The Japanese have a prettier common name for toad lilies: “hototogiso” which translates to "little cuckoo", a forest dwelling bird. The genus *Tricyrtis* is East Asian in origin. Their native range runs from China, Korea, and Japan in the North to Nepal, Taiwan and the Philippines in the South. *Tricyrtis* live in a wide range of conditions from mountainous regions such as the Himalayas to low-lying, humid, sub-tropical forests. They are always found in regions that receive plenty of rainfall. *Tricyrtis* is a member of the family Liliaceae which consisted of over 20 species [8].

Tricyrtis hirta (Thunb.) Hook

Tricyrtis hirta was renamed from *Uvularia hirta by* W.J. Hooker, in 1863 and published a description of it in Curtis' Botanical Magazine (Vol. 89 Tab. 5355). *Tricyrtis hirta* is an important endemic ornamental plant. *Tricyrtis hirta* is bisexual or monoecious plant. The capsules of *Tricyrtis hirta* were collected from Laitkseh, 25^{0}49”N, 0.91^{0}96”E at an altitude of 1462m above mean sea level in Nongstoin, Meghalaya. Climatologically this study area belongs to the sub-tropical wet climate regions. This region receives rainfall of over 900mm per year. In winter, temperature ranges from 7 ^{0}C – 10 ^{0}C and during summer mean temperature ranges from 18 ^{0}C – 22 ^{0}C.

Materials and Methods

The capsules of different sizes were collected from Laitkseh in the month of July-August and fixed in FAA (5ml of formalin and 5ml of acetic acid in 90ml of 70% alcohol) [9]. The plant samples fixed in FAA were used for microtomy by usual dehydration method using tertiary butyl alcohol series followed by impregnation with paraffin wax [9]. The paraffin block were trimmed and sectioned at a thickness of 7-10µm using Leitz rotatory microtome. The sections were then stained by safranin fast green. Photomicrographs were taken by using Olympus microscope (BX 43).

Result and Discussions

Structure of the flower

The flowers are showy, bisexual and actinomorphic. The 4-6" long leaves are arranged alternately on the stem, lanceolate to ovate in shape with acute or acuminate tips and a clasping, cordate base. All parts of the plant are covered with very fine, transparent hairs. The flower are formed on the axils of the leaf. The flowers are 2.5 cm (0.98 in) wide with six whitish to pale purple tepals that have dark purple spots. Each flower has 6 narrow tepals (3 petals + 3 sepals). The tepals open outward in a star-shaped pattern from the flower. The androecium usually consists of 6 fertile stamens. The gynoecium consists of a single compound pistil of 3 carpels, a single style commonly with 3 stigmas ovary with 3 locules, each containing several to numerous axile ovules. The fruit is a capsule. The orchid-like flowers typically have a white or yellow base color that is covered with hundreds of small purple spots (Figure 1).

Fig 1: Morphology of *Tricyrtis hirta* (Thunb.) Hook.

Structure of the ovule

Ovule is small and anatropous. Two integuments are seen in *Tricyrtis hirta*, thus it is bitegmic ovule, the inner integument differentiates earlier than outer integument. The inner integument consists of radial wall. The micropyle is formed by the outer integuments. (Figure 2)

Differentiation of the integumentary tapetum or endothelium was observed in *Tricyrtis hirta*, as it shows morphological variation from other cells (Figure 3). They show a pronounced radial elongation and sometimes become binucleate. The endothelium originates from the inner layer of inner integument and is confined to the micropylar end of the embryo sac. The endothelium has been recognized as being distinctive and rich in cytoplasm and tannin which are radially extended in early development and that in many cases are in direct contact with the embryo sac [10]. According to Tobe *et al.,* [11] the presence of tannins-like substance in endothelium could act as a protective layer rather than the nutritive function.

Hypostase was seen present on the chalazal portion (Figure 2, b). According to Van Tieghem [12], hypostase forms a sort of barrier or boundary for the growing embryo sac and prevents it from pushing into the base of the ovule. According to Tilton [13], while the chief function of the hypostase is in the translocation of nutrients, it may in some case act secondarily as a storage tissue. The presence of the hypostase at the chalazal region of the embryosac in *Tricyrtis* is indixcative of the water economy of the embryo sac, because the vascular supply of the ovule terminates at the chalaza.

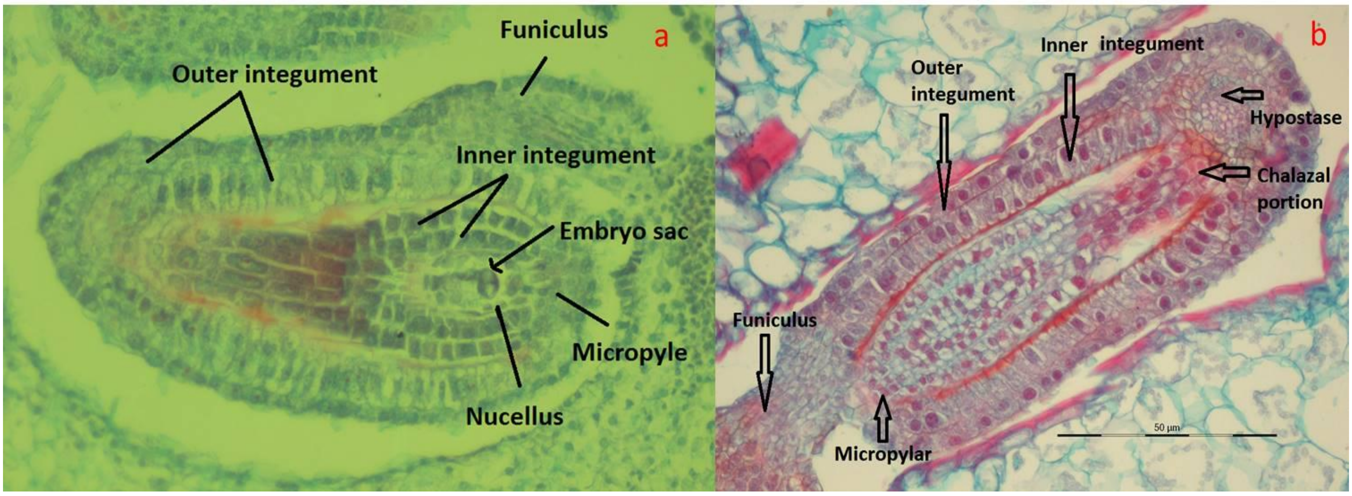

Fig 2: Ovule of *Tricyrtis hirta* (Thunb.) Hook.- a. Structure, b. Ovule with hypostase.

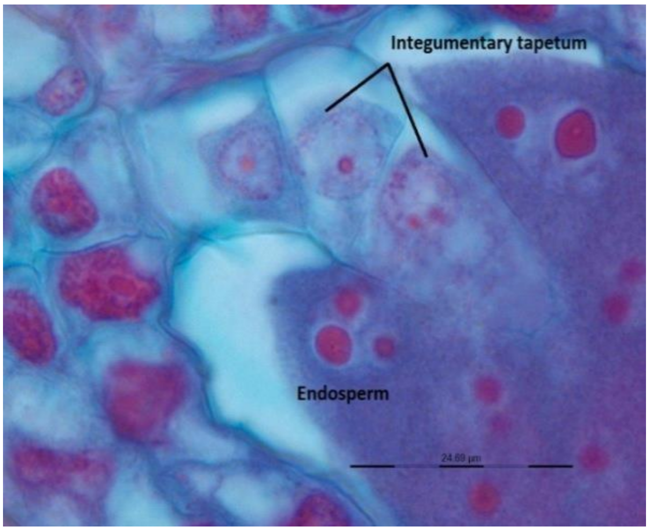

Fig 3: Endosperm with integumentary tapetum.

Organisation of the embryo sac

Embryosac consisted of 7 cells: the egg cell, two synergids, the central cells and the three antipodal cells. This type of embryo sac is generally referred to as "Normal or Polygonum type" (Figure 4, a). The extreme micropylar pole of the embryosac consists of the egg cell and two synergids known as the Egg Apparatus (Figure 4, b). At the extreme micropylar pole the wall is strongly thickened, forming a structure known as the Filiform Apparatus (FA). From the FA towards the base of the synergids the wall gradually thins out. The presence of filiform apparatus at the micropylar pole might played arole in pollen tube guidance and reception [14].

The antipodal cells show a linear arrangement and were placed in the funnel-shaped portion of the embryo sac at its chalazal end (Figure 4,c) and were very conspicuous by their extremely large size, large nuclei and intense staining. Ikeda [15], in connection with *Tricyrtis hirta*, claims the antipodal in the species are nutritively active from the full maturation of the sac to the formation of endosperm, after which they change in structure and gradually weakens and that during that period they not only provided food for endosperm formation, but also for the growth of the egg apparatus.

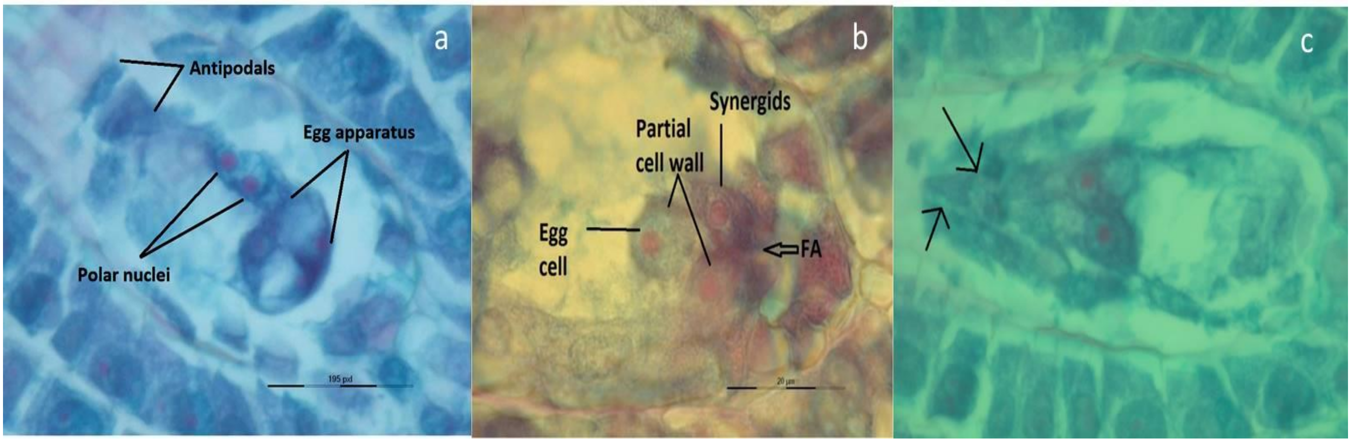

Fig 4: Embryo sac of *Tricyrtis hirta* (Thunb.) Hook., a. Normal type of embryosac, b. Egg apparatus, (FA) Filiform Apparatus, c. Antipodals placed in funnel-shaped portion (arrows).

Endosperm development

After the mitotic division of the primary endosperm nucleus (Figure 5,a) a wall forms between the two daughter nuclei and partitions the former central cell into two chambers: a large micropylar chamber and a significantly smaller chalazal chamber (Figure 5,c). Helobial endosperm development is common in monocots a feature most likely inherited from the shared common ancestor of all angiosperms [16].

Despite initially near-equal rates of free-nuclear development, the chalazal chamber, which is more densely cytoplasmic, remains the smaller of the two chambers. In *Tofieldia calyculata*, free-nuclear development in the chalazal chamber is limited from the onset in the vast majority of cases [17]. Endosperm haustoria were also seen at the chalazal portion which are finger like projections and usually help in the absorption of nutrients for the developing embryo (Fig 5, d). Hitochemical observations seem to suggest that starch, proteins and other metabolites pass into the endosperm through the haustorial [18].

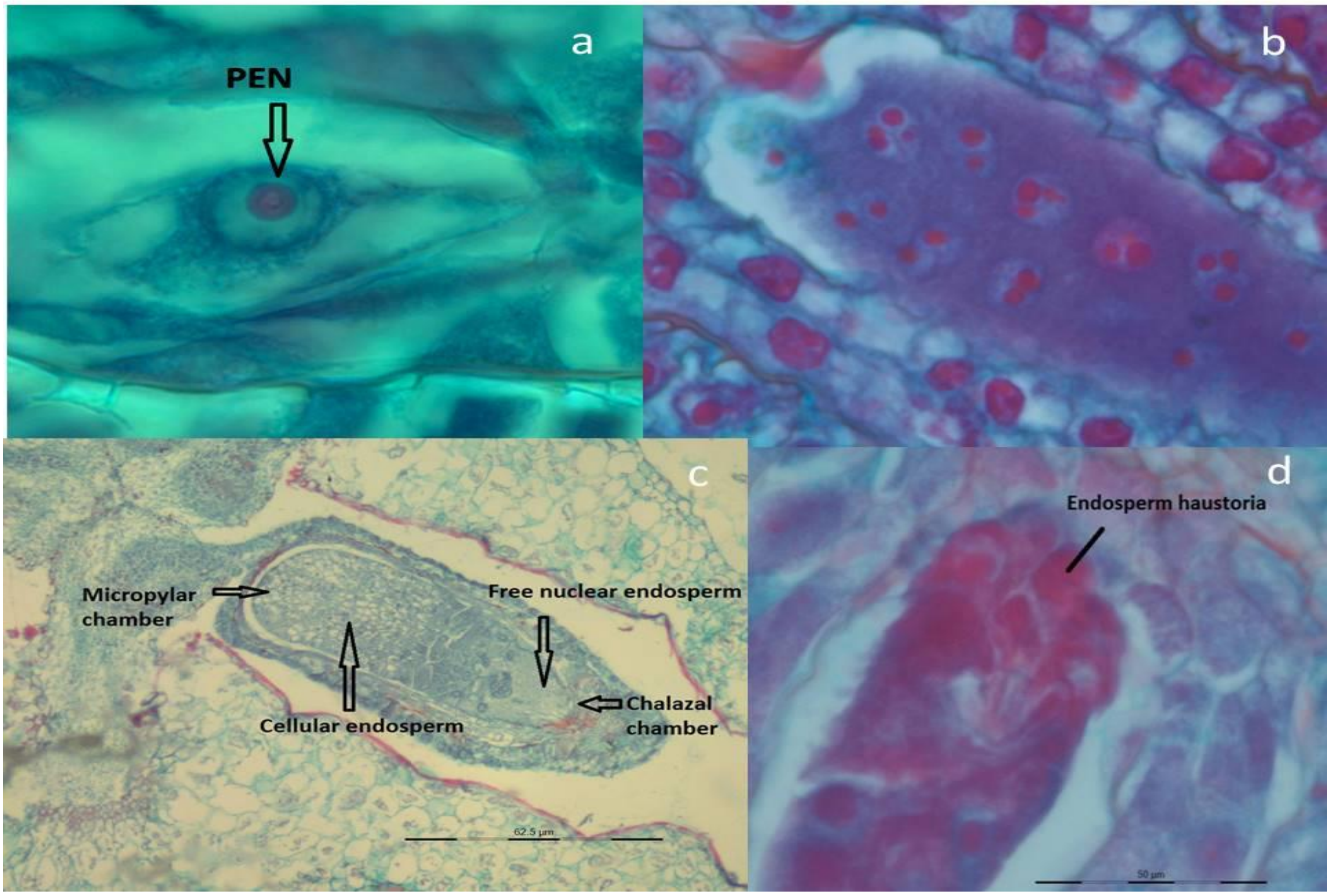

Figure 5: Endosperm development in *Tricyrtis hirta* (Thunb.) Hook., a. Primary Endosperm Nucleus (PEN), b. Initial division of PEN, c. Free nuclear and cellular endosperm, d. Endosperm haustoria

Embryo

The zygote divides transversly into apical cell (ca) towards the interior of embryo sac and basal cell (cb) towards the micropyle (Figure 6, a). Both the basal and the apical cell took part in the formation of embryo. While the basal cell divides and elongates to form the suspensor, the terminal cell becomes the cell embryo and on further division leads to the development of a globular embryo. From the phase contrast view of the photograph (Figure 6, b), a six celled filamentous proembryo was observed which confirmed to the chenopodiad type of embryogeny in *Tricyrtis hirta*. [19] Recognized five types of embryogeny which were Crucifer, Asterad, Solanad, Chenopodiad and Caryophyllad.

There are some traces of granules mainly the proplastids. According to Mansfield and Briarty [20], proplastids in meristematic cells were responsible for formation of chloroplast when the embryo develops upto the globular stage. The pro embryo is covered by a thin cuticle. According to Delude *et al*. [21], functional cuticle established early during embryo development and then maintained throughout plant development. Sucessive development from the octant stage to the globular embryo was also seen (Figure 7 a, b, c & d). With maturation of the embryo, the integument is completely absorbed so that only a thin seed coat is present.

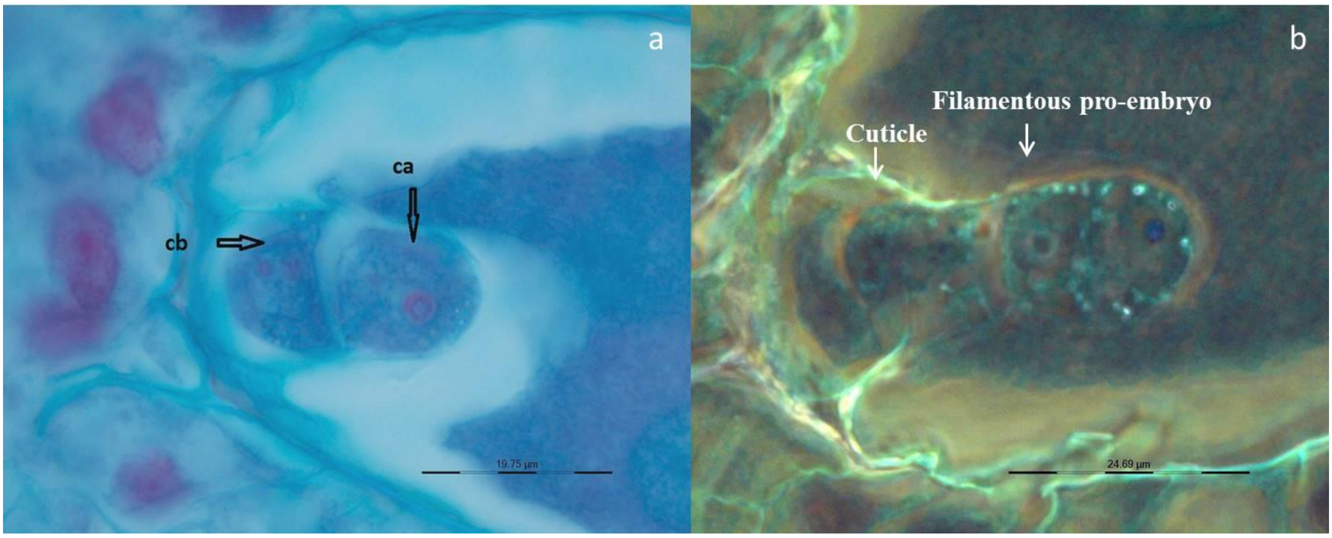

Fig 6: Embryo development in *Tricyrtis hirta* (Thunb.) Hook., a. Two celled embryo (ca - apical cell, cb - basal cell), b. Six celled filamentous pro-embryo.

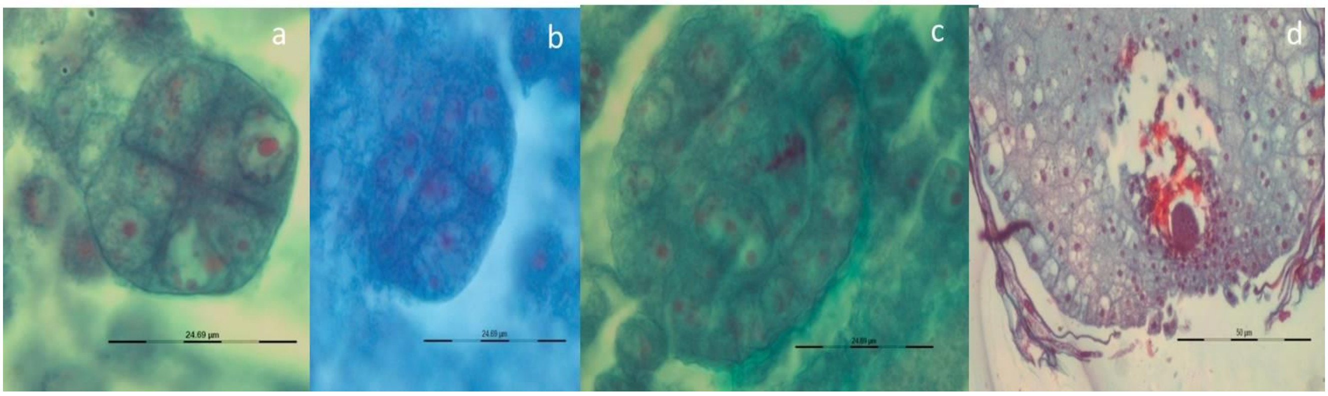

Fig 7: Embryo development in *Tricyrtis hirta* (Thunb.) Hook., a. Octant stage, b & c. Globular stage, d. Mature globular embryo.

Conclusion

Studies in reproductive biology help in developing strategies to preserve the genetic potential of rare species and are crucial for restoration and reintroduction [22]. Any conservation approach has to be based on in depth study of plant reproductive biology, as failure of reproductive processes to cope with the environmental changes is one of the fundamental reasons for species loss. Therefore these findings can help in developing certain protocols to combat the problems that impede regeneration**.**

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