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Cryptocotylarse mihypogeal germination and seedling morphology of *Polyalthia longifolia* (Sonn.) THW. (Annonaceae)

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SUMMARY

A study was undertaken to reinvestigate the germination pattern of *Polyalthia longifolia*. When the radicle is anchored in the soil and plumule protruded, the seed part consisting of testa, endosperm and cotyledons remained on the soil level and so the germination is semihypogeal. Since the cotyledons are abutted inside the endosperm within the testa and the cotyledonary petioles alone are emerged, the germination is cryptocotylar. The seedling excised from the testa and endosperm shows distinct radicle, collet, hypocotyl, plumule and white fragile cotyledons which are reserve (haustorial) in function. A pair of alternate cophylls are present above the cotyledonary node.

Key words : Collet, Cotyledonary petioles, Eophyll, Hypocotyl, Ruminate endosperm

Phanerocotylar germination is a general characteristic of the family Annonaceae and cryptocotyly is probably noticed in species with large seeds (Duke, 1969). It has been reported that only three of 90 forest species of Venezuela showed cryptocotylar type of germination (Ricardi 1999a, 1999b). Out of 20 humid forest species of Mexico, only five species are characterized by epigeal cryptocotylar germination and five functional types of seedlings are existing (Ibarra-Manriquez *et al.,* 2001) based on the terminology suggested by Garwood (Garwood, 1996). Examples of epigeal cryptocotylar germination are seemed to be rare.

Polyalthia Blume (Annonaceae) is an old world tropical genus comprising 100 species (Mabberley, 1997). An investigation on the germination and seedling morphology of *Polyalthia longifolia* (Sonn.) Thw. revealed that germination is hypogeal phanerocotylar (Saha(Das) *et al.,* 1998). The present authors made an attempt to reinvestigate the germination pattern of *Polyalthia longifolia* as a part of the studies on seed biology of Calicut University flora under Ph.D. program of the first author and germination behaviour of this taxa is found to be inconsistent with the views reported earlier (Saha(Das) *et al.,* 1998)

MATERIALS AND METHODS

Ripened fruits of *Polyalthia longifolia* were collected during 2005 - 2006 from the avenue trees of Calicut University campus. Fruits were depulped manually and seeds were washed thoroughly and air dried at room temperature.

Fresh seeds were kept for germination in Petri dishes lined with moist filter paper and germination was done under field conditions also. Time taken for germination was noted and seedling growth was observed until the seedlings attained growth up to 12 ± 2 cm length during a period of 10-15 days when the seedlings consisted of 4-5 leaves. Photographs of seeds, embryos, seedlings and excised seedling-parts were taken using Nikon D100 digital camera. Drawings were made using Wild Stereo Microscope and Prism type Camera lucida.

RESULTS AND DISCUSSION

Seeds of *P. longifolia* are endospermous with thick testa (Fig.1B-D). Seeds were considered germinated when the radicle protruded and attained 2 mm length. Seven to eighteen days were taken for germination. During germination embryonic axis alone was exposed and cotyledons, endosperm and testa remained intact as a unit. But prominent cotyledonary petioles were visible in very young seedlings (Fig. 1C). When the radicle and plumule were emerged, the testa and the cotyledons as abutted in the ruminate endosperm, remained on the soil surface without any hypocotyl growth.

Seedling morphology showed that the cotyledons were enclosed within the endosperm exposing prominent green coloured cotyledonary petioles (Fig. 1C) and the endosperm was ruminate type (Fig. 1D). During germination though the cotyledons grew inside the seed (Fig. 1 D-G), the testa and endosperm remained intact. Hence, the growth pattern and structure of the cotyledons

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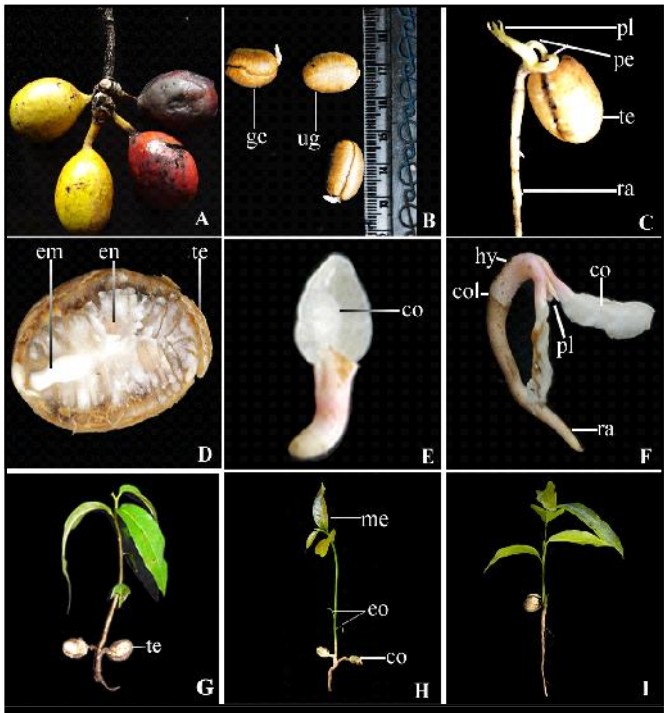

Fig. 1A-I:Different stages of germination of *Polyalthia longifolia* seeds A. Fruit (infructescens) with pericarp. B. Depulped seeds showing germination. C. Intact seedling with testa, endosperm and cotyledon. D. L.S. of seed showing testa, embryo and endosperm (ruminate) E. Excised embryo with cotyledons and axis. F. Excised embryo (developed stage) with radicle, plumule, cotyledons, hypocotyl and collet. G. Seedling with testa, cotyledons and endosperm. H. seedling with cotyledons (excised) showing eophylls and metaphylls. I. Intact (cryptocotylar) seedling

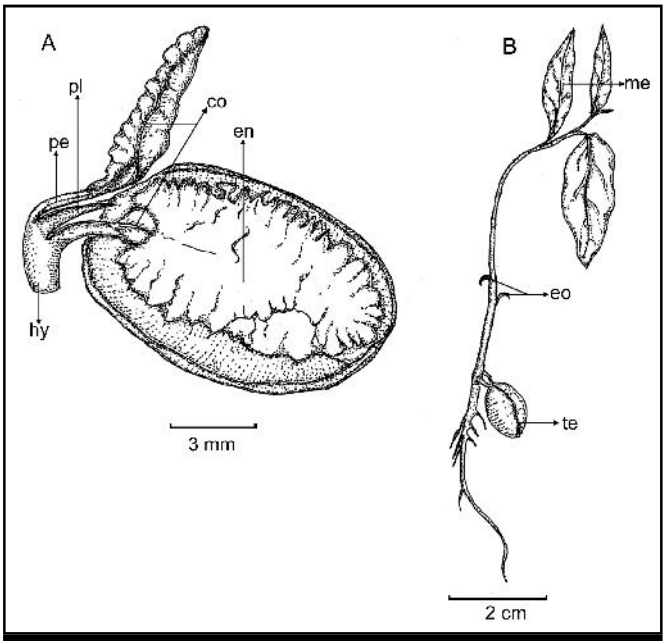

Fig. 2 (A-B) : A. Germinated seed with one cotyledon exposed by removing half of the testa and endosperm
B. Seedling with eophylls and attached seed enclosing the cotyledons and ruminate endosperm (co-cotyledon, col-collet, em-embryo, en-endosperm, eo-eophyll, ge-germinated, hy-hypocotyl, me-metaphyll, pe-cotyledonary petiole, pl-plumule, ra-radicle, te-testa, ug-ungerminated)

could be observed only after excision of the testa and endosperm (Fig. 2A).

The seedling excised from the testa and endosperm showed distinct radicle, collet, hypocotyl, plumule and cotyledons (Fig. 1F). Cotyledons were white in colour, thin, fragile with uneven surface without any clear venation except midrib with a thin layer of endosperm in between (Figs. 1G, H and 2A). The cotyledons did not survive when exposed after excision of the endosperm. A pair of alternate eophylls was present above the cotyledonary node (Figs. 1 H and 2B). In the fully developed seedlings by 30 $\pm$ 5 days of growth, the seed part consisting of the testa, endosperm and the abutted cotyledons remained intact and as a single unit, it was found attached to the seedling (Fig. 1G, I).

Eventhough the plumule and radicle were protruded after germination, the cotyledons were not emerged from the seeds. During seedling emergence, the radicle was anchored in the soil, plumule grew and the testa, endosperm and cotyledons as an intact structure, remained on the soil level and hence the germination was semi-hypogeal. This report is in consistent with the existing view on rare occurrence of semihypogeal type of germination as reported in some seeds (Baskin and Baskin, 2001).

Anonaceae species with large seeds show cryptocotyly (Duke, 1969). Since the cotyledons of *P. longifolia* are abutted inside the endosperm and during germination the cotyledonary petioles alone are emerged, the germination is cryptocotylar. So in *P. longifolia* seed germination was found to be semihypogeal-cryptocotylar.

Polyalthia longifolia seeds have already been reported as characterised by hypogeal phanerocotylar germination (Saha(Das) *et al.*, 1998). Nevertheless, those authors have not furnished any details of the position and nature of the cotyledons and so the occurrence of phanerocotyly is not at all evident. Those authors (Saha(Das) *et al.*, 1998) further suggested that a pair of rudimentary paracotyledons were present. The present investigation reveals that these 'paracotyledons' were actually the eophylls (Fig. 1H).

An unusual case of cryptocotylar germination in *Rollinia salicifolia* (Anonaceae) was reported and was

desginated as epigeal cryptocotyly since the cotyledons did not emerge from the seed coat and the growth of hypocotyl resulted in their emergence above the ground surface (Franceschini, 2004).

Cotyledons are of various types based on their functions in seedling development. According to Garwood (1996) cotyledons are classified cryptocotylar (C) means inside the seed coat, phanerocotylar (P) means free of seed coat; epigeal (E) means above the ground, hypogeal (H) means at or below the ground; foliaceous (F) means thin and photosynthetic and reserve (R) means storage or absorption.

Based on the above classification (Garwood, 1996), the cotyledons of *P. longifolia* were not foliaceous (photosynthetic), but came under reserve type of cotyledons. The reserve type is either storage or absorption in function (Garwood, 1996). In *P. longifolia* the cotyledons were involved in the absorption of nutrients from the endosperm which occured on both sides due to the presence of a thin layer in between (Figs. IG, I and 2A). The cotyledons were having midrib which was in close contact with the endosperm (Fig. IG and I). A recent study on *Cassava* (Pujol *et al.,* 2005) revealed that reserve cotyledons (R) can be of two types namely storage (s) *i.e*. thick fleshy cotyledons, which store reserves and translocate them directly to germinating seedling and haustorial (h) *i.e.* thin cotyledons, which absorb reserves from the abundant endosperm and translocate them to the germinating seedlings. Hence based on the characters described above, the cotyledons of *P. longifolia* seedling could be included under C-H-R (h) *i.e*., cryptocotylar, hypogeal, reserve (haustorial). Based on these findings, the present authors contradict the earlier view (Saha(Das) *et al.,* 1998) and suggest that the germination of *P. longifolia* seed was semihypogeal-cryptocotylar.

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