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Unusual germination and seedling development in two monocotyledonous dicotyledons

P A PHILOMENA* and C K SHAH

Botany Department, School of Sciences, Gujarat University, Ahmedabad 380009, India.

* Present address: Fredrick Institute of Plant Protection and Toxicology, Padappai 601 301, Chingleput District, Tamil Nadu, India

Abstract. Morphology of germination and seedling development have been studied in *Nymphaea lotus* Auct. var *pubescens* (Willd) HK. f. and Th. and *Trapa natans* L. var *bispinosa* (Roxb.) Makino. *Nymphaea* germination has been grouped as a separate type, namely, *Nymphaeal* and that of *Trapa* as *Trapael*. In *Nymphaea* and *Trapa* seedlings, the extension growth is at the mid portion of the cotyledon (mesocotyl) unlike at its base as in the monocotyledons.

Keywords. Germination; seedling development; *Nymphaea lotus* Auct. var *pubescens* (Willd) HK. f. and Th.; *Trapa natans* L. var. *bispinosa* (Roxb.) Makino; cotyledonary middle piece; mesocotyl; hypocotyl; *Trapa*; *Nymphaea*.

1. Introduction

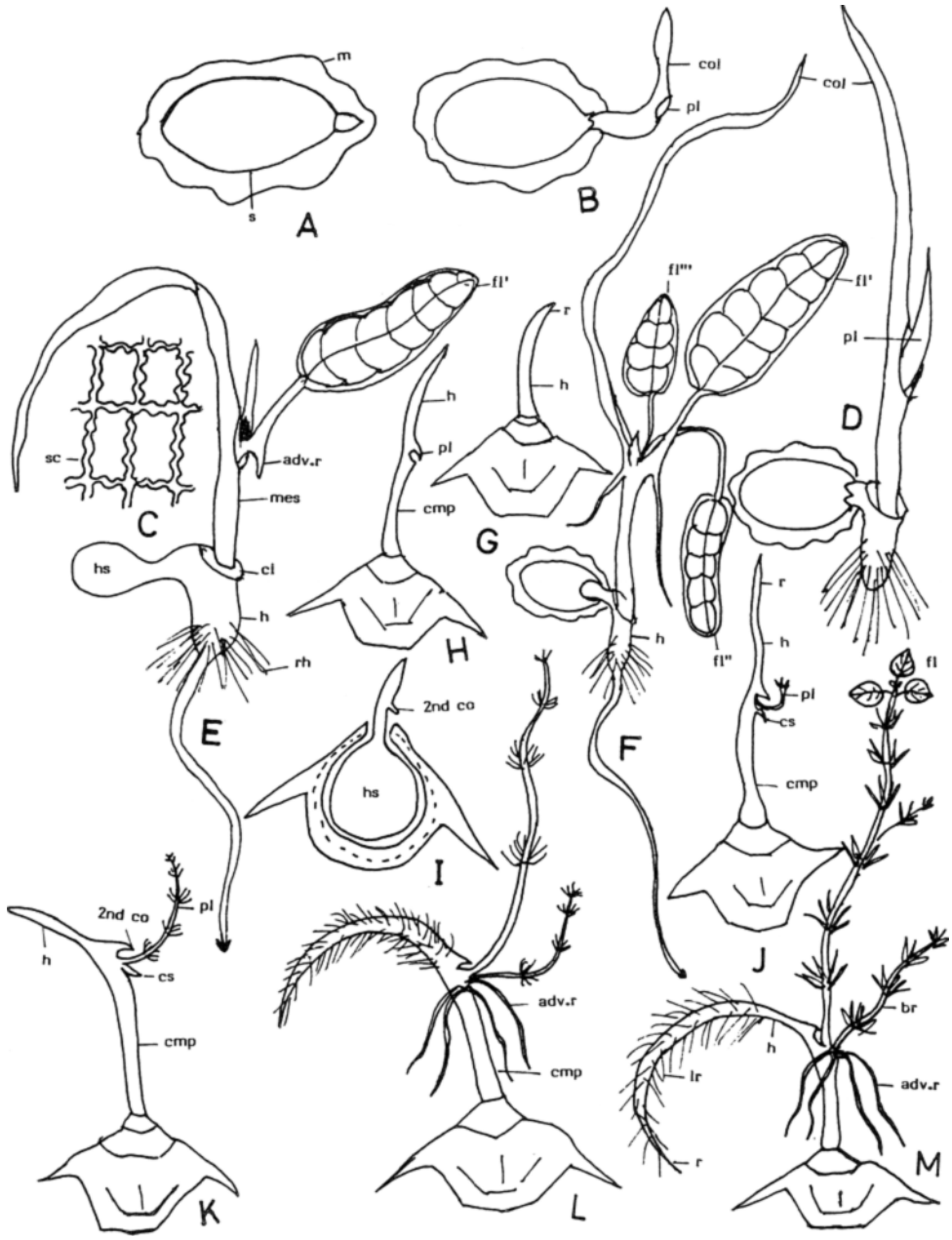
Certain dicots develop only a single cotyledon but the second remains rudimentary or suppressed. The present paper deals with the germination and seedling development in *Nymphaea* (Nymphaeaceae) and *Trapa* (Trapaceae). Hill (1938), Sculthrope (1967) and Cook *et al* (1974), have grouped them under dicots, while Haines and Lye (1975) include them among monocots seedling characters such as presence of coleoptile and mesocotyl. Thus, seedling characters are considered to be of taxonomic importance.

2. Materials and methods

Achenes of *Nymphaea lotus* Auct. var. *pubescens* (Willd) HK. f. and Th. collected from pond grown plants and of *Trapa natans* L. var. *bispinosa* (Roxb.) Makino from Chandola lake 37 km from Ahmedabad were used. Seeds of *Nymphaea* were germinated in Petridishes on moistured filter papers, as well as in a soil in beaker covered with 4″ of water. Once germination began, regular observations of seedlings were made under a binocular dissecting microscope and cameralucida sketches drawn. Seedlings were also cleared in KOH for better visualization of the regions like cotyledonary mid-piece and haustorium, mesocotyl, hypocotyl, etc.

3. Results and discussion

Nymphaea has floating leaves on long petioles. The seeds, 1–2 mm long, encapsulated by mucilage swells up considerably soon after imbibition (figure 1A). The seed coat has wavy margined cells (figure 1C). Germination begins 20–25 days or even more after soaking. Plumular sheath (coleoptile) is the first to come out (figure 1B) followed by a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very short hypocotyl and a radicle at its tip. A ring of long rhizoids develops around the base of the hypocotyl (figure 1D). Removal of the mucilage and the shell allows the separation of the haustorium formed by the combined ends of the two cotyledonary lobes ensheathing the plumule–radicle axis.

From the base of the sheathing coleoptile, where there is a plumular depression (figure 1B), the first foliage leaf makes its appearance followed later on by the second and the third (figures 1E, F). The base of the petiole of the first foliage leaf shows an angular protuberance of the first adventitious root (figure 1E). When the coleoptile reaches 2–3 cm in length the radicle also starts elongating. A root pocket protects the radicle at the extreme tip. In the meanwhile, the sheath and haustorial portion of the cotyledon are separated from one another by the development of an elongated cotyledonary middle piece. Haines and Lye (1975) call it the mesocotyl. The radicle and the adventitious roots increase in length and establish the seedling in the ground. The coleoptile by now stops elongation and senesces gradually. The mesocotyl length depends on the depth of sowing.

Trapa, free floating hydrophyte has rosettes of aerial and/or floating leaves and well developed submerged leaves. Fruit development is submerged. In Trapaceae, the fruit is a one-seeded drupe is lost its fleshy exocarp leaving a pyrene surrounded by the horned stony endocarp. The fruits of *Trapa* are fixed to the substrate by their own weight and by the spines of the fruit wall. During germination the radicle followed by the hypocotyl emerges from the micropyle, negatively geotropic (figures 1G, H). However, Sculthrope (1967) does not mention about the radicle at all in *Trapa*.

Of the two cotyledons, one is large and functional which remains within the seed while, the second abortive one is borne aloft on the negatively geotropic hypocotyl (figure 1I). The stalk of the functional cotyledon—the cotyledonary mid-piece—protrudes following the hypocotyl. It bears the cotyledonary sheath at the tip protecting the plumule (figures 1J, K). The plumular bud which is situated in the axil of

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Figure 1. **A–M.** Seed germination and seedling development in *Nymphaea lotus* and *Trapa natans*. **A.** *Nymphaea* seed after imbibition (× 20). **B.** Germinated *Nymphaea* seed showing the emergence of coleoptile and plumule prior to that of radicle (× 15). **C.** A portion of *Nymphaea* seed coat (sc) enlarged to show its ornamentation with wavy margins (× 50). **D.** *Nymphaea* seedling showing the emergence of the plumule from coleoptile and hypocotyl with the radicle at the tip (× 15). **E.** *Nymphaea* seedling cleared in KOH to expose the cotyledonary haustorium within the seed with the seed coat removed. The plumule has emerged through the flaps of cotyledonary lobes, elevated by a mesocotyl. Plenty of fine hairs, rhizoids can be seen around the hypocotylar base (× 5). **F.** *Nymphaea* seedlings showing the emergence of 3 foliage leaves and adventitious roots arising from the tip of mesocotyl (× 1). **G.** *Trapa natans*, initial stages of germination showing a hypocotyl with the radicle at tip (× 1). **H.** *Trapa* seedlings revealing the emergence of cotyledonary middle piece and plumule. Note the negatively geotropic hypocotyl and radicle (× 1). **I.** L.S. of *Trapa* fruit exposing the massive haustorium occupying the entire nut and the second rudimentary cotyledon (× 1). **J and K.** Seedling development of *Trapa* exhibiting the plumule curving towards light (× 1). **L and M.** Advanced stages of seedling development in *Trapa*. Note the curvature of hypocotyl and radicle becoming positively geotropic with many lateral roots developing around (× 1).

Abbreviations: adv. r, adventitious roots; br, branch; cl, cotyledonary lobes; cmp, cotyledonary middle piece; co, cotyledon; col, coleoptile; cs, cotyledonary sheath; fl, foliage leaf; fl′, fl″, fl‴, 1st, 2nd and 3rd foliage leaves; h, hypocotyl; hs, haustorium; lr, lateral roots; m, mucilage; mes, mesocotyl; pl, plumule; r, radicle; rh, rhizoids; s, seed; sc, seed coat.

the two cotyledons curves upward, elongates quickly and develops around the epicotyl arranged in whorls in a distichous manner, while foliage leaves of the adult develop later in a rosette shape. Secondary shoot often arises in the axil of the abortive cotyledon. The curvature of the epicotyl towards the water level causes the radicle and hypocotyl to bend in the opposite direction i.e. positively geotropic. Many lateral roots develop around the hypocotyl and the radicle and these, along with numerous adventitious roots developed from the axils of the two cotyledons, help in the establishment of the seedling in the ground (figure 1L).

The massive haustorial part of the cotyledon within the seed (figure 1I) by this time is exhausted of its reserve materials. It never leaves the interior of the nut but gradually decays with it when the seedling establishes completely. Endosperm is absent in *Trapa*.

Monocotyledons possess certain characteristic features, often better developed in the seedling than in the seed before germination, and these features can be used for classification purposes (Haines and Lye 1975). Even though the above authors group *Nymphaea* under hypogeal type of germination, *Nymphaea* combines the characters of epigeal, hypogeal and mesogeal blended in it along with some additional features such as two cotyledonary lobes, reticulate venation of the leaves, presence of a hypocotyl as well as mesocotyl and some unusual behaviour like the emergence of the coleoptile apriori to that of the radicle as reported earlier by Dakshini and Tandon (1970) in a graminaceous member, *Oropetium thomaeum*. All these characters render it necessary to separate *Nymphaea* from the rest and assign it to a separate type namely *Nymphaeal* type.

According to Takhtajan (1969) the only consideration that prevents the Nymphaeales being placed in monocotyledons is the supposed "presence of two cotyledons". In both *Nymphaea* as well as *Trapa*, the second cotyledon is present but remains rudimentary and suppressed. *Trapa* exhibits characters of different types blended in it like the presence of a hypocotyl (epigeal), hidden cotyledon, with massive haustorium (hypogeal) and presence of a cotyledonary mid-piece which simulates the mesocotyl of grasses (mesogeal), hence it is termed *Trapael* type.

Burtt (1972) reviewed the differences in germination types and concluded that in the monocotyledons extension growth is at the base of the cotyledon, while, in dicotyledons it is primarily in the hypocotyl and the seedling thus has exposed cotyledon and plumule. However, in *Nymphaea* and *Trapa*, the extension growth is chiefly in the mesocotyl and cotyledonary middle piece i.e. at the middle of the cotyledon instead of at the base distinguishing them as intermediary forms. Thus, these two monocotyledonous dicotyledons possess intermediary characters of monocotyledons as well as dicotyledons and stand out unique and deserve special rank.

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