MORPHOLOGICAL AND EMBRYOLOGICAL STUDIES IN NYMPHAEEACEAE

I. Euryale ferox Salisb.

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INTRODUCTION

Euryale of the Nymphaeaceae combines interesting morphological and embryological features—primitive as well as advanced. Cook (1902, 1906, 1909) described the embryo-sac and embryo in a number of members of the Nymphaeaceae. Schnarf (1931) has reviewed the earlier embryological literature on the family, while Johansen (1950) has summarised the data on its embryogeny. Recently, Leinfellner (1956 a, b) and Moseley (1958) have described the structure of the stamens and its significance in the taxonomy and phylogeny of the family. However, no work has been done on the genus Euryale which bears cleistogamous flowers and so is interesting. Euryale was, therefore, undertaken for the present study.

MATERIAL AND METHODS

The material was collected from Alwar, Rajasthan, India, in August and September, 1961-63. It was fixed in F.A.A and the usual methods of dehydration and embedding were followed. Sections cut 8–12 μ thick were stained with safranin-fast green and this gave excellent results.

OBSERVATIONS

Habitat.—Euryale is a densely prickled aquatic annual with a perennating root-stock. The leaves are alternate, peltate (Figs. 1, 2) and when mature prickled on the under-surface.

Flower.—The floral bud is covered by soft spiny outgrowths (Fig. 3). The flower is cleistogamous and remains under water throughout. It bears four persistent spiny sepals (Fig. 4) and numerous small violet petals which remain closed throughout. The inner petals are lorate and the outer ones obovate (Fig. 7). The stamens are numerous (Fig. 6). Each of the outer
stamens is dorsiventrally flattened, having a deltoid base (Fig. 5) and a distal appendage with embedded microsporangia. The inner stamens are of the conventional type. The change from the dorsiventrally flattened stamens to normal stamens is gradual and proceeds centrifugally.

The ovary is inferior, multicarpellary, syncarpous and 7–12 loculate (Figs. 9, 10), with 2–3 ovules on parietal placentae in each locule (Fig. 26). Usually, a single ovule matures in each chamber (Fig. 27) and the others abort.

The fruit is a berry crowned with the persistent sepals (Fig. 8). It is spinous and fusiform.

Microsporangium, Microsporogenesis and Male Gametophyte.—As studied earlier, the outer stamens are dorsiventrally flattened (Fig. 5). The microsporangia of these stamens are embedded in a massive sterile tissue. The anthers are four-loculate (Fig. 12) and become two-loculate at the time of dehiscence. Dehiscence is longitudinal and introrse.

The wall of the anther consists of an outer epidermis, an endothecium and 4–5 middle layers in the outer stamens (Fig. 13). There are 2–3 middle layers in the inner stamens. The tapetum is uninucleate to begin with, but finally becomes multinucleate (Fig. 13); it is of the secretory type and degenerates early (Fig. 14). The endothecial cells elongate radially.

The microspore mother cells undergo meiosis resulting in the formation of tetrahedral (Fig. 13) and isobilateral tetrads. The microspores are spherical and monocolpate. The exine is two-layered, the outer layer being thick and tubercled (sexine, Erdtman, 1952) and the inner one thin and hyaline (nexine, Erdtman, 1952). The intine is also thin and membranous (Fig. 16). The generative cell divides to form two sperm cells (Fig. 17). The pollen grains germinate in situ. The pollen tubes of these grains are of variable length and carry the two sperm cells surrounded by the inner hyaline exine (Fig. 18).

Ovule.—The ovule is anatropous, bitegmic, crassinucellate and receives a single vascular strand which terminates at the chalaza (Figs. 27, 31). The inner integument forms the micropyle. At the archesporial cell stage, four outgrowths arise from the funiculus which grow downward (Fig. 31) and finally cover the seed, forming an aril. During their development, the outgrowths fuse along the greater length of their length and remain free only in the apical region where they consist of four valves (Fig. 11).

The nucellus is very massive (Fig. 27). Its cells at the chalazal end and periphery are smaller in size as compared to those in the centre and the micropylar end at the eight-nucleate stage of the embryo-sac.
Fig. 12. Cross-section of anther, ×200. Figs. 13-15. A part of anther late ×1,620. Figs. 16-18. Pollen grains, ×2,125. Fig. 19. Archesporial cell, ×2,125. Fig. 20. Megasporangial mother cell, ×2,125. Figs. 21-22. Tetrads, ×2,125. Fig. 23. Functional megaspore, ×2,125. Fig. 24. Four-nucleate embryo-sac, ×2,125. Fig. 25. Embryo-sac, ×2,125. Figs. 26, 27. T.S. Ovary, ×170.

nuc, nucellus.
Megasporogenesis and Female Gametophyte.—The hypodermal archesporial cell (Fig. 19) divides to form an outer parietal cell and an inner megaspore mother cell. The parietal cell divides further anticlinally to form a single parietal layer (Fig. 20). The mother cell undergoes meiosis and linear and T-shaped tetrads result (Figs. 21, 22). The chalazal megaspore functions (Fig. 23) to form the embryo-sac. The nucleus of the functional megaspore undergoes three consecutive divisions to form an eight-nucleate embryo-sac (Figs. 24, 25). The organised embryo-sac is minute in size as compared to the size of the nucellus. The synergids are large and conspicuous (Fig. 25) and one of them persists after fertilization (Fig. 28). The antipodal cells are small and usually degenerate before fertilization. Rarely, they are persistent. The embryo-sac development conforms to the Polygonum type (Maheshwari, 1950).

Fertilization and Endosperm.—Double fertilization is simultaneous. Remnants of the pollen tubes (Figs. 31, 32) were observed at the micropylar end.

The primary endosperm nucleus undergoes free nuclear divisions (Fig. 28). The chalazal end of the embryo-sac elongates to form a tube-like outgrowth (Figs. 28–30) which penetrates deep into the nucellus bringing about the absorption of its tissue (Figs. 31, 32). The wall formation is initiated at the micropylar end before the division of the zygote. Wall formation is progressive (Fig. 29). The endosperm cells possess dense cytoplasmic contents in the beginning (Fig. 30) but become highly vacuolated later on (Fig. 32). The endosperm is almost completely consumed by the time the embryo matures.

Perisperm.—The massive nucellus stores the food reserves and forms the perisperm in seed. At the globular stage of the embryo, the cells of the perisperm get enlarged towards the micropylar end in the centre. Those situated centrally may become binucleate also. In seeds with mature embryos these cells are full of starch grains. The number of starch grains varies considerably in the cells of the perisperm (Fig. 33). They are comparatively a few in the cells of the micropylar end and those of the peripheral region, while the number is about a hundred in the centrally situated cells. The radially elongated cells of the micropylar region of the perisperm get cutinized on the outer and the radial walls. The perisperm forms the storage tissue in the mature seed.

Embryogeny.—The zygote (Fig. 34) divides by a transverse wall to form a terminal cell ca and a basal cell cb (Fig. 35). Ca and cb divide vertically (Fig. 36) and later on the derivatives of cb divide transversely forming ci
FIGS. 28–46. Fig. 28. Nuclear endosperm, ×1,620. Figs. 29–30. Cellular endosperm. Fig. 29, ×1,310. Fig. 30, ×800. Fig. 31. Anatropous ovule, ×90. Fig. 32. L.S. ovule showing inner integument, perisperm, endosperm and embryo. Mark the degenerating cells of perisperm, ×1,000. Fig. 33. Enlarged cells of perisperm showing starch grains, ×350. Figs. 34–44. Embryo development, ×1,000. Figs. 45–46. Mature embryos, ×800.

a, aril; i.i, inner integument; l.p, leaf primordia; o.i, outer integument; p, perisperm; p.t, pollen tube; sy, synergid; z, zygote.
and $m$ (Fig. 37). The derivatives of $ca$ divide vertically forming a quadrant $q$ (Figs. 37–39). Subsequently, $ci$ divides to form $n$ and $n'$ (Fig. 39).

Further divisions in the embryo could not be followed closely but soon it becomes globular (Figs. 40–43). The embryo gets flattened (Fig. 44) and a cotyledonary ridge is formed which overhangs the young shoot apex. Two saucer-shaped cotyledonary lobes (Figs. 45–46) arise from the ridge. Leaf buttresses are also organised forming leaf primordia in mature embryo (Figs. 45, 46). In some ovules polyembryony was seen.

Embryo development conforms to the Asterad type, Penea variation.

**SUMMARY**

*Euryale*, a densely prickled aquatic annual, with a thick perennating root-stock, is survived by a single species only in parts of China, Japan, North America, and India.

The leaves are alternate, peltate and prickled beneath. Epigynous flowers are submerged in water, having four spiny sepals, numerous small violet petals, numerous stamens—the outer dorsiventrally flattened with reduced and embedded microsporangia. The ovary is multicarpellary, syncarpous, 7-12-loculate with parietal placentation.

The anther wall consists of an epidermis, an endothecium, 2-5 middle layers and a multinucleate secretory tapetum. The endothecium develops characteristic fibrous thickenings at the time of dehiscence. The pollen grains are shed at the three-celled stage. The outer exine is thick and tubercled and the inner exine is thin and hyaline. The grains may germinate in situ.

The ovule is anatropous, bitegmic and crassinucellate with a massive vascular strand. Four outgrowths from the funiculus give rise to an aril. The hypodermal archesporial cell cuts off an outer prismatic cell and a megaspore mother cell. Linear and T-shaped tetrads are observed. The chalazal megaspore is functional; the embryo-sac is eight-nucleate and of Polygonum type.

Double fertilization is simultaneous. The endosperm is free nuclear. Wall formation is progressive. The endosperm is consumed by the time embryo matures. The food material is stored in the perisperm in the form of starch. The mature embryo possesses saucer-shaped cotyledons and 2-3 seminal leaves.
Figs. 1–11. Fig. 1. Young leaf, dorsal view, ×2. Fig. 2. Same, ventral view, ×2. Fig. 3. Bud, ×5·6. Fig. 4. Flower, ×2·6. Fig. 5. Outer stamen, ×90. Fig. 6. L.S. Flower, ×3. Fig. 7. Obovate petal, ×17. Fig. 8. Fruit, ×2·5. Fig. 9. T.S. A part of fruit, ×2·2. Fig. 10. T.S. Whole fruit, ×2·2. Fig. 11. Seed showing aril, ×19.

a, aril; se, sepal; pe, petal.
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