K. LAKSHMINARAYANA* & H. M. DEVI*: Embryology of Linociera intermedia Wt. (Oleaceae)

K. タクシンミナラヤナ*・H. M. デビ*：Linociera intermedia Wt. (モクセイ科の胚発生)

The Oleaceae is a small family of the flowering plants represented by 29 genera and 600 species (Willis 1966). Economically the family is very important because it provides many valuable ornamentals which are of much garden value. Though this family had attracted the attention of early embryologists, the embryological work in this family is very meagre and cover only a few members. Therefore it is felt that the family deserves further attention embryologically. In the present investigation the various aspects of the life history of Linociera intermedia starting from flower bud to fruit were investigated.

Material and methods The material Linociera intermedia Wt. was collected from the famous holy hills of Tirumala [Andhra Pradesh] by Prof. G. Rajeswara Rao was fixed in formalin acetic-alcohol. Dehydration and infiltration were followed according with the customary methods. The sections were cut between 5-12 μm in thickness and stained in Delafield’s hematoxylin.

Observations Linociera intermedia Wt. is a large tree with dense axillary panicles. The flower is tetramerous and consists of small, four lobed calyx, four short petals which are valvate in bud. The stamens are typically two in number, epipetalous and consist of short filament and broad connective. Ovary is 2-celled with two ovules in each cell. Fruit is an ellipsoid drupe with thin exocarp and bony endocarp.

Microsporangium, microsporogenesis and male gametophyte. The primary archesporium becomes differentiated in the four corners of the anther. The archesporial cells divide periclinally and an inner primary sporogenous and an outer primary parietal layers are formed. The latter by further periclinal divisions give rise to an anther wall which is 3-layered (Fig. 1. A). The inner most parietal layer develops as the anther tapetum. The subepidermal layer develops fibrous thickenings and forms the fibrous endothecium which is uni-

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seriate (Fig. 1C). However, these fibrous thickenings extend to some of the connective cells and there the fibrous endothecium becomes multilayered. The ephemeral middle layers become degenerated during the further growth of the anther. The tapetum is parietal in origin and is of the secretory type. The tapetal nuclei divide mitotically (Fig. 1. B) and produce 4-6 nucleate tapetal cells (Fig. 1. A).

The primary sporogenous cells undergo few mitotic divisions resulting in a moderately extensive sporogenous tissue (Fig. 1. A). The pollen mother cells undergo simultaneous divisions and produce isobilateral and tetrahedral tetrads of which the latter being more common (Fig. 1. D). Cytokinesis is by furrowing. The meiotic divisions in all the locules of the same anther are not synchronous. In the same anther one locule shows undivided pollen mother cells and the remaining locules show pollen tetrads. In some cases, pollen tetrads and pollen grains are seen in the different locules of the same anther.

The nucleus of the one nucleate pollen grain (Fig. 1. E) becomes displaced to the peripheral region by the formation of a large central vacuole. It undergoes mitotic division (Fig. 1. F) and cuts off a small lenticular generative cell and a large vegetative cell. The pollen grain are shed at the 2-celled stage. The pollen grains are triporate and occasionally tetraporate. The exine shows rod like thickenings and the intine is smooth (Fig. 1. E, F). The dehiscence of the anther is longitudinal and the pollen grains escape through the slit formed in the anther (Fig. 1. G).

In Linociera intermedia an interesting case of an abnormal anther inside the normal anther is observed (Fig. 1. I, J). This abnormal anther is seen in one of the anther locules of the normal anther. It protrudes into the adjacent locule of the normal anther by piercing through the connective (Fig. 1. I). This abnormal anther does not show the usual wall layers like epidermis, fibrous endothecium, etc. However it shows an extensive tissue towards outside which is irregular in shape and outline. This inner anther consists of an inner layer of tangentially elongated cells which form a circular ring around the pollen grains (Fig. 1. J). This layer superficially appears as the ‘anther tapetum’ with cells containing a single nucleus and dense cytoplasm. The abnormal anther had two sporangia. In one of them the pollen grains are agglutinised and form a mass and in the other normal pollen grains are present. The pollen grains of the abnormal anther are identical to the pollen grains of the normal anther.

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Fig. 1. A. Longitudinal section of anther showing sporogenous cells, tapetum (1-4-nucleate) and wall layers. B. Tapetal cell with dividing nucleus. C. Transverse section of part of anther showing fibrous endothecium, degenerating tapetum and pollen grains. D. Tetrahedral pollen tetrad. E. One nucleate pollen grain. F. Nuclear division in the pollen grain. G. Transverse section of anther showing dehiscing point (st. stomium). H. Transverse section of anther-sporangium enlarged to show agglutinated pollen grains (ag p). I. Longitudinal section of anther showing an abnormal anther (ab an) inside the normal anther. J. Portion of the 1, I enlarged to show the details.
in all respects such as the structure of exine, intine, etc. The normal anther containing this abnormal anther shows all the usual components namely epidermis, fibrous endothecium, pollen grains and connective tissue.

Pollen and pollen sac degenerations. Degenerations of pollen at various stages of development were observed. 1, 2, 3 or all the pollen grains of a tetrad degenerate quite frequently. In most of the cases one or more pollen sacs of one or both anthers degenerate. In still others, all the pollen sacs of both the anthers are seen degenerating. In a few cases the pollen grains become agglutinised and form large masses. Quite interestingly the anthers where the agglutinised condition of the pollen is present, the subepidermal layer does not develop fibrous thickenings (Fig. 1. H). But the fibrous endothecium is differentiated on the connective side.

Megasporangium, megasporogenesis and female gametophyte. The ovary is superior, bicarpellary, syncarpous and bilocular with two ovules in each locule on axile placentation. The ovule is orthotropus, unigamic and tenuinucellate (Fig. 2, A). An integumentary tapetum is differentiated which is uniseriate with uninucleate cells (Fig. 2. D). The hypodermal archesporium is unicelled and it directly functions as megaspore mother cell without cutting off a parietal cell (Fig. 2. B). The megaspore mother cell gives rise to a linear tetrad of megaspores (Fig. 2. D) after the meiotic divisions (Fig. 2. C). The chalazal one is functional and develops into an 8-nucleate embryo sac of the Polygonum type (Fig. 2. E, F). The mature megagametophyte is spindle-shaped (Fig. 2. F). The two synergids are flask-shaped. Two polars meet at the centre and fuse before the fertilisation. The antipodals are uninucleate and ephemeral.

Fertilisation. It is porogamous. Syngamy and triple fusion occur more or less simultaneously.

Endosperm. The endosperm is ab initio Nuclear and in latter stages shows rumination. Cell wall formation commences from the micropylar end and extends towards the chalazal end and ultimately filling the entire embryo sac with the cellular tissue. The rumination starts even when the endosperm is in a nuclear condition and by the time a globular embryo is formed the rumination becomes completed. The rumination here is due to the irregular ingrowth of the inner lining layer of the seed coat (Fig. 2. H). The endosperm extends into the grooves (Fig. 2. G). The endosperm cells are uninucleate and possess scanty cytoplasm.
Fig. 2. *Linociera intermedia* Wt. A. Transverse section of ovary. B. Megaspore mother cell. C. Megaspore dyad. D. Megaspore tetrad. Note the integumentary tapetum. E. 2-nucleate embryo sac. F. Mature embryo sac surrounded by the integumentary tapetum. G. A portion of the ruminate endosperm enlarged. H. Longitudinal section of massive seed coat, ruminate endosperm (end) and embryo.
Embryo. The embryo development could not be traced in detail due to the paucity of the material. However only a few stages of embryo development could be traced (Fig. 3. A–C). By a comparision of the avilable embryogenic stages with the stages obtained by the previous workers (Souéges 1942, Maheswari Devi 1958, 1975) it appears that the embryogeny in this species is also of the Solanad type. The suspensor is uniseriate and elongated.

Seed coat and fruit wall. The integumentary primordium arises simultaneously with the differentiation of the megaspore mother cell. It grows soon and covers the nucellus by the time the megaspore mother cell enters into the meiotic divisions. An integumentary tapetum makes its appearance by the time a megaspore dyad is formed in the ovule. It can be clearly seen at the megaspore tetrad stage. The integument is multiplicative and becomes massive at the post fertilisation stages. At the cellular endosperm stage the seed coat is very irregular in its inner side.

The ovary wall at the megaspore mother cell stage consists of 8–10 layers of parenchymatous cells which possess prominent nuclei and less cytoplasm (Fig. 3. D). At mature embryo sac stage, it consists of 14–16 layers of parenchymatous cells. All the cells are similar in appearance and have prominent nuclei but no cytoplasm (Fig. 3. E). The epidermal cells at this stage begin to accumulate tannins. The outer wall of the epidermis becomes thickened. After fertilisation, it grows extensively and becomes massive. At a few celled embryo stage the fruit wall is 25–30 layered thick and at this stage it can be demarcated into two distinct zones, the exocarp and the endocarp. The exocarp consists of 13–16 layers of somewhat large, loosely arranged, irregular cells. The endocarp consists of small, angular and closely arranged cells. By this stage the outer epidermal cells accumulate larger quantities of tannin. The outer wall of the outer epidermis is also very much thickened (Fig. 3. F).

Discussion The anther tapetum in Linociera intermedia is parietal in origin and is of the secretory type. The tapetal cells though uninucleate to start with becomes 4–6-nucleate due to mitotic divisions in the tapetal nuclei. Maheswari Devi (1958, 1975) also observed 3–4-nucleate tapetal cells in Nyctanthes arbor-tristis. Kapil & Vani (1966) reported nuclear divisions and fusions and ultimate formation of large irregular polyploid nuclei in the tapetal cells in N. arbor-tristis.

The presence of an abnormal anther inside the normal anther is reported in
Fig. 3. L. intermedius. A-C. Embryology. D-F. Fruit wall.
L. intermedia. In having pollen grains etc. the abnormal anther exactly resembles its normal counterpart. Such a condition is not reported in any other member of the family so far investigated. However, Andersson (1931) in *Syringa bretschneideri*, Messeri (1950) in *Olea europaea* recorded development of pollen sacs from the ovules, while Dutt (1950) in *Jasminum grandiflorum* and Patel (1965) in *N. arbor-tristis* and *J. flexile* reported carpelloid stamens. Thus in this family it appears that there are a few abnormalities in the development of anther and ovule.

In the presence of orthotoropous ovules *L. intermedia* differs with the rest of the members of the Oleaceae so far investigated. Uniseriate integumentary tapetum with uninucleate cells as reported in the present investigation was reported by Maheswari Devi (1958) in the species of *Jasminum* and *Noronhia emarginata*. She also reported biseriate integumentary tapetum at places in *Ligustrum confusum* and *N. arbor-tristis*. In the present study for the first time ruminate endosperm is recorded in the family Oleaceae. In *L. intermedia* the rumination is due to irregular inner lining layer of the seed coat.

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**References**

Linocieria intermedia Wt.

Endothecium tricorpate

Polygonum

Nuclear型で成熟すると種皮内部の不規則な成長によって胚乳表面にruminationがつくられる。胚形成の様式はSouëges (1942)やMaheswari Devi (1958, 1975)が他のモクセイ科の種で報告したものと同じである。