POST-FERTILIZATION STUDIES IN ANEILEMA PANICULATUM WALL.

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ABSTRACT

In Anetlema paniculatum the endosperm is of the nuclear type. Compound starch grains are abundant in all cells except in the outermost layer.

The embryogeny is of the Asterad type and is placed under Commelina variation. A single instance of polyembryony has been observed.

The seeds are pale brown, irregular and hard. The seed is divided into a narrow micropylar collar region and the broad basal region. The former is occupied by the embryo and the latter, by the endosperm. The nucellus is represented by a thin band of degenerated cells.

The seed coat is formed from both layers of the inner integument and inner epidermis of the outer integument.

INTRODUCTION

Although our knowledge of embryogeny in Commelinaceae dates back to 1839 when Jussieu gave an account of Commelina tuberosa, it is fragmentary. The later contributions are those of Sussenguth (1920), Parks (1935), McCollum (1939) and Maheshwari and Baldev (1958). A detailed account of the embryogeny is given by Souèges (1958 a, b) and Chikkannaiah (1962). Maheshwari (1958) and Chikkannaiah (1962) recorded the structure of the seed coat of some members of the Commelinaceae.

MATERIAL AND METHOD

Fresh opened flowers and seeds were fixed in formalin-acetic alcohol and preserved in 70 per cent alcohol. The usual process of microtomy was adopted, and staining was done with Heidenhain’s iron-alum-haematoxylin using, sometimes, erythrosin in 90 per cent alcohol as counterstain.
OBSERVATION

The development of the endosperm is of the nuclear type and the primary endosperm nucleus divides earlier than the zygote (Fig. 1). After a few free nuclear divisions the endosperm nuclei becomes peripheral (Figs. 2–4). When the embryo is at two-celled stage, the endosperm extends basally beyond the collar, enlarges and fills the lower part of the developing seed. Thus it gradually assumes an inverted top-shape (Figs. 3, 4). Wall formation is centripetal, and starts from the micropylar end towards the chalazal end. The endosperm becomes completely cellular when the embryo is at the globular stage (Figs. 5, 6).

In the outermost layer of the endosperm, the cells are almost narrow, rectangular, uninucleate and densely cytoplasmic. Sometimes, they divide

Figs. 1–10. Endosperm (emb, embryo; z, zygote); Figs. 1–4. Early stages in the development of endosperm; Fig. 4 is of whole mount. Fig. 5. L.s. seed showing cellular endosperm (hatched) and globular embryo. Fig. 6. Magnified view of endosperm marked a in Fig. 5. Figs. 7–9. Multinucleate endosperm cells from portion b marked in Fig. 5. Fig. 10. Enlarged view of chalazal region marked o in Fig. 5. Figs. 1–3, × 365; Fig. 4, × 152; Fig. 5, × 84; Figs. 6–10, × 830.
once periclinally to form two layers. At the chalazal end, however, these two layers show considerable modifications. In this region, their cells contain large irregular nuclei with several nucleoli (Figs. 5, 10). Unlike these cells of one or two layers, the internal endosperm cells are irregular, large, multinucleate and highly vacuolate (Figs. 6–9). Fusion and division of their nuclei are very common. All the cells of the endosperm except those of the outermost layer accumulate compound starch grains.

The zygote divides by an oblique wall to form the cells \( ca \) and \( cb \) (Figs. 11, 12). The segment \( ca \) divides vertically at right angles to the previous

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**Figs. 11–27.** Embryo (adv emb, adventitious embryo; cot, cotyledon; cot sh, cotyledonary sheath; cot st, cotyledonary strand; if, leaf primordium; pr, primary root; rc, root cap; sr, secondary root; st, stem apex; z emb, zygotic embryo). For explanation see text. Figs. 11–22, \( \times 553 \); Figs. 23–27, \( \times 243 \).
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wall forming two cells (Figs. 13, 14). Periclinal walls are laid down in this segment delimiting the dermatogen initials; a quadrant is, thus, eliminated (Figs. 15, 16). Meanwhile, the segment $cb$ also divides by a vertical-oblique wall forming two juxtaposed cells $ci$ and $m$. Of the latter, $ci$ divides transversely giving $n$ and $n'$; $m$ divides vertically to form two cells (Figs. 14, 15). Periclinal walls are laid down in $n$ and $m$ cutting of the dermatogen initials (Figs. 16, 17). The cell $n'$ divides into $o$ and $p$ (Figs. 17, 18). The embryo becomes globular due to irregular divisions (Figs. 18–21). When the embryo becomes subspherical due to more growth in the basal part, a lateral depression appears separating the derivatives of $ca$ from those of $m$ (Fig. 22). The derivatives of $ca$ give rise to a terminal cotyledon and those of $m$ contribute to the shoot apex, the cotyledonary sheath and one lateral part of the upper hypocotyledonary region. By further division $n$ contributes to the lateral upper part of the hypocotyl, and $o$ to the lower part of the hypocotyl and the root tip. Lastly, $p$ gives rise to the root cap with its initials (see Figs. 21–26).

The procambial strands for the primary root, the cotyledon and the stem apex are formed (Fig. 26). The lateral roots and first leaf primordium are differentiated at a later stage (Fig. 26).

The following sequence represents the development of the embryo: ($co$, root cap; $iec$, initials of central cylinder of root; $pco$, cotyledonary region; $phy$, hypocotyledonary region; $pvt$, shoot apex).

\[
\text{Zygote} \rightarrow \begin{array}{c}
\text{ca} \\
\text{rb} \\
\text{ci} \\
n' \\
p'
\end{array} \overset{\rightarrow}{\rightarrow} \begin{array}{c}
pco \\
pvt + phy \text{ (one lateral upper part)} \\
\overset{\rightarrow}{\rightarrow} \\
phy \text{ (other lateral upper part)} \\
\overset{\rightarrow}{\rightarrow} \\
phy \text{ (lower part)} + iec \\
\overset{\rightarrow}{\rightarrow} \begin{array}{c}
co
\end{array}
\end{array}
\]
Table showing the destination of the cells in different cell generation

1. First cell generation:
   - ca ~ pco
   - cb ~ pvt + phy + iec + co

2. Second cell generation:
   - ca ~ pco
   - m ~ pvt + phy (one lateral upper part)
   - ci ~ phy (other lateral upper part) + iec + co

3. Third cell generation:
   - ca ~ pco
   - m ~ pvt + phy (one lateral upper part)
   - n ~ phy (other lateral upper part)
   - n' ~ phy (lower part) + iec + co

4. Fourth cell generation:
   - ca ~ pco
   - m ~ pvt + phy (one lateral upper part)
   - n ~ phy (other lateral upper part)
   - o ~ phy (lower part) + iec
   - p ~ co

Thus, the embryogeny is of the Asterad type. A single instance of polyembryony has been observed. As indicated by its position the adventitious embryo might have been formed from one of the synergids (Fig. 27).

After fertilization the nucellar cells at the chalazal region enlarge and their walls become thickened forming hypostase (Figs. 28, 31). Some of its cells are filled with dark brown bodies (Fig. 35). This tissue persists in the mature seed (Fig. 37).

The nucellar epidermal cells at the micropylar region enlarge and their radial inner tangential walls become thickened during the development of the embryo-sac. After fertilization these thickenings become more pronounced. These cells form the epistase which persists till the globular stage of the embryo (Fig. 32). As the seed matures this tissue gets crushed.

At the time of organization of the embryo-sac a constriction is formed in the nucellus just below the micropylar end. After fertilization the inner
The seeds are pale brown, irregular and hard. Besides, the persistent hypostase and a crushed epistase, the nucellus are represented by a thin band of degenerated cells (Figs. 36, 37). The embryo surrounded by a few disintegrated endosperm cells occupies the entire collar whereas the basal region is filled with mealy endosperm.
Both the integuments contribute to the formation of the seed coat. The cells of the inner epidermis of the inner integument stretch as the seed develops (Figs. 28, 29, 31, 33). In a mature seed they become filled with brown material but the cross septa are still seen (Figs. 36, 37). The cells of the outer layer of the inner integument become vacuolated, and thickening proceeds in them from the outer tangential walls towards the inner tangential walls (Figs. 33, 37).

The cells of the inner epidermis of the outer integument are densely cytoplasmic (Fig. 29). As the seed develops, reticulate thickenings are formed in them and sometimes these cells are filled with refractive granules (Figs. 33, 37). The two outer layers of the outer integument at the basal region get stretched forming a thin strip (Fig. 37).

In the collar region also similar changes take place in the inner and outer integuments. But the cells of the outer integument except those of the innermost layer enlarge considerably filling the cleft around the collar (Fig. 36).

**DISCUSSION**

The endosperm formation follows the conventional type. The development of the endosperm has been described in *Commelina forskalaei* (Maheshwari and Baldev, 1958) and in *C. subulata, Murdania simplex, Floscopa scandens* and *Tinantia fugax* (Chikkannaiah, 1963, 1964, 1964–1965 a, b). In *Floscopa scandens* (Chikkannaiah, 1964–1965 a) enucleate button-shaped cytoplasmic vesicles appear frequently lining the cavity of the embryo-sac. In *Tinantia fugax* (Chikkannaiah, 1964–1965 b) there is only an aggregation of nuclei in the cytoplasm at the chalazal end of the embryo-sac. In *Aneilema paniculatum*, the two outer layers of the endosperm at the chalazal end are large and contain hypertrophied nuclei indicating high metabolic activities.

The development of the embryo in the Commelinaceae has never been the subject of complete investigation although some reports are available (see Murthy, 1934, 1938; Maheshwari and Baldev, 1958). Souèges (1958 a, b) and Chikkannaiah (1963, 1964, 1964–1965 a, b) have given a detailed account of the development of the embryo in some members of the Commelinaceae. Working on the embryogeny of *Commelina communis* and *Rhoeo discolor*, Souèges (1958 a, b) had brought out certain important features to give them a separate variation ‘Commelina variation’ under Asterad type. These features are (i) the juxtaposed position of the segments *m* and *ci*, (ii) the division of these two segments in different planes, (iii) the derivations of all the embryonic parts except the cotyledon from *cb* and (iv) the absence of suspensor. The embryogeny of *Commelina subulata, Murdania simplex,*
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Floscopa scandens and Tinantia fugax (Chikkannaiah, 1963, 1964, 1964–1965 a, b) follows the same sequence of those of Commelina communis and Rhoeo discolor (Souëges, 1958 a, b). The embryo development of Aneilema paniculatum is also of Commelina variation under Asterad type.

Although the segmentation in cb and its derivatives are similar in all plants so far investigated (see, Maheshwari and Baldev, 1958; Souëges, 1958 a b; Chikkannaiah, 1963, 1964, 1964–1965 a, b) the delimiting of the dermatogen initials from the segment ca is variable. It is at quadrant stage in Commelina forskalaei (Maheshwari and Baldev, 1958), Commelina communis and Rhoeo discolor (Souëges, 1958 a, b) and in Commelina subulata, Floscopa scandens and Tinantia fugax (Chikkannaiah, 1963, 1964–1965 a, b). In Murdania simplex (Chikkannaiah, 1964) it is at octant stage, while in Aneilema paniculatum, the plant under investigation it is at two-celled stage.

In a mature seed the basal region is filled with mealy endosperm and the micropylar region is occupied by the embryo surrounded by a few disintegrated endosperm cells. This apparently independent embryo from that of endosperm lead Parks (1935) and McCollum (1939) to believe that they had little influence upon one another at any time during their development. On the other hand, in Aneilema paniculatum the endosperm in the collar region is used by the developing embryo and the cotyledon is in direct contact with the endosperm in the basal region of the seed.

The occurrence of a collar is a characteristic in the seed of Commelinaeae. There are conflicting views regarding its formation. According to Parks (1935) and McCollum (1939) the outer integument is responsible for the initiation of the collar. Maheshwari and Baldev (1958) think that the collar is the result of inhibited growth of the nucellus at the micropylar end and enhanced growth at the other end. On the other hand, in Aneilema paniculatum it is the differential growth of the nucellus that initiates the collar formation and this, in turn, influences the integuments which play a secondary part in modelling the collar. Thus, there are at least two factors in the collar formation. The first is the differential growth of the nucellus which, perhaps, is intrinsic and the second is the spatial adjustment of the integuments.

In Commelina forskalaei (Maheshwari and Baldev, 1958) the seed coat is actually formed by the outer integument although the inner integument is transformed into a thin band. But the seed coat in the plant under study develops from both layers of the inner integument and the inner epidermis of the outer integument which is characteristic of this family (see Chikkannaiah, 1962). In Commelina subulata (Chikkannaiah, 1963) the cells of the inner
layer of the outer integument develop band-like thickenings; in *Floscopa scandens* and in *Tinantia fugax* (Chikkannaiah, 1964–1965 a, b) they are filled with white refractive granules. In *Aneilema paniculatum* they develop reticulate thickenings.

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