

Embryogenesis in sexual and asexual species of *Zeuxine* (Orchidaceae)

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Abstract. The development of the zygote following sexual union in *Zeuxine longilabris* exhibits a predetermined polarity and patternized sequence of developmental stages leading to the initiation of cotyledonary and epicotylary activity in adjacent sectors, both being terminal on the embryonal axis. The cotyledonary growth is incipient and the epicotylary sector is distinguishable only by histological distinctivity. The initiation and trend of growth of the asexual embryo totally deviates from that of its sexual counterpart. Although incipient and inconsistent polarity becomes established in the dividing nodular cell system eventually the embryos end up as a spindle-shaped structure of homogeneous cells where not even the initiation of root and shoot polar differentiation is seen either within or without. Under these circumstances the asexual embryos are broadly comparable to the earlier stages in the development of embryoids in free cell cultures.

Keywords. *Zeuxine*; embryogeny; Orchidaceae.

1. Introduction

Zeuxine longilabris Benth., a sexually reproducing species and *Z. strateumatica* Schltr., an asexually reproducing species possess somewhat overlapping distribution in South India. The materials of the former was collected from Setty thota, Mercara, Coorg District and of the latter from local environs. Customary methods of microtechnique and staining were followed in the preparation of serial microtome sections.

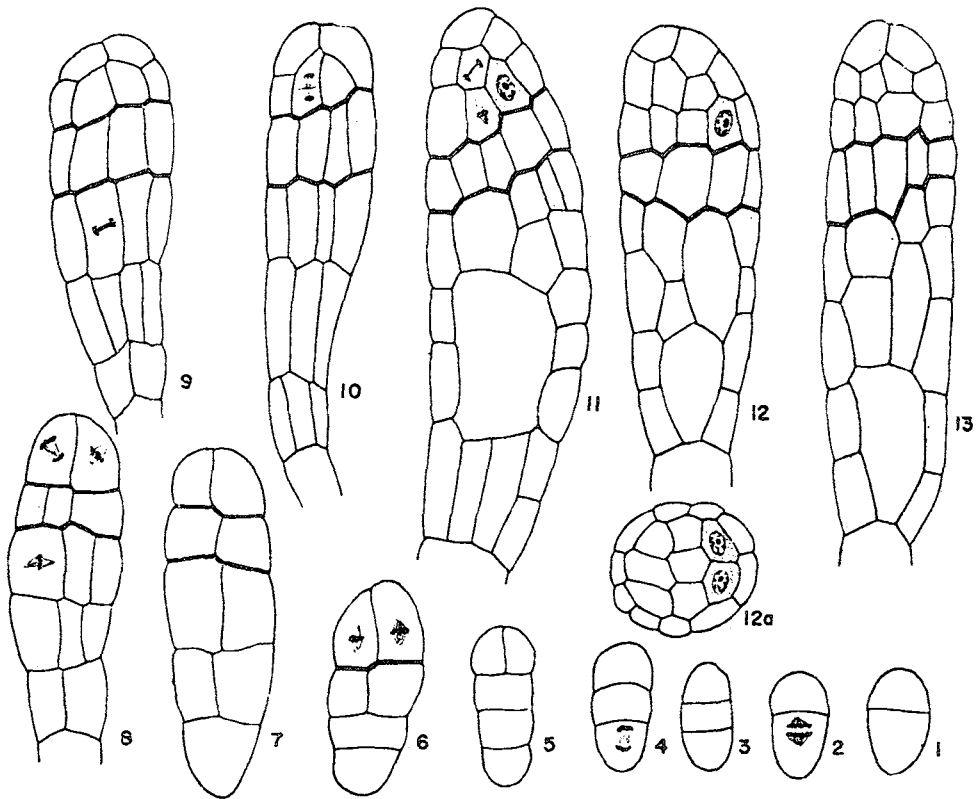
Considerable literature is available on the development of the embryo of orchids. Yet, more critical observations are necessary in order to establish the locus of origin of the shoot apex and to assess the degree of internal differentiation and organization. Attention is focussed on these aspects in the present study in regard to both sexual and asexual modes of embryonal development.

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2. Observations

2.1. *Zeuxine longilabris*

In the two-celled proembryo (figure 1), the basal cell, which almost abuts on the micropyle is slightly larger than its sister cell and divides transversely (figure 2) to result in a three-celled stage (figure 3). The cell next to the micropyle divides again (figure 4) in the same plane, resulting in a four-celled filament. The terminal cell now undergoes two successive divisions in vertical plane giving rise to a tier of four cells (figure 5). These cells divide in transverse plane (figure 6) giving rise to two superposed tiers of four cells each. By this time, the subjacent cells of the terminal tier also divide by vertical walls, each mother cell giving rise to a tier of four cells (figure 7). The last cell of the proembryo towards the micropyle, however, matures into an absorptive haustorial cell without undergoing division, a feature known for several orchid taxa (Swamy 1949; Wirth and Withner 1959).



Figures 1-13. *Zeuxine longilabris*. Longisections of successive stages in the development of embryo from 2-celled stage to maturity. Figures 10-12 are sections passing through cotyledon-epicotyl plane and figure 13 in paracotyledonary plane. Stippled cells in figures 11-12 represent the epicotylary sector. Figure 12a is transection of a stage similar to figure 12, the section passing through cotyledon and epicotyl sectors. Explanation in text.

As the orchid embryos are well-known to suffer continued development at any stage, the number of constituent cells remain small and the degree of differentiation weak. *Z. longilabris* is no exception. Both these conditions express themselves after the cell tiers between the terminal tier and the suspensor undergo one division by vertical walls (figures 8 to 10). Some of the resulting inner cells enlarge conspicuously and greatly disturb the tiered alignment (figures 11 to 13).

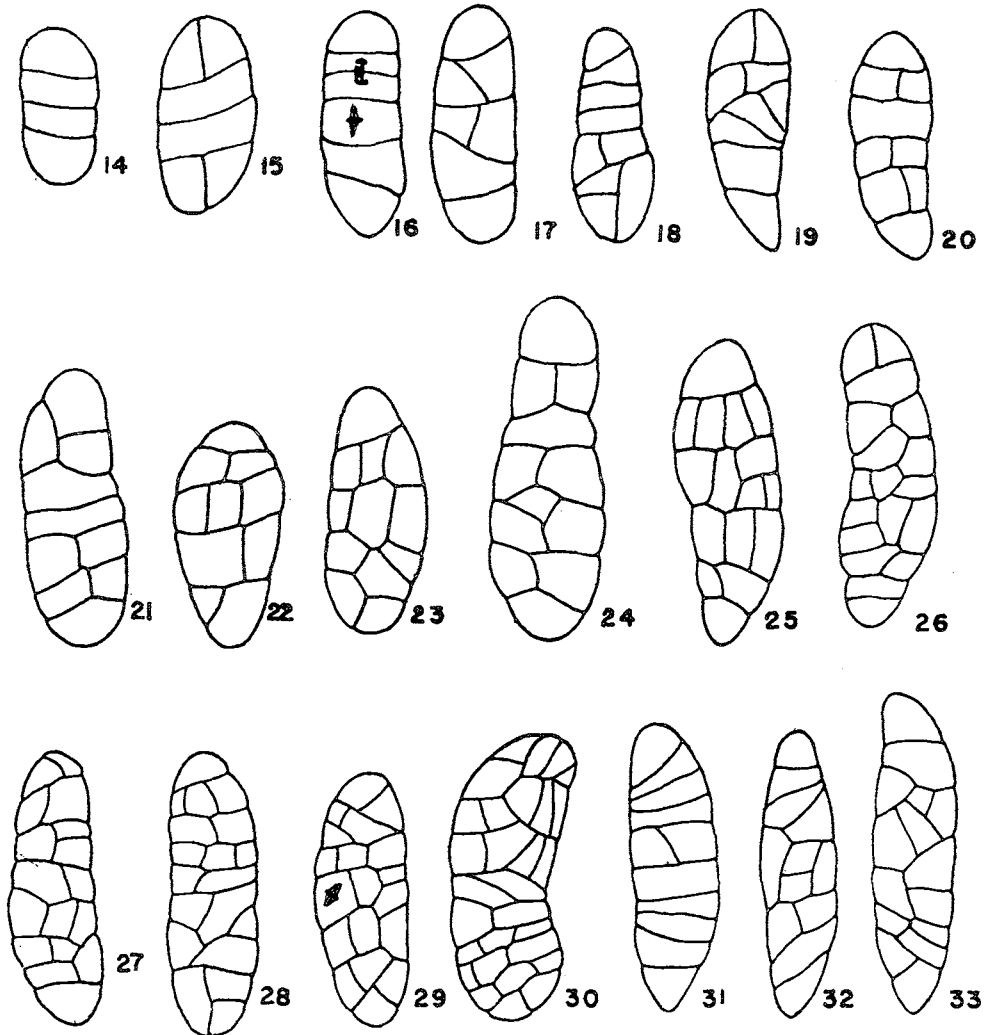
The two tiers derived from the terminal cell of the two-celled proembryo, however, remain relatively undisturbed during later ontogeny and also, the free tier exhibits a couple of decisive divisions leading to the organisation of an incipient shoot apex. After the cutting off of protoderm, two adjacent quarter sectors remain quiescent while in the other sector a pair of cells divides generally twice (figures 10 and 11) resulting in a small unit of cells (figure 12), thereby imparting asymmetry to the embryonic shoot apex, as seen in median longitudinal sections passing through the cotyledon-epicotyl plane. In median paracotylary plane, however, a symmetry is maintained (figure 13). The sector possessing the potentiality for division marks the locus of initiation of the cotyledon and the sector that remains quiescent signifies the epicotylary locus. Thus in *Z. longilabris* also the shoot apex is differentiated at the terminal point of the embryonal axis, and following the very first vertical division in the terminal cell the working sphere of the shoot apex is partitioned into two equal halves. One-half forming the initiation locus of the cotyledon and the other of the epicotyl, both being adjacent to each other.

2.2. *Zeuxine strateumatica*

An account of the apomictic phenomenon and the occurrence of polyembryony in this species has been published years ago (Swamy 1946) and a reexamination of fresh material in recent years only confirms the earlier conclusions. However, special attention has been focussed in the present investigation on the ontogeny of the adventive embryo in comparison with the sexual embryo of *Z. longilabris*.

The four-celled filamentous proembryo of the sexual species is derived from continuous divisions of the basal cell and its last derivative, while in the apomict (figure 14), both the basal and terminal cells divide once. The subsequent developmental pattern also significantly deviates from the norm of developmental sequence described above for *Z. longilabris*. Vertical walls may appear simultaneously in the end cells of the filament (figure 15). Or the filamentous stage continues further by the addition of new cells engendered by the intercalary cells of the filament (figure 16), resulting in an increased number of constituent cells (figure 31). Vertical and oblique divisions appear in these cells so that the embryonal body assumes an elongate spindle shape (figures 32 and 33).

More often, however, vertical or oblique walls appear when the filamentous proembryo is constituted of four or five cells. It is the intercalary cells of the filament that undergo such divisions (figure 17) and not the end cells. As a deviation from this norm the wave of such divisions may spread in one direction alone, as a result of which either the micropylar end or the chalazal end of the proembryo becomes swollen and the embryo as a whole assumes an obconical shape (figures 18 and 19). Figure 18 represents the normal orientation of the



Figures 14-33. *Zeuxine strateumatica*. Successive developmental stages of adventive embryos. Explanation in text.

normal embryo where the gradient of cell size and frequency of cell divisions are exactly the same as may be expected for asexually developed embryo; figure 19 represents the reverse situation, where the shoot apical pole of the embryo is directed towards the micropyle. It should be emphasized in this connection that the pattern and sequence of divisions at the shoot apical pole are neither comparable with the situation in the sexual embryo, nor is there the differentiation of suspensor in the adventive embryo.

The terminal cells remain unaffected for a long time while vertical divisions that were initiated in the intercalary cells of the filamentous proembryo normally spread in either direction (figures 20 to 25). Divisions in the intervening cells do not succeed in any definite sequence or according to a specified pattern in the

disposition of engendered cells. This situation is again a contrast where the concerned divisions give rise to axial tiers of eight cells, inner four and peripheral four. Although a few of the cells of a tier appear as if cutting off protodermal cells (figures 23 and 25), yet the inconsistency associated with this step is more obvious (figures 24, 26 to 30).

As stated already, the gamut of embryogenesis in the sexual species becomes arrested during the earlier stages of ontogeny itself. The developmental steps as seen in the adventive embryo—even as limited to the restricted gamut—are not comparable to the ontogenesis of the sexual embryo. The sole purpose of *Z. strateumatica* in producing only asexual embryos appears to obtain through any means a spindle-shaped mass of cells that are potentially meristematic. This means that there are no controlling factors of polarity or differentiation at work. The mature spindle shape results from a larger number of cell divisions in the intercalary tiers, where neither a patternized sequence nor a repeatable guideline is detected. It is true that an extremely weak bipolar gradient appears to be at work wherein the frequency of cell divisions is reduced from the equator to the pole. This apart, it must be emphasized that not even the earliest steps of endomorphic differentiations are seen, as a result of which neither root nor shoot apices are histologically detectable (figures 27 to 30).

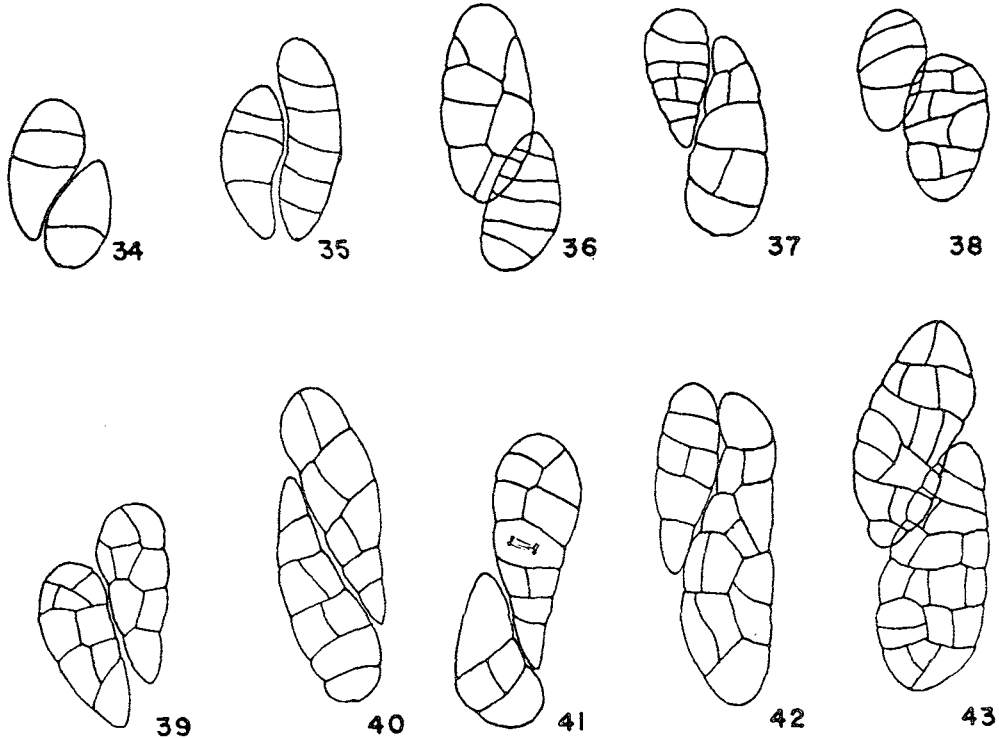
The positioning of the broadened end of embryos towards the micropyle has been referred to earlier. This situation is more frequently seen where more than one embryo develops in a seed. In such instances the reverse orientation is noticeable when the proembryos are in filamentous stage (figure 34) or one of the pair is in reverse orientation while the other fails to exhibit any directional trend (figure 35). The relative rates of development may be identical (figures 39, 40 and 43) or differential (figures 36 to 38, 41 and 42). Very rarely the orientation of the twins are identical, that is, the broader end facing the micropyle (figures 38 and 39), but the general condition is the mutually reverse orientations (figures 37, 40 to 42). Eventually, however, the retarded embryo also picks up speed and attains a similar state of development as its partner (figure 43).

3. Discussion

The essential results of the present study may be summed up as follows:

The development of the embryo in the sexual species *Zeuxine longilabris* follows a definite norm and after the delineation of the terminally located embryonic shoot apex and adjacently placed cotyledon and epicotyl loci suffers cessation of growth. Thus the ontogenetic events reflect a strongly polarized gradient and some of the morphogenetic steps are:

- (i) Attainment of the four-celled filamentous stage through the division of the basal cell or of its derivatives that are disposed next to the micropyle.
- (ii) The first vertical division in the terminal cell which signifies the division of the shoot apex into cotyledonary and epicotylary loci of equal volumes.
- (iii) Occurrence of periclinal divisions in all the cell tiers leading to the establishment of protoderm.
- (iv) Predominance of cell divisions in the cotyledonary sector although few imparts an asymmetry to the shoot apex.



Figures 34-43. *Zeuxine strateumarica*. Development stages of twin adventive embryos. Explanation in text.

None of these norms are encountered during the development of the asexual embryo in *Z. strateumarica*. On the contrary, the typical ontogenetic steps may be summed up as follows:

- (a) Although the two-celled proembryo often exhibits differential size, both the cells divide transversely to result in a four-celled filamentous stage. When this stage is prolonged, increase in length and in the number of constituent cells is achieved by transverse divisions in the intercalary cells.
- (b) The intercalary cells in the middle region of the filament divide by vertical walls and this wave decreases in opposite directions. Rarely, even when, the terminal cells of either end divide in vertical plane, the division is not related to morphogenetic involvement.
- (c) Occasionally the orientation of the embryo within the seed either may reflect the invariable norm seen in all instances of a sexually produced embryo or more often it is in the inverse direction, the broader end facing the micropyle. In instances of twin embryos, the most commonly encountered relates to the normal orientation for one embryo and the inverse orientation for the other.
- (d) Although the twin embryos often exhibit differential rates of development, eventually the retarded one picks up and both mature at the same time.
- (e) At the mature stage (when the capsule dehisces) the embryo is a spindle-shaped nodule-like body constituted of histologically homogeneous cells; there is no endomorphic differentiation; shoot and root polar distinctions are absent. All cells are potentially meristematic.

Thus the trends exhibited in the development of the asexual embryo are contrasting in every respect as compared with that of the sexually developed one.

The initial stages in the ontogeny of the asexual embryo often exhibit an oblong outline, thereby reflecting a blue-print of a unidirectional gradient on which the visible expression becomes superposed. However, it should be emphasized that the orientation of such embryos is not related to its position in the embryo sac, this structure having become defective, arrested and degenerating much earlier. The fact that the embryos grow outside the limits of the embryo sac suggest that if there is an agency that catalyzes a gradient, it must come into play by the built in capacity of the initial cells that grow into asexual embryos, thereby eliminating altogether the external environmental factors. That the parenchyma cells in general possess not only potentiality to revert to meristematism but also the capacity to initiate unidirectional flow of metabolites during specific conditions of growth is contemplated in connection with the development of embryoids (Steward *et al* 1969).

In reference to the asexual embryos of *Zeuxine strateumatica* the obovate shape is not consistent; nor is it permanent. This is readily seen in cases of twin embryos of an ovule. It may be argued that spatial factors within the ovule may, to some extent, bring about inverse orientation of one of the twins but a similar topography in regard to single embryos cannot be explained on the basis of the same factors. The evidence on hand strongly suggest the fact that the initial cells involved in the production of asexual embryos possess the capacity to synthesise and release unipolar gradients or bipolar gradients from the beginning. In the former example, the gradient begins to work in the reverse direction at some stage and eventually builds a cell-cluster identical in shape and structure comparable to the one that has developed from a bipolar gradient.

The rather precocious 'maturation' of the orchid embryo within the seed acts as a limitation for comparing later ontogenies of the sexually and asexually produced embryos. The capsules dehisce when the sexually developed embryo is in a stage represented in figure 12, the asexual embryo in figures 29, 30, 33 and the twin embryos in figure 43. However, it may be remarked that the general trend of development of the asexual embryo stands fair comparison with the initial stages in the *in vivo* growth of embryoids from free cells. The initial source in both is diploid somatic cells; they revert to meristematism and give rise to cell clusters. Polarity may set in at any stage by the built-in potency and from now on, the cluster body assumes an obovoid shape. In both, the direction of orientation of the nodular body is independent of the external influences and lack step-by-step differentiation.

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