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ALGORITHMS OF EMBRYO MORPHOGENESIS IN *AGAPANTHUS PRAECOX* WILLD. (ALLIACEAE) IN MONOCOTYLY, DICOTYLY AND TRANSITIONAL FORMS

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The study found that the monocot *Agapanthus praecox* from different cultivation localities has a stable tendency to develop dicotyledonous together with monocotyledonous and transitional forms, with various degrees of cotyledonary fusion. The morphogenetic events during embryogenesis leading to this diversity are presented and analyzed.

Key words: *Agapanthus praecox*, Alliaceae, embryogenesis, monocotyly.

INTRODUCTION

The ability of monocot plants to form dicotyledonous embryos is a question of great theoretical significance in connection with the problem of the origin of monocotyly. Earlier authors gave data on the spontaneous formation of typical dicotyledonous embryos in some Alliaceae (*Agapanthus umbellatus* – Coulter and Land, 1914), Amaryllidaceae (*Cyrthanthus sanguineus* – Farrell, 1914) and Araceae (*Colocasia antiquorum* – Suessenguth, 1921). For a long time these data have been criticized but never reinvestigated. A single work on the embryogenesis of *A. umbellatus* was published by Guignard and Mestre (1969), but it was devoted to the origin of the cotyledon and shoot apex in the embryo of this plant.

This study examines the formation of dicotyledonous embryos in *Agapanthus praecox* Willd. (= *A. umbellatus* L. Herit.), and analyzes the sequence of morphogenetic events during embryogenesis leading to mono- and dicotyledonous states.

MATERIALS AND METHODS

Mature seeds of *A. praecox* were collected from the Royal Botanical Gardens of Sydney, Australia (1998) and the greenhouses of the Komarov Botanical Institute, St. Petersburg, Russia (2000, 2001); material for study of embryogenesis was fixed at the Komarov Botanical Institute. Seed germination was monitored under laboratory conditions (18–20°C, natural illumination) in no less than 500 seeds from each locality of cultivation. For study of embryogenesis, flowers were artificially pollinated and the embryos were fixed. Material for light microscopy (LM) and SEM was fixed in FAA and 2.5% glutaraldehyde, respectively, and worked upon by standard methods. Sections 12 μm thick were stained with fuchsin/sulfuric acid, alcian blue and hematoxylin.

RESULTS

Structural analysis of seedlings obtained from seeds from both localities confirmed the spontaneous formation of dicotyledonous forms in

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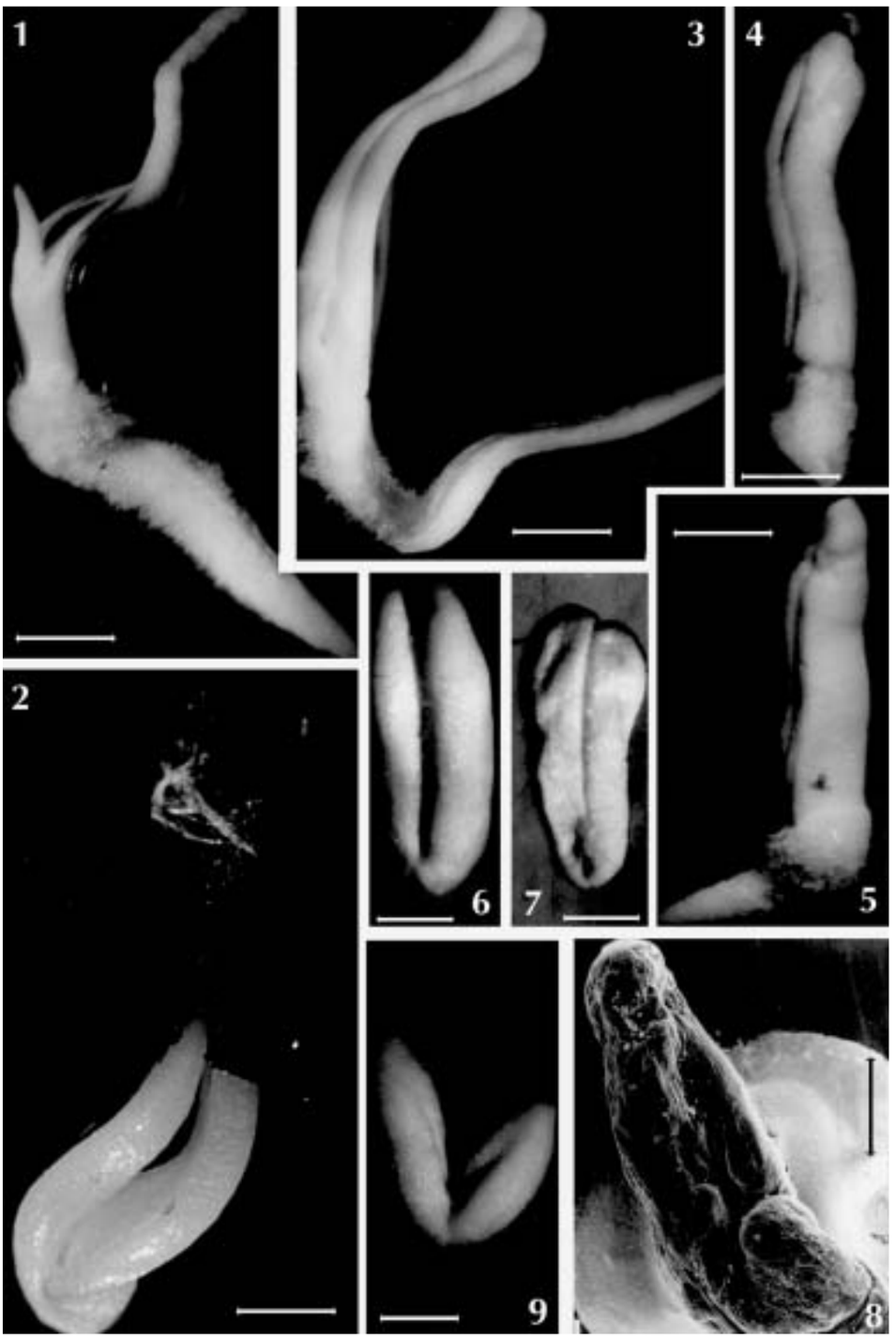


Figs. 1–9. Formation of three types of embryos in mature seeds and seedlings in *Agapanthus praecox*. **Fig. 1.** Monocotyledonous seedling. **Fig. 2.** Dicotyledonous seedling. **Figs. 3–9.** Seedlings (**3–5**) and embryos from ungerminated seeds (**6–9**) with full (**3–7**) and partial (**8, 9**) unimarginal congenital fusion of cotyledons; suppression of one of the cotyledon primordia is visible. Bars in Figs. 1–5 = 1 mm, in Figs. 6–7 and 9 = 0.5 mm, in Fig. 8 = 0.1 mm.

A. praecox together with monocotyledonous and different transitional forms, mainly with various degrees of unimarginal congenital fusion of cotyledons (Figs. 1–9). The percentages of dicotyledonous embryos and embryos with cotyledon fusion in seeds from Sydney and St. Petersburg were near 1% and 6%, respectively. The greater part of such embryos formed plantlets, but some of them were not able to germinate (Figs. 6–9).

Analysis of the sequence of morphogenetic events during embryogenesis of *A. praecox* showed that proembryo formation (1–25 DAP) occurs quite uniformly, according to the Onagrad type, in agreement with the data of Guignard and Mestre (1969). The differences were expressed after the globular stage of development. At the transition to organogenesis 26–27 DAP, the apical part of the embryo became flattened, changing its form from round to ellipsoidal, and the beginning of bilateral symmetry was observed (Fig. 10) ("triangular" stage according to Jurgens and Mayer, 1994). Then at 28–29 DAP, three types of embryos were noted, with different types of symmetry and modes of cotyledon formation: (1) embryos with continuing establishment of bilateral symmetry and the formation of two cotyledonary primordia (Fig. 11) with two vascular bundles in each; (2) embryos with dorsoventral symmetry and the formation of a single cotyledonary primordium (Fig. 12), usually with three vascular bundles in it; and (3) embryos also with dorsoventral symmetry but with partial congenital fusion of cotyledons (Fig. 13) and with diverse vascular system structure. In the kidney-shaped apical part of such embryos the unfused tops of two cotyledonary primordia with a common base were clearly distinguished; moreover, one of the primordia was bigger and more developed than the other.

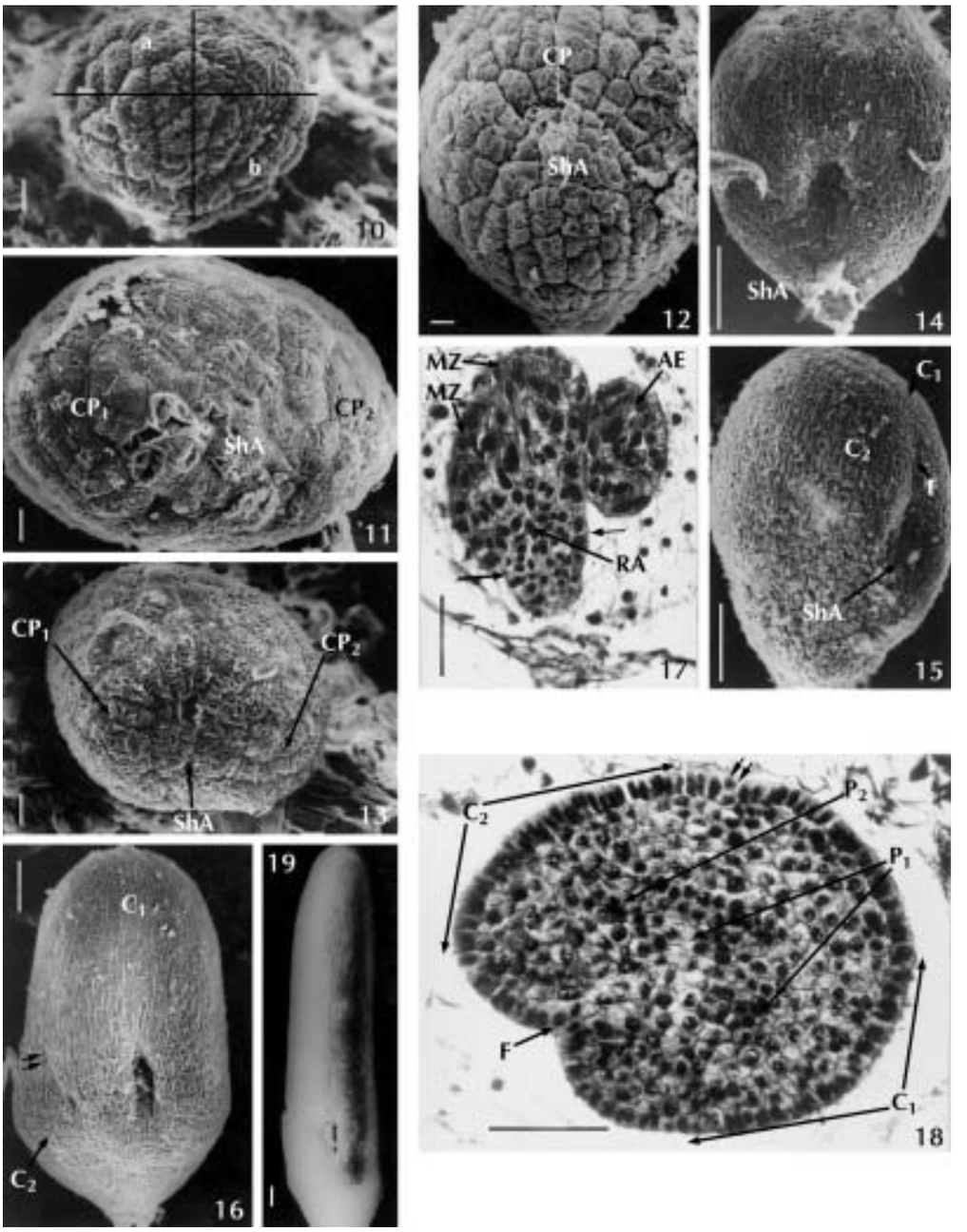
During the early stages of cotyledon development (30–45 DAP), traces of unimarginal congenital fusion of two initial cotyledons were almost always present in monocotyledonous embryos. Externally these were manifested in the general asymmetry of the integral cotyledon and in the presence of a furrow on its adaxial side in many cases (Figs. 14–16). Of particular interest are the difference in the positioning of the integral cotyledon margins on its opposite sides, and the specific curved orientation of the shoot apex zone. These can be seen in longitudinal sections of such embryos in the dorsoventral plane (Fig. 17) and in cross section (Fig. 18). Two meristematic zones of initial cotyledon primordia and the boundary of their fusion are visible as well. The suppression of one of these zones in development is the main reason for the asymmetricality of the integral cotyledon. In cross section, the vascular system of the integral cotyledon features two procambium bundles belonging to the bigger initial cotyledon, and one bundle belonging to the smaller, less developed one. Thus, the three vascular bundles in a single cotyledon of the monocotyledonous embryo apparently arise via suppression of one of two bundles in one of initial cotyledons in the dicotyledonous embryo.

In the final stages of embryo formation (46–60 DAP), all the above-described features of integral cotyledon development were lost: the asymmetry of its structure, the traces of two initial meristematic zones and the boundary of their fusion disappeared, and the margins lined up (Fig. 19). However, various deviations in integral cotyledon structure were often observed: a deep furrow on the adaxial side of the cotyledon (Figs. 3–7), unfused tops of the two initial cotyledons (Figs. 3, 4), frequently of very unequal sizes (Fig. 8), and two centers of very large cells in the apical part instead the usual single center.

The final observation regards the numerous abnormalities of nuclear division during the coenocytic stage of endosperm development and its cellularization in *A. praecox*. Especially characteristic was chromatin agglutination in the micropylar and chalazal parts of the endosperm. This peculiarity of development was correlated with the tendency to form additional embryos of integumentary origin, among which dicotyledonous forms also were noted (Fig. 9).

DISCUSSION

This study confirmed that the monocot *A. praecox* from different localities of cultivation can form dicotyledonous together with monocotyledonous embryos, as Coulter and Land (1914) first discovered. This tendency is stable, and involves a series of transitional forms with various degrees of cotyledonary fusion. The divergence in morphogenesis into three types of embryo formation begins after the globular stage of development, and finds expression in the establishment of different types of apical symmetry, with corresponding modes of cotyledon arrangement and conductive system development. Overcoming the "triangular" stage of development (the beginning of bilateral symmetry) apparently is what determines the preservation of traces of the initial dicotyledonous condition in monocotyledonous embryo structure. Analysis of the transitional



Figs. 10–19. Embryo development in *Agapanthus praecox*. **Figs. 10–13.** Changing apical form and symmetry during the transition to organogenesis and the formation of three types of embryos: "triangular" stage – beginning of bilateral symmetry establishment (a>b) (**10**), embryos with two cotyledonary primordia – bilateral symmetry (**11**), with a single cotyledonary primordium – dorsoventral symmetry (**12**), and with two congenital fused primordia (**13**). **Figs. 14–19.** Monocotyledonous embryo structure in early stages of cotyledon formation (**14–17**) and during completion of embryogenesis (**19**). Figs. 10–16 and Fig. 19. External view of embryo (SEM); Figs. 17–18. Embryo internal structure (LM) in longitudinal section in dorsoventral plane (17) and cross section on the level of the cotyledonary sheath (18). AE – adventitious embryo; C_1, C_2 – cotyledon; CP, CP_1, CP_2 – cotyledonary primordium; F – furrow on adaxial side of integral cotyledon; MZ – meristematic zone of initial cotyledons; P_1, P_2 – procambium strand; RA – root apex; ShA – shoot apex. Single arrows mark the different levels of margins of the integral cotyledon from opposite sides; double arrows marks the boundary of initial cotyledon fusion. Bars in Figs. 10–13 = 10 µm, in Figs. 14–16 and 19 = 100 µm, in Fig. 17 = 50 µm, in Fig. 18 = 100 µm.

forms showed that the monocotyledonous condition is derived from the dicotyledonous condition, and is achieved via unimarginal congenital fusion of two initial cotyledons, accompanied by various degrees of suppression of the meristematic zone of one of them – anisocotyly against the background of syncotyly. This allows some correction of Coulter and Land's (1914) hypothesis that "monocotyledony is not the result of the fusion of two cotyledons, or of the suppression of one; but it is simply the continuation of one growing point on the cotyledonary ring, rather than a division of the growth between two growing points." The divergence in the types of establishment of embryo apical domain symmetry in *A. praecox is* attended by different abnormalities of nuclear division during the coenocytic stage of endosperm development (chromatin agglutination, etc.) and its cellularization, and the tendency to polyembryony. Proceeding from modern data on regulation of these processes in plants (Liu et al., 1993; Fisher and Neuhaus, 1996 and others), the whole spectrum of phenomena mentioned for a given species evidently is connected with the changing hormonal status of the seed.

The present confirmation of the elements of dicotyledonous embryo formation in monocotyledonous *Agapanthus*, together with identification of the transitional forms between them, supports the concept of the homology of monocot and dicot cotyledons (see Burger, 1998). These findings are of key significance in solving the complex problem of the origin of monocotyly, which will be discussed in further publications.

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REFERENCES

BURGER WC. 1998. The question of cotyledon homology in angiosperms. *Botanical Review* 64: 356–371.

COULTER JM, and LAND WJG. 1914. The origin of monocotyledony. *Botanical Gazette* 57: 509–519

FARRELL ME. 1914. Ovary and embryo of *Cyrtanthus sanguineus*. *Botanical Gazette* 57: 428–436.

FISHER C, and NEUHAUS G. 1996. Influence of auxin on the establishment of bilateral symmetry in monocots. *Plant Journal* 10: 659–669.

GUIGNARD J-L, and MESTRE J-C. 1969. L'origine du cotyledon chez *l'Agapanthus umbellatus* L. Her. *Comptes Rendus Hebdomadaires des Seances de l'Academie de Sciences,* Paris 268: 3068–3070.

JURGENS G, and MAYER U. 1994. *Arabidopsis.* In: Bard JBL [ed.], *Embryos. Colour atlas of development*, 7–21. Wolf Publisher, London.

LIU CHUN-MING, XU ZHI-HONG, and CHUA NAM-HAL. 1993. Auxin polar transport is essential for the establishment of bilateral symmetry during early plant embryogenesis. *Plant Cell* 5: 621–630.

SUESSENGUTH K. 1921. Beitrage zur Frage des systamatischen Auschlusses der Monocotylen. *Beihefte zum Botanischen Centralblatt* 38: 1–79.