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Organogenetic Potencies of Isolated Apical Complexes of *Arabidopsis thalliana* (L.) Heynh. in Plants of Different Age

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Abstract—We studied the organogenetic potencies of the isolated apical complexes of *Arabidopsis thaliana* (L.) Heynh. in plants of different age. The explants directly continued their organogenesis in situ only provided that they were isolated from the plant in the vegetation period (the II phase of organogenesis). Taken from the plants turning to the formation of the generative organs, the apical meristem returns to the earlier phases of organogenesis. However, the "deep" reversion of development occurs only at phases III–IX. The degree of reversion decreases, up to the complete loss of the regeneration capacity, after the host plant has formed the seed rudiments.

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INTRODUCTION

One can use different approaches when studying plant development. The widely practiced approach is concerned with the observation of the intact plant paying attention to the structures being at the onset or in the end of morphogenesis (Akhundova et al., 1994). Unfortunately, this approach does not fit the evaluation of the organogenetic potencies of meristems of a different age and physiological state. Meanwhile, as N.P. Krenke (1950), N.I. Dubrovithskaia (1961), R.G. Butenko (1984), and A.G. Yusufov (1984) mentioned, the morphogenetic trend depends, not only on the plant species and environmental conditions, but also on the age of the maternal organism.

In order to solve this problem, one needs to have a model that is simpler than the plant organism as a whole. Both the physiology of plants and experimental botany apply the method of cultivation of the separate parts of the plants, both the organ and tissues (Leike et al., 1980; Butenko, 1984; Yusufov, 1988). This method provides just a model required for the fundamental investigations concerned with the regulation of development. Using of the isolated meristem complexes permits the solving of the problems concerned with the determination of the plant development, to evaluate the regeneration capacity and independence degree of buds, apical meristems, and organs of the flower (Rastogi, Sawhney, 1989).

Although the plant *Arabidopsis thaliana* (L.) Heynh. is the model object for genetics, biochemistry, and molecular and developmental biology, its tissues and organs were rarely cultivated in vitro. The plants genetically identical to the maternal ones are usually obtained from the calluses, by inducing of the shoot morphogenesis (Negrutiu et al., 1978; Gai, 2001). The calluses of *A. thaliana* can grow in vitro in the medium with no hormonal addition (Ondrej et al., 1984). This, however, is not suitable for the studying of the organogenetic potencies of the apical meristem. In the case of the shoot morphogenesis, all meristems are at the same stage of organogenesis (phase II). An introduction into the in vitro system of the *A. thaliana* apical meristems would provide an opportunity of obtaining of the explants from the plants being at different phases of the organogenesis. This encounters problems concerned with the small size of the meristems of *A. thaliana* (Vaughan, 1955). Even in most favorable conditions, it consists of a few hundreds of cells and is very sensitive to damage. Besides, even the intact meristem lacking the leaf or floral rudiments does not survive in culture, even provided that the medium contains the necessary set of nutrient substances, hormones, and vitamins. Regeneration occurs if the meristem is not separated from the subapical region including 2–3 floral or leaf rudiments. This is the minimal number of accessory organs allowing for the survival of the meristem in vitro (Sedova et al., 2002).

The goal of our experimental botanical work was to understand the mechanisms of developmental regulation based on how the ontogenetic stage of the host plant (the phase of organogenesis) influences the explants development. The aim was to evaluate the organogenetic potencies of the apical meristems of *Arabidopsis thaliana* (L.) Heynh. in vitro explanting these at different phases of organogenesis of the host plant.

MATERIAL AND METHODS

The host plants of *Arabidopsis thaliana* (L.) Heynh., the ecotype Columbia, were reared on the solid nutrient medium B5 (Gamborg et al., 1968), with no additions. When the host plant reached a definite phase of organogenesis, we separated the apical meristem of the main shoot together with 2–3 leaf or floral rudiments in ster-

Characteristic of organogenesis of regenerates from the apical meristems of *A. thaliana* in the plants of different age

Group	Stage of organogenesis of the host plant	Rosette leaves	Stem leaves	Mature flowers of the main inflorescence	Floral meristems	Axillary buds	Presumptive buds of the callus
1	II	8.91 ± 0.35	2.17 ± 0.13	2.77 ± 0.35	2.66 ± 0.25	1.46 ± 0.21	1.57 ± 0.24
2	III–VIII	5.59 ± 0.22	1.57 ± 0.14	4.48 ± 0.39	3.02 ± 0.18	1.25 ± 0.21	Single cases
3	IX	6.35 ± 0.39	0.56 ± 0.14	1.38 ± 0.35	1.05 ± 0.21	Single cases	–
4	X	–	1.88 ± 0.26	3.31 ± 0.49	2.06 ± 0.30	Single cases	–
5	XI	–	Single cases	3.20 ± 0.23	1.30 ± 0.15	–	–
6	XII	–	–	–	–	–	–

ile conditions and placed it into the B5 medium adding the sugarhouse (10 g/l) and kinetin (0.5 mg/l). As the explants grew out, they were transmitted into the medium with no additions. Their survival was about 80%. We analyzed the regenerates at 30–40 days after the beginning of cultivation.

The meristem complexes were subdivided into the groups corresponded to the characteristic features of the host plant morphogenesis (Krinitsyna and Murashov, 2006). In all the regenerates succeeded to survive (no less than 30 individuals in each group), we accounted the number of organs that arose because of the apical meristem activity. In each regenerate, we considered the stem and rosette leaves, buds in the rosette leaves axils, and additional buds that arose from the callus. In each group, we calculated the mean value of a character under consideration, standard deviation, and errors (table).

RESULTS AND DISCUSSION

The observation of the behavior of the shoot apical meristems of *A. thaliana* in vitro came to the following conclusions.

The regenerates of the explants whose host plants were at the II phase of organogenesis (Group 1) usually formed the rosette from 4–10 leaves. Occasionally, in the axils of rosette leaves, the meristem of an axillary bud arose, which, omitting the latent period, formed the new rosette of leaves. The trends of their further development varied. In 59% of the regenerates, the cells at the wound surface set to divide in disorder. This led to the formation of the callus with further differentiation of presumptive buds. Both the apical and secondary meristems continued to form the vegetal metameres. At the onset of our observations, they continued the vegetative organogenesis (Fig. 1a). In other regenerates, both the primary and secondary meristems turned to the prefloral organogenesis. In the apical meristem of these regenerates, we found the initiation of floral buds (the IV phase of organogenesis) and the beginning of differentiation of floral meristems (the V phase of organogenesis). However, these did not form the normal inflorescence pattern (Fig. 1b). In 41% of explants from the same group, regeneration turned to development of the generative organs, with no calluses at the wound surface. Both the apex and lateral meristems stopped to form the leaf rudiments turning to the prefloral, and then to the floral, organogenesis. When we started to analyze these explants, their inflorescences contained 6–12 flowers. Occasionally, the inflorescence axis had 1–2 stem leaves, with lateral inflorescence branches forming in their axils. The development of flowers usu-

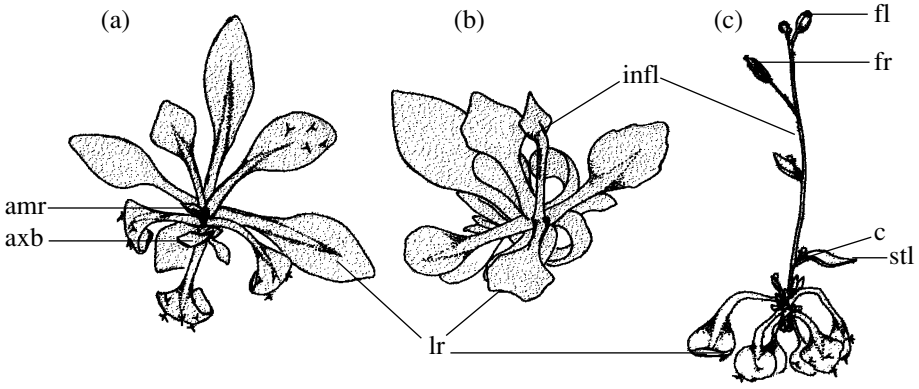

Fig. 1. Regenerates from the apical vegetative meristems of *A. thaliana* (the II stage of organogenesis of the host plant) at the 30th day of development: (a) preservation of the vegetative state, (b, c) transition to the generative organogenesis: lr, rosette leaf; stl, stem leaf; axb, axillary bud; amr, apical meristem of the regenerate; prc, paracladium; infl, main inflorescence; fr, fruit; fl, flower; br, bractea; flr, floral rudiment of the explant.

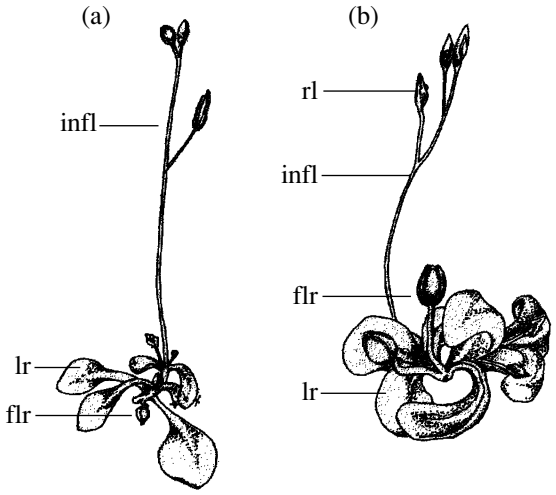

Fig. 2. Regenerates from the prefloral apical complexes of *A. thaliana* at the 30th day of development: (a) the III–VIII stages of organogenesis of the host plant, (b) the IX stage of organogenesis of the host plant (for designations, see Fig. 1).

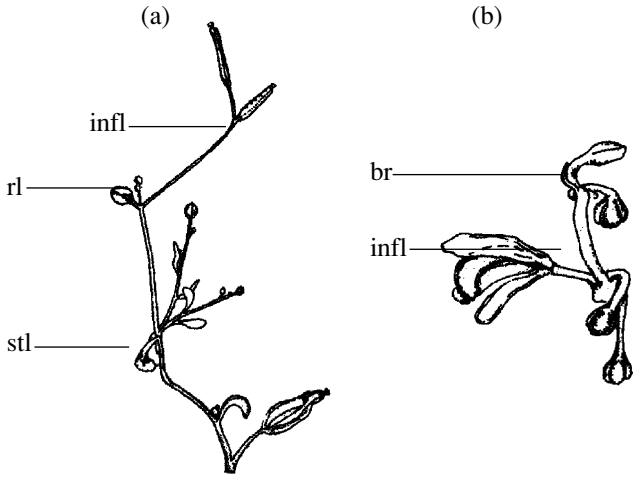

Fig. 3. Regenerates from the prefloral apical complexes of *A. thaliana* at the 30th day of development. The host plant was at the X (a) and XI (b) stages of organogenesis (for designations, see Fig. 1).

ally reached the VII phase of organogenesis (the closed flowers). The glowering of regenerates (corresponding to the IX phase) was rarely observed and we have registered only two regenerates that succeeded to form the fruits whose seeds were not viable (Fig. 1c). Prolongation of the vegetative period occurs in nature being the consequence of changing of the external conditions, for example, the photoperiod (Martinez-Zapater et al., 1995).

The behavior of regenerates originated from the explants whose host plants were at the III–VIII phases of organogenesis (Group 2) was tedious enough. The meristem, attempting to continue its developmental program to form 2–5 floral rudiments, failed to do this and regressed to the formation of the rosette. This always preceded the turning of the meristem to floral organogenesis. The apical meristem formed the axillary meristems in the rosette leaves axils, which, at the time of observation, were in the vegetative developmental phase. Some regenerates, similarly to that from Group 1, formed the calluses on their wound surface. They formed the stem leaves and the inflorescence with 4–9 flowers. The axils of stem leaves developed the lateral axes of inflorescence. The regenerates glowered, but we have not succeeded to get the viable seeds (Fig. 2a). The floral meristems of the subapical region of explants could perform floral differentiation, only provided that they were at the 3–4 developmental stages (Smith et al., 1990).

The regenerates grown from the meristems whose host plants were at the IX phase of organogenesis (Group 3) consistently formed many rosette leaves. The floral rudiments in the subapical explant region developed into the flowers whose pattern was not perfect, as the number of petals and sepals has often been reduced. We put off the task of tracing of the fate of floral meristems that were at earlier developmental stages. In contrast to the regenerates from Groups 1 and 2, we have never observed the formation of calluses at the wound surface (Fig. 2b). The explants formed the metameres of the shoot rosette region, up to the formation of the rosette, but the floral organogenesis did not occur in all the regenerates. In those forming the generative organs, paracladia appeared in the axils. Their further development looked like the development of the plant from the seed.

The regress to the earlier developmental stages in vitro conditions was observed in many monocotyledonous and dicotyledonous plants (in *Impatiens* sp. and *Anagulis* sp., see Battley and Lyndon, 1990; in *Glycine max*, see Washburn, 2000; in *Arabidopsis thaliana*, see Martinez-Zapater et al., 1995 and Okamuro et al., 1996). In *A. thaliana*, this has been observed in 0.4% of the plants (Okamuro et al., 1996). In all the cases, the host plants were at the III–IX phases of organogenesis.

The regenerates originated from the explants whose host plants were at the X phase of organogenesis (Group 4) continued to form the flowers. Occasionally, a new stem leaf formed at the regenerate base, producing in its axil a lateral inflorescence axis that failed to develop. These regenerates normally glowered, but the developmental cycle completing with the formations of fruits was observed only in those flowers that were in the direct contact with the nutrient medium. Some regenerates succeeded to form 2–3 short metameres and green leaves, which looked like a rosette. However, their shape was closer to that of the stem leaves. Matching the prefloral organogenesis to the vegetative one makes it clear that, in Group 4, the formation of stem leaves is the compensation of deficiency of the vegetative metameres, which do not develop in meristems taken from the host plant being at

the phase X of organogenesis (Fig. 3a). The behavior of the meristem in vitro confirms the idea that the normal floral organogenesis is possible only after the formation of the leaves (Sabinin, 1963).

In explants of meristems taken from the elder plants (the XI phase of organogenesis of the host plant, Group 5), the number of newly forming stem leaves was subject to decrease, as compared to the previous groups. At the same time, some individuals did form leaves that, in their shape, differ both from the stem and rosette leaves. A single flower developed in the axil of such a leaf. Therefore, these leaves correspond to the bracteas, which, even provided that they do form in the early ontogeny of *A. thaliana* in situ, reduce at the later developmental stages being absent in the adults. The developmental schedule of flowers in this group did not fit in to their position. Thus, in 40% of the obtained individuals, the fruits arose from the flowers situated in the middle of the peduncle, while the lower ones remained closed (Fig. 3b).

The explants from the plants that were at the XII stage of organogenesis did not regenerate at all, except for the rudiments of the subapical region. The apex per se died in vitro conditions, while the development of the floral meristems was arrested at the carpel stage (stage 9 according D.R. Smith, 1990).

Thus, we established that, in the isolated meristem complexes of *Arabidopsis thaliana* (L.) Heynh. developing in vitro, the formation of calluses at the wound surface of the explants attended by the formation of presumptive buds occurs, in general, only in the vegetative explants. With the disturbance of centralized developmental regulation, the apical meristem regresses to the earlier stages of organogenesis. However, the "deep" reversion of development is possible only at the III–IX phases of organogenesis. The formation of the seed rudiments in the host plants drastically decreases, up to the complete loss, the regeneration capacity of the apical meristem.

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