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Interconnection of the Organogenetic Processes in the Ontogeny of *Arabidopsis thalliana* (L.) Heynh.

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Abstract—We considered the interconnection of morphogenetic processes in the ontogeny of *Arabidopsis thaliana* (L.) Heynh. Our original observations, backed by the staging of organogenesis according to F.M. Kuperman, were matched to the data borrowed from literature on morphogenesis in Arabidopsis.

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INTRODUCTION

The problem of interconnection of morphogenetic processes is of crucial importance in the plant morphology (Batygina, 1974). Depending on the goal of the investigation, it can be considered at the levels of the organism, organ, tissue, and cell. Therefore, the morphogenetic studies of the plants pay much attention to the question on how morphological differentiation of the plant organism is concerned with the stage dependence, aging, and correlative changes that occur in ontogeny.

Although the plant *Arabidopsis thaliana* (L.) from the family of Crucifers is carefully studied being a model object for scientists all over the world (Meyrowitz, 2001), there is no clear-cut idea concerning the interconnection between organogenetic processes. Among the reviews, both of Russian and foreign authors, the most complete, to our opinion, is the monograph *Arabidopsis* (Cold Spring Harbor Laboratory Press, 1994) summing up the joint investigations of many authors. Besides, there are investigations that refer to the single periods, stages, and phases of the life cycle of this plant. These are the works concerned, for example, with the development of the flower as a whole (Smyth et. al., 1990) or its separate parts: gynoecia (Session, 1997; 1999), petals, stamens (Day, et. al., 1995; Irish, 1999), and fruits (Ferrandiz et. al., 1999). However, hitherto we have no unified description of microphenology of this plant, the phases and stages being separated by different criteria.

The aim of this work is to put together the versions of different authors, in order to display, as far as possible, the complete microphenology of the *Arabidopsis thaliana* matching it to the general staging of organogenesis in the higher plants (Murashov, 1982). This staging, based on the interconnection between the processes of morphological and embryological development, is widely practiced (Batygina, 1974). As the duration of developmental periods and stages of organogenesis crucially depends on the rearing conditions, our approach permits to compare the development of the plants in vivo and in vitro.

MATERIAL AND METHODS

The plants of *Arabidopsis thaliana* (L.) Heynh. (the ecotype Columbia) were reared in boxes with soil (in vivo) or in glasses with the solid nutrient medium B5 (Gamborg et. al., 1968), with no additions. The seeds before the planting, both into the soil or nutrient medium, had been kept for 2 days under 4 ± 1°C. Before introduction into the culture, the seeds had been sterilized by 10% hydrogen peroxide in 70% ethanol solution for 2–3 min, then washed three times by the sterilized distilled water and placed at the nutrient medium surface under the film.

The plants reared both in the ground and nutrient medium were illuminated by the luminescent daylight lamps. The intensity of illumination was 3000 lux/m^2 with a photoperiod of 16 (day)/8(night) h. The age of the plants, both in vivo and in vitro, was calculated from the day of germination of the seeds. In each plant, we counted the number of rosette and stem leaves and the number of flowers, fruits, and flower buds at different developmental stages.

RESULTS AND DISCUSSION

Summing up the long-term observations, we were urged to dismiss calendar dates, as far as the time of phenological phases in *A. thaliana* was subject to variation depending on both the year and season. Therefore, we tried to match all of our observations to the stages of organogenesis (Murashov, 1982), which are characteristic of the physiological state of the plants.

Phases XI–XII of the organogenesis of the maternal plant are those of accumulation of the nutrient substances in the seed and correspond to the stage 0 (Medford et. al., 1994) at which the apical meristem forms in embryogenesis.

Phase I of the organogenesis whose characteristic features are the setting apart of the primary meristem, the formation of the undifferentiated growth cone, the onset of histogenesis, and the embryonic organogenesis corresponds to stages 1–4 (Medford et. al., 1994). These are the stages at which, in the meristem of the rudimentary bud of *A. thaliana*, appears a cytological regionalization connected with the formation and differentiation of the leaf rudiments, from 1 to 4.

Phase II of the organogenesis (Murashov, 1982) is that of differentiation of the growth cone, its organogenetic activity concerning the formation of the first vegetative metameres of the plant. The growth of meristem is subject to acceleration, this allowing for the setting apart of the axillary meristems. This phase corresponds to stages 5–6 (Medford et al., 1994), that of the formation of the 5th and 6th leaf rudiments. At this phase, the bilateral symmetry of the meristem turns into the radial one.

The differentiation of the main axes of the rudimentary inflorescence and rudimentary covering leaves is the onset of the III phase of organogenesis (Murashov, 1982). Characteristic to the IV phase of organogenesis is the origination of lateral axes of the presumptive inflorescence in the axils of its presumptive lobes corresponding to the growth cones of the second order. The formation of proper leaves is arrested. The III phase of organogenesis corresponds to stage 7 (Medford et al., 1994), when the meristem turns to the formation of generative organs. To the same phase of organogenesis, one can refer to the stages of the flower development 7th [1]* and 8th [2] including the setting apart of the undifferentiated floral bud from the inflorescence meristem described by D.R. Smith et al. (1990), and so is stage I_1 of the inflorescence development (Ratcliffe et al., 1998). When turning to the formation of the generative organs, the shape of the meristem becomes subject to change. The rudiments of the stem leaves appear, their axils giving rise to the differentiation of paracladia (Ratcliffe et. al., 1998).

Stages from the 9th [3] to 14th [5] corresponding to the setting apart and growth of the primordial sepals, petals, stamens, and carpels (Smith et. al., 1990) are equivalent to phase V of organogenesis, that of the formation and differentiation of the flowers. At this stage, the rudiments of the lateral stamens start to differentiate and the anthers, as well as the stamen threads, become distinguishable. Stage I_2 of the inflorescence formation (Ratcliffe et. al., 1998) can be referred to that phase of organogenesis.

One can match the 15th [9] stage, whose characteristic feature is regional differentiation of the carpel (Smith et. al., 1990), to phase VI of the organogenesis (Murashov, 1982). Both stages correspond to the growth of the flower parts, when the stamen threads are still shorter than the anthers, the carpel extends, and the stigma develops, this being attended by the formation of macro and microspores, up to the formation of the microspore tetrads. All the organs are subject to growth, especially the stamens and pistils.

The formation of female and male gametophytes (phase VII of the organogenesis) proceeds in *Arabidopsis thaliana* almost simultaneously with the development of papilla at the stigma surface, the petals at that time acquiring the same length as the lateral stamens (stages 16 [10] and 17 [11]).

Phase VIII of the organogenesis (Murashov, 1982) is completed with the formation of all the parts of the flower. The last displays the characters inherent to a representative of the species, including coloration. In *A. thaliana*, this phase initiates when the petals and medial stamens acquire equal length, corresponding to stage 18 [12].

In brackets are the numbers of stages according D.R. Smith et al. (1990).

All the flowering process divided into three stages, the opening of the flower (19th [13]), lengthening of the stamens (20th [14]), and extending of the stigma (21st [15]) (Smith et. al., 1990) is in the limits of the IX phase of organogenesis corresponding to the flowering and fertilization and origination of embryonic endospermic tissues.

Phase X of the organogenesis is that of lengthening of the plant and formatting of the fruits. In *A. thaliana*, this starts from the drying out of petals and stamens, corresponding to stage 22 [16], and the formation of the green fruit corresponding to stage 23 [17] according D.R. Smith et al. (1990). At this time, the activity of the apical meristem of the inflorescence is arrested, yet its structural components are still recognizable (Hensel et al., 1994). This is the start of the development of the inflorescence lateral axes (paracladia).

Phase XI of the organogenesis is that of the accumulation of the nutrient substances in the seed. In *A. thaliana*, the initiation of this stage is manifested by turning of the coloration of the fruit folds from the green into the yellow ones, this corresponding to stage 24 [18] according D.R. Smith et al. (1990). Phase XII of the organogenesis, whose characteristic features are the attenuation of the growth processes inside the fruits and seeds and their desiccation. This corresponds to the coming of stage 25 [19], with the seeds setting apart from the mother plant (stage 29 [20]).

Thus, when matching different views, the life cycle of *Arabidopsis thaliana* can be subdivided into 29 stages, which naturally allocate among 12 stages of organogenesis (Murashov, 1982). To our opinion, the use of this staging is promising when analyzing the data concerned with the developmental biology of *Arabidopsis.*

In vitro, as we have observed, each of the developmental stages delayed for some days, as compared to the developmental schedule in vivo. We worked out the schedule of *Arabidopsis thaliana* development from the

* In brackets the stage number after D.R. Smith et al. (1990) is indicated.

The characteristic of the intact plants of *Arabidopsis thaliana* (L.) Heynh. at different phases of organogenesis

Group	Phase of organogenesis	Age (days from the onset of germination)	Rosette leaves	Buds in the rosette leave axils	Stem leaves	Flowers at different stages of differentiation	Floral rudiments	Fruits
1	II	8–20	4.69 ± 0.12	–	–	–	–	–
2	III–VIII	21–30	7.69 ± 0.31	Single cases	2.28 ± 0.16	3.38 ± 0.43	5.63 ± 0.17	–
3	IX	31–39	8.43 ± 0.22	1.67 ± 0.11	2.08 ± 0.09	6.10 ± 0.30	6.10 ± 0.16	–
4	X	40–54	7.63 ± 0.69	1.44 ± 0.13	2.56 ± 0.28	7.63 ± 0.39	5.86 ± 0.21	1.75 ± 0.48
5	XI	55–65	6.55 ± 0.23	2.2 ± 0.18	2.8 ± 0.14	7.50 ± 0.27	5.65 ± 0.17	5.45 ± 0.38
6	XII	92–95	7.38 ± 0.31	2.5 ± 0.28	2.44 ± 0.16	8.38 ± 0.20	4.69 ± 0.16	11.81 ± 0.59

seeds rearing in the nutrient medium in sterile conditions. The seeds germinated in 2–3 days after the planting. The formation of the rosette consisting of 6–8 leaves completed at the 20th–23rd day of development and the meristem initiated the prefloral organogenesis. The lower flowers of the inflorescence opened at the 33rd–35th day, the first fruits being formed between 40–45 days of development. The development of paracladia initiated just at that time. The initiation of shoots from axils of the rosette began at the 56th–60th day of development. Just at that time, the lower fruits turned yellow and the seed ripened. The activity of the apical meristem of the inflorescence was arrested at the 92nd–95th day.

Taking into account the characteristic features both of organogenesis in *A. thaliana* and the experimental device, we aged the plants as follows.

Group 1: The plants with the vegetative apical meristem (the II phase of organogenesis).

Group 2: The plants whose growth cone forms the florescence axial pattern. The lower (most developed) flowers are subject to differentiation and the flowers are still closed (the III–VIII phases of organogenesis). The apical meristem contemplates the general inflorescence pattern producing many floral rudiments that are ready for differentiation.

Group 3: The plants whose florescence has just formed. We determined this stage basing on the state of the most developed flower in the inflorescence.

Group 4: The plants in which the organogenetic activity of the terminal meristem of the main shoot is subject to decay, with the lower flowers forming the green fruits, while the paracladia start to grow (the X phase of organogenesis).

Group 5: The plants whose fruits originated from the lower flowers turn yellow. The activity of the main shoot apical meristem ceases eliminating the apical dominance and providing development of lateral shoots from the axils of the rosette leaves (the XI phase of organogenesis).

Group 6: The plants sewing the seeds originated from the fruits of the main inflorescence (the XII phase of organogenesis).

Both the state of the plants of each of these groups and the number of organs resulted from the activity of the apical meristem are shown in the table.

It is remained opened to question how the schedule of the apical meristem activity depends on the organogenetic state of the whole plant. To answer this question, one has to have isolated apexes of the shoot meristems and cultivate them in vitro. The advantage of this method is the opportunity of choice of rearing conditions, with no respect to the processes concerned with the "life program" of the plant (Butenko, 1999).

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