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Embryogenesis in *Sium latifolium* L. (Umbeliferae) under different water regimes

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Here we present our observations on embryo formation of *Sium latifolium* L. (Umbeliferae) air-aquatic and terrestrial plants. We noticed a significant similarity in the rate of embryogenesis at the early stages of development in both types of plants, whereas essential differences in the size of embryos and the degree of their differentiation from the beginning of cotyledon formation were revealed between the plants grown under different hydrological regimes. The size of mature embryos of terrestrial plants exceeds 1.7–1.8 times the size of air-aquatic ones.

Key words: embryo, differentiation, water regime

INTRODUCTION

It is known that the generative development of plants, directly connected with their productivity, depends on the action of different environmental factors, first of all on hydrological regime [1]. Water deficiency becomes apparent in the abnormalities that arise at different stages of the reproductive process, causing sometimes even the loss of egg cells [1] and finally to a reduction of plant productivity [2]. Galau with co-authors [3] showed that the embryo is directly subjected to the influence of water deficiency and can be an important determinant of embryo development. Moreover, zygotic and somatic embryos are both subjected to the effect of water deficit [4]. Additionally, embryos at different stages of their differentiation respond to the surrounding water deficit [3]. A clear correlation between seed biomass and number, as well as the stability of harvest under the effect of water deficiency testifies to a high sensitivity of plant development to water stress [5]. Therefore, studies of embryogenesis of plants grown under different hydrological regimes are important for understanding the peculiarities of embryo differentiation under different water regimes.

MATERIALS AND METHODS

As the object of our research we used *Sium latifolium* L. plants which grew in a river (in the Psel river of Poltava region) and on its bank. Due to the protandry of actinomorphic flowers of *Sium* species, the process of castration of anthers was not carried out. The age of embryos was identified by labelling flowers with coloured threads.

The embryos (by 30 units) on different stage of seed formation were isolated for definition of their size. The isolated embryos were measure with using of an eypiece-micrometer under 3x objective.

The ovules were fixed by the previously developed method [6]. Sections 1.5–3.0 mkm thick were prepared with an MX-TL ultramicrotome (Boeckeler Instruments, USA) and stained with toluidine blue, basic fuchsine and bright green [7]. The sections were visualized and photographed with Axioscope and Stemy-6V microscopes (Carl Zeiss, Germany).

RESULTS AND DISCUSSION

Previous investigations showed a significant similarity at the early stages of embryo formation in terrestrial and air-aquatic *Sium* plants [6]. *Sium* embryos develop according to the *Solanad* type (apical and basal cells after transverse division of the zygote produce a proper embryo and a suspensor). The endosperm in this species is formed according to the ceonocytic type, and cell formation begins from the apical part of the embryo sac. At early stages of embryo development they increase in size and after 6–8 days become globular with a narrow suspensor. The latter consists of one layer of cells.

Gradually, as a result of intensive cell division, the embryos change their form to pear-shaped one (10–15-day-old embryos) (Fig. 1, a, b). It should be noted that at this stage of development, endosperm cells around the embryo perform the trophic function and afterwards collapse. Due to this process, an empty zone around the embryo is formed. The rest of the embryo sac is filled with cellular endosperm.

From the moment of cotyledon formation (in 16–18-day-old embryos), essential differences in the size of embryos between terrestrial and air-aquatic plants were observed. In particular, the embryo of air-aquatic plants was smaller in size as compared with terrestrial ones.

During the further embryo development, the size of the cotyledons increases further. A comparative embryological analysis showed a clear difference in the size of 24–25-day-old

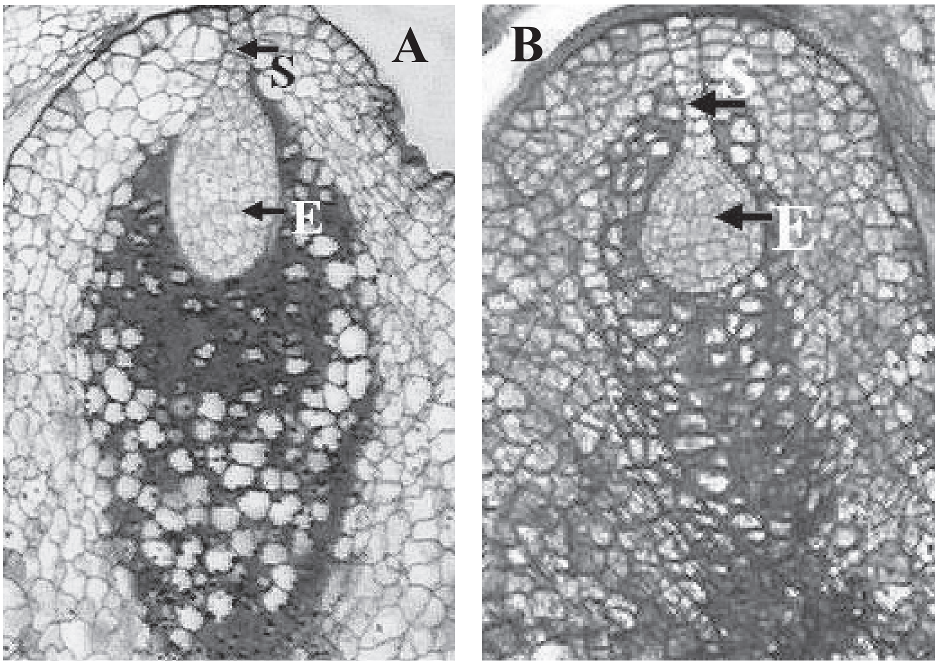

Fig. 1. Fragments of *Sium* embryo sac with 15-day-old embryos: air-aquatic plant (a); terrestrial plant (b).
Abbreviations: E – embryos; S – suspensor. Bar 10 μ.

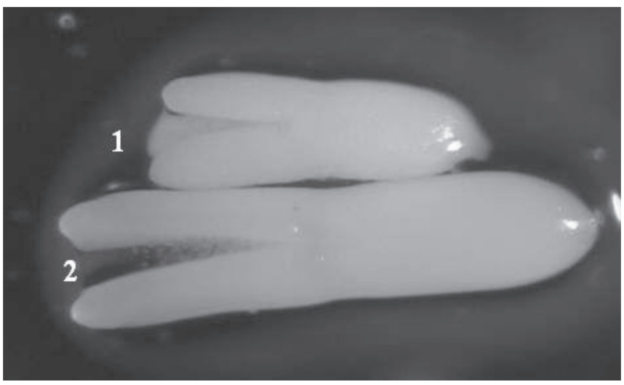

Fig. 2. Mature *Sium* embryos: the air-aquatic plant (1); terrestrial plant (2).
Abbreviations: E – embryos, C – cotyledon, R – radicle. Bar 10 μ.

embryos. In particular, embryos of terrestrial plants are 1.5–1.7 times larger than those of air-aquatic plants. The difference in embryo size among the study plants during their further development is preserved.

45-day-old *Sium* embryos are completely differentiated. They have a radicle, two long cotyledons and a rudiment of apical meristems between the cotyledons. Morphometric analysis showed that the maximum length of embryos in terrestrial plants is 1.2–1.3 mm, whereas in air-aquatic plants only 0.7–0.8 mm. Thus, embryos of terrestrial plants are almost 1.7–1.8 times longer than of air-aquatic ones (Fig. 2). Embryos of terrestrial plants, as a rule, have well developed cotyledons, their length exceeding that of the radicle. In contrast to this phenomenon, the cotyledons of air-aquatic embryos are frequently longer or shorter than the radicle.

However, mericarps (*Sium* fruits) of air-aquatic plants are larger than those of terrestrial ones due to overgrowth of the vascular system which is more developed in these plants. It should be noted that in terrestrial embryos the cotyledons are longer than the radicle. This relationship in air-aquatic embryos is not preserved, and cotyledons are frequently longer or shorter than radicles. In these embryos, underdeveloped or very short cotyledons are sometimes observed, which probably result from changes in the rate of their differentiation. Another peculiarity of mature seeds of the study plants is that seeds of air-aquatic plants have a very low degree of germination on the artificial substratum under laboratory conditions (1.5%) in comparison with terrestrial plants (98.5%), even after a 3-month stratification.

Thus, the presence of insignificant size fluctuations of the embryos at the globular stage of their development testifies to absence of essential differences in the rate of their formation in the early stages of embryogenesis. Possibly this is a result of sufficient water supply to terrestrial plants in spring. With the further development of embryos, clear differences in their size is noted. Actually, embryos in terrestrial plants were larger and more equalized in comparison with air-aquatic ones. The obtained data probably confirm the conventional opinion [8] that the reproductive development of plants under water deficit is accelerated.

Besides, it is known [9] that hormones play an important role in the metabolism of a developing embryo. The higher content of ABA in seeds of terrestrial plants of *Sium* in comparison with *seeds* of air-aquatic plants [10] probably confirms its regulative role in the adaptive response of plants to a decreased water supply. ABA is also able to inhibit the metabolic processes, to ensure the regulation of assimilate transport, the synthesis of reserve proteins in seeds, as well as the utilization of carbohydrates by the developing embryo [9]. Taking into account the above-mentioned data, the higher content of ABA may be regarded as a mechanism of adaptation to water deficit.

At the same time, we have revealed a decreased content of uncombined and associated cytokinins as ABA antagonists [10]. Their level in seeds of terrestrial plants is 1.5–2.0 times lower in comparison with air-aquatic ones. Taking into consideration the function of cytokinins [11], reduction in their activity correlates with inhibition of indices of growth rate in completely differentiated embryos of terrestrial plants.

Thus, formation of larger embryos in terrestrial plants, as well as the significant variability of air-aquatic embryos according to their differentiation is probably a result of their growth under different water regimes.

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