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Short Report

Early stages of pollen embryogenesis in barley anther cultures induced by pre-treatment with mannitol

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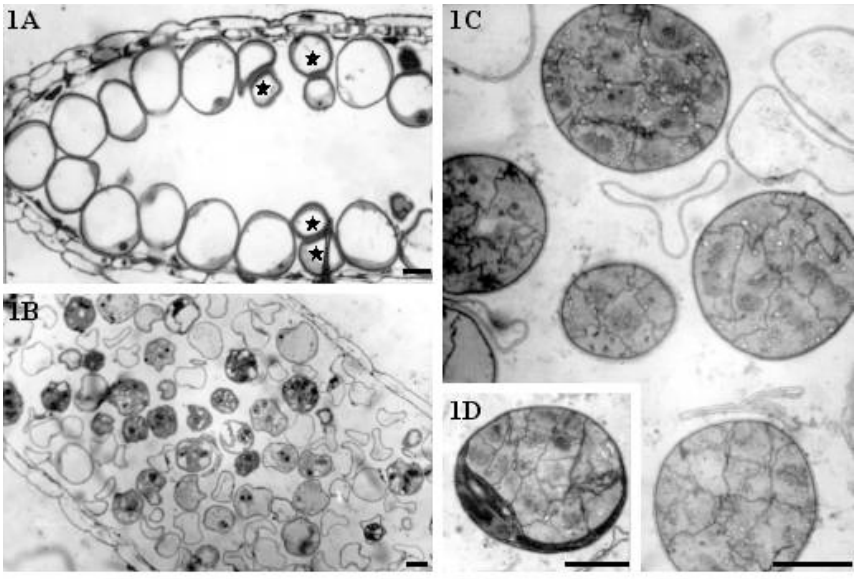
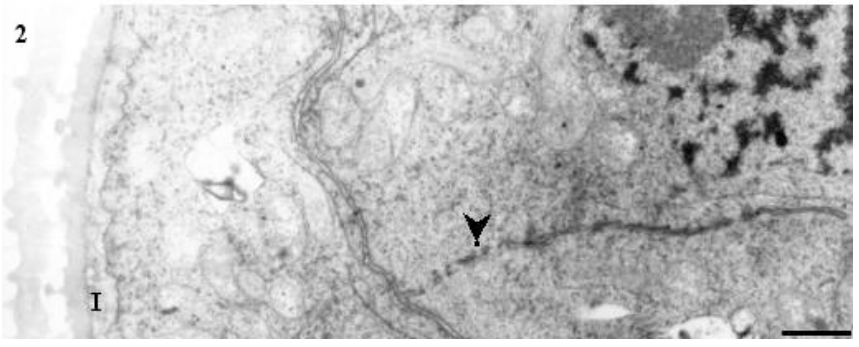
Introduction

Embryogenic development of male gametophyte can be induced by anther culture via androgenesis applying different stress pre-treatments. In barley *(Hordeum vulgare* L.) it was found that the most effective treatment to induce such androgenesis is the incubation of the anthers with mannitol. We describe here cellular changes in barley microspores during the early stages of androgenesis by using mannitol induction. This is the first time that the results of cytological analysis of androgenic embryos originated by this method have been reported. The main difference brought about by this method of inducing androgenesis is a disorganisation in the distribution of microspores within the anther, which may account for its greater success compared to other methods of inducing androgenesis.

Materials and Methods

Androgenesis was produced in anther cultures of *Hordeum vulgare* L. cv Igri plants by pre-treatment with 0.7 M mannitol according to Cistué *et al*. (1999). Anthers after androgenic induction treatment, anthers containing androgenic microspores cultured for 3 and 6 days and untreated control anthers were fixed, dehydrated in ethanol series and embedded in Unicryl (Bonet and Olmedilla, 2000) for their study by electron microscopy.





Results

In untreated, longitudinal, semi-thin sections of anthers containing vacuolated microspores we observed a peripheral ring of vacuolated microspores in close contact with the tapetum. Some smaller microspores were to be seen in a more central position in the locule and attached to other small microspores at the periphery of the anther (Fig. 1A). After pre-treatment with mannitol and several subsequent days' culture microspores appeared randomly distributed within the anther locule. Some of these were multicellular pollen grains and others dead or unchanged microspores (Fig. 1B). On the whole we found two types of embryo: one made up of very similar cells and another less frequent one containing a core of homogeneous cells surrounded by a ring of denser cells (Fig. 1 C,D).

Electron-microscope observations of the multicellular pollen grains revealed the formation of a

Fig.1. Longitudinal semi-thin sections of barley anthers at different stages during the induction of androgenesis (stained with toluidine blue). (A) *Control anther. Notice the small pollen grains (stars).* **(B)** *Anther after 3 days culture.* **(C,D)** *Multicellular pollen after 6 days of culture (homogeneous* **(C)** *and heterogeneous* **(D)** *embryos). Bars represent 5 μm.*

Fig. 2. Transmission electron micrograph of a multicellular pollen grain. *Notice the enlargement of the intine (I) and the formation of new cell walls (arrowhead). Bar represents 1 μm.*

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thicker intine sometimes forming intercellular walls. Rich cytoplasm with an abundance of ribosomes, mitochondria, dictyosomes and plastids were observed in these multicellular grains. In recently divided cells the chromatin was very condensed and it was possible to see the formation of new intercellular walls (Fig. 2).

Discussion

Pollen dimorphism observed in several species has been related to the possibility of identifying the potentially more embryogenic microspores (Heberle-Bors and Reinert, 1981). Our observations have confirmed the existence of pollen dimorphism in barley (Idzikowska *et al.*, 1982; Roberts-Oehlschlager and Dunwell, 1991). Nevertheless, in the serial longitudinal anther sections studied we found smaller dimorphic microspores not only at the centre of the locule, as previously described, but also at its periphery. The existence of these smaller microspores at the periphery of the locule, which have not been described before, might explain the presence of multicellular pollen grains detected in this position and also the higher number of these structures found compared to the number of smaller grains at the centre of the anther.

It has been shown that treatment with mannitol induces androgenesis more effectively in barley than other stress treatments such as cold or sucrose (Roberts-Oehlschlager and Dunwell,1990). We observed that the mannitol pre-treatment used in this study disrupted the organisation of the microspores in the anther locule more drastically than other pre-treatments. This observation supports the idea that when there is less contact and less synchrony between the microspores there is a greater possibility of deviation towards the sporophytic pathway.

The non-homogeneous response to the induction of androgenesis observed in our study seems to be a common characteristic, irrespective of the stress applied. This heterogeneous response, together with pollen dimorphism, are some of the reasons that lead us to suspect that androgenesis may not be originated by totipotency (Bonet *et al.,* 1998).

The embryogenic structures found after treatment with mannitol indicate that the microspores involved follow a similar pathway towards androgenesis as that followed after other induction methods (sucrose: Chen *et al.*, 1984 or cold: Sunderland *et al.,* 1979) i.e. a symmetrical division followed by further divisions which form a homogeneous embryo, and in rare cases, an asymetrical division followed by divisions both in the vegetative and generative cell. Multinuclear structures were also observed; their nuclei could fuse and be responsible for the ploidy increase observed in many plants obtained by this procedure. Ultrastructural characteristics found in microspores during the first days of culture after stress treatment were quite similar to those described elsewhere for the induction of androgenesis in barley using other methods (cf. Chen *et al.*, 1984). The enlargement of the intine found in other species is also significantly similar (Bonet and Olmedilla, 2000).

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