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Short Report

The switch of the microspore developmental program in Capsicum involves HSP70 expression and leads to the production of haploid plants

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ABSTRACT The switch of the gametophytic developmental program of the microspore towards embryogenesis to form a haploid plant represents an important tool in plant breeding for obtaining isogenic lines and new varieties through double-haploid plants. This process can be induced in microspore *in vitro* cultures by stress treatments, like starvation or heat-shock. Microspore embryogenesis also constitutes an interesting *in vitro* system for basic studies on cellular and molecular mechanisms controlling plant development, cell fate, stress response and signalling pathways. This process has been induced in many plant species but little is known about the mechanisms and factors involved in this change of developmental program. In this work, various ultrastructural *in situ* approaches have been performed to characterize changes in the subcellular organization and the localization of stress proteins, specifically the HSP70, during the induction and early microspore embryogenesis.

Microspore embryogenesis has been induced in C*apsicum annuum* L. (González-Melendi *et al.,* 1996) using heat treatment of 35ºC for the induction (Barany *et al.,* 2001) and a rich culture medium containing cytokinines (Mityko *et al.,* 1995). The appropiate selection of the microspore developmental stage to initiate the culture is a key feature since only a few stages are responsive to embryogenesis, the late vacuolate microspore stage (Fig. 1a) being one of the most efficient (González-Melendi *et al.,* 1996). After the inductive treatment some microspores were induced and initiated an embryogenesis pathway with the occurrence of numerous mitosis forming multicellular structures (Fig. 1 b,c). Twenty-thirty days after induction, small white globular and torpedo embryos were observed, which futher developed root, shoot and two green cotiledons, regenerating small haploid plantlets (Fig. 1d).

For microscopical analysis, samples at different stages of the *in vitro* culture were cryoembedded in Lowicryl K4M. This cryoprocessing provided a good ultrastructural preservation and kept the antigenic reactivity of the sample for immunocytochemical assays (Fig. 1 e-h) (Testillano *et al.,* 1995). After embryogenesis induction, the microspore underwent several mitosis forming multicellular structures, still surrounded by the special pollen wall, the exine (Fig. 1 b,e). In these proembryos some specfic features were observed: the inner layer of the microspore wall exhibited an important increase in thickness, cytoplasm of the individual small cells contained large vacuoles and numerous vesicles, organells, and walls separating cells showed heterogeneous thickness and wavy shape (Fig. 1e). After the breakdown of the exine, the proembryo increased its proliferative activity forming multicellular rounded masses (Fig. 1 c,f). The ultrastructural organization was quite different at this stage of microspore embryogenesis, polygonal small cells appeared separated by thin and straight walls; with large nuclei and dense cytoplasms containing abundant ribosomes, mitochondria, numerous plastids and small vacuoles (Fig. 1f). Nuclei showed small condensed chromatin patches (Fig. 1f) and nucleoli with abundant granular component, the typical nuclear organization of active proliferating cells.

The heat-shock proteins have been reported to be involved in Brassica microspore embryogenesis, in which a short heat-shock constitutes the inductive treatment (Cordewener *et al.,* 1995). Some immunolocalization data were also reported in Brassica microspores and embryogenesis (Testillano *et al.,* 2000, 2001).To evaluate the involvement of HSP70 in Capsicum system in which a much longer heat treatment is applied, immunogold labelling was performed. Differences in the presence and subcellular distribution of the protein have been observed in microspores at the beginning of the culture (Fig. 1g) and 1 day after the application of the inductive treatment (Fig. 1h). Immunogold labelling was very scarce in microspores just after plating, a few gold particles were observed in the nucleus and cytoplasm (Fig. 1g), probably localizing the constitutive form of the protein. 1 day after the stress treatment, HSP70 labelling density increased, many gold particles being localized in some regions of the cytoplasm and the nucleus, specifically on the interchromatin region (Fig. 1h). These results suggested that the reprogramming of the microspore to embryogenesis involved the induction of HSP70 expression one day after the initiation of the culture, the cell response to the heat treatment probably in combination with other factors is needed for the switch to the embryogenesis in Capsicum.

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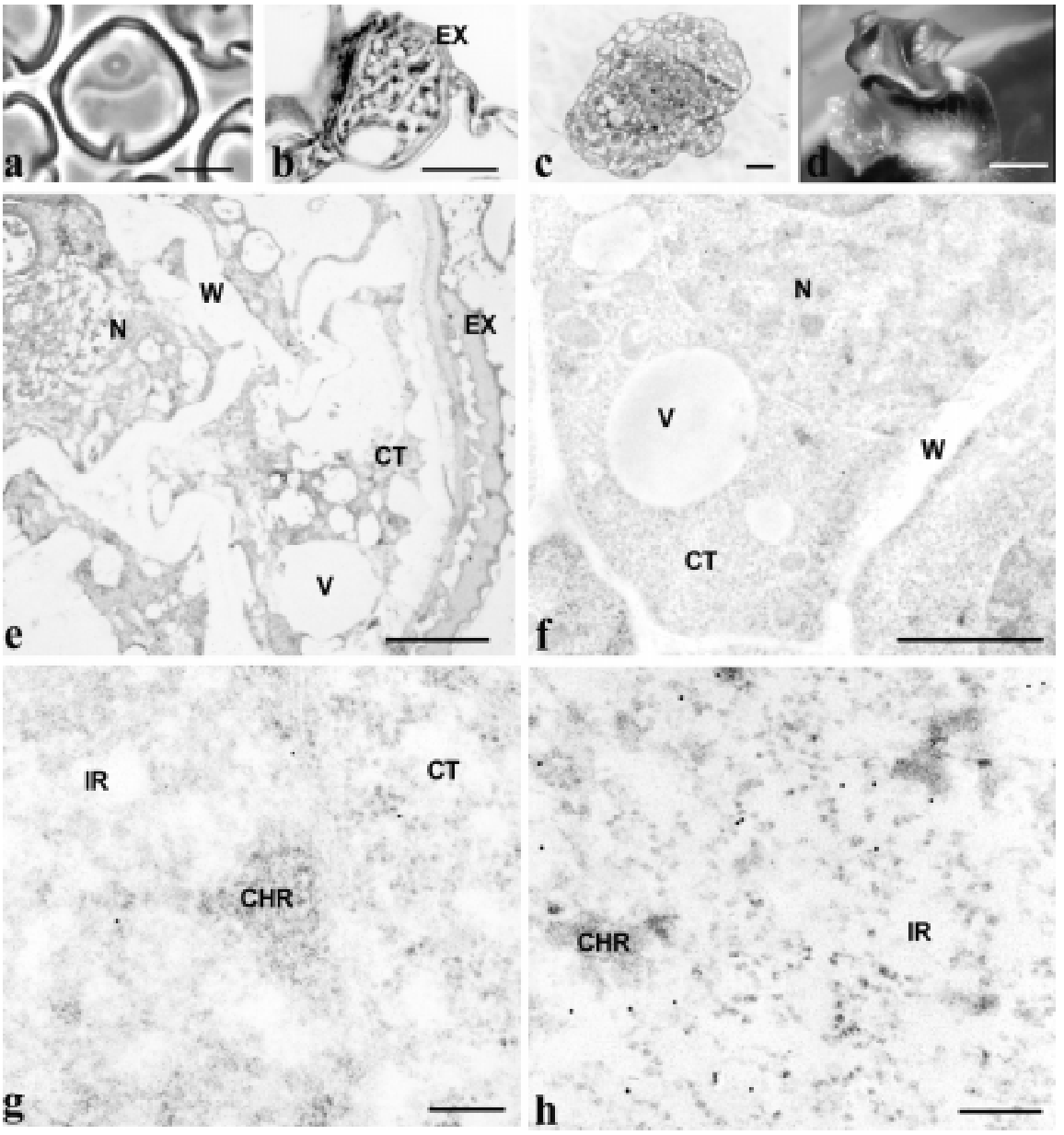

Fig. 1. Microspore embryogenesis in Capsicum. (a-d) *Different stages of the embryogenesis culture from the beginning (a) to the haploid plantlet regeneration (d). a, vacuolate microspore; b, multicellular structure surrounded by the exine; c, multicellular proembryo; d, Haploid green plantlet.***(e,f)** *Ultrastructure of the multicellular proembryos before (e) and after (f) the exine breakdown.* **(g,h)** *HSP70 immunogold labelling in microspores before (g) and 1 day after (h) the application of the inductive treatment. N, nucleus; CT, cytoplasm; V, vacuole; W, cell wall; EX, exine. IR, interchromatin region; CHR, condensed chromatin. Bars in a,b, 10 µm; c, 20 µm; d, 10 mm; e,f, 2 µm; g,h, 0.2 µm.*

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