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ISOLATED ENDOSPERM TISSUE AS A MODEL FOR EXPERIMENTAL BOTANY

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Abstract. Experiments conducted on isolated endosperm under *in vitro* conditions have opened new possibilities to investigate this specific and unique plant tissue. Results are important as well for basic knowledge as for agriculture practice.

Key words: cereals, kiwifruit, regeneration, triploid plants

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Endosperm is the ephemeral tissue, however it plays an important role in the life cycle of angiosperms plant. In most angiosperms, endosperm is formed during the process of double fertilization as a product of the fusion of a haploid sperm nucleus with two haploid polar nuclei. It results in a triploid structure formation, which develops into the tissue extreme specialized (Costa *et al.* 2004) and consumed by embryo during embryogenesis or seed germination. Interesting, there are no reports concerning *in vivo* endosperm differentiation resulted in plant regeneration. But it was proved that endosperm under *in vitro* cultures has the potential to proliferate, differentiate and finally even plant regenerate.

Both, mature and immature endosperm tissues revealed ability to proliferate *in vitro*. Factors like proper stage of endosperm development and culture conditions (e.g. plant growth regulators, light conditions) are important for successful plant regeneration. The capacity for tissue proliferation and morphogenetic response differ among species. There could be observed the correlation between nutrition status of plant and a kind of morphogenetic reaction. Usually, direct organogenesis is typical for parasitic and semi-parasitic species. The semi-parasitic *Exocarpus cupressiformis* Labill. was indeed the first species ever to show the totipotency of endosperm (Johri & Bhojwani 1965). The endosperm of autotrophic plant indicated indirect organogenesis and callus stage is necessary for next steps of differentiation. Continuously growing non-morphogenic callus cultures, without organ regeneration, have been obtained from the endosperm of such important commercial species like maize (*Zea mays* L.), cocos (*Cocos nucifera* L.), tomato (*Lycopersicon esculentum* Mill.) or cucumber (*Cucumis sativus* L.) (Thomas & Chaturvedi 2008). Plants regenerated this way show very often 3C level of nuclear DNA content. Successful 3C plant regeneration from endosperm-derived callus has been reported actually in 19 species, e.g. in acacia (*Acacia nilotica* (L.) Delile), walnut (*Juglans regia* L.), papaya (*Carica papaya* L.).

Kiwifruit (*Actinidia deliciosa* (A. Chev.) C.F. Liang et A.R. Ferguson var. *deliciosa*) is one of the important crops in interests of our team. We described efficient protocol for plant regeneration from endosperm-derived callus (Góralski *et al.* 2005). Kiwifruit is hexaploid (2n=6x=174) and because of that flow cytometry was used to determine the ploidy of callus and regenerated organs. We investigated the differences (e.g. the presence of the plant extracellular matrix, cutin, pectins) between morphogenic and non-morphogenic

endosperm-derived callus in kiwifruit (Popielarska *et al.* 2006; Popielarska-Konieczna *et al.* 2008, 2011).

The other object of our research is cereals. Its isolated immature endosperm develops under *in vitro* conditions in similar way like in caryopsis *in planta*. Recently (Popielarska-Konieczna *et al.* 2013) we conducted detailed histological and ultrastructural studies on isolated endosperm of bread wheat (*Triticum aestivum* L.), durum wheat (*T. durum* Desf.) and triticale (*Triticosecale* Wittm.). Endosperm development of cereals is strongly connected with the starch accumulation and programmed cell death (PCD) (Sabelli & Larkins 2009). In triticale induction of PCD starts in 16 days post anthesis (Li *et al.* 2010). Our data (not published) revealed that cultured endosperm tissue of triticale, which accumulated starch granules under *in vitro* conditions showed the viability during 3-4 months of the culture, what was confirmed using Evans blue staining procedure (according to Zhou *et al.* 2009).

Recent reports (Carciofi *et al.* 2012; Li & Berger 2012) pointed that experimental research concerning endosperm-tissue are still needed and could be convenient platform to manipulation of the development and study the molecular and biochemical mechanisms regulating seed storage accumulation in the endosperm.

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