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PRELIMINARY STUDIES ON PLANTS REGENERATED FROM ENDOSPERM-DERIVED CALLUS OF KIWIFRUIT (*ACTINIDIA DELICIOSA* VAR. *DELICIOSA*)

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Abstract. To show differences between plants of *Actinidia deliciosa* var. *deliciosa* regenerated from endosperm-derived callus (with 3C amounts of DNA) and those obtained from seeds, observation of their stomata and leaf hairs density was carried out. Stomata and leaf hairs are the features which are often related to ploidy status of plants. Our observation revealed that for plants, which represents 3C DNA level, stomata density was higher than for plantlets showing 2C DNA content. Additionally, density of leaf hairs seems to be also higher in regenerants. This is the first morpho-histological studies of plants regenerated from kiwifruits endosperm tissue.

Key words: *Actinidia deliciosa* var. *deliciosa*, guard cells, in vitro cultures, kiwifruit, leaf hairs, stoma, trichomes, triploid plants

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The isolated mature and immature endosperm of different species has possibility to proliferation, differentiation and finally – regeneration of triploid plants (for review see: Thomas & Chaturvedi 2008; Hoshino *et al.* 2011). The number of plant species successfully regenerated from endosperm is limited. However studies for obtaining new triploid plants, especially crop-plants are still reported (Sun *et al.* 2011; Thammina *et al.* 2011; Tian *et al.* 2012). Regenerated plants were studied mainly for ploidy determination. In some cases it was done through chromosome observation and counting (Sun *et al.* 2011). However in plants with small size (< 1μm) and high chromosome number, like *Actinidia deliciosa* with 2n = 6x = 174, cytological studies are very difficult. Thus the ploidy level was analyzed with using flow cytometry method (Góralski *et al.* 2005). In a few cases only, number of chloroplasts in guard cells and stomata density were studied (Sun *et al.* 2011) in regenerated plants.

Studies by Bauer & Fischer (2011) have shown that there are differences in genomic DNA methylation patterns between embryo and endosperm. Also analytical models proposed by Cailleau *et al.* (2010) showed that doubled maternal contribution is favored when deleterious mutations alter the function of endosperm. This point of view is fascinating when we take into consideration plants regenerated from endosperm tissue.

So far, there was only the study about nucleus DNA content of regenerated plants to confirm their ploidy (Góralski *et al.* 2005). Other features, at cytological, morphological, biochemical or molecular levels were not yet analyzed. In presented paper we focused on cytological features of leaves, especially stomata and trichomes density.

Commercially available fruits of *Actinidia deliciosa* (A. Chev.) C.F. Liang et A.R. Ferguson var. *deliciosa* A. Chev. cv. *Hayward* were used as source of explants. Mature endosperm was isolated from seeds as described previously (Góralski *et al.* 2005) and cultured under conditions reported by Popielarska-Konieczna *et al.* (2011). Shoots developed from endosperm-derived callus were transferred to half-strength Murashige and Skoog (MS) medium (Murashige & Skoog 1962). Kiwifruit plantlets were obtained from intact seeds germinated on half-strength MS medium. Regenerants as well as plantlets were cultured in Magenta™ vessels (Sigma).

For observations in scanning electron microscopy (SEM) leaves of five selected regenerants (containing 3C amount of DNA; nuclear DNA content established by flow cytometry in the other paper, unpublished) and five plantlets (with 2C of DNA) were prefixed in 5% glutaraldehyde (0.1 m phosphate buffer, pH 7.2) for 24 h at room temperature. After dehydration through a graded ethanol series, the samples were dried, sputter-coated with gold and observed with scanning electron microscope as described previously (POPIELARSKA-KONIECZNA *et al.* 2011). Abaxial parts of leaves were observed and numbers of stomata were determined in 500 μm^2 areas.

Differences in stomata and leaf hairs density in *A. deliciosa* var. *deliciosa* cv. *Hayward* between regenerated plants received from endosperm-derived callus and plants obtained from seeds were observed. Range number of stomata for plantlets was 7-9 per 500 μm^2 (Fig. 1 a). However, 12-15 stomata per 500 μm^2 were revealed in regenerants (Fig. 1 b). Number and length of leaf hairs in regenerants were much greater but thinner than in plants from seeds (Fig. 1 c, d). Results correspond to the DNA content and are related to the origin of plants: these from endosperm-derived callus represent 3C DNA level (Fig. 1 b, d) and plants from seedling show 2C DNA content (Fig. 1 a, c).

Features mentioned above are often connected with the ploidy level of plants (DOWNS & BLACK 1999; MENG *et al.* 2014). Results of our observations confirm this view. Not only stomata density but also size of stomata could be an effective parameter for analysis of ploidy levels (PRZYWARA *et al.* 1988; VANDENHOUT *et al.* 1995; MARINHO *et al.* 2014). DOWNS & BLACK (1999) and MENG *et al.* (2014) suggested that plants with lower ploidy level had smaller but greater number of trichomes. This conclusion disagrees with results of our trichomes observation (Fig. 1 c, d). Because of preliminary character of these studies more details concerning size of guard cells, ratios of guard cells length to width, histological sections of leaves surface with stomata as well as measurements of trichomes and the higher number of analyzed plants are needed. Investigations of other features of regenerated plants (e.g. sex determination of plants) are in progress.

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References

BAUER M.J., FISCHER R.L. 2011. Genome demethylation and imprinting in endosperm. *Curr. Opin. Plant Biol.* **14**: 162–167.

CAILLEAU A., CHEPTOU P.O., LENORMAND T. 2010. Ploidy and the evolution of endosperm of flowering plants. *Genetics* **184**: 439–453.

DOWNS J.L., BLACK R.A. 1999. Leaf surface characteristics and gas exchange in *Artemisia tridentata* subspecies wyomingensis and tridentate. *USDA Forest Serv. P. RMRS* **11**: 108–112.

GÓRALSKI G., POPIELARSKA M., ŚLESAK H., SIWIŃSKA D., BATYCKA M. 2005. Organogenesis in endosperm of *Actinidia deliciosa* cv. *Hayward* cultured *in vitro*. *Acta Biol. Cracov. Ser. Bot.* **47**: 121–128.

HOSHINO Y., MIYASHITA T., THOMAS T.D. 2011. *In vitro* culture of endosperm and its application in plant breeding: Approaches to polyploidy breeding. *Sci. Hort.* **130**: 1–8.

MARINHO R.C., MENDES-RODRIGUES C., BONETTI A.M., OLIVEIRA P.E. 2014. Pollen and stomata morphometrics and polyploidy in *Eriotheca* (Malvaceae-Bombacoideae). *Plant Biol.* **16**: 508–511.

MENG F., PENG M., PANG H., HUANG F. 2014. Comparison of photosynthesis and leaf ultrastructure on two black locust (*Robinia pseudoacacia* L.). Biochem. *Syst. Ecol.* **55**: 170–175.

MURASHIGE T., SKOOG F. 1962. A revised medium for rapid growth and bioassay with tobacco tissue cultures. *Physiol. Plantarum* **15**: 473–497.

POPIELARSKA-KONIECZNA M., KOZIERADZKA-KISZKURNO M., BOHDANOWICZ J. 2011. Cutin plays a role in differentiation of endosperm-derived callus of kiwifruit. *Plant Cell Rep.* **30**: 2143–2152.

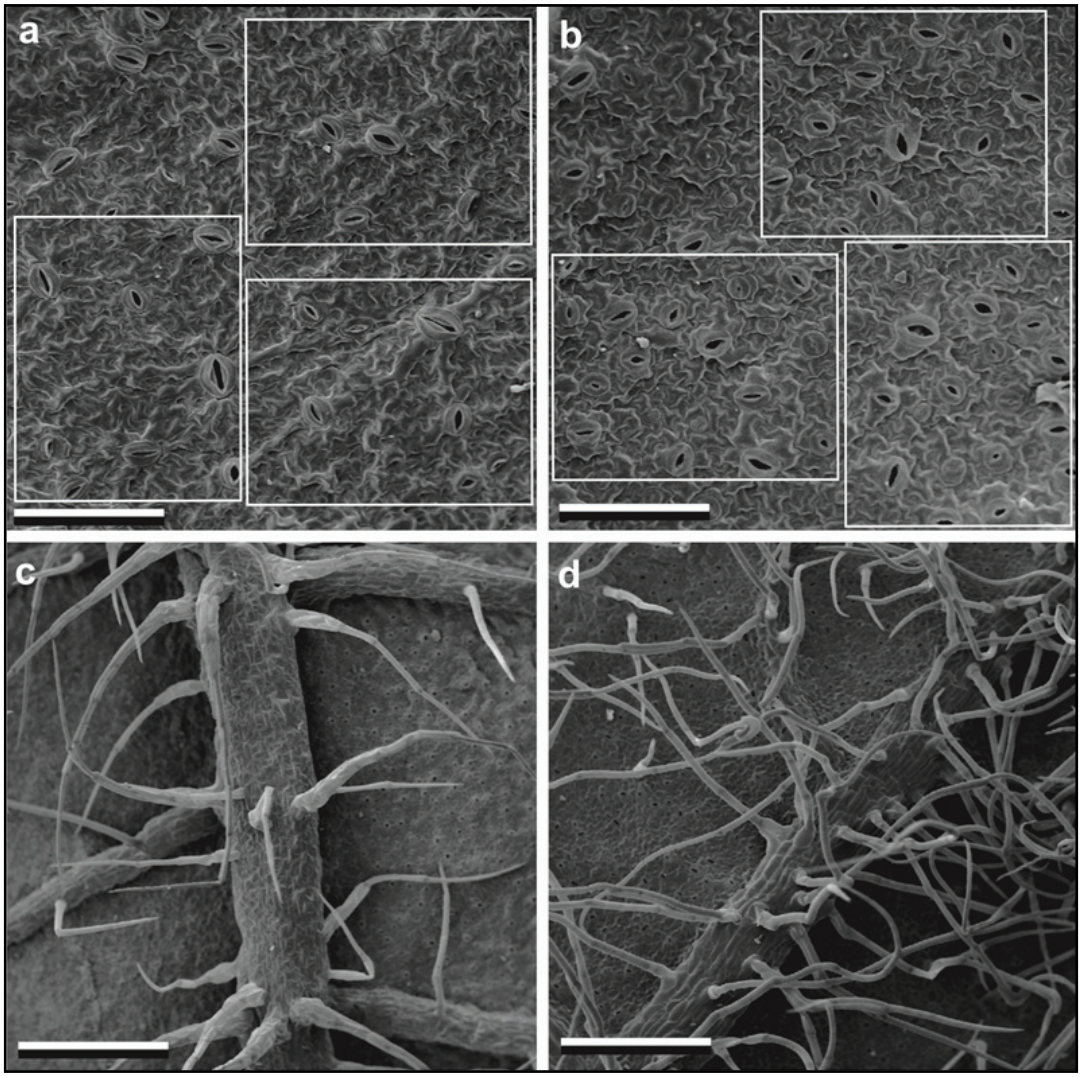

Fig. 1. SEM images of abaxial leaves surface of plantlets germinated from seeds (**a, c**) and endosperm-derived regenerants (**b, d**). **a, b** – epidermis with guard cells; white squares show area of 500 μm^2; **c, d** – vascular veins with leaf hairs. Bars: 130 μm (**a, b**), 600 μm (**c, d**).

Przywara L., Pandey K.K., Sanders P.M. 1988. Length of stomata as an indicator of ploidy level in *Actinidia deliciosa*. *New Zealand J. Bot.* **26**: 179–182.

Sun D.Q., Lu X.H., Liang G.L., Guo Q.G., Mo Y.W., Xie J.H. 2011. Production of triploid plants of papaya by endosperm culture. *Plant Cell Tiss. Org. Cult.* **104**: 23–29.

Thammina Ch., He M., Lu L., Cao K., Yu H., Chen Y., Tian L., Chen J., McAvoy R., Ellis D., Zhao D., Wang Yu., Zhang X., Li Y. 2011. *In vitro* regeneration of triploid plants of *Euonymus alatus* '*Compactus*' (Burning bush) from endosperm tissue. *Hort. Sci.* **46**: 1141–1147.

Thomas T.D., Chaturvedi R. 2008. Endosperm culture: A novel method for triploid plant reproduction. *Plant Cell Tiss. Organ Cult.* **93**: 1–14.

Tian L., Ke Y., Gan S., Chen Y., Chen Y., Yang Z., Wang X. 2012. Triploid plant regeneration from mature endosperms of *Sapium sebiferum*. *Plant Growth Regul.* **68**: 319–324.

Vandenhout H., Ortiz R., Vuylsteke D., Swennen R., Bai K.V. 1995. Effect of ploidy on stomatal and other quantitative traits in plantain and banana hybrids. *Euphytica* **83**: 117–122.