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MOLECULAR, HISTOLOGICAL AND EMBRYOLOGICAL ANALYSIS OF REGENERANTS OBTAINED DURING *IN VITRO* CULTURE OF IMMATURE EMBRYOS OF APOMICTIC *TARAXACUM BELORUSSICUM* VAL. N. TIKHOM.

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Abstract. Dandelion as a model herbal plant has a wide application in analysis of genetic, molecular background of apomictic reproduction. *Taraxacum belorussicum* Val. N. Tikhom. is a triploid species belonging to *Taraxacum* sect. *Palustria*. As an obligatory apomictic plant it presents attractive experimental material for apomixis and somatic embryogenesis aspects. Here we present the preliminary studies showing direct organogenesis and genetic stability of the regenerants achieved during *in vitro* culture of dandelion along with their embryological analysis.

Key words: *Taraxacum belorussicum*, apomixis, somatic embryogenesis, parthenogenetic embryos, regenerants, RAPD, genetic stability

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Introduction

Apomixis, the process of asexual seed formation, has been documented in about 44 families of flowering plants including both monocots and eudicots, however, it is especially prevalent within Asteraceae, Poaceae and Rosaceae (Bicknell & Koltunow 2004; Noyes 2007). In the recent years, the genus *Taraxacum* (Asteraceae) has been widely investigated as a model group for the analysis of embryological, genetic and molecular aspects of apomictic mechanisms (for review see Van Dijk *et al.* 2009). Moreover, dandelions have long been used as important medicinal herbs due to the content of components demonstrating among other anti-inflammatory, anti-oxidative and anti-carcinogenic activities (Ilu & Kitts 2003). The *Taraxacum* species have also been studied under *in vitro* conditions (Jamshieed *et al.* 2010 and references therein, Trejgell *et al.* 2013).

Apomixis in *Taraxacum* includes meiotic diplospory, parthenogenesis and autonomous endosperm formation (Asker & Jerling 1992). *T. belorussicum* Val. N. Tikhom., an object of this study, is a triploid species (2n=3x=24) belonging to *Taraxacum* sect. *Palustria* (Marciniuk *et al.* 2010). As an obligatory apomictic species, forming parthenogenetic embryos, it presents an attractive experimental material for comparative research aspects of parthenogenesis and somatic embryogenesis in diplosporous plants. Somatic embryogenesis is the process by which somatic cells develop into differentiated plants through characteristic embryological stages without fusion of the gametes (Williams & Maheswaran 1986). Based on totipotency of plant cell, somatic embryogenesis may be induced almost in all plants and each kind of plant tissues and cells can be used as an explants. Only differences in an efficiency of this process may occur.

Here we present the studies showing direct organogenesis induction during *in vitro* culture of immature parthenogenetic embryos of *T. belorussicum*. Moreover, we present the preliminary results of the molecular and embryological analysis of regenerants achieved during *in vitro* culture of dandelion embryos.

Material and methods

Single flowers were collected from the older capitula of *T. belorussicum*, and then immature parthenogenetic embryos (IPE) were isolated

from ovules. The cut and uncut IPE, after standard sterilization, were inoculated on MS based media supplemented with IAA and BAP, and on MS based media supplemented with BAP with standard and an enlarged sugar concentration according to Jach & Przywara (2000). Another part of explants were maintained on MS based media containing 2,4-D and KIN at the same light conditions.

Genomic DNA was isolated from the leaves of donor plants and also from 15 randomly selected regenerated plants using CTAB method. Genetic uniformity between the mother plant and *in vitro* regenerated plantlets was assessed by random amplified polymorphic DNA (RAPD) analysis. RAPD assay was performed using eight primers. The amplified samples were analyzed by electrophoresis in 1% agarose gel using 1xTBE buffer and stained with ethidium bromide. A 100-bp DNA ladder was used as a molecular standard. The bands were transformed into a binary character matrix, "1" for presence and "0" for absence of band. Cluster analysis was performed using dendro UPGMA.

For histological and embryological investigations, the explants, callus as well as whole capitula at the different developmental stages sampled from regenerants were fixed in 5% glutaraldehyd and embedded according Technovit procedure in Technovit 7100 (Heraeus Kulzer). The material was cut on 5 μm slides, stained and examined using a Nikon 400 Eclipse microscope.

Results and discussion

Both cut and uncut cultures resulted in adventitious shoot formation and the induction of organogenesis was observed at the same time (7 days of culture). The differences between these cultures occurred in the efficiency of shoot number, the cut embryos produced much more adventitious shoots than uncut ones. The application of IAA and BAP, described previously for *in vitro* propagation of leaf segments of *T. officinale* (L.) Weber (Jamshieed *et al.* 2010) occurred suitable for IPE in our experimental model as well. Regenerated shoots rooted well when subcultured on MS media containing IBA, next transferred to soil and then to the field. The last ones were in good conditions and produced inflorescences.

Histological analysis confirmed the organogenic character of structures occurring on the media used and showed mostly direct (without callus formation) organogenesis observed on IPE of studied dandelion. In the explants tissue coming from long term culture (47 day of culture) we observed some fibrillar structure located between the cells which was similar to ECM (extracellular matrix) what may confirm the morphogenetic competence of this tissue like in another plants (Popielarska-Konieczna 2008). The pictures of SEM showed the presence of membranous structure covering explants surface deriving from the same tissue culture conditions.

With the 8 analyzed primers a total of 102 distinct bands (with an average of 12.75 bands per primer) in the size range of 260-3500 bp were observed. The number of bands for each primer varied from 11 in RAPD8 and RAPD10 to 16 in RAPD4 primer. Among 102 scorable fragments only 7 were polymorphic (6.86%). Despite the stability of DNA profiles, regenerated plants showed changes in chromosome number.

Embryological analysis showed the presence of well-developed ovaries and stamens in flowers of regenerants. Although Marciniuk *et al.* (2010) noted that *T. belorussicum* is a male sterile dandelion, in the analyzed material, microspores as well as two-celled pollen grains with well developed sporoderm were observed within anthers. However, it should be pointed that in triploid dandelions, as a result of disturbed meiosis, most of the produced pollen is sterile (Musiał *et al.* 2013). Ovaries in the flowers of regenerants exhibited a structure typical for the members of Asteraceae and contained anatropous, tenuinucellate and unitegmic ovules. Preliminary analysis revealed the occurrence of dyad of megaspores and one- or two-nucleate female gametophytes in the studied ovules. It therefore appears that in the flowers of dandelion regenerants the

embryological processes are similar to that in apomictic *Taraxacum* species but to confirm this, further detailed studies are required.

Although only organogenesis was achieved under the conditions used so far, the results seem to be beneficial for the future analysis of aspects of somatic embryogenesis in the obligatory autonomous apomicts.

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