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MORPHOLOGICAL AND HISTOLOGICAL EVENTS IN THE PRELIMINARY TISSUE CULTURE OF HAPLOID AND DIPLOID *PELARGONIUM ZONALE* VAR. *'KLEINER LIEBLING'*

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Pelargonium species are know and popular as the ornamental plants valued for their colorful, showy flowers and attractive leaf shape. Furthermore, some of these species are used in the pharmacy, aromatherapy, perfumery and cosmetic industry as well (Moyo *et al.* 2012). Most of approximately 280 species of the genus *Pelargonium* (fam. Geraniaceae) are native for South Africa (Mithila *et al.* 2001). Some of them such as *P. zonale* (L.) L'Hér. ex Aiton were brought to Europe as early as in 1609. *P. zonale* var. *'Kleiner Liebling'* is one of the pelargonium dwarf varieties with chromosomes number nine, and it is true haploid (monoploid) (Daker 1966), which makes it an interesting model plant.

The aim of our study was the observation of *in vitro* culture of haploid and diploid plants of *P. zonale* var. *'Kleiner Liebling'*. It seems that this is the first attempt to establish an efficient protocol for regeneration in tissue culture conditions for these plants. These studies may serve as a start point for further studies of concerning plants at different ploidy levels.

To obtain the plant regeneration, in the tissue culture conditions, we used explants both from mature haploid and diploid. Fragments of stems, parts of leaf petioles and leaf lamina were maintained on MS (Murashige and Skoog) basal media supplemented by several plants growth regulators. The first fodder contained 1 μM 2,4-D and 1 μM KIN; the second – 18,2 μM TDZ and 0,57 μM IAA; the third – 0,44 μM BAP and 0,54 μM NAA; and the fourth – 5,4 μM NAA and 0,44 μM BAP.

The morphological and histological examinations showed the differences in morphogenetic response of haploids and diploids in applied tissue culture conditions. The response time for haploid and diploid *P. zonale* var. *'Kleiner Liebling'* plants was also different. First of all, the callus formation on diploid stem explants was observed on eleventh day of culture on the medium with NAA and BAP combination. Two types of callus were observed on both haploid and diploid explants: endogenous and exogenous. The efficiency of callus production varied depending on type of explants used. The most frequent callus formation was found on fragments of stems and leaf blades (they responded in 100%), while leaf petioles were less efficient, 70% in diploids and 50 % for haploids.

Organogenesis (mainly direct) was achieved in three types of explants; however, the response was different for haploids and diploids. Shoots were observed in most cases on haploid petiole leafs, whereas direct organogenesis in diploid explants was observed on stems and leaf blades.

The optimal combination of plant hormones for the induction of adventitious shoots were TDZ and IAA (second fodder), while in the medium supplemented with 2,4-D and KIN organogenesis was not recorded at all. Regenerated adventitious shoots were transferred to solid 1/2 MS medium, where

their multiplication and successful development were observed.

Direct rhizogenesis was induced only on stem explants. For haploids, roots were obtained on the medium containing TDZ with IAA and 2,4-D with KIN (the first and the second fodders), for diploids third and fourth media (supplemented with NAA and BAP in different concentrations) were suitable.

The histological analysis confirmed the direct organogenesis and rhizogenesis, as well as heterogeneous origin of callus in diploid and haploid plants.

In conclusion the crucial factor for organogenesis induction seems to be TDZ with combination with IAA application, what correlates with other experimental reports, where TDZ has been reported to induce adventitious shoot buds in number of plant species (Acharjee *et al.* 2012)

In our experiments the somatic embryogenesis induction was not observed, although it is described in Pelargonium (Wojtania *et al.* 2004). However the SEM analysis showed the ECM (extracellular matrix) presence in diploid and haploid explants of leaf petioles, which may suggest the embryogenic potential of this experimental system. Future investigations are necessary for improving the culture protocols to obtain somatic embryos, plant regeneration and improvement of shoots formation.

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