2. Abstract

Viola stagnina Kit. is endangered in most parts of its European distribution range. In the present study a protocol for *in vitro* culture and cryopreservation was developed for *ex situ* protection of this rare violet. The frequency of cells with different DNA content in the explants was established allowing to choose the most suitable explant (with the highest number of 2C cells) for culture establishment. Leaf blade and petiole fragments were cultured on MS medium solidified with agar and supplemented with different concentrations and combinations of auxins and cytokinins. Direct organogenesis was induced with the highest frequency on 0.5 mg l^{-1} TDZ, while direct and indirect (*via* callus) on 1 mg l^{-1} TDZ, what was confirmed in histological analysis and SEM observations. Adventitious shoots were rooted on half-strength MS medium with 2% sucrose and 0.5 mg l^{-1} IAA. Regenerants were transferred to garden soil and acclimatized in laboratory conditions. Sixty-five of the regenerated plants (72% of isolated shoots cultured on rooting medium) after moving to field conditions at Jagiellonian University experimental plot survived in good condition several seasons. Plants developed chasmogamous and cleistogamous flowers morphologically similar to flowers of plants from natural populations which were used for culture establishment and set seeds from both flower types in the first, second and third seasons. Estimated by histochemical tests, the pollen viability of chasmogamous flowers of regenerated plants was, in majority of plants, very high (over 90%), similar to that of the plants from natural habitat.

Adventitious shoot buds of *V. stagnina* on different developmental stages were used as an initial plant material for different cryopreservation methods. All this methods resulted in callus proliferation and indirect shoot formation with various efficiency in post thawing culture. Encapsulation/osmotic dehydration was the most efficient technique for cryopreservation of *V. stagnina* material in liquid nitrogen.

An assessment of genetic fidelity of micropropagated and cryopreserved clones was performed based on ISSR molecular markers. Genetic variation of the species in two natural populations was estimated and used as reference for somaclones. Among recovered *via* organogenesis plants individuals 'true-to-type' as well as plants genetically distant from initial plants were detected, while cryopreserved plants revealed genetic compatibility with maternal plant genotype, what was shown on PCoA and diagrams of genetic relations. In all groups of clones values of genetic parameters such as number of genotypes and polymorphic markers as well as H_j, H_T and H_S were lower than in natural populations. Flow cytometry revealed in recovered by organogenesis and after cryopreservation plants genome size uniformity with plants from natural habitats, with the exception of only one autopolyploid plant regenerated after PVS3 treatment.

This is the first report on micropropagation and cryopreservation of *V. stagnina* with efficient protocols developed for use in *ex situ* conservation of this endangered species. This is also the first detailed genetic analysis of recovered plants based on commonly used methods in population genetics and first data of genome size of this species.

Kraków, 27.02.2017 r.