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Genetic variation of *Astrantia major* population using for restoration of natural Belarus coenopopulations

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Key words: AFLP, *Astrantia major* L., critically rare species, restoration of natural coenopopulation.

Astrantia major L. is critically rare species of the category I. The natural range of this species covers mainly the mountainous regions of Central Europe. Individual locations are known in Eastern Europe: Lithuania, Latvia, Ukraine and Moldova. The only one location in the Belovezhskaya Pushcha National Park (BPNP) coenopopulation is known in Belarus at the time of the examination (May 23, 2018), which is located in the grove of the hornbeam and consisting of 14 individuals developing into a right-sided type populations with no signs of generation. This site of growth in BPNP was described for the first time in the early 1970-ies and this population was quite numerous having at about 120 individuals, although even then it was represented by underdeveloped plants with irregular flowering. Based on the results of the age spectrum studies of this coenopopulation conducted over a number of years, a trend toward its slow extinction has been found. In order to preserve the biodiversity of BPNP and restore this population, protection translocation measures are planning to implement according to the task "Foundation of scientific base for the formation of a national reserve gene pool of rare and endangered plant species of Belarus natural flora and identify ways of their conservation and repatriation" within the framework of the State Nature Management and Ecology Program.

A sample of about 20 plants obtained from seeds of BPNP coenopopulation is growing in the collection of rare and endangered plant species of Belarus natural flora of the Central Botanical Garden (CBG) of the National Academy of Sciences of Belarus. Comprehensive ecological, morphological, anatomical and carpological studied have been carried out of this sample during the last five years. The aim of this investigation was to perform comparative DNA analysis of CBG sample to ensure possibility of its use for restoration natural coenopopulation in BPNP.

To analyse genetic variation of *A. major* population

planning for restoring natural coenopopulation, the following samples were included in AFLP analyses: (i) BPNP natural coenopopulation, (ii) BPNP artificial population used for restoration in 1980 (origin of the plants used is unknown), (iii) CBG collection of rare and endangered plant species, (iv) sample from National Botanical Garden (Ukraine, Kyiv) and (v) *A. major* commercial variety using for estimation of possible range of species genetic diversity. Leaf material was collected in summer 2018. The leaves were dried in silica gel and DNA was extracted using QIAGEN DNeasy 96 Plant Kit to maintain the quality of DNA as high as possible. 300 ng of genomic DNA was digested with *EcoRI* and *MseI*, and double-stranded *EcoRI* and *MseI* adapters were ligated to the ends of the fragments. Six primer combinations were used in the last amplification using C1000 Touch™ Thermal Cycler (BioRad). Fragment analysis was performed on GenomeLab GeXP (Beckman Coulter) using 3' fluorescently labeled primers. Genetic distances was estimated using GenA1Ex ver. 6.502. A dendrogram was created using unweighted pair group method with arithmetic mean.

The results obtained proved genetic closeness of BPNP natural coenopopulation and CBG collection sample planning for restoring. Some genetic difference in these samples could result from species allogamous mechanism leading to intrapopulation genetic variation rather than evolution (population) changes during cultivation in CBG conditions. It was detected that plants used for restoration of BPNP coenopopulation in 1980 were not closed to natural *A. major* population. The commercial variety significantly differs from natural samples as it was predicted to estimate possible range of species genetic diversity. The closeness of Kyiv sample to BPNP and CBG population was detected that needs to be additionally investigated as could result from collecting near BPNP or common genetic structure of Belarus and Ukraine *A. major* populations.