The prospect of using clonal micropropagation to conserve the gene pool of natural *Tulipa suaveolens* (Liliaceae) populations

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Key message. Despite the morphological correspondence between the regenerated and natural *Tulipa suaveolens* plants, the ISSR analysis identified a relatively high degree of somaclonal variability between them.

Keywords: biodiversity conservation, morphogenesis, somaclonal variation, ISSR

Tulipa suaveolens Roth (= *T. schrenkii* Regel) is a decorative bulbous polycarpic of the Liliaceae family and the hypothetical ancestor of garden tulips. Violation of natural steppes leads to a reduction in the number of its populations.

The goals of the study were to obtain *T. suaveolens* regenerated plants *in vitro*, to study the characteristics of their morphogenesis, and to assess their genetic stability using the ISSR markers. Natural *T. suaveolens* specimens were introduced in culture *in vitro*. As the donor material for clonal micropropagation, we used the seedlings obtained from rape seeds that had been cultivated under low temperatures for three months. The selection of the clonal micropropagation protocol stemmed from the data recorded in literature. For testing the somaclonal variability with the ISSR markers, we used the fully formed regenerant plants obtained from the samples of the donor population from the Ozinsky district of the Saratov province.

The plants developed on the covering scales by direct organogenesis. The average number of shoots per seedling was 4.8 ± 0.8 pcs for the nutrient medium containing 0.2 mg/L BAP + 0.5 mg/L NAA (Var1) and 7.9 ± 0.5 pcs for the medium containing 0.5 mg/L BAP + 1.0 mg/L NAA (Var2). The rate of shoot formation was 65.7%. In the control group, a single bulblet developed on the inner surface of the seedling.

In the course of the ISSR analysis, we conducted the PCR of the DNA samples extracted from the initial natural *T. suaveolens* population as well as from the regenerated plants grown in two variants of nutrient media (Var1 and Var2) and obtained 101 bands 300–2500 bp long. The ISSR pattern of the donor plants and *in vitro* cultured regenerated plants were similar but not identical. In the ISSR pattern of the regenerated plants, we found the bands which were absent both in the specimens of the donor population and in the specimens of the adjacent populations studied earlier. Their share was 13.9% for the Var1 regenerants and 15.8% for the Var2 regenerants. Other four bands (3.9%), on the contrary, were present in all donor genotypes and were completely absent in the regenerants. In general, the degree of genetic identity between the regenerants obtained in different variants of the experiment was higher than the degree of identity between the regenerants and the original donor plants. Hence, we suggested that in *in vitro* culture *T. suaveolens* genotypes undergo selection or similar changes.

On the UPGMA dendrogram, regenerants formed a separate cluster that was significantly different (bootstrap index 100%) from the cluster consisting of the samples of the donor population. The natural samples, despite their intra-population polymorphism, formed a clear homogeneous cluster.

The ISSR analysis showed a fairly high level of somaclonal variability in *T. suaveolens* regenerated plants obtained as a result of one complete cycle of clonal micropropagation. Furthermore, we noted that the proportion of somaclonal variability increases with an increase in the number of micropropagation cycles.

Thus, we concluded that the fact that during clonal micropropagation *T. suaveolens* genotypes undergo selection and modification contradicts the main mission of germplasm banks. First, the selection of genotypes takes place already at the level of seedlings, and 34.3% of them do not respond to the influence of exogenous growth regulators and do not produce microshoots. Secondly, the bulblets obtained as a result of regeneration have unique bands that are not found in the ISSR pattern of the initial natural population and the populations closest to it, which were studied earlier. For these reasons, we believe that the creation of an *in vitro* germplasm bank for the conservation of the *T. suaveolens* gene pool, at least with the use of clonal micropropagation according to the protocol tested in this work, is impractical.

Taking into account the susceptibility of tulips grown in *ex situ* collections to viral diseases, we propose that the main measures to conserve the *T. suaveolens* gene pool should be the creation, maintenance, and monitoring of the protected natural areas with the restoration of the species population. Also, in order to develop a technology facilitating the efficient preservation of the *T. suaveolens* gene pool in an *in vitro* germplasm bank, it is necessary to identify the plant growth regulators and their combinations that would not have a mutagenic effect on the genome.

This work was supported by the Russian Foundation for Basic Research (grant no. 16-04-00142).