# BIOTECHNOLOGY METHODS USING IN SUGAR BEET BREEDING

KORNIENKO A.V., ZHUZHZHALOVA T.P., ZNAMENSKAYA V.V., FEDULOVA T.P., PODVIGINA O.A., BOGOMOLOV M.A., CHERKASOVA N.N.

*The A..L. Mazlumov All-Russian Research Institute of Sugar Beet & Sugar (VNIISS) Ramon, 396030, Voronezh region, Russia*

Successful fulfillment of breeding programs to obtain highly heterozygous sugar beet varieties depends on development of starting material with genetic traits valuable for breeding. The outcome of these works becomes more when modern biotechnological approaches are used which development is currently central.

Using modern biotechnology methods, the experiments have been carried out for the purpose of directional searching forms with the most valuable traits for breeding as well as their obtaining by an experimentation to increase starting material diversity and variability of plats.

The investigations have resulted in origination of effective and reliable system for genetic transformation in sugar beet that includes induction of regeneration from plant leaf petioles and usage of Ti-plasmids and Agrobacterium tumifaciens, bearing genes of resistance to herbicides of new generation, as vector system. Transformation rate is 6,25 % in this case. Using this method, transgenic sugar beet plants with the gene of resistance to glyphosate have been obtained for the first time in Russian Federation

An affective technology to obtain dihaploid sugar beet breeding material *in vitro* is suggested that involve haploid induction from unfertilized ovules, their diploidization by colchicine and homozygous dihaploid lines forming. It has been shown that the main factors that stimulate haploid morphogenesis are physical and chemical actions upon female generative system. Exposure to x-rays (1000-5000 roentgen), low temperature (+4 +6$^{\circ}$C) and hormonal composition of nutrient media (gibberelline – 1 mg/l, 6-BAP – 0,2 mg/l, IBA – 10,1 mg/l) induce both direct regeneration from sex cells and secondary one from hypocotyl tissue that makes the outcome of haploids 6-10 times more. The greatest amount of dihaploids have been obtained when treating regenerants with colchicine in concentration of 0,01 %.

Micropropagation of the most mature dihaploids and *in vitro* mass selection have provided formation of 10 lines with high level of homozygousity and valuable traits for breeding during last 1,5-2 years.

The carried out experiments resulted in absolutely new method for rapid development of apomictic beet lines, based on controlled pollination with wild species pollen, exposed to gamma-rays (1500-2000 Gr.), and on screening of plants by cytological, morphological and genetic traits together with apomictis propagation. Using the suggested method, 10 gamma-lines combining apomictic way of propagation and high monogermity (93-100 %) and productivity (beet

root mass is 635-900 g, sugar content is 18,2-20,0 %) have been developed. Using the gamma-line RF-2113, the heterosis hybrid RMS-90 has been developed which crop yield is 18,6 % higher and sugar yield is 17,3 % more than the standard ones.

The method for identification of sugar beet breeding materials, varieties and hybrids on the basis of isozyme marking has been devised that, using cluster analysis, enables to determine genetic distance and to identify breeding materials different in origin. The given method allows using isozyme markers widely to estimate breeding materials and to carry out directional hybridization when creating highly productive hybrids. In the whole, more than 1000 breeding lines have been determined and marked by 7 isozymes – Me-1, Mdh-1, Mdh-2, Gdh-1, Idh-1, Idh-2, Adh-1.

The investigations have resulted in that the method for microclonal propagation of valuable starting and breeding materials is devised and promoted. This method has a high resolving ability and reduces time needed for some phases of breeding process in one half.

Thus, usage of modern biotechnology methods will allow to develop competitive varieties and hybrids that satisfy the world standards.