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AGROBACTERIUM-MEDIATED GENETIC TRANSFORMATION OF SORGHUM USING TISSUE CULTURE-BASED AND POLLEN-MEDIATED APPROACHES

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Genetic transformation is a powerful tool for genetic improvement of arable crops. Genetic engineering approaches are especially important for modification of starch and protein contents, vitamin and micronutrient concentration, improvement of nutritive value of protein fractions, and increase tolerance to environmental stresses. Application of transgenic technologies for genetic improvement of sorghum, a highly productive heat tolerant and drought resistant crop, is extremely important since climate aridization in many regions all over the globe hampers sustainable production of traditional cereals, such as wheat, maize and barley. However, sorghum, in spite of great number of investigations, is one of the most recalcitrant crop species to genetic modification. The most frequently reported problems are a low frequency of transformation and silencing of transgenes. Using the *A. tumefaciens* strain AGL0/p35SGIB with the bar and gus-intron genes under the nos and CaMV35S promoters, respectively, we studied different methods of *Agrobacterium*-mediated genetic transformation of the grain sorghum: *in vitro* culture-based techniques, by inoculation of immature embryos or embryo-derived calli, and pollen-mediated approach, by inoculation of flowering panicles. Four lines of grain sorghum – Milo-10, [9E] Milo-10 (CMS-line), KVV-114, and KVV-45 – were used. In both approaches, for activation of vir-genes agrobacterial cell suspension was grown in the AB or modified AB media with acetosyringone at room temperature. *In vitro* culture approach was effective for obtaining transgenic plants in the lines Milo-10 and KVV-45, which were able to produce embryogenic callus from immature embryos after their co-cultivation with agrobacterial cell suspension. Callus cultures tolerant to glufosinate ammonium (GA) and capable to plant regeneration were obtained. The frequency of immature embryos producing PCR-positive transgenic plants varied in different experiments from 4.5% to 5.4%. Cultivation conditions increasing embryogenic potentials of cultured tissues were the key factors for obtaining of transgenic plants. In the Milo-10, transgenic plants were regenerated also from established embryogenic cultures after their co-cultivation with agrobacterial cell suspension, their frequency was 1.7%. Immature embryos of KVV-114 did not produce embryogenic callus, and in this line transgenic plants were obtained by inoculation of flowering panicles at anthesis. In the progeny of each inoculated panicle the frequency of fertile PCR-positive transgenic plants survived BASTA application was approx. 1%. In the progeny of the [9E] Milo-10 panicle, which was obtained by its pollination with the Milo-10 pollen following agrobacterial inoculation, the frequency of PCR-positive plants survived BASTA application was 3.4%. In the self-pollinated progeny (T1) of KVV-114 and Milo-10 transgenic plants (T0), the seedlings that grew on the GA-containing medium were found, while the leaves of adult plants were sensitive to BASTA application. Nevertheless, PCR analysis confirmed the inheritance of the transgene. This work was funded partly by the Russian Foundation for Basic Researches, grant 10-04-00475.