Approaches to the Elaboration of Regeneration and Transformation Systems for Elite Kazakh Cotton Varieties

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The development and wide application of genetic transformation for cotton improvement are restrained by the unresolved problem of genotype dependence in regeneration in vitro. High embryogenic and regenerative potential have been obtained for limited number of Coker type genotypes, which often had no commercial importance. An efficient transformation protocol was elaborated for model Coker 310 variety by the use of long-term embryogenic callus as a recipient system. However, a high level of somaclonal variations in plants regenerated from cotton callus culture has been established. Different approaches to avoid genotype limitation and somaclonal variations have been used including shoot meristem transformation and pollen transformation; however, the efficiency of transformation was significantly low in comparison with the use of embryogenic callus lines. As each of above mentioned systems have their own advantages and disadvantages, we use various commonly used recipient systems (long-term embryogenic calli, cotyledons, hypocotyls, shoot meristems, pollen) for transformation of local Kazakh cotton varieties. For the purpose of overcoming genotype dependence in regeneration in vitro, we have generated an approach consisting on the revealing of morphological type of primary calli, which is common for various genotypes (Maktaaral-4003, Maktaaral-4005, Maktaaral-4006, Maktaaral-4007, Maktaaral-4011, Makta-aral-4019, Pakhtaaral -3044) and valuable in relation to morphogenesis. From three types of primary tissues, type I - grayish-white callus, was found as most typical for all seven investigated genotypes and two type of explants (hypocotyls, cotyledons). This common tissue type was very responsive to the changes in media composition and used as a source for the induction of long-term friable embryogenic calli for two cultivars (Maktaaral-4005, Maktaaral-4006). This approach is in process and embryogenic callus lines are being induced from other local genotypes in the same conditions. Long-term embryogenic calli of cv. Maktaaral-4005 were bombarded by plasmid pAHC25 containing reporter gene -glucuronidase (GUS) and selective marker gene, Bar, for phosphinotricin resistance. GUS-gene expression in callus cells was obtained during the optimization of ballistic transformation. Pollen transformation was carried out for cv. Maktaaral-4005. Pollen was transformed by Agrobacterium strain containing plasmid with reporter gene GUS and selective marker gene, nptII, for resistance to kanamycin. The treated cotton bolls were produced from flowers that had been emasculated the previous day and pollinated by transformed pollens. Seedlings produced from these treated bolls were tested on GUS-gene expression. From 140 pollinated flowers 19 bolls were produced; 600 seeds were obtained from these bolls and 25 of them gave rise to plantlets with positive reaction to histochemical GUS-assay. Progeny of these plants (T_1) is investigated for resistance to kanamycin and to obtaining molecular biology evidence of foreign gene insertion.