1438

TRANSFORMATION AND REGENERATION OF CARROT

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Carrot (*Daucus carota* L.) is one of the most important vegetables, with 13.5 million tons grown in 1991 for human consumption, representing 3% of world-wide vegetable production. Of commercial interest are plant lines with high yield and high sugar for producing fuel alcohol or sugar, and high resistance to diseases, insects and nematodes. Furthermore, of high interest are plants expressing antibodies or synthetic vaccines to be used orally as passive immunization. A protocol is presented for the efficient transformation of carrot by *Agrobacterium tumefaciens*. The binary vector contained the marker gene ß-glucuronidase (GUS), driven by the 35S promoter of cauliflower mosaic virus, and the *nptII* gene, which confers kanamycin resistance. Highest T-DNA transfer rates were obtained by co-cultivating bacteria with hypocotyl segments of dark-grown seedlings on solidified B5 medium containing naphthalene acetic acid and 6-benzylaminopurine. After 2 days, bacterial growth was stopped with antibiotics. Two weeks later, the explants were placed on agar containing the kanamycin derivate geneticin; antibiotic-resistant calli developed during the following 4 weeks. Suspension cultures were obtained from resistant calli and plants regenerated via somatic embryogenesis in liquid culture. The majority of plants were phenotypically normal. About equal levels of GUS activity were found in different organs of young plants up to 6 weeks post-embryogenesis. In leaves of older plants, GUS activity was markedly reduced, whereas the activities in phloem and xylem parenchyma cells of developing tap roots were still high and fairly uniform. Thus, the 35S promoter may be a useful tool to drive the expression of transgenes in developing carrot storage roots. Currently, we are using the transformation protocol to generate transgenic carrot, expressing genes for the synthesis of polyfructans or an anti-idiotype antibody for the treatment of allergies.

1439

CELL FUSION-MEDIATED DNA TRANSFER TO *MEDICAGO TRUNCATULA*

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Medicago truncatula is an annual nitrogen fixing plant, that is used as a model nitrogen fixing species by many researchers. A regenerable line of *M. truncatula* as a recipient species, was fused with incompatible wild *Medicago* species - *M. scutellata, M. rugosa, M. intertexta* and *M. granadensis*. Modifications of previously published protocols were required to increase the numbers and quality of purified protoplasts for fusion. The selection of fused cells is based on treatment of *M. truncatula* mesophyll cells with iodoacetamide (which influences mitochondrial metabolism) and the donor protoplasts were non-regenerable and in some treatments gamma-irradiated. Colonies from fused cells were produced using an agarose droplet technique. In all fusion combinations regenerated fertile plants without donor chromosomes were obtained and DNA transfer was demonstrated using amplified fragment length polymorphism (AFLP). The morphology of the majority of the plants with transferred DNA were identical to recipient but a few plants had some donor characteristics. The protocol attempts to combine the methods of cybridisation where all donor chromosomes are eliminated and somatic hybridisation to transfer nuclear DNA.

1440

CALLUS INDUCTION AND PLANT REGENERATION IN TROPICAL MAIZE (*Zea mays* L.) GENOTYPES

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Genetic transformation experiments with maize (*Zea mays* L.), using either biolistics or *Agrobacterium sp.* infection, require well-defined protocols on callus induction and plant regeneration. For this purpose, embryogenic callus formation hability of 60 tropical maize hybrids and inbred lines was evaluated, by cultivating immature embryos on four distinct culture media. Basically, these media were similar to that proposed by Chu *et al.* (1975), but Dicamba, L-Proline and silver nitrate were added in different concentrations. Calli formed were classified either as Type I (compact) or Type II (friable). Embryogenic calli from 26 genotypes, formed in these referred media, were transferred to Magentas™, containing regeneration medium MS (Murashige & Skoog, 1962), with no growth regulators added. Promising genotypes regarding to embryogenic callus formation and plant regeneration were selected and used in *Agrobacterium* transformation studies.

1441

PRODUCTION OF ZUCCHINI YELLOW MOSAIC VIRUS (ZYMV) RESISTANT CUCUMBER BY *AGROBACTERIUM*-MEDIATED TRANSFORMATION OF COAT PROTEIN GENE OF ZYMV.

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Although zucchini yellow mosaic virus (ZYMV) belonging to the Potyvirus group causes a serious problem in cucumber production, few successful results have been acquired in conventional breeding for resistance to ZYMV. As coat protein-mediated protection (CPMP) has been reported in a number of plant species, it is expected to provide an alternative method for developing crops resistant to viral diseases. In the present study, we introduced