Generation of instantly homozygous transgenic tobacco plants by gene transfer to haploid cells

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Doubled haploid plants are valuable in fundamental and applied research for mainly two reasons: (a) each chromosomal locus is represented by no more than one genetic variant so that the phenotype unequivocally reveals the genotype, and (b) the genetic variability contained in a given mother plant can be readily dissoluted in a population of genetically fixed meiotic recombinants. Likewise, the stable introduction of genetic modifications, be it mutations or recombinant DNA, can be performed using haploid cells which will then give rise via whole genome doubling to plants instantly homozygous for the alternation made. To take advantage of this principle in tobacco (*Nicotiana tabacum* cv. SNN), leaf explants of microspore culture–derived haploid plants were used for *Agrobacterium*-mediated genetic transformation. Regenerated plantlets were checked for transgene integration by PCR and DNA gel blot and ploidy using flow-cytometry, and positive transgenic haploid plants were transferred to the green-house. About 40% of the primary transgenic plants set seed, indicating spontaneous genome doubling (as haploid plants are generally infertile). Analyses of T1 populations did not reveal any transgene segregation. This indicates that the approach pursued enables the generation of instantly homozygous and genetically homogeneous (non-chimeric) primary transgenic plants. The method established will be a valuable tool in functional genomics as well as for the biotechnological improvement of tobacco.

Key words: haploid technology, chimerism, plant regeneration, genetic transformation.