Agrobacterium tumefaciens-mediated transformation in shallot (*Allium cepa* L.)

Agus Purwito [1], Si-Jun Zheng[2]

[1] Laboratory of Plant Biotechnology, Dept. of Agronomy and Horticulture, Bogor Agricultural University, Kampus IPB Darmaga, Bogor 16780, Indonesia. [2]Plant Research International, Wageningen University and Research Center, P.O. Box 16, 6700 AA Wageningen, the Netherlands

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ABSTRACT

This paper describes the use a reliable protocol for producing transgenic plants of 2 cultivars of shallot from mature zygotic embryos. Embryos were isolated and cultured in MS medium containing 1 mg/l 2,4-D for producing embryogenic callus. Three weeks old calli were inoculated with Agrobacterium tumefaciens AGL0 pCAMBIA 1301-cry1Ca, and AGLOpCAMBIA 1301-ho4. Calli were co-cultivated in MS medium supplemented with 100 μM acetosyringone and examined their transient expression of after 4 days culture. The infected calli were then transferred to selection medium MS containing 1 mg/l 2,4-D, 400 mg/l cefotaxime, 100 mg/l vancomycin and 50 mg/l hygromycin for 2 months which they were subculture in the same medium every 2 weeks. The resistant calli were transferred to regeneration medium MS with 1 mg/l kinetin and 50 mg/l hygromycin. Characterizations of transgenic plants were done by PCR analysis by amplifying hpt gene and genomic DNA flanking T-DNA by means of adaptor ligation PCR (AL-PCR). GUS transient expression after 4 days co-cultivation were up to 75 percent in shallot. From total of 2 836 embryos of shallot, 2 244 callus lines were transferred to selection medium and 291 of them were able to survive and transferred to regeneration medium. Plants were formed either by organogenesis or formation of somatic embryos after one-week culture. In shallot cv. 'Tropix' (pCAMBIA 1301-cry1Ca), 34 shoots were produced from individual callus of 18 lines from a total of 36 lines cultured in regeneration medium. In the case with pCAMBIA 1301-ho4 26 plants were produced from individual callus of 16 lines from a total of 41 lines cultured in regeneration medium. Amplifying the hpt gene by specific primers produced single band of 1.2 kb indicating that all examined plant samples were transgenic. The AL-PCR confirmed the result.