Shoot regeneration and genetic transformation by Agrobacterium tumefaciens of Hydrangea macrophylla Ser. leaf discs

Titre	Shoot regeneration and genetic transformation by Agrobacterium tumefaciens of Hydrangea macrophylla Ser. leaf discs
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Résumé en anglais	A reproducible procedure was developed for genetic transformation of Hydrangea macrophylla Ser. cv. Blaumeise by Agrobacterium tumefaciens following the development of an efficient regeneration system using leaf discs excised from 12 to 15 weeks old meristem-derived vitroplants. Explants were cultivated on solid B5 medium complemented with maltose 110 mM, BAP 10 μM and NAA 0.5 μM. A low light regime of 17 μmol m−2 s−1 improved regeneration frequency up to 86%. For transformation, leaf discs were inoculated and co-cultivated with two disarmed A. tumefaciens strains, EHA 101 and LBA 4404, both carrying the binary vector pFAJ3000 which contained the nptII selectable gene and the GUS reporter gene. A pre-culture period of 3 days and a short co-cultivation duration (1 day) improved the efficiency of transformation. Inoculation of only 10 min with agitation including (or not) vacuum infiltration was sufficient. If selection on kanamycin containing medium was applied after a 2 weeks culture period on shoot regeneration medium, the percentage of explants forming kanamycin-resistant shoots increased from 3.3 to 13.3%. Integration and expression of the introduced transgene were confirmed by histochemical GUS assay, PCR and Southern blot analysis. Flowering of transgenic plants in glasshouse occurred 10 months after acclimatization.
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