

DEVELOPMENT OF AGROBACTERIUM TRANSFORMATION SYSTEMS FOR SELECTED CROP VARIETIES IN SRI LANKA

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Agrobacterium rhizogenes-mediated root transformation system is an efficient and useful technique for functional characterization of root genes. The transformation efficiency depends on many factors such as genotype, age and type of explant and the *Agrobacterium* strain used for transformation. Therefore, it is important to optimize the system under local condition before its application. In the present study, *A. rhizogenes*-mediated transformation system was tested for the most popular local varieties of soybean (PB-1), tomato (T-245) and chilli (MI-2). Five different sterilization methods were tested for each crop to establish *in vitro* system for hairy root culture. These were; (1) 70% ethanol (ETOH) for 5 min and 50% commercial bleach (CB) for 20 minutes, (2) 70% ETOH for 1 min and 25% CB 30 min, (3) 70% ETOH for 3 min (4) running tap water and tween-20 for 10 min, and (5) 50% CB and 0.1% Triton-X-100 for 30 min. After sterilization, 30 seeds from each crop were cultured in MS medium supplemented with 3% sugar and maintained at 25°C with light. Percentage of germination and contamination were recorded after two weeks. Soybean and chilli respectively showed a significant germination percentage of 93%, and 63% with method 1 whereas tomato showed the highest germination (31%) in method 2. With the germinated seedlings, several experiments were setup for testing the best explant type and age for transformation. A 24 h old *A. rhizogenes* strain MSU440 harboring pBIN-YFP vector grown on solid MGL medium was used for all experiments with water as the control. Transformed plants were maintained on MS medium with 3% sugar at 16°C for 10 days before transferring to culture room at 25°C. Different explant types at different ages were tested for the transformation efficiency of each crop. Soybean showed significantly higher degree of transformation compared to other crops studied. Though there was no significant difference in transformation efficiency between growth stages, certain explants exhibited significant transformation efficacy. In soybean, hypocotyls gave the highest transformation efficiency (84%) followed by radical (39%) and cotyledons (18%). Fifty-four percent of the transformed soybean hypocotyl was transgenic with YFP. In chilli, 15-day-old hypocotyl showed the highest transformation efficiency (24%) whereas tomato confirmed 12-day-old hypocotyl to produce the highest transformation efficiency.

Keywords: *Agrobacterium rhizogenes*, Hairy roots, Strain MSU440, Transformation