

High – Efficiency *Agrobacterium* – Mediated Transformation of Coffee (*Coffea canephora* Pierre ex. Frohner) Using Hypocotyl Explants

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SUMMARY

An efficient regeneration and *Agrobacterium* mediated transformation system was developed for two commercially important cultivars (c.v S.274 and CxR) of robusta coffee. Hypocotyl explants obtained from the *in vitro* grown seedlings and embryogenic calli gathered from the hypocotyl explants were co-cultivated with *Agrobacterium tumefaciens* strain EHA 101/p BECKS that harbored genes for β -glucuronidase (GUS), kanamycin (*npt II*) and hygromycin (*hpt*) resistance. Prior to co-cultivation the target explants were incubated on MS medium containing 0.1 mg/l 2,4-dichlorophenoxyacetic acid, 1 mg/l Indole -3 Acetic Acid and 4 mg/l Kinetin for 3 days in darkness. Several factors which significantly affected the t-DNA transfer were evaluated and optimized co-cultivation was performed at 22°C in an acidic medium (pH 5.5) in the presence of 50 μ M acetosyringone for 3 days. After 2-3 months of culture on hygromycin (30 mg/l) supplemented selection medium, hygromycin resistant somatic embryos were isolated and separately cultured on germination medium containing 0.5 mg/l IAA and 2 mg/l kinetin and 50mg/l hygromycin. Putative transformed plantlets of two cultivars were obtained after rooting on half strength MS medium supplemented with 0.5 mg/l kinetin and 40 mg/l hygromycin and subsequently hardened in the polybags containing soil mixture. Stable transformation of the recovered plants was verified by histochemical GUS assay and molecular analysis.