

Transgenic Roots for Functional Genomics of Coffee Resistance Genes to Root-Knot Nematodes

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SUMMARY

The possibility of rapid validation and functional analysis of nematode resistance genes is a major objective for coffee. We developed an *Agrobacterium rhizogenes*-mediated transformation protocol for *Coffea arabica* enabling efficient and rapid regeneration of transformed roots from hypocotyls of zygotic embryos, and subsequent production of composite plants (transformed roots induced on non-transformed shoots). Embryos from two *C. arabica* varieties, resistant or susceptible to *Meloidogyne exigua*, were transformed with *A. rhizogenes* A4RS strain armed with binary vectors possessing *gus* or *gfp* reporters genes. Transformed roots with *A. rhizogenes* were regenerated at the inoculum site for 90% of infected embryos. These roots did not exhibit 'hairy' disturbed phenotype. Putative transformed roots could be identified and further selected by histochemical GUS assay and fluorescence microscopy without using herbicide or antibiotic selection. Transgene incorporation was confirmed by PCR. Co-transformation frequencies [i.e. co-integration of both T-DNA from "root inducing" Ri plasmid and binary plasmid] ranged from 40-80%. Composite plants, bearing a single co-transformed ramified root were obtained 12 weeks after transformation (all non co-transformed or weakly ramified roots were eliminated). Nematode infection in nursery conditions of 16 weeks-old composite plants from the Caturra susceptible variety resulted in the development of numerous big sized-galls, while very few small galls were observed in the IAPAR-59 resistant variety. No difference was found in the number of extracted nematodes between the transformed and non-transformed roots ($P < 0.95$) for both varieties, demonstrating that transformed roots retained the resistance/sensibility phenotype of varieties from which they are derived. These results suggest that composite plants constitute a powerful tool for studying genes involved in nematode resistance and root development.