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Regeneration and transformation of cassava (*Manihot esculenta* Crantz)

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Objectives

The goals of our research are establishment of stable tissue culture system and improvement of *Argrobacterium*- and biolistic- mediated transformation system of cassava (*Manihot esculenta* Crantz).

Materials and Methods

1. Material

Plant – *Manihot esculenta* Crantz, Cassava cultivar TMS60444

Agrobacterium strain – GV3101/pKB2, LBA4404/pKB1, pKB3 and EHA105/pKB3

2. Methods:

Cassava cultivar was maintained through micropropagation by shoot cuttings cultured on MS medium supplemented with 2% sucrose (MS2). To obtain organized embryogenic structure(OES), unfolded leaf lobes were cultured on MS2 medium supplemented with 50 μM picloram (MS2 50P). Friable embryogenic callus was induced by transferring these embyogenic structure(OES) to Gresshoff & Doy basal medium supplemented 2% sucrose and 50 μM picloram (GD2 50P). These friable embryogenic callus (FEC) were used as target tissues for regeneration and transformation of cassava.

Results and Discussion

Cassava (*Manihot esculenta* Crantz) is a perennial vegetatively propagated root crop, which serves as important food in tropic area. To develop growth performance of cassava, we have established regeneration and transformation system of cassava. Since maintenance of healthy friable embryogenic callus is a key factor for transformation of cassava, we set up the stable FEC induction and maintenance system. FEC of the cassava was transformed by particle bombardment and *Agrobacterium* harboring pCAMBIA vector containing oat *S598A phytochrome A*, *nptII*, and *uidA* marker genes. Transformed FEC was selected using paromomycin instead of kanamycin, because callus proliferation was more efficient by paromomycin. Before regeneration step, survived callus was histochemically analyzed in X-gluc solution to eliminate false positive FEC. We obtained 4 lines of putative transgenic cassava by biolistic method, and 17 lines by *Agrobacterium* mediated method.

The putative transgenic plants were conformed by PCR analysis and GUS histochemical assays. Young transgenic lines in culture medium showed GUS activity in stem and leaves and two months old transgenic lines after transfer to soil showed shorter height and had more leaf numbers compare to wild-type cassava.

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