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Transformed Cell Suspensions of Wild Cherry Tomato with Kanamycin Resistance Marker

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ABSTRACT. *The nptII gene which confers resistance to kanamycin monosulphate (km) has been effectively used in somatic hybridization studies of the genus Lycopersicon as a hybrid selection marker. Aim of the present study was to incorporate this gene into wild cherry tomato (Lycopersicon esculentum var. cerasiforme) via Agrobacterium - mediated transformation and establish cell suspensions from the transformed materials. These cell suspensions were prepared to isolate protoplasts with the nptII marker gene.*

Stem pieces (1.0 - 1.5 cm) from axenic plant cultures of L. esculentum var. cerasiforme were inoculated with A. rhizogenes strain R1601. Putative transformed roots, appeared from the inoculated sites were multiplied in high km (100 μg ml^{-1}) medium. DNA was extracted from transformed root cells and the presence of the nptII gene was confirmed using PCR and electrophoresis techniques. Calli were initiated from transformed roots in UM medium (Uchimiya and Murashige, 1974) and subsequently cell suspensions were initiated from these calli. Protoplasts were produced from these cell suspensions through enzyme digestion. Viability of suspension cells and resultant protoplasts were detected by FDA staining.

Each stem piece produced 3-4 roots from the inoculated sites. Such roots were able to grow in the presence of km and DNA analysis confirmed the presence of the nptII gene. Calli originated from these roots developed into a cell suspension. Viability and growth of these cell suspensions were maintained over 10 months. Large numbers (approximately 13×10^6 protoplasts per g.f.wt. suspension cells) of clean and intact protoplasts were isolated from these cell suspensions with a high level of protoplast viability (around 70 %). These protoplasts were expected to be utilized in somatic hybridisation programmes with the km resistance in hybrid cells as a half - selection criteria.

OBJECTIVE

The aim of this study was to establish cell suspensions of wild cherry tomato (*Lycopersicon esculentum* var. *cerasiforme*) to produce protoplasts with resistance to the antibiotic, kanamycin monosulfate (km). Protoplasts obtained from these suspensions were expected to be used in somatic hybridization studies where km resistance in hybrid cells can be a half-selection criteria.

In somatic hybridization (*via* protoplast fusion) of the genus *Lycopersicon*, the *npt*II marker gene has efficiently been used to select hybrid cells (Wijbrandi *et al.*, 1990; Latif, 1993; Schoenmakers *et al.*, 1994). This gene which confers resistance to km can be incorporated into protoplasts through *Agrobacterium* - mediated gene transformation.

MATERIALS AND METHODS

Agrobacterium-mediated transformation of *L. esculentum* var. *cerasiforme*

Stem pieces (1.0 - 1.5 cm) from axenic plant cultures of *L. esculentum* var. *cerasiforme* were inoculated with *A. rhizogenes* strain R1601 (Pythoud *et al.*, 1987) and cultured in a selection medium containing a relatively high concentration of km (100 $\mu g\ ml^{-1}$). Roots which appeared from the inoculated sites were separated and cultured in the same medium in petri dishes to facilitate the growth of transformed root cultures. Presence of the *npt*II gene in these root cells was confirmed by DNA extraction according to Edwards *et al.* (1991) followed by Polymerase Chain Reaction (PCR) and electrophoresis.

Establishment of cell suspensions and isolation of protoplasts

Vigorously growing roots in selection medium were cultured in UM medium (Uchimiya and Murashige, 1974) with 75 $\mu g\ ml^{-1}$ km to initiate callus. Soft and friable calli were transferred to liquid UM medium in Erlenmeyer flasks and incubated (25°C/dark) on a continuous rotary shaker (80 cycles/min). Cell suspensions were maintained by repeatedly subculturing in the same medium at 7d intervals. Protoplasts were produced and isolated according to Latif (1993) with slight modifications followed by sequential sieving (80 µm, 64 µm, 45 µm) and washing. Viability of suspension cells and protoplasts was assessed by fluorescein diacetate (FDA) staining.

RESULTS

Genetic transformation of *L. esculentum* var. *cerasiforme*

White, hairy, putatively transformed roots appeared from the inoculated stems of *L. esculentum* var. *cerasiforme*. Once transferred to root growing medium with 100 μg ml^{-1} km, these roots continued to grow fast and produced a dense root mat within 3 - 4 weeks. Normal (non-transformed) roots obtained from seedlings on the other hand, turned necrotic when cultured in the same medium. Analyses of extracted root DNA indicated the presence of the *npt*II gene, thereby confirming the transformed nature of surviving roots.

Establishment of cell suspensions

Cell suspensions were initiated by the fourth month but higher settled cell volumes (SCV) recorded after 7 months indicated that the cell suspensions were well established and capable of sustained cell division at that time. The cells were highly viable and continued to multiply for a period of 6 - 21d prior to the onset of cell deterioration. Therefore, subculturing was undertaken every 7d.

Protoplast production

Clean, spherical and intact protoplasts were isolated from cell suspensions with high viability rates (Table 1).

Table 1. Yield and viability of protoplasts produced by *L. esculentum* var. *cerasiforme* cell suspensions grown in 75 μg ml^{-1} kanamycin medium.

Source	Yield (number / ml suspension)	Viability (%)
Cell suspension - derived protoplasts	1.4×10^6	70 ± 7

DISCUSSION AND CONCLUSION

Protoplast-derived selectable markers introduced *via* gene transformation have been utilized in hybridization as well as in genetic studies based on foreign DNA transmission and stability (Negrutiu *et al.*, 1992). In the present study, efficient transformation rates (85%) were obtained for *L. esculentum* var. *cerasiforme* by inoculating stem segments with *A. rhizogenes* strain R1601. This could be attributed to the presence, in trans, of PTVK2911, a plasmid containing part of the *vir* region of the supervirulent pTiBo542 plasmid in R1601 (Lipp Joao and Brown, 1994). Continued multiplication of cells in the presence of km in suspension cultures suggested that they were resistant to kanamycin. These cells of tested plant species in suspension were capable of producing a large number of viable and intact protoplasts which could be used as fusion partners for somatic hybridization, where km resistance of hybrid cells will act as a selection criteria.

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