AGROBACTERIUM – MEDIATED TRANSFORMATION OF SOME ROMANIAN CULTIVARS OF TOMATO (*LYCOPERSICON ESCULENTUM*)

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The organogenetic capacity of two Romanian tomato cultivars *Lycopersicon esculentum var Vidra 533* and *L. esculentum var Roxana* after *Agrobacterium tumefaciens* (carrying *npt II* marker gene) mediated transformation was investigated. Both varieties regenerated shoots after 3–4 weeks of incubation on zeatin-contained medium. A comparative analysis of the presence of neomycin phosphotransferase (*npt II*) gene was performed in transformed and untransformed plants, analysis that shows a transformation efficiency of 18.7%, which is a value a little higher than the reported ones for the same transformation technique. It is not clear if this regeneration efficiency is due to the tomato genotypes used, the *Agrobacterium tumefaciens* strain GV3101 (pMP90), or to some other reasons.

Key words: tomato cultivars, *Agrobacterium tumefaciens.*

INTRODUCTION

Transformation of tomato and other dicotyledoneous plants via *Agrobacterium tumefaciens* is still far from routine and there is not a universal procedure suitable to transform different cultivars within each species. Availability of an efficient regeneration system is usually a prerequisite to genetic modification of a particular plant species. Dicotyledoneous species differ widely in their organogenic potential and amenability to genetic transformation.

Tomato is considered more difficult to be transformed than species such as *Petunia hybrida* and *Nicotiana tabacum* and can show widely different success rates, possibly depending on cultivar, *Agrobacterium* strain, antibiotic selection, and/or the personnel performing experiments (1).

The current protocols used for tomato transformation are based on shoot regeneration from leaf disc tissue co-cultivated with disarmed *A. tumefaciens* harboring binary vectors (2). The efficiency of such procedures is generally low (3, 4) because most of the transformed leaf/cotyledons cells do not develop into shoots.

Even if this transformation procedure via *Agrobacterium tumefaciens* through adventitious shoot regeneration is not considered to be a real problem, the interest to obtain more efficient, reliable, simple, rapid and universal methods for tomato transformation is well documented in the literature (2, 4, 5, 6, 7).

Previously reported methods are, in general, tedious and time consuming, with variable transformation efficiencies (4–12%) (3, 7).

In the present work we investigated the competence of two Romanian cultivars of tomato (*Lycopersicon esculentum cvs. Vidra 533* and *Roxana)* to regenerate shoots from leaf explants transformed via *A. tumefaciens*.

MATERIALS AND METHODS

Plant materials

Seeds of tomato (*Lycopersicon esculentum* cvs. Vidra 533 and Roxana) were at the surface sterilized by 20 min immersion in 20% v/v commercial bleach (5% available sodium hypochlorite) plus 0.1% Tween 20 and rinsed in sterile distilled water three times. Seeds were germinated on MS medium (8) with Gamborg vitamins (9) (MS) plus 20 g l^{-1} sucrose and 8 g l^{-1} agar (Sigma-Aldrich, Taufkirchen, Germany) in a growth room at 24°C, with a photoperiod of 16h/day. Young leaves from 6 weeks old plants were cut near the proximal end (an additional cut surface near the distal end can be made) and used as explants.

Agrobacterium strains and plasmids

The *Agrobacterium tumefaciens* strain GV3101 (pMP90) (10) was used for transformation. The construct contains a NOS-driven neomycin phosphotransferase (nptII) plant selection gene (11).

The bacteria were grown overnight in LB medium (12) with 40 mg l^{-1} rifampicin plus 50 mg l^{-1} kanamycin, diluted to $OD_{600} = 0.1$ and grown to $OD_{600} = 0.4–0.5$. Bacterial suspensions were used to infect the explants.

Transformation procedure

The bacterial suspensions in liquid LB medium were placed into sterile glass jars (10 cm diameter, 10 cm height), containing the explants, and gently shaken for 10 or 15 min. Infected explants were blotted dry on sterile filter paper and co-cultivated for 2 days on fresh MS medium. The explants were then transferred for callus formation and differentiation to solid MS, with 500 mg l^{-1} carbenicillin and hormones in different combinations and concentrations. These comprise auxins (indoleacetic acid, 2,4-dichlorophenoxyacetic acid) and cytokinins (kinetin, 6-benzylaminopurine, zeatin) in a concentration between 0.1–2 mg l^{-1}. Infected explants were transferred every three weeks on fresh MS medium with hormones and lowering concentration of carbenicillin. After 4–5 weeks, elongated shoots emerging from the cut surfaces were excised and placed on MS for rooting.

Transformation frequency was expressed as the percentage of inoculated explants producing transformants. A transformant was defined as a shoot

Fig. 1– Generation of transgenic tomato plants (*L. esculentum cv. Roxana*).
A. Plant regeneration from callus tissue on MS medium with zeatin 2mg/l.
B. Transgenic tomato plants on MS medium for rooting.

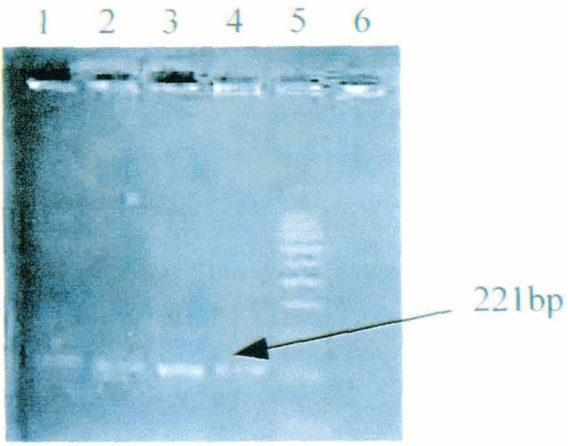

Fig. 2 – Molecular analysis of wild-type and regenerated tomato plants after transformation via *A. tumefaciens*. The agarose gel shows the results of amplification with the *nptII* primers. The tracks are as follows: 1–4 – DNA amplified from leaves of *L.e. cv. Roxana* (1,2) and *cv. Vidra 533* (3, 4) plants regenerated after transformation; 5 DNA marker (100 bp ladder); 6 - DNA amplified from leaves of untransformed tomato plants.

regenerated on hormone containing MS medium and presenting the nptII gene integrated in its genome (revealed by PCR analysis, see below).

Plant DNA analysis

Genomic DNA was isolated from transformed and untransformed plants (young leaves) according to Vallejos *et al.*, 1992.

PCR analysis (14) for the presence of nptII gene from T-region of pMP90 was used as an indication of transgenic tissues. The amplification was carried out using a Perkin Elmer (GeneAmp PCR System 2400) PCR station. The reaction mixture was made up by 0.5U REDTaq™ DNA Polymerase (Sigma-Aldrich, Taufkirchen, Germany), 200 μM dNTP (Sigma-Aldrich, Taufkirchen, Germany), 1X concentrated REDTaq DNA Polymerase PCR buffer (Sigma-Aldrich, Taufkirchen, Germany), 1 μM each of the two primers and 50 ng genomic DNA, in a total volume of 25 μl. The following cycling conditions were performed: 5 min melting at 95°C, 35 cycles of 45 sec melting at 95°C, 40 sec annealing at 56°C and 2 min elongation at 72°C, followed by a final elongation step for 5 min at 72°C. The oligonucleotides used were 5'-GCA TAC GCT TGA TCC GGC TAC C-3' and 5'-TGA TAT TCG GCA AGC AGG CAT-3', yielding a fragment of 221 bp. PCR products where electrophoretically separated by ordinary 2% agarose gel electrophoresis and visualized on a standard transiluminator, after staining the gel with ethidium bromide (0.5μg/ml) for 30 minutes.

RESULTS AND DISCUSSIONS

Tomato is a natural host for *Agrobacterium*, amenable to current plant transformation techniques. In addition, tomato has been used extensively as a model system for Agrobacterium-mediated transformation (15). In this experiment, two Romanian cultivars of tomato (*Lycopersicon esculentum cvs. Vidra 533* and *Roxana)* were tested for their ability to regenerate shoots from leaf explants transformed via *Agrobacterium tumefaciens.* The bacterial strain used for the T-DNA transfer was GV3101 containing a nopaline Ti plasmid (pMP90) (10) carrying the *nptII* marker gene.

The most efficient medium for dedifferention and redifferention of infected explants from both cultivars was the zeatin contained medium (zeatin 2mg l^{-1}). The results in Figure 1 show the stages of shoot elongation on zeatin contained medium (A), respectively regenerants on MS medium (B). The phenotype of the transformed plants showed no significant differences, as compared with the normal plants.

The criterion for scoring positive transformants in this study was the presence of the inserted *nptII* gene in the plant genome as revealed by PCR analysis (Figure 2).

For *L. esculentum cv. Vidra 533* the frequency of transformation was 18.7% (n = 30), while for *L. e. cv Roxana* the transformation frequency was slightly below this value.

Hamza and Chupeau (1993) and van Roekel *et al.* (1993), reported transformation efficiencies of 4–12% for the leaf disc protocol transformation via *A. tumefaciens*. Pozueta-Romero *et al.* (2001), reported a higher transformation frequency (21.6%) in tomato, using a different method (flamingo-bill explants). The results presented in this study show a higher regeneration efficiency (18.7%) for the classic method of transformation (leaf disc explants) than the one previously reported in the literature (3, 7). We cannot say for the moment if this result is due to the genotype of these Romanian tomato cultivars, the genetic peculiarities of the *Agrobacterium tumefaciens* strain GV3101 (pMP90), or some other reasons.

CONCLUSIONS

We have tested two Romanian tomato cultivars for their ability to regenerate transformed plants after infection with *Agrobacterium tumefaciens*.

The two cultivars (*L. esculentum cv Vidra 533* and *cv Roxana*) showed a positive response to transformation using leaf disc protocol and both have regenerated transformed plants after callus formation. The technique used is considered by some authors to be more like "an art" (1), as compared to other transformation procedures.

The transformation efficiency obtained is slightly higher than the one previously reported in literature for the protocol used (3, 7). The reasons for this results are not known, but the use of the specific tomato genotypes and the specific *Agrobacterium* strain could be involved, as suggested by other authors (1, 4) who noticed the high variability of the process of regeneration of tomato transformed plants mediated by *A. tumefaciens*. Due to the relatively higher transformation efficiency, these cultivars can be used in further studies to introduce genes of interests in their genome via *Agrobacterium tumefaciens* and can be tested also for *Agrobacterium rhizogenes* transformation.

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