

## OPTIMIZATION OF DIRECT GENE TRANSFER IN TOMATO

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## 1. INTRODUCTION

The successful transformation of callus derived protoplasts of the tomato genotype MSK93 was recently described (2, 4). Transformants were obtained with plasmid DNA containing the kanamycin resistance gene from Tn5 with expression signals from the *nos* gene as a selectable marker (plasmid pneo $\Delta$ 18). In addition a (0.8 kb) tomato sequence which promoted autonomous replication in yeast was cloned into pneo $\Delta$ 18 resulting in pCTW22 (2). Because thus far only a limited number of transformants were obtained (exclusively with pCTW22) and no diploid plants could be regenerated from these callus derived protoplasts, the transformation procedures were further optimized with leaf derived protoplasts of MSK93.

## 2. MATERIAL AND METHODS

The origin and properties of the easily regenerable tomato genotype MSK93 has been previously described (3, 4). The plasmids used and the DNA isolations are described by Jongsma et al. (2).

DNA transformation of protoplasts was performed according to the modified Krens procedure (2) and the MgCl<sub>2</sub>/PEG procedure of Negrutiu et al. (5). Aliquots of  $5 \cdot 10^5$  protoplasts (1 ml) were used with 30  $\mu$ g plasmid DNA in all experiments. The plasmids were linearized by cutting the plasmids at their unique *Hind*III site. In some experiments a heat-shock treatment (5) was applied. In addition the transformation procedure of Hain et al (1) with the PEG treatment and dilution scheme of the modified Krens procedure and electroporation with the media of respectively Riggs and Bates (7) and Shillito et al. (8) were tested.

Protoplasts were isolated and cultured in 2 ml liquid TMP (5 cm dishes) as described previously (4). On the 3th day after transformation the protoplasts were embedded in 0.6% Sea Plaque agarose (4 ml). Agarose parts were placed into liquid medium 3 days later. Selection started on day 10 when the liquid medium was replaced with 6 ml dilution medium (4) containing 100 mg l<sup>-1</sup> kanamycin.

Growing microcalli were subsequently transferred to callus growth, shoot induction and rooting media all containing 100 mg l<sup>-1</sup> kanamycin.

## 3. RESULTS AND DISCUSSION

The modified Krens procedure and the method of Negrutiu et al. (1987) gave fairly reproducible frequencies of 0.1% (range 0-0.6%) kanamycin resistant calli among the colony forming units (RTF) (see Table 2).

The modified Hain procedure resulted in comparable RTF values, although survival was very much reduced. A heat-shock treatment and the use of linearized plasmid DNA appeared to enhance the transformation frequency. The two electroporation procedures (7, 8) yielded transformants, although at a lower frequency than the chemical methods.

TABLE 2. Plating efficiencies and transformation frequencies obtained with MSK93 leaf protoplasts.

Plasmid	Linearized	Transform. procedure <sup>1</sup>	Number of exp.	Average PE <sup>2</sup>	RTF(x10 <sup>-4</sup> ) <sup>3</sup>	ATF(x10 <sup>-5</sup> ) <sup>4</sup>
pneo $\Delta$ 18	-	K	5	1.9	5.2	1.0
	+	K	4	2.7	7.4	2.0
	-	N	7	2.2	10.3	2.3
pCTW 22	-	K	5	2.7	8.1	2.2
	+	K	4	2.8	13.1	3.7
	-	N	7	2.8	10.8	3.0
control		untreated	8	7.9	0	0

<sup>1</sup>) K: Modified Krens procedure

N: Negrutiu procedure

<sup>2</sup>) PE: Plating efficiency

<sup>3</sup>) RTF: Relative transformation frequency

<sup>4</sup>) ATF: Absolute transformation frequency

Plasmids pCTW22 and pneo $\Delta$ 18 were used in all experiments and comparison of transformation frequencies showed that pCTW22 gives a 1.5 higher transformation frequency than pneo 18. Tested with a sign test this was significant at  $P = 0.05$ .

In 9 transformants that were tested, the kanamycin gene was integrated into the nuclear genome as shown by Southern blotting.

The slightly higher transformation frequency of pCTW22 might be explained by a (temporary) more stable or more abundant presence within the cell before integration takes place and not by an improved chance for integration since Jongsma et al (2) found no indication for homologous recombination in 7 pCTW22 transformants.

For 20 independent transformants (10 of each plasmid were tested) resistance to kanamycin at the callus level was still present after 4 weeks on non-selective medium.

Regeneration was tested for 67 resistant calli. After 3 months on shoot induction medium 66 transformants had formed shoots. Out of 51 plants regenerated from 24 transformants 8 plants were diploid, 42 tetraploid and 1 probably octaploid as determined by counting the number of guard cell chloroplasts. Five of the 24 transformants regenerated diploid plants.

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