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Agrobacterium tumefaciens-mediated transformation of *Lycopersicon esculentum* cv. Pusa ruby with *Sclerotium rolfsii* lectin gene*

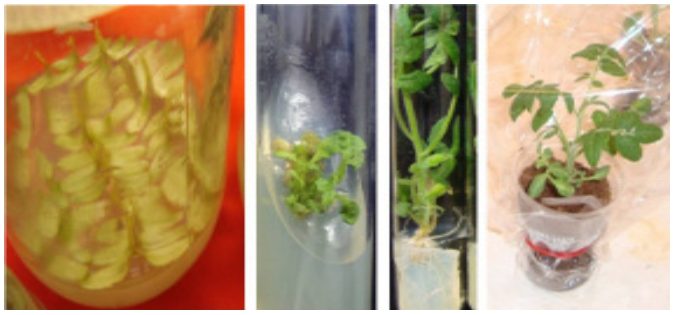
Lectins are the proteins that bind to polysaccharides or glycoproteins (Goldstein *et al.,* 1980). In general, they are involved in the defense mechanisms of the plant against viruses, bacteria, fungi, nematodes and insect pests (Etzler, 1985; Chrispeels and Raikhel, 1991; Van Damme *et al.,* 1994a; Van Damme *et al.,* 1994b). Previous efforts at the Department of Biotechnology, UAS Dharwad has led to molecular cloning of a lectin coding gene (*srl1*) from *Sclerotium rolfsii* (Chandrashekar, 2007). *srl1* had high structural homology with *Xerocomus chrysenteron* lectin gene, which is insecticidal (Trigueros *et al.,* 2003). *srl1* when expressed in *Escherichia coli*, the extract showed nematicidal activity against the common root knot nematode, *Meloidogyne incognita* (Bhat *et al.*, 2010). *srl1* was transferred to tomato, and the transgenic plant was found to be moderately resistant to *M. incognita* (Bhagat, 2010). Hence, an effort was made to generate a large number of transformation events.

Seeds of Pusa ruby genotype were sown in pots in the greenhouse. Leaf discs collected from the young cotyledonary leaves of 7-8 days old seedlings were surface sterilized with 0.1% $HgCl_2$ for 1 min, and rinsed with sterile distilled water for 2-3 times to remove traces of $HgCl_2$. Leaf discs were dried on sterilized blotting paper in Petri plate for 10 min. Completely dried leaf discs were transferred in inverted position to culture bottles containing MS medium and kept under light for 2 days for pre-incubation. *Agrobacterium tumefaciens* strain LBA4404 carrying pNR68 with *srl1* (GenBank Acc. No. FJ211419) cloned in between CaMV 35S promoter and NOS terminator of the binary vector pHS100 (Kamble *et al.,* 2003) was inoculated to YEM broth containing appropriate antibiotics ($Streptomycin_{100}$ + $Rifamycin_{25}$ + $Kanamycin_{50}$) and incubated. Overnight culture with an OD_{600} of 1.0-1.5 was centrifuged at 3,000 rpm for 15 min. Supernatant was discarded and the pellet was re-suspended in MS liquid medium. Pre-incubated explants were soaked in *Agrobacterium* suspension for 20 min. Explants were then blot-dried on filter paper and transferred to MS medium in culture bottles and incubated at 28°C in dark for 2 days for co-cultivation. Such leaf discs were washed in sterile water containing 200 mg/l cefotaxime to check the over-growth of *Agrobacterium*, and transferred to callus induction MS medium supplemented with Zeatin (1 mg/l), IAA (0.1 mg/l), Kanamycin (50 mg/l) and Cefotaxime (200 mg/l). Calli were sub-cultured on the same medium at 8-10 days of interval for 4-5 times. Calli with green growth initials were transferred to shoot regeneration MS medium supplemented with Zeatin (0.1 mg/l), IAA (0.1 mg/l), Kanamycin (50 mg/l) and Cefotaxime (200 mg/l). Shoot initials were sub-cultured on the same medium once after 10-12 days of regeneration. Well developed shoots were aseptically transferred to rooting MS medium supplemented with NAA (0.5 mg/l). The rooted plants were grown in 3% sucrose solution overnight, and then transferred to cups containing the mixture of sand and vermicompost (1:1) and kept in the incubation room for 10 days to facilitate further root development and hardening. Well established plants (15-25 cm length) were transferred to bigger pots (30 cm diameter) containing a mixture of soil, farm yard manure and sand (4:3:1), and kept in green house till maturity.

Genomic DNA was isolated from young leaf tissues of sT_0 and T_1 plants by following a modified CTAB method (Sambrook and Russell, 2001). A forward primer (5' ATGGATCCATGACTTATAAGATTACCGT 3') and a reverse primer

(5' CGAGCTCTCACCCGATAATGACGTT 3') were used to amplify *srl1* gene. PCR product was electrophoresed on 1% agarose gel.

In total, 900 leaf disc explants were co-cultivated with *Agrobacterium tumefaciens* carrying pNR68, and care was taken



A B C D

Fig 1. *In vitro* regeneration of SRL1 transgenic tomato plants

A: Cotyledonary leaf-disc explants, B: Regenerating shoots, C: Rooted shoots and D: Plants established in the cups.

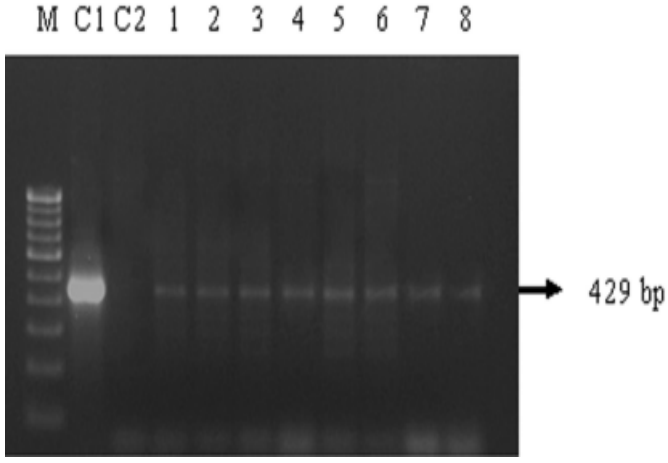


Fig 2. PCR confirmation of SRL1 transgenic plants

M: 100 bp DNA ladder, C1: Plasmid DNA (pNR68), C2: Non-transgenic tomato plant, 1-8: SRL1 transgenic tomato plants

to transfer a single regenerant from each explant to rooting medium (Fig 1). Ninety regenerants were obtained and they were established in the green house. Of them, 40 plants could

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amplify 429 bp product with *srl1*-specific PCR (Fig 2). Since these plants originated independently *in vitro*, they represented independent transformation events. Thus the efficiency of generating independent transformation events was 4.44% (40 PCR positive plants from 900 explants).

Previous reports have indicated vast differences in the expression level of the transgene among transgenic events generated under identical conditions (Peach and Velten, 1991; Bennet, 1993; Birch, 1997; Bhat and Srinivasan, 2002; Butaye *et al.,* 2004). This variation was attributed to site of integration (position effect), transgene structure, transgene copy number etc. Hence such an efficient transformation protocol is useful in developing an elite transgenic event by generating a large number of independent transformation events.

Copy number of the transgene was checked in a selected primary transgenic plant, SRL1-T_0(7). Randomly chosen 50 T_1 seeds were grown and segregation was analysed. In total, 33 plants were PCR positive and 17 were negative for *srl1*-specific PCR. It followed 3:1 segregation ratio with a chi square value of 2.16 at 0.05 probability, indicating the single copy integration. The transgenic plants generated in this study are being tested for resistance to *M. incognita* and white fly.

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