

Tomato (*Lycopersicon esculentum* Miller) is one of the most important and popular winter vegetable of Bangladesh. But its yield is hampered by various biotic and abiotic factors. To make it resistance towards these factors different transgenic approaches have been reported. In the present study, an efficient transformation method was developed with three locally grown tomato varieties, namely Bahar (BR), Bina tomato 5 (B-5), Bina tomato 3 (B-3) and one Indian commercial variety, Pusa Ruby (PR). A reliable and reproducible *in vitro* regeneration protocol for these varieties was obtained as a prerequisite for transformation. Cotyledonary leaf explants of tomato were collected from 8-10 days old *in vitro* germinated seedling. To increase germination, agitation of seeds following sterilization was found to be effective. Different concentrations and combinations of growth regulators were added to MS media to observe shoot initiation and root induction. MS media containing 2 mg/l BAP showed best shoot regeneration with maximum number of shoots (-6 shoots/ explant) for all four varieties and obtained 91%, 86%, 83% and 93% regeneration percentage in BR, B-5, B-3 and PR variety respectively. Rooting was best in half strength MS media supplemented with 0.2 mg/l IAA. The regenerated plantlets successfully acclimatized in soil, where they flowered and formed fruits. Seeds collected from these fruits were found to be viable in germination tests. For transformation, a genetically engineered *Agrobacterium* strain LBA4404 containing binary vector pBI121 was used to transform cotyledonary leaf explant of all the four tomato varieties and found to be susceptible towards it. Further studies showed that an OD<sub>600</sub> of 0.8 with 10-15 mins of incubation and 3 days of co-cultivation period was the best to achieve maximum transformation ability. B-3 showed highest transformation ability (96.7%), whereas BR, B-5 and PR showed 83.3%, 93.3% and 93.3% as confirmed by transient GUS assay. Carbenicillin and cefotaxime were used as bacteriostatic antibiotics while kanamycin was used for the selection of transformed explants. Immediate selection pressure with 200 mg/l kanamycin in the regeneration media following co-cultivation was found to be the best selection condition to obtain transformed shoots. Putative transformed shoots that survived under selection pressure were transferred to 1/4 strength of MS media containing 0.5 mg/l IBA and reduced concentration of bacteriostatic antibiotics to obtain rooting avoiding callus formation.

With respect to transgenic B-3 plantlets which rooted in soil, efficiency of transformation

was found to be 11.11%. Following rooting GUS histochemical assay was showed stable incorporation of GUS gene in these putatively transformed plantlets. Among the four varieties B-3 was chosen as it showed the highest transformation ability. Transformed B-3 plantlets were successfully acclimatized in soil and flowered normally. Different parts of the flower were found to be GUS positive and the flowers produced fruit. Seeds from the fruit have been collected for checking inheritance of the antibiotic resistant and GUS genes.