Abstract

Chickpea (*Cicer arietinum* L.), an important pulse crop in the world, provides a unique source of dietary protein especially for the people of developing countries. Several biotic and abiotic factors are responsible for the loss of its productivity. Severe damage caused by pod borer (*Helicoverpa armigera* Hubner) has been recognized as the major one. Developing a pest tolerant cultivar by conventional breeding techniques could not succeed due to paucity of genomic resources. Therefore, genetic engineering approaches are adopted to develop pod borer tolerant cultivars but with limited success due to lack of efficient regeneration and transformation protocol of this recalcitrant crop.

To overcome this, the present study was undertaken to develop highly reproducible, efficient regeneration and transformation protocol with three chickpea genotypes which broadened the scope of genetic manipulation of this popular pulse crop. Basal media was manipulated for direct *in vitro* organogenesis where 2XMS media fortified with 1.5 mg/L BAP was found to be most efficient for enhanced shoot proliferation (maximum 23 shoots per explant) irrespective of all three test cultivars. While normal MS with B5 macro salts resulted in highest elongation of micro shoots, addition of 0.05 mg/L IAA enhanced micro shoot length of 5.0 cm within two weeks. Rhizogenesis and outdoor establishment of plantlets were achieved by direct rooting in 1/2MS basal media supplemented with either 0.125 mg/L IAA or 0.05 mg/L IBA and thereafter growing the rooted plantlets in sand: soilrite (2:1) pot mixture.

Efficient Biolistic and *Agrobacterium* mediated transformation protocols were identified by optimizing several physical factors such as culture condition of explants, suitable target tissue and amenable genotypes. Strategic manipulation of sectorial sectioning was adopted for maximum elimination of chimeric distribution of transformed cells to achieve large number transgenic events.

Finally, pod borer resistant chickpea by overexpressing codon optimized *Cry1*Ab gene under green tissue specific RuBisCo small subunit (*rbcS*) promoter with a chloroplast target sequence was achieved up to T_2 generation. The direct sequence of asymmetric LoxP recognition site was used at two distant ends of marker genes (*bar* and *gfp*) for further application of marker-free approach exploiting cre-lox mediated recombination phenomenon.

Keywords: *Agrobacterium*, *Bacillus thuringiensis*, Biolistic, Bt-toxin, *Cicer arietinum, Cry1*Ab, Grafting, *Helicoverpa armigera, in vitro*, multiple shooting, rooting, transformation, T_1 transgenic line, T_2 transgenic line.