Development of selectable marker free transgenic pigeonpea conferring resistance against *Helicoverpa armigera*

ABSTRACT

Production of high yielding pigeonpea (*Cajanus cajan* (L.) Millsp.**)** cultivars has gained priority to satisfy the global demand of growing population in developing countries. Despite of its immense importance as grain legume, pigeonpea production has remained stagnant during last decade due to prevalence of several biotic constraints. The most crucial yield constraint of pigeonpea is susceptibility to pod borer *Helicoverpa armigera* which causes extensive damage and severe economic losses every year. However, due to non-availability of genetic resources, the applied conventional breeding strategies were not successful. Several in vitro tissue culture based transformation strategies were applied for development of transgenic pigeonpea, but the success of these methods were not satisfactory due to lack of reproducibility. Present study was designed to develop a novel *Agrobacterium* – mediated transformation method in pigeonpea. We designated it as plumular meristem transformation method, which involved culture based *Agrobacterium*-infection, culture independent plant establishment and PCR based selection of primary transformants. Selectable marker, used for T_1 screening, enabled establishment of progeny plants with highest frequency of stable transgenic development, reported so far. The method was further successfully applied to another leguminous crop chickpea and 60 % transformation frequency was achieved. Using this novel transformation method Cre/*lox* recombination mediated marker elimination strategy was applied for the first time in pigeonpea. Bio-activity of *cry1Ac* gene was compared on the basis of integration and expression driven by two promoters, constitutive *CaMV35S* and green tissue specific *ats1A,* in the developed transgenic events. The transgenic events also contained selectable marker gene, *nptII* flanked by *loxP* sites. The constitutive expression of Cry1Ac protein was found to be more effective for conferring resistant activity against *H. armigera* larvae in comparison to green tissue specific expression. Independent transgenic events expressing *cre recombinase* gene along with linked *bar* selection marker were also developed. Constitutively expressing Cry1Ac T_1 events were crossed with Cre recombinase expressing T_1 events. Crossing based Cre/*lox* mediated marker elimination developed *nptII* free Cry1Ac expressing T_2 events, which were allowed to self-fertilize. In T_3 generation, 6 Cry1Ac expressing transgenic pigeonpea events were devoid of *nptII* marker as well as *cre-bar* genes. *H. armigera* larval mortality was found to be 80-100 % on those marker free T_3 events. Development of such marker free Cry1Ac expressing pigeonpea transgenics would greatly support the sustainable transgenic development program for pod borer resistance in pigeonpea.

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