*Asian J. of Bio Sci. (2006) Vol. **1** No. 2 : 140-143*

PCR amplification and cloning of proteinase inhibitor gene in cotton (*Gossypium species*)

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(Accepted : June, 2006)

The main objective of the work was to see presence of the three different Proteinase inhibitor genes of soybean Viz. Kti3, CI-II and PI-IV genes in cotton by synthesizing specific primer from already published data. DNA was extracted from different cotton spp. and was used for PCR amplification by the different specific primers for the Kti3, CI-II and PI-IV genes. Results have shown expected fragments of 651 bp for Kti3 gene and 250 bp for CI-II and PI-IV genes. These PCR fragments were were electroeluted from the Agarose gels and then used for cloning. These genes were then cloned in plasmid vector. Selection of transformed colony was done on the basis of Blue-White screnning. Plasmid was isolated and results were reconfirmed by doing PCR amplification of the Kti3, CI-II and PI-IV genes using specific primers. Results showed that the expected fragments were successfully cloned in the plasmid

Key words : Proteinase Inhibitor, Cotton, Protinase Inhibitor, Polymarase Chain Raction, Kunitz Trypsin Inhibitor, DNA isolation

INTRODUCTION

BOTANICALLY, cotton belongs to the family Malvaceae and the genus *Gossypium*. This genus comprises of about 50 species among which 4 are commercial cultivated. They are *Gossypium arboreum, G. herbaceum, G. hirsutum and G. barbadense*In India alone on an average 6-8 insecticidal sprays are applied in cotton crop. More than 10% of the world's pesticides and nearly 25% of the world's insecticides are used in cotton farming (Khadi, 2003). In general, transgenic plants develop using proteinase inhibitors digestive enzymes with a view to control crop pest and designed not to kill the insect that feed, but to retard their development. Presumably, this is the fundamental difference between this strategy and chemical pest control and use of Bt toxins are aimed at complete control through pest mortality. PIs are primary gene products and they are excellent candidates for engineering pest resistant into plants. Inhibitor genes of plant origin are particularly promising. Gatehouse *et al.* (1985) reported that insects belonging to both Lepidoptera and Coleoptera can over express existing gut proteinases or induce the production of new types that are insensitive to the introduced PIs to overcome the deleterious effect of PIs ingestion .The first were transgenic plants using cowpea trypsin inhibitor cDNA clone. Hilder *et al*. (1987). The different PIs that have been used for development of insect resistant transgenic plants are cowpea serine PIs (CpTI), potato serine inhibitors (PI-I and PI-I), sweet potato inhibitors, rice cysteine PIs, soybean Kunitz trypsin inhibitors, corn cysteine PIs, mustard trypsin inhibitor (TI), tobacco PI and bean a- amylase inhibitors. Strong inhibitory activity against insect gut proteases of *Sitioplilus zeamais* and a wide inhibitory spectrum against various cysteine proteinases of insects Irie *et al*. (1989). A cDNA clone of multidomain PI from *Nicotiana spp* was transfer into tobacco and peas under the control promoter from ribulose 1,5-biphosphate carboxylase gene . Transformed tobacco plants with gene coding tomato and potato inhibitor proteins and the transgenic plants found resistant to *Manduca sexta* Johnson *et al.* (1989). Field trials carried out in the US showed that the expression of CpTI on tobacco provided significant protection in field against *Helicoverpa zae* (Hoffman *et al.* (1992). The gut digestive enzymes are not the only targets effected by PIs, they can also effect the water balance, molting and enzyme regulation of insects Boulter (1993). Mcmanus *et al*. (1994) transferred the chymotrypsin inhibitor gene from potato to tobacco and these plants were also resistant to *Chrysodeixis eriosoma*. Shade *et al.* (1994) reported that the transgenic pea expressing bean á-amylase inhibitor from bean (*Phaseolus vulgaris*) confers resistance to the bruchid beetle, *Callosobruchis maculates* and *C. chinensis*. Shroeder *et al*. (1995) reported that bean alfa-amylase inhibitor-expressing pea was also resistant to *Bruchus pisorum.* The expression of bean á-amylase inhibitor in azuki bean conferred resistance to three species of bruchids (Ishimoto *et al*. 1996). However the bi-functional á-amylase / proteinase inhibitor genes are more useful for developing insect- resistant transgenic plants.

Duan *et al*. (1996) introduced PI-II gene from potato in several Japonica rice verity and this transgenic plants were found to insect resistant in green house trials. Wound inducible PI-II promoter with the first intron of rice actin I gene was able to give high-level expression of PI-II gene in transgenic rice plant. These transgenic plants expressing PI gene were found resistant to pink stem borer (*Sesamia inferens*). Thus, introduction of plant derived PI gene in to serial plant was successful for control of insect pests. Xu *et al.* (1996) reported the constitutive expression CpTI genes in transgenic rice plants, which confined resistant to two species of stem borers. Expression of CpTI gene, driven by the constitutive active promoter of the rice actin - I gene resulted in high-level accumulation of CpTI protein in transgenic rice plants. The trypsin inhibitor in transgenic plants was biologically active and the plants showed increased resistance against two species of stem borers. Sane *et al.* (1997) isolated cowpea trypsin inhibitor gene and cloned the fragment in a plant expression vector coupled with CaMV 35S promoter and NOS terminator and used for tobacco transformation. The efficiency of transgenic tobacco plants express CpTI against *Spodoptera litura* in feeding trials under laboratory conditions and found reductions to extent of 50% in the biomass of *S. litura* larvae fed on transgenic leaving expressing 3-5 µg CpTI /g fresh leaves. Yeh *et al.* (1997) reported the transgenic tobacco plants expressing the sweet potato TI and conferred resistance against *S. litura*.Transfer of sweet potato gene to cauliflower. cDNA gene encoding a cysteine PI isolated from rice was introduced into tobacco, potato, poplar, oilseed-rape but only results reporting the toxicity of such plants against a beetle feeding on poplar have been published. PI genes from different sources have been transferred to rice and insect resistance has been tested in detail. Schuler *et al.* (1998) reported that although a number of transgenic crop plants have been developed to determined the

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