



ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2018; 7(8): 551-552
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www.thepharmajournal.com
Received: 19-06-2018
Accepted: 23-07-2018

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Transgenic *Bt* chickpea: India's most required GM pulse crop

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Abstract

Chickpea is one of the ten economically important grain legumes which represents valuable source of protein. There are many factors responsible for low yield, among which insect pests appear to be the most vital. Chickpea is attacked by more than 36 species of insect pests in India. Among these pests, the pod borer *Helicoverpa armigera* is the most serious one. Crop management practices such as application of bio-pesticides, insecticides and integrated pest management are less effective to control this devastating pest. Breeding for development of resistant lines is restricted by lack of resistant sources within the gene pool. Therefore, application of gene technology for chickpea improvement appears to be appropriate approach for development of *Helicoverpa* resistant lines. Genetic transformation of chickpea using various versions of *Bacillus thuringiensis* (*Bt*) insecticidal genes have been carried out and found to confer resistance to pod borers in the laboratory bioassays. The most preferred genetically modified (GM) chickpea for field release is pyramided lines having two or more *Bt* genes with diverse mode of action for effective management of *Helicoverpa*. Here we discuss about the rationale for generation of *Bt* chickpea to enhance production.

Keywords: transgenic, India's, GM, pulse crop, chickpea

Introduction

Chickpea (*Cicer arietinum* L.) is the second most important and major food legume crop after pigeonpea cultivated worldwide in over 40 countries. It is grown widely in India because the seeds are rich source of protein for human consumption and feed for livestock. Besides being of high dietary protein value providing 33% of nitrogen requirement, it also helps in soil fertility management through nodular nitrogen fixation. Nutritionally chickpea is relatively free of various anti-nutritional factors, while being richer in high protein, phosphorus and calcium than other pulses and caloric source of feed for animals. Chickpea is of two types Desi and Kabuli. Desi is small in size, dark in colour with rough seed coat, while Kabuli is large in size, light in colour with smooth seed coat. India, Pakistan, Turkey, Iran, Myanmar, Africa and Mexico are major chickpea growing countries. The major chickpea growing states in India are Madhya Pradesh, Uttar Pradesh, Maharashtra Rajasthan, Andhra Pradesh, Karnataka and Gujarat.

Why *Bt* required in chickpea

Bacillus thuringiensis, commonly known as *Bt*, is a naturally occurring, gram-positive, spore-forming soil bacterium. *Bt* has been known to be reservoir of several insecticidal proteins, such as δ -endotoxins, cytolytic proteins, vegetative insecticidal proteins, etc. Among these, δ -endotoxins have been more efficiently utilized for protection of a variety of crops from various insect-pests. Crystal (cry) proteins derived from the soil bacterium *Bacillus thuringiensis* (*Bt*) play an important role in controlling infestation of *Helicoverpa armigera*, which has been considered a serious problem in chickpea productivity. The genome of the *B. thuringiensis* constitutes genes that encode several insecticidal protein that accumulate during sporulation are known as crystalline inclusion bodies (Cry proteins) and produced during vegetative growth are known as vegetative insecticidal proteins (Vips). Demand and the global yield of chickpea is reduce and slow in progress for last two decades primarily due to large number of biotic and abiotic stresses and slow progress of genetic improvements for yield parameters. *Helicoverpa armigera* is a cosmopolitan pest having high mobility, high reproductive rate, short generation time and high polyphagy. Chickpea is infected by nearly 60 insect species, of which the major damage is caused by pod borer the insect mainly attacks or infests the young pods and foliages of chickpea causing 20–30 per cent yield losses in India.

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Development of improved insect pest-resistant varieties of chickpea by conventional breeding is difficult due to its narrow genetic base, limited genetic diversity for this trait, barriers for sexual incompatibility and high degree of autogamy. Development of improved insect pest-resistant varieties of chickpea by conventional breeding is difficult due to its narrow genetic base, limited genetic diversity for this trait, barriers for sexual incompatibility and high degree of autogamy. Hence, limited success of conventional breeding, hazardous chemical means development of resistance in pest have directed for a better, potential choice of insect resistance by incorporating cry gene derived from *Bacillus thuringiensis* into chickpea.

Bt Gene Introduce In Chickpea

Fonseca and Co-workers (1993) [3] have reported success on transgenic plant regeneration in of chickpea through *Agrobacterium* mediated transformation. The first successful genetic transformation of nuclear genome of chickpea was reported in 1997 using the *cryIac* gene. Transgenic chickpea stacked with *Bt* genes such as *CryIA* along with *Vip3A* or hybrid *Bt* protein in combination with *Vip3A*, could be a suitable combination for Indian Agriculture. The first successful genetic transformation of nuclear genome of chickpea was reported in 1997 using the *CryIac* gene. A second gene, *Cry2Aa*, was also introduced in chickpea to facilitate pyramiding with existing *CryIac* lines generated pyramided *CryIac* and *CryIab* gene chickpea. Latest study carried out at jorhat on transgenic chickpea that the expression of *Cry* proteins derived from the soil bacterium *Bacillus thuringiensis* (*Bt*) have proved to be an effective method in controlling pod borer (*Helicoverpa armigera*) infestation in crops such as chickpea. Researchers at Assam Agricultural University in collaboration with Commonwealth Scientific and Industrial Research Organisation (CSIRO), Australia have developed marker free transgenic chickpea plants expressing *Bt* genes (*Cry2Aa*) which provide resistance to pod borer infestation. Sanyal *et al.* (2005) carried out quantitative assessment of expression for *CryIac* protein in different T₀ chickpea plants monitored in cell-free extracts in ELISA assay using *CryIac* specific polyclonal antibodies. B8 genotype having highest δ -endotoxin and C1 having lowest. Indurker *et al.* (2007) [5] carried out genetic transformation of chickpea with insecticidal crystal protein gene in chickpea using particle gun bombardment. Among different explants, epicotyle has more transformation frequency and embryonic axis having less frequency.

Anwar *et al.* (2009) carried out research on chickpea regeneration and reported that chickpea improvement and application of genomics tools to study the chickpea genome will be enhanced through the use of genetic transformation.

Mehrotra *et al.* (2011) [8] carried out research on expression of *Bt*-toxin in T₀ and T₁ chickpea transgenic plants and toxicity to *H. armigera* larvae and mortality by using various vectors. Among *thn-Bt*) by ELISA in leaf twig and on pod. T₂/1/28 having higher expression of *bt* toxin in leaf twig and T₁/9 having higher expression of *bt* toxin in pod.

Khatodia *et al.* (2014) [7] introduced *cryIac* gene using tissue culture in *desi* type chickpea cvs. C-235 and HC-1 by using microbial vector *Agrobacterium tumefaciens*. TH-141 having higher expression of *Bt* toxin in cv. HC-1 and TC-81 having higher expression of *Bt* toxin in cv. C-235.

Methods for development of Bt chickpea plant

1. Microbial Vectors
2. Particle Bombardment
3. Electroporation
4. Micro-Injection Method

Conclusion

Having great loss of yield due to insect-pest into chickpea therefore requirement to develop transgenic plant. The conventional methods of protecting chickpea for insect pest are inadequate to meet the challenges of the present agricultural scenario in India. The limitation of conventional technologies are lack of resistant germplasm, enhanced susceptibility of high yielding varieties to pests, barriers to cross cultivated varieties with wild relatives to acquire resistant genes. In order to protect the chickpea yield from losses due to pest infestation resistant gene transfer across the sexual barriers through recombinant DNA technology is mostly preferred. However, selection of suitable gene or combination of genes for genetic modification of chickpea may be found critical to protect chickpea from *H. armigera* damage.

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