DOI: 10.55278/CSVG8009

Molecular approach towards resistance to chickpea pod borer : *Helicoverpa armigera*

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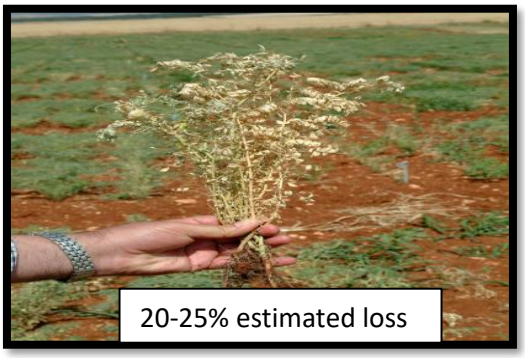
Chickpea (*Cicer arietinum* L.) is a rabi season legume crop and third most extensively cultivated pulse crop globally. *Helicoverpa armigera* is considered as one of the most devastating key pest severely affecting chickpea, due to its high rate of reproduction with short life span (Kumar and Singh, 2014). It is a vigorously feeding, polyphagous pest and is also called as corn ear worm, American cotton boll worm, tobacco bud worm, fruit borer of tomato, carnation worm *etc*. The prime reason for the lower yield of chickpea is infestation by pod borer (*Helicoverpa armigera*) from the vegetative phase to pod formation period (Dhingra *et al*., 2003). Single larva of the *Helicoverpa* damages 30-40 pods prior to its maturity. Yearly pod damage caused by *Helicoverpa* alone is about 150 to 200 million tonnes. With the predictable yield loss only due to gram pod borer is 10-90 per cent.



Even though efforts have been made towards developing resistant genotypes through conventional method, especially conferring pod borer resistance it has not been successful up to requisite level due to the lack of genetic resource conferring resistance to chickpea pod borer. This is the similar case in cotton boll worm also where successfully genetic engineering was used to transfer genes from bacteria, *Bacillus thurengiensis* known to produce proteins toxic to *Helicoverpa armigera*. Now, in the world more than 95 per cent of the commercial cotton growing area is covered with genetically modified cotton, called as *Bt* cotton, resistant to boll worms. Similar efforts have been made in chickpea at Assam Agricultural University, Jorhat.

Although the potential productivity of chickpea has been scientifically or experimentally proved to be 20 – 22 quintals

per hectare, farmers are harvesting only 5 – 10 quintals per hectare. This low and variable productivity level of chickpea in farmers' field is due to loss of crop because of incidence of pod borer and *Fusarium* wilt disease. If these two biotic stresses are not managed, then the extent of loss will be 80 – 90 per cent. To decrease the cost of cultivation and reducing environmental pollution by limiting the application of pesticides and fungicides to manage these two biotic stress problems, developing genotypes tolerant or resistant to pod borer (*Helioverpa armigera*) and *Fusarium* wilt (*Fusarium oxysporum* f. sp. *ciceri*) is the best alternate option.

Thus it was planned to introgress pod borer and *Fusarium* wilt resistance in Chickpea by involving *Bt* event of Chickpea (Acharjee *et al.,* 2010) and wilt resistant Super Annigeri-1 genotypes by using Simple Sequence Repeats (SSR markers for screening specific to SA-1).

An overview of experimental site

Hybridization experiment was conducted at transgenic green house during rabi season, when donor parents (Bt events having Cry genes resistance to pod borer pest) and recipient parent (Super Annigeri-1) were sown. Crossing for artificial hybridization was carried out using recipient parent as female parent. F_1 seeds obtained from these crosses were harvested. The harvested F_1 seeds were sown in pots during late kharif season then checked for the presence of Cry genes in the hybrids.

The genomic DNA was extracted from young leaves of 20 days old seedlings of both parents and hybrids using CTAB method and quality of DNA for each sample was assessed on agarose gel. DNA amplification was carried out using pair of 177 SSR primers developed by Nayak *et al*. (2010) in polymerase chain reaction (PCR). Whereas, *Cry* genes specific

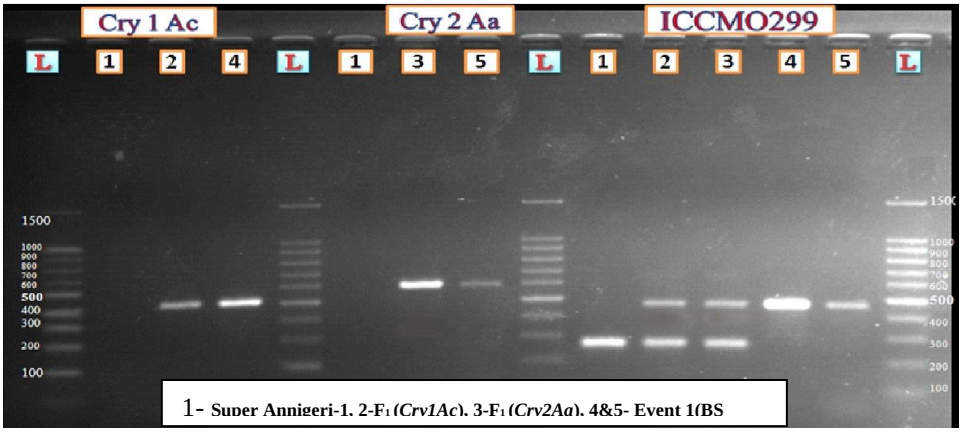
markers namely *Cry1Ac* and *Cry2Aa* were also involved in the study in order to confirm the presence of cry genes in hybrids. The hybridity of F1 plants was confirmed when they showed presence of both male and female parent alleles with the help of parental polymorphic markers along with cry gene specific markers.

Donor parents (1 and 2) having cry genes resistance to pod borer pest, recipient parent Super annigeri-1

F_1 hybrid plant crossed between Super annigeri-1 × Bt event BS 100B (having cry gene resistance to pod borer)

The presence of both the parental alleles in hybrid plants confirmed their origin from the two parents used in the present study, as well as genuineness of hybrid plants. Plants were confirmed as true hybrids on the basis of amplification pattern of SSR marker. Results of the present study are in agreement with the conclusions of Hipi *et al.,* (2013) in maize. Sharma *et al*. (2018) confirmed the hybridity of Indian mustard (*Brassica juncea* L.) using SSR markers. Out of 20 random SSR primers used for the screening of parental polymorphism, 5 primers were found polymorphic. BR_A04_9627743 and

BR_A01_13393871 were identified as the specific markers for parents RSPR-01 and Donskaja-IV which enable to distinguish and identify hybrid form their parental lines.



Confirmation of true F_1 using gene specific primers (cry genes)

F_1 hybrid was confirmed with both donor gene and recipient gene through one of the parental polymorphic marker ICCM0299

This study showed that SSR markers are more reliable and robust for assessing genetic purity as compared to morphological marker. The results of study are expected to be useful in the verification of genetic purity of hybrid seeds in chickpea accurately. Identified polymorphic markers between parents are good source for recovering recurrent parent genome in early backcross generations.

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MS Received 15 April 2022
MS Accepted 30 April 2022