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Smitha S and I. S. Katageri

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Inheritance Study of *cry1Ac* Conferring Pod Borer Resistance in Chickpea (*Cicer arietinum* L.)

Smitha S*[1]. and I. S. Katageri[2]

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A B S T R A C T

The use of genetically modified (*Bt*) crops expressing lepidopteron-specific Cry proteins derived from the soil bacterium *Bacillus thuringiensis* is an effective method to control the polyphagous pest *Helicoverpa armigera* (Hubner). As *H. armigera* potentially develops resistance to Cry proteins, *Bt* crops should be regarded as a tool in integrated pest management. The F_2 population derived from the cross Super Annigeri-1 and *cry1Ac* event (BS 100B) were subjected to chi-square test for inheritance study of *cry1Ac* gene which was found significant to accept 3:1 Mendelian ratio. Hence, the observed ratios are in good fit with the expected Mendelian ratio of 3 *cry1Ac* positive: 1*cry1Ac* negative. These results confirmed that Event-1, BS 100B is having a single copy of *cry1Ac* gene and significant chi-square test for 3: 1 ratio for *cry1Ac* gene in F_2 indicates the presence of single copy of a gene which will be useful in transferring to Super Annigeri-1, a wilt resistance gene through backcross breeding easily.

Key words: *cry1Ac,* Inheritance study, *Helicoverpa armigera, Bacillus thurengiensis,* Chickpea

Chickpea (*Cicer arietinum* L.) is the chief *rabi* season grain legume crop. It is the third most essential pulse crop globally and extensively cultivated in several subtropical and warm-temperate areas. It is self- pollinated and diploid (2n = 2x = 16) with a genome size of 740Mb. Chickpea is the most primitively cultivated legume crop. It is an annual plant, much branched herbaceous plant rarely exceeding height of 60cm and can ends its life span in 90 to 180 days duration depending upon existing agro-meteorological environment [1]. Papilionaceous flowers having five sepals, five petals and solitary in nature present in axils of leaves. Globally, chickpea is grown on 14.56 million hectares area producing 14.77 million tones with an average yield of 9560 kg per hectare [2]. India is the largest producer of Chickpea accounting for about 72 per cent around the world chickpea production. Although the potential productivity of Chickpea has been scientifically or experimentally proved to be 20–22 quintals per hectare, farmers are harvesting with a productivity of 5–10 quintals per hectare. This low and variable productivity level of Chickpea in farmers field is due to loss of crop due to incidence of pod borer and fusarium wilt disease. Chickpea legume is spoiled by a pod borer (*Helicoverpa armigera)* and it is a vigorously feeding, polyphagous pest and also called as corn ear worm [3]. The pest attack begins right from middle of vegetative phase and continues further up to maturity stage of the crop. The foremost reason for the lower yield of chickpea is the spoil caused by *Helicoverpa armigera* from vegetative to podding stage [4]. Independently managed the extent of loss is almost 10-90 per cent, globally. To decrease the cost of cultivation and reducing the environmental loss by limiting the application of pesticides and fungicides to manage this problem, is only possible by developing genotypes resistant to pod borer (*Helioverpa armigera*).

Even though hard works have been made to develop resistant varieties through conventional method especially for pod borer resistance, it was not successful to the request level due to the lack of genetic resource for pod borer resistance in Chickpea. This is the similar case in Cotton boll worm also, successfully genetic engineering was used to transfer genes from bacteria, *Bacillus thurengensis* known to produce proteins toxic to *Helicoverpa armigera*. Now commercially in the world more than 95 per cent of the Cotton growing area is covered with genetically modified cotton, called as *Bt* cottons, resistant to boll worms similar efforts have been made in Chickpea at Assam Agricultural University, Jorhat and developed *Bt* Chickpea events [5]. Thus, it was planned to pyramid *cry1Ac* gene into Super Annigeri-1, wilt resistant cultivar developed by University of Agricultural Sciences, Raichur through Marker-assisted backcross breeding [6] as a recipient parent in the study. The objective of the present study was to investigate the inheritance pattern of *cry1Ac* gene conferring pod borer resistance in chickpea through *cry1Ac* gene specific molecular marker.

* **Smitha S.**
✉ smithaswathickm@gmail.com

[1] Department of Biotechnology, College of Agriculture, Vijayapura - 586 102, Karnataka, India

[2] Professor of Genetics and Plant Breeding, Editor, Publication center, University of Agricultural Sciences, Dharwad - 580 005, Karnataka, India

MATERIALS AND METHODS

Plant materials

Bt event *cry1Ac* (BS 100B) used as a donor parent conferring pod borer resistance developed by Assam Agricultural University, Jorhath [7] and Super Annigeri-1, a wilt resistant and a high yielding cultivar recently developed by University of Agricultural Sciences, Raichur [8] with the aid of molecular marker.

DNA extraction and polymerase chain reaction

The genomic total DNA was extracted from young leaf tissues of 20-days old seedlings of both parents and hybrids using CTAB method [9] and quality of DNA for each sample was assessed on 0.8% of agarose gel and then stored at 4ºC for further use. DNA amplification was carried out using pair of *cry1Ac* gene specific marker was used in polymerase chain reaction (PCR) tubes containing 20 µL reaction mixture. and also involved in the study in order to confirm the presence of cry gene in hybrid. The reaction mixture contained 1 µl of template DNA, 2µl of 2.5 mM, 1 µl of each forward and reverse primers, 5 U of Taq polymerase, 2µL of 10X PCR buffer with $MgCl_2$. Amplification cycle comprised of initial denaturation for 5 min at 94ºC; 30 cycles of 94ºC for 1min, annealing depending on primers used for 1 min and extension at 72ºC for 2 min.; followed by a final extension at 72ºC for 7 min. in Master Cycler Gradient. The products of amplification were stored at 4ºC and resolved by electrophoresis in horizontal agarose gel system at 65 V for 2 hour on 3% agarose gel stained with ethidium bromide (10mg/ml) using 1X TAE buffer. The amplified products were visualized under gel documentation system and the size of amplicons was estimated with the help of 100bp ladder. Genetic polymorphism of SSR markers used in study was recorded on the basis of relative size of bands and hybridity confirmation was done by using 100bp ladder [10].

The harvested F_1 seeds were sown in pots during late *kharif* of 2018, checked for both pod borer resistance (presence of *cry* genes) and *Fusarium* wilt resistance in the hybrid, F_2 selfed seeds were also harvested, those F_2 seeds were sown during late February, 2019 to test the inheritance pattern for *cry1Ac* gene in F_2 population.

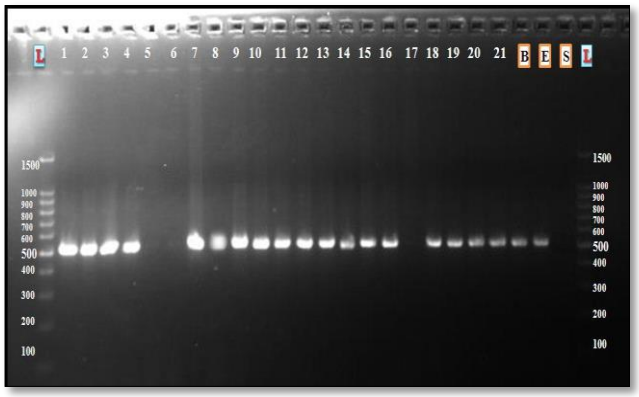
F_2 seeds obtained from F_1 hybrid derived from the cross Super Annigeri-1 × BS 100B were only sown during February, 2019 to raise F_2 population. In this population, segregants for *cry1Ac* gene was recorded and subjected to chi-square test.

DNA was isolated from each F_2 plants with parents and subjected to PCR to amplify *cry1Ac* gene with marker specific to that gene. Amplicons from each plant was recorded and used to analyze segregation pattern. Amplicon presence or absence was noted. Presence of amplicon indicated the positive plants (presence of *cry1Ac* gene) and absence of amplicon indicates negative plants (absence of *cry1Ac* gene).

$$\chi^2 = \Sigma \left[\frac{\text{Observed} - \text{Expected}}{\text{Expected}}\right]^2$$

RESULTS AND DISCUSSION

39 plants of Super Annigeri-1 × BS 100B were raised in February 2019, to study the *cry1Ac* gene inheritance. Based on the PCR amplification of *cry1Ac* (Plate 1) genes F_2 plants were classified in to two classes as *cry1Ac* positive and *cry1Ac* negative plants. Out of 39 plants, 30 *cry1Ac* positive (amplified at 533 bp and 615 bp) and 9 *cry1Ac* negative plants were grouped into these two classes. Results were fit with the expected Mendelian ratio of 3 *cry1Ac* positive: 1 *cry1Ac* negative and calculated χ^2 value, 0.07693 was less than the table χ^2 value (3.841) at 5% level of significance (Table 1).



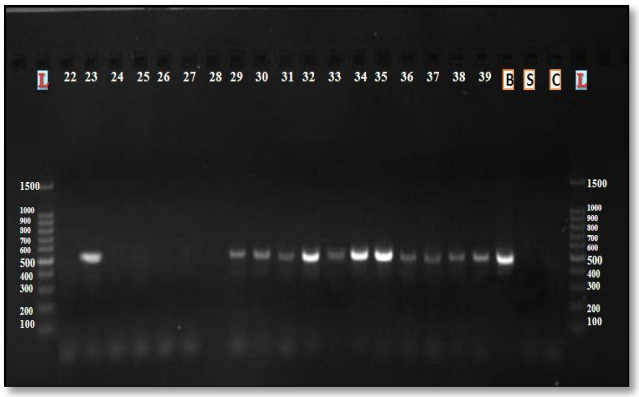


Table 1 χ^2 tests for *cry1Ac* gene inheritance in Super Annigeri-1 × BS 100B cross

cry1Ac gene	Observed (O)	Expected (E)	(O-E)	$(O-E)^2$	$\chi^2 = (O-D)^2/E$
Positive	30	29.25	0.75	0.5625	0.019231
Negative	9	9.75	-0.75	0.5625	0.057692
Total	39	39		$\chi^2 = 0.05$, p = 3.841	0.076923

Segregation for *cry1Ac* gene among the F_2 segregants of the cross Super Annigeri-1 × BS 100B (*cry1Ac* event) on individual plant basis where data were recorded and analyzed with the help of chi-square test which is commonly used to test hypothesis concerning the frequency distribution to fix 3: 1 ratio hypothesis in order to see the *cry1Ac* gene inheritance in chickpea. The calculated chi-square value of F_2 segregants is 0.0769 which is less compared to table chi-square value 3.841 at 5% level of significance. Hence, the observed ratios are in good fit with the expected Mendelian ratio of 3 *cry1Ac* positive: 1*cry1Ac* negative. These results confirmed that Event-1, BS 100B is having a single copy of *cry1Ac* gene.

CONCLUSION

The wide studies on the stable inheritance of foreign genes in various transgenic plants have been noted earlier. *cry1Ac* gene inheritance in backcross lines of crosses (Mara and EE-1) and (DLP and EE-1) and found significant Mendelian phenotypical ratios of 1: 1 and 3: 1 in F_1 and F_2 plants

respectively, for a dominant single gene by demonstrating cry1Ac gene can stably inherit by means of back-crossing. Stable integration through Significant segregation of pyramided *cry1Ac* and *cry2A* genes in *Gossypium hirsutum* (cotton) showed 3: 1 Mendelian inheritance pattern in T_1 generation for resistance of *Heliothis.* Whereas, both significant Mendelian as well as distortion segregation ratios in selfed and crossed F_2 plants, respectively in *cry1Ab* gene segregation between Japonica intra-subspecies and inter-subspecies and concluded that gene is stably inherited in intact through sexual generations.

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