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Research Note

Field evaluation of UASD Bt-cotton Event-78 based early segregating generations for cotton leaf hopper

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Abstract
UASD Bt cotton Event-78 based early segregating generations (F_2 & F_3) derived from three diverse crosses were evaluated for field incidence of cotton leaf hopper during *kharif*-2021 & 2022 at Agricultural Research Station, UAS, Dharwad. A total 600 F_2 plants from the crosses *viz.,* UASD Bt-78 x DHS-29, UASDBt-78 x Suvin and Suvin x UASD Bt-78 were screened of which 103,100 and 102 plants respective to the three crosses were found to be resistant. The segregation of F_2 population for the jassid tolerance deciphered the implicit "Inhibitory epistasis" mechanism of host plant resistance. Further evaluation of F_3 families of these crosses leads to identification of 103 resistant lines from all the three crosses, the mean leafhopper population ranges from 0.67- 2.67 leafhoppers / 3 leaves with the LHRI score of 1.

Keywords: Segregating generations, Cotton leaf hopper, Inhibitory epistasis, Host plant Resistance

Cotton (*Gossypium spp.*) also known as the "White Gold" is a major cash crop and a crucial part of the Indian economy as the country's textile industry is predominantly cotton-based. The textile industry represents an important component of the country's industrial production and is one of the largest sources of employment. India is the only nation that grows all the four domesticated species of cotton *viz., Gossypium arborerum, G. herbaceum, G. hirsutum,* and *G. barbadense* as well as their inter-specific and intra-specific hybrids.

World's first cotton hybrid (H-4) was developed by Dr. C. T. Patel from Gujarat Agricultural University in 1970 and it was later commercially grown in Gujarat and Maharashtra. This marked the beginning of the hybrid cotton era. Globally 31.36 million hectares of land is under cotton cultivation with a commercial production of 24.09 million tones and yielding 768 kg/ha. USA, China, India, Pakistan, Uzbekistan, Egypt, Argentina, Australia, Greece, Brazil are major cotton-growing countries in the world (Avinash *et al*., 2022). The entire cotton value chain in India has altered since *Bt*-cotton was introduced in 2022. India is the largest country in terms of area (12.56 mha) having around 40% of world's cotton area and second largest in the production (5.7 million tones), after China (6.7million tones) but the productivity is 487 kg/ha, which is quite low against world's cotton productivity (768 kg/ha)(COCPC2022–23). The major states of cotton cultivation include Maharashtra, Gujarat, Telangana, Rajasthan and Karnataka (Ranjit Kumar *et al*., 2019). Most of the cotton cultivating area is rain-fed exacerbating climate changes undermine the yield potential.

The cotton microclimate harbours several insect pests, approximately 1326 insect and mite pests worldwide (Hargreaves, 1948), and around 200 of them being reported in India (Anandhi *et al.,* 2019), 17 of which were designated as the major insect pests of the crop and are categorized into borers (such as bollworms - American bollworm, Pink bollworm, Spotted bollworm, Stem weevil, shoot weevil, etc.), defoliators (including Tobacco cutworms, semi loopers, ash weevil, hairy caterpillars, cotton grasshoppers, etc.), flower feeders (like blister beetles, flower weevils), and sucking pests (such as jassids, thrips, whiteflies, aphids, cotton mealybugs, Red cotton bugs, Dusky cotton bugs, etc.). Transgenic cotton competently effective in controlling the bollworm complex but the calumnious incidence of sucking pests in the recent years such as leaf hopper, whitefly, and mealybugs critically affect the cultivation.

The cotton leafhopper, (*A. biguttula biguttula*) is a polyphagous pest which attacks from seedling to end-season. However, vegetative stage of the crop is more sensitive to leafhopper infestation. It completes 12-15 generations in one year. Every female lays, on average, 20-25 eggs during the season, ideally on the leaf veins. Adults and nymphs suck the sap from leaves causing downward curling and yellowing of leaves.

Two jassids or nymphs per leaf is the economic threshold level of the pest at which chemical control is inevitable. Under severe infestation characteristic phytotoxic hopper burn symptoms could be observed resulting in complete desiccation of foliage in addition, the honeydew excreted by leafhoppers facilitates the growth of black mold, further impairing photosynthesis.(Madhu *et al.,* 2023). In many cotton-growing regions Economic Threshold Level (ETL) for leafhopper is arbitrarily taken for insecticide applications even under the diverse climatic condition, varieties, pest dynamics and population pressure (Chaudhari *et al*., 2018). On an average of 18.78 per cent yield loss was reported (Sarma *et al*., 2021; Santhoshi *et al*.,2022). Higher soluble nitrogen in the growing tissues, supported by high humidity due to rains and irrigations, triggers the pest outbreak. Often, insecticides are employed to control cotton leaf hoppers, which eventually result in pest resurgence due to increased resistance to pesticides. Therefore Host plant resistance is a crucial element of integrated pest management as it allows plants to withstand, avoid or recover from insect pest attacks (Painter, 1951).

The public sector *Bt*-cotton event of University of Dharwad, UASD Event No-78 has been developed with Cry-1Ac gene driven by double promoter. The gene construct was developed by International Centre for Genetic Engineering and Biotechnology New Delhi. The event is in the genetic background of RAH-100, a released variety belonging to *G. hirsutum* L., with good agronomic characters and resistance to leaf hopper (Prasad Rao *et al*., 2015). Molecular characterization for the selection of event-78 was accomplished by a series of molecular assays. Southern blot analyses indicated a single copy of T-DNA integration through transformation. The Cry-toxin expression in different plant parts of UASD *Bt*-78 was found to be significantly higher than the BG-II, providing effective control over boll worms throughout the cropping season (Manjunath Swamy, 2014; Manjula *et al*., 2022). The cultivar is also tolerant to sucking pests such as leaf hopper, white flies and aphids (Patil, 2019).

In the present investigation UASD *Bt*-78 based early segregating generations (F_2 and F_3) derived from three diverse crosses were screened for field incidence of leaf hopper under unprotected conditions. Cross-I consisted of an intra-hirsutum cross between the jassid-tolerant *Bt*-cultivar UASD Bt-78 and the jassid-susceptible, non-*Bt* genotype Dharwad hirsutum selection-29. Cross-II was an inter-specific cross between UASD *Bt*-78 and the jassid-susceptible extra-long staple *G. barbadense* L., Suvin. Cross-III consisted of the reciprocal cross of the inter-specific cross (Suvin x UASD *Bt*-78). Individual plant selection was carried out in the F_2 generation with the objective to obtain the *Bt*-homozygous, jassid resistant transgressive segregants, and to understand the inheritance pattern of host plant resistance against jassid. In the subsequent season selected resistant, *Bt*-transgressive segregants were again evaluated as progeny-rows to advance most productive, jassid resistant F_3 lines to develop potent *Bt*-cotton varieties.

The experimental material consisted of early segregating (F_2) generation developed from selfing of F_1s of crosses *viz.,* UASD*Bt*-78 × DHS-29, UASD*Bt*-78 × Suvin, Suvin × UASD*Bt*-78 (**Table 1**). The segregating generations (F_2 & F_3) were evaluated during the *kharif* -2021 & 2022 at Agricultural Research Station, Dharwad, Karnataka, India. All the recommended agronomic practices were followed with the exception of the plant protection measures.

Screening of F_2 plants: A total of 600 F_2 plants from each cross, along with leafhopper resistant (NLDH 1938) and susceptible checks (DCH-32), were assessed for field incidence of leaf hopper in fifteen blocks using augmented block design with a 90 cm row x 40 cm plant spacing. Each F_2 plant was graded at 30, 60, 90, and 120 DAS based on the symptoms of leaf hopper injury observed following the criteria provided by the Indian Central Cotton Committee (Sikka *et al*., 1966; Rao, 1973) (**Table 2**). The plants were then broadly categorised into resistant and susceptible plants.

Chi-square test for determination of genetic basis of jassid resistance in the host plant: Based on the segregation pattern of F_2 populations for jassid infestation, the genetic basis determining the resistant phenotype in the host plant confirmed by chi-square test (goodness of fit).

H_0 : The segregation pattern of population for jassid incidence fit into the ratio 13:3

H_1 : The segregation pattern does not fit the ratio(13 :3)

$$\chi 2 \ \ \textit{value} \text{ (Test statistic) } = \sum_{i=1}^{n} \frac{(O_i - E_i)^2}{E_i}$$

O_i is the observed frequency in the category i
E_i is the expected frequency in the category i
n is the number of categories

Critical values were determined at 5% level of significance at n-1 degrees of freedom. On comparing with critical value, if the test statistic value was less than the critical value, null hypothesis was accepted otherwise rejected.

Assessment of leaf hopper population and Hopper burn Injury in F_3 lines: In the subsequent season (*Kharif*-2022) 40 F_3 lines each from the interspecific cross (UASD *Bt* -78 x Suvin) and the reciprocal cross (Suvin x UASD *Bt*-78) and 103 F_3 lines of *intra-hirstum* cross (UASD *Bt*-78 x DHS-29), were screened for field incidence of leaf hopper along with resistant and susceptible checks. Leafhopper population density was recorded on five randomly selected plants in each of the F_3 lines at 30, 60, and 90 days after sowing. Three leaves were examined from the top, middle, and bottom of each plant, and the average population per three leaves was noted. Simultaneously each F_3 line was graded based on symptoms of hopper burn injury.

Based on the observations on symptoms of hopper burn injury on five tagged plants in each of the F_3 line, each line was graded at 30 DAS, 60 DAS, 90 DAS and 120 DAS. Leaf hopper Injury Index (LHRI) was calculated according to the formula given by Nageswara Rao (1973).

$$\text{LHRI} = \frac{G1 \times P1 + G2 \times P2 + G3 \times P3 + G4 \times P4}{P1 + P2 + P3 + P4}$$

Where,

G - Leafhopper Injury Grade of ICCC (Sikka *et al.,* 1966 and Rao, 1973),

P - The plant population under the grade for each category.

Based on leafhopper resistance index (LHRI), the lines were classified into highly resistant (1.0), moderately resistant(2.0), susceptible (3.0) and highly susceptible (4.0)

Of the total 600 F_2 plants, each from the crosses *viz.,* UASD *Bt*-78 x DHS-29, UASD*Bt*-78 x Suvin, and Suvin x UASD *Bt*-78, 103, 100, and 102 plants exhibited the resistant phenotype (grade I or II) comparable to the resistant check (NDLH-1938) with grade I, while 497, 500, and 498 plants showed susceptible reaction(grade III or IV) comparable to the susceptible check (DCH-32) with grade IV respectively.

Based on the segregation pattern of F_2 population, various epistatic interactions and their standard ratios were analyzed. The studied population data certainly fitted into the modified dihybrid ratio 13:3 (13 susceptible, and 3 resistant types), confirmed by the chi-square test for goodness of fit (**Table 3**). In all the three different segregating populations the calculated chi-square value was less than the critical value (3.84) at 5 % level of significance with one degree of freedom. The test revealed that there was no significant difference between the observed and expected ratio, and the deviations observed in the studied populations were due to the chance factor.

The obtained segregation ratio of 13:3 explains dominant-inhibitory gene action controlling jassid resistance in the host population. In this type of gene interaction, the first gene(A) in dominant condition produces the relevant phenotype, while its recessive allele (a) produces the contrasting phenotype. The second gene (B) in dominant condition inhibits the expression of the first gene. Consequently, the phenotype generated by two dominant genes coexist is identical to the phenotype produced by the recessive homozygote of the first gene. Thus susceptible phenotypes are governed by the genotypic configurations; A-B-(9), aaB-(3) and aabb (1) and resistant phenotype governed by the genotype A-bb(3). The obtained results are in accordance with the

Table 1. The experimental material used in the study

S.No	Plant type	Pedigree	Population Size(F_2)	F_3 lines
Cross-I	*Bt* x Non-*Bt*	UASD-78 x DHS-29	600	103
Cross-II	*Bt* x Non-*Bt*	UASD-78 x Suvin	600	40
Cross-III	Non-*Bt* x *Bt*	UASD-78 x Suvin	600	40

Table 2. Assessment of Leaf hopper severity (Sikka *et al.,* 1966 and Rao, 1973)

Grade	Symptoms	Degree of Susceptibility
I	Leaves will be normal/ little downward curling.	Resistant (R)
II	Crinkling, curling, slight yellowing in few leaves on lower portion of the plant	Moderately Resistant (MR)
III	Crinkling, curling, yellowing, browning and bronzing in the middle and lower portion.	Susceptible (S)
IV	Extreme crinkling, curling, yellowing, browning, bronzing and drying of leaves, defoliation and stunted growth.	Highly Susceptible (HS)

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Table 3. Segregation Pattern of UASD *Bt*-78 derived F_2 populations for leaf hopper under unprotected conditions.

F_2 plants of the cross		Reaction to leaf hopper		Total	χ2 value (Test statistic)	χ2 value (critical value)	Expected Ratio	P value
		Susceptible Grade(III+IV)	Resistant Grade(I+II)					
UASD *Bt*-78 x DHS-29	Observed	497	103	600	0.987	3.84	13:3	0.320
	Expected	488	112					
UASD *Bt*-78 x Suvin	Observed	500	100	600	1.709	3.84	13:3	0.191
	Expected	488	112					
Suvin x UASD *Bt*- 78	Observed	498	102	600	1.206	3.84	13:3	0.272
	Expected	488	112					

Level of significance(α) =0.05, Degrees of freedom = 1

Table 4. Field Evaluation of selected $F_{2:3}$ progeny rows for Leafhopper

Cross	F_3 lines	No. of lines	Average leafhopper/3 leaves	LHRI	Phenotype
UASD *Bt*-78x DHS-29 (103 lines)	3,7,8,10,13,14,17,19,21,23,24,25,26,28,30,31,32,34,35,36,37,38,39, 41,43,45,46,48,50,51,54,55,57,58,60,61,63,64,66,68,71,72,74,78,79 ,81,83,84,85,87,88,90,92,94,95,103	58	0.67-1.87	1	R
	1,2,4,5,6,9,11,12,15,16,17,20,22,27,29,33,40,42,44,47,49, 52,53,5 6,59,62,65,67,69,70,73,75,76,77,80,82,86,89,91,93,96,98,99,100,1 01,102	45	2.73-3.53	2	MR
	Total	103			
UASD *Bt*-78 x Suvin (40 lines)	1,2,4,5,6,7,10,11,13,14,15,17,19,20,22,23,24,25,26,28,32,33,38	23	0.67-2.53	1	R
	3,8,9,12,16,18, 21, 27,29 30,31,34.35,36,37,39,40	17	2.8-3.13	2	MR
	Total	40			
Suvin x UASD *Bt*-78 (40 lines)	2,3,4,5,7,,8,9,10,11,12,13,14,2122,25,27,28,31,33,36,39,40	22	0.73-2.67	1	R
	1,6,15,16,17,18,19,20,23,24,26,29,30,32,34,35,37,38	18	2.67-3.00	2	MR
	Total	40			
Resistant Check (NDLH-1938)			0.67	1	R
Susceptible Check (DCH-32)			5.27	4	S

R- Resistant, MR- Moderately resistant, LHRI- Leaf hopper Injury Index

earlier studies made by Mahal (1978), Radhika *et al.* (2004), Pushpam and Raveendran, (2005), Murugesan and Kavitha, (2010), Zhang *et al.* (2013), Venkatesha (2014), and Yaksha *et al*. (2022).The resistant (F_2) plants advanced to the next generation as progeny rows were evaluated for jassid infestation along with resistant and susceptible checks.

Of the total 103 F_3 (UASD *Bt*-78 x DHS-29) lines, 58 F_3 lines were resistant with mean of 0.67 - 1.87 leafhoppers / 3 leaves and leaf hopper injury grade (LHRI) of one and remaining 45 lines were moderately resistant with the mean population of 2.73-3.53/ 3 leaves, and injury grade of two. Out of the 40 productive, F_3 lines of the inter-specific cross (UASD *Bt*-78 x Suvin), 23 lines were true to type for jassid-resistance with mean population of 0.67-2.53/ 3 leaves, and LHRI grade one, while the remaining 17 lines were moderately resistant with the population density of 2.8-3.13/3 leaves. 22 F_3 lines of the cross, Suvin x UASD *Bt*-78 were resistant with the average jassid population of 0.73-2.67/ 3 leaves and leafhopper injury index one, while another 18 lines were moderately resistant with mean jassid population of 2.67-3/3 leaves and injury index two. All the resistant lines of the three crosses were on par with resistant check (NDLH-1938), which had mean of 0.67 leafhoppers/3 leaves with the injury index of one. The susceptible check had average 5.27 leafhoppers/3 leaves with the leaf hopper injury index four (**Table 4**). Similar results were demonstrated by other studies (Dhillon and Sharma, 2013; Manivannan *et al.*,2017; Patel and Radadia, 2018; Sasikumar and Rathika , 2020; Sivaram Krishna and Rama Reddy, 2020; Avinash *et al*., 2022; Gangavati and Maralappanavar, 2022 ; Senguttuvan *et al*.,2022).

In conclusion**,** the present study based on screening of segregating generations of cotton reveals the dominant-inhibitory epistasis mechanism governing the host plant resistance to leaf hopper. The resistant homozygous *Bt*-plants identified in the F_2 generation were evaluated as $F_{2:3}$ progeny rows for jassid resistance. Of the total 103 F_3 lines from cross-I, 40 F_3 lines each from cross-II, & cross - III evaluated, 58, 23 and 22 F_3 lines from the respective crosses were categorized as resistant lines with the average leafhopper population ranged from 0.67 to 1.87, 0.67 to 2.53, 0.73 to 2.67 leafhopper/3 leaves/plant and injury index of one respectively. *Bt*-cotton has become increasingly susceptible to sucking pests, particularly leafhoppers in recent times. Therefore, selection of novel plant-types in the early segregating generations that are both productive and resistant to the pest is crucial for developing productive, transgenic-insecticidal cultivars or to use as parent material for future breeding programs as a leafhopper resistant source. Pre-breeding programs using jassid resistant wild Gossypium species such as G.armourianum (Imtiyazahmed *et al.,* 2020) would extend the genetic base of high-yielding cultivars. Development and deployment of resistant cultivars is a cost-effective and ecologically sustainable method of managing insect pests.

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