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Identification of SSR Molecular Markers for Jassid Resistance in Cotton

Sridhar Venkatesulu[1,2], Satya Prasad Makula[1], Mahantesh Basetteppa Satihal[1], Satish Kumar Puligundla[1], Koigoora Srikanth[2]

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ABSTRACT

Background: This study has focussed on identifying the Simple Sequence Repeat (SSR) markers associated with the sucking pests (more precisely on jassid) tolerance/resistance loci. The study was initiated with 4348 mapped SSR markers to differentiate between resistant (R-1) and susceptible (BS-1) genotypes. Out of 4348 markers (retrieved from cotton microsatellite database and cottongen.org) used,17 markers were showing polymorphism between the parental lines.

Methods: The F_1 population was generated by crossing Resistant line (R-1) and the elite Susceptible line (Bs-1). F_1 progeny were selfed to obtain F_2 population. Following the screening, to identify the SSR markers associated with trait, Single Marker Analysis was performed.

Result: The results indicated that two of the seventeen polymorphic markers - BL1646 and DOW047 had significant LoD of 4.5 and 7.2 respectively and PVE% of 6.6 and 10.3 respectively. Hence, these were identified as the two markers associated with jassid resistance trait having the potential for their use in marker assisted selection (MAS).

Key words: Cotton crop, Jassid resistance, Phenotyping, SSR markers.

INTRODUCTION

Cotton is one of the important fibre crops in the world. This crop is also termed as 'White gold'. There are about 50 species alone in the genus *Gossypium* and of which only 4 are commercially used (Sahu and Samal 2020). The sucking pest could destroy the products of agriculture at various stages that cause a total loss of 30 to 40%. Therefore, insect pests are the predominant factors for reduction in the quantity and quality of yield (Sahu and Samal 2020). In general, cotton attracts nearly 1326 insect species worldwide causing severe damage to the plant from the sowing stage to the maturity stage (Blaise and Kranthi, 2019). The damage is caused due to both chewing and sucking-type pests.

During the early stages of cotton crop, mostly sucking pests, *e.g.*, whitefly (*Bemisia tabaci*), thrips (*Thrip stabaci*), Jassids (*Amrascabiguttula*) and aphid (*Aphis gossypii*) are the main perpetrators of damage. The most common way of controlling the pests by the farmers is by use of insecticidal sprays that are quick in action (Soomro *et al*., 2000). Indiscriminate use of such chemicals poses serious harm to the human health and adds resistance to these insects against these insecticides. In addition to this, the environment is also polluted by them (Palumbo *et al*., 2001). These chemicals are non-degradable and quite hazardous to non-target organisms or predators also (Da Silva *et al*., 2012).

The present study utilized a wild relative of cotton, *i.e*., 7076 and 7082 followed by crossing of these lines with BS-1 line (susceptible to sucking pest). Near Isogenic Lines (NILs) were derived by crossing BS-1 with 7076 and 7082 with respect to sucking pest resistant trait. An alternative approach to transgenic plants is to search for wild resistant genotypes and then make cross for transferring desirable traits/loci. Selective breeding approaches like, using wild relatives and do wide hybridization to develop Recombinant cotton Inbred Lines (RILs) and utilize to get NILs against the commercially important traits. With the advent of the molecular marker technology, marker assisted selection has become one of the potential candidate approaches for the improvement of cotton cultivar through the exploitation of genetic diversity of cotton genotypes (Mishra and Fougat, 2013). Quantitative Trait Locus (QTLs) linked to sucking pesttrait can be identified using molecular markers.

[1]Division of Biotechnology, Nuziveedu Seeds Ltd., Gundlapochampally, Ranga Reddy, Hyderabad-501 401, Telangana, India.
[2]Department of Biotechnology, Vignan's Foundation for Science Technology and Research, Deemed to be University, Vadlamudi-522 213, Guntur, Andhra Pradesh, India.

Corresponding Author: Satish Kumar Puligundla; Koigoora Srikanth, Division of Biotechnology, Nuziveedu Seeds Ltd., Gundlapochampally, Ranga Reddy, Hyderabad-501 401, Telangana, India; Department of Biotechnology, Vignan's Foundation for Science Technology and Research, Deemed to be University, Vadlamudi-522 213, Guntur, Andhra Pradesh, India.
Email: puligun@yahoo.com; koigooras@gmail.com
Orcid id: 0000-0002-8547-4680

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Hence, the construction of molecular linkage map using DNA markers have been recognised as an essential tool for plant molecular breeding studies (Wu *et al*., 2009).

The objective of the study is to analyse the genetic variation between resistant and susceptible genotypes and to identify SSRs and possible candidate genes underlying 'sucking pest resistance' in cotton (Idrees and Irshad, 2014). The genic and EST-SSRs used for tagging sucking pest trait were taken from earlier consensus map (Blenda *et al*., 2013). Identification of these molecular markers linked to sucking pest trait will accelerate the selection and breeding for traits.

MATERIALS AND METHODS

Plant material

Plant type *Gossypium hirsutum* was used as parents. The F_1 generation is obtained from crossing between two lines of cotton R-1 (Resistance line) and BS-1(Susceptible line). The trait segregating population was generated by selfing F_1 plants, which was further screened for different phenotypic traits and genotypic variations. A total of 1478 F_2 plants have been obtained for screening. The resulted population were then screened for genotypic and phenotypic traits against sucking pests.

Phenotyping

The mapping population was screened against jassid tolerance. For phenotyping, 267 F_2 plants were selected. All these plants were grown in the field. The field is located at 17.59′ N, 78.49′ E and is about 577 meters from sea level with an average temperature of 32°C. The typical sucking pest (jassid) damage symptoms were considered for phenotyping the plants. These plants were scored according to the criteria described in Table 1. Each criterion describes the effect of pest on plant in different forms like crackling, curling and yellowing of leaves from minimum to maximum damage.

Mapping of population

F_2 mapping population was generated by selfing F_1 pants derived from a cross between R-1 vs. BS-1. A total of 1478 seeds were sown in the open field during kharif session. Phenotyping was carried out against jassid resistance. After successful evaluation, 267 plants were selected based on the jassid damage severity index. Eighty nine plants from each grade were selected for SSR genotyping.

Isolation of plant DNA and PCR

DNA was isolated from leaves of cotton seedlings by means of a cetyltrimethylammonium bromide (CTAB) procedure (Paterson *et al*. 1993). Each PCR reaction was carried out in a volume of 10 μL containing 1X PCR buffer, 1.5 mMMgCl_2, 0.20 mMdNTPs, 2.5 μM each of upstream and downstream primers and 0.5U Taq polymerase. PCR conditions were as follows: 94°C initial denaturation for 7 min; 94°C for 30 s, 55-58°C for 30 s and 72°C for 1 min for 34 cycles followed by an extension at 72°C for 5 min. PCR products were analysed by 2.5% agarose gel electrophoresis. The gels were EtBr-stained to visually differentiate bands among different samples. Primers used for PCR are shown in Table 2.

SSR marker analysis

The marker identification was carried out after screening of F_2 population. The DNA was extracted using CTAB method (Stefanova *et al*., 2013).

As the trait that we try to identify in this study was the sucking pest (jassid) resistance, the plants were screened for susceptible and resistance genotypes. To identify polymorphic SSR markers between resistance and susceptible lines, 4348 markers were used (Varshney *et al*., 2008). This gives an accurate and precise data of the polymorphism present in the particular trait present in a locus. Between the resistance and susceptible lines, a total of 29 polymorphic markers were obtained in the parental genotypes. From these 29 polymorphic markers, 17 polymorphic markers were used for further analysis of F_2 population scoring and categorizing. The F_2 generation is then scored using the 17 polymorphic SSR markers. The PCR amplified product is visualized on agarose gel for the number of bands specific for a given marker to score the genotypes as-resistant, susceptible or hetero (Supplementary Information, S1). This scoring was used to identify the genotypes of F_2 generation plants which eventually lead to sucking pest (jassid) resistance trait marker association.

All the current work was carried out in the year 2022 and the work was executed at Nuziveedu Seeds limited, Hyderabad, Telangana, India.

RESULTS AND DISCUSSION

Phenotypic data

The phenotyping of jassid injury has been observed in 1st BED plants (Fig 1). The plants were divided into several groups and have been plotted according to their arrangement in the field. The groups were 36 '7076 Donor Parents' arranged in 4 rows, 35 '7082 Donor Parents' arranged in 4 rows, 38 'BS-1 susceptible check plants' arranged in 4 rows, 51 'R-1 resistance check plants' in five rows. Every plant in 1ST BED has been observed for the

Table 1: Grading scores adopted to categorize progeny based on phenotype.

Grade I	Entire foliage free from cracking and curling with no yellowing.
Grade II	Crinkling and curling of few leaves in the lower portion of plant + marginal yellowing of leaves.
Grade III	Crinkling and curling of leaves almost all over the plant and plant growth hampered.
Grade IV	Extreme curling, crackling, yellowing bronzing and drying of leaves.

jassid made injuries and were scaled according to the damage caused to the plants. Among the 36 '7076 donor parent plants' 32 plants have been observed with no damage on leaves and rest 4 with low or negligible damage. Among the 35 '7085 donor parent plants' 30 plants have been noticed devoid of any damage and the rest 5 with low damage. Fig 2, depicts the damage in terms of individual leaves.

Two plants exhibited no damage, 20 plants low damage, 14 plants medium damage and 4 plants high damage among 35 BS-1 susceptible check plants. Among the 51 'R-1 resistance check plants' 34 were observed with no damage and 17 with low damage. Phenotypic variations of these 1478 F_2 generation plants were screened and recorded.

Based on the observed phenotype grade, 89 plants from each grade have been selected for genotyping with SSR markers. All the data regarding the phenotypic variations observed in a population of 267 plants is for a single trait *i.e.*, sucking pest trait. Table 3 represents the phenotypic statistics of 'jassid pest impact' over 267 samples. The mean impact of jassid is obtained as 2.0075 which is almost susceptible to jassid as per the scoring table (Table 1). The values for skewness and kurtosis have not been

Table 2: Primers for PCR amplification of the selected marker genes.

Primer name	Forward sequence	Reverse sequence
BNL2440	TGTTAAGCATACATTAGTTTCACTCG	CCGGCACCACAAAAGTAAAT
BNL3280	GCAGAACTGCCACTTGTTTG	AGAAAATGGGTTGTGCTTGG
BNL3443	CTGTGGCTACTATAGCTTGATGC	TCAGACCCCACTCTCATTCC
BNL3594	AGGGATTTTGATTGTTGTGC	TGAATTCAAAACAAATGTTAGCC
CM45	GATGCCAGTAAGTTCAGGAATG	GCCAACTTATATTCGGTTCCT
HAU1321	ACTCAGGGAATGATGCAAAT	CCGGTTCACTCTCTCTCACT
HAU2748	GCAACTAAGGCCACCCCCAA	AGAAATGCCAGGCCAGGGTG
JESPER235	GAGCAAGGATGAGGAACGAG	CAAATTACTCAAGTGTCCCATCTC
NAU2443	CGTTGAGAAGGAAAGCCTAA	AGCCTGCTTCATGTTCTTTT
MUSB1166	AGACGTGGAACTTATGACACCCA	GCTTGATGGGTGAAAACACTGCA
BNL1646	TTAAAGGGCAACAAAAGTTCAA	CATGTGATGTAACCTCTCTCTCTCTC
BNL1227	CATCAAGATCTATCTCTCTCTATACCG	TTTACCCTCCGATCTCAACG
DPL0442	TTACGGTGGCTAATGTAATATCCC	ATTCTTGAGAGTTCACCAGGAAAG
TMB0471	AAGAATTAGCGGAAGTGGTCA	TTTGACAAAACATGGATGGA
BNL1694	CGTTTGTTTTCGTGTAACAGG	TGGTGGATTCACATCCAAAG
DOW047	TTCGGACATCCAAAACCTACAAAGA	TGATGGTGGCAAAGGATGATAATGAT
HAU0876	ACAAACGCTGTCACTACGAA	CCATCCTTGTTTTCCAACTC

Table 3: Phenotypic statistics of jassid pest impact over 267 samples.

Trait ID	Trait name	Sample size	Mean	Variance	Std error	Skewness	Kurtosis	Minimum	Maximum	Range	W-test	P-value
1	Jassid_score	267	2.01	0.669	0.818	-0.014	-1.45	1	3	2	0.77	0.00E+00

Fig 1: R-1 is showing no jassid damage in 50-day-old cotton plant in the open-field experiment. BS-1 is showing extreme jassid infection after 50-days of growth in the cotton plant in the open-field experiment.

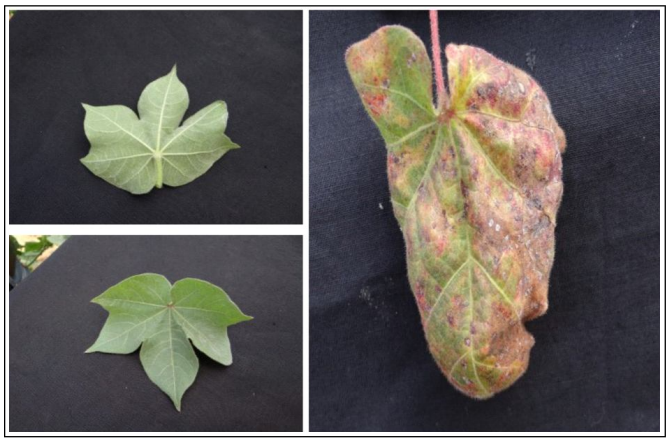

Fig 2: Individual leaf damage after jassid infection for R-1 and BS-1 plants in the open-field experiment.

Table 4: Chi-square analysis.

Marker ID	Marker Name	Chromosome	Position	Size (2/12)	Size (1)	Size (0/10)	Size (-1)	Chi-Square	Pr>Chi Sq	HetBand
1	BNL2440	15	0	153	75	39	0	148.618	0	Codominant
2	BNL3280	18	0	66	107	94	0	16.3933	0.0003	Codominant
3	BNL3443	14	0	142	82	43	0	113.1498	0	Codominant
4	BNL3594	25	0	48	116	103	0	27.2472	0	Codominant
5	CM45	20	0	59	128	80	0	3.7566	0.1529	Codominant
6	HAU1321	12	0	108	97	62	0	35.809	0	Codominant
7	HAU2748	13	0	104	102	61	0	28.7154	0	Codominant
8	JESPER235	22	0	60	124	83	0	5.3146	0.0701	Codominant
9	NAU2443	18	0	57	113	97	0	18.2809	0.0001	Codominant
10	MUSB1166	18	0	49	122	96	0	18.5281	0.0001	Codominant
11	BNL1646	8	0	135	99	33	0	95.764	0	Codominant
12	BNL1227	12	0	73	112	82	0	7.5318	0.0231	Codominant
13	DPL0442	20	0	65	127	75	0	1.382	0.5011	Codominant
14	TMB0471	17	0	57	114	96	0	17.0899	0.0002	Codominant
15	BNL1694	7	0	44	132	91	0	16.5805	0.0003	Codominant
16	DOW047	12	0	27	134	106	0	46.7528	0	Codominant
17	HAU0876	3	0	44	135	88	0	14.5356	0.0007	Codominant

Table 5: Summery and significant marker trait association table.

Trait ID	Trait name	Chromosome	Position	Marker name	LOD	PVE (%)	Add	Dom
1	Jassid_score	1	0	BNL2440	0.2588	0.4494	-0.0452	0.074
1	Jassid_score	1	0	BNL3280	2.0235	3.422	-0.1594	-0.1562
1	Jassid_score	1	0	BNL3443	0.3736	0.6453	0.0317	-0.1171
1	Jassid_score	1	0	BNL3594	2.145	3.6234	-0.1644	-0.1515
1	Jassid_score	1	0	CM45	0.8994	1.5393	-0.1067	-0.1183
1	Jassid_score	1	0	HAU1321	1.9278	3.2632	-0.1834	-0.1423
1	Jassid_score	1	0	HAU2748	2.1731	3.6695	-0.1908	-0.1729
1	Jassid_score	1	0	JESPER235	0.7433	1.2745	-0.1056	-0.0873
1	Jassid_score	1	0	NAU2443	1.4917	2.5357	-0.1052	-0.1851
1	Jassid_score	1	0	MUSB1166	1.2197	2.0788	-0.0781	-0.1847
1	Jassid_score	1	0	BNL1646	4.508	7.457	-0.3505	-0.1444
1	Jassid_score	1	0	BNL1227	0.9742	1.665	-0.0138	-0.212
1	Jassid_score	1	0	DPL0442	0.5777	0.9928	-0.1051	-0.0518
1	Jassid_score	1	0	TMB0471	0.287	0.4973	-0.068	-0.0417
1	Jassid_score	1	0	BNL1694	2.0725	3.5035	-0.048	-0.2828
1	Jassid_score	1	0	DOW047	7.2297	11.6847	-0.2301	-0.3578
1	Jassid_score	1	0	HAU0876	1.6904	2.8677	-0.1364	-0.1645

obtained as perfect but are very close to representing a normal distribution. The slight negative value indicates a left side weightage, but since skewness is almost '0', a normal distribution is implied. Kurtosis value signifies the heaviness of the tail and the observed value is not significant enough to imply a very sharp peak in distribution. Although there were four categories based on Table 1, but all the plants showed variations to a maximum category of 3, *i.e.*, none of the plants showed any extreme phenotypic traits due to the pests (category 4). The W-test, which provides an epistatic insight between the control and the test, indicates that the pest resistant trait in study is not significantly affecting the other traits.

Single marker analysis

A total of 4348 markers (https://www.cottongen.org) were used to identify the polymorphic markers between resistance and resistance gene. Among the 4348 markers, 29 markers have been found to be polymorphic between parental genotype, *i.e.*, RS-1 vs BS-1, but only 17 have been found to be useful for further analysis. These 17 markers that were recognised for further genotypic analysis were BNL2440, BNL3280, BNL3443, BNL3594, CM45, HAU1321, HAU2748, JESPER235, NAU2443, MUSB1166, BNL1646, BNL1227, DPL0442, TMB0471, BNL1694, DOW047 and HAU0876. Using these markers, a genotypic analysis has been carried out for 267 plants and the results after chi-square analysis has been shown in Table 4. All the markers have shown co-dominance except CM45, JESPER235 and DPL0442 and all other markers indicated significance at a minimum of 95% confidence interval.

Marker trait association

The marker trait association was performed for all the screened 17 markers and the results showed that two markers *viz.*, BNL1646 and DOW047 had significant variance. The markers have shown high LOD values of 4.508 and 7.2297 respectively. Also, they have shown high PVE values as 6.5784 and 10.308 respectively, as compared to other markers. The Marker Trait Association results are tabulated and displayed in Table 5. The observed significant markers are presented in bold. The results obtained from the Marker Trait Association analysis indicates that BNL1646 and DOW047 are the markers which can be considered for 'jassid pest control' among the various markers used for screening and selection.

Marker correlation

Further, marker correlation has been analysed to check the links between the same and to identify any underlying connection. The resultsare shown in Table 6 and indicates significantly good positive correlation between the following markers: CM5 and JESPER235, HAU1231 and HAU2748, JESPER235 and DPL0442; while a moderate positive correlation was observed between the following markers: BNL3280 and NAU2443, NAU2443 and MUSB1166. These correlations have been marked in light brick for distinction.

Table 6: Marker correlation.

Marker ID	Marker name	BNL2440	BNL3280	BNL3443	BNL3594	CM45	HAU1321	HAU2748	JESPER235	NAU2443	MUSB1166	BNL1646	BNL1227	DPL0442	TMB0471	BNL1694	DOW047	HAU0876
1	BNL2440	1.00																
2	BNL3280	0.17	1.00															
3	BNL3443	0.06	0.14	1.00														
4	BNL3594	-0.01	-0.07	-0.05	1.00													
5	CM45	0.07	0.02	-0.02	0.13	1.00												
6	HAU1321	-0.06	-0.01	-0.02	-0.04	0.06	1.00											
7	HAU2748	-0.02	0.03	-0.01	-0.05	0.04	0.80	1.00										
8	JESPER235	0.04	0.04	-0.01	0.02	0.80	0.01	0.00	1.00									
9	NAU2443	0.08	0.57	0.00	0.04	0.09	0.07	0.11	0.08	1.00								
10	MUSB1166	0.10	0.29	0.13	0.08	0.09	0.00	0.08	0.01	0.42	1.00							
11	BNL1646	-0.05	-0.01	-0.07	-0.02	-0.13	-0.01	-0.03	-0.11	-0.17	-0.15	1.00						
12	BNL1227	0.05	0.03	-0.02	0.08	-0.02	0.05	0.08	0.01	0.01	0.00	0.11	1.00					
13	DPL0442	0.07	0.09	0.04	0.08	0.75	-0.01	0.00	0.71	0.06	0.03	-0.14	-0.09	1.00				
14	TMB0471	0.12	0.02	0.08	0.15	0.03	0.09	0.07	0.02	0.05	0.10	-0.03	0.11	0.04	1.00			
15	BNL1694	-0.01	0.02	0.13	-0.05	0.12	0.04	0.02	0.09	0.04	0.05	-0.11	0.09	0.13	0.01	1.00		
16	DOW047	-0.05	0.05	0.05	0.09	0.12	0.08	0.07	0.11	0.19	0.00	-0.11	0.16	0.10	0.12	0.16	1.00	
17	HAU0876	0.07	0.02	0.05	0.06	-0.01	-0.06	-0.10	-0.06	0.04	0.10	-0.03	0.05	-0.03	0.08	0.01	0.09	1.00

Very weak negative correlations have been noticed among few of the markers, but none of them were significant. No significant marker correlation has been observed for BNL1646 and DOW047, which ascertains no underlying divergence from the observed results from Marker Trait Association. The rows and columns corresponding to both BNL1646 and DOW047 are marked in light green colour.

CONCLUSION

Jassid pest control has been a matter of concern for the farmers. Although we have been successful in increasing the yield of cotton production, over time, compromised on the other factors like resistance of the plant. This study has brought into light two distinct markers, BNL1646 and DOW047 that can be used while screening 'jassid resistant' plants. This will help many breeders in their attempts of such screenings. In addition to reducing the time consumed in screening such resistant plants, the identification of these markers are now open to genetic engineers and breeders for designing a high yielding cotton crop with jassid resistance.

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