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Rajkumar BK
Main Cotton Research Station (MCRS), Navsari Agricultural University (NAU), Athwa Farm, Surat, Gujarat, India

Preeti R Parmar
Main Cotton Research Station (MCRS), Navsari Agricultural University (NAU), Athwa Farm, Surat, Gujarat, India

RD Patel
Main Cotton Research Station (MCRS), Navsari Agricultural University (NAU), Athwa Farm, Surat, Gujarat, India

HR Ramani
Main Cotton Research Station (MCRS), Navsari Agricultural University (NAU), Athwa Farm, Surat, Gujarat, India

DH Patel
Main Cotton Research Station (MCRS), Navsari Agricultural University (NAU), Athwa Farm, Surat, Gujarat, India

HR Desai
Main Cotton Research Station (MCRS), Navsari Agricultural University (NAU), Athwa Farm, Surat, Gujarat, India

Corresponding Author:
Rajkumar BK
Main Cotton Research Station (MCRS), Navsari Agricultural University (NAU), Athwa Farm, Surat, Gujarat, India

Evaluation of biochemical host plant resistance traits against leafhopper in cotton using recombinant inbred population

Rajkumar BK, Preeti R Parmar, RD Patel, HR Ramani, DH Patel and HR Desai

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Abstract
Sucking pest, Indian cotton jassid *Amrasca biguttula biguttula* has became serious threat to Bt cotton cultivation in India. It's prevalent throughout the cropping season causes phenomenal yield losses and effects quality also. A total of 140 Recombinant Inbred Lines (RILs) were developed from cross of Coker-310 (jassid susceptible) × GISV-218 (jassid resistance) and field evaluated under augmented design to study genetic basis and to determine relationships among biochemical host plant resistance (HPR) traits towards jassid resistance and yield of cotton. Biochemical analysis across RILs revealed that population of jassid and jassid injury decreased with higher biochemical content (tannin, total phenol and gossypol) and lower reducing sugar. Further, significant positive association among biochemical traits (total phenol, gossypol and tannin) and with seed cotton yield per plant and negative association with jassid count and jassid injury supported with high estimates of PCV, GCV, heritability and genetic advance in this RIL populations clearly emphasises studied biochemical traits offers resistance to jassids in cotton. Further, potent transgressive segregation identified with desirable biochemical traits, low jassid count and jassid injury with significantly higher seed cotton yield compared to GISV218, superior parent, our RIL population clearly paves the way for improvement of jassid resistance and yield in cotton.

Keywords: Biochemical host plant resistance (HPR), jassid, recombinant inbred lines (RIL), and transgressive segregation

Introduction
Cotton is grown as fibre, feed and food crop; and the primary source of natural fibre for the textile industry in India. In India, about 95% area in cotton is under the Bollgard-II. Adaption Bt technology has contributed largely to cotton production and productivity. Further, hirsutum Bt hybrids that are grown in India are highly vulnerable to sap-sucking insects (Kranthi and Stone 2020) [13] which poses a great threat to Bt cotton cultivation. Among the sucking pests of cotton, *Amrasca biguttula biguttula* known as Indian cotton jassid is an alarming pest throughout the season. Both nymphs and adults suck the sap from leaves and cause phytotoxic symptoms known as hopper burn leading to significant yield loss. Severe infestation leads to poor crop stand, stunted growth and seed cotton yield loss to the tune of 25-45% with negative impact on fibre quality (Kalyan *et al*. 2017) [11]. Thus, development of jassid resistant genotype is the eco-friendly and cost effective measures for Integrated Pest Management (IPM).

Biochemical and morphological host plant resistance (HPR) traits play major role in the resistance mechanism against pests and also contribute to differential survival of pests on resistant and susceptible cultivars (Singh and Agarwal, 1988) [29]. Morphological HPR characters such as thickness of leaf lamina, length of hair, trichome density, hairiness of leaves and toughness of leaf veins were reported to contribute for resistance against for sucking pests in cotton (Parnell *et al*, 1949, Meagher *et al* 1997) [21, 18]. Few studies on biochemical analysis also reported lower jassid incidence for genotypes having higher content (tannin, gossypol, phenol) and lower content (reducing sugar, soluble proteins), and higher chlorophyll index (Rohini 2010, Mandhania *et al*. 2010, Deb *et al*. 2015, Harijan *et al*., 2017) [23, 16, 5, 8].

But all these studies have focused on few genotypes and advanced lines. Thus, characterization large breeding population for jassid resistance would give clear understanding of role biochemical HPR traits in governing jassid resistance in cotton. Therefore, a large breeding population of 140 RILs were screened for jassid resistance in the present study.

Breeding for jassid resistance in cotton through conventional approach has been challenging due to the intricate nature of the cotton genome and the complex inheritance patterns associated with jassid resistance. Moreover, the lack of sufficient resistance sources against jassids has further complicated the breeding process. Previous efforts at our Main Cotton Research Station (MCRS), NAU Surat, we successfully identified and released a jassid tolerant line, GISV 218 in 2014 (). Furthermore, GISV 218 has been utilized as a parent to develop a mapping population (with the susceptible parent being Coker-310), resulting in a set of 140 RILs that have been meticulously bred and maintained at our centre. Thus, present investigation was undertaken to characterize biochemical HPR traits (tannin, total phenol, reducing sugar and gossypol) and to determine the relationships among contributing traits in imparting resistance to jassid in cotton.

Materials and Methods

Field evaluation and observation

A set of 140 recombinant inbred lines (RILs) of F_7 generation derived from an intraspecific cross of *G. hirsutum* var. Coker-310 (jassid susceptible) and GISV-218 (jassid resistance) was grown in the field under natural infestation conditions during *Kharif* 2020 at Main Cotton Research Station (MCRS), NAU, Surat. Plant protection measures were not adopted in order to expose the genotypes to insect attack. Augmented design with 14 blocks was used to evaluate140 RILs and parents. Individual entries were sown with a spacing of 120 x 45 cm in single rows, accommodating 10 plants per row.

The biochemical parameters *viz*., tannin (g/100 g of dry weight) [Folin-Denis method, Schanderi, 1970], total phenol (mg/g of tissue) [FCR method, Malick and Singh (1980)], reducing sugar (mg/g of tissue) [DNSA method, Miller, 1972] and gossypol (mg/g of tissue) [Sood and Singh, 1973] analysis was carried across 140 RILs and parents from three tagged plants using standard biochemical methods during October (42-43 standard week) when jassid population incidence was at peak level.

The jassids population (jassid count) and injury on plants were recorded to screen the RILs for jassid resistance. Observation on jassids population was recorded from five tagged plants of each RIL and parents from each block at weekly interval from August to December 2020 (32 to 52 standard weeks). The density of jassid population was counted by visually observing the abaxial surface of the leaves. In each plant three leaves one each from the top, middle and bottom parts of the plant were considered for recording observations for jassids population. The average value of three leaves per plant recorded during peak jassid incidence or at its ETL i.e., average of 2 nymphs per leaf (37 to 49 standard weeks) considered for the statistical analysis. Observation for jassid injury grade was recorded based on resultant symptoms upon infestation (1-4 grades) as per Indian Central Cotton Committee (ICCC, 1960) [9].

Statistical analysis

The data collected for biochemical traits (tannin, total phenol, reducing sugar and gossypol), jassid count, jassid injury and seed cotton yield were subjected to analysis of variance [Burton and Devane (1953)] [4]. Heritability and genetic advance estimates were calculated following the method of Allard (1960) [2] and Johnson *et al* (1955) [10], respectively. The phenotypic and genotypic correlations matrix was computed between pairs of characters using OPSTAT Free BioTools (http://www.202.141.47.5/opstat/index.asp)

Results

Biochemical host plant resistance (HPR) traits have gained increasing importance in recent times and information on biochemical basis of resistance to jassid in cotton is scarce. Thus, in the present study our study aimed at study genetic basis and determine relationships among biochemical HPR traits towards jassid resistance and yield of cotton

ANOVA revealed significant difference between checks (parents) for all the characters studied (Table 1). The susceptible parent, Coker-310 had high mean number of jassid (9.57) with injury grade of 3.62, minimum tannin (5.95 g/100 g), total phenol (4.23 mg/g), gossypol (4.91mg/g) and high reducing sugar (12.42 mg/g), and low seed cotton yield per plant (22.57). Contrastingly, the resistant parent, GISV 218 had low mean number of jassid (3.37) with injury grade (1.43), maximum tannin (10.69 g/100 g), total phenol (12.86 mg/g), gossypol (11.17 mg/g of tissue), low reducing sugar (3.42 mg/g), and maximum seed cotton yield per plant (91.46 g/plant) (Table 2). The contrasting parents that are diverse with respect to jassid count and injury, biochemical traits along with yield under study indicate the proper choice of parents for the development of RILs.

ANOVA also revealed significant differences across RILs for all the biochemical traits, jassid count, jassid injury and seed cotton yield (Table 1). The RILs had wide range for all the character studied (Table 2). The range of tannin (1.96-17.03 g/100 g), total phenol (2.85 g-20.49 mg/g), reducing sugar (0.56-22.49 mg/g), gossypol (3.18-15.63 mg/g), jassid count (1.96-11.86), jassid injury (1-4) and seed cotton yield (15.56-133.33 g/plant) recorded indicate sufficient genetic variability among the RILs for these characters (Table 1). Further, the frequency distribution based on the mean data of all the studied traits showed near normal distribution pattern (Fig. 1) indicating polygenic inheritance and proper development for RILs for genetic studies.

The genotypic variance (σg^2), phenotypic variance (σp^2), Phenotypic Coefficient of Variation (PCV), Genotypic Coefficient of Variation (GCV), heritability and genetic advance as per cent of mean were worked out for all the traits (Table 3). Estimation of environmental components of variance in relation to their genotypic counterpart revealed that the estimation of σg^2 were higher than σe^2 for the all characters, which indicated narrow difference between genotypic and phenotypic components of variance. High values of PCV (26.82-68.08%) and GCV (26.54-67.82%) observed for all the biochemical traits (tannin, total phenol, reducing sugar, and gossypol), jassid count, jassid injury and seed cotton yield in RIL population emphasizes that variation in these characters contributed markedly to the total variability.

Further, high estimates of heritability (95.05% to 99.25%) coupled with genetic advance as per cent of mean (54.09% to 139.19%) for the studied traits indicated the genotypic variance for these traits were primarily due to additive genetic effect. Therefore, these traits are key factor in determining the jassid resistance. Similar kind of high values of GCV, PCV and heritability for jassids/leaf, jassid injury along with leaf pubescence traits in RIL population derived from the cross DS-28 x SBYF-425 was reported by Sankeshwar *et al.,* (2018) [25]. Similar trend of high values genetic parameter reported for number of leafhoppers/leaf, gossypol glands, total soluble sugar and trichome density by Murugesan and Kavitha (2010) [20] and Aherkar *et al*. (2023) [1] and for seed cotton yield per plant by Erande *et al* (2014) [6], Kumar *et al*. (2019) [14], Gnanasekaran *et al*. (2020) [7]

Correlation

The jassid count and jassid injury revealed negative significant correlation with amount of tanin (-0.340,-0.357), total phenol (-0.401,-0.434), gossypol (-0.158,-0.276) and seed cotton yield (-0.408,-0.708) but positive significant association with the amount of reducing sugar (0.132, 0.405) (Table 4). Likewise, tannin, total phenol and gossypol displayed negative association with reducing sugar. Seed cotton yield per plant positively associated with tannin, total phenol, gossypol and on the contrary negatively related with reducing sugar, jassid count and jassid injury. The correlation results implicated that the biochemical HPR traits (tannin, phenol, and gossypol) highly negatively correlated with reducing sugar, jassid count and injury but positively significant with seed cotton yield thereby directly effecting the jassid resistance and yield in cotton.

Earlier studies have also revealed negative and significant correlation between biochemical trait and jassid susceptibility (Rohini 2010, Mandhania *et al*. 2010, Deb *et al*. 2015, Harijan *et al*., 2017, Subhashini *et al*. 2022) [23, 16, 5, 8, 31]. Similar findings for higher the amount of reducing sugar more the susceptible cultivar (Singh *et al*., 1988) [29]; and higher the phenolic content the lower jassid incidence (Shinde *et al*., 2014) [27]. Bhoge *et al* (2019) [3] and Rohini *et al*. (2011) [22] also reported the higher the phenolic and gossypol in cotton plant would result in a highly resistant plant. Keerthivarman *et al*., (2022) [12] suggested genotypes with high phenolic content, low reducing sugar, lower soluble proteins, higher chlorophyll index and higher trichome density the number of jassids per plant would be lower for such genotypes.

Transgressive segregation

A total of 16, 15, 17, 26, 13, 9, and 11 transgressive segregation (RILs) were identified for tannin, total phenol, reducing sugar and gossypol, jassid count, jassid injury and seed cotton yield, respectively. A total of 18 RILs with either significantly higher and/or on par seed cotton yield compared to GISV218, and more than one desirable biochemical trait for jassid resistance and yield were identified in this RIL population. Further, six potential RIL with desirable biochemical traits for jassid resistance with higher seed cotton yield compared to GISV218, superior parent *viz*., RIL-24, RIL-6, RIL-28, RIL-31, RIL-88, and RIL-94, can be used for further bred for jassid resistance and high yield (Table 5).

Discussion

GISV 218 (tolerant parent) recorded higher content of tannin, total phenol, gossypol, low reducing sugar, low mean number of jassid with injury grade, and higher seed cotton yield per plant establishes a close relationship between jassid resistance and biochemical HPR traits. Further, wide range for the studied traits among parents and the RILs not only provides preliminary idea about the variability and also gives good chance to select RILs with desirable jassid resistance traits.

Normal distribution, higher value PCV, GCV, heritability, and genetic advance for studied biochemical HPR traits, jassid count, jassid injury and seed cotton yield suggests polygenic inheritance of traits and the genotypic variances for these traits were primarily due to additive genetic effect. Therefore, these traits are key factor in determining the jassid resistance.

Correlation coefficients among jassid resistance traits in RIL population revealed significant and practical associations. The correlation results implicated that the biochemical HPR traits (tannin, phenol, and gossypol) highly negatively correlated with reducing sugar, jassid count and injury but positively significant with seed cotton yield thereby directly effecting the jassid resistance and yield in cotton. This trend of association emphasise that these parameters could be utilized as selection criteria for jassid resistance in cotton. The per se performance of transgressive RILs gives ample opportunity to select and breed these potential RILs for jassid resistance and yield in cotton.

Table 1: Analysis of variance showing mean square value for biochemical traits, jassid count, jassid injury and seed cotton yield in the RIL population

Sources of variation	Blocks	Treatment	Checks	RILs	C v/s V	Error
Df	13	141	1	139	1	13
Tannin (g/100 g of dry weight)	0.119	6.591**	157.463**	5.509**	6.050*	0.116
Total phenol (mg/g of tissue)	0.937	16.162**	521.079**	12.095**	76.435**	0.394
Reducing sugar (mg/g of tissue)	0.601	25.221**	566.910**	21.179**	45.477**	0.159
Gossypol (mg/g of tissue)	0.376	15.331**	274.001**	13.128**	62.927**	0.479
Jassid count (Jassid/3 leaves)	0.167	8.630**	268.770**	6.818**	0.337**	0.052
Jassid Injury Grade (JIG)	0.062	1.119**	33.376**	0.894**	0.151**	0.014
Seed cotton yield per plant (g/plant)	14.427	935.151**	33222.215**	687.817**	3027.412**	34.027

*,** Significant at 0.05 and 0.01 level, respectively

Table 2: Mean performance and range for biochemical traits, jassid count, jassid injury and seed cotton yield in the RIL population

Sr. No	Characters	Parental lines		RILs			C.D. at 5%				C.V. %
		Coker-310	GISV 218	Range		Mean	Between two control mean	Between two RILs in same block	Between two RILs not in same block	Between RIL and control	
				Min	Max						
1	Tannin (g/100 g of dry weight)	5.95	10.69	1.96	17.03	8.83	0.278	1.039	1.273	0.932	3.89
2	Total phenol (mg/g of tissue)	4.23	12.86	2.85	20.49	10.36	0.513	1.919	2.350	1.720	6.25
3	Reducing sugar (mg/g of tissue)	12.42	3.42	0.56	22.49	6.53	0.325	1.217	1.491	1.091	5.90
4	Gossypol (mg/g of tissue)	4.91	11.17	3.18	15.63	9.68	0.565	2.114	2.589	1.895	7.35
5	Jassid count (Jassid/3 leaves)	9.57	3.37	1.96	11.86	6.35	0.186	0.698	0.855	0.625	3.58
6	Jassid Injury Grade (JIG)	3.62	1.43	1.00	4.00	2.44	0.096	0.358	0.439	0.321	4.81
7	Seed cotton yield per plant (g/plant)	22.57	91.46	15.56	133.33	68.41	4.763	17.822	21.827	15.976	8.77

C.D. = Critical Difference, C.V. = Coefficient of Variation, NS = Non-significant

Table 3: Estimation of variability parameters for biochemical traits, jassid count, jassid injury and seed cotton yield in the RIL population

Sr. No.	Characters	Components of variation		Coefficient of variation		h2 (%)	GA (%)	GAM (%)
		σg	σp	GCV (%)	PCV (%)			
1	Tannin (g/100 g of dry weight)	5.393	5.509	26.54	26.82	97.89	4.73	54.09
2	Total phenol (mg/g of tissue)	11.701	12.095	34.04	34.60	96.74	6.93	68.96
3	Reducing sugar (mg/g of tissue)	21.020	21.179	67.82	68.08	99.25	9.41	139.19
4	Gossypol (mg/g of tissue)	12.649	13.128	37.80	38.50	96.35	7.19	76.42
5	Jassid count (Jassid/3 leaves)	6.766	6.818	40.83	40.99	99.24	5.34	83.80
6	Jassid Injury Grade (JIG)	0.880	0.894	38.13	38.44	98.43	1.92	77.94
7	Seed cotton yield per plant (q/ha)	653.790	687.817	38.44	39.43	95.05	51.35	77.21

σg^2-Genotypic variance, σp^2-Phenotypic variance, GCV-Genotypic coefficient of variance, PCV-Phenotypic coefficient of variance, h^2-Broad sense heritability, GA-Genetic advance, GAM-Genetic advance as per cent of mean

Table 4: Correlation coefficient value for biochemical traits, jassid count, jassid injury and seed cotton yield in RILs population

	Tannin	Total phenol	Reducing sugar	Gossypol	Jassid /3 leaves	Jassid Injury Grade	Seed cotton yield per plant
Tannin	1	0.420**	-0.404**	0.200**	-0.340**	-0.357**	0.276**
Total phenol		1	-0.254**	0.175*	-0.401**	-0.434**	0.458**
Reducing sugar			1	-0.153*	0.132*	0.405**	-0.187*
Gossypol				1	-0.158*	-0.276**	0.202**
Jassid /3 leaves					1	0.328**	-0.408**
Jassid Injury Grade						1	-0.708**
Seed cotton yield per plant							1

*, ** Significant at P= 0.05 level and P= 0.01 level respectively

Table 5: Promising transgressive RILs with desirable biochemical traits for jassid resistance

S. No.	RIL No.	Tannin (g/100 g)	Total phenol (mg/g)	Reducing sugar (mg/g)	Gossypol (mg/g)	Jassid count	JIG	SCY	RILs with desirable traits
1	RIL-94	9.18	8.25	1.11	13.57	2.68	1.00	115.71	RS, G, JC, JIG, SCY
2	RIL-88	9.54	16.00	4.17	5.80	2.97	1.00	111.66	P, JIG, SCY
3	RIL-31	5.26	8.41	3.05	14.48	1.99	1.00	127.50	G, JC, JIG, SCY
4	RIL-28	7.13	14.78	4.72	7.17	2.87	1.00	111.00	P, JIG, Scy
5	RIL-6	9.54	9.57	18.60	8.45	2.68	1.00	125.00	JC, JIG,SCY
6	RIL-24	5.79	9.49	8.89	9.86	1.97	1.00	133.33	JC, JIG,SCY
7	RIL-96	10.25	15.77	2.78	14.41	3.72	1.40	96.66	P, G, SCY
8	RIL-106	12.39	9.27	2.78	7.47	2.27	1.20	100.00	JC, T, SCY
9	RIL-108	10.52	14.84	9.16	15.35	3.88	1.40	94.28	P, G, SCY
10	RIL-25	6.06	10.71	1.94	3.18	2.17	1.20	106.87	RS, JC, SCY
11	RIL-107	10.16	19.90	13.05	8.06	2.97	1.20	100.00	P, SCY
12	RIL-113	10.70	19.94	3.89	10.18	3.88	1.40	98.00	P, SCY
13	RIL-114	7.93	18.91	6.94	8.31	3.99	1.40	91.66	P, SCY
14	RIL-36	9.27	10.89	3.89	11.53	2.67	1.20	110.00	JC, SCY
15	RIL-4	9.36	10.71	18.05	11.49	1.96	1.40	98.89	JC, SCY
16	RIL-95	8.74	9.60	0.56	10.18	3.57	1.40	98.00	RS, SCY
17	RIL-69	10.87	10.58	1.39	7.09	2.52	1.80	83.75	RS, JC
18	RIL-54	5.79	12.30	12.22	13.03	2.71	1.20	110.00	JC, SCY
	GISV 218	10.69	12.86	3.42	11.17	3.37	1.43	91.46	
	Coker 310	5.95	4.23	12.42	4.91	9.57	3.62	22.57	
	CD @ 5%	0.932	1.721	1.091	1.895	0.625	0.321	15.976	

T-Tannin; RS-Reducing sugar; P-Total phenol; G-Gossypol; JC-Jassid/3leaves; JIG-Jassid Injury Grade; SCY-Seed cotton yield

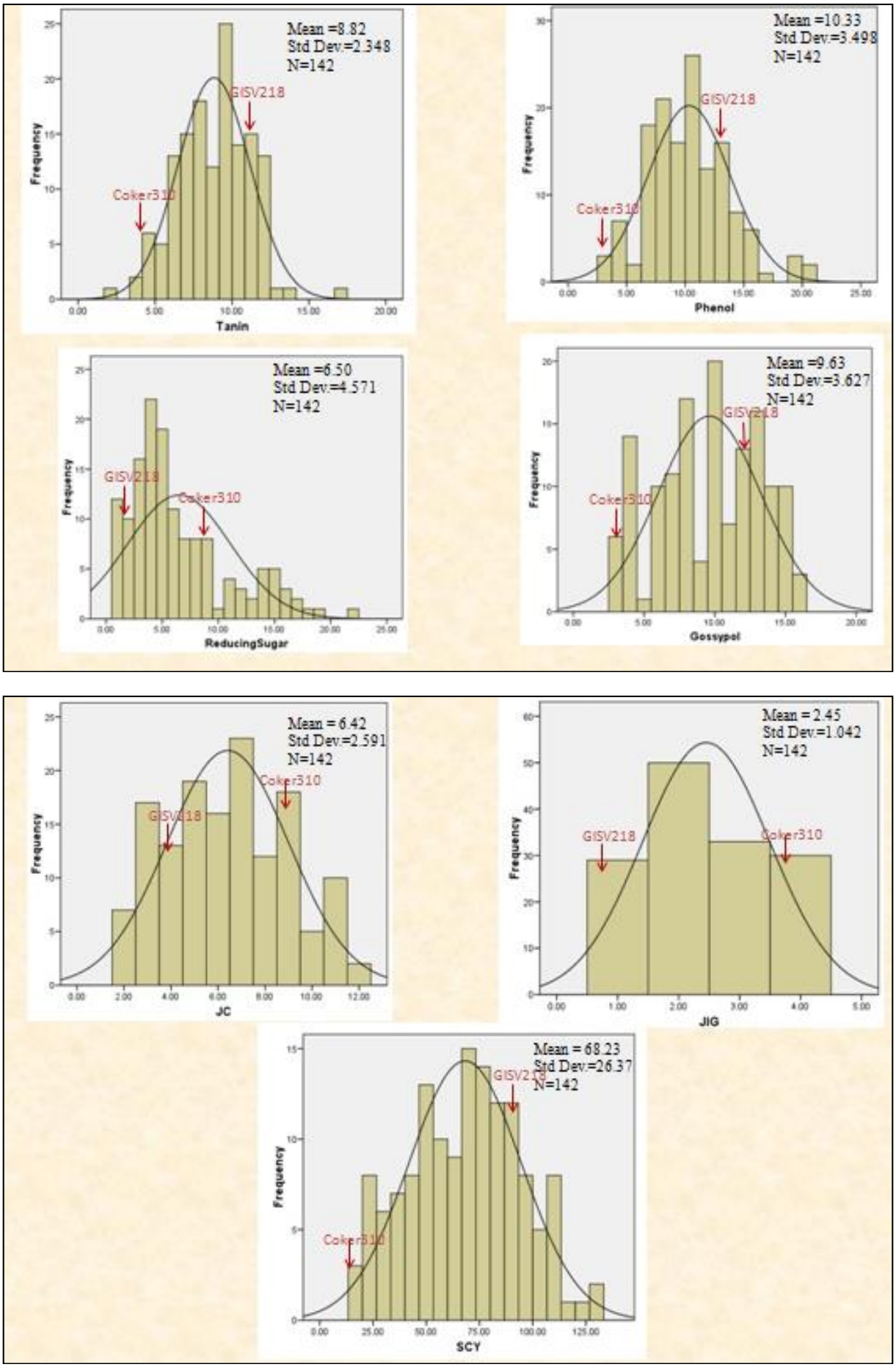

Fig 1: Frequency distribution of the mean values of for biochemical traits, jassid count, jassid injury and seed cotton yield in the RIL population

Conclusion

Biochemical analysis across RILs revealed that population of jassid and jassid injury decreased with higher biochemical content (tannin, total phenol and gossypol) and lower reducing sugar. Further, significant positive association among biochemical traits (total phenol, gossypol and tannin) and with seed cotton yield per plant and negative association with jassid count and jassid injury supported with high estimates of PCV, GCV, heritability and genetic advance in this RIL populations clearly emphasise biochemical traits studied offers resistance to jassids in cotton. Further, transgressive segregation identified can be bred for jassid resistance and high yield.

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