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# Evaluation of cotton germplasm for morphological and biochemical host plant resistance traits against sucking insect pests complex

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## Abstract

**Background:** Sucking insect pests cause severe damage to cotton crop production. The development of insect resistant cotton cultivars is one of the most effective measures in curtailing the yield losses. Considering the role of morphological and biochemical host plant resistance (HPR) traits in plant defense, 12 cotton genotypes/varieties were evaluated for leaf area, leaf glanding, total soluble sugars, total soluble proteins, total phenolics, tannin and total flavonoids against fluctuating populations of whitefly, thrips and jassid under field conditions.

**Results:** The population of these insects fluctuated during the growing season and remained above threshold level (whitefly > 5, thrips > (8–10), or jassid > 1 per leaf) during late June and early July. Strong and negative association of whitefly ( $r = -0.825$ ) and jassid ( $r = -0.929$ ) with seed cotton yield was observed. Mean population of insects were the highest in Glandless-1 followed by NIA-82 and NIA-M30. NIAB-Kiran followed by NIAB-878 and Sadori were the most resistant, with the mean population of 1.41, 1.60, 1.66 (whitefly); 2.24, 2.32, 2.53 (thrips) and 0.37, 0.31, 0.36 (jassid), respectively. The resistant variety NIAB-Kiran showed less soluble sugars ( $8.54 \text{ mg}\cdot\text{g}^{-1}$ ), soluble proteins ( $27.11 \text{ mg}\cdot\text{g}^{-1}$ ) and more phenolic ( $36.56 \text{ mg}\cdot\text{g}^{-1}$ ) and flavonoids ( $13.10 \text{ mg}\cdot\text{g}^{-1}$ ) as compared with the susceptible check Glandless-1. Moreover, all insect populations were positively correlated with total soluble sugars and proteins. Whitefly populations exhibited negative response to leaf gossypol glands, total phenolics, tannins and flavonoids. The thrips and jassid populations had a significant and negative correlation with these four biochemical HPR traits.

**Conclusion:** The identified resistant resources and HPR traits can be deployed against sucking insect pests' complex in future breeding programs of developing insect resistant cotton varieties.

**Keywords:** *Gossypium*, Insect resistance, Plant defense, Plant breeding, Resistant traits, Sucking insects

## Introduction

Cotton (*Gossypium hirsutum* L.) as a major crop has a 1.0% share in GDP and 5.5% in agriculture value addition in Pakistan. During 2017–2018, the cotton crop in Pakistan was cultivated on 2.699 Mha. The production stood at 11.935 million bales (One bale = 170 kg),

which is 15% off the target of 14.04 million bales (Government of Pakistan 2017–2018). A decline in production has several reasons in which the sucking insect pests played a major role. Sucking insect/pest complex especially whitefly, jassid and thrips have caused severe damages to genetically-modified cotton systems in Pakistan. Notable yield losses have occurred in previous years (Razaq et al. 2013) and are expected in the future due to lack of resistant cultivars, increased insect pressure and pesticide resistance (Amjad and Aheer 2007).

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Genetic modification (GM) permitted the incorporation of toxins from bacteria (*Bacillus thuringiensis*, Bt) into cotton to control key Lepidopteron pests. However, sucking insect pest complex (whitefly, jassid and thrips) which is not controlled by Bt cotton has emerged as a new problem. This sucking complex causes significant loss in yield and fiber quality, and their management is also a key challenge for cotton growers. Losses up to 40% are reported due to sucking complex (Oerke 2006; Aslam et al. 2004; Nizamani et al. 2002). Significant losses > 12% were estimated even after taking control measures (Oerke 2006). Current control measures include the use of different chemicals as pesticides which is associated with high cost, pesticide resistance and environmental risks (Costa et al. 2003). Alternative methods should be opted for the control of these sucking insects. Host plant resistance (HPR) offers an opportunity for effective control of sucking insect pests as an economically and environmentally safe strategy in the GM cotton system (Khan et al. 2003). Khalil et al. (2017) studied the role of some morphological plant traits against sucking insect pest complex in six genotypes of cotton. They identified the negative response of whitefly to gossypol glands on leaf lamina, midrib and vein. Aslam and Saeed (2004) studied the comparative performance of 22 cotton genotypes against sucking complex. They investigated the relationship between leaf morphological traits and the population of sucking insects. Perveen et al. (2001) investigated the biochemical HPR basis of insect resistance in cotton, and found that the resistant varieties showed more phenolics and tannin as compared with the susceptible varieties; the negative correlations were witnessed between total phenols and the insect attack.

Therefore, the deployment of morphological and biochemical HPR traits as a cotton plant defense mechanism provides an alternative management strategy and controls these insect pests. Cotton cultivars having HPR against these insects would entail fewer pesticide applications, reducing costs and risks associated with pesticide resistance and beneficial the insect populations. Previously, researchers have focused on morphological HPR traits with only one or two reports on biochemical HPR (phenolics and tannins) in cotton. Compared with these studies, we used a much broader range of germplasm covering the morpho-biochemical HPR traits from toxic to attractive to insects, such as higher contents of soluble proteins and sugars. Further, genotypes used in our study are also advanced and new genetic resources. Therefore, keeping in view the above facts, the present study investigates the response of some advanced lines and cultivars against sucking insect pest complex and identifies the morpho-biochemical basis of insect resistance by exploiting host plant resistance traits.

## Materials and methods

### Study site and experimental materials

The research work was conducted at Plant Breeding and Genetics Division, Nuclear Institute of Agriculture (NIA), Tando Jam, Pakistan during the Kharif season, 2018–2019. The experimental material consisted of 12 diverse cotton advanced lines and cultivars (NIAB-878, NIA-M30, NIA-H24, NIA-HM48, NIA-Bt.30, NIA-H32, NIA-H67, NIAB-112, Sadori, NIAB-Kiran, and NIA-82) including one glandless genotype (Glandless-1) as the susceptible check.

### Evaluation of cotton genotypes against sucking insects under field conditions

#### Field experiment design

The experimental materials were sown in Randomized Complete Block Design (RCBD) blocks during May, 2018. The plot size was 6.1 m × 3.0 m with four rows. Plant × plant and row × row distance was 30 cm and 75 cm, respectively. Three replicates, each of 12 genotypes, produced a total of 36 plots. Each plot comprised four rows of 20 cotton plants for a total of 80 plants. Standard agronomic practices were performed throughout the growing season. Plant protection measures were not adopted in order to expose the genotypes to insect attack. The experimental materials were harvested during October, 2018.

#### Estimation of sucking insect pest infestation

The population of adults per leaf of cotton whitefly (*Bemesia tabaci*), thrips (*Thrips tabaci*) and jassid (*Amrasca devastans*) were recorded at weekly intervals, early in the morning, from 20 days after sowing up to the picking of seed cotton. Five plants were tagged randomly in each replication of the treatment. From each plant, data were recorded from three leaves, one from the upper leaf, one from the middle and one from the lower portion of the plant and then converted into per leaf basis (Ahmad et al. 2011). A hand magnifying lens was used for counting the sucking insect population. A total of 11 observations were recorded for about 3 months during the study.

#### Estimation of morphological and biochemical HPR traits

**Leaf area and glanding** The above randomly selected five plants were also used to measure morphological HPR traits (leaf area and leaf glanding). Five leaves per plant from different positions (upper, middle and lower) of each plant were cut, bagged, and carried to the laboratory. The leaf area (cm<sup>2</sup>) of 25 randomly selected leaves was measured with a leaf area meter and means were computed. The leaf glanding was also estimated from

the lower side of these leaves and scored by using the following scale given by Romano and Scheffler (2008).

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0	= Glandless
2	= Very few gland on the interveinal region
4	= Few number of interveinal glands (between very few glands = 2 and normal glanding = 6)
6	= Normal glanding on throughout the interveinal area and on the margins and veins

Note = If categories 0, 2 or 4 had normal glanding on leaf margins or veins, 1.0 was added to score.

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**Total soluble sugars (TSS)** The amount of TSS ( $\text{mg}\cdot\text{g}^{-1}$ ) in fully expanded youngest leaves randomly collected from different plants of each genotype was estimated by phenol sulphuric acid reagent method given by Dubois *et al.* (1951). Leaf samples (0.05 g) were extracted in 95% methanol solution (2 mL) for 48 h at room temperature. Assay solution was prepared by using 96% sulphuric acid, 5% phenol and sample extract. A 1 mL of leaf extract, 0.5 mL phenol solution, and 2.5 mL sulphuric acid were added in glass tubes. Each tube was gently agitated during acid addition and then kept in a water bath at 26–30 °C for 20 min. Then absorbance of the colored solution was measured at 490 nm using spectrophotometer VIS-1100 (BMS, Canada). Different concentrations (0.1–3 mg with 0.5 mg interval and 3–6 mg with 1 mg interval) of glucose were prepared in 4 mL distilled water. After mixing the standards and placing them in the water bath at 26–30 °C for 20 min, absorbance was measured at 490 nm. A standard curve was drawn by plotting glucose concentration on the *x*-axis against their absorbance at 490 nm on the *y*-axis.

**Total soluble proteins (TSP)** The TSP was measured using Bradford protein estimation assay (Bradford 1976). Briefly, 0.5 g fully expanded youngest leaves randomly collected from different plants were ground and homogenized in 10 mL of ice-cold potassium phosphate buffer. Then samples were centrifuged for 100 min at 15000×*g*. The supernatant (5  $\mu\text{L}$ ) was then mixed with a 1 mL reaction solution containing Coomassie Brilliant Blue G-250 (0.02 g), which was dissolved in 10 mL of 95% ethanol. To this solution, 20 mL of 85% phosphoric acid was added. The resulting solution was diluted to a final volume of 200 mL, and bovine serum albumin was used as a standard.

**Total phenolics (TP)** The TP was measured using the Folin-Ciocalteu reagent following a method adapted from Singleton and Rossi (1965). The fully expanded youngest leaves of 0.05 g randomly collected from different plants were extracted in a 95% methanol solution

(500  $\mu\text{L}$ ) for 48 h at room temperature. The extract was centrifuged at 14 000  $\text{r}\cdot\text{min}^{-1}$  for 10 min. The supernatant (100  $\mu\text{L}$ ) was added to a 100  $\mu\text{L}$  of 10% Folin-Ciocalteu reagent, after 2 min, 800  $\mu\text{L}$  of 7.5%  $\text{Na}_2\text{CO}_3$  solution was added. After shaking, the solutions were maintained at room temperature for 1 h. Absorption was measured at 765 nm using the spectrophotometer VIS-1100. Gallic acid standard solutions were used to calibrate the method and the results were expressed as gallic acid equivalents in milligram per gram in fresh weight.

**Tannins** Fresh leaf samples (0.05 g) were extracted in a 95% methanol solution (500  $\mu\text{L}$ ) for 48 h at room temperature with occasional stirring. The extract was centrifuged at 14 000  $\text{r}\cdot\text{min}^{-1}$  for 10 min. The supernatant was used to estimate tannins following the method described by Burns (1971). Tannin in the sample was measured at 765 nm using tannic acid as standard. The total tannins were expressed as milligram per gram fresh weight.

**Total flavonoids (TF)** The TF were determined following the method described by Chang *et al.* (2002). The fully expanded youngest leaves of 0.05 g randomly collected from different plants were extracted in a 95% methanol solution (500  $\mu\text{L}$ ) for 48 h at room temperature. The TF assay was performed using an extract (200  $\mu\text{L}$ ) dissolved in 800  $\mu\text{L}$  distilled water, 10% aluminium chloride (50  $\mu\text{L}$ ), 1  $\text{mol}\cdot\text{L}^{-1}$  potassium acetate (50  $\mu\text{L}$ ), and 1.4 mL distilled water. After shaking, the mixtures were incubated at room temperature for 40 min and the absorbance was then measured at 415 nm using the spectrophotometer VIS-1100. The TFs were expressed as rutin equivalents in milligram per gram in fresh weight.

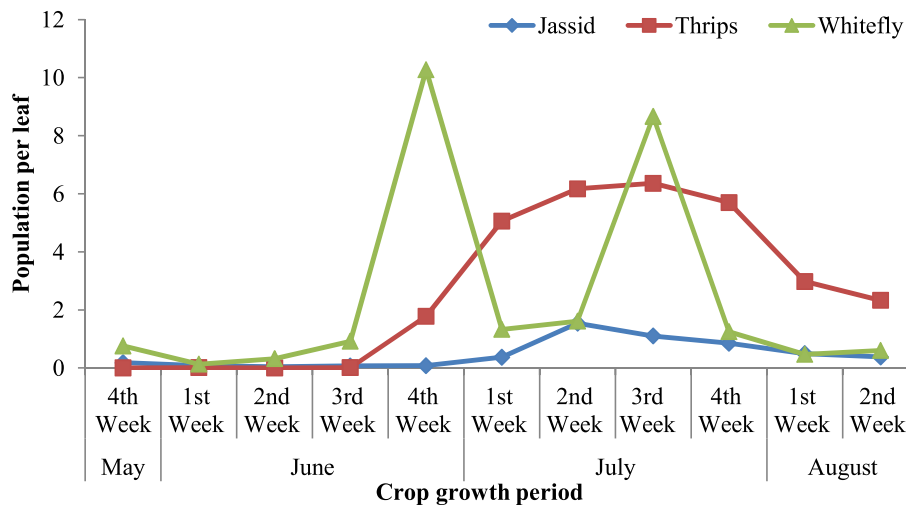
#### Statistical analysis

The data collected were analyzed separately for each parameter and subjected to analysis of variance following Steel *et al.* (1997). The means were compared using least significant differences (LSD) test ( $\alpha = 0.05$ ). Correlation matrix was computed using XLSTAT 2012. The values presented are mean of three replicates  $\pm$  standard error (SE), respectively.

## Results

### Population dynamics of sucking insect pests

The population of sucking insect pests (average of the 12 genotypes) fluctuated throughout the cotton crop growth season during 2018/2019 (Fig. 1). Whitefly infestation increased from the 3rd week of June and exceeded the threshold level and reached up to 10 whiteflies per leaf during the 4th week of June and the 3rd week of July. Thrips infestation also exceeded the



**Fig. 1** Fluctuation in sucking insect pest complex population (data from the average of 12 cotton genotypes) during the growing season 2018/2019

threshold level (> 6 thrips per leaf) from the 2nd to 4th week of July. A similar trend was also observed for jassid infestation. The jassid population increased from the 1st week of July (> 1 jassid per leaf) till the 4th week of July. The months of June and July were the most favorable for sucking insect pest infestation as their peak abundance was recorded in these months.

#### Impact of cotton genotypes on sucking insect pests

Significant differences among genotypes were observed for sucking insects per leaf (Table 1). The cultivars, NIAB-Kiran followed by NIAB-878 and Sadori, showed less infestation of whitefly, i.e., 1.41, 1.60, and 1.66 per leaf, respectively. The genotype Glandless-1 was the most

susceptible having a whitefly infestation of 4.95 per leaf. Next higher numbers of whitefly were observed on NIA-82 (4.07 per leaf) followed by NIA-M30 (2.89 per leaf). The genotypes Glandless-1 and NIA-82 were also found susceptible to thrips, having 3.70 and 2.96 thrips per leaf, respectively. Thrips infestation was less in NIAB-Kiran (2.24 per leaf) followed by NIAB-112 (2.29 per leaf), NIAB-878 (2.32 per leaf) and NIA-H32 (2.35 per leaf). Regarding jassid infestation, the cultivars NIAB-878 (0.31 per leaf) followed by Sadori (0.36 per leaf) and NIAB-Kiran (0.37 per leaf) were less attacked. Jassid infestation was higher in genotypes Glandless-1 (0.78 per leaf) followed by NIA-H24 (0.57 per leaf). Overall, NIAB-Kiran, NIAB-878 and Sadori were less attacked by sucking insect pest complex.

**Table 1** Mean population of sucking insect pests (number/leaf) on different genotypes of cotton

Genotypes	Whitefly	Thrips	Jassid
NIAB-Kiran	1.41 ± 0.21 f	2.24 ± 0.21 e	0.37 ± 0.06 cd
NIAB-878	1.60 ± 0.21 f	2.32 ± 0.22 de	0.31 ± 0.01 d
Sadori	1.66 ± 0.12 ef	2.53 ± 0.08 bcde	0.36 ± 0.05 cd
NIAB-112	1.96 ± 0.12 def	2.29 ± 0.30 e	0.48 ± 0.04 bc
NIA-H32	2.21 ± 0.14 cdef	2.35 ± 0.30 de	0.53 ± 0.06 bc
NIA-Bt.30	2.46 ± 0.13 cde	2.69 ± 0.23 bcde	0.43 ± 0.04 bcd
NIA-H67	2.50 ± 0.42 cd	3.03 ± 0.07 b	0.56 ± 0.08 b
NIA-HM48	2.58 ± 0.10 cd	2.98 ± 0.13 bc	0.48 ± 0.04 bc
NIA-H24	2.72 ± 0.57 cd	2.40 ± 0.17 cde	0.57 ± 0.08 b
NIA-M30	2.89 ± 0.19 c	2.89 ± 0.29 bcd	0.56 ± 0.06 b
NIA-82	4.07 ± 0.40 b	2.96 ± 0.16 bc	0.52 ± 0.04 bc
Glandless-1 (Susceptible Check)	4.95 ± 0.26 a	3.70 ± 0.13 a	0.78 ± 0.08 a

These are the means of the season in which population of the sucking insect pests were recorded  
Means sharing similar letters in columns are not significantly different by LSD test at the 0.05 probability level ( $P > 0.05$ )

### Morpho-biochemical HPR traits in different genotypes of cotton

Highly significant differences among cotton genotypes were observed for the studied traits including leaf area, leaf glanding, total soluble sugars and proteins content, total flavonoids content, total phenols content, and seed cotton yield except tannins content, which does not differ significantly in most materials (Table 2). Leaf area of studied genotypes ranged from 88.68 cm<sup>2</sup> for NIA-M30 to 55.88 cm<sup>2</sup> for NIA-82. NIAB-112 possessed higher leaf gossypol glanding (5.00) followed by NIAB-878 (4.87), NIA-H24 (4.80) and NIAB-Kiran (4.67). Glandless-1 genotype showed very few leaf gossypol glands (0.67). However, the Glandless-1 (the susceptible check) showed higher total soluble sugars content, i.e., 22.67 mg·g<sup>-1</sup>, followed by NIA-82 (17.38 mg·g<sup>-1</sup>), NIA-H24 (15.27 mg·g<sup>-1</sup>), NIAB-112 (15.07 mg·g<sup>-1</sup>), NIA-HM48 (10.87 mg·g<sup>-1</sup>), NIAB-878 (9.53 mg·g<sup>-1</sup>), and NIAB-Kiran (8.54 mg·g<sup>-1</sup>). Total soluble proteins content was ranked in decreasing order as NIA-82 (57.09 mg·g<sup>-1</sup>) followed by Glandless-1 (55.55 mg·g<sup>-1</sup>), NIA-M30 (48.11 mg·g<sup>-1</sup>), NIAB-878 (30.22 mg·g<sup>-1</sup>), and NIAB-Kiran (27.11 mg·g<sup>-1</sup>). NIAB-Kiran showed higher total phenols content (36.56 mg·g<sup>-1</sup>) and tannins content (3.30 mg·g<sup>-1</sup>). Next to NIAB-Kiran, NIAB-878 showed 34.58 mg·g<sup>-1</sup> of total phenols content and 3.13 mg·g<sup>-1</sup> of tannins content. Total flavonoids content of NIAB-Kiran (13.10 mg·g<sup>-1</sup>), NIAB-878 (11.21 mg·g<sup>-1</sup>), and Sadori (9.80 mg·g<sup>-1</sup>) were higher than that of the susceptible check Glandless-1. Overall, considerable variations existed among genotypes for studied HPR traits.

### Correlation between morpho-biochemical HPR traits and the sucking insects population

Correlations among studied HPR traits and sucking insect pests are presented in Table 3. Leaf gossypol glanding, total phenolics, tannins and total flavonoids had a significant and negative relation with the whitefly infestation, whereas total soluble sugar content and total soluble proteins content correlated positively with the whitefly population. There was no significant correlation between leaf area and the whitefly population. Similar trend for correlation of studied HPR traits with thrips and jassid infestation were observed.

### Effect of sucking insect pest's population on seed cotton yield of different cotton genotypes

Sadori produced seed cotton yield of 2 237 kg·ha<sup>-1</sup> followed by NIAB-878 (2 209 kg·ha<sup>-1</sup>), NIAB-Kiran (2 186 kg·ha<sup>-1</sup>), and NIA-Bt. 30 (2 129 kg·ha<sup>-1</sup>). The susceptible check, i.e., Glandless-1 produced seed cotton yield of 1 011 kg·ha<sup>-1</sup> (Table 2). As correlation of whitefly with seed cotton yield is concerned, it had strong and negative relation with seed cotton yield. Jassid infestation

had a highly strong and negative association with seed cotton yield, too (Table 3). Overall, above threshold level of sucking insects significantly reduces seed cotton yield. Only genotypes, Sadori, NIAB-878, NIAB-Kiran, and NIA-Bt.30 showed less penalty on seed cotton yield under severe attack of sucking insects.

### Discussion

The sucking insect pest population exceeded threshold level during June and July. This trend persisted from the last couple of years and expected to continue in the coming future. This is mainly due to the favorable weather conditions (high temperature and relative humidity) for sucking insects, lack of resistant cultivars and pesticide resistance (Amjad and Aheer 2007; Razaq *et al.* 2013; Nemade *et al.* 2018). In present study, two peaks of the whitefly population have been observed during the growing season. The possible reason for this fluctuation might be the change in weather condition. As whitefly is a highly movable insect, most influenced by rainfall, humidity and wind speed specifically in our field conditions (Tando Jam, Pakistan). Population fluctuation trend observed in present study is also in line with the results of Arshad and Anjum (2010) and Saleem *et al.* (2018) who observed sucking insects peak infestation in late July. In present investigation, 12 genotypes and cultivars including one susceptible check Glandless-1 were evaluated against sucking insects. Significant differences among genotypes were observed for sucking insects per leaf. The cultivars NIAB-Kiran, NIAB-878 and Sadori were less attacked by sucking insect pest complex. Several studies have been carried out to evaluate the response of cotton germplasm to sucking pest's resistance (Khalil *et al.* 2017; Pathan *et al.* 2007; Saleem *et al.* 2018). Aslam and Saeed (2004) evaluated 22 cotton genotypes for their comparative resistance to whitefly, jassid and thrips where the minimum mean population of whitefly was 0.5 per leaf on genotype BH-121 and CRIS-467, thrips 0.8 per leaf on CIM-499 and jassid 0.6 per leaf on MNH-635.

HPR investigation of resistant resources from present study has revealed the mechanism behind their resistance to sucking insect infestation. The sucking insects population negatively correlated with leaf gossypol glands, which is in agreement with the findings of Arif *et al.* (2006), Irfan *et al.* (2008) and Khalil *et al.* (2017) who observed leaf gossypol glands as a source of resistance against whitefly, thrips and jassid. This negative association indicates the presence of high gossypol content in gossypol glands present on the leaves of studied genotypes. However, the results of Khan *et al.* (2010) and Zia *et al.* (2011) were contradictory, as they observed positive response of whitefly with gossypol contents. This might be due to

**Table 2** Morphological and biochemical host plant resistance traits in different genotypes of cotton

Genotypes	Leaf area /cm <sup>2</sup>	Leaf glanding	Total soluble sugars content / (mg.g <sup>-1</sup> )	Total soluble proteins content / (mg.g <sup>-1</sup> )	Total phenolic content / (mg.g <sup>-1</sup> )	Tannin content / (mg.g <sup>-1</sup> )	Total flavonoids content / (mg.g <sup>-1</sup> )	Seed cotton yield / (kg.ha <sup>-1</sup> )
Glandless-1 (Susceptible check)	73.49 cd	0.67 g	22.67 a	55.55 a	14.89 g	2.05 c	04.47 f	1 011 d
NIA-82	55.88 g	3.00 f	17.38 b	57.09 a	22.97 f	2.56 bc	05.42 ef	1 398 c
NIA-M30	88.68 a	4.13 de	14.07 cd	48.11 b	25.97 ef	3.06 ab	07.78 cde	1 547 c
Sadori	62.70 f	4.00 e	12.24 def	35.22 efg	28.97 de	3.04 ab	09.80 bc	2 237 a
NIA-H67	69.68 de	4.33 cde	12.37 cdef	32.78 gh	30.24 cd	3.17 ab	07.83 cde	1 478 c
NIA-Bt.30	73.80 cd	4.53 bcd	12.90 cde	45.90 bc	30.50 cd	3.10 ab	06.85 def	2 129 ab
NIA-H32	58.76 fg	4.07 e	14.02 cd	34.42 fg	31.04 cd	2.99 ab	08.82 bcd	1 409 c
NIA-H24	80.87 b	4.80 ab	15.27 bc	37.67 ef	31.80 bcd	3.18 ab	07.45 cde	1 461 c
NIAB-112	71.27 d	5.00 a	15.07 bcd	39.00 de	32.24 bcd	3.01 ab	08.69 bcd	1894 b
NIA-HM48	81.78 b	4.20 de	10.87 efg	42.78 cd	32.85 bc	3.20 ab	10.17 bc	1 618 c
NIAB-878	79.62 bc	4.87 ab	09.53 fg	30.22 hi	34.58 ab	3.13 ab	11.21 ab	2 209 a
NIAB-Kiran	63.51 ef	4.67 abc	08.54 g	27.11 i	36.56 a	3.30 a	13.10 a	2 186 a

Means sharing similar letters in columns are not significantly different by LSD test at the 0.05 probability level ( $P > 0.05$ )

**Table 3** Correlation coefficient values between sucking insect pests population (number/leaf) and morpho-biochemical host plant resistance traits across different cotton genotypes

Host plant resistance traits	Whitefly	Thrips	Jassid
Leaf area /cm <sup>2</sup>	0.020 <sup>ns</sup>	0.153 <sup>ns</sup>	0.132 <sup>ns</sup>
Leaf glanding	-0.865 <sup>a</sup>	-0.836 <sup>a</sup>	-0.730 <sup>a</sup>
Total soluble sugars content /(mg·g <sup>-1</sup> )	0.887 <sup>a</sup>	0.661 <sup>b</sup>	0.856 <sup>a</sup>
Total soluble proteins content /(mg·g <sup>-1</sup> )	0.883 <sup>a</sup>	0.729 <sup>a</sup>	0.622 <sup>b</sup>
Total phenolic content /(mg·g <sup>-1</sup> )	-0.915 <sup>a</sup>	-0.838 <sup>a</sup>	-0.776 <sup>a</sup>
Tannin content /(mg·g <sup>-1</sup> )	-0.870 <sup>a</sup>	-0.723 <sup>a</sup>	-0.701 <sup>a</sup>
Total flavonoids content /(mg·g <sup>-1</sup> )	-0.870 <sup>a</sup>	-0.694 <sup>b</sup>	-0.773 <sup>a</sup>
Seed cotton yield /(kg·ha <sup>-1</sup> )	-0.825 <sup>a</sup>	-0.684 <sup>b</sup>	-0.929 <sup>a</sup>

*ns* Non-significant

<sup>a</sup>Correlation is significant at the 0.01 level

<sup>b</sup>Correlation is significant at the 0.05 level

the presence of low gossypol content in the gossypol glands of the studied genotypes. Similarly, total phenolics and tannins content had negative correlation with sucking insect's infestation which is in confirmation with Butter *et al.* (1992) and Perveen *et al.* (2001) who advocated the role of phenolics content in cotton plant defense against different insects. They observed direct correlation between insect attack and total phenols and tannins content. Butter *et al.* (1992) identified that cultivars with greater total phenolic and tannin contents in leaves support fewer whitefly eggs. Dixit *et al.* (2017) reported that phenolics accumulation upon insect infestation varied in cotton genotypes. Phenols are kinds of plant secondary metabolites involved in plant defence against insects (War *et al.* 2012). They either directly affect insect growth and development or indirectly by acting as oviposition deterrents (Dixit *et al.* 2017). However, these results are in contradiction with Acharya and Singh (2008) who reported non-significant negative correlation of total phenol and tannin with the whitefly population. No specific reason was provided for this non-significant negative correlation, but one can infer that there may be some other defensive traits contributing in host plant resistance of these genotypes.

In our study, total flavonoids content also exhibited negative correlation with the sucking insect's population and this is in confirmation with the results of Butter *et al.* (1992) who observed negative correlation of total flavonoids content with the population density of whitefly. In present study, total soluble sugars and proteins content were positively correlated with the population densities of whitefly, thrips and jassid. Genotypes with high soluble sugars and proteins content were observed to be more susceptible to the sucking insect pests. These results are in confirmation with Athar *et al.* (2011) and Sonalkar (2020) who reported positive association of leaf soluble proteins and sugars content with whitefly infestation.

However, Butter *et al.* (1992) observed negative correlation between total sugars content and population density of whitefly. Involvement of total soluble sugars content, proteins content and some micronutrients in having more insects feeding on plants has been reported in different plant insect interaction studies (Athar *et al.* 2011; Sonalkar 2020). As for as yield is concerned, Sadori produced maximum seed cotton yield of 2 237 kg·ha<sup>-1</sup> followed by NIAB-878 (2 209 kg·ha<sup>-1</sup>), NIAB-Kiran (2 186 kg·ha<sup>-1</sup>) and NIA-Bt.30 (2 129 kg·ha<sup>-1</sup>). Our results showed that, NIA-Bt.30 have higher yield but lower phenolics, tannins, flavonoids and higher soluble proteins content compared with NIA-HM48. This might be also due to high gossypol content because NIA-Bt.30 leaves possess higher gossypol glanding and less insect infestation as compared with NIA-HM48.

## Conclusion

Populations of the sucking insects, viz., whitefly, thrips and jassid are influenced by numerous biochemical constituents present in cotton genotypes, positively or negatively. Cotton varieties less attacked/preferred by these pests would help in pest management of cotton. It can be concluded that considerable variation exists among cotton genotypes having morpho-biochemical host plant resistance traits, which can be deployed for the breeding of sucking insect pest resistant cultivars.

## Authors' contributions

Rizwan M conceived the idea, recorded data in field and laboratory, prepared draft and finalized the manuscript. Abro S supervised the work, helped in the execution of field experiment. Asif MU recorded insect pest data and helped in data analysis. Hameed A helped in the estimation of biochemical parameters. Mahboob W helped in formulating methodology for estimation of biochemical parameters. Deho ZA helped in the execution of field experiment. Sial MA validated the idea and provided technical inputs in writing the manuscript and proof reading. The author(s) read and approved the final manuscript.

## Availability of data and materials

The data generated and materials used in this study are available from the corresponding author on reasonable request.

## Declarations

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

All co-author has consent for submission of manuscript.

### Competing interests

The authors declare that there are no competing interests.

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