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Full Length Article

Resistance to Thrips (*Thrips tabaci*) in Bt Cotton Genotypes in Punjab, Pakistan, Based on Population Dynamics and Plant Morpho-chemical Properties

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Abstract

The present study was conducted to evaluate twenty Bt-cotton genotypes for their resistance to thrips, *Thrips tabaci* (Lind.) under field conditions in Faisalabad, Pakistan. Thrips population seedling^{-1} and leaf^{-1} was recorded from each genotype in 2008 and from these data, six genotypes were selected for final screening trial in 2009. Thrips density per leaf was correlated with abiotic factors, morphological and chemical characteristics to establish basis of resistance mechanism. Results showed that AA-802 and VH-259 with 3.40 and 4.45 thrips per leaf were resistant and susceptible genotypes, respectively and two had significant difference between each other ($p<0.05$). Weather factors were non-significantly correlated with leaf thrips populations except relative humidity, which was positively and significantly correlated ($r=0.451$; $p = 0.014$). Hair density on leaf midrib ($r=0.850$) and vein ($r=0.762$) were positively whereas gossypol gland on leaf lamina ($r=0.462$) were positively correlated with leaf^{-1} thrips population. Moisture contents, total minerals, protein, lipids, reducing sugar, calcium, magnesium, and zinc were significantly correlated with thrips population. These findings are discussed in comparison with basis of resistance on non-Bt-cotton varieties reported earlier, in the study area.

Keywords: Bt-cotton; Resistance; Thrips; Morphology; Chemistry; Abiotic factors

Introduction

Thrips tabaci (Lind.), is the sucking insect pest in cotton growing areas of Sindh and Punjab in Pakistan. alongwith Jassid (*Amrasca devastans* Dist.) and whitefly (*Bemisia tabaci* Gen.). Since its attack on lower side of cotton leaves start with beginning of growth of cotton, so farmers are advised to be vigilant as attack's severity increases in dry weather. A study in Pakistan revealed 37.6% loss in yield of seed cotton by combined attack of Thrips (14.6 leaf^{-1}) and jassid (4.6 per leaf^{-1}) (Attique and Ahmad, 1990). Two species of thrips viz., *Thrips tabaci* (Lind.) and *Scirtothrips dorsalis* Hood have been recognized, but *T. tabaci* is considered ubiquitous (Naqvi *et al.*, 1988).

The highest and lowest population of thrips (4.28 and 2.21 leaf^{-1}, respectively) was found on Empire WRD and Rode Okra varieties of cotton (Syed *et al.*, 2003). Genotypes CRIS-168, CRIS-9, NIAB-78 and CIM-482 were moderate in degree of resistance against insect pest complex whereas CRIS-468 was highly resistant having maximum yield of 1021 Kg ha^{-1} (Pathan *et al*., 2007). The population of thrips on the cultivar SLH-257 resisted thrips' population to a greater extent (5.01 leaf^{-1}) (Salman *et al*., 2011). Among nine varieties of cotton viz., CRIS-125, CRIS-9, B.T, CIM-506, DNH-105, CIM-554, BH-167, GOMAL-93 and DNH-57; DNH-105 and CIM-506 were found relatively resistant to sucking insect pest as they showed least infestation and higher seed cotton yield (Khan *et al*., 2011).

Non Bt-cotton varieties despite possessing morpho-chemical characters for resistance to sucking insect pests demanded heavy application of insecticides to prevent their populations reaching to injury level (Syed *et al*., 1996; Rafiq and Shah, 1998; Aheer *et al*., 1999; Syed *et al*., 2003; Abro *et al*., 2004; Arif *et al*., 2004, 2006; Ali and Aheer, 2007). In one of above studies, hairiness and gossypol contents in cotton leaves are important in this regard. Hair density on midrib of leaves of CIM 109 played a positive and highly significant role towards thrips population. Length of hair on veins exerted negative and significant correlation with thrips population. Gossypol glands on midrib and veins were correlated significantly showing negative response to thrips population (R^2 value for this effect was 0.756) (Arif *et al*., 2004).

These characters have also been recognized in Bt varieties. Genetically engineered cotton, *Gossypium hirsutum* L., genotypes (*Bt*-121, *Bt*-196, *Bt*-313, *Bt*-333, *Bt*-496, *Bt*-703, *Bt*-802, *Bt*-1524, *Bt*-3701, *Bt*-W1) were evaluated for their resistance against whitefly, *Bemisia tabaci* (Genn.), during the cropping seasons 2008 and 2009. In 2008, lamina thickness and gossypol glands were

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positively correlated with mean *B. tabaci* population $leaf^{-1}$ (r= 0.66; r = 0.67, respectively), whereas in 2009, hair length and gossypol glands showed negative and positive correlations, respectively (r = -0.65; r = 0.78) (Khan *et al.*, 2010). There are no studies on Bt resistance against thrips. The present study was, thus, conducted to find out the role of biotic and abiotic factors for resistance in Bt-cotton varieties to *Thrips tabaci* under field conditions.

Materials and Methods

The experiment was conducted on resistance of Bt-cotton varieties to thrips (*T. tabaci*) at a farmer's fields in chak No. 60 JB, Shahbazpur, District Faisalabad, Punjab, Pakistan and in the Laboratories of Entomological Research Institute, Faisalabad, from 2008 to 2010, for correlation with morphological and chemical characters of cotton plants with thrips population.

Host Plant Resistance

Preliminary screening trial: This study was conducted during 2008 to screen the genotypes for sorting out Randomized Complete Block Design (RCBD) with three replications. The plot size was kept at 7.64-m × 9.17-m and row to row distance 75 cm. Twenty genotypes of Bt-cotton viz., AA-802, BH-178, MN-121, Bt-2131, Bt-141, CBS-1, FH-114, FH-113, FH-4243, GM-2085, IR-3, IR-4, IR-901, IR-824, IUB-212, IUB-222, IUB-2009, Subhan-2001, Tarzan-2 and VH-259 were sown on May 1, 2008 and based on per seedling and leaf density data of the pest 6 genotypes (VH-259, Tarzan, BH-178, FH-4243, GM-2085 and AA-802) were selected for final screening.

Final Screening Trial

In this trial, six abovementioned genotypes, every two, VH-259 and Tarzan-2, AA-802 and GM-2085 and BH-178, and FH-4243 showing susceptible, resistant and intermediate response were sown on May 13, 2009 in the same farmer's field, where preliminary study was conducted, following RCBD with three replications.

Data Collection

Data regarding thrips population were recorded at weekly intervals by randomly selecting 10 seedlings from each plot and by selecting 15 leaves from randomly selected five plants in such a way that one leaf from top portion of 1^{st} plant, second leaf from middle portion of 2^{nd} plant and third leaf from bottom portion of 3^{rd} plant and so on.

Collection of weather data for the studies on effect of abiotic factors: The data on temperatures, relative humidity and rainfall were obtained from the observatory of Physiology Division, Agronomy Research Institute, Faisalabad, which were processed for simple correlation simple and multiple linear regression analyses to ascertain the contribution of these factors individually and in their combinations on the population fluctuation of thrips for both the study years i.e., 2008 and 2009. The IBM Compatible Computer with M-Stat package was used for the analysis.

Morphological and Chemical Characters of Bt-cotton Plants in Relation to Effect on Thrips Population

Hair density: One leaf each from top, middle and bottom portion of three plants was plucked and the numbers of hairs were counted under a stereo binocular microscope. The number of hair was counted from midrib, vein in 10 mm length and lamina in 1 cm^2 iron ring square.

Length of hair: Length of hair was measured by an ocular micrometer from midrib, vein and lamina by counting six hairs using stereo binocular microscope in the samples used for counting hair density.

Thickness of leaf lamina: A cross section was made with the help of a fine sharp razor and the thickness of leaf lamina was measured from three different places of each leaf under a stereo binocular microscope with the help of an ocular micrometer. One leaf each from top, middle and bottom portion of three plants was taken into account.

Gossypol glands: One leaf each from top, middle and bottom portion of three plants was plucked and the number of gossypol glands was counted under a stereo binocular microscope, from midrib, vein and lamina from three different places of each leaf.

Chemical Factors

Leaf moisture contents: Three leaf samples each of 10 gm each, from the top, upper and lower parts of various plants were plucked from every plot. All leaves, under experiment, were cleaned with distilled water and placed on tissue paper unless moisture on leaves drained out, weighed, classified and kept into a drying oven, run at (100±5°C), for 12 h. The dry matter of leaves was weighed and put back into the oven, at the same temperature, for another six hours. After the weight of the dry material, became constant, the moisture percentage was calculated, according to the following formula:

$$\text{Moisture contents (\%)} = \frac{\text{Weight of fresh leaves} - \text{Weight of dry leaves}}{\text{Weight of fresh leaves}} \times 100$$

Samples weighing 500 grams of top, bottom and middle leaves of each selected genotype of Bt-cotton were taken from each plot on August 07, 2009. These samples were brought in to the laboratory, washed with distilled water and kept into open air under shade for 3 h. These were then dried in a drying oven run at 70 ± 5°C for 12 h. The oven-dried material was cut into pieces, and passed through 1-mm mesh sieve. The samples were stored in dry polyethylene bags for working out their chemical analysis.

Total Minerals and N along with protein contents were determined by methods of Ranganna (1977) and Winkleman *et al.* (1986). Lipids and Reducing Sugars were determined

Table 1: Comparison of thrips population seedling^{-1} and leaf^{-1} on different genotypes of Bt-cotton in 2008

Genotypes	Thrips population seedling^{-1}	Thrips population leaf^{-1}
AA-802	0.33 e	2.04 g
BH-178	1.38 bc	4.48 c
MN-121	0.30 e	2.16 fg
Bt-131	0.83 de	3.59 e
Bt-141	0.87 cd	3.57 e
CBS-1	0.77 de	2.53 e
FH-114	1.67 ab	4.59 bc
FH-113	0.30 e	2.28 f
FH-4243	0.93 cd	3.78 d
GM-2085	0.32 e	2.12 g
IR-3	1.70 ab	4.59 bc
IR-4	1.88 ab	4.54 bc
IR-901	1.87 ab	4.55 bc
IR-824	1.05 cd	3.73 d
IUB-212	1.82 ab	4.54 bc
IUB-222	1.12 cd	3.75 d
IUB-2009	2.05 a	4.61 bc
SAEBAN-2001	1.85 ab	4.76 a
TARZAN-2	1.85 ab	4.63 b
VH-259	1.75 ab	4.77 a
	LSD, 0.476; SE,±0.169	LSD, 0.118; SE, ±0.042

Means sharing similar letters are not significantly different by DMR Test at $p<0.05$

Table 2: Comparison of Thrips Population seedling^{-1} and leaf^{-1} on various selected genotypes of Bt-cotton during 2009

Genotype	Thrips population	
	seedling^{-1}	leaf^{-1}
VH-259	1.31 a	4.45 a
Tarzan-2	1.14 b	4.36 b
BH-178	0.91 c	3.95 c
FH-4243	0.88 c	3.81 d
GM-2085	0.58 d	3.54 e
AA-802	0.52 d	3.40 f
	SE±0.045	SE±0.019
	LSD=0.128	LSD = 0.053

Means sharing similar letters are not significantly different in a column by DMR Test at P=0.05

by methods of Firestone (1995) and AOAC (1975), respectively. Micro and Macro nutrients were carried out by Wet Digestion of plant tissue for macro and micro nutrient analysis following methods of Jackson (1958), Wright and Stuczynski (1996).

Results and Discussion

Preliminary Screening of Bt-Genotypes in 2008

The genotype FH-113 having minimum population (0.30 seedling^{-1}) did not differ significantly from those observed on MN-121, GM-2085, AA-802, CBS-1 and BT-131 with 0.30, 0.32, 0.33, 0.77 and 0.83 thrips, respectively. The genotype IUB-2009 with maximum population (2.05 seedling^{-1}) differed significantly from BH-178 (1.38), IUB-222 (1.12), IR-824 (1.05), FH-4243 (0.93) and BT-141 (0.87). The latter genotypes represent intermediate number of thrips. The genotype VH-259 with maximum population of thrips per leaf^{-1} (4.77) did not show significant difference with Saeban-2001 (4.76 thrips leaf^{-1}). The genotype AA-802 was comparatively resistant showing minimum population of thrips leaf^{-1} (2.04) and did not show significant difference with those of found on MN-121 and GM-2085 with 2.16 and 2.12 thrips, respectively (Table 1).

Final Screening in 2009

VH-259 showed maximum thrips population (1.31 seedling^{-1}) and had statistical difference with all other genotypes. Tarzan-2 categorized as second susceptible genotype showing 1.14 thrips seedling^{-1} and also differed significantly with all other genotypes. The genotypes AA-802 and GM-2085 had significantly least thrips counts (0.52 and 0.58 seedling^{-1}). In between former two genotypes, BH-178 and FH-4243, (0.91 and 0.88 thrips seedling^{-1}, respectively) were statistically at par with each other. Varietal difference based on thrips leaf^{-1} showed that all the genotypes had significant difference among them, being maximum (4.45 thrips laef^{-1}) on VH-259 and minimum (3.40) on AA-802 (Table 2).

These results are largely in confirmation to the earlier studies with which level of infestation (thrips seedling^{-1} and laef^{-1}) and period of infestation were comparable (Syed *et al*., 1996; Rafiq and Shah, 1998; Aheer *et al*., 1999; Syed *et al*., 2003; Abro *et al*., 2004; Arif *et al*., 2004, 2006; Ali and Aheer, 2007). However, minor differences in these parameters may be due to different ecological conditions.

The data recorded at different times of the season showed that thrips had a peak at in August 31, 2008 and this month seems the most favorable period for the development of the pest (data not shown). The present findings are not in conformity with those of Nizamani *et al*. (2002) who reported that the population of thrips rapidly increased between June 27 June to July 11 and then sharply declined up to 8th August, which is contrast to that reported here. The present findings are in conformity with those of Sewify *et al*. (1996) who reported that maximum population densities of sucking insect pests occurred during July, August and September, whereas Gupta *et al*. (1997) observed that the highest population of thrips during the last week of July to mid August. In 2009, the month of August was favorable for thrips population peak, population trend being similar to the one year that in 2008. The variation of peak population may be due to genotypes used in those studies.

Population of Thrips per leaf in relation to Weather Factor

Relative humidity in 2009 and on cumulative basis (2008 and 2009) was positively and significantly correlated with thrips' population ($p<0.05$). Temperatures and rainfall had non-significant relation with pest population (Table 3). The previous studies have shown non-significant and negative relation of relative humidity with thrips population (Ali *et al*., 1993; Patel *et al*., 1997; Khan *et al*., 2008).

Table 3: Relationship of weather factors with population fluctuation of thrips in 2008 and 2009 on Bt-Cotton genotypes

Year	Parameter	Temperature (°C)			RH (%)	Rainfall (mm)
		Maximum	Minimum	Average		
2008	r-value	0.343	0.258	0.399	0.103	-0.052
	SE	±0.135	±0.229	±0.112	±0.416	±4.219
	P-value	0.274	1.00	0.198	1.00	1.00
2009	r-value	-0.277	0.081	0.065	0.555*	0.013
	SE	±0.314	±0.399	±0.266	±0.667	±2.677
	P-value	0.282	1.00	1.00	0.020	1.00
Cumulative	r-value	-0.153	0.130	0.103	0.451**	0.004
	SE	±0.257	±0.329	±0.215	±0.625	±3.299
	P-value	1.00	1.00	1.00	0.014	1.00

* = Significant at $P \leq 0.05$; ** = Significant at $P \leq 0.01$; r = Correlation Coefficient

Table 4: Relationship of morphological characters with population fluctuation of thrips in final selected genotypes of Bt-Cotton

Plant Characters		Parameters		
		r value	SE	p value
Hair Density	midrib	0.855**	±2.81	0.00
	vein	0.762**	±0.439	0.00
	lamina	0.405 ns	±1.94	0.09
Length of Hair	midrib	-0.238 ns	±1.64	1.00
	vein	0.388 ns	±6.88	0.169
	lamina	-0.059 ns	±3.87	1.00
Thickness of leaf lamina		-0.164	±10.64	1.00
Gossypol Glands	midrib	-0.189	±0.18	1.00
	vein	0.114	±0.16	1.00
	lamina	-0.462*	±0.20	0.05

* = Significant at $P \leq 0.05$; ** = Significant at $P \leq 0.01$; ns = Non-significant; r = Correlation Coefficient; SE = Standard Error; p = Probability Value

Table 5: Linear Regression Models for effect of cotton morphological characters on thrips population of in finally selected genotypes of Bt-Cotton

Significance	Regression Equation	R^2	100-R^2	Impact (%)	SE	F value
**Y	$1.36 + 0.15X_1$**	0.730	73.0	73.00	0.05	43.31
**Y	$1.31 + 0.38X_1$**- $0.19X_2$**	0.819	81.9	8.90	0.04	33.87
**Y	$2.11+0.34X_1$**- $0.16X_2$**-$0.11X_3$**	0.888	88.8	6.9	0.03	36.82

X_1 = Hair Density on Midrib (cm^{-1}); X_2 = Hair Density on Vein (cm^{-1}); X_3 = Gossypol Glands on Lamina (cm^{-2}); R^2 = Coefficient of Determination; SE = Standard Error; ** = Significant at $P \leq 0.01$

The present results of non significant relation of temperatures and rainfall have been contradicted by Li *et al*. (1992), Panicker and Patel (2001), Khan *et al*. (2008) and Shivanna *et al*. (2009), however Ali *et al*. (1993) had reported non-significant relation of temperatures with thrips density. In the present study rainfall showed non significant effect on the population of thrips. The present findings are contradicted with the findings of Mabbett *et al*. (1984) who reported that heavy rain provided appreciable natural control of thrips on cotton. Similarly Ali *et al*. (1993) reported negative and significant correlation between rainfall and thrips density, while Khan *et al*. (2008) reported non-significant positive correlation and Shivanna *et al*. (2009) found negative correlation.

Morphological Characters and Pest Population

Hair density on leaf midrib and vein were significantly correlated with thrips population, whereas non-significantly on leaf lamina, though latter relation was positive. The length of hair and thickness of leaf lamina had non-significant relation. Gossypol glands on leaf lamina had negative relation with thrips population significantly than on leaf midrib and vein (Tables 4 and 5). Hair density on midrib and vein and gossypol glands on lamina affected pest population (100 R^2 = 88.8).

The present findings are in conformity with those of Quisenberry and Rummel (1979) who reported that pilose was associated with high level of resistance to thrips. The present findings are also in conformity with those of Riaz *et al*. (1987) who also reported positive correlation between thrips population and hair density on leaf vein. Contrary to Ali *et al*. (1995), the gossypol glands on lamina showed negative and significant correlation with the pest density while these glands on vein and midrib were not as important as above.

Chemical Characters and Pest Population

Moisture contents, nitrogen, protein, lipids, reducing sugars, calcium, magnesium, and zinc were positively and significantly related with thrips population, whereas total

Table 6: Relationship of chemical plant characters with population fluctuation of thrips in final selected genotypes of Bt-Cotton

Plant Characters	Parameters		
	r-value	SE	p value
Moisture Contents	0.571**	±0.02	0.01
Total Minerals	-0.864**	±0.12	0.00
Nitrogen	0.837**	±0.02	0.00
Protein	0.837**	±0.04	0.00
Lipids	0.807**	±0.18	0.00
Reducing Sugar	0.501*	±0.25	0.03
Calcium	0.890**	±0.07	0.00
Magnesium	0.508*	±0.01	0.03
Phosphorous	-0.216 ns	±5.97	1.00
Potassium	0.068 ns	±0.13	1.00
Copper	0.094 ns	±2.01	1.00
Zinc	0.849**	±0.15	0.00
Manganese	0.299 ns	±0.33	0.23
Ferrous	-0.219	±0.13	1.00

*= Significant at $p \leq 0.05$; **= Significant at $p \leq 0.01$; r= Correlation Coefficient; ns = Non-significant; SE = Standard Error; p = Probability Value

Table 7: Linear Regression Models for effect of chemical characters on thrips population of in finally selected genotypes of Bt-Cotton

Significance	Regression Equation	R^2	$100-R^2$	Impact (%)	SE	F value
**Y	$-14.57+1.84X_1$**	0.328	32.8	32.8	0.081	7.81
**Y	$6.25–1.16X_1+3.81X_2$**	0.743	74.3	41.5	0.052	21.72
**Y	$6.38–1.15X_1+2.89X_2+0.33X_3$	0.745	74.5	0.2	0.053	13.61
**Y	$3.03–0.66X_1+4.12X_2–0.64X_3+0.15X_4$**	0.852	85.2	10.7	0.042	18.75
**Y	$3.4–0.7X_1+5.45X_2–1.24X_3+0.17X_4$**$+0.06X_5$	0.865	86.5	1.3	0.042	15.39
**Y	$1.21–0.33X_1+4.76X_2–1.26X_3+0.11X_4+0.06X_5+0.19X_6$	0.874	87.4	0.9	0.042	12.73
**Y	$1.31–0.33X_1+4.77X_2–0.98X_3+0.12X_4+0.06X_5+0.09X_6–1.12X_7$	0.878	87.8	0.4	0.044	10.27
**Y	$5.77–0.60X_1+4.28X_2–1.19X_3+0.15X_4+0.04X_5–0.11X_6–2.23X_7+0.28X_8$	0.884	88.4	0.6	0.045	8.56
**Y	$0.95–0.22X_1+3.23X_2–0.56X_3+0.16X_4+0.01X_5–0.03X_6–3.37X_7+0.41X_8+0.27X_9$	0.898	89.8	1.5	0.045	7.82

X_1 = Moisture Contents (%); X_2 = Nitrogen (%); X_3 = Protein (%); X_4 = Lipids; X_5 = Reducing Sugars; X_6 = Calcium; X_7 = Magnesium; X_8 = Zinc; X_9 = Total Minerals; ** = Significant at $P \leq 0.01$

minerals were negatively significantly related (Tables 6 and 7).

It is to be noted that all the chemicals were significantly enhanced in the resistant varieties (data not shown). The present findings are in conformity with those of Zia and Chaudhry (1993) who reported that moisture contents in the cotton leaves had significant and positive correlation with the thrips population. The present findings are contradicted with Ali *et al*. (1995) who reported that moisture in the leaves of various genotypes of cotton was not important for thrips.

This study has indicated clear differences in morphological and chemical characters in tested Bt genotypes, which helped them resist thrips population so that injury levels can be reduced. Environment has played a little role in contributing to the resistance. Infestation levels on these Bt genotypes will provide baseline data for further screening as market is flooded with selection of Bollgaurd I type genotypes. These studies will also help to reset economic level for curative and preventive measures and integration of insecticides with other control measures.

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