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Full Length Research Paper

Morphological and chemical characteristics of tomato foliage as mechanisms of resistance to *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) larvae

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Morphological characters and chemical composition of tomato (*Lycopersicon esculentum* Miller) leaves were measured and compared among nine tomato varieties (Roma VFN, NARC-1, Fs-8802, Tommy, Pant Babr, Rio Grande, Nova Mecb, Pakit and Sahil) exhibiting varying levels of host plant resistance to *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) as based on fruit infestation. The variety, Sahil, was resistant, whereas Roma VFN was the susceptible variety. Hair length and hair density on lower leaf surface, as well as thickness of leaf lamina significantly correlated with larval population and fruit infestation. Leaf hair density accounted for 92.0% of the variation in fruit infestation and 77.0% of the variation in larval population. Ferrous (Fe^{2+}) and phosphorous content in the leaves were negatively correlated with fruit infestation and larval population; whereas, nitrogen, calcium, magnesium, manganese and zinc content were positively correlated with fruit infestation and larval population. The resistant variety, Sahil, produced the maximum yield as compared to susceptible variety, Roma VFN.

Key words: *Helicoverpa armigera*, tomato fruit borer, host plant resistance, *Lycopersicon esculentum*.

INTRODUCTION

Tomato (*Lycopersicon esculentum* Miller) is an important solanaceous crop grown throughout the world, including Pakistan where total production was 468,146 MT on 46,200 ha in 2005 to 2006 (Agricultural Statistics of Pakistan, 2007). Yield of Pakistan-grown tomatoes is limited by arthropod pests including *Helicoverpa* sp., *Spodoptera* sp., and tomato russet mites [*Aculops lycopersici* (Massee)] (Hartz et al., 2008). *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) feeds primarily on the tomato fruit causing reported yield losses as high as 35% in Pakistan (Ahmad and Mohsin, 1969; Latif et al., 1997) and 38% in neighboring India (Selvanarayanan and Narayanasamy, 2006). Insecticides use to manage *H. armigera* poses health, safety, environmental and economic concerns. Host plant resistance has proven to be a valuable tool for crop protection (Kennedy, 1984) and may provide a viable pest management tactic for managing *H. armigera* in Pakistan-grown tomatoes (Jallow et al., 1998), with reducing deleterious effects of uncontrolled use of pesticides.

Cultivation of *Helicoverpa*-resistant tomato cultivars is limited due to a lack of data on potential genetic sources and plant mechanisms (antixenosis) of resistance. Later-instar *H. armigera* larvae feed on developing tomato fruit, while neonates and early instars usually start feeding on tender leaves before moving to the fruit (Liu et al., 2004; Perkins et al., 2009) and are often impacted by leaf characteristics (Sheloni et al., 2010, Simmons et al., 2004). Role of physio-chemical factors is important to identify a source of resistance in plants against pests (Dhilllon et al., 2005). Based upon preliminary screening data (Sajjad and Ashfaq, unpublished data), Roma VFN, NARC-1, and Fs-8802 were found susceptible, Nova Mecb, Pakit and Sahil were resistant, and Tommy, Pant Babr, and Rio Grande exhibited intermediate levels of

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resistance based on the fruit-infestation data. Therefore, the study reported herein was undertaken to: (1) determine the relative resistance of these nine commercially-available tomato varieties to *H. armigera*, (2) correlate morphological and chemical characteristics of leaves with the observed resistance to *H. armigera*, and (3) compare marketable yield of resistant and susceptible varieties.

MATERIALS AND METHODS

Varietal infestation screening

Based upon preliminary screening data (Sajjad and Ashfaq, unpublished data), nine varieties of tomato were planted in field trials to screen for possible *H. armigera* resistance. These were; Roma VFN, NARC-1, Fs-8802, Tommy, Pant Babr, Rio Grande, Nova Mecb, Pakit and Sahil (Ali Akbar Group, Pakistan). Nursery-grown seedlings of these varieties were transplanted on the 17th of March 2008 and 15th of March 2009, in a randomized complete block design with four replications per variety. Individual plots were 6.11 × 12.23 m with a row width of 102 cm and a distance of 26 cm between adjacent plants within a row. Standard cultivation practices were used throughout the study, and no plant protection measures were used.

Plants were monitored weekly starting one week after transplanting. At each observation, the number of H. armigera larvae per plant from five randomly-selected plants in each plot was recorded. Once tomato fruit appeared on the plants, the numbers of damaged and undamaged fruits were recorded weekly from five randomly-selected plants in each plot to calculate percent of fruit infestation.

Leaf morphological factors

Three leaves (top, middle and lower portions of the plant) from each of three randomly-selected plants per plot were excised and transported to the laboratory. Six areas on the upper surface of each leaf and six areas on the lower surface of each leaf were microscopically examined. The density of leaf hairs and length of the hairs was measured at each location. In addition, a cross-section of the leaf lamina was cut using a razor blade to measure the thickness of the lamina at six locations on each leaf. All measurements were made with a Carl Zeiss™ binocular microscope equipped with an ocular micrometer. Non glandular trichomes were recorded in the present study.

Leaf chemical factors

To determine selected chemical content of the leaves, leaf samples weighing 500 g each were removed from the top and bottom portions of randomly-selected plants in each plot. These samples were transferred to the laboratory, washed with distilled water, air dried under shade for 3 h, and oven-dried at 70 ± 5°C for 12 h. The oven-dried material was cut, then ground and passed through a 1-mm sieve. These ground samples were stored in dry polythene bags. Furthermore, for each of these samples, 2 g of dried and ground leaf tissue was placed in a boron-free, silica-fused crucible and then placed in a muffle furnace at 600°C for 5 h. The combusted material was weighed and returned to the furnace for an additional 5 h. This was repeated until the material was completely combusted yielding a grayish-white ash with a constant weight. The percentage of total minerals in the leaf tissue was determined by comparison of weight of combusted ash to weight of the dried leaves.

The Kjeldahl method was used to determine % nitrogen in the leaves. Individual 0.5 g samples were removed from the dried and ground leaf tissue for digestion, distillation and titration. Magnesium and phosphorous content was determined using calorimetric methods, calcium and potassium content was determined using photometric analyses, while copper, manganese and zinc content were measured using spectrophotometric analyses. Percentage lipid content was determined using a soxhlet extractor. Carbohydrate content and fiber content were estimated using formulas based upon residuals remaining after measurement of other major components of the leaves.

Statistical analyses

Larval population and fruit damage as response to tomato varieties were subjected to analysis of variance using MSTAT statistical software. Where applicable, treatment means were separated by Tukey's honestly significant difference (HSD) tests for paired comparisons (P = 0.05). SPSS software was used for data analysis (one-way ANOVA) of morphological and chemical leaf characters (O'Connor, 2000). Larval population and fruit damage data were also correlated with the various morphological and chemical leaf factors using correlation analysis and stepwise multiple regression analysis.

RESULTS AND DISCUSSION

Varietal infestation screening

There were significant differences (F = 45.7, 61.2; df = 8; P < 0.05) among the nine tomato varieties with respect to *H. armigera* larval population per 10 plants and % tomato fruit damaged by *H. armigera* larvae (Table 1). Both characteristics were similarly expressed across the nine varieties. Varieties 'Sahil', 'Pakit' and Nova Mecb, seem to be more resistant to *H. armigera* than other varieties. Less than one-half of the larvae were found on the most susceptible varieties 'Roma VFN' and NARC-1 followed by FS-8802, while less than one-third of the fruit damage incurred on Roma VFN, NARC-1 and FS-8802. This result is in accordance with the Khanam et al. (2003) and Mishra et al. (1988). Numerous studies have also shown variation among the varieties for their susceptible/ resistant response towards the pest infestation (Sarfraz et al., 2007; Ashfaq et al., 2010).

Influence of physical leaf characteristics on larval population and fruit infestation

Significant variations were recorded for some physio-morphic leaf characteristics among tomato varieties (Table 2). Hair density and hair length on lower surface, and thickness of leaf lamina ranged from 49.8 to 23.7 per ½ cm^2, 32.5 to 19.4 micron, and 6.1 to 4.9 micron, respectively. These values were higher for 'Sahil', 'Pakit', and Nova Mecb than others varieties. However, there were no differences between tomato varieties in hair density and hair length on upper leaf surface.

Table 1. Comparison of average means of the data for 2008 and 2009 regarding larval population per 10 plants and the fruit-infestation (%), caused by the fruit-borer in various varieties of tomato.

Variety	Fruit infestation (%)	No. of larvae per 10 plant
Roma VFN	36.0[a]	5.7[a]
NARC-1	32.0[ab]	5.7[a]
FS-8802	29.3[b]	5.6[a]
Tommy	22.7[c]	3.5[b]
Pant Bahr	20.3[c]	3.5[b]
Rio Grande	19.1[c]	3.5[b]
Nova Mecb	11.7[d]	1.4[c]
Pakit	10.8[d]	1.3[c]
Sahil	10.7[d]	1.3[c]

Means sharing same letter are not significantly different (*P* = 0.05).

Table 2. A comparison of means for the data on physio-morphic characteristics of the leaves between selected varieties of tomato.

Variety	Hair density (no. per ½ cm^2)		Length of hair (μM)		Thickness of Leaf Lamina (μM)
	Upper leaf	Lower leaf	Upper leaf	Lower leaf	
Roma VFN	22.6[ns]	23.7[h]	12.9[ns]	19.4[f]	4.9[c]
NARC-1	23.2	25.8[g]	12.8	21.9[e]	4.9[c]
FS-8802	23.7	27.4[f]	12.7	23.7[d]	4.9[c]
Mommy	24.0	33.9[e]	12.7	25.3[c]	5.3[b]
Pant Babr	24.1	36.3[d]	12.7	26.0[c]	5.3[b]
Rio Grande	24.1	37.2[d]	12.6	27.8[b]	5.5[b]
Nova Mecb	24.4	43.7[c]	12.6	31.7[a]	5.9[a]
Pakit	24.4	47.0[b]	12.3	32.1[a]	6.0[a]
Sahil	24.5	49.8[a]	12.1	32.5[a]	6.1[a]

Ns, Non-significant; means sharing similar letter are not significantly different (*P* = 0.05).

Table 3. The correlation coefficient (r) for different physical leaf characteristics, on the larval population of fruit borer and fruits infestation in tomato.

correlation coefficient (r)	Fruit Infestation	Larval population
Larval population	0.89**	
HDus	-0.22[ns]	-0.31[ns]
HDls	-0.96**	-0.88**
HLup	0.42[ns]	0.33[ns]
HLls	-0.95**	-0.86**
Tlf	-0.91**	-0.87**

**, Significant at P≤0.01; *, significant at P≤0.05; ns, non-significant. HDus, Hair density on upper surface; HLup, hair length on upper surface; HDls, hair density on lower surface; HLls, hair length on lower surface; Tlf, thickness of leaf lamina.

Furthermore, the correlation coefficient values between physical leaf characteristics and fruit infestation and larval population are presented in Table 3. The hair density and length of hair on the lower surface showed a significant negative correlation with the fruit-infestation and larval population, showing r-value of -0.96**, -0.88**; -0.95**

Table 4. Stepwise regression model showing effect of different physical leaf character on larval population and fruit infestation.

Regression equation	R^2
Larval population	
Y = 10.6 - 0.19X1	0.77
Percentage fruit infestation	
Y **=** 57.2 - 0.99X1	0.92

X1, Hair density.

Table 5. A comparison of means for the data on chemical characteristics of the leaves of various selected varieties of tomato.

Chemical trait	Tomato Variety								
	Roma VFN	**NARC-1**	**FS-8802**	**Tom-my**	**Pant Babr**	**Rio Grande**	**Nova Mecb**	**Pakit**	**Sahil**
Total Minerals (%)	7.40^{h}	7.47^{h}	7.70^{g}	7.98^{f}	8.26^{e}	8.32^{d}	8.34^{c}	8.53^{b}	8.62^{a}
Nitrogen (%)	2.73^{a}	2.66^{b}	2.61^{bc}	2.59^{cd}	2.58^{cd}	2.57^{cde}	2.55^{cde}	2.54^{de}	2.52^{e}
Phosphorous (%)	0.36^{d}	0.36^{d}	0.36^{d}	0.37^{cd}	0.37^{cd}	0.38^{bc}	0.38^{bc}	0.40^{b}	0.43^{a}
Potassium (%)	2.73^{e}	2.79^{d}	2.79^{d}	2.80^{cd}	2.82^{bcd}	2.83^{abc}	2.84^{ab}	2.84^{ab}	2.86^{a}
Calcium (%)	0.33^{a}	0.22^{ab}	0.22^{ab}	0.21^{abc}	0.21^{abc}	0.21^{bc}	0.20^{c}	0.20^{c}	0.18^{d}
Magnesium (%)	0.29^{ns}	0.28	0.28	0.28	0.28	0.27	0.27	0.27	0.27
Ferrous (%)	0.14^{c}	0.15^{c}	0.15^{c}	0.16^{bc}	0.16^{bc}	0.17^{ab}	0.18^{a}	0.18^{a}	0.18^{a}
Manganese (%)	0.39^{a}	0.38^{ab}	0.37^{bc}	0.36^{cd}	0.35^{de}	0.35^{de}	0.35^{de}	0.33^{ef}	0.33^{f}
Zinc (%)	0.13^{ns}	0.13	0.12	0.12	0.12	0.12	0.12	0.11	0.11
Fat (%)	2.60^{d}	2.62^{c}	2.62^{c}	2.64^{b}	2.64^{b}	2.65^{ab}	2.66^{ab}	2.67^{a}	2.67^{a}
Crude Fiber (%)	16.27^{b}	16.45^{ab}	16.50^{ab}	16.60^{ab}	16.64^{ab}	16.67^{a}	16.71^{a}	16.74^{a}	16.76^{a}
Carbohydrate (%)	48.76^{c}	48.64^{c}	49.37^{b}	49.55^{ab}	49.58^{ab}	49.58^{ab}	49.64^{ab}	49.68^{a}	49.71^{a}

Ns, Non-significant; means sharing similar letter are not significantly different (*P* = 0.05).

and -0.86** respectively.

These findings are in lined with the findings of Juvik et al. (1982), Selvanarayanan and Narayanasamy (2006) and Peter (1995). This may be due to the reason that exudates of trichomes interfere in larval feeding (Dimoch et al., 1983; Kennedy and Sorenson, 1985) and reduced larval weight (Sunitha et al., 2008). Thickness of leaf lamina is negatively correlated with fruit infestation (r = -0.91**) and larval population (r = -0.87**). Similar findings were also reported by Coley (1983) and Morrow (1983). According to Larsson and Ohmart (1988), tough leaves prohibit feeding of early larval instars and reduce their development (Clissold et al., 2006). Moreover, stepwise regression analysis indicated that hair density on lower surface explained 92.0 and 77.0% variation in fruit infestation and larval population respectively (Table 4). These can be used as marker trait to develop resistant varieties against tomato fruit worm.

Influence of chemical traits on larval population and fruit infestation

Nitrogen, calcium and manganese ranged from 2.52 to 2.73%, 0.18 to 0.33% and 0.33 to 0.39%, respectively, with values significantly lower in resistant varieties and higher in susceptible varieties. Contrarily, phosphorous and ferrous ranged from 0.43 to 0.36% and 0.18 to 0.14%, respectively and were higher in resistant varieties than in susceptible varieties (Table 5). According to Table 6, fruit infestation and larval population were positively correlated with nitrogen (r = 0.74**, 0.65**), calcium (r = 0.59**, 0.59**), magnesium (r = 0.44*, 0.49**), manganese (r = 0.75**, 0.72**) and zinc content (r = 0.57**, 0.49**). In contrast, phosphorous and ferrous was negatively correlated with fruit infestation (r = -0.43**, -0.81** respectively) and larval population (r = -0.46**, -0.73** respectively). Stepwise regression analysis indicated that ferrous and calcium explained 74.0 and 65.0% variation in fruit infestation and larval population, respectively (Table 7). Therefore, these can be selected as marker traits to develop resistant varieties against *H. armigera* in tomato crop.

There are no available data on relation between *H. armigera* and chemical leaf characteristics investigated in this study; therefore, it is discussed with reference to other systems. Our study showed that high concentration of nitrogen, manganese and calcium increases fruit

Table 6. Effect of chemical plant characteristics (%) on the larval population and infestation (%) of fruit borer on tomato crop.

Chemical factor	Larval population	Fruit infestation (%)
Total Minerals	-0.07^{ns}	-0.11^{ns}
Nitrogen	0.65**	0.74**
Phosphorus	-0.46*	-0.43 *
Potassium	-0.31^{ns}	-0.20^{ns}
Calcium	0.59**	0.59**
Magnesium	0.49**	0.44 *
Ferrous	-0.73**	-0.81**
Manganese	0.72**	0.75**
Zinc	0.49**	0.57**
Fat	-0.22^{ns}	-0.07^{ns}
Crude fiber	0.03^{ns}	-0.00^{ns}
Carbohydrate	-0.30^{ns}	-0.36^{ns}

**, Significant at $P \leq 0.01$; *, significant at $P \leq 0.05$; ns, non-significant.

Table 7. Stepwise regression model showing effect of different chemical leaf characteristics on larval population and fruit infestation.

Regression equation	R^2
Larval population	
Y = 4.4 - 72.8X1 + 52.0X2	0.65
Percentage Fruit Infestation	
Y = 38.6 - 392.1X1 + 219.9X2	0.74

X1, Ferrous (Fe^{2+}); X2, calcium.

infestation and larval population density. This is similar to findings of Grafton-Cardwell and Ouyang (1996) who stated that high concentration of nitrogen and manganese increases the reproduction of predaceous mite, and Khaliq et al. (2001) who reports the similar kind of relationship between calcium and *Chilo partellus* (Swinhoe) infestation. Moreover, the decrease of concentration of zinc (Phelan et al., 1995) and magnesium (McKinnon et al., 1999) decreases the pest's oviposition. This supports the findings of our present study that fruit infestation and larval population can be decreased with the decrease in concentration of zinc and magnesium.

Marketable fruit-yield of tomato

An analysis of variance revealed a significant difference in the yield among the varieties at *P*>-0.01 (Figure 1). The variety, Sahil, which showed a resistant trend against the fruit-borer, showed a maximum fruit-yield of 99.56 kg/plot. The variety, Roma VFN, showing a susceptible trend, against the fruit-borer, had a minimum yield of 39.50 kg/plot. Generally, varieties Sahil, Pakit and Nova Mecb provide higher fruit yield than others. These results are similar to findings of Heinrichs (1994) who reports that resistant varieties show high yield response.

Effect of the larval population and infestation of the fruit borer on the yield of tomato

Table 8 shows the results regarding the effect of larval population and infestation on the yield of tomato. The larval-population and fruits infestation exerted a significant and negative effect on the fruit-yield, with r-values of -0.81** and -0.86**, respectively, and this was in line with findings of Phillips et al. (1979). Our results clearly suggest that resistant varieties', having less fruit infestation than susceptible varieties, is due to physical and chemical leaf characteristics.

Conclusion

The presented study indicates that morphological characteristics such as leaf hair density, and chemical traits

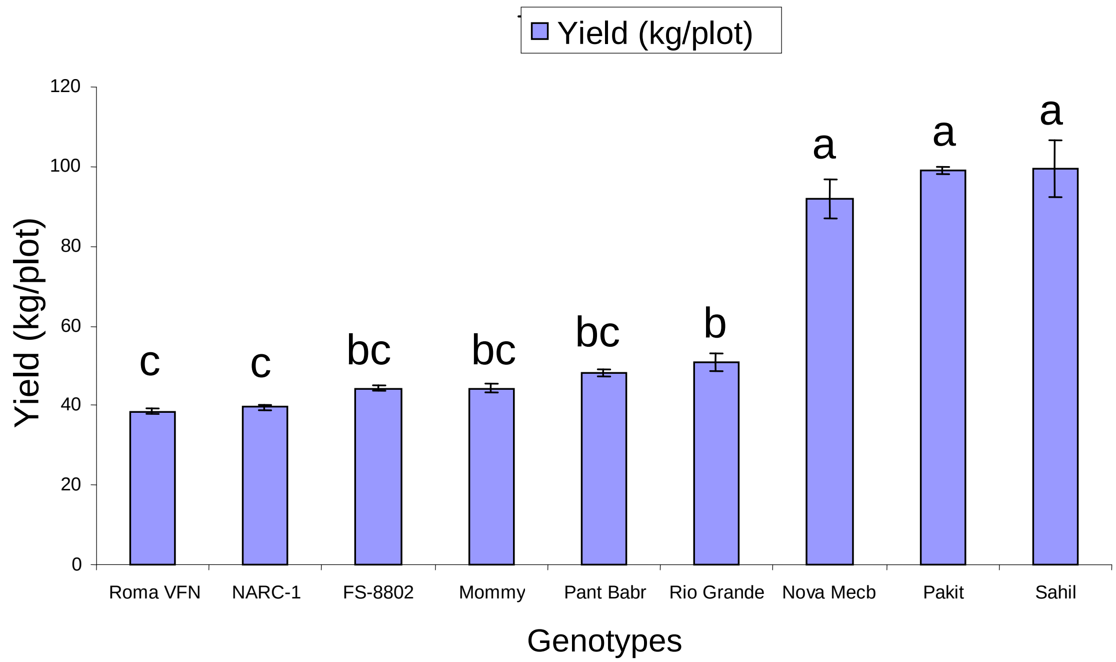

Figure 1. Marketable fruit-yield of different tomato varieties.

Table 8. Effect of the larval population and fruit infestation on the yield of tomato.

Independent factor	Yield (kg/plot)
Larval population	r = -0.81 **
Fruit infestation (%)	r = -0.86 **

**, Significant at P ≤ 0.01.

like iron and calcium concentration, can be used as maker traits to develop insect resistant varieties of tomato through breeding programs. Further research is needed to study the physical and chemical characteristics of tomato fruit in relation to *H. armigera* and its natural enemies, so that combination of host plant resistance and biological control can be used to attain maximum yield.

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