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Variability analysis in F6 lines of tomato for the role of trichomes for resistance against fruit borer (*Helicoverpa armigira)*

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Abstract

Use of pesticides to control the pest is unsafe practice for environment and human health therefore the present era scientist are seeking to adopt host-plant resistance which is a natural strategy to control the pests on crops. Our study aims to find the role of trichomes in conferring resistance against fruit borers(*Helicoverpa armigira)* in tomato breeding lines.F6 lines were transplanted in the field and data were collected on trichomes density, No of fruits affected by fruit borers(*Helicoverpa armigira)* and total number of fruits/plant. The lines having higher density of trichomes (6.5 on the scale of 10) were found to be less affected (1.33 Nos fruits affected) by fruit borers (*Helicoverpa armigira)*as compared to the lines with lower trichome density .Highest value of Va(5.3) was reported for Number of fruits/plant. Heritability % was found to be lower for all the parameters. The degree of resemblance was found to be moderate for all the parameters. Vp (Phenotypic variance) and Va values within the lines exceeded the Vp and Va values of the whole population. The high difference was found between the Vp and Va within the lines. The PCV (Phenotypic Coefficient of Variation) within the lines for fruits affected/plant, for non-glandular trichomes type V and for glandular trichomes type VI was found to be higher. The above mentioned findings depict that trichomes confer resistance to host plant against fruit borers and can be relayed upon to some extent for crop protection.

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Introduction

Tomato is the second most important vegetable crop after potato. It is vulnerable to insects and disease which leads to loss in its production. Among the insects,fruit borer (*Helicoverpa armigira)* is a polyphagous insect which inflicts heavy loss on tomato.Its young larvae feed on leaves while mature instars bore into fruits (GopalaKrishnan, 2006).Worldwide annual crop losses due to *Helicovepra armigera* alone are approximately 5 billion US dollars (Sharma *et al*, 2009). In Pakistan, 32-35% fruit infestation was observed in tomato due to this pest (Haider *et al*, 2006, Inayat Ullah *et al*, 2007). Severity of the pest incidence can be judged from the fact that in Pakistan 80% of the total insecticides are used against this (Kamil *et al.*, 2011). The use of pesticides to control the pests is not only expensive but also hazardous to natural environment and human health(Souza and Reis, 1999). Therefore under the global environmental conditions, the present world is seeking the natural ways to control the pests. Among the natural ways host plant resistance is the reliable strategy to address the problem. Tomato has various biochemical and physical factors which contribute in insect resistance such as trichome density, calyx area, thickness and toughness of fruit rind; however among all these factors trichomes density has significant and negative correlation with larval feeding (Selvanarayanan,2015). There are two kinds of trichomes: (i). glandular trichomes and (ii) Non glandular trichomes. Both glandular and non-glandular trichomes can coexist in the same genotype (Romeis *et al,* 1999).

Glandular trichomes are common in night shade families like tomato and potato. They secrete several groups of chemicals including phenols, quinines, resins and volatiles.

They inhibit insect damage by reducing the insect population growth, limit mobilization of insects by creating traps or by repelling insects to come near host.TypeA glandular trichome of wild potato produce phenolic exudates. On their oxidation with polyphenol oxidase they produce a cement like substance resulting in entrapment and death of aphids (Kowalski *et al,* 1993).

Non-glandular trichomes also play an important role in hindrance the attack of the pests. Presence of high density of these trichomes in pigeon pea helps in prevention of pod borer *(Helicoverpa armigera)*, which is the most serious pest of the crop(Maiti *et al,* 2012).

The purpose of our study is to find the relation between trichomes and fruit borer *(Helicoverpa armigera)* infestation in F6 lines of tomato and to find the level of variability for the parameters under study and to screen the best performing lines for resistance to the insects.

Material and methods

1. *Nursery sowing and transplantation*

11 NosF6 lines were sown as nursery at Hazara agricultural research station Abbottabad, Pakistan and after 45 days, plantlets were transplanted in the field. Plant to plant distance was kept to be 50 cm while row to row distance was maintained to be 100 cm. Data were collected on No of fruits/plant,No of fruits affected/plant, density of glandular trichomes type VI and non glandular trichomes type V.

Data Collection

The density of trchomes type V and Type VI was estimated by following method. Four samples of 1mm piece of pedicle and leaf were taken from each line and were observed in the binocular microscope. 1-10 scale was fixed to record the density. 1 value indicates very low density while10 value indicates the highest density of the trichomes. Type VI (grandular trichomes) and Type V (Long trichomes) are shown in images 1 and 2 respectively as observed through microcope.

Data analysis

Additive Variance (Va) for the means of F6 lines was calculated by using the approach of Wrike and Weber (1986) as following:

Vg between means of lines in F6=Va, as variance in F4 and onward generations is contributed by only additive gene action and there is no role of Vd (dominant gene action).

Heritability in F6 lines was calculated by using the formula Hb=Vg/Vp, Since Vg=Va in F6, therefore Hb=Va/Vp.

Heritability% was calculated by using the following formula used by Ahmad *et al*, (2017), in their study. Hb%=Va/Vp x 100. Where Hb= broad sense heritability, Va=additive variance, Vp=phenotypic variance.

Degree of resemblance between lines and population was calculated by using the formula of Falconer (1960), as following:

$t=\sigma^2B/(\sigma^2B+\sigma^2W)$ where t= degree of resemblance, σ^2B= Standard deviation between the lines, σ^2W= Standard deviation within the lines.

Phenotypic coefficient of variation was computed by using the method of Butron and Devane (1953) as following:

Phenotypic coefficient of variation (PCV%)= $\sqrt{Vp}/\bar{X} \times 100$.

Results and discussion

1.*Mean and range*

The values of mean for No of fruits/plant and Fruits affected/plant shows that the attack of fruit borers on the overall population was noted to be 10% and none of the line showed complete resistance to the borer attack.

Table 1. Mean, Range, Phenotypic variance (Vp), Environmental variance (Ve), Additive variance (Va), Phenotypic coefficient of variation (PCV),Heritability(H) and Heritablility percentage(H%) for number of fruits/plant (NoF), number of fruits affected/plant (NoFEP), non-glandular trichome type V, glandular trichome type VI and percentage of fruits affected/plant in F6 population .

Entry	Mean	Range Min-Max	Vp	Ve	Va	PCV	H	H %
NoF	25.81	18-33	21.36	16	5.36	17.90	0.01	1.70
NoFEP	2.89	1-5	1.024	1	0.024	34.96	0.02	2.41
Trichome V	3.03	1.6-4	0.53	0.5	0.031	24.05	0.05	5.87
Tirchome VI	3.29	0.6-5	3.10	3.05	0.059	53.44	0.01	1.89
%FEP	10.29	4.6-16	12.19	12	0.19	33.93	0.01	1.59

The lowest No of fruits affected/plant was recorded in entry B10;the same entry had the highest density of non-glandular trichomes Type-Vand glandular trichomes type-VI as determined according to scale (1-10). Entry No B1 followed B10 in lowest No of fruits affected by borers/plant and higher density of glandular tichcomes type VI(Table 2).

Table 2. Mean,range and degree of resemblance (DOR) for number of fruits/plant (NoF),number of fruits affected/plant(NoFEP), non-glandular trichomes type V and glandular trichomes type VI in F6lines.

Lines	NoF			NoFEP			Trichome-V			Trichome-VI		
	Mean	Range	DOR	Mean	Range	DOR	Mean	Range	DOR	Mean	Range	DOR
B1	33	11-48	0.21	1.55	0-5	0.39	2.2	1-4	0.45	6	5-7	0.63
B2	25	11-52	0.28	2.16	0-8	0.27	3.3	1-5	0.33	4.5	1-8	0.26
B3	18	5-49	0.27	1.69	0-5	0.39	3.4	1-7	0.27	2.3	0-7	0.30
B4	28	12-43	0.35	2.46	0-10	0.28	3.2	1-7	0.27	4	0-8	0.30
B5	25	11-42	0.31	2.91	0-7	0.32	2.2	1-8	0.28	5.6	1-9	0.29
B6	29	10-44	0.44	3.69	0-12	0.28	3.6	1-6	0.34	3.33	0-8	0.29
B7	19	7-33	0.44	2.37	0-10	0.28	3.6	1-8	0.29	5.3	3-8	0.41
B8	27	19-45	0.38	3.33	1-9	0.27	1.6	1-4	0.40	2	0-4	0.46
B9	22	12-33	0.42	2.63	0-8	0.28	2.7	1-5	0.36	5	1-7	0.33
B10	28	11-48	0.34	1.33	0-8	0.29	4	1-6	0.31	6.5	2-8	0.28
B11	30	7-46	0.33	4.88	0-10	0.25	2.7	1-6	0.29	0.66	0-1	0.75

Correlation

The correlation results show that non-glandular trichomes Type-V and glandular trichomse type-VI are negatively correlated to No of fruits affected/plant (Table 3).

Table3. Correlation among number of fruits/plant (NoF), number of fruits affected/plant (NoFEP), non-glandular trichomes type V, glandular trichomes type VI and percentage of fruits affected/plant (%FEP)in F6population.

	NoFEP	NoF	Trichome VI
NoF	0.24		
Trichome VI	-0.49	0.2330	
Trichome V	-0.31	-0.2881	0.0369

This may be due to reason that secretions of trichomes inhibit larvae feeding (Dimoch *et al*, 1983). Duffey and Isman (1981) found that glandular trichomes are rich in phenolic compounds such as flavonol glycoside and these secretions serve as inhibitors for the larval growth of fruit worms. Farrar *et al.* (1992) found that type VI glandular trichomes possess secondary metabolites such as methyle ketones (2-tridecanon and 2-undecanone) which confer resistance against insects. Sultani and Omid (2013) also found negative correlation between trichome density and larvae on fruit. Sahhu *et al.* (2005) found negative correlation between No of trichomes and fruit infestation.

Table 4. Phenotypic variance (Vp), additive variance (Va) and phenotypic coefficient of variation for number of fruits/plant (NoF), number of fruits affected/plant (NoFEP), non-glandular trichomes type V and glandular trichomes type VI in F6lines.

Lines	NoF			NoFEP			Trichome V			Trichome VI		
	Vp	Va	PCV	Vp	Va	PCV	Vp	Va	PCV	Vp	Va	PCV
B1	414.32	158.79	51	6.82	2.48	95	0.75	0.11	38	1	0.13	16
B2	268.95	13.425	47	11.3	6.98	77	2.02	0.52	42	24.5	15.73	95
B3	271.52	15.995	64	6.6	2.31	65	3.83	1.28	57	16.3	7.53	99
B4	197.50	0.28	29	10.5	6.17	72	3.77	1.22	59	16	7.23	91
B5	232.58	.945	39	8.7	4.44	72	3.43	0.87	81	17.3	8.56	73
B6	199.73	.793	28	10.9	6.65	69	1.95	0.60	38	17.3	8.56	93
B7	161.14	0.27	30	11.0	6.74	94	3.10	0.55	48	6.33	0.43	47
B8	183.24	0.288	26	11.1	6.78	78	1.15	0.40	64	4	0.77	90
B9	165.77	0.10	27	10.7	6.45	96	1.61	0.93	46	12	3.23	69
B10	204.99	0.14	31	9.9	5.62	177	2.42	0.12	38	19	10.23	98
B11	215.68	0.23	31	13.1	8.81	60	3.06	0.51	63	0.33	0.10	86

Variability analysis

Higher value of Va in F6 population was noted for No of fruits/plant while other parameters showed the lower values.No of fruit/plant is quantitative trait and many genes has their contribution,therefore variability in this trait may be higher than other traits even in F6 generation. Highest PCV was calculated in F6 population for Type VI glandular trichomes. Heritability and heritability percentage was recorded to be much lower (1.5-5.8 %) for all the parameters (Table 1). Decrease in heritability in advanced breeding generation is often observed.

This may be due to increase in homozygosity and decrease in heterozygosity in these generations (Wallace, 1972).

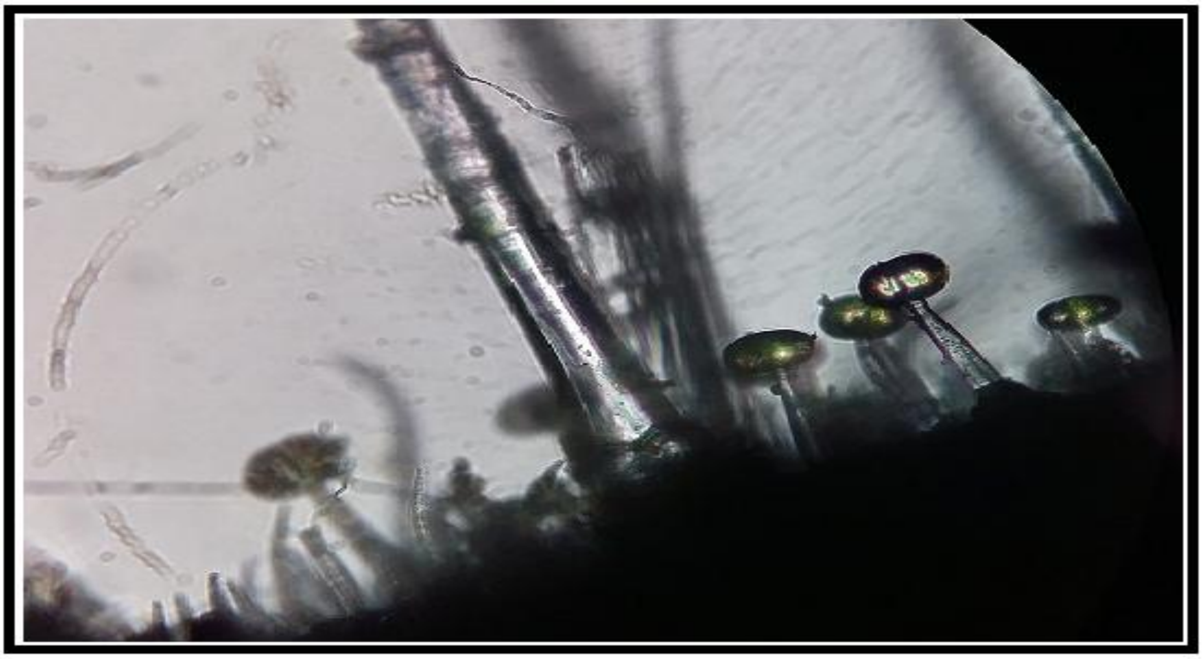

Fig. 1. Microscopic view of trichomes type VI.

For No of fruits/plant the range of degree of resemblance of the lines to the whole F6 population was found to be 0.21-0.44, for fruits affected/plants it was 0.25-0.39, for non-glandular trichome type V it was found to be 0.27-0.45 and for glandular trichome type VI it was estimated to be 0.28-0.63 (Table-2).

Fig. 2. Microscopic view of trichomes type V.

The degree of resemblance of lines to the population for all the parameters is moderate which indicates that there is diversity between the lines.

The findings also indicate that the lines may be different at F2 pedigree level.

Vp and Va values for number of fruits within some lines like B1, B2 and B3 exceeded the values of Vp and Va of the whole population (Table 4). Likewise the Vp and Va for No of fruits affected and non- glandular trichome type V within the lines exceeded the values of Vp and Va of the whole population. For glandular trichomes type VI, all the Vp and Va values within the lines were found to be higher than Vp and Va value of all the population except entry No 1. The results show that there is still some variation and heterozygosity within the lines for the mentioned traits and the lines cannot be fixed at this stage. High difference between Vp and Va within the lines for all parameters except non-glandular trichomes type V was found which shows the role of environmental effect within the lines. The PCV within the lines for No of fruits/plant ranged 28-68%,for fruits affected /plant it was 69-95%, for non-glandular trichome type V it was 31-81% and for glandular trichome type VIit was found to be16-99 % (Table 4). The results shows that the later three parameters possess phenotypically high variability, however, it should be kept in mind that phenotypic coefficient of variation is contributed by both environmental as well as genetic variation.

Conclusion

Host plant resistance though not confers full protection to plants against insects; however it can play its role to certain limits. In this regard breeding and selection for trichome density (glandular and non-glandular trichomes) can help develop insect resistant varieties.

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