PEST MANAGEMENT



Plant Resistance to the Moth *Tuta absoluta* (Meyrick) (Lepidoptera:Gelechiidae) in Tomato Cultivars

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Abstract

The resistance of 11 tomato cultivars (Ps-6515, Berlina, Poolad, Petoprid-5, Zaman, Matin, Golsar, Sandokan-F1, Golshan-616, Sadeen-95 and Sadeen-21) to the tomato moth, *Tuta absoluta* (Meyrick) (Lepidoptera:Gelechiidae) was investigated under field conditions. A randomized complete block design was used with three replications. Data analysis indicated that there were significant differences (P < 0.05) among cultivars regarding leaflet damage, leaf damage, overall plant damage, number of mines per leaf, number of holes on the stem, and fruit. Our findings revealed that the cultivars Berlina, Golsar, Poolad, and Zaman were less suitable cultivars, suggesting that they are more resistant to the tomato moth than the other cultivars. The high density of leaf trichomes present in the cultivars Berlina, Zaman, and Golsar can be one of the possible causes of resistance to *T. absoluta*. Knowledge of the extent of susceptibility or resistance of cultivars to a pest on a crop is one of the fundamental components of integrated pest management (IPM) programs for any crop.

Introduction

The tomato moth, *Tuta absoluta* (Meyrick) (Lepidoptera:Gelechiidae), is a devastating insect pest affecting tomato production in Iran and many other countries (Baniameri & Cheraghian 2011, Gharekhani & Salek-Ebrahimi 2014). The larvae produce galleries in the leaves, stems, terminal buds and fruits. The main damage is caused through larvae feeding on the parenchyma between the epidermal layers of the leaves, reducing the photosynthetic capacity of the plant with subsequent reduction of the yield (Desneux *et al* 2010, 2011). Under heavy infestation, the yield loss between 80–100% is common (Gebremariamd 2015).

In many agronomic and vegetable cropping systems, the primary strategy employed to control this pest involves the use of chemical insecticides. However, it has serious problems such as destruction of natural enemy populations (Campbell *et al* 1991), build-up of insecticide residues on tomato fruits (Walgenbach *et al* 1991) and in the environment, and, especially, evolution of *T. absoluta* resistance to many

of the active ingredients available on the market (Siqueira *et al* 2000a, b, Lietti *et al* 2005, Silva *et al* 2011, Campos *et al* 2015, Roditakis *et al* 2015).

One of the important control methods for sustainable management of T. absoluta to minimize development of pesticide resistance is the use of resistant host plants. The use of resistant plants can be a useful component of an integrated pest management (IPM) system that could affect pest population density, herbivore damage, and decrease pesticide applications in agricultural ecosystems. In addition, in many cases, even partial resistant cultivars are useful to enhance the effects of beneficial natural enemies (Hare & Andreadis 1983, Bong et al 1991, Cogni et al 2002, Kaplan 2007, Kaplan & Thaler 2010). Plant resistance to a pest can be caused by antixenosis, a mechanism employed by the host plants, deters the insects from oviposition, feeding, seeking shelter, and colonization (Oyetunji et al 2014); antibiosis, which has a direct influence on the life history of a pest (Ofomata et al 2000, Li et al 2004); and tolerance, the plant's capacity of keeping its production under attack for herbivore insect (Vargas 1970, Stowe *et al* 2000, Stevens *et al* 2008). Many researchers have investigated the resistance of host plants to tomato moth (Gilardón *et al* 2001a, Leite *et al* 2001, Suinaga *et al* 2004, Silva 2009, Gharekhani & Salek-Ebrahimi 2014). In this study, we present data on susceptibility of 11 tomato genotypes to *T. absoluta*. The data obtained from these experiments are used to understand the mechanism of population build-up of this pest on different tomato genotypes to develop a comprehensive pest management program for tomato.

Material and Methods

The experiment was performed in a field in the Faculty of Agriculture, Persian Gulf University, Bushehr province, Borazjan region, Bondarooz (Southern Iran) (29°12'54.1" N, 51°13'57.1" E, and elev. 99 m) from February 2014 to June 2015. Eleven cultivars of tomato were used in this study, including five cultivars Ps-6515, Berlina, Poolad, Petoprid-5, Zaman from FALAT Co., Iran; two cultivars Matin and Golsar from GOLSAM Co., Iran; and four cultivars Sandokan-F1, Golshan-616, Sadeen-95 and Sadeen-21 from BEHTA Co., Iran. The tomato seeds were planted in plastic transplant trays containing peat moss soil and perlite on November 2014. With the appearance of the first true leaves, the seedlings were transplanted into the main field. The evaluation of the resistance was performed in a randomized block design with three replications. A total of 20 plants per cultivar were planted in each replicate plot (plot area = 24 m^2) in two 150cm spaced rows. The space between plants in each row was 50 cm, and the space between plots was 150 cm. The cultivars were exposed to the natural infestation by indigenous population of tomato leaf miner in the field.

Leaflet damage, leaf damage, and overall plant damage caused by the insect were evaluated at days 20, 40, and 60 after infestation. Five plants were randomly selected in each plot and marked notes and the different characteristics on these plants were measured. Leaflet and leaf damage was evaluated based on the percentage of leaflet or leaf area affected by T. absoluta. In this case, three leaves were selected from the upper third of each of the five selected plants and the damaged area of each whole leaf and its leaflets was recorded. The overall plant damage estimates were also performed for each of the five selected plants. In addition, the number of mines per leaf, holes on the stem, and holes per fruit were assessed at the last sampling date. The number of mines per leaf was counted on three leaves selected from the upper third of each of the five randomly selected plants. The number of holes on the stem and the number of holes on the fruit were evaluated by performing a direct counting of these features throughout the stem and five fruits from each of these five selected plants, respectively. In addition,

	Leaflet damage			Leaf damage			Overall plant dama	ıge	
	Sampling time			Sampling time			Sampling time		
Cultivar	20	40	60	20	40	60	20	40	60
PS6515	11.33 ± 3.36a	30.00±5.47a	53.67 ± 7.30b	11.33 ± 3.40a	36.67±6.00a	63.67±8.oobc	12.67 <u>±</u> 3.50a	36.oo±6.ooa	55.67 ± 7.40a
Berlina	3.oo±1.73d	8.67±2.94d	14.33±3.80f	3.33±1.80e	11.00±3.30e	20.67±4.50 g	7.33 ± 2.70bcd	15.67 ± 4.00bc	26.33±5.10c
SandocanF1	9.33±3.05ab	33.67 ± 5.80a	58.33 ± 7.60ab	9.67±3.10abc	34.00±5.80ab	67.00±8.20abc	10.00±3.20abc	31.33 ± 6.00ab	48.00±7.00ab
Matin	11.33 ± 3.36a	33.00±5.74a	65.00±8.00a	12.33 ± 3.50a	33.67±5.80ab	71.00 ± 8.40ab	12.33 ± 3.50ab	31.33 ± 6.00ab	50.00±7.00a
Sadeen95	7.00±2.64bc	21.00 ± 4.58b	36.67±6.00d	6.67±2.60bcde	22.33±4.70 cd	43.67±6.60de	7.33 ± 2.7obcd	22.67 ± 4.70abc	37.00±6.00abc
Zaman	5.67±2.38 cd	14.33 ± 3.78c	24.00 ± 4.90e	6.00±2.50bcde	17.66 ± 4.20de	35.67±6.ooef	5.33±2.30 cd	13.67±4.00c	25.67±5.00c
Petopride5	10.67±3.26a	34.00 ± 5.83a	63.00±7.90a	10.33 ± 3.20ab	36.33±6.00a	74.67±8.60a	9.00±3.00abcd	30.67±5.50ab	49.67±7.00a
Golsar	3.67 ± 1.90d	10.67±3.26 cd	19.00±4.30ef	4.33 ± 2.10de	15.00 ± 3.90e	29.33±5.40 fg	4.67±2.10d	16.00 ± 4.00bc	27.00±5.20c
Golshan616	9.33±3.05ab	24.33±4.90b	45.00±6.70c	9.33±3.00abc	27.33 ± 5.20bc	58.00±7.60c	10.67±3.30ab	23.33 ± 4.8oabc	37.67±6.10abc
Poolad	5.00±2.24 cd	13.00±3.60 cd	22.00 ± 4.70ef	5.67±2.30cde	17.33 ± 4.20de	30.33 ± 5.5of	7.33 ± 2.7obcd	19.67 ± 4.40bc	28.67±5.30bc
Sadeen21	8.00 ± 0.00abc	20.67 ± 4.54b	37.33 ± 6.10d	8.33±2.90abcd	24.33±4.90 cd	46.67 ± 6.8od	8.00 ± 2.80abcd	16.67 ± 4.00bc	28.67±5.30bc

Table 2 The mean number of mines on the leaf, number of holes on the stem, and number of holes on the fruit of tomato cultivars submitted to infestation of *Tuta absoluta*.

Cultivar	Number of mines on the leaf	Number of holes on the stem	Number of holes on the fruit
PS6515	6.47±2.50a	3.67±1.90a	11.67 ± 3.40ab
Berlina	3.27±1.80b	1.33 ± 1.00c	4.07±2.00e
SandocanF1	5.20a ± 2.30b	2.80 ± 1.60abc	11.00 ± 3.30ab
Matin	5.73±2.40a	3.13 ± 1.70ab	9.67±3.10bc
Sadeen95	4.60 ± 2.10ab	2.33 ± 1.50abc	6.87±2.60cde
Zaman	3.20 ± 1.80b	1.33 ± 1.10c	5.53 ± 2.30de
Petopride5	6.13±2.50a	3.33 ± 1.80ab	13.00 ± 3.60a
Golsar	3.53 ± 1.80b	1.40 ± 1.10c	5.20 ± 2.30de
Golshan616	4.67 ± 2.10ab	2.40 ± 1.50abc	7.67±2.70 cd
Poolad	3.47±1.80b	1.40 ± 1.10c	7.00 ± 2.60cde
Sadeen21	3.47±1.80b	1.90 ± 1.10c	8.67 ± 2.90bc

Means followed by the same letters in columns do not differ by the Duncan test ($\alpha = 0.05$).

three leaves were selected from the upper third of each of the five randomly selected plants for counting the density of total trichomes and type VI glandular trichomes on the leaves. Then, three leaflets separated from each leaf and trichomes were counted using a stereomicroscope (×40) on three 2-cm² regions of each leaflet. The total number of trichomes and type VI glandular trichomes were also counted on different 2-cm² sections of the stem.

The normality of data was assessed with Shapiro-Wilk's test (Proc Univariate, SAS Institute (2003), Cary, NC, USA). Data which needed to be normalized were transformed before being analyzed. The percentage data were subjected to arcsin square root transformation; however, no count data transformation was performed before analysis because there was no evidence of non-normality within these data. Analysis of variance was performed using the general linear model (GLM) procedure in the SAS software and means were compared using Duncan's multiple range test. Total damage index for each cultivar was presented as thesum of the indices gained for different evaluated traits. Index for each trait was

calculated by dividing the lowest recorded number in a certain cultivar to the greatest recorded number for that trait in all cultivars. Pearson's correlations (5% significance) were used to evaluate the relationships between traits. Data were then subjected to stepwise regression with overall plant damage at 60 day as the dependent variable. Cultivar comparison and selection was accomplished by cluster analysis according to Ward's method using SAS software.

Results and Discussion

According to data of leaflet damage and leaf damage, and overall plant damage, we found that the cultivars Berlina, Golsar, Poolad, and Zaman more effectively avoided damage caused by *T. absoluta* in the three evaluation periods (Table 1). In contrast, Matin, Petopride5, SandocanF1, and PS6515 showed significantly greater damage rates than other evaluated cultivars (Table 1). There were also significant differences in the number of mines on the leaf (F=3.33, df=10,

Table 3 Estimates of damage indices for different plant parts at day 60 after infestation and total damage index for tomato cultivars submitted to infestation of *Tuta absoluta*.

Cultivar	Leaflet damage index	Leaf damage index	Plant damage index	Number of holes on the stem index	Number of mines on the leaf index	Number of holes on the fruit indx	Total damage index
PS6515	0.78	0.84	0.80	0.78	0.74	0.67	4.60
Berlina	0.21	0.28	0.38	0.28	0.37	0.23	1.75
SandocanF1	0.84	0.88	0.70	0.60	0.60	0.63	4.24
Matin	0.94	0.93	0.72	0.66	0.66	0.56	4.47
Sadeen95	0.53	0.57	0.53	0.49	0.53	0.40	3.44
Zaman	0.35	0.47	0.37	0.28	0.37	0.33	2.15
Petopride5	0.91	0.98	0.71	0.70	0.70	0.75	4.76
Golsar	0.27	0.39	0.39	0.30	0.40	0.30	2.05
Golshan616	0.65	0.76	0.54	0.51	0.53	0.44	3.05
Poolad	0.32	0.40	0.41	0.30	0.40	0.40	2.23
Sadeen21	0.54	0.61	0.41	0.30	0.40	0.50	2.76

Table 4 Average densities (number/2 cm²) of total trichomes and type VI glandular trichomes on the leaf and stem for the plants used in the bioassay.

Cultivar	Leaf		Stem	
	Total trichomes	Type VI glandular trichomes	Total trichomes	Type VI glandular trichomes
PS6515	38.27±6.20 cd	28.67±5.30c	64.70 ± 8.00a	20.00 ± 4.47abc
Berlina	55.33 ± 7.40ab	34.70 ± 5.90b	78.30 ± 8.80a	18.00 ± 4.24 cd
SandocanF1	43.00 ± 6.50c	29.33 ± 5.40c	70.40 ± 8.40a	19.70 ± 4.43bc
Matin	36.40±6.00 cd	23.70 ± 4.80c	84.10±9.20a	16.00 ± 4.00d
Sadeen95	60.4 ± 7.70a	37.70 ± 6.10a	87.7±9.30a	21.70 ± 4.65ab
Zaman	50.13 ± 7.00b	34.33 ± 5.80b	101.7±10.00a	21.33 ± 4.62ab
Petopride5	26.80 ± 5.10e	17.70 ± 4.20e	86.8±9.30a	22.00 ± 4.69ab
Golsar	55.33 ± 7.40ab	37.70 ± 6.10a	110.00 ± 10.40a	22.33 ± 4.72a
Golshan616	42.27 ± 6.50c	30.70±5.50c	94.50 ± 9.70a	20.00 ± 4.47abc
Poolad	34.33 ± 5.90d	23.33 ± 4.80d	72.30 ± 8.50a	16.70 ± 4.08d
Sadeen21	38.20±6.20 cd	25.67 ± 5.00d	69.30 ± 8.30a	18.00 ± 4.24 cd

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Means followed by the same letters in columns do not differ by the Duncan test (α = 0.05).

20, P = 0.0107), number of holes on the stem (F = 3.37, df = 10, 20, P = 0.0110), and fruit (F = 9.10, df = 10, 20, P < 0.0001) caused by *T. absoluta* among the tomato cultivars (Table 2). The lowest number of mines on the leaf and holes on the stem were observed in the cultivars Berlina, Zaman, Golsar, Poolad, and Sadeen21. The cultivars Berlina, Zaman, Golsar, and Poolad also had the lowest number of holes on the fruit (Table 2).

Total damage index for evaluated tomato cultivars has been presented in Table 3. Based on the results, the cultivars Berlina, Golsar, Zaman, Poolad, and Sadeen21 with the lowest total damage index (1.75, 2.05, 2.15, 2.23, and 2.76, respectively) were the most resistant cultivars against *T. absoluta*. The greatest total damage index was obtained for the cultivars Petopride5, PS6515, Matin, and SandocanF1 (4.76, 4.6, 4.47 and 4.24, respectively) sustained less damage from pest (Table 3). Genetic variability is one of the characteristics of the germplasm bank subsamples that gives higher or lower susceptibility to pest insects (Fernandes *et al* 2012). So, observed differences between the levels of damages caused by *T. absoluta* on different tomato cultivars in the present study may have occurred because of genetic variability among them. Resende *et al* (2006), Gonçalves *et al* (2008), Oliveira *et al* (2009), Gonçalves Neto *et al*. (2010), Maciel *et al* (2011), and Gharekhani & Salek-Ebrahimi (2014) have also observed resistance to *T. absoluta* as non-preference and antibiosis in some evaluated tomato cultivars.

The genetic diversity of tomato cultivars may display inappropriate morphophysiological features to oviposition of *T. absoluta* adults and/or restrict the larvae feeding (Sobreira *et al* 2009). Trichome density is the most important structural feature of plants known to confer resistance to insect pests (Sharma *et al* 2009, He *et al* 2011). In the present study,

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
(1) Total number of trichomes on the stem	_	_	_	_	_	_	_	_	_
(2) Number of Type VI glandular trichomes on the stem	0.19	-	-	-	_	-	-	-	-
(3) Total number of trichomes on the leaf	0.32	0.30	-	-	_	-	-	-	-
(4) Number of type VI glandular trichomes on the leaf	0.30	0.30	0.91*	-	-	-	-	-	-
(5) Number of mines on the leaf	0.04	0.09	-0.40*	-0.40*	-	-	-	-	-
(6) Number of holes on the stem	0.02	0.1	-0.40*	-0.40*	0.10*	-	-	-	-
(7) Number of holes on the fruit	-0.10	-0.03	-0.63*	-0.61*	0.75*	0.74*	-	-	-
(8) Leaflet damage	-0.17	-0.02	-0.58*	-0.58*	0.76*	0.76*	0.82*	-	-
(9) Leaf damage	-0.14	0.04	-0.58*	-0.56*	0.73*	0.73*	0.82*	0.97*	-
(10) Overall plant damage	-0.07	0.08	-0.42*	-0.40*	0.10*	0.10*	0.73*	0.78*	0.76*

Table 5 Estimates of Pearson's correlations among the evaluated characteristics in tomato cultivars submitted to infestation of Tuta absoluta.

*Significant at 5% by the *t* test.

Table 6 Results of stepwise multiple regression analysis	Variable	Parameter estimate	Model R-square	C(p)	F value
between overall plant damage at day 60 (y) and the evaluated	Number of mines on the leaf	8.85	0.92	2.30	360.29*
characteristics.	Total number of trichomes on the stem	-0.06	0.93	0.40	5.04*

*Significant at $P \leq 0.05$.

significant differences were observed in the total number of trichomes on the leaf (F = 21.47, df = 10, 20, P < 0.0001), number of type VI glandular trichomes on the leaf (F = 46.58, df = 10, 20, P < 0.0001), and number of type VI glandular trichomes on the stem (F=7.54, df=10, 20, P<0.0001) among the cultivars (Table 4). However, differences in the total number of trichomes on the stem were not significant $(P^{\circ}0.05)$ (Table 4). The cultivars with more total trichome density on the leaf were Sadeen95, Berlina, and Golsar. The most density of type VI glandular trichomes on the leaf was observed in Sadeen95 and Golsar (Table 4). Golsar, Sadeen95, Zaman, Petopride5, PS6515, and Golshan616 had the greatest number of type VI glandular trichomes on the stem. The lowest total number of trichomes and also type VI glandular trichomes on the leaf was observed in Petopride5. The cultivars Matin, Poolad, Berlina, and Sadeen21 had the lower number of type VI glandular trichomes on the stem (Table 4).

Pearson's correlations revealed that the total number of trichomes on the leaf had negative and significant correlations with the overall plant damage (r = -0.42), leaf damage (r = -0.58), leaflet damage (r = -0.58), number of mines on the leaf (r = -0.40), number of holes on the stem (r = -0.40), and number of holes on the fruit (r = -0.63) (Table 5). Person's correlation estimates between type VI glandular trichomes on the leaf with overall plant damage (r = -0.40),

leaf damage (r = -0.56), leaflet damage (r = -0.58), number of mines on the leaf (r = -0.40), number of holes on the stem (r = -0.40), and number of holes on the fruit (r = -0.61) were also negative and significant (Table 5). These results suggest trichomes may have direct negative influence on both larval feeding and oviposition by insects (Handley et al 2005), result in the lowest number of larvae and consequently lower damage to leaves and plants. Gilardón et al (2001a) and Neves et al (2003) also reported significant positive correlation between the density of trichomes on the leaves and resistance to Tuta species, as well as its relation to trichomes type VI. Thus, the high density of leaf trichomes present in the cultivars Sadeen95, Berlina, and Golsar can be one of the possible causes of resistance to T. absoluta known as the antixenosis mechanism. Oliveira et al (2009) also observed that the HGB 1497 subsample of Solanum lycopersicum L. presented resistance by antixenosis to the tomato plant miner T. absoluta. The high density of trichomes on tomato leaves can be extremely important for a cultivar to avoid the presence of T. absoluta. In addition, different metabolites are secreted from trichomes on the stems and leaves of the tomato plants, which cause different resistance against T. absoluta (Gilardón et al 2001b). Compounds such as tridecan-2-one and undecan-2-one, especially secreted by type VI glandular trichomes on the tomato leaves, perform as physical and chemical barriers for



Fig 1 Dendrogram of 11 tomato cultivars for six studied variables using hierarchical cluster analysis (Ward's method).

insects and pathogens (Farrar & Kennedy 1991, Eigenbrode & Espelie 1995, Justus *et al* 2000, Picoaga *et al* 2003). Such features can be used in plant breeding programs aimed at resistance to pests with selections toward genes that express a higher number of trichomes. An exception was Poolad with a low number of trichomes on the leaf (Table 4), which showed high resistance to *T. absoluta* (Tables 1, 2 and 3). Also, Sadeen95 with the most density of its trichomes (Table 4), showed partially resistance to the pest (Tables 1 and 3). This result can be explained by the presence and role of other potential resistance factors such as allelochemicals that confer resistance to *T. absoluta* as shown by Leite *et al* (1999) and Suinaga *et al* (2004).

Person's correlation estimates between leaf and leaflet damage with the number of mines on the leaf, number of holes on the stem, and fruit were positive and significant (Table 5). Overall plant damage also had positive and significant estimates of correlation with leaf damage (r=0.76), leaflet damage (r=0.78), number of mines on the leaf (r=0.10), number of holes on the stem (r=0.10), and number of holes on the fruit (r=0.73) (Table 5). The number of holes on the fruit had positive and significant correlations with the number of mines on the leaf (r=0.75) and number of holes on the stem (r=0.74) (Table 5). The number of holes on the stem had positive and significant correlation with the number of mines on the leaf (r=0.10) (Table 5). The number of holes on the stem had positive and significant correlation with the number of mines on the leaf (r=0.10) (Table 5).

Stepwise regression is an automated tool used in the exploratory stages of model building to identify a useful subset of predictors. The process systematically adds the most significant variable or removes the least significant variable during each step. In order to remove the effect of non-effective characteristics in the regression model on grain yield, stepwise regression was used. The results of the stepwise regression analysis are presented in Table 6. The number of mines on the leaf (x_{4}) was the variable that best explained overall plant damage at day 60 after infestation (y) as shown by stepwise regression (Table 6). The total number of trichomes on the stem (x_1) was the second variable that exerted influence on overall plant damage. Parameter estimates showed that the number of mines on the leaf had positive significant effect, while the number of trichomes on the stem negatively affected overall plant damage at day 60 (Table 6).

The results of cluster analysis separated 11 evaluated tomato cultivars in three distinctive categories including Petopride5, Matin, SandokanF1, and PS6515 as susceptible; Golshan616, Sadeen21, and Sadeen95 as partially resistant; and Berlina, Zaman, Golsar, and Poolad as resistant cultivars (Fig 1).

Conclusions

for use in backcrosses in processing tomato breeding programs. However, our results are preliminary and require future studies for identifying the other resistance factors, other than trichome density, associated with these cultivars. Also, additional analyses with molecular markers will be needed for indicating the probable genetic variation between these tomato cultivars.

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