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Antibiosis Resistance of Plum Cultivars and Genotypes to the Plum Moth *Grapholita funebrana*

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ABSTRACT

Grapholita funebrana is one of the most dangerous pests for plum cultivation. This research used 26 promising plum cultivars and genotypes to evaluate several antibiosis indicators, i.e., the Antibiosis index (ABI), Larval Population Growth Rate (MRCR), and Population Age Structure Index (PASI). The quantitative and qualitative characteristics of plum germplasms were examined in laboratory conditions. Cluster analysis separated the genotypes according to their susceptibility to the plum fruit moth. Different fruit characteristics defined the antibiosis resistance index, analyzed by correlation. ABI and MRCR had large positive and negative values, respectively. The plum germplasms of Zojelo, Gholaman, Gr-Rezaeyeh, Laroda, Queen roza, Bokhara, Ghalo, Black star, Zard Kordestane, Feriyar, Genotype 19, G99, Black Amber, Ghomi, Mortini, G98, and G-balck had the highest antibiosis resistance to plum moths. Cluster mean values of ABI, MRCR, and PASI were 1.39, 0.28, and 75.43, respectively. The ABI correlated significantly with fruit diameter, TSS, fruit tissue firmness, kernel thickness, harvest time, dry weight to fresh fruit weight ratio, MRCR and fruit length, fruit width, fruit fresh weight, dry fruit weight, fruit diameter, fruit surface area, fruit tissue firmness, and PASI. Therefore, these characteristics were effective in antibiosis reactions in plum germplasms. The results of this research are scientifically valuable as supplementary information for selecting suitable plum cultivars and developing plum cultivation.

Introduction

Plum (*Prunus domestica* L.) is a stone-fruit tree with significant economic value (Cummins and Aldwinckle, 1995). It is crucial to investigate the genotypic and phenotypic diversity of plant characteristics, including nutritional quality, architecture, physical attributes, and resistance or tolerance to pest damage, while aiming to minimize pest populations in agricultural systems. (Wetzel et al., 2016). Plant resistance to biotic stress is an essential aspect of specific selection targets for plant breeders (Khan and Korban, 2022).

Manipulating the defense pathways of plant crops is a promising approach to controlling horticultural pests and improving crop-induced resistance (Bektas and Eulgem, 2015). The need to produce fruit tree cultivars with pest resistance is essential, especially in countries where gardeners can not withstand the economic failure caused by pests (Arora, 2017).

The plum fruit moth (*G. funebrana*) is one of the most hazardous pests in plum cultivation. The high yield is affected by the migration of larvae in the epidermis of the fruit, and by consuming their inner part; the fruits are damaged in terms of organoleptic and visual effects.

Understanding the reason for climate change and global warming, the increase in distribution, the damage severity, and the economic significance of this pest is close to being predicted (Torriani et al., 2010). To date, the extent of damage and reduced yield of fruit trees due to the moth is an estimated 30 and 70%. (Lu et al., 2012).

The plum moth had three generations on the late Italian plum cultivars Pozegaca, and two on Bilska Rana. The population of first-generation larvae on Italian plum cultivars was lower than on Pozgaca and Bilska Rana. The pest damage rate on Bilska Rana fruit was 37.96%, whereas on Italian and Pozgaca late cultivars, it was 43.78 and 51.45%, respectively (Batinica and Muratovic, 1972).

In other moths of the plum moth family, researchers evaluated the cultivar effect on the oviposition choice site in field conditions on (Denis and Schiffermüller) *Lobesia botrana* (L.), *Eupoecilia ambiguella* (Hübner), *Grapholita molesta* (Busck), and *Cydia pomonella* (L.) (Myers et al., 2006; Sharon et al., 2009; Wearing, 2016; Pavan et al., 2018).

Previous research considered the physical characteristics of fruits, such as the shape, size, texture, and color of *L. botrana* and *E. ambiguella* (Markheiser et al., 2018). In addition, the difference in larval development time, fecundity, egg size, and egg hatchability of the moth *L. botrana* in different grape cultivars has been investigated (Moreau et al., 2006a; 2007b; Thiéry and Moreau, 2006). In the Stanley cultivar, the hunting rate of pheromone traps was 70% lower than in other cultivars (Mitrea and Bancă, 2011). In another study, the least affected fruits by the plum fruit moth (*G. funebrana*) were of the Herman cultivar (Głowacka and Rozpara, 2014). However, the plum cultivars Empress, Veriti, Kachanska Najbulja, and Dabrovica were the most infected with pests. The cultivars were affected to varying degrees each year. However, the Weller, Vajuka, and Stanley cultivars showed the lowest damage incidence by the pest.

The infection of plum fruit nightshade is not dependent on yield or harvesting time (Pluciennik et al., 1999). In another study, the new Bulgarian plum cultivar, Ostromila, which is resistant to plum pox virus, also tolerated the damage caused by the eastern fruit moth (*Cydia molesta* Busck) (Zhivondov and Milusheva, 2015).

The low correlation between egg-laying preference and larval feeding performance on a host emanates from at least five factors, including the scarcity of the preferred host, the unsuitability of the host for egg-laying, the inadequacy of the food source for larval growth, the presence of secondary defense compounds, and the reduction of intra-species competition (Lill et al., 2007). Providing plum cultivars resistant to plum fruit moth can reduce orchard costs and increase fruit yield per unit area. It is vital to introduce plum cultivars resistant to the plum moth for effective pest management.

Material and Methods

Place and time of research

This research was carried out in the research orchard of the temperate and cold fruits research center located in Kamalshahr, Karaj (2019-2021). This station is located at an average height of 1350 m above sea level, 48 km west of Tehran. The geographic coordinates of this location is 51" 2' E and 35" 48' N.

Plum germplasms

Twenty-six promising plum cultivars and genotypes were used, namely, Kh-Mashhad, Sosormi, G-balk, G98, Mortini, No 17, Ghomi, Black Amber, G99, Geontype 19, Feriyar, Zard-Kordestane, Black Star, Ghalo, Bokhara, Queen rose, Laroda, Gr-Rezaeyeh, Gholaman, Zojelo, Uromieh 20, Santarza, G-Malayer, Kermanshah, Angelo, and G100 (Fig. 1).

Sampling method

Data collection started in the period of pest activity, i.e., in the middle of April. The data were collected once every seven days until the middle of October. For this purpose, four tree directions were considered, comprising the south, north, east, and west per tree. Twenty fruits were selected per direction.

Then, the samples were transferred to the laboratory, and active larvae were counted inside the fruits separately. The density of fruit moths in the sampling intervals showed a mutual relationship between their population density and fruit injury. Thus, the number of larvae was considered throughout the days when the plums were exposed to the larvae. The estimate was based on larval day (Ld) and calculated as equation 1 (Machlitt, 1998). In this relationship, A (i-1) and Ai are the number of larvae in the previous and present sampling, respectively, and "t" is the interval time (d) between the two samples.

Equation 1: $\mathrm{Ld} = \frac{\mathrm{A(i-1)-Ai}}{2} \times \mathrm{t}$

Fig. 1. Fruit pictures of studied plum cultivars/genotypes.

Antibiosis indicators

Antibiosis index (ABI)

The antibiosis index was calculated using equation 2. Here, GPD is the maximum larval population density in each cultivar/genotype during the season and tGPD is the time (d) to reach the maximum population in each cultivar/genotype (Inayatullah et al., 1990).

Equation 2: $\text{ABI} = \frac{\text{GPD}}{\text{t}_{\text{GPD}}}$

Larval population growth rate index (MRCR)

The population growth index was calculated using equation 3. In this relationship, C1 and C2 were the density of the total effective larval population in two consecutive samples and t is the range between the two samples (Dahlin and Ninkovic, 2013).

Equation 3: $\text{MRCR} = \frac{(\log \text{C2} - \log \text{C1})}{\text{t}}$

Population age structure index (PASI)

The population age structure index was calculated using equation 4. In this regard, Pnx is the percentage of larval population abundance in each cultivar or genotype compared to the average of all cultivars/genotypes, and Nmy is the larval population density in the cultivar/genotype (Bell, 2015).

Equation 4: $\text{PASI} = \frac{\sum \text{PnxNmy}}{100}$

Calculation of quantitative and qualitative characteristics

Ten fruits of each plum germplasm were randomly selected and transferred to the laboratory to examine their quantitative and qualitative characteristics, including fresh and dry weight, percentage of dry matter (using an accurate scale and oven), length, width, and thickness of the fruit. Measurements required a digital caliper (mm) and the ratio of length to diameter for each of the samples. The fruit size index was calculated by measuring the length or width through ($\boldsymbol{D_a = \frac{L+W+T}{3}}$). The average fruit diameter was calculated through $\boldsymbol{D_g = \sqrt[2]{L \times W \times T}}$, the sphericity index was calculated through $\boldsymbol{\varphi = \frac{D_g}{L} \times 100}$, and the surface area of the fruit was calculated through $\boldsymbol{\pi (D_g)^2}$. Total soluble solids (%) were measured via a Refractometer, acidity using TA titration method, fruit juice pH with a pH meter device, and fruit flesh texture firmness with a penetrometer device. Also, we used Vernier calipers to measure axial dimensions, i.e., length L (longest diameter), thickness T (shortest diameter), and width W (Sabzi et al., 2022).

Data analysis

The Kolmogorov-Smirnov test was used before statistical analysis to determine the normal distribution. The results showed the hypothesis of data normality ($P \leq 0.05$). The first step involved factor analysis to identify the unobservable factors of the effective combination of the antibiosis indices based on the set of observable indicators. The resulting linear combinations made it possible to describe the indicators of resistance and their relation to those calculated with it. Factors were extracted from the correlation coefficient matrix. Factors were rotated to maximize the relationship between indicators and factors. Then, we calculated the factor load (factor score) to determine the desired factors (Hamzehzarghani et al., 2005).

The second step involved cluster analysis as a function of the degree of susceptibility of plum fruit to the moths, aiming to separate the genotypes. The genotypes/cultivars that were similar in terms of antibiosis characteristics were divided into different groups based on a specific Euclidean distance. The data collected in this research were analyzed using the hierarchical grouping method via the IBM SPSS Statistics 27.0.1.0 software. After selecting the similarity criteria, we prepared a tree diagram (Dendrogram) representing the internal structure of the variables. Thus, we selected a suitable method to connect the clusters. The criteria for determining the best location were selected based on the Euclidean distance (Frades and Matthiesen, 2010), Linear discriminant analysis (LDA), the normal discriminant analysis (NDA), and generalization of Fisher's linear discriminant. These parameters enabled evaluations of germplasm clustering accuracy (Fraley and Raftery, 2002). The third step involved relationship analysis between the quantitative and qualitative characteristics of the fruit with the occurrence of antibiosis resistance in the studied germplasms. Correlation analysis revealed how effective each fruit characteristic was in determining antibiosis resistance indices (Gogtay and Thatte, 2017).

Results

Antibiosis reaction in plum germplasms

In this research, the occurrence of antibiosis resistance in the studied plum germplasms were used for the simultaneous investigation of antibiosis resistance indices, together with antibiosis resistance index (ABI), larval population growth rate index (MRCR), and population age structure index (PASI) and their location (Fig. 2).

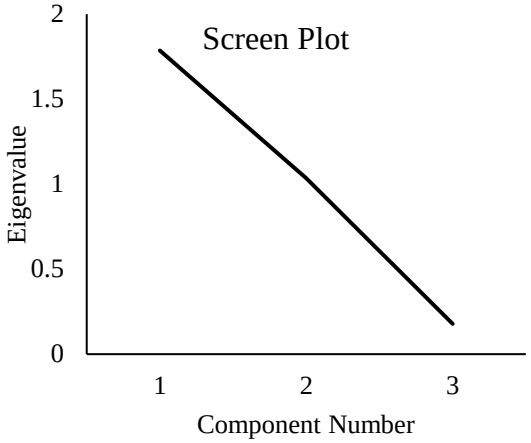

Fig. 2. Plot of the main components in the rotating space in the studied plum germplasms.

Based on the relationship between the indicators, three factors with roots greater than one explained 100% of the variation in the data. The first two factors had the greatest variations and accounted for 59.53 and 34.53% of the total variations, respectively (Table 1). Considering the period of the factors with varimax rotation (Table 2), which maximizes the variance between the factors and simplifies the interpretation of the factors, the factors that justify a higher percentage of index changes in the occurrence of antibiosis resistance are more important and should be investigated. Therefore, the effective indicators in each factor were identified, and the factors were named based on the most effective characteristics. According to Table 2, in the first factor, three indices were antibiosis resistance index (ABI), larval population growth rate index (MRCR), and population age structure index (PASI). These factors had positive and large coefficients in the occurrence of antibiosis resistance. According to the factor load, ABI and MRCR had large positive and negative values, respectively.

The clustering dendrogram of the studied plum genotypes and cultivars based on unobservable combined factors affecting the indices of antibiosis resistance was based on the set of observable and calculated indicators (Fig. 3).

Table 1. Factors that affect antibiosis resistance in the studied plum germplasms.

Component	Initial Eigenvalues			Extraction Sums of Squared			Rotation Sums of Squared		
	Total	% Variance	Cumulative %	Total	% Variance	Cumulative %	Total	% Variance	Cumulative %
1	1.786	59.527	59.527	1.786	59.527	59.527	1.552	51.743	51.743
2	1.036	34.529	94.056	1.036	34.529	94.056	1.269	42.314	94.056
3	0.178	5.944	100.000						

Table 2. Decomposition roots of factors after varimax rotation to identify combined unobservable factors affecting the type of antibiosis resistance indicators in the studied plum germplasms.

Indicators	Component	
	1	2
Antibiosis Index	0.565*	0.804*
Larval Population Growth Rate Index	0.744*	-0.624*
Population Age Structure Index	0.955*	0.012

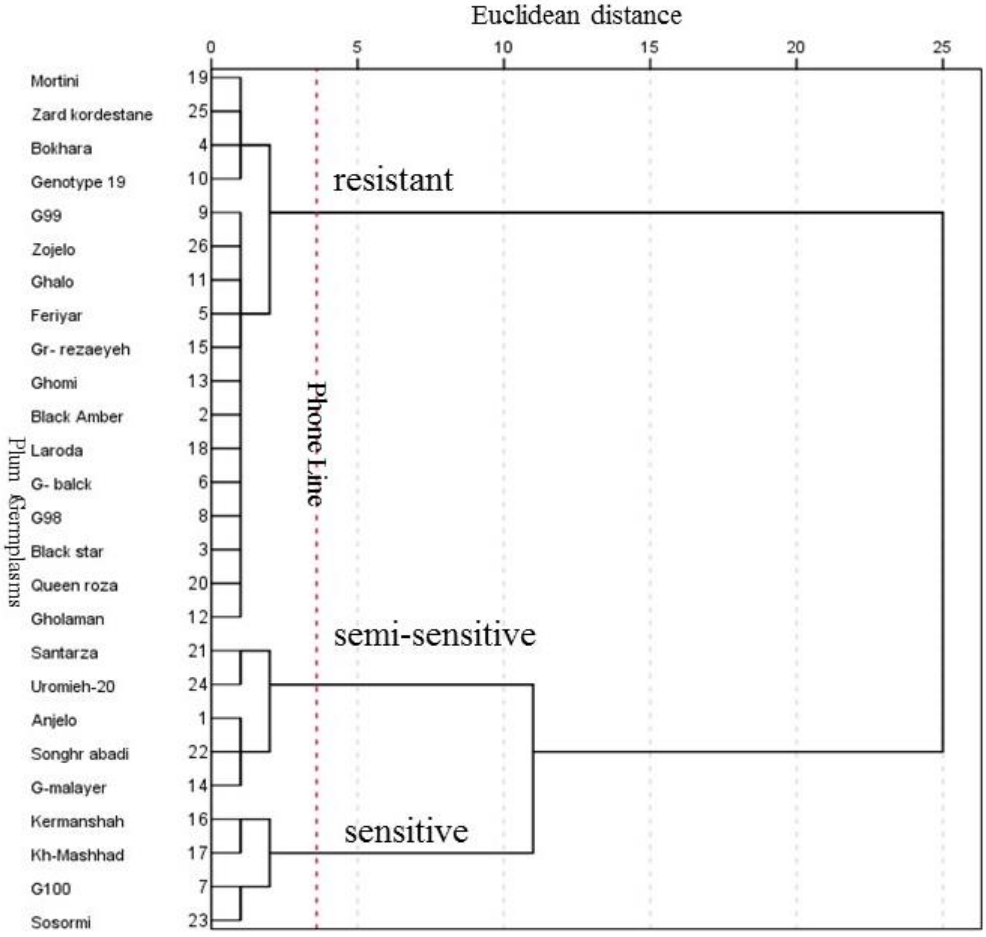

Fig. 3. Clustering of plum cultivars/genotypes based on antibiosis resistance reaction to plum moth.

In the clustering, three separate groups were obtained. The correlation coefficient between the Euclidean distance matrix and the dendrogram outlet matrix was 0.95. It showed the acceptable cultivar group in terms of antibiotic resistance indicators. The figures for each cluster are given in Table 3. In cluster 1, there are four plum germplasms named Sosormi, G100, Kh-Mashahd, and Kermanshah. These germplasms had the highest rank in terms of antibiosis resistance to

plum moths, so they were the most sensitive germplasms to plum moths. The average ABI, MRCR, and PASI were 1.39, 0.28, and 75.43, respectively.

Table 3. Characteristics of the studied plum germplasm grouping based on antibiosis resistance reaction to plum moth.

Rank of Antibiosis			Mean	Std. Deviation	Valid N (listwise)	
Clusters	Germplasms	Indices			Unweighted	Weighted
1.00	Sosormi, G100, Kh-Mashahd, Kermanshah	Antibiosis index	1.3950	0.51306	4	4.000
		Mean rate Population Growth	0.2750	0.03697	4	4.000
		Population Age Stacture	75.4350	6.64017	4	4.000
2.00	G-Malayer, Songhor Abadi, Anjelo, Uromieh 20, Santarza	Antibiosis Index	0.9400	0.21366	5	5.000
		Mean rate Population Growth	0.2400	0.03240	5	5.000
		Population Age Stracture	48.8160	5.39994	5	5.000
3.00	G- black, G98, Mortini, Ghomi, Black Amber, G99, Genotype 19, Feriyar, Zard Kordestane, Black star, Ghalo, Bokhara, Queen roza, Laroda, Gr-Rezaeyeh, Gholaman, Zojelo	Antibiosis Index	0.8224	0.33793	17	17.000
		Mean rate Population Growth	0.2176	0.02658	17	17.000
		Population Age Stracture	19.9335	5.45650	17	17.000

In the second cluster, there are five plum germplasms known as Anjelo, Santarza, G-Malayer, Uromieh 20, and Songhor Abadi. These germplasms were semi-sensitive to the plum moth in terms of antibiosis resistance. The mean of ABI, MRCR, and PASI were 0.94, 0.24, and 48.82, respectively. Finally, the most resistant germplasms were placed in cluster three. These genotypes/cultivars were Zojelo, Gholaman, Gr-Rezaeyeh, Laroda, Queen roza, Bokhara, Ghalo, Black star, Zard Kordestane, Feriyar, Geontype 19, G99, Black Amber, Ghomi, Mortini, G98, and G-black, i.e., 17 germplasms. The mean ABI, MRCR, and PASI were 0.82, 0.22, and 19.93, respectively. Comparing the dendrogram obtained from the cluster analysis using Fisher's linear detection function analysis indicated that Ward's criterion was able to group the plum germplasms with a probability of 96.8% accuracy and the difference between plum germplasms. The first function captured 99.3% of the variance. The closer Wilks's lambda is to zero, the better it is for separating groups. The first function had the lowest Wilks's lambda (0.054) and was more suitable than the other functions.

Relationship between quantitative and qualitative fruit characteristics and resistance indices

Relationships between quantitative and qualitative fruit characteristics with the occurrence of genetic resistance mechanisms were determined among the plum germplasms. The degrees of effect of different fruit characteristics on the resistance level were analyzed by calculating the correlation coefficient (Fig. 4).

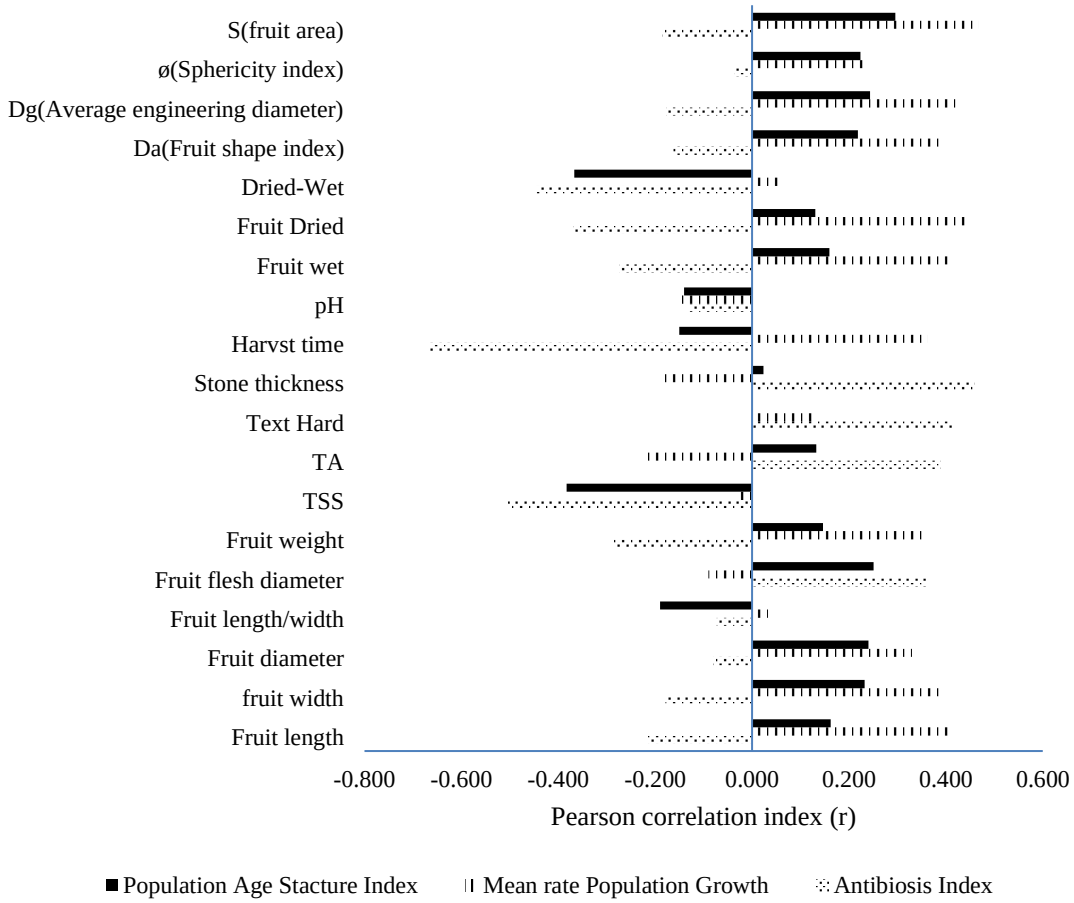

Fig. 4. Correlation coefficients of plum germplasm quantitative and qualitative fruits characteristics with antibiosis resistance indicators to plum moth.

Among the studied characteristics, the ABI correlated significantly with fruit diameter, TSS, fruit tissue firmness, kernel thickness, harvest time, ratio of dry fruit weight to fresh weight, MRCR, fruit length, fruit width, fruit fresh weight, fruit dry weight, fruit diameter, fruit surface area, fruit tissue firmness, and PASI. Therefore, the desired characteristics were effective in the occurrence of antibiosis reactions in plum germplasms.

Discussion

This research was conducted during pest activities (May to late October). There were differences in the resistance to antibiosis compared to the natural infection of *G. funeberana*. This difference occurred due to the differentiation of the pest (plum moth) based on antibiosis indices, including antibiosis index, larval population growth rate index, and population age structure index. The results showed a wide range of reactive levels. The most resistant germplasms were Zojelo, Gholaman, Gr-Rezaeyeh, Laroda, Queen roza, Bokhara, Ghalo, Black star, Zard-Kordestane, Feriyar, Genotype 19, G99, Black Amber, Ghomi, Mortini, G98 and G-black. The most susceptible plums were Sosormi, G100, Kh-Mashahd, and Kermanshah. The indices ABI, MRCR, and PASI were effective in the occurrence of antibiosis resistance. However, ABI and MRCR had a positive and negative relationship, respectively, in the incidence of antibiosis reactions among the studied germplasms.

The larvae that feed on more resistant germplasms have a higher antibiosis index and lower growth rate index and age structure of the population than the larvae that feed on other germplasms. The results obtained by other researchers in the study of the antibiosis response of host plants to pest moths in free-choice conditions show similar findings (Rodriguez-Saona et al., 2011). A previous study showed that the larvae feeding from some cultivars had a lower weight than others, which indicated a decrease in feeding, hence reduced damage (Parrott et al., 1978). A significant cross-

correlation was observed between a two-time series of seasonal changes in population density and the amount of damage. This correlation was stronger in sensitive germplasms than in other germplasms. However, since any evidence of antibiosis becomes more reliable with pupation and the emergence of adults, future research can examine this evidence, which can be useful in completing the information on the antibiosis response of plum germplasms (Latifian et al., 2023). Short-term changes in larval growth rates may not accurately reflect changes in the entire insect population. Other factors may influence changes in correlations between population changes among these two developmental stages at the pupal stage.

Another crucial point is that differences in the growth rate of the larval population alone may not be a specific predictor of host plant resistance. This issue was investigated when studying the resistance of other host plants to moths, thus confirming our conclusion (McMahan et al., 2017). Of course, other ecological factors, including bioclimatic factors, have decisive effects on the process of changes in the population of butterfly larvae. Increasing the necessary knowledge and information in managing the plum moth population is also essential in this field (Fonseca-Medrano et al., 2020).

The results showed that the antibiosis index had a significant correlation with the fruit diameter, TSS, the fruit tissue firmness, the thickness of the kernel, the harvest time, the ratio of dry weight to the fresh fruit, the growth rate index of the larval population and the length of the fruit, fruit width, fruit fresh weight, fruit dry weight, fruit diameter, fruit surface area, and fruit tissue firmness. The larval population age structure had a significant relationship with fruit tissue firmness. Therefore, the desired characteristics are effective in antibiosis reactions for plum germplasms. Studies conducted in recent years have shown that various quantitative and qualitative fruit characteristics are effective in the response of fruit tree cultivars to fruit-eating pests (Gogorcena et al., 2020). Other researchers have investigated the relationship of secondary plant compounds, such as the total concentration of phenolic acids or flavonols, regarding the antibiosis response of cultivars to pest moths (Isman et al., 1982; Stevenson et al., 1993). We suggest further research to investigate the role of secondary metabolites in plum germplasms on the intensity of antibiosis response to the plum moths.

Conclusion

This study presented a first-phase plum germplasm breeding program for obtaining cultivars resistant to the plum moth. Focusing solely on selecting fruits based on quality characteristics and not considering how this selection impacts the resistance of the fruit to pest damage we may end up using more pesticides in the future. It is important to revisit the potential consequences of our decisions to promote sustainable farming practices. The results of this research can offer supplementary information for selecting suitable plum cultivars when developing the cultivation of this product. Highly resistant cultivars increase the emergence rate of resistant biotypes and affect the level of pest resistance to pesticides. Therefore, it is crucial to select an exact level of antibiosis and know how to combine the resistant cultivar with other preventive methods against pests. It is necessary to conduct detailed and supplementary studies to gather information for the rational utilization of resistant plum cultivars in future research.

Author contributions

All authors read and approved the final manuscript.

Conflict of Interest

The authors indicate no conflict of interest for this work.

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