

Impact of morphologic characters in natural resistance of *Vicia faba* against *Aphis fabae*

Original Article

Abstract:

Vicia faba is one of the most important leguminous crops worldwide. *Aphis fabae* is considered the most common insect pest of this crop. The first aim of the present study was to assess the level of resistance of 68 broad bean genotypes to *A. fabae* in the field. To identify possible relationships between the external appearance of genotypes and their resistance levels to *A. fabae*, measurements were taken on the stem, foliage, flower, pod, and dry seed of all genotypes. Among 68 genotypes tested, 11 were extremely vulnerable, while 19 of them were resistant. Only leaflet width and dry seed weight differ significantly among all characters between the resistant and highly susceptible genotypes. This least had wider leaflets and a low weight of dry seeds. The taller genotypes had more stems and pods. The longer leaflets were wider. Genotypes with more flowers produce larger pods and a higher dry-seed weight. The resistant genotypes had narrow leaflets and high seed dry weight, with healthy aspects.

Key words:

biotic stress, broad bean, genetic sources, morphological characters, tolerance

Apstrakt:

Uticaj morfoloških karakteristika na prirodnu otpornost *Vicia faba* prema *Aphis fabae*

Vicia faba predstavlja jednu od najvažnijih mahunarki na globalnom nivou. *Aphis fabae* se smatra najčešćim insektnim štetočinom ove kulture. Prvi cilj ove studije bio je procena nivoa otpornosti 68 genotipova boba na *A. fabae* u poljskim uslovima. Radi identifikovanja mogućih odnosa između spoljašnjeg izgleda genotipova i njihovog nivoa otpornosti na *A. fabae*, vršena su merenja stabljike, lisne mase, cveta, mahune i suvog semena kod svih genotipova. Od 68 ispitivanih genotipova, 11 je bilo izrazito osetljivo, dok je 19 pokazalo otpornost. Samo širina listića i masa suvog semena pokazali su statistički značajne razlike između otpornih i visoko osetljivih genotipova. Ovi poslednji imali su šire listiće i manju masu suvog semena. Viši genotipovi imali su više stabljika i mahuna. Duži listići bili su i širi. Genotipovi sa više cvetova formirali su veće mahune i imali veću masu suvog semena. Otporni genotipovi imali su uske listiće i veliku masu suvog semena, uz zdraviji spoljašnji izgled.

Ključne reči:

biotički stres, bob, genetički resursi, morfološke karakteristike, tolerancija

Introduction

Broad bean (*Vicia faba* L.) (Fabaceae) is one of the most widely cultivated field crops globally, producing 6,073,526.15 t of dry matter, and covering approximately 2,782,564 hectares (Faostat, 2023).

The application of chemical pesticides is the predominant strategy for mitigating crop losses from insect pests and diseases, with an annual valuation exceeding USD 250 billion (Padmavathi & Padmaja, 2022).

The unscreened use of hazardous pesticides has led to the emergence of non-target pests, the development of resistance in target pests, and

considerable environmental risks (Padmavathi & Padmaja, 2022). Host plant resistance is essential for reducing crop losses in major crops and enhancing their economic efficiency by reducing input requirements. Insect-resistant varieties can be adopted by farmers without additional technical expertise or financial investment. Consequently, significant progress has been made in developing agricultural cultivars resistant to major insect pests affecting various field crops (Tooker & Frank, 2012; Padmavathi & Padmaja, 2022; Zhang et al., 2025).

Plant resistance refers to the proportion of heritable traits in a plant that influence the degree of damage caused by an insect (Painter, 1951).

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According to Smith (2005), host plant resistance encompasses all constitutive, genetically inherited traits that confer greater resistance to injury in a specific cultivar or species of plant compared to a susceptible plant that lacks these traits.

Any breeding effort focused on developing insect-resistant variants must commence with identifying the factors contributing to resistance through reliable phenotyping techniques. Quantifying plant traits associated with insect infestations is a key component of the phenotyping process for insect pests (Padmavathi & Padmaja, 2022).

Many native landraces and farmer-selected cultivars possess genetic traits that confer insect resistance. Salkathi, a landrace resistant to the brown planthopper, exemplifies this approach (Mohanty et al., 2017). Additionally, Rathuheenathi, a variety of guinea sorghum resistant to head bugs, is cultivated in West Africa (Padmavathi et al., 2007), along with shot fly-resistant sorghum (landrace variation Maldandi) grown in India during the post-rainy season (Sharma, 1993).

Several studies have investigated the sources of resistance in plants against insects, including the works of Bayoumi et al. (2017), Pinto & Ongaratto (2019), and Warghat et al. (2023).

Regarding faba bean seeds, Algeria has a

significant amount of local plant material. For example, in the Biskra region, each farmer grows his own seeds, selecting them from the robust plants of the previous harvest (Laamari et al., 2008).

To optimize the use of these local plant genetic resources, surveys were conducted across several regions of eastern Algeria to collect a diverse array of provenances. Consequently, the primary objective of the current study was to assess the resistance levels of 68 faba bean genotypes against various insect pests, including *A. fabae* (Aphididae), under field conditions. To achieve this objective, specific traits of the stems, foliage, flowers, pods, and dry seeds of the genotypes were measured to identify potential correlations between external morphological characteristics and the levels of resistance exhibited by these genotypes.

Materials and methods

Plant material

The plant material used in the present study comprised 68 genotypes of broad bean (*V. faba major* L.) collected from several localities in Spain and various locations in Algeria (Khenchela, Batna, Biskra, and Ouargla; **Tab. 1**).

Table 1. Codes and origins of the various broad bean tested cultivars

Code	Origin	Code	Origin	Code	Origin	Code	Origin
1	Spain	103	Biskra	123	Biskra	140	Khenchela
2	Spain	104	Biskra	124	Biskra	141	Biskra
4	Biskra	105	Biskra	125	Biskra	142	Khenchela
5	Biskra	107	Biskra	126	Biskra	143	Khenchela
6	Biskra	108	Biskra	127	Khenchela	144	Khenchela
7	Biskra	119	Biskra	128	Biskra	145	Khenchela
8	Biskra	110	Biskra	129	Biskra	146	Khenchela
9	Biskra	111	Biskra	130	Khenchela	147	Khenchela
10	Biskra	112	Biskra	131	Biskra	148	Khenchela
11	Biskra	113	Biskra	132	Biskra	149	Khenchela
12	Biskra	114	Biskra	133	Biskra	150	Khenchela
16	Biskra	115	Biskra	134	Khenchela	151	Batna
18	Biskra	116	Biskra	135	Khenchela	152	Batna
19	Biskra	117	Biskra	136	Khenchela	153	Biskra
100	Biskra	118	Biskra	137	Ouargla	155	Biskra
101	Biskra	119	Biskra	138	Ouargla	156	Biskra
102	Biskra	120	Biskra	139	Khenchela	157	Biskra

Aphids

The adults of *A. fabae* used in the study were obtained from a single-winged adult collected from a field bean crop in the Biskra region, eastern Algeria. The aphid breeding was carried out under greenhouse conditions of 20 ± 1 °C, natural photoperiod, and $42 \pm 5\%$ relative humidity [20 ± 1 °C; (L13: D11) h; $42 \pm 5\%$]. The collected aphid species was identified and verified by the expert Professor Malik Laamari, University of Batna 1-Hadj Lakhdar, Algeria.

Evaluation of the resistance in the field

In this trial, bean seeds representing 68 genotypes were produced at a rate of 10 seeds per genotype. The row spacing was set at 40 cm, and the plant spacing at 20 cm. At growth stage 65 (full flowering) (Mier, 2001), one apterous adult was placed on each plant (El-Defrawi et al., 1991). The aphid colonies on each plant were counted at 10, 20, and 30 days post-infestation to identify the most resistant genotypes to *A. fabae* and assess the persistence of this resistance over time (Meradsi, 2009; Lebbal, 2010). Genotypes were categorized according to the following scale proposed by El-Defrawi et al. (1991):

- Class 1: ≤ 5 aphids; resistant genotype
- Class 2: 6 – 20 aphids; tolerant genotype
- Class 3: 21 – 50 aphids; susceptible genotype
- Class 4: > 50 aphids; highly susceptible genotype

Evaluation of morphological characters of genotypes

To elucidate the relationship between the morphological traits of the genotypes and their level of resistance, a study was conducted to characterize the various plant organs. Measurements were taken on the stem, leaflet, flower, and pod of plants from 68 genotypes grown in the field. The dry seed weight of all cultivars was also measured and recorded. This assessment was applied according to the standards proposed by the UPOV guide (2003). In accordance with the same guidelines (UPOV, 2003), measurements of morphological characteristics should be conducted at specific phenological stages. Characteristics 3, 4, 6, 9, 10, 13, and 15, according to the UPOV guide (2003), must be assessed at full flowering. On the other hand, characteristics 21, 23, 24, and 27, according to the same guide, can be assessed during the fruit development stage. Characteristic 31 was calculated after harvest. In addition, it was noted that characteristics 9, 10, 13, 15, 23, 24, and 27 were estimated at the level of the secondary flowering node.

Statistical analysis

Normality was assessed using the Shapiro-Wilk

test, which is appropriate for samples across all morphological character parameters.

The application of a general linear model, such as the MANOVA test, aims to study the differences between the resistant and susceptible genotypes concerning the morphological parameters: plant height, stem number, leaflet length, leaflet width, flower number, flower length, pod number, pod length, pod width, ovule number, and dry seed weight. The means were separated using the least significant difference (LSD) test at the $p \leq 0.05$ level, after checking for homogeneity of variances using the Levene test.

Principal component analysis (PCA) was used to highlight possible relationships between morphological characters. An ascending hierarchical classification (AHC) was used as a complementary analysis to determine the number and composition of each homogeneous group of broad bean genotypes.

All statistical analyses and graphs were performed using SPSS version 27 software (SPSS, 2020).

Results

Resistance in the field

In the first count, 35 genotypes were classified as resistant, with numbers ≤ 5 aphids per plant. In the second count, only 23 maintained this status (resistant genotype). During the third count, this figure fell to 29 genotypes (Tab. 2).

Fig. 1 shows the morphology of 30 seed sizes from various bean genotypes, classified as resistant or highly susceptible after field harvesting.

Comparison between the morphology and the level of resistance of genotypes

The Manova test showed a significant difference ($p=0.050$; $p=0.039$) only for leaflet width and dry seed weight characters respectively (Tab. 3). The resistant genotypes had narrow leaflets and a high weight of dry seeds in comparison to the highly susceptible cultivars (Tab. 3). For the other characters, both types of cultivars were not significantly different ($p>0.05$) (Tab. 3).

Relationship between morphology and the level of resistance of genotypes

According to the circle of correlations (Fig. 2), the plant height, number of stems and pods were correlated positively with r ranged from 0.52 to 0.74 (Fig. 2). The leaflet length was positively correlated with the leaflet width ($r=0.68$; Fig. 2). The characters of flowers number, pod width and dry seed weight were correlated positively with r ranged from 0.55 to 0.66 (Fig. 2).

The 30 tested genotypes were divided into two

Table 2. Distribution of 68 broad bean cultivars during the three counts (10, 20 and 30 days after infestation) according to the number of *A. fabae* trained (n = 10 plants per cultivar).

Classe	Counts		
	10 days	20 days	30 days
Resistant	124 1 126 113		
	111 145 116 144	126 113 111 145	126 111 12 145 144
	149 127 12 129	144 127 12 129	149 127 2 142 10
	146 2 142 141	2 142 141 143	4 141 143 131 130 7
	143 131 130 156	131 130 148 133	156 133 117 6 114
	117 6 114 120	6 114 120 135	120 137 135 110
	135 109 103 100	110 105 119	104 105 101 119
	16 110 150 157		
	105 101 119		
Tolerant	134 4 7 19 148	134 152 112 156	103
	133 137 108	117 103	
Susceptible	132	4	19
Highly susceptible		107 102 118 125 8	107 102 124 118
	107 125 8 139	139 9 136 140 123	125 8 139 1 9 136
	140 123 11 147	11 132 116 151 18	140 123 11 132 116
	115 5 155	128 146 147 115	151 18 128 146
		100 108 5 155 157	147 115 100 108 5 155 157
Heterogeneous	9 10 18 102 104	1 7 10 16 19 101	16 109 112 113 129
	112 118 128 136	104 109 124 137	134 138 148 150
	138 151 152 153	138 149 150 153	152 153

homogeneous subgroups according to the Ascending Hierarchical Classification (**Fig. 3**). The first subgroup, composed of genotypes 107, 114, 8, and 6, has high levels of plant height, number of stems, length and width of leaflet, number and width of pod. The second subgroup, consisting of the 26 other genotypes, has particularly low levels of plant height, number of stems, leaflet length and width, and number of pods (**Fig. 4**).

Discussion

The broad bean genotypes under investigation were categorized based on their degree of resistance to *A. fabae* using the three counts. Eleven genotypes were

extremely vulnerable, while 19 were resistant. Numerous studies have already conducted on broad beans’ ability to withstand black aphid infestations. Using resistance scales based on the number of insects found on plants, El-Defrawi et al. (1991) successfully identified 114 broad bean cultivars resistant to *Aphis craccivora* (Aphididae) from a study of 7,156 cultivars conducted over four years in field conditions in Egypt. Additionally, over 1,000 lines were evaluated in this country, of which 36 were classified as resistant (Bond et al., 1994).

Furthermore, Khelfa (2004) found that among 48 faba bean cultivars collected from various locations in Biskra, Algeria, 5 exhibited varying levels of resistance to *A. craccivora* in field tests. In a separate study, Lebbal (2010) identified 16 resistant genotypes out of 62 examined in his investigation into the resilience of broad beans against the black alfalfa aphid.

However, several factors can influence field results. These include the

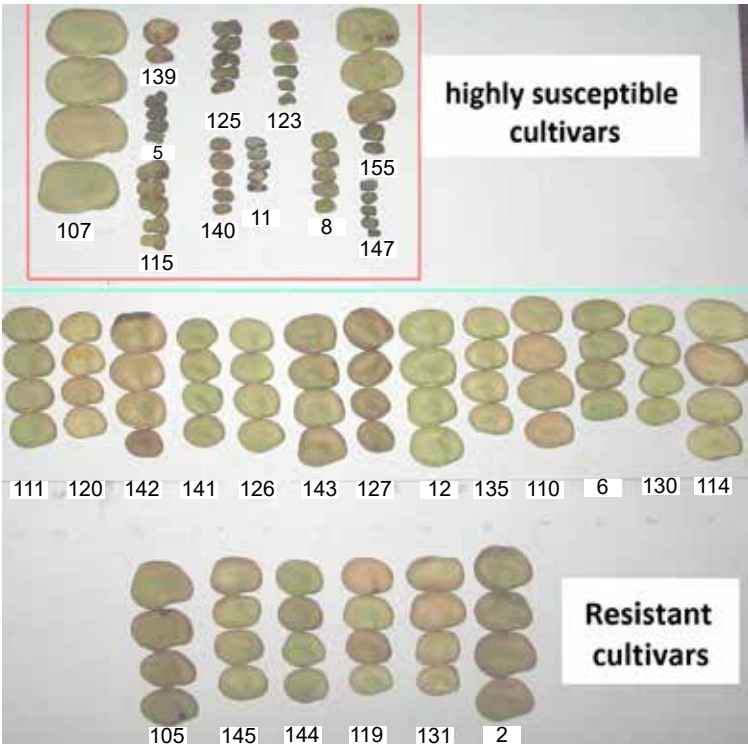


Fig. 1. External appearance and seed size of various broad bean genotypes classified as resistant (bottom) and highly susceptible (top) after resistance field test

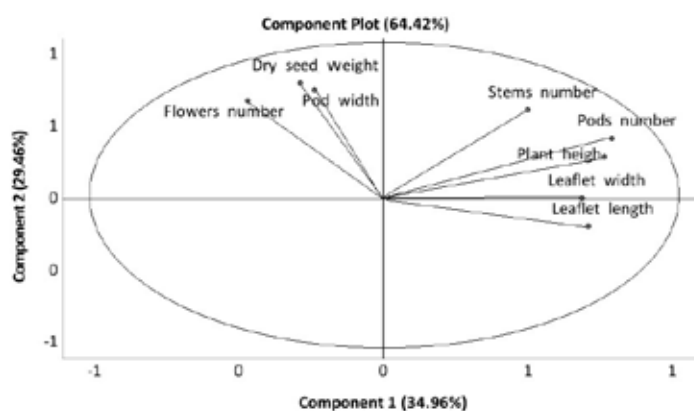
Table 3. Comparaision of morphological and agronomic characters between resistant and highly susecptible cultivars (Mean $\pm$ SE).

Morphological parameters	Level of resistance	n	Mean $\pm$ SE	F	p
Plant height cm (3)	RC	19	24.07 $\pm$ 1.20a	0.902	0.350 ^{ns}
	HSC	11	25.83 $\pm$ 1.24a		
Stems number (4)	RC	19	2.27 $\pm$ 0.14a	0.331	0.570 ^{ns}
	HSC	11	2.45 $\pm$ 0.33a		
Leaflet length cm (9)	RC	19	4.84 $\pm$ 0.16a	1.192	0.284 ^{ns}
	HSC	11	5.12 $\pm$ 0.18a		
Leaflet width cm (10)	RC	19	2.45 $\pm$ 0.08b	4.177	0.050*
	HSC	11	2.72 $\pm$ 0.08a		
Flowers number (13)	RC	19	2.74 $\pm$ 0.15a	0.001	0.978 ^{ns}
	HSC	11	2.75 $\pm$ 0.20a		
Flower length cm (15)	RC	18	3.05 $\pm$ 0.05a	0.073	0.789 ^{ns}
	HSC	9	3.08 $\pm$ 0.07a		
Pods number (21)	RC	19	4.48 $\pm$ 0.63a	0.879	0.357 ^{ns}
	HSC	11	5.67 $\pm$ 1.25a		
Pod length cm (23)	RC	16	10.54 $\pm$ 0.53a	0.094	0.762 ^{ns}
	HSC	7	10.23 $\pm$ 0.95a		
Pod width cm (24)	RC	15	2.57 $\pm$ 0.08a	0.018	0.894 ^{ns}
	HSC	7	2.60 $\pm$ 0.25a		
Ovules number (27)	RC	15	1.98 $\pm$ 0.27a	0.244	0.627 ^{ns}
	HSC	7	1.75 $\pm$ 0.32a		
Dry seed weight g (31)	RC	19	0.84 $\pm$ 0.07a	4.702	0.039*
	HSC	11	0.49 $\pm$ 0.17b		

* : $p \leq 0.05$. ns : not significant. n : number of repetitions. RC : Resistant cultivars. HSC: Highly susceptibles cultivars. Means with the same letters in the same morphological parameter were not significantly different at $p \leq 0.05$ according to LSD test's.

aphid biotype used to artificially infest the plants, and the phenological stages of the plants at the time of infestation and counting. Additionally, it is important to note that the field-grown plants were not covered, which facilitated the arrival of emigrating adults and their landing on the most attractive genotypes (El-Defrawi et al., 1991).

The resistance of the same plant can change depending on its stage of development. Apters of the green corn aphid, *Rhopalosiphum maidis* (Aphididae), raised on *Miscanthus sinensis* (Poaceae) plants at the two leaves stage were shown to be significantly less fecund than those kept on plants at the five leaves stage in a study

**Fig. 2.** Circle of correlation of morphological and agronomic characters of broad bean genotypes, projection of all characters on the factor (1,2).

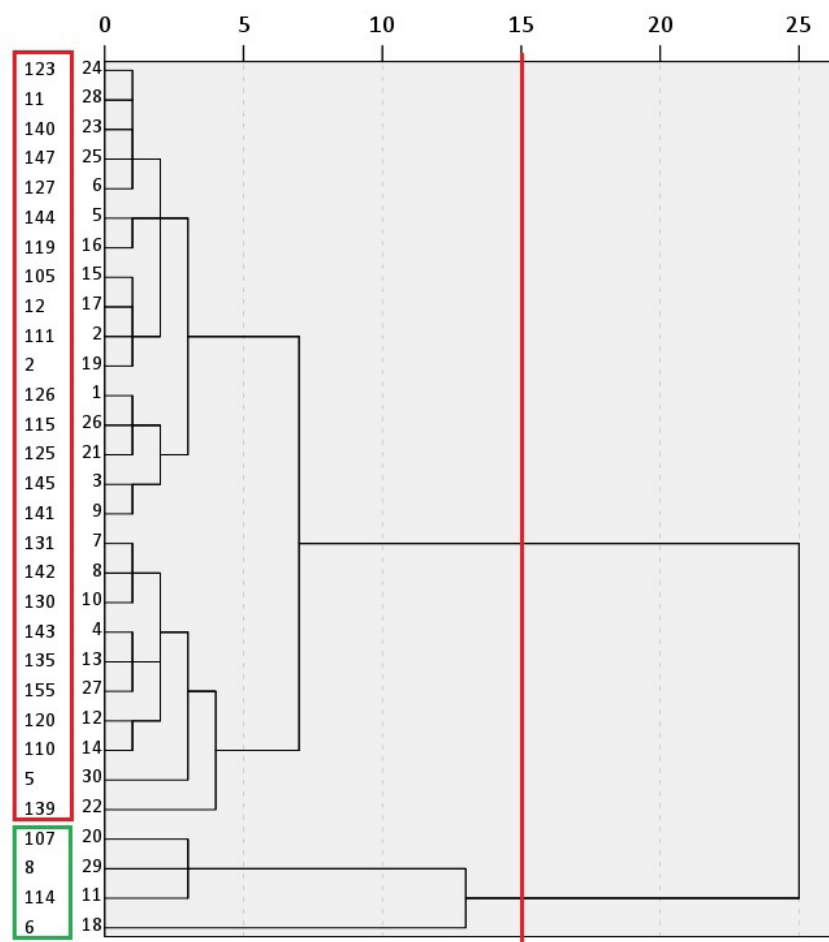


Fig. 3. Dendrogram using the average distance between 30 genotypes of broad bean according to the ascending hierarchical classification (AHC)

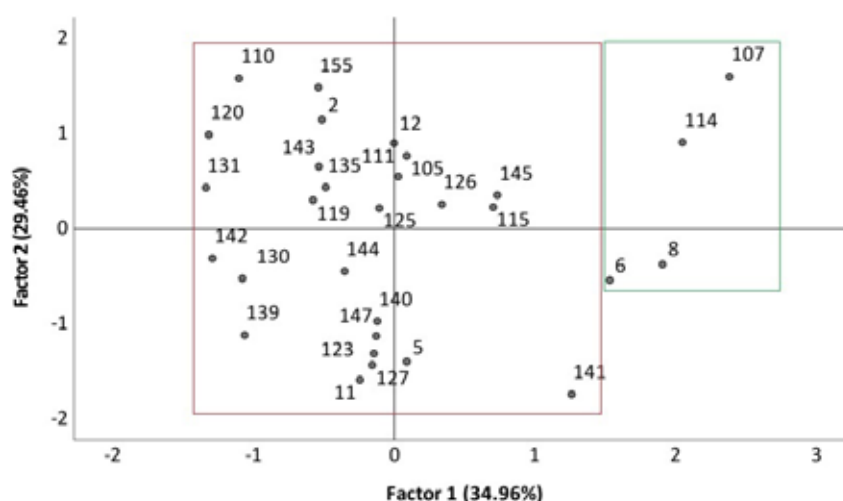


Fig. 4. Projection of 30 genotypes of broad bean on the factors (1,2)

conducted by Huggett et al. (1999). Similarly, certain genotypes of *Solanum* spp. (Solanaceae) showed resistance to green peach aphid *Myzus persicae* (Aphididae) in young leaves, while senescent leaves were susceptible to the disease (Alvarez et al., 2006).

Singh (2006) demonstrated that alfalfa's resistance to certain pests attacking the pod was correlated with morphological traits, including husk thickness, peduncle length, and seed position within the pod.

The results clearly showed that *A. fabae* attacks

Additionally, some grasses exhibit resistance only at a later stage of plant development (Smith, 2005).

In this field study, it was observed that among the highly susceptible genotypes, only cultivar 107 maintained a healthy vegetative state and green appearance. Resistance through the tolerance mechanism does not prevent aphid infestation or hinder their development and reproduction, but helps reduce the damage caused by the pest.

Numerous scientific studies have demonstrated that morphological and agronomic traits influence the level of plant resistance to pests. This is supported by several conducted experiments (Sharma et al., 2003; Dogimont et al., 2010; Smith & Chuang, 2014; Züst & Agrawal, 2016; Evgeny et al., 2022). Only the leaflet width and the weight of dry seed were included in the resistance of cultivars. The resistant genotypes had narrower leaflets and higher seed dry weight than the highly susceptible genotypes. It appears that the broad bean leaflet characteristic associated with resistant genotypes was specific to the genotypes examined in this study. In contrast, another study found that both resistant and highly susceptible bean genotypes shared the same morphological characteristics, including leaflet width (Lebbal, 2010). On the other hand, our previous study (Meradsi & Laamari, 2016) reported that both resistant and highly susceptible broad bean genotypes had similar leaflet widths, with the resistant genotypes also exhibiting longer leaflets. Indeed, Edwards &

negatively affected dry seed weight. Among the 11 genotypes identified as highly susceptible, 10 had lower dry seed weights than the resistant genotypes. For other traits such as plant height, number of stems, leaflet length, number of flowers, flower length, number of pods, pod length, pod width, and number of ovules, both resistant and highly susceptible genotypes exhibited similar levels.

It may be that other criteria, not quantified in this study, were responsible for the difference in resistance level between genotypes. These may be morphological, such as wax thickness, or chemical, such as the presence of certain primary or secondary metabolites and the nutritional value of the sap. Components within a plant species differ in terms of quantity and quality (Bernays & Chapman, 1994). More so, nutritional quality is one of the factors that determine plant exposure to phytophagous insects (Bernays & Wcislo, 1994). Aphid growth and reproduction were heavily influenced by nutrition, and the resistance of aphids may be affected by the nutrient and sugar content in plants. Various plant-produced substances can influence aphid physiology, behavior, and metabolism, potentially reducing aphid populations on resistant plants (Golawska, 2007). Secondary metabolic compounds in plants are critical biochemical constituents that contribute to their resistance to insects (Cai et al., 2004). In *Lupinus angustifolius* (Fabaceae), the alkaloids lupanine and 1-hydroxylupanine have been associated with resistance to *M. persicae* (Berlandier, 1996). Additionally, glucosinolates and their decomposition products play a role in plant defense against a wide range of potential threats. The primary products of glucosinolate breakdown, isothiocyanates, are toxic to certain herbivores (Wittstock et al., 2004). Indeed, it has been reported that the enzymes phenylalanine ammonia-lyase, polyphenol oxidase, and peroxidase are components of plant defense against insects (Felton et al., 1992; Stout et al., 1999; Chaman et al., 2001; Ni et al., 2001; Chaman et al., 2003). For instance, phenylalanine ammonia-lyase converts phenylalanine into trans-cinnamic acid, which can be further hydroxylated and methylated to yield compounds that are toxic to herbivores and pathogens (Cole, 1984; Leszczynski et al., 1989; Verpoorte & Alfermann, 2000; Morello et al., 2005; Wang et al., 2006). The activity of phenylalanine ammonia-lyase is associated with lettuce resistance to the aphid *Pemphigus bursarius* (Aphididae) (Cole, 1984).

The substances that play a role in insect resistance may be constitutive or induced, and may be present in plants, but their levels increase after insect attack. Wheat cultivars resistant to the ear aphid *Sitobion avenae* (Aphididae) showed

higher constitutive phenylalanine ammonia-lyase activity than susceptible cultivars during some phenological stages (Han et al., 2009). Han et al. (2009) found that infestation by this aphid increased phenylalanine ammonia-lyase levels in both resistant and susceptible cultivars.

Finally, it was interesting to identify resistant genotypes that combine quality, yield, and natural pest resistance, as in the research of Boissot et al. (2016). It should be noted that a genotype resistant to aphids does not necessarily protect it from viruses transmitted by these insects. Accidental transmissions (test bites) by other aphid species, not infesting the broad bean, may be responsible for virus transmission.

Conclusion

The field resistance results showed that 19 genotypes maintained their *A. fabae* resistance throughout the three counts. Conversely, 11 genotypes proved to be highly susceptible throughout the experiment.

Twelve morphological and agronomic characters were measured on the stem, foliage, inflorescence, pod, and dry seed of the 30 genotypes selected as resistant or highly susceptible after three counts throughout the broad bean development cycle.

The dry seeds of resistant genotypes retain a good external appearance, compared with the remarkably weathered seeds of highly susceptible genotypes. For the other characters (plant height, number of stems, leaflet length, number and length of flowers, number of pods, length, width, and number of ovules), both genotypes (resistant and highly susceptible) had the same levels.

The PCA results indicated that taller cultivars had more stems and pods, including genotypes 107, 114, 8, and 6. In terms of foliage, the longer leaflets were wider. Genotypes with more flowers produce larger pods and a higher dry-seed weight. The AHC indicated that the 30 genotypes were divided into two subgroups, with the genotypes 107, 114, 8, and 6 in the first subgroup. The second subgroup comprises the remaining 26 genotypes. These latest genotypes had particularly low levels of plant height, number of stems, leaflet length and width, and number of pods.

The present investigation indicated that resistant genotypes displayed narrower leaflets and heavier, healthier dry seeds. Consequently, more in-depth studies are needed to confirm the significance of these traits for integration into breeding programs focused on improving resistance to *A. fabae*. Additionally, this research recommends potential sources of resistance to *A. fabae* that can be leveraged in advanced *V. faba* breeding programs.

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