

Research Article

Identification of sources of resistance to the black bean aphid, *Aphis fabae* Scopoli, in Faba bean, *Vicia faba* L., accessions

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Abstract: In several parts of the world, important yield losses are caused to Faba bean due to attack by the black bean aphid *Aphis fabae* Scopoli. Although resistance could be a good alternative to chemical control, its genetic basis is still largely unknown; and its phenotypic expression is still poorly described, making crucial the permanent evaluation of genetic resources available in different countries; together with using adequate agronomic parameters and statistical inference. In this study, two Faba bean collections provided by Faba bean breeding programs of Tunisia and Spain were evaluated for resistance to *A. fabae* during two consecutive years in the field, based on infestation rates and three yield parameters: number of grains (NG), weight of grains (WG) and weight of pods (WP). Principal components analysis (PCA) showed that the most effective parameters contributing to response variation were the weight of pods (WP) and the number of grains (NG). Genotypes were grouped based on clustering patterns inferred from PCA and UPGMA, both showing the existence of two distinct groups: a resistant group characterized by high WP; and in most cases high NG values; and a susceptible group characterized by a low WP; and in most cases a low NG + WG. Furthermore, tolerance to infestation was identified in one genotype from the Spanish collection. Our results demonstrate that WP is the most reliable parameter for resistance selection; and that the genotypes V1, V9 and V16 from the Tunisian collection and V2e and V7e from the Spanish one should be exploited for their resistance potential.

Key words: *Aphis fabae*, *Vicia faba*, resistance, tolerance, screening

Introduction

Legume crops are mainly recognized for two characteristics: (1): their ability to fix atmospheric nitrogen by symbiosis with *Rhizobium* bacteria,

which makes their use in rotation with other crops a particularly efficient farming practice, leading to decreased weed infestation in the following season; and (2): their high protein content, making food legumes the best substitute for meat, for lower income populations. Faba bean, *Vicia faba* L., is one of the most important legume crops in the world. In the Mediterranean basin region, this legume plays key roles in human consumption, animal nutrition and farming systems.

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In Tunisia, food legumes are grown in approximately 80,000 ha, mainly in the north of the country under pluvial conditions, most of them are Faba bean (around 70%). Minor growing areas are also allocated to this crop in the centre and south of the country under irrigated conditions (Kharrat *et al.*, 1991). However, the total area dedicated to Faba bean farming is decreasing, as it is the case at global scale (Jensen *et al.*, 2010). One reason for this decrease is the susceptibility of this crop to several diseases and insect pests. Aphids such as *Acyrtosiphum pisum* Harris, *Aphis craccivora* Koch. and *Aphis fabae* Scopoli, inflict important yield loss to this crop (Bouhachem-Boukhris, 2002). Black bean aphid, *A. fabae* is the most damaging aphid in Tunisia. This aphid inflicts a destructive damage to Faba bean, on one hand through direct phloem feeding and on the other hand by transmission of several viruses, such as FBNYV and BLRV (Najar *et al.*, 2000). Currently chemical control is used effectively to control this pest, but it has numerous drawbacks, such as enhancement of the virulence of aphids, remaining of pesticide residues on the crop and the high cost of phytosanitary products. Therefore, breeding for pest resistance is, now, considered as the most efficient, cost-effective and environment-friendly controlling method. However, in most resistance reactions described, so far, in Faba bean, there are mainly two major problems: firstly; the genetic basis of resistance is still largely unknown; and secondly; the phenotypic expression of resistance is usually poorly described. As a consequence, there are currently few resistant sources available in the world.

The present study was conducted to be a contribution towards a better determination of sources of resistance to *A. fabae*, among Mediterranean Faba bean genotypes. Our aims were to define effective parameters enabling a reliable description of resistance to *A. fabae* in Faba bean and to assess two collections of Faba bean lines from the breeding program of Tunisia and Spain for their resistance level and productivity under artificial infestation in field.

Materials and Methods

Insect and plant materials

Samples of black bean aphid, *A. fabae* were collected in December 2010 from private fields of Faba bean, in Douala in the North-East of Tunisia (36.80 N; 10.64 E). Species identification was made in the laboratory, using taxonomic keys described by Blackman and Eastop (1984). Aphids were reared on potted Faba bean plants inside a growth chamber, at 23°C, 16L: 8D, and relative humidity of 80% to obtain enough population. Two sets of Faba bean genotypes, one consisting in 25 genotypes from the breeding program of Tunisia (kindly provided by Prof. Mohamed Kharrat, Laboratoire de Grandes Cultures, Institut National de Recherches Agronomiques de Tunisie, INRAT, Tunisia) and one consisting in 7 genotypes from Spain (kindly provided by Prof. Diego Rubiales, CSIC-IAS, University of Cordoba, Spain), were evaluated in field at Ariana station in the National Agriculture Research Institute of Tunisia (INRAT) station, in 2011 and 2012 (Table 1).

Experimental design

The experiment was conducted in a randomized complete block design with four replications. Each genotype was planted in a plot which consisted of 1 row of 1 m length with 1m inter row spacing. Ten seeds of each genotype per row were sown in early December 2010. When plants reached the 4-leaf stage (4 weeks after planting), top of each plant was artificially infested with five apterous adult aphids using a fine brush. In addition to the three infested blocs, another was planted and kept free of infestation. Using this control bloc allowed comparing infested and non infested plants, to identify the impact of the aphid on yield.

Assessment of resistance in field

The infestation level of each tested plant was estimated during the month of February of the years 2011 and 2012. To measure the infestation, we used a four-level scale corresponding to the percentage of plant

surface covered with aphids, as follows: 1: no infestation; 2: weak infestation (less than 20% of the plant was colonized); 3: average infestation (almost half of the plant was colonized) and 4: high infestation (almost all the plant was colonized). Subsequently, individual notes (1 to 4) were transformed into a mean infestation index (IF), applying to each tested genotype, following examination of 30 plants. Yield estimation was performed following harvest of whole plot, in early June of the years 2011 and 2012, by measuring three parameters of each plant: number of grains (NG), weight of grains (g/plant) (WG) and weight of pods (g/plant) (WP), and by considering average values, based on 30 plants.

Statistical analyses

For each of the three yield parameters, analysis of the variance was performed individually, using Proc ANOVA procedure and mean comparison test of Student-Newman-Keuls (SNK) in SAS software version 9.32 (2012), in order to define level classes among the tested Faba genotypes. Correlation between infestation and yield variables was performed based on the Spearman's (D) coefficient, using PAST software version 2.17b (Hammer *et al.*, 2001). Principal Component Analysis (PCA) was performed using PAST 2.17b, based on combined infestation indices and yield parameters. Cluster analysis was conducted based on combined infestation indices and yield parameters, using the UPGMA method (Sneath and Sokal, 1973) with Euclidian distance, in PAST version 2.17b.

Results

Analysis of individual yield parameters

Estimated parameters had significantly ($p < 0.01$) different values between tested samples. IF ranged between 1.65 and 4, with an average value of 3.14. WP ranged between 5.93 and 23.46, with an average value of

12.67 g/plant. NG ranged between 5.44 and 20.13, with an average value of 12.02. WG ranged between 1.46 and 13.49, with an average value of 6.51 g/plant (Table 1). The yield parameters (WP, NG and WG) showed high significant differences between genotypes ($p < 0.01$) however the differences between blocs were not significant. For IF, no significant differences between genotypes were observed.

No significant correlations were found between the studied parameters used although the highest correlation coefficient ($D = 0.78$) was detected between WP and NG (Table 2). IF was negatively correlated with WP (-0.49) and NG (-0.45); however, WG was not affected by IF. The Tunisian large seed variety 'Chahbi' (V11) had the highest IF (4) affecting the grain yield, since the variety was classified in the lowest yielding group. However the Tunisian small seed variety 'Bachaar' (V9) had the highest yield (WG = 17.73 g/plant) and low value of IF (2.44).

Classification of genotypes based on cluster analyses

In order to understand the contribution of each studied parameter to the total variation, PCA was conducted. PCA showed that the first two components explained 91.15% of total variation used for clustering lines (Table 3). The most effective parameter in the first component was WP; while the second component was mostly affected by NG. The PCA scatter plot (Fig. 1) showed the existence of two distinct groups. The first group (cluster I) is divided in two sub-clusters: (a) sub-cluster I-a, included genotypes V1 and V2 from the Tunisian collection, and genotypes V2e and V7e from the Spanish set; and (b) sub-cluster I-b represented by a single genotype V16. The second group (cluster II) comprised all the remaining genotypes from Tunisia and Spain. This distribution was perfectly in agreement with the topology of the UPGMA tree obtained using Euclidian distances (Fig. 2).

Table 1 Faba bean genotypes tested in the study and their responses to artificial infestation by *Aphis fabae*, as estimated by the infestation index (IF), weight of pods (WP), number of grains (NG) and weight of grains (WG).

Label	Genotypes	IF	WP (g/plant)	NG/plant	WG (g/plant)
<i>Tunisian collection</i>					
V1	Sel97 lat 97.107.1	2.70	22.48 bc	10.98 ab	14.21 cde
V2	R40	2.70	12.19 abc	12.62 ab	9.37 abcde
V3	XBJ90.04-6-2-1-1-4C	3.48	13.18 abc	14.17 ab	8.41 abcd
V4	XBJ92.13-9-2-1	3.92	11.21 abc	10.17 ab	7.80 abcd
V5	GIZA4	3.74	10.55 abc	10.42 ab	7.90 abcd
V6	Malti [#]	2.61	14.75 abc	11.14 ab	11.04 abcde
V7	XBJ92.13-6-3-1	3.83	9.65 ab	9.10 ab	5.81 abc
V8	XBJ92.10-46-1-3-1-2-1-1-1-6A	2.44	6.46 a	6.18 a	7.42 abcd
V9	Bachaar [#]	2.44	2.35 c	16.28 ab	17.73 e
V10	Baraca	3.39	11.87 abc	11.72 ab	8.96 abcd
V11	Chahbi [#]	4.00	11.22 abc	9.23 ab	7.00 abcd
V12	FRYT99-43	4.00	9.89 ab	9.34 ab	7.61 abcd
V13	BPL710-1	4.00	6.09 a	7.43 a	3.92 ab
V14	XBJ92.10-46-1-3-2-1-8A	4.00	11.06 abc	12.38 ab	7.77 abcd
V15	XBJ90.02-18-5-1-1-1-7A	3.74	10.17 ab	12.68 ab	6.82 abcd
V16	Sel.lat.98.304.4	2.87	17.83 abc	20.13 b	13.50 bcde
V17	FRYT98-14-2	3.13	13.06 abc	10.13 ab	7.52 abcd
V18	XBJ90.04-2-3-1-1-1-2A	3.48	10.03 ab	9.04 ab	7.54 abcd
V19	XBJ92.13-14-3-1	2.52	12.25 abc	13.87 ab	10.33 abcde
V20	FRYT98-48	2.44	7.53 a	7.89 ab	5.24 abc
V21	XBJ90.03-16-1-1-1A (Najeh) [#]	3.05	12.09 abc	15.34 ab	8.86 abcd
V22	Sel97.lat.98.134-1	4.00	4.85 a	6.25 a	3.21 a
V23	FRYT98-44	2.70	10.77 abc	11.00 ab	6.24 bc
V24	Badii [#]	3.22	6.36 a	10.51 ab	5.48 abc
V25	XBJ91.16-5-1-3-1-2-1	3.22	9.72 ab	11.38 ab	6.89 abcd
<i>Spanish collection</i>					
V1e	IX13/3-47	2.00	11.07 abc	11.82 ab	8.49 abcd
V2e	Baraca	3.22	21.20 bc	16.36 ab	14.80 cde
V3e	Av1099-2	2.35	16.03 abc	15.24 ab	12.31 abcde
V4e	IX 12-1-23	2.44	15.37 abc	14.87 ab	12.56 abcde
V5e	V-1108	2.26	13.47 abc	12.47 ab	8.19 abcd
V6e	IX10/1-19	1.65	16.63 abc	15.67 ab	12.07 abcde
V7e	V-1164	2.52	21.56 bc	18.73 ab	16.16 de
Overall mean		3.14 ± 0.82	12.67 ± 4.66	12.02 ± 3.38	6.51 ± 2.18

Means followed by the same letter(s) are not significantly different at the 0.01 level by the Student-Newman-Keuls' Test.

[#]: Tunisian commercial varieties.

Table 2 Spearman's D correlation ($p < 0.05$) coefficients between four infestation and yield parameters used to assess response of Faba bean to *A. fabae* infestation.

	IF	WP	NG	WG
IF	0			
WP	-0.491	0		
NG	-0.456	0.781	0	
WG	0.105	0.245	0.195	0

IF: Infestation index; NG: Number of grains; WG: Weight of grains; WP: Weight of pods. The highest correlation between WP and NG is indicated in bold.

Table 3 Principal component analysis for 4 studied parameters in Tunisian and Spanish Faba bean collections artificially infested by *A. fabae*.

Parameter	PC1	PC2	PC3	PC4
IF	-0.070	0.019	0.097	0.992
WP	0.823	-0.392	0.410	0.025
NG	0.548	0.829	-0.728	0.103
WG	0.128	0.396	0.539	-0.060
Eigenvalue	30.23	5.01	2.90	0.51
% variance	78.19	12.96	7.51	1.32
% cumulative	78.19	91.15	98.66	99.98

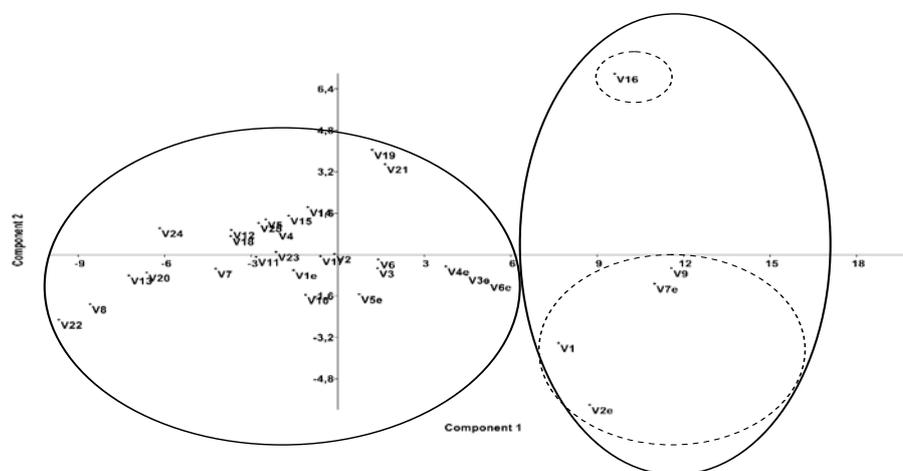


Figure 1 PCA scatterplot of 32 Faba bean genotypes artificially infested by *A. fabae* and studied with 4 parameters.

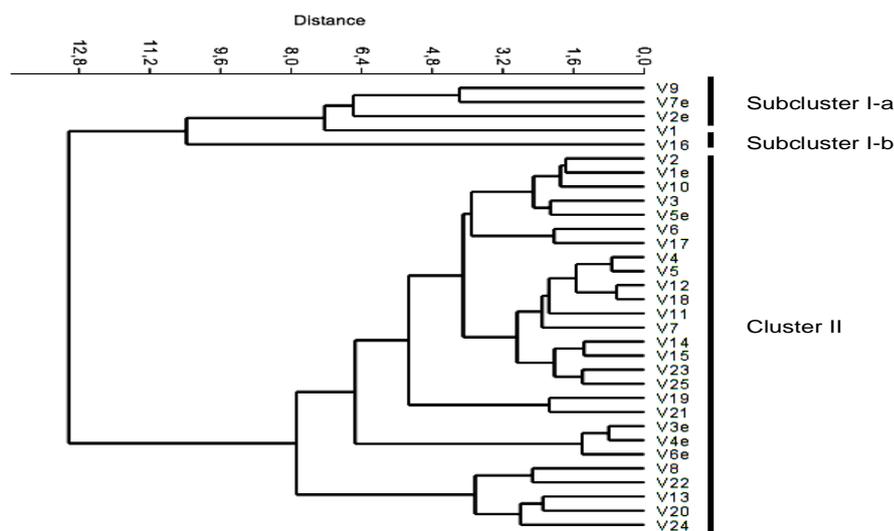


Figure 2 Tree diagram of 32 Faba bean genotypes submitted to artificial infestation by *A. fabae* and studied with 4 parameters (UPGMA method and Euclidean distance).

Selection of parameters describing resistance

By comparing the obtained PCA/UPGMA clustering patterns with the ranking of genotypes according to individual yield parameters, described above, at least four observations could be made; (i): genotypes belonging to cluster I (I-a and I-b) correspond to those showing the best WP values; (ii): in four out of five genotypes belonging to cluster I (V9, V2e, V7e and V16), the high WP performance was coupled to a high NG performance; (iii): the unique V16 genotype belonging to subcluster I-b was the only genotype among both Tunisian and Spanish collections that was characterized by a high yield performance based on all three yield parameters; and (iv): all genotypes belonging to cluster II were characterized by low WP performances. Therefore, both parameters WP and NG were crucial in developing the two major clusters related to *A. fabae* resistance: cluster I characterized by high WP and in most cases high NG values; and cluster II characterized by a low WP performance and in most cases a low NG + WG performance. Therefore, genotypes belonging to sub-cluster I-a can be considered as resistant, the unique genotype V16 belonging to sub-cluster I-b as highly resistant; while the remaining genotypes included in cluster II are susceptible.

Relationship between infestation and yield

Among Tunisian collection, genotypes V11, V12, V13, V14 and V22 exhibited the highest infestation index (4.00), followed by V4 and V7. None of these heavily infested genotypes was classed among genotypes displaying the best yield performances, with respect to WP, NG or WG. In contrast, the genotype V2e from the Spanish collection was the most infested but had high WP and NG values, suggesting that this genotype is tolerant to infestation.

Discussion

The first aim of the current study was to define effective parameters enabling a reliable description of resistance to *A. fabae* in Faba

bean. The second aim was to assess two collections of Faba bean lines from Tunisia and Spain for resistance level and productivity under high infestation. The most effective parameters, as shown by PCA, were the weight of pods (WP) and the number of grains (NG). Both parameters were decisive in developing the clustering patterns inferred from PCA and UPGMA. Two major clusters were defined.

The response of a number of legumes to infestation by aphids was previously studied by several authors and various parameters were used for the assessment of plant response. For instance, Klingler *et al.* (2005) studied the response of *Medicago trunculata* to *Acyrtosiphum pisum* Harris, based on the number of pods per plant, presence or absence of necrotic patches, population growth rate, total aphids weight and their percent of survival and scale of damages. Mian *et al.* (2008) studied response of soybean, *Glycine max* L., to *Aphis glycines* Matsumura, in the field and greenhouse, based on the severity of infestation. Laamari *et al.* (2008) used distinct protocols designed to assess, separately, antibiosis, tolerance and antixenosis mechanisms, in the evaluation of Faba bean resistance to *Aphis craccivora* Koch. Basky (2009) studied the effect of three aphid species, namely *Aphis fabae*, *Brachycaudus helichrysi* Kaltentbach and *Myzus persicae* Sulzer, on the common ragweed, *Ambrosia artemisiifolia* L., using three parameters: plant height, plant dry weight and pollen emission. The great variability of parameters used across these studies, in order to characterize legume responses to aphid infestation, reflects the symptomatic and phenotypic plasticity that usually accompanies legume-aphid interaction (Ferrari *et al.*, 2001; Fordyce, 2006). Such plasticity could be explained by the multiple facets of the infestation process, encompassing direct effect through plant injury, honeydew excretion and fungal attack; and finally the transmission of several damaging viruses.

The present study characterized five novel sources of resistance of Faba bean to *A. fabae*: 3 from Tunisia and 2 from Spain. Among Tunisian

commercial varieties, 'Bachaar' (V9), registered in Tunisia in 2003 and characterized by its high yield potential and its partial resistance to rust *Uromyces viciae-fabae* Pers. and stem nematode *Ditylenchus dipsaci* Kühn, could be a valuable source of resistance, to be immediately considered in breeding program since it showed low IF and high yield parameters. Although promising in terms of resistance diagnosis and resistance sources identification, the findings reported in this paper should be regularly discussed in light of continuous re-conducting of resistance tests, the enlargement of tested collection and, especially, linking variety response to the complex genetic structure of *A. fabae* populations in Tunisia, which is governed by a mixed effect of subspecies and geographical region as reported in Béji et al. (2013).

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تعیین منابع مقاومت در ژنوتیپهای مختلف باقلا *Vicia faba* L. به شته سیاه باقلا *Aphis fabae* Scopoli

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چکیده: در کشورهای مختلف دنیا شته سیاه باقلا *Aphis fabae* Scopoli موجب کاهش مهم عملکرد در گیاه باقلا میشود. گرچه ارقام مقاوم میتواند جایگزین کنترل شیمیایی شود اما هنوز اساس ژنتیکی مقاومت بهخوبی مشخص نشده است. بنابراین ارزیابی منابع ژنتیکی مقاومت در کشورهای مختلف ضروری بهنظر میرسد. همچنین لازم است ارزیابی مقاومت با سایر پارامترهای زراعی و تفسیرهای آماری همراه باشد. در این مطالعه دو سری از ژنوتیپهای باقلای تهیه شده از بانک ژن کشورهای تونس و اسپانیا در یک برنامه اصلاح ارقام باقلا برای تعیین مقاومت به شته سیاه باقلا در دو سال زراعی متوالی در مزرعه انجام گرفت. در شرایط آلودگی طبیعی پارامترهای مرتبط با عملکرد محصول نظیر تعداد دانه، وزن دانه و وزن غلاف اندازهگیری شد. روش تجزیه به مؤلفه‌های اصلی *Principal Component Analysis* (PCA) نشان داد که موثرترین پارامتر مقاومت ارقام باقلا وزن غلاف و تعداد دانه در غلاف میباشد. براساس کلاستر بندی بهروش *UPGMA* و *PCA* ژنوتیپهای مورد مطالعه در دو گروه مجزا شامل ژنوتیپهای مقاوم و ژنوتیپهای حساس قرار گرفتند. ژنوتیپهای مقاوم دارای وزن غلاف بالا و تعداد دانه بیشتری بودند. این درحالی است که ژنوتیپهای حساس تعداد غلاف کم و در بیشتر موارد وزن و تعداد دانههای کمتری داشتند. بهعلاوه یک ژنوتیپ متحمل از اسپانیا مورد شناسایی قرار گرفت. نتایج اثبات میکند که وزن غلاف مهمترین شاخص برای انتخاب ارقام مقاوم بوده است. همچنین نتایج نشان داد که ژنوتیپهای V1، V9 و V16 از تونس و ژنوتیپهای V2e و V7e از اسپانیا میتوانند برای تولید ارقام مقاوم به شته سیاه باقلا مورد استفاده قرار گیرند.

واژگان کلیدی: *Vicia faba*، *Aphis fabae*، مقاومت، تحمل، غربالگری