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Full Length Research Paper

Resistance source to cowpea aphid (*Aphis craccivora* Koch) in broad bean (*Vicia faba* L.) Algerian landrace collection

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The present study evaluated the resistance of 7 varieties of the broad bean *Vicia faba* L. to cowpea aphid, *Aphis craccivora* Koch, 1854. These landraces from the region of Biskra (in the south of Algeria) were selected in an initial field trial and subjected to further testing in the greenhouse. Landrace V51 proved to be the most interesting, in that it resisted aphid attack by mechanisms involving tolerance and antibiosis. Aphid infestation of this landrace resulted in a dry weight loss of only 3.09% (comparison with uninfested plants). The antibiotic resistance of this landrace also significantly decreased the biological potential of the cowpea aphid. In addition to the agronomic and heritage value, some of the landraces of the Biskra region displayed tolerance and antibiosis characteristics suggesting that they might serve as an important genetic resource in future bean selection programs. All tested landraces are attractive to *A. craccivora* but only this preference is marked more for varieties V23 and V24.

Key words: Bean, *Aphis craccivora*, land varieties, tolerance, resistance to deleterious factors.

INTRODUCTION

Bean (*Vicia faba* L.) remains the most important legumenous food crop in Algeria, covering an area of 58,000 hectares and generating a total yield of 254,000 tons (Anonymous, 1990). The Biskra region in the south satisfies almost 70% of national fresh bean requirements. The cowpea aphid, *Aphis craccivora* Koch, is the principal pest of bean crops in this region. Indeed, this pest causes major yield losses, due to the transmission of two major viruses, FBNYV and BLRV (Oufroukh, 1997) and commercial losses due to its production of large amounts of honeydew, rendering the pods unsightly and unsaleable.

Most of the farmers in this region produce their own seed, from vigorous plants in the previous season's crop.

This has led to the selection, over a number of generations, of a large number of varieties specifically adapted to this region. The Remafève network (a North-African bean research network) and the Mediterranean food legume research group have therefore decided, in collaboration with the ITDAS (The Technical Institute for the Development of Saharan Agriculture at Biskra), to collect as many of these landraces as possible and to identify those of major agronomic value, with the aim of conserving this heritage of plant resources. We tested 50 of these landraces for resistance to *Aphis craccivora*. A preliminary evaluation was carried out in the field (unpublished results), leading to the selection of seven landraces for further testing. A second study was then carried out in the greenhouse, to confirm the performance

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Abbreviations: FBNYV, Faba bean necrotic yellows virus; BLRV, Bean leafroll virus.

of these seven landraces and to determine the source of this resistance.

MATERIALS AND METHODS

Biological material

The seven landraces studied were supplied by the Technical Institute for the Development of Saharan Agriculture in Biskra (in the south of Algeria). These landraces were named V19, V23, V24, V26, V48, V19 and V51.

The aphids used for artificial infestation were obtained from a single strain reared continually for two years on beans in the zoology laboratory of the Agronomy Department of Batna University (Algeria). This strain was established from a colony collected from bean plants growing in the Biskra area.

Evaluation of tolerance

Tolerance was evaluated by comparing the dry weight of infested plants with that of healthy plants. The seven landraces were grown in pots containing 5 kg of soil, with one seed sown per pot. We planted 10 seeds in total for each landrace; ten for infestation and ten for use as negative controls (non infested). The plants were arranged in the greenhouse in a randomized block design. At the three-leaf stage, five apterous virgin *A. craccivora* females were placed on each of the plants to be infested. The plants were then individually covered with Tulle netting, to prevent external contamination. The plants were checked every two days. If necessary, individual aphids were moved from one plant to another, to ensure that the same level of infestation was maintained on all infested plants, throughout the experiment. After one month, the plants were cut at the level of the collar and placed individually in labeled plastic bags. They were transferred to the laboratory, where they were placed in an oven at 75°C for 72 h. Their dry weight was then determined.

We carried out an analysis of variance to determine the effects of landrace, infestation and the interaction of these two variables on dry weight. Fishers PLSD test was then used for pair-wise comparisons of all the landraces.

The decrease in dry weight due to infestation was determined as follows:

$$\text{Decrease in dry weight (\%)} = [(W_1 - W_2) \times 100]/ W_1,$$

Where W_1 is the dry weight of non-infested plants, in grams, and W_2 is the dry weight of infested plants, in grams.

Evaluation of resistance by antibiosis

For each landrace, we sowed a single seed into each of ten pots, each containing 5 kg of soil. At the two- to three-leaf stage, an apterous virgin female of *A. craccivora* was placed on each plant (Zeng et al., 1994). The female was removed after she had released two larvae, along with any additional larvae released. Once the two larvae maintained on the plant had reached the adult stage and had begun to reproduce, they were removed. All but one of the larvae of the second generation were removed. This individual was observed daily, to determine the duration of larval development, the duration of post-reproductive life, the duration of the imago stage, the duration of reproductive life and overall fertility (total number of larvae produced).

We calculated the multiplication rate (MR) and the intrinsic rate of natural increase (rm), using the following formulas:

MR = mean birth rate per female (number of larvae)/total lifespan (days) (Kashyap et al., 1988).

rm = 0.738 (log Fe) / Tpr (Asin and Pons, 2001)

Where Fe = effective fertility = number of larvae produced during a period equivalent to Tpr and Tpr = pre-reproductive time = number of days from the birth of the aphid until production of the first larva.

An analysis of variance was carried out for each variable, to define the impact of landraces on the various biological parameters of *A. craccivora.* Fisher's PLSD test was then used for pair-wise comparisons of all the landraces.

Evaluation of antixenotic resistance (non preference)

Seven seeds, one for each landrace, were sown at random in the same bag for each replicate; there were ten replicates in total. The seeds were allowed to germinate and the plants were allowed to grow for one month. We then placed 70 apterous virgin females in the center of each bag. After 72 h, we counted the number of aphids present on each plant in each of the 10 replicates.

Statistics

All statistical and graphical analyses were carried out with Statview V.5.0.

RESULTS

Resistance by tolerance

The analysis of variance showed that the various landraces reacted differently to infestation ($F_{6,70}$= 6.618 ; $P < 0.0001$). Landraces V48 and V51 were much more tolerant to *A. craccivora* than the other landraces. Infestation decreased dry weight by only 8.35% for V48 and 3.09% for V51 (Table 1 and Figure 1). The other landraces tested displayed decreases in dry weight of 37.21% (V19) to 65.25% (V24) (Figure 2). Landraces V48 and V51 also had a significantly higher dry weight ($P < 0.05$) than most of the other landraces in cases of infestation. Only V19, despite its low tolerance (37.216% decrease in dry weight) did not have a significantly lower dry weight than V48 and V51 after infection. This was due to a significantly higher dry weight of the non-infested controls ($P < 0.001$) for this landrace.

Resistance by antibiosis

The mean total fertility of *A. craccivora* females was between 36.5 and 80.2 larvae. Analysis of variance revealed a very strong effect of landrace ($F_{6,32}$ = 6.592; $P = 0.0003$). Fertility levels were highest for female aphids reared on V19 (mean of 80.2 larvae). On V51 and V23, the mean total fertility of female aphids was significantly lower ($P < 0.0001$), at about half the number of larvae released on V19 (Table 2).

Larval development lasted between 10.2 and 13 days.

Table 1. The dry weight of infested and non-infested seedlings and the decrease in dry weight due to infestation.

Landrace	Mean of dry weight of non infested plants (g)	Mean of dry weight of infested plants (g)	Decrease in dry weight (%)
V19	3.536 ± 0.054	2.22 ± 0.164	37.22
V23	3.03 ± 0.142	1.864 ± 0.174	38.48
V24	3.094 ± 0.081	1.075 ± 0.166	65.25
V26	2.78 ± 0.019	1.44 ± 0.056	48.20
V48	2.632 ± 0.086	2.412 ± 0.123	8.35
V49	3.252 ± 0.019	1.87 ± 0.257	42.49
V51	2.198 ± 0.009	2.13 ± 0.104	3.09

Values are mean ± SE (Standard error).

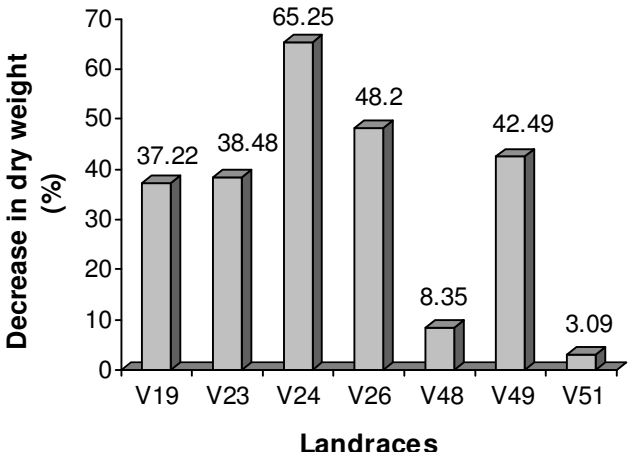

Figure 1. Decrease in dry weight (%) of broad bean landraces.

Figure 2. Comparison of a non-infested plant (on the right) with a plant infested with *Aphis craccivora* (on the left), for landrace V49.

Analysis of variance revealed an effect of landrace ($F_{6,32}$ = 12.201; P < 0.0001). V51 and V24 were the only landraces displaying a significant difference in the duration of larval development, at 13.33 and 11.6 days, respectively (P < 0.05). The prolongation of larval development was therefore more marked for V51. Individual aphids reared on this landrace were at the fourth stage of larval development when adults were emerging, or even starting to reproduce on the other landraces.

Landrace also had an effect on adult longevity ($F_{6,32}$ = 12.033; P < 0.0001) and the duration of reproductive life ($F_{6,32}$ = 16.693; P < 0.0001). On V51 and V23, adult longevity (10.75 and 15.25 days, respectively) and the duration of reproductive life (7.6 and 12.7 days, respectively) were significantly shorter (P < 0.05) than on the other landraces. Conversely, these periods were significantly longer (P < 0.05) on V19. There was no significant difference between V51 and V23 in terms of adult longevity, but the duration of reproductive life was significantly shorter (P = 0.0372) on V51 than on V23.

The multiplication rate and the intrinsic rate of natural increase are synthetic values designed to reflect the potential of colonies to develop. Analysis of variance identified a significant effect of landrace for these two variables [$F_{6,31}$ = 3.781; P = 0.0081 for MR, $F_{6,32}$ = 11.04, p < 0.0001 for rm]. The lowest multiplication rate (1.4) was obtained with V23, followed by V51 (1.56) and V48 (1.72). The highest multiplication rate was obtained on V24 (2.29). The intrinsic rate of natural increase (rm) was lowest on V51, at 0.081. The aphid was the most productive (rm = 0.116) on V19.

Antixenotic resistance

All tested landraces are attractive to *A. craccivora* but only this preference is marked more for varieties V23 and V24 (Figure 3).

Table 2. Mean of biological and demographic parameters of *A. craccivora* on seven broad bean landraces.

Landrace	Fertility – total number of larvae	Duration of larval development in days	Adult longevity in days	Duration of reproductive life in days	Intrinsic rate of increase	Multiplication rate
V19	80.2 ± 10.175	10.2 ± 0.2	29.4 ± 1.631	28.4 ± 1.661	0.116 ± 0.001	2.04 ± 0.272
V49	72.5 ± 3.524	10.25 ± 0.25	22 ± 1.817	20.250 ± 1.887	0.111 ± 0.002	2.19 ± 0.090
V24	69.4 ± 3.356	11.6 ± 0.51	18.8 ± 1.393	17 ± 2.739	0.098 ± 0.005	2.29 ± 0.081
V26	68.8 ± 8.206	10.2 ± 0.2	21 ± 1.140	18.2 ± 1.715	0.107 ± 0.004	2.18 ± 0.185
V48	53.8 ± 8.405	10.4 ± 0.245	20.4 ± 2.4	19.4 ± 2.182	0.098 ± 0.005	1.72 ± 0.212
V23	36.5 ± 3.4	10.75 ± 0.25	15.25 ± 1.436	12.7 ± 1.493	0.095 ± 0.002	1.4 ± 0.056
V51	38 ± 2.470	13 ± 0.316	10.75 ± 0.479	7.6 ± 0.678	0.081 ± 0.002	1.56 ± 0.129

Values are mean ± SE (Standard error).

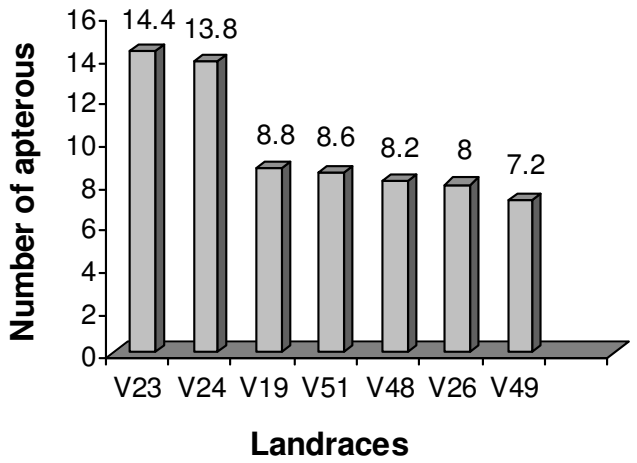

Figure 3. The attractivity of *A. craccivora* by the seven broan bean landraces.

DISCUSSION

Landraces V51, V48 and V19 produced the largest amounts of dry matter when infested with *A. craccivora.* V51 and V48 displayed a high level of tolerance to infestation, with a very small decrease in dry weight in the presence of aphids; however, this was not the case for V19, which was simply much more productive in the absence of infestation, in terms of dry matter, than the other landraces. Bean landraces in which aphid infestation caused only a small decrease in dry matter (V48, V51) suffered little damage due to *A. craccivora.* The plants remained green and in good condition until the end of the experiment. Loss of vegetative tissue due to infestation was much greater in the least resistant landraces. Landraces V51 and V48 should therefore be conserved and/or multiplied for production, or could be used in future selection programs aiming to develop bean plants tolerant to *A. craccivora* infestation.

Although tolerance makes it possible to achieve satisfactory yields even in the presence of aphids, only antibiosis can decrease the number of aphids present on the plant; the production of honeydew by aphids makes the pods unsightly and unsaleable, and therefore commercial losses would be reduced by minimizing the number of aphids.

Landrace V51 seemed to display the highest level of antibiosis-mediated resistance to the aphids, regardless of the biological parameter considered. Some landraces had a limited effect on larval development. This was the case for V24. Others affected only the adult phase. This was the case for V23, which was the only landrace that gave an MR lower than that for V51. *A. craccivora* performed significantly better on V19 than on any of the other landraces.

The significantly lower total fertility, MR and duration of reproductive life and the significantly longer duration of larval development on some landraces may reflect poor aphid nutrition. This may result from the absence of certain essential nutrients or the presence of substances or barriers inhibiting food intake. The presence of a thick layer of wax in the cuticle of V23 and V51 probably made it harder for the aphids to gain access to food. Some studies have suggested that substances synthesized after aphid infestation may play a role in resistance through antibiosis (Fidantsef et al., 1999; Vancanneyt et al., 2001; Brewer and Webster, 2001). More detailed studies of V23, V24 and, above all, V51, should make it possible to determine more accurately the source of this antibiosis.

Finally, all the landraces were attractive to *A. craccivora*; none of the landraces displayed resistance by antixenosis (lack of attractiveness). Research into possible antixenosis in other landraces is required. Thus, the problem of transmission by this aphid of viral diseases in bean landraces remains unsolved. When aphids are at a distance, their perception of the form (silhouette) of the plant allows them to recognize their host, whereas color plays a greater role when the aphid is closer (Rat-Morris, 1995). Aphids tend to be more attracted to large leaves than to narrow leaves. Searches of the Remafève network collection for landraces with particular characteristics in terms of leaf color and shape might lead to the discovery of new landraces displaying resistance by antixenosis.

Conclusion

Landraces V51 and V48 were the most tolerant to *A. craccivora.* They grew and developed normally during infestation, remaining green and in good condition. In addition to having a high level of tolerance, V51 showed the highest level of resistance by antibiosis, affecting various biological parameters of the aphid. V51 was therefore the landrace with the best performance. In terms of resistance through antixenosis, all the landraces were attractive to the aphid, so the problem of virus transmission remains unsolved. The selection of their own seeds by farmers in the Biskra region has generated a large number of landraces adapted to this region. In addition to their agronomic and heritage value, some of these landraces display tolerance and antibiosis, and may therefore constitute a useful genetic resource for future bean selection programs.

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