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Diallel analysis of pea aphid resistance in alfalfa seedlings

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SUMMARY – A 6-parent diallel crossing design was performed to analyse the genetic inheritance of the resistance of alfalfa seedlings to the pea aphid. Resistance was measured using no-choice laboratory screening techniques. Two aphid infestations were made and stopped 13 days after the first infestation. The dry weight of surviving seedlings was recorded 26 days after the first infestation. Three parents (CUF101, Monarca, the experimental cultivar Milfeuil-P4) were previously tested as resistant, two were susceptible (Europe and Diane) and one was intermediate (Cinna). General combining ability (GCA) was significant and much more important than specific combining ability (SCA). These results indicated that breeding for pea aphid resistance in alfalfa should produce a high genetic progress.

Key words : *Medicago sativa*, *Acyrthosiphon pisum*, diallel, resistance, combining ability.

RESUME – *"Analyse diallèle de la résistance des plantules de luzerne au puceron du pois". On a réalisé un plan de croisements diallèle 6 6 pour analyser le déterminisme génétique de la résistance des plantules de luzerne au puceron du pois. La résistance a été évaluée en conditions d'infestation contrôlée. Deux infestations successives ont été réalisées les premier et sixième jours, et arrêtées le 13ème jour. Le poids sec des plantules survivantes est mesuré le 26ème jour. Trois parents (CUF101, Monarca et le cultivar expérimental Milfeuil-P4) avaient été évalués précédemment comme résistants, deux comme sensibles (Europe et Diane) et un de niveau intermédiaire (Cinna). L'aptitude générale à la combinaison (AGC) est significative et beaucoup plus élevée que l'aptitude spécifique à la combinaison (ASC). Ces résultats indiquent que la sélection pour la résistance au puceron du pois, évaluée dans un test sur plantules, devrait conduire à un rapide progrès génétique chez la luzerne.*

Mots-clés : Medicago sativa*,* Acyrthosiphon pisum*, diallèle, résistance, aptitude à la combinaison.*

Introduction

The pea aphid (P.A.), *Acyrthosiphon pisum* Harris, is a well-known pest of forage production of alfalfa in France (Bournoville, 1989) as in the rest of Europe (Bournoville, 1976). Sometimes, it may pullulate in alfalfa seed production (Hacquet, 1997). To prevent its damages, varietal resistance of alfalfa has been developed mainly in America and Oceania. More recently we improved a standardized seedling test of resistance (Girousse *et al*., 1999; Bournoville *et al.,* 1999b) at the request of French plant breeders. This method evaluates the resistance of the seedlings to aphid infestation under controlled conditions and allows the ranking of cultivar resistance to the French P.A. populations. According to the percentage of seedling mortality, three classes of cultivars were defined: susceptible cultivars (less than one third of surviving seedlings), resistant (over two thirds surviving) and intermediate.

Previously, we evaluated alfalfa pea aphid resistance by insect responses to plants, by estimating the net reproductive rate of 4th stage larvae bred during two weeks (Girousse and Bournoville, 1994). This test was an antibiosis test in which some biological criteria of the aphids (mortality, reproduction, larval development, adult and larvae biomass) were followed. This kind of test, which is reliable but constraining, is not convenient when a great number of cultivars must be tested. In a previous study (Bournoville *et al.,* 1984) based on the antibiosis test, we found that the inheritance of P.A. resistance in alfalfa was mainly additive, and assumed that the resistance was determined by recessive alleles. The former information published in the US, also performed with antibiosis tests (Glover and Stanford, 1966), concluded that the genetic basis of alfalfa resistance to the P.A. was related to a single dominant gene. The present study examines the genetics of the resistance expressed in the seedling test in a diallel crossing design.

Materials and methods

The plant parental material used in this study was six alfalfa cultivars. The cultivars varied for their geographical origins and for their resistance to the pea aphid. CUF101 is a US cv., known as resistant to the P.A. in the US (Nielson and Lehman, 1977) as in France (Girousse and Bournoville, 1994). Monarca has been selected in Argentina for its resistance to aphids including *A. pisum* (Spada, 1996) and is resistant to P.A. in France (Landré *et al*., 1999). Cinna is a French cv. adapted to southern regions which ranked as intermediate for P.A. in our tests (Landré *et al.*, 1999). In these tests, the last cultivars of French origin, Diane and Europe ranked as susceptible to the P.A. An experimental cultivar (Milfeuil-P4) was obtained from 3 cycles of selection within the French cultivar Milfeuil for resistance to the P.A. (Bournoville *et al*., 1999a). We noticed an increase of resistance in P4, close to the resistant check (CUF101). Diallel crosses were made by bumblebees in glasshouse, with 21 plants for each cultivar. The seeds were collected on both parents and were used for the seedling tests.

The seedling tests were carried out according to the procedure of Bournoville *et al*. (1999b) who evaluated under growth chamber conditions (20ºC, 16:8 L:D) the survival of seedlings infested twice (on days 1 and 6) by a fixed biomass of aphids. On day 13, aphid infestations were stopped and the seedling survival was assessed on day 27. At that time, the dry weights of the surviving seedlings were measured. Seven replicates of 50 seedlings were used to test each diallel progeny and one control of 50 non-infested seedlings was added to ensure the quality of the growing conditions. As the different progenies of the diallel were evaluated in three successive tests, a common check (a susceptible cultivar, Milfeuil) was also included in each test. The infesting aphids originated from a clone of *A. pisum* multiplied on alfalfa shoots of a susceptible cv. In the successive tests, 70 to 120 adults of aphids were weighed with a microbalance, before each infestation, to be sure of the quality of the aphid stock.

The percentage of surviving seedlings and the dry weights of the surviving seedlings were subjected to analyses of variance. In a first step, the effect of progeny for the variable *Y* was tested as:

$$Y_{ij} = \quad + P_i + R_{ij}$$

with P_i the effect of the progeny *i* and R_{ij} the residual effect for progeny *i* and replicate *j*. In a second step, the diallel analysis was performed using the following model with fixed effects (Griffing, 1956):

$$Y_{ijk} = \quad + G_i + G_j + S_{ij} + R_{ijk}$$

with G_i and G_j the General Combining Ability (GCA) of the parents *i* and *j*, respectively, and S_{ij} the Specific Combining Ability (SCA) of the progeny *ij*. Mean squares of GCA effects were tested against SCA mean squares. SCA was tested against the residual. GCA of each parent and SCA of each progeny were calculated.

Results and discussion

The dry weights of the common check (Milfeuil) were very close for the three tests, and their means (0.195 g; 0.207 g; 0.194 g) did not differ ($p = 0.871$). It allows the general analyses of the diallel. The mean weight of the adults of P.A. were high (3.61 mg), which proved that the clone we used was well fitted to alfalfa.

The progeny effect was highly significant for the dry weight of surviving seedlings (Table 1). The effect of GCA and SCA were significant, but the mean square of GCA effect was far higher than that of SCA effect (Table 1). The inheritance is thus mainly additive, and suggests a possible rapid genetic progress in P.A. resistance of alfalfa cultivars.

The GCA of each parent is presented in Table 2. Two cultivars (CUF101 and Monarca) had a positive GCA, two (Europe and Diane) had a negative GCA, and the other two (Milfeuil-P4 and Cinna) had GCA close to 0.

Table 1. Diallel analysis (mean squares) for the dry weight of the surviving seedlings (DWS) of alfalfa after infestation by the pea aphid

Source	Df	DWS
Progeny	14	0.214***
GCA	5	0.525***
SCA	9	0.042***
Residual	90	0.007
Mean		0.362

***Significant at P = 0.001.

Table 2. GCA of six alfalfa cultivars in a diallel design for the dry weight of the surviving seedlings (DWS) after pea aphid infestation

Cultivar	DWS
CUF101	0.24
Milfeuil-P4	-0.05
Monarca	0.06
Cinna	0.00
Europe	-0.14
Diane	-0.11

Considering the SCA effects (Table 3), two crosses had a positive SCA (CUF101 Cinna and Milfeuil-P4 Europe) and two had a negative SCA (CUF101 Europe and Milfeuil-P4 Cinna). The genetic origin of the cultivars could explain a part of this feature: CUF 101 is an US cultivar, and could have genes or alleles of resistance different from those of the French cultivar Cinna. At the opposite, Milfeuil and Cinna were selected by the same breeder and could share the same genes or alleles of resistance. The other crosses had SCA values close to 0.

Table 3. SCA of the 15 F1-progenies obtained in a diallel design with six alfalfa cultivars as parents, for the dry weight of the surviving seedlings (DWS) after pea aphid infestation

	CUF101	Milfeuil-P4	Monarca	Cinna	Europe	Diane
CUF101		0.03	-0.02	*0.14*	*-0.10*	-0.05
Milfeuil-P4			-0.02	*-0.11*	*0.07*	0.03
Monarca				0.01	0.02	0.02
Cinna					-0.02	-0.02
Europe						0.03
Diane						

Conclusions

We have studied the resistance in alfalfa to the pea aphid by traits related to the aphid action to the plants, which are different from our previous studies made on insect reproduction. Soper *et al*. (1984) published diallel analysis of potato leafhopper resistance among alfalfa genotypes using yellowing injury (feeding damage), leafhopper oviposition, and nymphal survival. They concluded that these three characteristics should be considered separately in a breeding program. In our present study, the inheritance of the resistance was mainly additive, and genetic progress through breeding should be rapid.

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