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COMBINING ABILITY OF *Phaseolus vulgaris* L. FOR RESISTANCE TO COMMON BACTERIAL BLIGHT*

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ABSTRACT

Many diseases limit dry bean and snap bean yields. Common bacterial blight (CBB), caused by *Xanthomonas axonopodis* pv. *phaseoli*, is one of the most serious bacterial diseases in dry bean and snap bean that cause crop losses. Since there is no satisfactory chemical control, the use of resistant cultivars is an important control measure. Genetic studies of resistance are important for choosing appropriate breeding methods. Combining ability was determined for disease resistance in three snap bean genotypes (Alessa, Hab 52 and Hab 198) and two dry bean genotypes (Bac-6 and A-794). Plants were inoculated with highly pathogenic isolate CNF 15, using a razor blade procedure in leaves and needle punctures in pods. They were evaluated 7 days after inoculation. Leaves were evaluated on a 1 to 5 scale and pods by lesion diameter. Diallel analysis was conducted using Griffing's model. General combining ability (GCA) was significant for both leaf and pod infection, whereas specific combining ability (SCA) was significant for disease reaction in pods. Bac-6 and A-794 were considered superior genotypes for leaf resistance. Nonadditive effects were predominant in pod reactions, and Alessa x Bac-6, Alessa x A-794 and Hab 52 x Bac-6 were the best combinations.

INTRODUCTION

One of the most important diseases that affect snap and dry bean crop yields is common bacterial blight (CBB) caused by *Xanthomonas axonopodis* pv. *phaseoli*. This disease is also very important because of seed transmission, inefficient chemical control, and unsatisfactory resistance levels in available cultivars (Zapata, 1996). The main difficulties for obtaining resistance to CBB involved the identification of resistance sources, different leaf and pod response reactions and inoculation and assessment methodologies.

Knowledge of the genetic control of CBB resistance is needed for the determination of the breeding methods to be adopted. Parent selection for hybridization and formation of segregating populations should consider other traits besides disease resistance. Quality for consumption and/or industrialization and adaptation of the lines for cropping in specific regions are examples of characteristics to be considered (Zimmermann *et al*., 1996).

The diallel is a genetic-statistical methodology that assists in the selection of parents, based on their combining ability and potential to produce promising segregating populations. Diallel analysis also explains genetic control of traits, which further guides breeding and selection methods (Ramalho *et al*., 1993; Cruz and Regazzi, 1994).

The objective of this study was to determine the genetics of CBB resistance in bean crosses.

MATERIAL AND METHODS

Parents and their F_1 diallel progeny were studied for genetic control of CBB resistance in leaves and pods. Five *Phaseolus vulgaris* L. cultivars, three snap bean cultivars susceptible to CBB (Alessa, Hab 52 and Hab 198) and two dry bean cultivars resistant to CBB (Bac-6 and A-794), were used. Parents were chosen based on their preliminary CBB response tests and on divergent morphological-agronomic characteristics, which would allow morphological identification of F_1 individuals.

Crosses between the five parents (p) were produced in full diallel, with no reciprocals, resulting in 10 F_1 hybrids [p(p - 1)/2]. Hybridizations were made in greenhouses from May to October 1995. Hybrid seeds were obtained by artificial pollination done in the morning (6:30 to 10:00 a.m.) and afternoon (4:30 to 6:00 p.m.). Plants were hybridized using the emasculation with protected stigma method: the flower bud of the female parent is emasculated. Sepals are kept to protect the bud, and pollination quickly follows (CIAT, 1977).

Plants were grown in five-liter pots containing a soil-manure mixture (1:1) previously treated with methyl bromide. Nitrogen was added to the plants 20 and 40 days after emergence. Isolated or associated (depending on the cross) markers such as flower color, growth habit, and pod type (flat or round) were used to screen against self-pollinated seeds.

Fifteen genotypes (treatments) including parents and hybrids were cultivated in greenhouses from April to June 1996. The experimental design was a randomized complete block design with five replications of single plant plots (one plant per pot).

The highly pathogenic CNF 15 bacterial isolate (Rava, 1984; Rava *et al*., 1990), provided by the National Center for Beans and Rice Research - Embrapa/CNPAF,

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was cultivated in DYGS liquid medium (Rodrigues Neto *et al.*, 1986) for about 30 h. The bacterial suspension was smeared on Petri dishes containing solid DYGS. After approximately 36 h, bacterial colonies were suspended in a 0.8% saline solution, and cell concentration was adjusted to 10^7 cells/ml, using a spectrophotometer at 640-nm absorbance (Arnaud-Santana *et al.*, 1994). Freshly prepared inoculate was used.

Leaves were inoculated 25 days after sowing using the razor blade method (Pastor-Corrales *et al.*, 1981). Two folioles from the same leaf received the bacterial suspension, while a third received a saline solution and was used as a control.

Pods were inoculated by pipetting 10 µl of the inoculate at the height of the first seed from the insertion point in the plant in holes previously made with a hypodermic needle. Two green pods with full seed development were inoculated on each plant.

CBB leaf reaction was assessed 7 days after inoculation using the following scale: 1.0 = no symptoms; 2.0 = 1 to 5% necrosis; 3.0 = 6 to 25%; 4.0 = 26 to 50%; 5.0 = > 50% of the inoculated area presenting symptoms. The final score was the arithmetic mean of three independent assessments.

Pod lesion size (mm), measured from the point of inoculation, was assessed 7 days after inoculation, using the following classifications: resistant = $0 < x < 1$ mm; moderately resistant = $1 < x < 2$ mm; moderately susceptible = $2 < x < 3$ mm; susceptible = $3 < x < 4$ mm; highly susceptible = $x > 4$ mm.

Combining ability analyses were conducted using Griffing's (1956) method 2, in which parents and their F_1 hybrids are included, in $p(p + 1)/2$ genotypes. Statistical model 1, where cultivars are fixed effects and only experimental error is a random effect (Ramalho *et al.*, 1993), was adopted.

RESULTS AND DISCUSSION

F-tests of the analysis of variance for CBB reaction in leaves and pods of parents and F1 hybrids in the diallel of the assessed traits were significant at $P < 0.01$, indicating genetic variability among the 15 cultivars (five parents and 10 crosses). Average CBB infection was 2.27 for leaves and 1.78 for pods. Leaf (Table I) and pod (Table II) genotypes with lower mean values were more resistant to CBB, since the scale used lower values for more resistant genotypes.

Genotypes displaying CBB leaf reaction values above 2.5 were discarded as not having a satisfactory resistance level. Of the 10 hybrids obtained, only three were below the selection cutoff level, which was half of the maximum value of the adopted scale (Table I).

Using the value 2.0 for CBB pod reaction as a selection criterion, which was half the maximum value of the adopted scale for level of infection, the genotypes with above average resistance to CBB infection were selected (Table II). The cross of Alessa x Hab 198 would be selected for leaf reaction (2.40; Table I), but would be discarded for pod reaction (2.10; Table II). Hab 52 x A-794 would be selected for pod reaction (1.25), but not for leaf reaction (2.62). Alessa x Hab 52 and Hab 198 x Bac-6 would not be selected, for either leaf or pod resistance to CBB.

Table I - Mean values for parent and F1 hybrid reactions to common bacterial blight infection in *Phaseolus vulgaris* L. leaves measured on a scale of 1 to 5.

Parents	Alessa	Hab 52	Hab 198	Bac-6	A-794
Alessa	-	2.75	2.40	2.45	1.60
Hab 52	-	-	2.40	1.50	2.62
Hab 198	-	-	-	2.48	2.15
Bac-6	-	-	-	-	1.50
A-794	-	-	-	-	-
Mean of the parents	2.85	3.00	3.12	2.00	1.30

Least significant difference = 1.36.

Table II - Mean values for parent and F1 hybrid reactions to common bacterial blight infection in bean pods measured by lesion size (mm).

Parents	Alessa	Hab 52	Hab 198	Bac-6	A-794
Alessa	-	2.10	2.10	1.50	1.50
Hab 52	-	-	1.75	1.15	1.25
Hab 198	-	-	-	2.05	1.65
Bac-6	-	-	-	-	1.40
A-794	-	-	-	-	-
Mean of parents	2.70	1.75	3.40	1.20	1.20

Least significant difference = 0.91.

CBB reaction means in leaves were distributed into five groups by the Tukey test, with the A-794 parent representing extreme resistance and parents Hab 52 and Hab 198 extreme susceptibility. The remaining genotypes had intermediate resistance/susceptibility levels. Bac-6 was considered resistant by some authors (Mohan and Mohan, 1983; Aggour and Coyne, 1989), but Bac-6 was in an intermediate group, and its reaction was classified as moderately resistant, which is similar to the results reported by Arnaud-Santana *et al.* (1993) and Arnaud-Santana *et al.* (1994).

Pod reaction to CBB was classified into six groups. The hybrid Hab 52 x Bac-6 had extreme resistance (value near 1.0). Hab 198 parent had maximum susceptibility and by the adopted scale was classified as moderately susceptible. Bac-6 performed similarly to A-794, and both were classified as moderately resistant.

Based on the significance of the F-test, the sum of squares for treatments was partitioned into sum of squares for general combining ability (GCA) and specific combining ability (SCA), according to method 2, model 1, proposed by Griffing (1956) (Table III). GCA mean square values were highly significant ($P < 0.01$) for both leaf and pod infection. Differences among the GCA effects of the genotypes suggest that additive gene effects were expressed for CBB infection. SCA mean square for CBB pod reaction was highly significant ($P < 0.01$), suggesting that nonadditive gene effects were involved in the expression for CBB infection of pods. The finding of additive gene effects for these traits suggests the possibility of obtaining new cultivars from segregating populations from crosses among the tested parents. The estimates of the general combining ability (g_i) of each parent for reaction to CBB infection of leaves and pods and the standard deviation (SD) among any two parent g_i values suggest that the parents did not differ much from the mean of all the crosses in the diallel (Table IV). Estimates of significantly positive or negative g_i values indicate superior or inferior parents compared with the other parents in the diallel.

Table III - Estimates of the mean squares among the *Phaseolus vulgaris* L. genotypes (parents and their F1 hybrids of the diallel), the general and specific combining abilities (GCA and SCA) and the error variance and the estimates of the square values of the mean combining ability effects and error variance for reaction to common bacterial blight (CBB) in leaves and pods, according to method 2, model 1 of Griffing (1956).

SV	d.f.	Mean square	
		CBB leaves	CBB pods
Genotypes	14	1.491**	1.935**
GCA	4	3.847**	2.174**
SCA	10	0.549	1.840**
Error	56	0.590	0.263
Mean square of the effects			
GCA		0.093	0.055
SCA		0.008	0.315
Error		0.589	0.263

** Significant by the F-test, at 1% probability level. SV, Source of variation.

Table IV - Estimates of the general combining ability (g_i) for reaction to common bacterial blight (CBB) in leaves and pods assessed in five *Phaseolus vulgaris* L. genotypes and the standard deviations (SD) of the effects of two different parents.

Genotypes	Assessed characteristics	
	CBB leaf	CBB pod
1. Alessa	0.150	0.325
2. Hab 52	0.275	0.053
3. Hab 198	0.260	0.103
4. Bac-6	-0.202	-0.154
5. A-794	-0.483	-0.326
SD (g_i - g_j)	0.184	0.123

GCA values indicate the importance of genes with predominantly additive effects. Parents with the greatest GCA are potentially superior and may be included in breeding programs to select new inbred lines in advanced generations (Ramalho *et al.*, 1993). The lower g_i values correspond to superior parents, as lower g_i values indicate greater CBB resistance in leaves and pods.

Only Bac-6 and A-794 had negative g_i values for CBB reaction in leaves and pods, which means that these parents contribute to greater CBB resistance. A-794 was superior to Bac-6, since g_5 was greater than g_4 and about 2.6 times the magnitude of the g_i standard deviation for leaves and pods. Alessa, Hab 52, and Hab 198 had positive g_i values and did not contribute to resistance in the crosses in which they were included. The range of variation, given by the difference between the largest and smallest effect (g_2 - g_5), corresponded to about four times the standard deviation for CBB reaction in leaves and approximately 5.3 times CBB reaction in pods.

The g_i estimates are only valid for the set of parents tested in this study. If any of the parents described here were used in another diallel, that is, in combination with other genotypes, its GCA may be different, depending on the genetic structure of the other parents used (Vencovsky and Barriga, 1992).

SCA is due to nonadditive gene effects (Cruz and Regazzi, 1994) and depends on genes which show dominance and/or epistatic effects. SCA effects are the deviations in the performance of a hybrid compared with what would be expected based on the GCA of the parents. Table V

Table V - Estimates of the specific combining ability (s_{ii} and s_{ij}) effects for common bacterial blight (CBB) reaction in leaves and pods of *Phaseolus vulgaris* L. and the standard deviations (SD) of the effects for two F1, with and without common parents, and between two parents.

Effects (S_{ij} and S_{ij})$^{1/}$	Assessed characteristics	
	CBB leaves	CBB pods
1 x 1	0.2414	0.2714
1 x 2	0.0171	-0.4071
1 x 3	-0.3186	1.1929
1 x 4	0.1943	-0.7500
1 x 5	-0.3757	-0.5786
2 x 2	0.1429	0.2143
2 x 3	-0.4429	0.1643
2 x 4	-0.3800	-0.1786
2 x 5	0.5199	-0.0071
3 x 3	0.2914	-0.2357
3 x 4	0.1143	-0.5786
3 x 5	0.0643	-0.3071
4 x 4	0.0971	0.5786
4 x 5	-0.1228	0.3499
5 x 5	-0.0429	0.2743
SD (S_{ii} - S_{jj})	0.3180	0.2123
SD (S_{ij} - S_{ik})	0.4497	0.3003
SD (S_{ij} - S_{kl})	0.4105	0.2741

$^{1/}$(1)Alessa, (2) Hab 52, (3) Hab 198, (4) Bac-6 and (5) A-794.

includes the estimates of the SCA effects (s_{ii} and s_{ij}) and the standard deviations of the effects of two F_1 hybrids with and without a common parent and among two parents. s_{ii} indicates the presence or absence of unidirectional dominance, with genetic significance in terms of magnitude and signal. Negative s_{ii} shows the presence of deviations from unidirectional dominance, and consequently the presence of positive heterosis in the hybrid combinations. The magnitude of s_{ij} indicates genetic divergence of the parent in relation to the mean of the other parents tested in the diallel. The greater the absolute s_{ij} value, the greater will be the effect of the varietal heterosis, which is manifested in all of its hybrids (Cruz and Vencovsky, 1989).

A-794 had a negative s_{ij} reaction to CBB in leaves (Table V) but with an absolute value of small magnitude (0.0429). Hab 198 had a negative s_{ij} value for CBB reaction in pods. This shows that, in general, these traits have negative heterosis in hybrid combinations. Small s_{ij} values indicate that the F_1 hybrid performance can be predicted by parent performance. High absolute s_{ij} values indicate that the performance of a particular hybrid is better or worse in relation to the expected value based on the parental GCA. Low SCA values for CBB reaction in leaves and pods indicate greater resistance (better results).

CONCLUSIONS

The results obtained are inherent to the test conditions and cannot be extrapolated to other environments or populations. With that in mind, the following conclusions can be drawn:

- Bac-6 and A-794 had the greatest resistance to CBB. Furthermore, both parents performed well in terms of number of pods and seeds per plant.
- Griffing's analysis (1956) showed that only additive effects are involved in the genetic control of resistance to CBB in leaves, indicating that this resistance may be fixed in following generations.
- Dominant effects predominated over additive ones for CBB reaction in pods, especially in the combinations Alessa x Bac-6 and Alessa x A-794.

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RESUMO

Entre as várias doenças que causam problemas às culturas do feijão-de-vagem e do feijão comum, uma das mais importantes é o crestamento bacteriano comum (CBB), causado pela bactéria *Xanthomonas axonopodis* pv. *phaseoli*, que pode ocasionar grandes perdas na produção. O controle químico não é eficiente, e entre as medidas de controle recomendadas, destaca-se a resistência genética. Estudos genéticos da resistência são básicos para a definição dos métodos de melhoramento a serem adotados para cada caso. Avaliou-se a capacidade de combinação de três genótipos de feijão-de-vagem (Alessa, Hab 52, Hab 198) e dois de feijão comum (Bac-6 e A-794) quanto à resistência ao CBB, em folhas e vagens. A análise dialélica foi feita utilizando-se o esquema de cruzamentos dialélicos segundo o método II, modelo I, de Griffing. Para inoculação nas folhas empregou-se o método das lâminas paralelas e, nas vagens, o inóculo foi depositado com auxílio de micropipeta. Utilizou-se o isolado bacteriano CNF 15, procedente da Embrapa-CNPAF. A reação ao CBB foi avaliada sete dias após a inoculação por meio de escala de notas de 1 (resistente) a 5 (suscetível) para folhas. Para as vagens, avaliou-se o tamanho da lesão em mm. A capacidade geral de combinação foi significativa para ambos os caracteres avaliados. Efeitos significativos para a capacidade específica de combinação foram observados para resistência ao CBB em vagens. Constatou-se que os efeitos de aditividade foram superiores aos de dominância para resistência em folhas, identificando-se os genótipos Bac-6, A-794 e Alessa como os mais promissores. Para resistência em vagens, os efeitos de dominância se sobrepuseram aos de aditividade, permitindo-se destacar as combinações Alessa x Bac-6, Alessa x A-794 e Hab 52 x Bac-6.

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