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CROP BREEDING AND
APPLIED BIOTECHNOLOGY

Genetic divergence in snap bean based on agronomic traits and resistance to bacterial wilt

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ABSTRACT - *The objectives of this research were to estimate the genetic divergence among genotypes of bush snap bean with based on morphoagronomic descriptors and evaluation of resistance to bacterial wilt; to determine the relative importance of the distinguishing traits of genotypes and to indicate potential parents to establish a snap bean breeding program for resistance to bacterial wilt. Two experiments were conducted, the first in the field and the second in a greenhouse, both with 15 genotypes in a randomized block design with three replications. Seven traits were studied in the field experiment: days to flowering; days to harvest; mean pod length; number of pods; number of pods per plant; total pod weight and number of seeds per pod. In the greenhouse, the reaction of bacterial wilt was assessed based on two variables, one based on a descriptive grade scale and the other based on the Area Under the Disease Progress Curve (AUDPC). For the evaluation of genotype resistance to bacterial wilt the isolate Feij – 2634 of* Curtobacterium flaccumfaciens pv. flaccumfaciens *was inoculated. The statistics of pseudo t^2 indicated the formation of four groups by the UPGMA method, the same number of groups as indicated by the use of canonical variables. The traits that contributed most to genetic divergence were days to harvest, pod length, pod diameter and AUDPC.*

INTRODUCTION

Snap bean is affected by the same diseases and pests as common bean, since both crops belong to the same species, *Phaseolus vulgaris* L.. It is therefore possible to breed snap bean using common bean genotypes, e.g., snap bean breeders frequently use common bean as disease resistance source or other traits of interest in breeding programs. Among the main diseases of common bean, the bacterial wilt, caused by *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*

(*Cff*), is considered one of the most harmful to common and snap bean. In Brazil, this disease was first reported in common bean in 1995 (Maringoni and Rosa 1997) and to date there are no studies on the identification of resistance sources in snap bean.

The determination of genetic divergence among genotypes allows the choice of parents to obtain heterotic effect and to increase the probability that superior genotypes are found in the segregating generations. To estimate genetic divergence, different multivariate methods can be applied, as analyses by

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principal components, canonical variables and clustering methods. When the objective is to identify parents for crosses in a breeding programs, the mean Euclidean distance and especially Mahalanobis generalized distance are routinely used (Cruz et al. 2004).

As also for other species, the techniques of multivariate analysis are being used in snap bean to obtain information on the genetic divergence among accessions. Abreu et al. (2004) estimated the genetic divergence in 25 snap bean accessions with indeterminate growth habit of the genebank of the Universidade Estadual do Norte Fluminense Darcy Ribeiro. Five of the accessions, with highest divergence, were crossed by Silva et al. (2004) who studied the combining ability and identified promising parents and combinations to start a breeding program for climbing snap bean.

The objectives of this study were: a) to estimate the genetic divergence in bush snap bean genotypes based on morpho-agronomic descriptors and on the evaluation of resistance to bacterial wilt, b) to determine the relative

importance of traits in the discrimination of the genotypes, and c) to indicate potential parents for a snap bean breeding program for resistance to bacterial wilt.

MATERIAL AND METHODS

Two experiments were conducted in the experimental area of the Universidade do Estado de Mato Grosso (UNEMAT), in Tangará da Serra, MT, along the highway MT 358, km 07 (lat 14° 39' S and long 57° 25' W; alt. 321 m asl).

In experiment 1, the treatments consisted of 15 snap bean genotypes (Table 1) grown in the field from November 2006 to January 2007, in a randomized block design with three replications. The experimental plots consisted of two 3.0 m rows, spaced 0.8 m between rows and 0.2 m between plants. Thirty plants per plot were evaluated, without using the border plants. The cultural treatments were performed according to crop–specific recommendations (Filgueira 2008).

Table 1. Identification, origin and traits of 15 snap bean genotypes studied for the genetic divergence regarding reaction to bacterial wilt and morphoagronomic descriptors

Genotypes	Traits[1]					Origin
	PT	FC	PCOL	SCOL	HB	
Alessa	Manteiga	White	Green	White	Determinate	PESAGRO[2]
Andra	Macarrão	White	Green	White	Determinate	PESAGRO
Cota	Manteiga	White	Green	White	Determinate	PESAGRO
UEL-1	Macarrão	White	Green	White	Determinate	UEL[3]
UEL-2	Macarrão	White	Green	White	Determinate	UEL
Amarelo Baixo	Manteiga	Lavender	Golden	Black	Determinate	ISLA[4]
UENF-1467	Manteiga	Lavender	Green	Beige	Indeterminate	UFV[5]
UENF-1480	Manteiga	Lavender	Green	Black	Indeterminate	UFV
Manteiga Baixo	Macarrão	White	Green	White	Determinate	ISLA
Macarrão Baixo	Macarrão	Lavender	Golden	Black	Determinate	ISLA
UENF-1466	Manteiga	White	Green	Brown	Determinate	UFV
UENF-1453	Manteiga	Lavender	Green	Black	Indeterminate	UFV
UENF-1579	Macarrão	White	Green	Black	Determinate	CIAT[6]
UENF-1469	Manteiga	White	Green	Purple	Indeterminate	UFV
Novirex	Macarrão	Lavender	Green	Black	Determinate	EPAMIG[7]

[1] PT = Pod type; FC = flower color; PCOL = pod color; SCOL = Seed color; HB = growth habit
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[3] Universidade Estadual de Londrina
[4] Seed companies
[5] Universidade Federal de Viçosa
[6] Centro Internacional de Agricultura Tropical
[7] Empresa de Pesquisa Agropecuária de Minas Gerais

At sowing 625 kg ha⁻¹ of NPK (5-25-15) fertilizer was applied in the furrow, according to soil analysis. Twenty five days after plant emergence 30 kg ha⁻¹ of NPK (5-25-15) was applied. The following morphoagronomic descriptors were studied: days to flowering (DF) - number of days from planting to flowering observed (50% of the plants of a plot had newly opened flowers); days until harvest (DPH) - number of days from planting to harvest (Harvest time was considered ideal when the pods reached maximum development, before becoming fibrous. In this way, the pods are harvested when still tender and the turgid tips can be bent and broken off without effort); mean pod length (PL) - in a sample of 50 pods per plot, expressed in cm; total number of pods (TNP) - count of all pods per plot; number of pods per plant (NPP) - dividing the total number of pods per plot by the number of plants per plot; pod weight (PW) - total pod weight in grams; and number of seeds per pod (NSP) - ratio between the total number of seeds produced by the number of pods, considering a sample of 50 pods per plot.

The second experiment was conducted to evaluate the reaction of 15 bush snap bean genotypes to bacterial wilt. In this experiment the genotypes were grown in a greenhouse from January to March 2007. The plants were grown in pots containing a volume of approximately 2 liters of previously autoclaved soil. The experiment was conducted in a randomized block design with three replications. Each plot was represented by three plants, one per pot.

A portion of the stock bacterial culture of isolate Feij – 2634 of *Cff* (supplied by Professor Antonio Carlos Maringoni, UNESP / Jaboticabal), was transferred with a platinum spatula to Petri dishes containing solid Kado medium (Kado and Heskett 1970). The bacteria grew for 96 hours at 26 °C before inoculation, as proposed by Maringoni (2002). The plant stems of all plants were inoculated just below the pair of cotyledonary leaves eight days after emergence by inserting toothpicks, previously autoclaved and brought in contact with *Cff* colonies grown on the dishes, (Leite Junior and Behlau 2001).

The disease severity was evaluated by a descriptive grade scale described below: 1.0 = up to 10% of the plant wilted, 2.0 = 10% to 25%, 3.0 = 25% to 50%, 4.0 = 50% to 75% and 5.0 ≥ 75%. The disease severity was evaluated 12 times, every two days, beginning four days after inoculation. The grades were used to calculate the area under the disease progress curve (AUDPC), according to Campbell and Madden (1990).

Data were submitted to analysis of variance, and the means compared by the grouping test of Scott-Knott (Scott and Knott 1974), at 5% probability. Mahalanobis generalized distance (D^2) was used to quantify the genetic divergence among genotypes. The following methods were used: hierarchical clustering UPGMA (Unweighted Pair-Group Method using an Arithmetic Mean) and the canonical variables. The cophenetic correlation coefficient (CCC) was calculated to test the efficiency of UPGMA clustering, and pseudo-F (Calinski and Harabasz 1974) and pseudo t^2 statistics (Duda and Hart 1973) to determine the optimal number of groups. The relative importance of variables was also analyzed by the method of Singh (1981). The statistical analyses were performed using the programs GENES (Cruz 2006) and SAS (SAS Institute 1988).

RESULTS AND DISCUSSION

Significant differences were detected among the genotypes means (P<0.01) for all traits. By the Scott-Knott test, the means were classified into two groups for days to flowering, with eight genotypes in each group (Table 2). The genotypes Andra, UEL 1, UEL 2, Amarelo Baixo, Manteiga Baixo, Macarrão Baixo and Novirex flowered first, between 36 and 38 days after planting (DAP). The other genotypes flowered on average around 40 and 45 DAP. For the trait days until harvest, two groups were formed, the first with three later accessions, which flowered on average within 63 and 66 days after sowing. In the other 13 genotypes the harvest season was earlier, beginning 58 DAP for some genotypes. The genotype Novirex, although flowering first, was classified in the group of later harvest (mean of 59 DAP). This age is the ideal time to harvest this genotype (Pinto et al. 2001). In general, a longer cycle can result in higher labor cost and higher consumption of chemicals due to the increased possibility of occurrence of pests and diseases (Peixoto et al. 2002).

The variability among genotypes was greatest for pod length and the genotypes were classified into four groups (Table 2). The pod length of the genotypes Alessa, Cota, Macarrão Baixo and Novirex was greatest. Genotypes Alessa, Andra, Macarrão Baixo, UENF 1453 and UENF 1469 produced most seeds per pod. Two

yield-related traits, number of pods per plant and total number of pods, formed two groups and the genotypes UENF 1469 and UENF 1480 were present in the groups with the highest means for these variables. For total pod weight only one group was formed, based on the means.

The genotypes UEL 1, Amarelo Baixo and Novirex were the most promising in terms of *Cff* resistance, probably due to some resistance mechanism that resulted in a delay or prevention of *Cff* colonization. Common bean plants resistant to bacterial wilt were described by Schuster et al. (1964) who found that resistance is expressed as a smaller quantity of bacteria in the plant xylem. The other genotypes were highly susceptible to bacterial wilt (Table 2).

The AUDPC values ranged from 20.43 to 49.20, and the AUDPC values of the genotypes UEL 1, Amarelo Baixo, Novirex, Manteiga Baixo and Macarrão Baixo were the lowest, indicating some level of disease resistance (Table 2).

There are no studies on the performance of national snap bean genotypes challenged with this disease. Based on the results of this study, the genotypes identified as resistant, which are already commercial cultivars, may be recommended to producers and included in breeding programs to obtain cultivars with resistance along with other traits of interest.

The cluster analysis of genotypes by the UPGMA method, based on Mahalanobis generalized distance (D^2) as dissimilarity measure, had a CCC value of 0.89, demonstrating good reliability of the dendrogram. According to Sokal and Rohlf (1962), values above 0.8 indicate a good fit of the dissimilarity matrix and the dendrogram.

The optimal number of groups could not be determined by the pseudo F analysis, because the calculated F increased constantly with the number of groups formed, suggesting the absence of a 'natural' structure of data partition (Table 3). However, the analysis of the statistics of pseudo t^2 showed that the peak (56.6) was reached with the formation of four groups, indicating that this peak value is related to the lower probability of significance of the test, and would therefore indicate the interruption of the grouping process. Four groups are therefore considered ideal for UPGMA clustering analysis (Figure 1).

Table 2. Means of seven agronomical traits and reaction to bacterial wilt of 15 snap bean genotypes

Genotypes	Traits[1]								
	DF	DPH	PL	NVP	TNP	TPW	NSP	GRADE	AACPD
UENF 1480	41.67 a[2]	64.33 a	7.43 d	17.47 a	502.33 a	1269.33 a	5.37 b	3.89 a	43.05 a
UENF 1453	45.00 a	63.67 a	8.08 d	10.99 b	281.08 b	769.67 a	6.80 a	4.33 a	43.88 a
UENF 1469	43.00 a	61.00 b	9.22 c	16.94 a	463.99 a	1636.67 a	5.93 a	4.11 a	42.27 a
UENF 1579	41.33 a	66.00 a	11.28 b	5.45 b	129.87 b	537.33 a	5.40 b	4.22 a	46.72 a
UENF 1467	41.33 a	61.00 b	8.27 d	11.59 b	307.74 b	916.33 a	5.57 b	3.78 a	39.19 a
Alessa	41.33 a	59.33 b	12.87 a	7.70 b	231.00 b	901.33 a	5.17 b	4.55 a	48.39 a
Cota	40.00 a	59.33 b	13.00 a	8.03 b	232.33 b	1021.33 a	5.47 b	3.66 a	38.00 a
UENF 1466	40.33 a	59.33 b	10.07 c	10.39 b	303.73 b	856.33 a	5.13 b	3.89 a	43.30 a
UEL 2	37.00 b	58.00 b	11.87 b	3.99 b	73.91 b	284.33 a	4.30 b	4.33 a	43.83 a
Andra	38.33 b	58.67 b	11.33 b	5.59 b	138.15 b	725.00 a	6.07 a	3.22 a	35.55 a
UEL 1	37.00 b	58.00 b	10.48 b	9.21 b	276.20 b	918.67 a	5.10 b	1.50 b	19.83 b
Amarelo Baixo	36.00 b	58.00 b	11.07 b	9.01 b	222.05 b	850.00 a	4.93 b	2.00 b	25.33 b
Manteiga Baixo	38.67 b	59.33 b	11.23 b	5.44 b	144.89 b	541.67 a	4.57 b	3.11 a	32.77 b
Macarrão Baixo	36.00 b	58.00 b	12.90 a	6.98 b	209.30 b	798.67 a	6.53 a	3.22 a	31.55 b
Novirex	36.00 b	59.00 b	12.37 a	8.40 b	231.90 b	780.00 a	4.47 b	2.00 b	24.42 b
Mean	39.53	60.20	10.76	9.15	249.9	853.78	5.39	3.45	37.21
CV (%)	6.17	2.38	8.32	38.21	45.38	32.59	13.35	24.71	25.40

[1] DF = days to flowering; DPH = days from planting to harvest; PL = mean pod length (cm); NVP = mean number of pods per plot; TNP = total number of pods per plot; TPW = Total pod weight (g); NSP = mean number of seeds per pod; GRADE = reaction to bacterial wilt obtained in the 12th evaluation, 24 days after inoculation (DAI); AACPD = area under the disease-progress curve
[2] Means followed by the same letter in the same column belong to the same group, according to the Scott-Knott test, at 5% probability

Table 3. Determination of the ideal number for genotype grouping by the statistics pseudo F and pseudo t² based on the UPGMA method

No. of groups	Pseudo F	Pseudo t²
14	164.0	.
13	120.0	.
12	83.0	.
11	68.0	.
10	47.0	12.4
9	42.1	3.1
8	41.6	3.3
7	40.4	2.9
6	39.8	8.8
5	26.0	7.9
4	24.0	56.6
3	26.5	12.7
2	17.5	17.5
1	.	.

Figure 1. Dendrogram by the UPGMA method based on genetic dissimilarity measures of seven morphoagronomic traits and reaction to bacterial wilt in 15 snap bean genotypes

Considering the three yield components (number of pods per plant, total number of pods and pod weight) the genotypes UENF 1469 and UENF 1480 in group 1, were the most productive, could be used for crosses, mainly the cultivars with indeterminate growth habit. However, one must keep in mind that the difficulty of management and harvesting is greater for taller plants (Peixoto et al. 2002). Besides, these accessions have colored seeds, a qualitative trait which, in general, is not of interest on the snap bean consumer market, where white seeds are preferred, because they do not stain the pods with colored spots when cooked.

The first two canonical variables explained approximately 80% of the total variation (Figure 2). The results of grouping based on the canonical variables were similar to those of the UPGMA clustering technique, which also identified four genotype groups.

The four main variables that contributed most to the divergence among the genotypes were days to harvest, pod length, days to flowering and AUDPC (25.9%, 24.5%, 14.5% and 11, 3%, respectively) (Figure 3). The AUDPC was an important trait of the genetic divergence among accessions, since all resistant genotypes were clustered in one group (group 4).

In a breeding program, the use of parents with high genetic diversity is recommended to increase the probability of finding superior segregating populations in advanced generations and to expand the genetic base. However, the genotype choice should also be based on the performance *per se*. Thus,
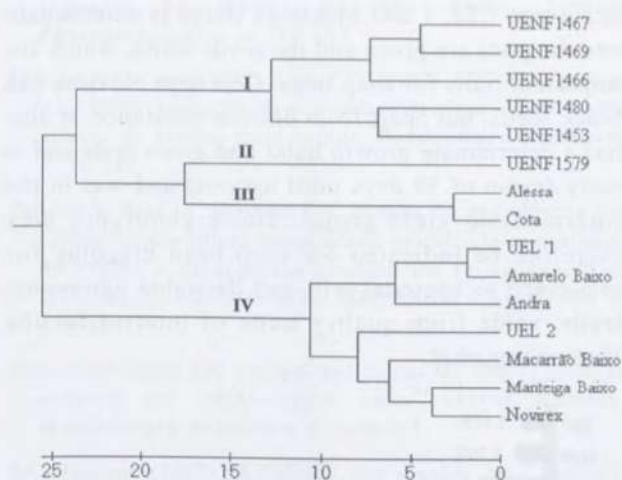
divergent genotypes should be used for crosses, with different desirable agronomic traits that meet the goals of the program (Maluf and Ferreira 1983, Oliveira et al. 1999, Cruz et al. 2004).

The genotypes of groups 1, 2 and 3 (Figure 2) were susceptible to bacterial wilt (Table 2). The genotypes Alessa and Cota (group 3) have a determinate growth habit, white seeds, green pods of the butter type, which are traits of interest for the consumer market. Therefore, both can be used for crossing with wilt-resistant genotypes of group 4. In this group, only genotypes UEL 2 and Andra were susceptible to bacterial wilt (Table 2). Besides the resistance to this disease, the growth habit of the
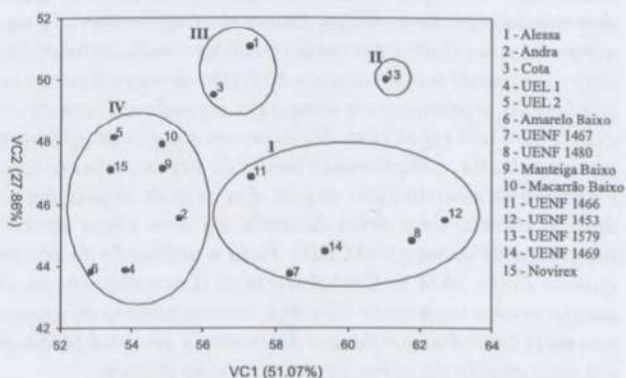

Figure 2. Graphic dispersion of 15 snap bean genotypes, in relation to the two first canonical variables, established by the linear combination of seven agronomical traits and reaction to bacterial wilt

genotypes UEL 1 and Manteiga Baixo is determinate and the pods are green and the seeds white, which are important traits for snap bean. Genotype Novirex has black seeds, but apart from disease resistance, it also has a determinate growth habit and green pods and is early (mean of 59 days until harvest) and was in the intermediate yield group. These genotypes may therefore be indicated for snap bean breeding for resistance to bacterial wilt and desirable agronomic traits, aside from quality traits of interest for the consumer market.
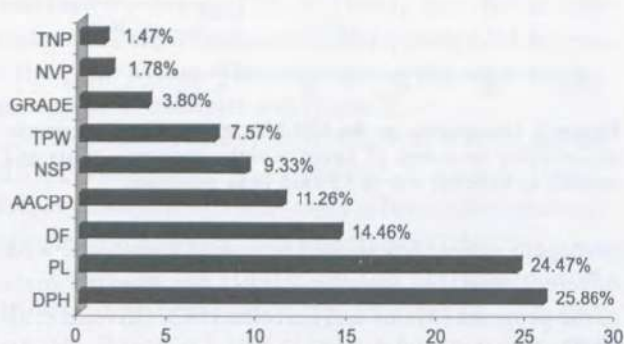

Figure 3. Relative contribution (%) of the traits evaluated in 15 snap bean genotypes

CONCLUSIONS

Of the four groups formed by cluster analysis, one joined all genotypes with some level of resistant to bacterial wilt, which were the genotypes UEL 1, Novirex, Amarelo Baixo, Manteiga Baixo and Macarrão Baixo.

The traits that contributed most to genetic divergence were days to harvest, pod length, days to flowering and area under the disease-progress curve.

The genotypes UEL1, Manteiga Baixo and Novirex had good agronomic performance with regard to yield-related traits and showed some level of resistance to bacterial wilt and can be indicated as parents in a snap bean breeding program for resistance to bacterial wilt.

Estimativa da divergência genética em feijão-de-vagem com base em características agronômicas e de resistência à murcha-de-curtobacterium

RESUMO - *Os objetivos desta pesquisa foram estimar a divergência genética entre genótipos de feijão-de-vagem de hábito determinado com base em descritores morfoagronômicos e na avaliação da resistência à murcha-de-curtobacterium; determinar a importância relativa das características estudadas na distinção dos genótipos e indicar potenciais genitores para estabelecer um programa de melhoramento do feijão-de-vagem para resistência à murcha-de-curtobacterium. Dois experimentos foram conduzidos, o primeiro em campo e o segundo em casa de vegetação com 15 genótipos, ambos no delineamento de blocos ao acaso, com três repetições. No experimento de campo foram estudadas sete características: dias para o florescimento; dias para a colheita; comprimento médio da vagem; número total de vagens; número de vagens por planta; peso total de vagem, e número de sementes por vagem. Em casa de vegetação, a reação à murcha-de-curtobacterium foi avaliada em função de duas variáveis, uma delas baseada em uma chave descritiva de notas e a outra baseada na área abaixo da curva de progresso da doença (AACPD). Para a avaliação da resistência dos genótipos à murcha-de-curtobacterium inoculou-se o isolado Feij – 2634 de* Curtobacterium flaccumfaciens pv. flaccumfaciens. *A estatística do pseudo t² indicou a formação de quatro grupos no método UPGMA, mesmo número de grupos indicado com o uso das variáveis canônicas. As características que mais contribuíram para a divergência genética foram dias para a colheita, comprimento da vagem, diâmetro da vagem e a área abaixo da curva de progresso da doença.*

Palavras-chave: *Curtobacterium flaccumfaciens* pv. *flaccumfaciens, Phaseolus vulgaris* L., resistência genética, estatística pseudo F e pseudo t².

W Krause et al.

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