Inferências genéticas em cultivares diferenciadoras de feijoeiro comum ao *Colletotrichum lindemuthianum* raça 69

Genetic inferences in common bean differential cultivars to *Colletotrichum lindemuthianum* race 69

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Abstract

Anthracnose caused by the *Colletotrichum lindemuthianum* Sacc. et Magn fungus, is one of the most important diseases and can result in heavy yield losses to the common bean (*Phaseolus vulgaris* L.). Genetic inferences about resistance of cultivars: Michelite, Michigan Dark Red Kidney, Perry Marrow, Cornell 49-242, PI 207262, AB 136, G 2333 and their 21 diallel hybrids were obtained in relation to the reaction to 69 race by using Hayman's method. The results showed that dominance effects were higher than additive effects to resistance of the related race. The order of parents in relation to dominant genes concentration obtained was: G 2333, AB 136, PI 207262, Cornell 49-242, Michigan Dark Red Kidney, Perry Marrow and Michelite. G 2333, AB 136 and PI 707262 parents are the most indicated for breeding programs to obtain anthracnose resistant cultivars.
Key Words: diallel analysis, genetic control, differential cultivars, common bean.

Resumo

A antracnose, causada pelo fungo *Colletotrichum lindemuthianum* Sacc cet Magn, é uma das mais importantes doenças e pode causar severas perdas ao cultivo do feijão comum (*Phaseolus vulgaris* L.). Inferências genéticas sobre a resistência de sete cultivares diferenciais de feijão comum (Michelite, Michigan Dark Red Kidney, Perry Marrow, Cornell 49-242, PI 207262, AB 136, G 2333) e seus 21 híbridos dialélicos foram obtidas em relação à raça 69, por meio da metodologia de Hayman. Os resultados mostraram que os efeitos dominantes foram superiores aos aditivos para resistência à referida raça. A ordem dos parentais em relação à concentração de genes dominantes obtida foi: G 2333, AB 136, PI 207262, Cornell 49-242, Michigan Dark Red Kidney, Perry Marrow e Michelite. Os parentais G 2333, AB 136 e PI 707262 são os mais indicados para programas de melhoramento visando à obtenção de cultivares resistentes à antracnose.
Palavras-chave: análise dialélica, controle genético, cultivares diferenciadoras, feijoeiro comum.

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Introduction

Anthracnose caused by *Colletotrichum lindemuthianum* (Sacc. & Magn.) Scrib. is one of the most widespread and economically important fungal diseases of common bean (*Phaseolus vulgaris* L.). Genetic resistance is the most effective method of controlling anthracnose in common bean and as new resistance sources become available (MAHUKU et al., 2002) their genetic characterization is essential to ensure novelty from previously characterized sources. Eleven independent resistance loci, *Co-1* to *Co-11*, that condition resistance to anthracnose have been identified and mapped to different linkage groups of the bean genome and allelic series are known to exist at the *Co-1, Co-3* and *Co-4* loci (KELLY; VALLEJO, 2004). The 12-member differential series (PASTOR-CORRALES, 1991) used to differentiate races of *C. lindemuthianum* has proved to be a valuable source of resistance genes for breeders. According to Young et al. (1998), it is confirmed that G 2333 carries three independently inherited resistance genes, one of which is a second resistance allele, *Co-4²*, at the *Co-4* locus. The finding of different alleles at the same locus conferring differential resistance to races of *C. lindemuthianum* is not unique (FOUILLOUX, 1979).

Since this fungus is a seed borne pathogen that exhibits extensive physiological variability, the use of genetic resistance has been the most effective control strategy implemented in Europe and North America (FOUILLOUX, 1979; TU, 1992; KELLY et al., 1994).

In despite of the extensive number of information that may be offered to plant breeders, diallel crosses analysis has received emphasis in many breeding programs in several crops (AMARAL JÚNIOR, 1996; CRUZ et al. 2004). According to Cruz et al. (2004), Hayman's method (1954) brought valuable contributions to genetic breeding, since makes possible an efficient study of genic action involved in characteristics determination, as well as identifies the presence of epistatic interactions. According to the same authors, data related to resistance disease may be studied by using this methodology, since a note scale provides quantitative variables. This article reports the genetic control obtained from Andean and Mesoamerican differential cultivars for their reaction to 69 race of *C. lindemuthianum*.

Material and Methods

Plant Material

The seeds of the differential cultivars were supplied by Embrapa-CNPAF (Brazilian Agricultural Research Corporation – National Research Center of Beans and Rice at Goiânia). Table 1 shows the characterization of the cultivars.

Table 1. Common bean (*Phaseolus vulgaris* L.) cultivars used in this study.

Cultivar	Characteristics			
	Origin	Growth habit*	Seed size	Seed color
Dark R. Kidney	Andean	I	Large	Red
Perry Marrow	Andean	II	Medium	White
Michelite	Mesoamerican	III	Small	White
Cornell 49-242	Mesoamerican	III	Small	Black
PI 207262	Mesoamerican	III	Small	Brown
AB 136	Mesoamerican	IV	Small	Red
G 2333	Mesoamerican	IV	Small	Red

* I = determinate; II = indeterminate, erect bush; III = Indeterminate, weak- stemmed, semi climber; IV = Indeterminate, weak-stemmed, climber (Singh, 1982).

Twenty one hybrids derived from a diallel among Michelite and Perry Marrow (susceptible to the 69 race), Dark Red Kidney, Cornell 49-242, PI 207262, AB 136 and G 2333 (resistant to the 69 race) cultivars were obtained.

Preparation of C. lindemuthianum isolates

The physiological 69 race (epsilon) was supplied by the fungi collection at the Federal University of Viçosa and Embrapa-CNPAF. This race was chosen because it is frequent in Parana State and more information on the inheritance of resistance is needed.

Monosporic culture of this race was transferred to test tubes containing Mathur et al. (1950) culture medium. These cultures were incubated at at 20ºC ± 2°C for ten days in BOD. After sporulation, the pathogen culture was kept in a refrigerator at 5ºC and used as a culture stock for the later experiments. The isolates were inoculated in the set of 12 differential cultivars for anthracnose to confirm their phenotypes (PASTOR-CORRALES, 1988).

Inoculation and incubation

Plants with their first trifoliate leaf completely developed were transferred to a humid chamber at approximately 22ºC ± 2°C. Inoculation of 30 parent plants and also 30 F_1 plants of each one of the 21 crosses were carried out.

The protocol for inoculation was as follows: 15-day-old bean plants with fully developed first trifoliate leaves were spray-inoculated with a spore suspension (1.2 x 10^6 spores per mL) of 69 race of *C. lindemuthianum*. After the inoculation, the plants were maintained in a mist chamber for 48 hours at 20ºC ± 2°C, under controlled light (12 hours with 680 lux illumination alternated with 12 hours of darkness) and approximately 100% relative humidity. Seven days after inoculations the plants were evaluated for their disease reaction using a scale from 1 to 9 (BALARDIN et al., 1990). Plants with disease reaction scores of 1-3 were considered resistant, whereas plants that rated 4-9 were considered susceptible.

The plants were kept in the same chamber for 96 hours after inoculation, at 20ºC, controlled light (12 hours with 689 lux illumination alternated with 12 hours of darkness) and approximately 100% relative humidity. The plants were then transferred to tables, in a suitable environment at 22ºC with artificial light, where they stayed until the assessment has been begun. Four replications of the parents and their F_1 generation from the 21 crosses were evaluated for the physiological race.

The parents and hybrids, in a total of 28 treatments, were assessed in greenhouse conditions at the Nucleus for Applied Studies on Agriculture (Nupagri) of the Department of Agronomy, State University of Maringa (PR), from September to November, 1997. The experiments were conducted as full randomized block design with four repetitions.

Hayman's diallel analysis (1954): The parents were considered different in a **T/t** locus, with an u_t proportion of favorable alleles and another v_t of unfavorable alleles (CRUZ et al., 2004), disabling the appliance of Hayman's method (HAYMAN, 1954). The Genes computer program (CRUZ, 2001) was used for this.

The components of variance were obtained from the diallel table and by association among them and the genetic resistance parameters of the cultivars and their hybrids to the race 69 of the pathogen were estimated. This model was adopted because the diallel analysis of the parents presented anthracnose symptoms classified from 1 to 9.

Results and Discussion

Assuming that the genetic effects are the results of the additive and dominant action of genes, Hayman (1954) imposed a series of restrictions which required

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the application of tests to verify the sufficiency of the model. The application of test for the race data demonstrated no significant in test F, hence the assumptions imposed were valid and indicated that the additive-dominant model was adequate to the genetic study.

The positive correlation among $\hat{Y}_{ii}$ and ($\hat{W}_i + \hat{V}_i$), of magnitude equal to 0.9763, show that the genes which act to increase the presence of the disease are, in its totality, recessive. In this case, Michelite, Michigan Dark Red Kidney and Perry Marrow, which were closer to the recessive end of the regression of $\hat{W}_i$ over, $\hat{V}_i$ would be unsuitable for breeding programs to obtain resistant cultivars to anthracnose. Thus, the most appropriate parents are, in order, G 2333 and AB 136, because they are closer to the limit where the concentration of dominant alleles predominates.

Table 2 shows the possibility of obtaining even more dominant segregant populations. The results showed that dominance effects prevailed over additive for resistance to 69 race. The order of the parents in relation to dominant genes concentration is: G 2333, AB 136, PI 207262, Cornell 49-242, Perry Marrow, Dark Red Kidney, and Michelite. This classification order is presenting in agreement with the importance resistance index order that the cultivars possess. In view of this, G 2333 and AB 136 parents are the most indicated for breeding programs, by using gene introgression or pyramiding to obtain anthracnose resistant cultivars. The perspective of obtaining resistant cultivars is noted by the fact of the selection limit to reach higher genotypes is $\hat{W}_D + \hat{V}_D = -0.1058$.

The genetic components estimates are showed in Table 3. The results demonstrated the more importance of the associated components to dominant effects rather than the additive ones, because the negative value (– 1.4103) of the component that expresses the difference among the additive and dominant effects ($\tilde{D} - \tilde{H}_1$).

Table 4 shows the predominance of the dominant genetic effects, since the estimates of the mean degree of dominance (1.2282), indicated that over dominance effects were responsible for allelic interaction was observed in this study. The differences among heritability magnitudes in narrow sense (0.4418) and broad sense (0.9764) make evident the superiority of the dominant component over the additive one (Table 4). Narrow sense heritability value, although of medium magnitude, shows the possibility of success on obtaining resistant genotypes to this pathogen.

Table 2. Values of the sum of the covariance among parents means and means of the ith line ($\tilde{\mathbf{D}}$) with the variance among means of the ith line ($\hat{V}_i$) and rank of the magnitude of the sum for seven common bean parents, for resistance to anthracnose, according to Hayman's method (1954).

Parents	$\hat{W}_i + \hat{V}_i$	Classification
G 2333	5.8333	1st
AB[W] 136	5.5476	2nd
PI 207262	2.8333	3rd
Cornell 49-242	0.1190	4th
Michigan Dark Red Kidney	0.1190	5th
Perry Marrow	0.0000	6th
Michelite	0.0000	7th

Table 3. Estimates of the genetic components and information for the assessed race, according to Hayman's method (1954).

Estimates of genetic components	Reaction to race 69
$\tilde{D}$ (Additive)	2.7724
$\tilde{H}_1$ (Dominance)	4.1827
$\tilde{H}_2$ (Dominance)	3.3606
$\tilde{h}^2$ (Dominance)	1.4786
$\tilde{D} - \tilde{H}_1$ (Difference of the additive and dominant effects)	-1.4103
$\tilde{E}$ (Enviromental)	0.0376

Table 4. Estimates of genetic information to the characteristic by using Hayman's method (1954)

Genetic parameter	Reaction to race 69
$\sqrt{\tilde{H}_1 / \tilde{D}}$ = Mean degree of dominance	1.2282
$\tilde{H}_2 / 4\tilde{H}_1$ = Distance of the alleles symmetry	0.2008
$\tilde{h}^2 / \tilde{H}_2$ = Number of genes with dominance	0.4399
$\tilde{h}_R^2$ = Narrow sense genotypic determination coefficient	0.4418
$\tilde{h}_A^2$ = Wide sense genotypic determination coefficient	0.9764

The medium dominance degree, with value of 1.2282, indicates the occurrence of complete dominance in the inheritance to this fungus in common bean, which makes easy the use of breeding programs with the goal of obtaining resistant cultivars. It is notes that there was allelic symmetry in parents distribution in the regression of the Wi over Vi, in despite of the value 0.2008, so that, near of 0.2500 to this standard. In studies with such cultivars, Gonçalves-Vidigal et al. (1997), Young and Kelly (1996) and Poletine et al. (1999), identified that the resistance to anthracnose is conferred by dominant genes. In Brazil, according to Gonçalves-Vidigal et al. (1999), and Alzate-Marín et al. (2003) the *Co-4³* allele in PI 207262 is potentially source of resistance to anthracnose. Therefore, breeders should focus their attention on the *Co-4²* allele as the best resistance source available at the *Co-4* locus (BALARDIN; KELLY, 1998). The *Co-4²* allele was confirmed to be the only allele in the 3-gene pyramid in G 2333 to afford resistance to the highly virulent race 2047 (SILVÉRIO et al., 2002). The dominance effects were higher than additive effects to resistance of the 69 race. The order of parents in relation to dominant genes concentration is: G 2333, AB 136, PI 207262, Cornell 49-242, Michigan Dark Red Kidney, Perry Marrow and Michelite. The G 2333, AB 136 and PI 207262 cultivars should be recommended in order to obtain a resistant cultivar to anthracnose by backcross programs due to their the highest proportion of resistant dominant alleles.

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