Crop Breeding and Applied Biotechnology 4:422-426, 2004
Brazilian Society of Plant Breeding. Printed in Brazil

Genetic constitution of anthracnose-resistance in common bean lines

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Received 19 September 2004

Accepted 5 December 2004

ABSTRACT - *Aiming at the identification of common bean lines with anthracnose-resistance, 256 lines obtained from the backcrossing program of the donor parent G2333 (carrier of the alleles* Co-4^2, Co-5, *and* Co-7*) with the recurrent parents ESAL 696 and CI 140 were used. These lines were inoculated with the three races 2047, 1545, and 73. In the lines with allele* Co-4^2, *resistant to race 2047, we could not identify other resistance alleles, although* Co-5, Co-7 *or both might be present in some of them. In the lines without allele* Co-4^2 *(susceptible to race 2047) and inoculated with the races 1545 and 73, some lines were identified with pyramid Co-5 and* Co-7, *some with* Co-5 *only, and others with* Co-7 *only. Lines carrying allele* Co-7 *only can be used for tagging.*

Key words: *Colletotrichum lindemuthianum*, *Phaseolus vulgaris*, gene pyramid, selection, plant breeding.

INTRODUCTION

Using resistant common bean cultivars is one of the most efficient ways of controlling anthracnose (C*olletotrichum lindemuthianum*), mainly for not increasing production costs and contributing to avoid the polluting and environmentally damaging chemical control.

There are a number of independent genes and in each there are one or more alleles which confer resistance to several pathogen races (Pastor-Corrales et al. 1994, Young and Kelly 1996) This makes the development of resistant cultivars with two or more alleles of different genes, i.e., an allele pyramid possible. This alternative is regarded as efficient to increase the useful lifetime of resistance alleles, which protect the cultivar for a few years when used singly, as in the case of cultivar Carioca 80, carrier of the *Co-2* allele (Balardim and Pastor-Corrales 1990, Kelly and Miklas 1998).

One difficulty of pyramiding alleles in a line is the non-availability of a set of races which allows the identification of all resistance alleles. Depending on the number of alleles of the pyramid, some races have not even been identified yet, making the genotype identification of the line impossible. However, this difficulty can be overcome with the use of molecular markers such as RAPD, which, when closely linked to the resistance alleles, allow the selection of lines, regardless of the number of alleles in the pyramid, eliminating the need for inoculation. A

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number of resistance alleles have already been identified by markers (Young and Kelly 1997, Young et al. 1998, Castanheira et al. 1999, Alzate-Marin et al. 2000, Alzate-Marin et al. 2001, Awale and Kelly 2001, Vallejo and Kelly 2001, Vigo et al. 2002, Silva et al. 2003). One of the most important alleles is *Co-4²*, present in cultivar G2333 (Young et al. 1998) for conferring resistance to all races identified in Brazil (Rava et al. 1994). In addition to *Co-4²*, cultivar G2333 possesses two more resistance alleles, *Co-7* and *Co-5* (Young et al. 1998). In spite of the great amount of information in literature on the different resistance alleles, documentation is scarce regarding *Co-7*. One of the causes for this lack of information is because the allele was originally identified in cultivar G2333 along with *Co-4²*, which confers resistance to a very large number of races and practically covers its effect (Pastor-Corrales et al. 1994). Although G2333 is not adequate as cultivar because it has several undesirable phenotypes, the resistance alleles in it can be used efficiently through its cross with agronomically superior parents that may be employed as recurrent in a backcrossing program.

In this context, the objective of this study was to identify lines carrying pyramids of anthracnose resistance alleles present in the donor parent G2333 and lines carrying only the *Co-7* allele.

MATERIAL AND METHODS

A total of 256 lines were evaluated, 15 derived from a segregating F_5 family of the first backcrossing [(G2333 x ESAL 696) x ESAL 696] and 241 selected from three segregating F_3 families of the second backcrossing {[(G2333 x ESAL 696) x ESAL 696] x CI 140}.

G2333 is a Mexican line with several unfavorable phenotypes, i.e., type IV growth habit, red grains, and photoperiod sensitivity although this line is carrier of an allele pyramid (*Co-4²*, *Co-5*, and *Co-7*) which confers resistance to all *C. lindemuthianum* races prevalent in Brazil. Line ESAL 696 has some favorable phenotypes such as type II growth habit, Carioca cultivar-similar grains and *C. lindemuthianum* resistance due to the *Co-5* allele. This line is derived from cross L3272 x Line (Carioca x TU) and is anthracnose-resistant like line 'Carioca x TU'. Line CI 140 comes from a recurrent selection program and stands out for the excellent grain type similar to that of the Carioca cultivar.

Genotype identification of the lines for anthracnose reaction

The lines were inoculated with three different races (2047, 1545 and 73) of *C. lindemuthianum* from monosporic cultures. They were transferred into test tubes containing agar-water medium and bean pods in aseptic conditions and incubated in a growth chamber at a temperature of 20 °C for 15 days. A spore suspension of each race, containing about 1.2×10^6 conidia per millimeter was prepared from this culture.

Ten seeds of each line were sown on a plastic tray containing plantimax substrate and the inoculation was set up around 10 days later, when the plants presented the completely developed primary leaves. The spore suspension was sprayed onto both leaf surfaces and the plant stem. After inoculation, the plants were kept in a chamber at 100% relative air humidity and a temperature of 20 °C for three days with 12 hours of light, alternating with 12 hours of dark. The severity of the disease was evaluated in a score diagram of 1 to 9 (Rava et al. 1993). The lines with an average score of up to 3.6 were considered resistant and the others susceptible.

At first, one sample of each line was inoculated with race 2047. Samples of all susceptible lines were then inoculated twice, one with race 1545 and the other with race 73.

Identification of lines with a resistance allele pyramid

The lines with allele *Co-4²* (resistant to race 2047) were analyzed with a SCAR marker through primer SAB3 (Vallejo and Kelly 2001). The pyramid involving both *Co-5* and *Co-7* alleles was identified by means of inoculations with the races 73, 1545, and 2047.

Each race was identified by a number according to the binary procedure recommended for *C. lindemuthianum* (Pastor-Corrales 1992). So, the name (number) of each race is given by the expression $\sum_i (2^{d_i - 1})$, and d_i is the number of order of the differential cultivar matched by the race. It is necessary to remember that the differentials have to be used in a fixed and crescent order from 1 to 12 (Pastor-Corrales 1992). Therefore, race 2047 corresponds to $\sum_i (2^{d_i - 1})$, in which only the last differential is resistant, namely: $2047 = 2^0 + 2^1 + 2^2 + 2^3 + 2^4 + 2^5 + 2^6 + 2^7 + 2^8 + 2^9 + 2^{10}$.

DNA Extraction and SCAR Analysis

The DNA of the lines was extracted according to the procedure used by Silva et al. (2003). Each SCAR reaction was also performed following the procedure used by Silva et al. (2003). The primer was SAB3 (Vallejo and Kelly 2001), which amplifies a DNA fragment of about 450 base pairs (bp) and the recombination frequency with allele *Co-5* is 12.98%.

The amplification reactions were performed in an Eppendorf thermocycler with amplification programs recommended by the manufacturers. The amplified DNA fragments were separated in 1% agarose gel in TBE buffer (Tris, boric acid and EDTA) in 40-50V and stained with ethidium bromide (0.5 $\mu g\ mL^{-1}$) for 30-50 minutes.

RESULTS AND DISCUSSION

In bean breeding aiming at the vertical resistance against anthracnose the most recommended strategy is the use of a resistance allele pyramid (Kelly and Miklas 1998, Alzate-Marin et al. 1999). In the present study, one of the objectives was the transfer of the pyramid found in the donor parent G2333, represented by the resistance alleles *Co-4²*, *Co-5* and *Co-7* to lines that associate other agronomical phenotypes (Hagiwara et al. 2001). Information on the genotypes of the lines relative to the resistance alleles is therefore required since they recombined during the backcrossings and the conduction of the segregating populations.

The results of the three inoculations and the SCAR analysis identified lines with different resistance allele combinations (Table 1). Taking Flor (1971)'s gene-for-gene theory into consideration, which explains the genetic base of the pathogen x host interaction when the resistance is vertical (Vanderplank 1968), there is a correspondent avirulence allele in the pathogen (De Witt 1997) for each resistance allele in the host. So, only race 2047 does not match the resistance allele *Co-4²*, which may be identified by $2^{11} = 2048$. Nevertheless, that allele should be matched by a race equal or superior to 2048, which has fortunately not yet been identified in Brazil. Consequently, the lines resistant to race 2047 are carriers of the *Co-4²* allele and one cannot, by means of inoculations, verify if they also possess the *Co-5* and/or *Co-7* alleles coming from the donor parent G2333.

Table 1. Allelic combinations identified in the lines by means of inoculation

Resistance alleles	Reaction to races[1]		
	2047	1545	73
*Co-4²**	R	-	-
Co-5, *Co-7*	S	R	R
Co-5	S	S	R
Co-7	S	R	S
None	S	S	S

*It was not possible to identify if these lines possess the *Co-7* and *Co-5* alleles or not; [1]R =Resistant; S = Susceptible

Among the 256 evaluated lines, 29 are carriers of the *Co-4²* allele (Table 2). Amongst these, 15 were selected on the basis of other traits, mainly for their Carioca-similar grain type, which is the preferred consumer type. Among those 15 lines, one was derived from backcrossing 1 and therefore also possesses the *Co-5* allele, since the recurrent parent was ESAL 696 which possesses that allele. The other 14 lines are descendent from backcrossing 2, therefore, they may or may not be carriers of the *Co-5* allele, which is not present in the second recurrent parent, CI 140. The fastest way of verifying the presence of the allele in these lines is by a molecular marker such as SCAR through the primer SAB3 (Vallejo and Kelly 2001). Besides these 15 lines, 14 other genotypes were also used in the SCAR reactions, of which 11 are lines carrying the *Co-5* allele, identified by inoculations with the races 73 and 1545; two are the resistant tester (G2333 and TU) and one is the susceptible tester (Carioca cultivar). Surprisingly, except for G2333 and TU no other genotype exhibits the SCAR band, even in those 11 lines with allele *Co-5*. Considering that the 15 lines with *Co-4²* had been selected within only three families and that the distance between the SCAR marker and the *Co-5* allele is 12.98 cM, there is a high chance that this marker had been lost in the three plants that generated the segregating families. The absence of the SCAR marker in the 11 lines with *Co-5* is also an indication that the marker had been lost. So, we could not identify any other allele in the lines with *Co-4²*, although some of them might have *Co-5*, *Co-7* or both.

Table 2. Number of lines identified with each allele combination

Allelic combinations	Number of lines
*Co-4²**	29
Co-7, *Co-5*	50
Co-7, *Co-5*(?)	27[a]
Co-7	20
Co-5	55
Co-5 (?)	56[b]
None	19

*It was not possible to verify the presence of *Co-7* and *Co-5*; [a]Lines not inoculated with race 73 (may also possess *Co-5*); [b]Lines not inoculated with race 73 (may have *Co-5*)

According to Young et al. (1998), the resistance to races 1545 and 521 in populations derived from the cross of G2333 with a completely anthracnose-susceptible cultivar is controlled by two genes. As those races match the *Co-5* allele, it is clear that the alleles which confer resistance to those races in that cross are *Co-4²* and *Co-7*. The resistance conferred by the *Co-5* allele is matched by the races that have the value $2^9 = 512$ in their genotypic constitutions and are carriers of the virulence allele of the same number (Robinson 1987).

Therefore, by using race 1545 for the inoculation of the race 2047-susceptible lines, it was possible to identify the carriers and non-carriers of the *Co-7* allele because this race matches the resistance conferred by the *Co-5* allele and does not match that conferred by *Co-7*.

Alzate-Marin et al. (2001) working on a population derived from a cross of a completely anthracnose-susceptible cultivar with G2333 obtained results which point out that the resistance to races 73 and 89 is controlled by two genes. As it is already known that the *Co-4²* and *Co-5* alleles confer resistance to those races, it follows that the resistance of allele *Co-7* was matched by the same ones. Therefore, lines not carrying the *Co-*

4² allele inoculated with race 73 identified the resistant lines due to allele *Co-5* and susceptible lines which do not have *Co-5*.

By comparing the results of the inoculations with races 73 and 1545 (Table 1), 50 lines with the pyramid *Co-5* and *Co-7*, 55 with *Co-5* only, 47 with *Co-7*, and 75 without resistance alleles were identified. However, among the lines without *Co-4²*, only 144 were inoculated with race 73. Thus, out of the 47 lines carrying *Co-7*, 27 can also be carriers of *Co-5*, and amongst the 75 apparently without any resistance allele, 56 possibly present *Co-5*. Then, only 20 lines with only *Co-7* and 19 completely susceptible ones were identified (Table 2).

According to Alzate-Marin et al. (2001), the resistance due to allele *Co-7* is matched by races which have virulence allele 64 (2^6) in their genotypes. Therefore, such an allele may be the same present in the differential cultivar Mexico 222 (Pastor-Corrales 1992). It is necessary to examine this allele further by means of an allelism test. Besides, when using one of the lines which has only *Co-7*, it is important to identify it by means of a marker that will allow verifying its presence in the pyramid lines with other alleles.

So, the genotypes of 173 lines were identified, 29 of them with the *Co-4²* resistance allele. These lines are the most promising for disease control and some of them might have *Co-5*, *Co-7*, or both. Other 50 lines possess the two-allele pyramid *Co-5* and *Co-7* and may also be useful in the case of presenting favorable phenotypes in agronomic traits. Most of those lines possess similar grains to the ones of the Carioca cultivar, although with a broad variation. So, if some of them show off other agronomical traits they may become a new cultivar or else be used in new crosses in breeding programs. In addition, there is the possibility of identifying lines carrying only *Co-4²*, which may be used instead of the differential cultivar G2333, which is not ideal as such because it carries other resistance alleles, and also because it is not adapted to most of Brazil's cultivation conditions.

ACKNOWLEDGEMENTS

This research was supported by a grant from FAPEMIG. H S Pereira was supported by a CNPq scholarship.

Constituição genética de linhagens de feijão com resistência à antracnose

RESUMO - *Um total de 256 linhagens de feijão obtidas do programa de retrocruzamento entre o genitor doador G2333 (portador dos alelos* Co-4², Co-5 *e* Co-7*) e os genitores recorrentes ESAL 696 e CI140 foram estudadas com o objetivo de identificar linhagens com resistência à antracnose. Essas linhagens foram inoculadas com três raças 2047, 1545 e 73. Nas linhagens com alelo* Co-4², *resistentes à raça 2047, não foi possível identificar outro alelo de resistência, entretanto* Co-5, Co-7 *ou ambos podem estar presentes em algumas delas. Nas linhagens sem alelo* Co-4² *(suscetíveis à raça 2047), inoculadas com as raças 1545 e 73 foram identificadas linhagens piramidadas com alelos* Co-5 *e* Co-7, *outras com* Co-5 *apenas e ainda outras com* Co-7 *somente. Linhagens com apenas alelo* Co-7 *podem ser usadas para que este seja marcado.*

Palavras-chave: *Colletotrichum lindemuthianum*, *Phaseolus vulgaris*, pirâmide de genes, seleção, melhoramento genético.

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