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ORIGINAL ARTICLE

Genetic diversity of *Pseudocercospora griseola* resistance *loci* in common beans

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Abstract

Common bean (*Phaseolus vulgaris* L.) is one of the most widely grown legumes in the world. Although the crop has high yield potential, average yields in Brazil are low due to several diseases. Angular leaf spot (ALS), caused by *Pseudocercospora griseola*, is among the most important diseases. A set of 81 accessions from the Instituto Agronômico (IAC, Campinas, SP) germplasm bank were evaluated for ALS resistance and genotyped by 12 microsatellites previously associated with ALS QTL resistance. Allele frequencies, number of alleles per *locus*, expected heterozygosity (He), and Shannon's Information Index (I) were calculated. The average Ho was 0.12, and the He was 0.54. The STRUCTURE analysis and UPGMA clustering based on Nei's genetic distance indicated a moderate degree of genetic diversity, with 4 and 5 main groups, respectively. Evaluation of the severity of ALS showed that 17% of the accessions had resistance. Cultivars were recommended for breeding crosses aimed at gaining in genetic diversity and resistance to ALS.

Keywords *Phaseolus vulgaris* L. · *Pseudocercospora griseola* · Population structure

Introduction

The common bean (*Phaseolus vulgaris* L.) is an important source of protein, natural fiber, and calories that, together with rice, constitutes a staple food with nutritional and economic importance for the Brazilian population (Broughton et al. 2003; Hefni et al. 2010; Bellucci et al. 2014). Brazil is the largest consumer and the third largest producer with estimated production of about 2360 thousand tons (CONAB 2019). Brazil may be considered a secondary center of common bean diversification (Valentini et al. 2018). However, the major constraint to bean production is the occurrence of several diseases caused by fungi, viruses, bacteria, and nematodes (Schwartz and Pastor-Corrales 1989; Schwartz et al. 2005).

Angular leaf spot (ALS) caused by fungus, *Pseudocercospora griseola* (Sacc.) Crous & Braun, is one of the most serious diseases of common bean in tropical and subtropical countries and losses in the crop due to ALS can be as high as 80% (Stenglein et al. 2003; Miklas et al. 2006; Crous et al. 2006; Singh and Schwartz 2010). More than 40 races of *P. griseola* have been identified (Wagara et al. 2004; Nay et al. 2019a) and a successful resistance breeding strategy requires a thorough understanding of the host-pathogen interaction and the characterization of genetic and phenotypic resistance (Correa-Victoria et al. 1989; Liebenberg and Pretorius 1997).

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P. vulgaris accessions are classified into two distinct gene pools, the Andean and the Mesoamerican (Schmutz et al. 2014), and *P. griseola* isolates have the same classification, due to the process of co-evolution between pathogen and host (Guzmán et al. 1995; Wagara et al. 2004). Isolates from the Mesoamerican group have greater genetic variability and infect both Mesoamerican and Andean bean cultivars, while isolates from the Andean group only infect beans of the same origin (Pastor-Corrales and Jara 1995; Wagara et al. 2004).

The currently approved ALS resistance *loci* include three dominant and independent *loci* (*Phg*-1 - Carvalho et al. 1998; *Phg*-2 - Sartorato et al. 2000; *Phg*-3 - Gonçalves-Vidigal et al. 2013), as well as two major QTLs, *Phg-4* and *Phg-5* (Souza et al. 2016; Oblessuc et al. 2012; Keller et al. 2015; Bassi et al. 2017). In addition to these resistance *loci*, some cultivars are sources of resistance and have been reported on studies of phenotypic resistance evaluations (Aggarwal et al. 2004; Sartorato 2004; Tryphone et al. 2016; Pereira et al. 2019a, b).

The use of fungicides to control ALS is an efficient measure; however, it is expensive mainly for smallholder farmers (Nay et al. 2019a). The most cost-effective and environmentally friendly management strategy to control the disease is through resistant cultivars (Pastor-Corrales et al. 1998). The characterization of new sources of resistance became indispensable for the genetic improvement of the species (Oblessuc et al. 2012). However, resistance inheritance of angular leaf spot in common beans has shown to be complex in some situations due to the oligogenic nature of the ALS resistance (Oblessuc et al. 2012; Keller et al. 2015; Bassi et al. 2017; Perseguini et al. 2016). The development of common bean cultivars with durable ALS resistance is difficult and requires pyramiding of several ALS QTL (Quantitative Trait *Loci*, Mendonça et al. 2003; Oblessuc et al. 2012).

Common bean breeding programs usually focus exclusively on breeding lines and/or cultivars, using a narrowed-based germplasm which may be a limiting factor for genetic progress (Valentini et al. 2018). Success in developing new cultivars with gain in genetic diversity is highly dependent on the choice of divergent parents to compose the crosses in a breeding program (Perseguini et al. 2011). The accessions selected for the crosses must have the desirable agronomic traits and the greatest possible genetic divergence to exploit the maximum genetic variability. Thus, it is necessary to know the genetic and phenotypic variability of the available core collection before using it (Pereira et al. 2019a, b).

Therefore, the aim of the present study was to characterize 81 accessions from the core collection of the Instituto Agronômico (IAC, Campinas, SP) regarding resistance to ALS and the degree of genetic diversity using microsatellites previously associated with ALS resistance. The characterization of genetic and phenotypic resistance to ALS was used to select specific accessions for recombination, to obtain lines with high resistance and genetic diversity for ALS resistance *loci*.

Material and methods

Plant material, DNA extraction, and SSR genotyping

A total of 81 accessions were selected from the Active Germplasm Bank (BAG) of the Instituto Agronômico (IAC, Santa Elisa's Farm, Campinas, SP, Brazil) to represent the genetic and phenotypic variability of the core collection proposed by Perseguini et al. (2015). The set exhibits wide variability for agronomic characteristics such as grain size, growth habit, yield, tolerance to abiotic stress, and disease resistance. The set includes several accessions used as parents in genetic mapping populations (Oblessuc et al. 2012; Sanglard et al. 2013; Bassi et al. 2017; Briñez et al. 2017; da Silva et al. 2018). All information on accessions, such as genealogy, commercial class, grain size, and institution, as well as the classification of the degree of ALS resistance and the genotype matrix, is available in the full list in the Supplementary Material 1.

For DNA extraction, three seeds from each accession were planted in pots kept in a greenhouse. The first trifoliate leaf was collected from plants at V3 phenological stage, frozen in liquid nitrogen, and lyophilized for 72 h. After lyophilization, the plant samples were ground in a mechanical mill (Cyclotec-1093 Sample Mill, Tecator), packed in hermetically sealed vials, and stored at − 20 °C. Total DNA from each sample was extracted from lyophilized plant material using the CTAB protocol proposed by CIMMYT (2005). DNA was quantified on a broad-spectrum spectrophotometer (Nanodrop 2000), diluted to 10 ng/μL. Integrity and quality were confirmed on 1% agarose stained by GelRed (Biotium, Inc.).

A set of 12 microsatellite (SSR) molecular markers associated with ALS resistance QTL (Oblessuc et al. 2012, 2013) were selected for genetic diversity analysis (Table 1). PCR were performed on the BioRad My Cycler thermocycler. For each reaction, 2.5 μL Milli-Q water, 7.5 μL Master Mix (Promega), 1 μL of each primer (0.8 pmol/μL - forward and reverse), and 30 ng of genomic DNA were used, in a final volume of 15 μL. The amplification conditions were 1 min at 94 °C, followed by 30 cycles of 1 min at 94 °C, 1 min at each SSR specific annealing temperature, and 1 min at 72 °C, with a final extension of 5 min at 72 °C. The quality of the amplifications was visualized on 3% agarose stained by GelRed (Biotium, Inc.). The PCR product was visualized in automated genotyping Fragment Analyzer™ 96 (Plant Genetic Resource Center, Instituto Agronômico, Campinas, SP, Brazil) using the DNF-905 double-stranded DNA Reagent Kit (Advanced analytical Technologies, Ames, IA, USA). The PROSize™ 2.0 software included in the advanced Fragment Analyzer™ 96 system was used to visually select strong, clear polymorphic DNA bands for scoring. The genetic matrix containing the fragment size of each reaction was converted to a GenAlEx format file.

Table 1 Microsatellites (SSRs) used in the present study for diversity analyses of the 81 accessions of common bean (*Phaseolus vulgaris* L.) from the Instituto Agronômico (IAC, Campinas, SP, Brazil). All SSRs were associated with angular leaf spot resistance QTL from previous studies (Oblessuc et al. 2012, 2013)

SSR	LG	QTL	Reference	Sequence forward/reverse	AT (C°)
PVBR106	2	ALS2.1^{UC}	Oblessuc et al. (2012)	CCTTACGTTCCTAAACCACCAG GAGCAATGGGGATTATGATGTT	56
SSRIAC134	2	ALS2.1^{UC}	Oblessuc et al. (2012)	TGGAAACAGCAGAGCGATACT TTGGTCCCTAAATTCTTCTCAT	56
PVBR21	3	ALS3.1^{UC}	Oblessuc et al. (2012)	GTGAGGGTTCTGTGGATTCTTC ATTGACACAACAGCCAATATGC	56
BM159	3	ALS3.1^{UC}	Oblessuc et al. (2012)	GGTGCTGTTGCTGCTGTTAT GGGAGATGTGGTAAGATAAT GAAA	52
SSRIAC52	4	ALS4.$^{1GS/UC}$	Oblessuc et al. (2012)	TGCTGTATGTAGGCGGTTTA GTGGCTTTTGCTTTTGTAGTCA	56
PVBR128	4	ALS4.2$^{GS/UC}$	Oblessuc et al. (2012)	GAAGAGCTCTCATCGCAACG CTAGCTCCCTCCCCTCGTAA	56
PVBR124	5	ALS5.1^{UC}	Oblessuc et al. (2012)	CCTAAAAACCAGGTGCGAGA TGGGAAACCTAGCCAAACAC	56
BM175	5	ALS5.2^{UC}	Oblessuc et al. (2012)	CAACAGTTAAAGGTCGTCAAATT CCACTCTTAGCATCAACTGGA	50
SSRIAC261	5	ALS5.2^{UC}	Oblessuc et al. (2012)	TTCCCAAACACCACACCTAAGT TCACCGCGCACGAGATAA	60
PVM22	10	ALS10.1$^{DG/UC}$	Oblessuc et al. (2013)	ACTCTCACAATGGCGGAATC GGCGTTTTCTCCCTCTTCTT5	60
PVM127	10	ALS10.1$^{DG/UC}$	Oblessuc et al. (2013)	AACTTTCTTTGACCCTCTC GCTTTGTCTTGTTCTTCCA	60
SSRIAC137	10	ALS10.1$^{DG/UC}$	Oblessuc et al. (2013)	GCAGCAGCAAATACAAC CCCCTAAACAATACAGC	60

LG, linkage group; *AT*, annealing temperature. (C°) (C°)

Evaluation of resistance to ALS

The evaluation of resistance to ALS was carried out using an isolate of *P. griseola* (provided by Dr. Elaine Aparecida de Souza from the Genetics and Plant Breeding Department, UFLA, MG, Brazil) characterized as physiological race 31-31, according to the series of differentiating cultivars proposed by Pastor-Corrales and Jara (1995). The high virulence isolate classified as a Mesoamerican race was chosen due to the capacity to infect both Andean and Mesoamerican beans with the same intensity. The experiment was performed in an inoculation chamber (Santa Elisa's Farm – Instituto Agronômico), in a randomized block design with four replications. Each plot consisted of a pot with two plants. Thus, a total of 8 seeds of each accession were pre-germinated and transplanted to plastic pots (11 × 8 × 9 cm) containing plant substrate (Plantmax®), in a greenhouse, where they remained for 15 days. Upon reaching the V3 phenological stage, the pots were transferred to an inoculation chamber, where they remained under controlled temperature (24 °C), relative humidity (95–100%), and photoperiod (12 h/12 h) conditions.

For inoculation, the monosporic isolate 31-31 was subcultured in Petri dishes containing V8 medium (1.61% agar, 0.25% calcium carbonate, 61.94% distilled water, and 36.2% Campbell's V8 vegetable juice) 3 days after planting the accessions and incubated at 24 °C for 14 days, with 12 h of photoperiod, to induce sporulation. The inoculum suspension was prepared by the addition of 5 mL of sterile water to each plate containing the pathogen colonies, followed by scraping for conidia release. The final suspension was filtered and diluted to a concentration of 4×10^4 conidia mL^{-1} and, before inoculation, a drop of Tween 20 (0.1 mL/L^{-1}) was added. Inoculation occurred 24 h after the plants were transferred to the inoculation chamber with the aid of *De Vilbiss* atomizer powered by an electric air compressor spraying on both the adaxial and abaxial surfaces of each leaf. The inoculation chamber consists of four large glass aquariums (4 × 1 × 1 m) with individual humidification system in a room with temperature control and artificial light. Five days after inoculation, the plants were transferred to a greenhouse and maintained with relative humidity and temperature around 75% and 28 °C, respectively. Symptoms were evaluated 15 days after inoculation using the diagrammatic scale (1–9) proposed by

Van-Schoonhoven and Pastor-Corrales (1991), where plants with infection scores from 1 to 3 are considered resistant, from 4 to 6 moderately resistant, and from 7 to 9 susceptible.

Statistical analysis

Molecular data were used to calculate expected heterozygosity (He) and Shannon's Information Index (I) using GenAlEx 6.5 (Peakall and Smouse 2012). Polymorphic Information Content (PIC) was estimated by the CERVUS 3.0.7 program (Kalinowski et al. 2007). Principal component analysis (PCA) was performed using the ADE 4 package (Dray et al. 2007), and genetic distance according to Nei (1978) was grouped by the UPGMA using the POPPR package (Kamvar et al. 2015) in the software R. For population structure analysis, the STRUCTURE v2.3.1 program (Pritchard et al. 2000) was used, with a burn-in period of 20,000 and 50,000 MCMC interactions, in a continuous series of groupings (K) ranging from 2 to 10 in 15 repetitions. The best K (DELTA K) was estimated by the method proposed by Evanno et al. (2005) through the STRUCUTURE HARVESTER program (Earl and Vonholdt 2012). The graphic was generated by the POPHELPER package (Francis 2017).

The final resistance evaluation score of each access in the four repetitions was understood by the arithmetic average of the two plants in each plot. The score values obtained were used for analysis of variance (ANOVA), the normality test (Shapiro and Wilk 1965), and the Scott and Knott (1974) means test by the EXPDes package (Ferreira et al. 2014). Broad-sense heritability was estimated by the RBio program (Bhering 2017) and the lower and upper limits for the confidence intervals were obtained according to Knapp et al. (1985).

Results

Resistance to ALS

Angular necrotic spots delimited by the leaf veins, symptoms that are typical of ALS disease, began to appear 12 days after inoculation. On the day of evaluation, the difference in severity between accessions was clear, being easy to distinguish plants with the presence of a few small nonsporulating lesions (resistant, score < 3) from plants with several sporulating lesions (susceptible, score > 3). Analysis of variance (ANOVA) revealed significance for accessions (Table 2), allowing separation of cultivars through the means test. The significance for blocks is probably due to the variation of the relative humidity between the 4 aquariums of the inoculation chamber, highlights the importance of using the statistical design. Due to the quantitative nature of the disease, even with the well experiment conduction, the coefficient of variation was moderate (27%).

Table 2 Analysis of variance (ANOVA) for 81 accessions of common bean (*Phaseolus vulgaris*) evaluation of resistance to angular leaf spot (*Pseudocercospora griseola*)

SV	DF	SS	QM	Fc	Pr>Fc
Accessions	80	997.74	12.4718	6.5938	0.00001***
Blocks	3	28.45	12.8163	6.7759	0.00021***
Residue	240	453.95	1.8914		
Total	323	1490.14			
CV%	27.49%				
Shapiro-Wilk	0.83				
h^2	0.85 (0.79–0.89)				

SV, source of variation; *DF*, degrees of freedom; *SS*, sum of squares; *MS*, mean squares; *Fc*, *F* value; *CV%*, coefficient of variance; h^2, heritability (lower and upper limits for the confidence intervals)

***Significant at 0.001

Data normality was tested by the Shapiro-Wilk test, which was not significant ($P = 0.830$). High broad-sense heritability estimated in 84% (confidence intervals = 79 and 89%) is considered relatively high, allowing the selection of superior accessions to ALS resistance. Regarding the Scott-Knott means test, four groups were formed, revealing high variability for resistance to ALS. The first group (a) was comprised of only 14 resistant accessions (Fig. 1).

Genetic diversity and population structure

The general diversity estimated for the 12 SSR markers associated with 7 ALS resistance *loci* showed that the mean number of alleles identified was 4 (2 to 6), the allele frequency ranged from 0.01 to 0.91, and the polymorphic information content (PIC) ranged from 0.13 to 0.72 (mean 0.51). Regarding the diversity parameters of the 81 accesses, the results evidenced moderate diversity for the markers used, with expected mean heterozygosity (He) and Shannon's Information Index (I) estimated at 0.54 and 1.05, respectively.

Inference of genetic structure by Bayesian analysis (STRUCTURE) indicated division of the accessions into five groups (Fig. 2a). The grouping from STRUCTURE analysis with $K = 5$ is represented by Fig. 2b. The first group (blue cluster) was composed of mostly elite lines from the Instituto Agronômico (IAC), together with the AND 277 and CAL 143 cultivars. The second group, consisting mainly of lines from breeding programs, was the largest and corresponded to 99% membership coefficient of the IAC Carioca cultivar (the first carioca cultivar released). The third and fourth groups were mainly composed of older cultivars, such as IAC Pyatã, IAC Alvorada, IAC Aysó, IAC Apuã, IAC Maravilha, IAPAR 3, IAPAR 81, and Carioca MG. The last group (gray cluster) consisted of only 10 accessions, including BRS-Requinte and BRS Pérola, both released by EMBRAPA.

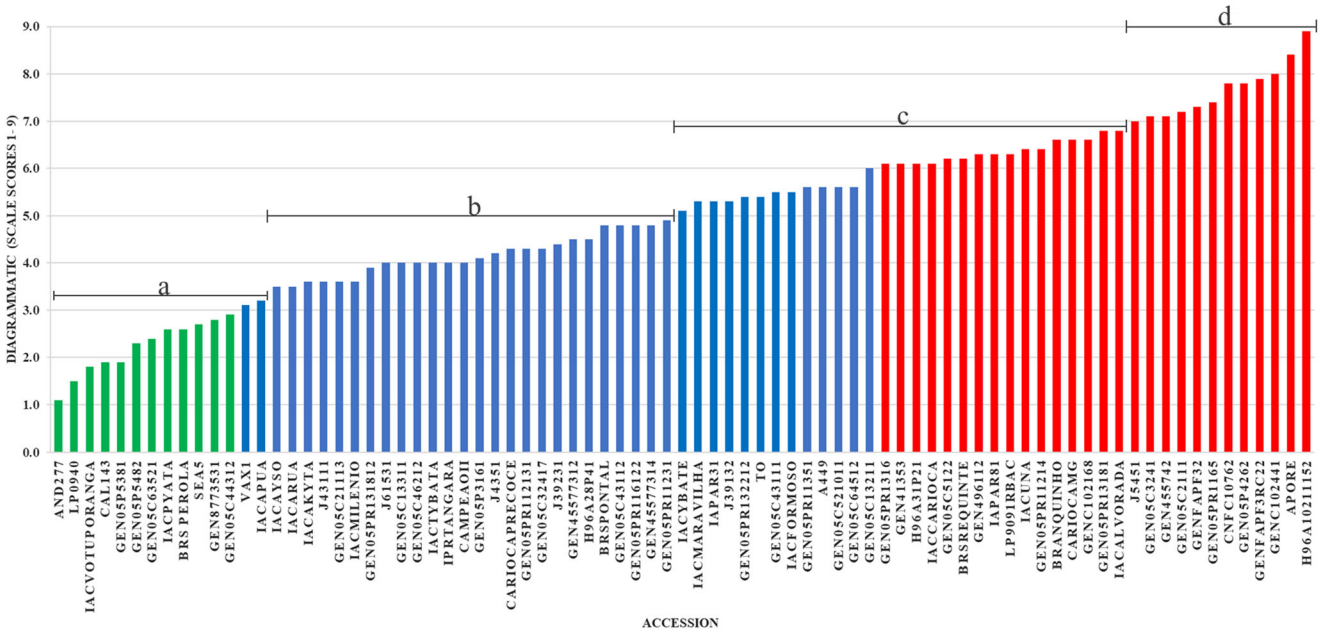

Fig. 1 Mean scores (1 to 9) for each accession in relation to evaluation of resistance to angular leaf spot, grouped by the Scott-Knott means test (indicated by the letter above the bars). Means for the same letter do not differ significantly by the Scott-Knott test (P value < 0.05). The colors of the bars classify the degree of resistance of the accessions according to Van-Schoonhoven and Pastor-Corrales (1991); green, blue, and red corresponding to the resistant (scores from 1 to 3), moderately resistant (scores from 4 to 6), and susceptible (scores from 7 to 9) accessions, respectively

Interestingly, the cultivars IAC Akytã and IAC Pyatã came from the same cross but were in different groups.

The UPGMA dendrogram based on Nei's genetic distance matrix (Fig. 3) revealed considerable genetic variability of the set evaluated by the formation of four main groups in the dendrogram, the second group being the largest one. Cultivars resistant to ALS (red color) were distributed throughout all groups, allowing the selection of accessions as parents recommended for crosses aiming to obtain lines with high resistance and genetic diversity for the evaluated QTL. Genealogy information was also confirmed through the dendrogram, such as Gen05Pr11214, Gen05Pr1316, Gen05C43111, Gen05C63521, Gen05C44312, Gen05C32417, and Gen05C64512 lines, which all came from the same breeding program.

Selection of accessions for breeding

To obtain lines with gain in genetic variability, higher ALS resistance, and agronomic performance, 11 accessions and 9 parents' pairs were selected (Table 3). The pairs were formed only from resistant accessions, and the cultivars' pairs did not belong to the same group formed by the structure analysis (Fig. 2) and/or the dendrogram (Fig. 3). Aiming at reduction of the linkage drag and the segregation for grain color promoted by recombination of cultivars from different commercial classes, the pairs formed were limited to the commercial grain types.

The AND 277 (brindle grain) x SEA5 (red grain) cross was strategic for breeding of special bean varieties,

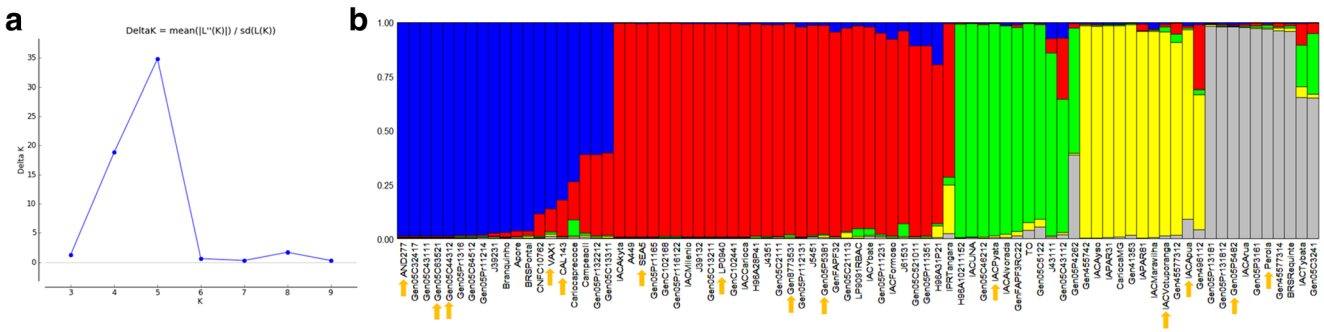

Fig. 2 **a** Evanno analysis results for determination the best number of clusters (DELTA K). **b** Population structure analyzed for 81 accessions and grouped by $k = 5$. Each accession is represented by a vertical bar and the clusters are indicated by different colors. The cultivars in group "a" (resistant) according to the mean test for severity data are indicated by an orange arrow

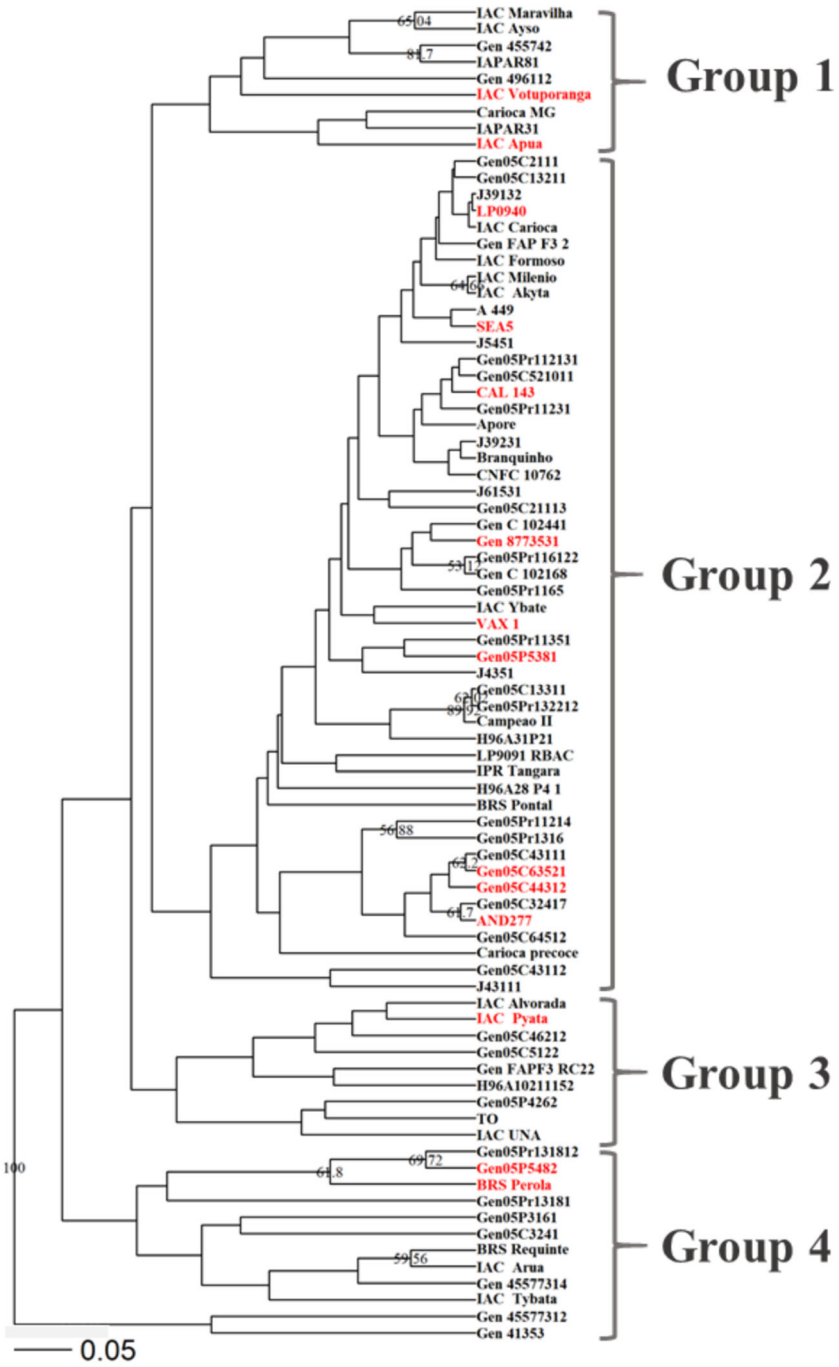

Fig. 3 Nei's (1978) genetic distance estimated for the 81 accessions and grouped by UPGMA. The cultivars in group "a" (resistant) according to the mean test for severity data are highlighted (red color)

since both have high resistance, wide genetic divergence, different genetic structure, and seed color classified as special commercial group. Gen05P5381 x Gen05P5482 improved lines were recommended to obtain new advanced lines from the black commercial group. The other seven pairs are recommendations for improvement of the carioca variety. Among them, the combination of the elite line LP0940 with the IAC Votuporanga and BRS Pérola cultivars, with 1.5, 1.8, and 2.6 severity scores, respectively, is the most outstanding.

Discussion

The most important agronomic trait for the common bean production chain is grain yield, as the financial return depends mainly on the quantity of grains produced (Pereira et al. 2019a, b). For this reason, the genetic breeding programs have as main goal the increase in productivity of the new bean cultivars to be released. Several other secondary traits are also improved with the aim of adding value, as plant size, grain tolerance to shelf storage, reduced cooking time, biofortification, heat and drought tolerance,

Table 3 Selection and recommendation of common bean (*Phaseolus vulgaris*) cultivars for recombination pairs (cross breeding) aiming to obtain lines with high resistance to angular leaf spot and gain in genetic diversity. Combinations from number 1 to 6 designate pairs for breeding the carioca variety, number 8 and 9 for black bean and special commercial grain, respectively

N°	GD	PS	ALS	Parent 1[1]		Parent 2[1]	ALS	PS	GD
1	1	4	1.8	IAC Votuporanga	X	LP0940	1.5	2	2
2	3	2	2.6	IAC Pyatã	X	IAC Apuã	3.2	4	1
3	1	4	3.2	IAC Apuã	X	BRS Pérola	2.6	4	5
4	5	4	2.6	BRS Pérola	X	LP0940	1.5	2	2
5	2	1	2.9	Gen05C44312	X	IAC Votuporanga	1.8	4	1
6	1	4	1.8	IAC Votuporanga	X	BRS Pérola	2.6	5	4
7	2	1	3.1	VAX1	X	IAC Pyatã	2.6	2	3
8	2	2	1.9	Gen05P5381	X	Gen05P5482	1.9	5	4
9	2	1	1.1	AND 277	X	SEA5	2.7	2	2

N°, recombination pair number; *GD*, genetic distance according to Nei (1978) and grouped by the UPGMA (4 groups); *PS*, population structure analyzed by the STRUCTURE v2.3.1 program (5 groups); *ALS*, resistance to angular leaf spot (*Pseudocercospora griseola*) (1–9 scoring scale from CIAT)

[1] The choice of maternal and paternal parent was not considered, so reciprocal cross is suitable as well

and resistance to diseases. Yield is a complex trait influenced by biotic and abiotic factors, and it is a real challenge for breeders. However, it is possible to improve traits that are simpler and positively correlated with yield.

Incidence of diseases such as ALS can lead to serious production losses, depending on environmental conditions, the degree of cultivars resistance, and the virulence of the pathogen (Schwartz et al. 1981; Rava et al. 1985; 66 Jesus-Junior et al. 2001). Therefore, obtaining ALS-resistant cultivars directly influences yield and productivity. However, the basis of a breeding program for disease resistance is the genetic variability present in the germplasm bank, which can only be explored when well characterized and evaluated.

In the case of germplasm bank with a lot of accessions, the variability characterization becomes costly, and the best way is to select a reduced number of accessions that represents almost all the diversity of the active germplasm bank, so that the characterization becomes viable. According to Colombari Filho et al. (2010), the characterization of diversity may be more useful when evaluated in combination with genotype and phenotypic information of interest. Even though there are several studies on the genetic (Corrêa et al. 2001; Mahuku et al. 2011; Miller et al. 2018; Nay et al. 2019b) and phenotypic (Sannazzaro et al. 2003; Reis-Prado et al. 2006; Chataika et al. 2010; Pereira et al. 2019a, b) characterization of ALS resistance, as well as evaluation of the general genetic diversity of common bean accessions (Campa et al. 2018; Raatz et al. 2019; Valentini et al. 2018; Gioia et al. 2019), this is the first study involving phenotypic resistance evaluation in combination with genetic diversity of *loci* associated with ALS resistance.

Variance analysis of ALS phenotypical evaluations, using physiological race 31-31, in controlled inoculation condition (greenhouse), confirmed great variability among accessions shown by highly significant values of F tests. We found high heritability (84%) for ALS that corroborates previous studies. Oblessuc et al. (2012) estimated 69% of heritability in the evaluation of ALS resistance in a greenhouse and 81% under natural infection conditions. According to Amaro et al. (2007), the high heritability usually estimated for the ALS resistance allows phenotypic selection in early generations.

The groups formed by the Scott-Knott mean test are according to the resistance classification proposed by Van-Schoonhoven and Pastor-Corrales (1991), with group "a" composed of resistant cultivars, groups "b" and "c" of moderately resistant, and group "d" constituted by susceptible cultivars. Low number of ALS resistant cultivars was also reported in a recent phenotypic evaluation study using 144 cultivars, where only 7% of accessions showed resistance to both 63-63 and 63-23 isolates (Pereira et al. 2019a, b). Other studies have reported the high resistance of the AND 277 and CAL 143 cultivars and their importance as sources of genetic resistance (Gonçalves-Vidigal et al. 2011; Oblessuc et al. 2012; Bassi et al. 2017). However, both cultivars belong to the Andean gene pool, which makes it difficult to use them in the improvement of Mesoamerican cultivars, such as the carioca bean, that represents 70% of the varieties consumed in the country (Pereira et al. 2019a, b). Thus, the high resistance of the carioca cultivars IAC Votuporanga, BRS Pérola, IAC Pyatã, IAC Apuã, and LP0940 makes them extremely important sources of ALS resistance.

Regarding the diversity of the 12 SSRs used, our results were close to studies carried out on common beans with a larger number of markers. Perseguini et al. (2011) evaluated 70 SRRs in a set of carioca bean cultivars and identified an average PIC of 0.47 and several alleles ranging from 2 to 6. More recently, Delfini et al. (2017) analyzed a set of 39 Brazilian cultivars genotyped by 17 SSRs and reported a mean PIC of 0.33 and a mean number of alleles of 3.4 per *locus*. Furthermore, our results showed a higher degree of diversity

for accessions than the ones reported by Delfini et al. (2017), with an average value of 0.69 for carioca cultivars and 0.65 for black tegument cultivars. The authors concluded that the evaluated set had moderate genetic diversity. Probably, the greater diversity found in the present study is mainly because this set of accessions was not limited to Brazilian commercial cultivars.

The genetic distance and STRUCTURE analysis confirmed the moderate degree of diversity of the evaluated group, with the formation of 4 and 5 groups, respectively. Interestingly, although the IAC UNA and IAC Alvorada cultivars do not have common parents, they were grouped together in the present study, agreeing with Perseguini et al. (2015) who analyzed the genetic distance with 180 accessions. Resistant accessions were grouped in all groups, which allow the selection and recommendation of nine cultivar pairs with high resistance and genetic divergence for recombination. According to Perseguini et al. (2011), the success of a breeding program in order to increase genetic diversity depends on the choice of divergent parents for the crosses. However, the bean breeder normally exploits only a small fraction of the genetic diversity available, and due to the difficulty in improving quantitative traits, recombinations tend to be only with elite cultivars to exploit additive inheritance (Pereira et al. 2019a, b). The problem is that the frequent use of elite cultivars in the bean breeding process leads to a high degree of kinship, diminishing the genetic base of commercial cultivars (Cooper et al. 2001).

In this sense, the hybridization pairs formed by the 11 selected accessions have great potential for the genetic improvement of the species, not only aiming at gaining ALS resistance but also to other agronomic traits of interest. In addition to the higher ALS resistance, the selected accessions showed genetic diversity accessed by 12 resistance *loci* evaluated, to increase genetic diversity and the diversification of alleles responsible for the resistance of the lines to be obtained by hybridization. Another advantage of the proposed pairs is the reduction of linkage drag and segregation for grain color since they were separated into commercial classes.

Between the selected pairs, the combination of the LP0940 elite line with the IAC Votuporanga and BRS Pérola cultivars is the most outstanding. In a study of yield, adaptability, and multiple resistance with 22 lines, Azevedo et al. (2015) found that LP0940 line (IAPAR) showed higher adaptability and yield than the standard cultivar (IAC Alvorada) for water and winter harvest, in addition to resistance to anthracnose and Fusarium wilt. In relation to the BRS Pérola cultivar, its approval by the producer market was so extensive that it changed the sieve yield standard, causing the carioca commercial variety to go from grain size 4.36 to 5.15 mm. On the other hand, the IAC Votuporanga cultivar was introduced in the 2000s aiming at grain quality and high yield (2853 kg/ha), which was higher than the BRS Pérola cultivar in the VCU evaluated over 3 years (2001, 2002, and 2003) (Chiorato and Carbonell 2014).

In summary, the characterization of ALS resistance on the 81 accessions, together with analysis of diversity, structure, and genetic divergence using the 12 microsatellites previously associated with the QTL resistance, allowed selection and recommendation of cultivars for breeding resistance to ALS with wide genetic diversity for the resistance *loci* evaluated. This fact not only enables resistant lines to be obtained but also ensures allelic variability to generate superior cultivars with durable resistance.

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