Agronomical Characters and Rapd Markers Associated with the Resistant Allele to the *Erysiphe Polygoni* in Common Bean

Viviane Ferreira Rezende[1]; João Bosco dos Santos*[1]; Magno Antônio Patto Ramalho[1] and Daniel Furtado Ferreira[2]

[1] Departmento de Biologia da Universidade Federal de Lavras (UFLA), Caixa Postal 37, CEP 37200-000, Lavras – MG, Brasil; [2] Departmento de Ciências Exatas da UFLA, Caixa Postal 37, CEP 37200-000, Lavras – MG, Brasil. (* Corresponding Author)

ABSTRACT

Aiming at verifying whether the common bean allele of resistance to *Erysiphe polygoni* is associated with undesirable agronomic characters and identifying RAPD markers linked to the resistance allele of the line ESAL 686, the reaction of 64 $F_{1:2}$ families of the backcross (Jalo x ESAL 686) x Jalo to the pathogen was evaluated. The $F_{1:3}RC_1$ families were evaluated, in the absence of oidium, by grain yield (g/ plot), weight of 100 seeds (g) number of days to flowering and grain aspect. The association noticed of the reaction gene with grain aspect should not prevent the selection of resistant lines with grains similar to Jalo. DNA of F_1RC_1 plants, resistant and susceptible, were pooled making up two bulks and amplified by RAPD. The four markers identified, amplified by the primers OPAC15, OPC14, OPC18 and OPL16, are closely linked among them and relatively far from the resistance allele, having little usefulness for indirect selection of resistant plants.

KEY WORDS: Bean, Resistence allele, Erysiphe Polygoni.

INTRODUCTION

In Brazil, common bean (*Phaseolus vulgaris* L.) of the Jalo type, with yellow and large grains, have a great commercial acceptance in some regions and in general possess a higher market price. However, those beans bear some problems which make their cultivation difficult such as susceptibility to pests and some pathogens specially *Erysiphe polygoni*, causal agent of oidium.

Oidium is a disease of importance in the common bean when it is cultivated at the times of "drought" and fall-winter, when environmental conditions favor the occurrence of that pathogen. The losses of grain yield ascribed to oidium may reach up to 69% depending on the cultivar and environmental conditions (Shwartz et al., 1981). In a work performed in the South of Minas Gerais State there were reductions in yield of 50% in the cultivar Eriparza and 40% in Rio Vermelho (Arriel et al., 1991).

Although there are efficient alternatives to disease control with chemical products, the use of resistant cultivars is the control measure most advised for not increasing production costs or causing environmental unbalance and pollution (Rava and Sartorato, 1993). A large number of cultivars with high resistance to the pathogen are known, however, most of the resistant cultivars belong to the mesoamerican groups, with medium to small sized grains and indeterminate growth habit (Schwartz et al., 1981; Sartorato et al., 1993). ESAL 686 is one of the few lines with medium grains, determinate habit and resistant (Rezende et al., 1996).

As the Jalo cultivar presents crossing incompatibility with most cultivars of the Mesoamerican group (Singh and Gutierrez, 1984; Vieira et al., 1989) the introduction of the allele of resistance to *E. polygoni* in that cultivar is difficult. With the identification of the pathogen resistance in the line ESAL 686, which is compatible to large grained cultivars, the possibility of the introduction of the resistance allele into the cultivar Jalo appeared.

In the development of a breeding program, obtaining information on the genetic control and association among the characters help breeders in decision making. Another aspect to be taken into account is that the pathogen is obligatory and its occurrence is not frequent through the year which may delay the selection program. One alternative is the selection of resistant genotypes by indirect way through markers linked to the resistance allele (Ferreira and Grattapaglia, 1995; Kelly, 1994). RAPD (Random Amplified Polymorphic DNA) is one of the most utilized markers. In this context, the objectives of the present work were to verify whether the oidium resistance allele is associated with undesirable agronomic characters and identify RAPD markers linked to the resistance allele of the line ESAL 686 to *Erysiphe polygoni*.

MATERIAL AND METHODS

The experiments were carried out at the Universidade Federal de Lavras, located in South of Minas Gerais State (21º58' S, 45º22' W and 910 m of altitude), with dark red latosol and CWb climate according to Koppen climate classification.

Parents and crosses

One of the parents utilized was the Jalo cultivar, with large yellow grains, type III indeterminate growth habit and high oidium susceptibility but with a grain type well accepted by consumers. The other parent was the line ESAL 686, with medium sized grains and dark yellow in color, type I determinate growth habit and resistant to oidium. Each plant from the first backcrossing generation F_1RC_1 with the susceptible parent [(Jalo x ESAL 686) x Jalo] was utilized for DNA extraction, obtaining $F_{1:2}RC_1$ families.

Evaluation of oidium reaction in the $F_{1:2}RC_1$ families

Sixty four $F_{1:2}RC_1$ families were evaluated, aiming at identifying those segregating ones in regards to oidium reaction and also those which were completely susceptible. An 8 x 8 simple lattice design was utilized, the plot being made up of a one meter row with 15 plants. Twenty days before the experiment was set up in the winter of 1998, two rows were sowed with Jalo around the experiment, which served as a border and inoculum source. Twenty days after the sowing of the experiment, an inoculation with the pathogen was done through the contact of pieces of infected leaves with healthy leaves in addition to the natural inoculation done by the wind.

The first evaluation of oidium incidence was performed two months after sowing by using a score scale of 1 to 10 (Rezende et al., 1999). After six days, another evaluation was performed. During the evaluation of each plot, whether the plants were evenly susceptible or whether segregation occurred was recorded. An average score from two evaluators at two scoring times was utilized for variance analysis by decomposing the source of variation treatments into susceptible families (group 1), segregating families (group 2) and between groups.

The heritability (h^2) among means of families, in the wider sense, was estimated, utilizing the methodology presented by Vencovsky and Barriga (1992). Also, the h^2 confidence intervals estimation was obtained, by the expressions presented by Knapp et al. (1985) with 95% confidence ($1 - \alpha = 0.95$). The relationship between the genetic and the experimental coefficient of variation was estimated (Vencovsky and Barriga 1992) as well. The number of segregating families and susceptible families observed was compared with the expected one, monogenic segregation being considered by the χ^2 test.

Evaluation of the $F_{1:3}RC_1$ families in the absence of oidium

The same 64 families were evaluated in the $F_{1:3}RC_1$ generation, in the rainy season of 1998/1999 in a 8 x 8 triple lattice design. Each plot was made up of two rows 2m long, 0.5 meters apart. For that generation, a weekly control of oidium was done by utilizing a fungicide based on sulphur. The objective of that control was to verify the effect of the resistance allele, in the absence of disease, on

the following characters: number of days to flowering, grain yield (g/plot), weight of 100 seeds (g) and grain aspect. This last character was evaluated by utilizing a score scale of 1 to 5 in which: 1 – grains of uniform aspect and yellow, similar to Jalo; 2 – grains similar to Jalo in shape and differing in color; 3 – smaller–sized grains, rather round and dark; 4 – round and dark grains and, 5 – grains completely different from Jalo.

The analyses of variance were conducted for each character decomposing the treatment source of variation treatments into families within groups, and between groups of families with and without the resistance allele identified in the $F_{1:2}RC_1$ (Fujimaki, 1978). Two heritability coefficients were estimated, one including the total genetic variation (h^2) and other omitting the variation among groups (H). Their respective intervals of confidence at 95% ($1 - \alpha = 0.95$) was estimated as well. Genetic correlation was estimated utilizing the means of $F_{1:2}RC_1$ and $F_{1:3}RC_1$.

DNA extraction

DNA extraction was made from F_1RC_1 plants by means of a procedure similar to that employed by Nienhuis et al. (1995). DNA concentration was determined by using a fluorometer (Hoeffer Scientific, San Francisco, Ca, U.S.A).

DNA bulks and RAPD analysis

Two bulks were built from DNA samples extracted from leaves of F_1RC_1 plants previously identified as to their reaction to *Erysiphe polygoni*. One of the bulks contained equimolar amounts of DNA extracted from seventeen resistant F_1RC1 plants and the other bulk seventeen susceptible F_1RC_1 plants. DNA of the bulks was amplified with 700 decamer primers (Operon Technologies Inc. Alameda, Ca, USA) to identify polymorphisms between the bulks.

The RAPD reactions were prepared in volumes of 10 µl in a way similar to the procedure used by Nienhuis et al. (1995). The reactions were performed in glass capillary tubes in an air-refrigerated thermal-cycler (Idaho Technology, Idaho Falls, ID, USA). The thermal cycler was programmed for 40 cycles under the following conditions: 1) DNA denaturation at 91^0C, for 60s, in the first 2 cycles, and 1s in the next 38 cycles; 2) Elongation at 72^0C, for 70s; 3) Primer annealing at 42^0C, for 7s. After amplification, the reaction products were separated by eletrophoresis in 1% agarose gel, stained with ethidium bromide, viewed in ultraviolet light transiluminator and photographed.

Analysis of genetic distances

The potential RAPD markers detected in segregating bulks were firstly tested in the constituent individuals of each bulk to check the co-segregation between those markers and the resistance/susceptibility phenotype determined in 64 F_1RC_1 plants. Chi-square tests were utilized to compare the observed and expected phenotypic ratios under the supposition of independent assortment.

To determine the positions of the identified markers relative to the resistance allele of the ESAL 686 line the MAPMAKER program (Lander et al., 1987) version 3.0 with minimum lod score of 0.83 was used. The map-funtion of Haldane (1919) was employed to calculate the genetic distances in centimorgans (cM). The recombination frequency (r) was estimated by using an iterative process through the non-linear least square procedure (Mendonça et al., 1998), by utilizing the PROC NLIN (SAS®, 1990) procedure. The model adjustment was evaluated by the determination coefficient.

RESULTS AND DISCUSSION

Genetic control of oidium resistance

The summary of the analysis of variance of the oidium incidence scores is presented in Table 1. Initially it is worthwhile to point out that practically no lattice efficiency was found which suggests that the distribution of the inoculum was uniform. Along with that, the experimental precision, calculated by the CV (%), was superior to those reported with other common bean characters (Abreu et al., 1994) as well as to those reported by Marques

Júnior (1997) by utilizing scores in the evaluation of diseases.

It was found that the families evaluated differed in relation to pathogen incidence ($P \leq 0.01$). When proceeding the decomposition of the families into two groups (segregating and susceptible), no significant differences were observed among the susceptible ones. The opposite took place in the case of the segregating families. As it was expected, a significant difference was detected ($P \leq 0.01$) among groups, highlighting the highest incidence of disease occurred among the susceptible families. The non-significance of the susceptible families is due to the fact that those families come from the self-fertilization of homozygous plants with the susceptibility allele, while the segregating families come from the self-fertilization of heterozygous plants to the gene that control the reaction to oidium having, therefore, genetic variation possible to be explored for this character.

The 35 segregating families for the 29 susceptibles are very close to the 1:1 ratio, as expected in the backcrossing considering the involvement of a single gene ($\chi^2 = 0.5625$).

In a previous work involving the same crossing, but with other segregating generations, the hypothesis which two genes with double recessive epistasis interaction was tested (Rezende et al., 1999). However, the segregation observed in this research shows that as a matter of fact, only one gene is involved. Results of the literature leave doubt about the gene number involved and the sort of gene action (Dundas, 1936; Dundas, 1942; Bett and Michaels, 1995). It is probable that this difference occurs in terms of the criterion of scores utilized in each case and further, the score from which a plant or a family must be considered as resistant or susceptible. In addition, in the evaluation of families by Rezende et al. (1999), an average score was given and it was not taken into consideration whether there was or not segregation within the families as was the case of this work.

The high estimate of heritability in the wide sense as well as the relationship between the coefficient of genetic and experimental variation are a clue that the genetic control of oidium reaction is due to one or few genes (Rezende et al., 1999). Those values show the existence of genetic variability among families and a reduced environmental variation which allows to foresee the success with the selection of resistant plants and/or families in segregating populations.

Association of oidium resistance allele with agronomical characters

The summary of the analysis of variance of agronomical characters is presented in Table 2. It is found that the lattice design was of little efficiency

Table 1 - Analysis of variance of the oidium incidence scores in $F_{1:2}$ families of the cross (Jalo x ESAL 686) x Jalo of bean in the winter of 1998.

Sources of variation	DF	MQ	F probability
Replications	1	8.508	
Families	63	6.515	0.000
Susceptible families	28	0.264	0.954
Segregating families	34	1.104	0.004
Between groups	1	365.5	0.000
Effective error	49	0.481	
Lattice efficiency		100.5	
Average		6.281	
CV (%)		11.05	
$\hat{h}^2$ (%)		92.62	
Lower and upper limits of $\hat{h}^2$ (95%)		(87.30; 93.83)	
B		2.503	

relative to the randomized blocks, specially for 100 seeds weight and days to flowering. The coefficient of variation (CV%) was less for the number of days to flowering (3.43%) and larger for grain aspect (18.85%). Even this last value is of magnitude similar or inferior to that normally reported in experiment with common bean crop (Marques Júnior, 1997; Abreu et al., 1994), standing out that the families were evaluated with precision.

Genetic differences were found among families for all the characters evaluated ($P \leq 0,01$). It was still observed that the plot of the total genetic variation due to groups was significant only for the weight of 100 seeds ($P \leq 0.05$) and grain aspect ($P \leq 0.01$), indicating that those characters may be associated with the oidium resistance allele. The genetic variation of the families within groups was significant ($P \leq 0.01$) for all the characters.

The heritability estimates including total genetic variation (h^2) and omitting the variation between group (H), were distinct only for grain aspect, showing a more marked effect of the allele resistance for the formation of undesirable grains. Nevertheless, there is an overlapping between the two estimates when taking the intervals of confidence of each of them into consideration, which suggests that the unfavorable effect in grain aspect will not be able to prevent the selection of resistant lines and with grains similar to Jalo. In addition, such similarity took place probably due to the recombination of the resistance allele relative to one or more genes which affect grain aspect, by the fact of eight families with grain aspect close to the ideal and carriers of the resistance allele having been detected. Therefore, the association of the oidium resistance allele with those which condition the grain undesirable aspect may be due to genic linkage (Fujimaki and Comstock, 1977; Fujimaki, 1978).

On the other hand, the differences among the average weights of 100 seeds of the two groups cannot be interpreted as due to gene linkage or pleiotropy (Table 6) because the mean of the families with the resistance allele (2.855g) was slightly superior to the mean of the susceptible families (2.532g), a result opposite to the average weight of 100 seeds of the two parents. The resistant line ESAL 686 possess seeds smaller than Jalo, the susceptible parent. Therefore, no difficulty in the selection of resistant families and of large seeds as Jalo is expected.

The estimates of the correlations between the score ascribed to the incidence of the pathogen with the other characters evaluated were of little magnitude, nevertheless, when involving grain aspect it was rather superior (Table 3). As the occurrence score of the pathogen was given in an environmental condition different from that in which the other characters were evaluated, the estimated covariance is genetic, the same occurring with correlation which is a measure of genetic association among the characters and may be due to linkage of genes or pleiotropy (Falconer, 1987). Thus, it may be inferred that there is an association of the oidium reaction gene with those responsible for grain aspect. That result confirms what was previously reported with analysis of variance, showing that Fujimaki's methodology (1978) is effective for detecting the association of the characters. Similar results were obtained in wheat (Cox et al., 1997) and rice (Fujimaki, 1978).

In addition, in the present results, the high estimates of heritability of all characters evaluated allowed to foresee the success with the selection of plants/ families resistant to oidium, early, high yielding, with large grains and similar to Jalo.

RAPD markers linked to the oidium resistance allele

Four potential RAPD markers at the coupling phase were identified. Figure 1 shows the amplification patterns of the parents and of the DNA bulks with the four primers which enabled the detection of the RAPD markers. It was found that the four markers (arrows) exhibited higher intensity in the parents which are homozygous relative to the resistant bulks because the F_1RC_1 plants are heterozygous (Table 7).

Table 2 - Analyses of variance of the number of days to flowering, grain yield (g/plot), weight of 100 seeds (g) and grain aspect of the $F_{1:3}$ families of the crossing (Jalo x ESAL 686) x Jalo, in the rainy season of 1998/1999.

Sources of variation	DF	Medium Square			
		Days to flowering	Yield (g/plot)	Weight of 100 seeds (g)	Grain aspect
Replications	2	0.563	4090	9.180	1.595
Families	(63)	16.00**	3856**	14.08**	1.016**
Between groups	1	3.266 ns	1326 ns	12.62*	4.965**
Families within groups	62	16.20**	3897**	14.10**	0.952**
Effetive error	105	1.969	1277	3.016	0.261
Lattice Efficiency		100.3	102.5	100.1	107.0
Média		40.91	282.7	36.51	2.710
CV (%)		3.430	12.64	4.760	18.85
$\hat{h}^2$ (%)		87.69 (81.03; 92.21)[1/]	66.89 (48.96; 79.05)	78.57 (66.98; 86.44)	74.32 (60.41; 83.74)
H		88.97 (81.24; 92.33)	67.94 (49.41; 79.32)	78.75 (66.98; 88.50)	68.00 (57.67; 82.70)

[1/] lower and upper limits.

* and ** – significant at the level of 5% and 1% by the F test; ns – non-significant;

Table 3 - Genetic correlations (r_G) involving the oidium incidence scores in the $F_{1:2}$ families, the number of days to flowering, grain yield (g/plot), weight of 100 seeds (g) and grain aspect of the $F_{1:3}$ families of the crossing (Jalo x ESAL 686) x Jalo.

Pairs of characters	r_G	Probability > |t|
Oidium x number of days to flowering	- 0.046	0.474
Oidium x grain yield	- 0.199	0.099
Oidium x weight of 100 seeds	- 0.216	0.017
Oidium x grain aspect	- 0.390	0.000

The segregation analysis of the markers of 1/2 presence of bands: 1/2 absence of bands in the 64 individuals of the F_1RC_1 population was consistent with the dominant monogenic inheritance expected for the four markers which characterize them as genetic markers (Table 4).

To confirm whether the markers were really linked to the oidium resistance allele, the co-segregation analysis between each marker and the resistance/ susceptibility allele was done. A co-segregation analysis between the markers two by two was also conducted (Table 5). It is found that the four markers are closely linked and all of them are relatively distant from the resistance allele.

The genetic distances, LOD scores (log of odds–ratio) as well as the recombination frequency, standard error, and determination coefficient (R^2) are in Table 6. The least value of LOD score presented in Table 6 (0.88) exceeds the minimum LOD score of 0.83. Therefore, it may be

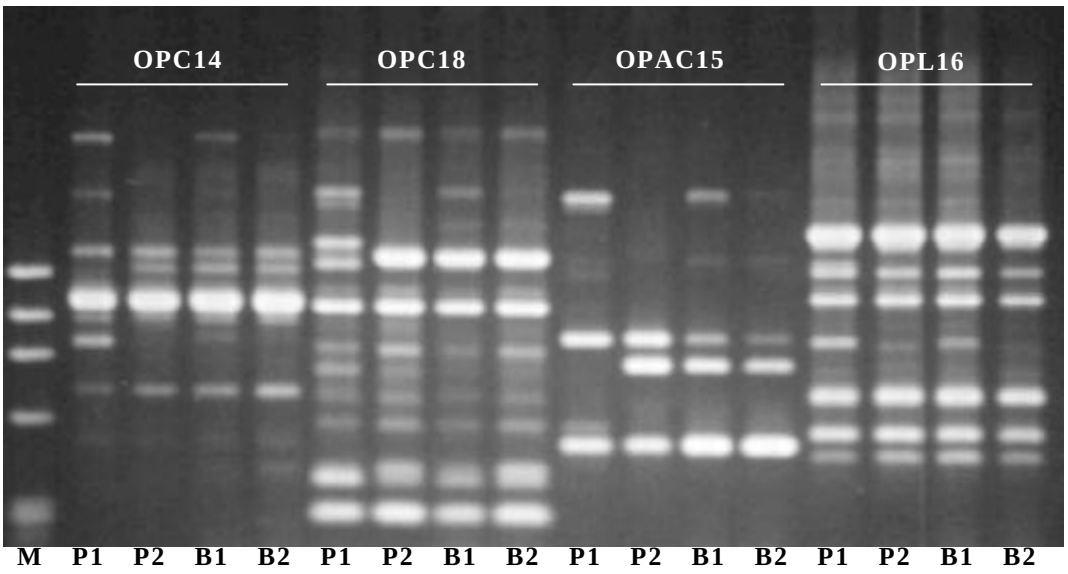

Figure 1 - RAPD markers (arrows) obtained by DNA amplification with the primers OPC14, OPC18, OPAC15 and OPL16 in which: P1 is the resistant parent ESAL 686, P2 is the susceptible parent Jalo, B1 is the resistant bulk and B2 is the susceptible bulk. The column M corresponds to the lambda DNA digested with the restriction enzyme Hae III.

considered that all the markers are linked to the oidium resistance allele with 95% confidence. The high values of R^2 shows that the estimated frequencies fit those observed in RC_1, showing the good adjustment of the model.

Table 4 - Segregation analysis of the markers OPC14, OPC18, OPAC15 and OPL16 in the F_1 generation of the crossing (Jalo x ESAL 686) x Jalo.

Tested locus	Observed frequency	Expected ratio[1/]	c^2	Probability
OPC14	34:30	1:1	0.250	0.617
OPC18	35:29	1:1	0.562	0.453
OPC15	35:29	1:1	0.562	0.453
OPC16	34:30	1:1	0.250	0.617

[1/] presence of band: absence of band, considering monogenic inheritance.

According to the results on Table 5 and 6, the four markers are closely linked among each other and weakly linked to the resistance allele. Taking into account the marker closest to the resistance allele, amplified by the primer OPAC15, the recombination frequency estimated is of 32.51%, which means that if the indirect selection of plants is performed with the marker in a F_2 population, 18% of the selected plants will be susceptible (Table 7). This frequency of erroneously selected plants by means of the marker shows that it has little utility in aiding selection.

Table 5 - Segregation analysis of the four marker loci and oidium reaction gene, taken two by two and considering independent assortment in the F_1 generation of the crossing (Jalo x ESAL 686) x Jalo.

Tested loci	Observed frequency	Expected ratio AB: Ab: aB: ab	χ^2	Probability
Resistance allele / OPC14	23:12:11:18	1:1:1:1[a]	5.875	0.118
Resistance allele / OPC18	24:11:11:18	1:1:1:1[a]	7.375	0.061
Resistance allele / OPAC15	24:11:10:19	1:1:1:1[a]	8.375	0.039
Resistance allele / OPL16	23:12:12:17	1:1:1:1[a]	5.125	0.163
OPC14 / OPC18	34:0:1:29	1:1:1:1[b]	60.88	0.000
OPC14 / OPAC15	33:1:1:29	1:1:1:1[b]	56.75	0.000
OPC14 / OPL16	34:0:1:29	1:1:1:1[b]	60.88	0.000
OPC18 / OPAC15	34:1:0:29	1:1:1:1[b]	60.88	0.000
OPC18 / OPL16	34:1:1:28	1:1:1:1[b]	57.38	0.000
OPAC15 / OPL16	33:2:1:28	1:1:1:1[b]	53.38	0.000

[1/] AB (presence of the resistance allele and marker), Ab (presence of the resistance allele and absence of the marker). AB (presence of the susceptibility allele and marker), ab (presence of the susceptibility allele and absence of the marker).
[2/] AB (presence of the band in the two markers), Ab (presence of the band in the first marker and absence in the second marker), ab (absence of the band in the two markers).

The magnitude of the error expected in the selection reduces proportionally as the marker is closer to the allele of interest. This is the case, for example, of the Co.5 allele, which confers resistance to some races of *Colletotrichum lindemuthianum* in common bean, where only 7.2% of the plants selected indirectly in F_2 by means of the marker amplified by the primer OPF10 (r=0.115), will be susceptible (Castanheira et al., 1999).

Table 6 - Distance and frequency of recombination of RAPD markers and the oidium resistance allele in the F_1 generation of the crossing (Jalo x ESAL 686) x Jalo.

Locus	Distance[1/] (cM)	LOD *Score*	r (%)[2/]	Standard Error	R^2
Resistance allele/OPL16	69.30	0.880	37.49	3.830	78.05
Resistance allele/OPC14	63.40	1.110	35.93	3.250	86.19
Resistance allele/OPC18	58.20	1.380	34.39	3.830	84.75
Resistance allele/OPAC15	53.40	1.680	32.81	3.250	90.30
OPL16/OPC14	1.600	17.03	1.560	3.250	98.67
OPL16/OPC18	3.200	15.40	3.130	2.550	99.12
OPL16/OPAC15	4.900	14.01	4.690	3.250	99.98
OPC14/OPC18	1.600	17.03	1.560	3.250	98.67
OPC14/OPAC15	3.200	15.40	3.130	2.550	99.12
OPC18/OPAC15	1.600	17.03	1.560	3.250	98.67

[1/] Total distance=58.20 cM (Haldane);
[2/] Recombination frequency.

It is noticed, in Table 7, that the other markers are slightly less efficient for indirect selection purposes than OPAC15, due to they are farther from the resistance allele. Considering the distance of the markers identified relative to the resistance allele, efforts must be done to identify others closer, which will be useful for indirect selection specially to fasten the selection program in times of the year when the disease generally does not develop.

CONCLUSIONS

Association of the oidium resistance allele only with grains aspect was observed however, that association must not prevent the selection of resistant lines and grains similar to Jalo.

Four RAPD markers amplified by the primers OPAC15, OPC14, OPC18 and OPL16 which are closed linked and relatively distant from the resistance allele were identified, therefore they have little usefulness to the indirect selection of plants with resistance allele.

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Table 7 - Estimate of the frequency of susceptible plants (EFSP) selected as resistant in F_2 by the four markers, the respective number of pair of bases (pb) of each marker, each one amplified by a primer of known sequence.

Primer	Base sequence	pb of the marker	r (%)	EFSP (%)
OPAC15	5'TGCCGTGAGA3'	2239	32.81	18.28
OPC18	5'TGAGTGGGTG3'	2239	34.39	18.98
OPC14	5'TGCGTGCTTG3'	3020	35.39	19.65
OPL16	5'AGGTTGCAGG3'	912	37.49	20.31

RESUMO

Caracteres Agronômicos e Marcadores RAPD Associados ao Alelo de Resistência à *Erysiphe polygoni* em Feijão

Com o objetivo de verificar se o alelo de resistência ao *Erysiphe polygoni* em *Phaseolus vulgaris* L. está associado a caracteres agronômicos indesejáveis e de identificar marcadores RAPD ligados ao alelo de resistência da linhagem ESAL 686, foi avaliada a reação de 64 famílias $F_{1:2}$ do retrocruzamento (Jalo x ESAL 686) x Jalo ao patógeno. Na geração $F_{1:3}RC_1$ foram avaliados, na ausência de oídio: produtividade de grãos (g/parcela), peso de 100 sementes (g), número de dias para o florescimento e aspecto do grão. A associação observada do alelo de resistência com o aspecto do grão não deverá impedir a seleção de linhagens resistentes e com grãos semelhantes ao Jalo. DNA de plantas F_1RC_1, resistentes e suscetíveis, foram misturados formando dois bulks e amplificados por RAPD. Os quatro marcadores identificados, amplificados pelos primers OPAC-15, OPC-14, OPC-18 e OPL-16, estão intimamente ligados entre si e relativamente distantes do alelo de resistência, tendo pouca utilidade para a seleção indireta de plantas resistentes.

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Genetic Inheritance of Aluminum Tolerance in Oat

Caroline Moor Wagner; Sandra Cristina Kothe Milach* and Luiz Carlos Federizzi
Departamento de Plantas de Lavoura da Universidade Federal do Rio Grande do Sul (UFRGS), Caixa Postal 776, CEP 91501-970, Porto Alegre – RS, Brazil. (* Corresponding Author. E-mail: milach@vortex.ufrgs.br)

ABSTRACT

Aluminum toxicity is an important factor that limits crop productivity in acid soils. Understanding the inheritance of this trait may help breeding programs to develop aluminum-tolerant cereal crops. The objectives of this work were to study this trait in oat (*Avena sativa* L.) genotypes not yet investigated and to determine the allelic relationships between aluminum tolerant genotypes. Three tolerant (UFRGS 17, UFRGS 93605 and UFRGS 15), two sensitive genotypes (UFRGS 911715 and UFRGS 93598), and their respective F_2 populations were evaluated for root regrowth in the presence of 20 ppm of aluminum. One dominant gene for tolerance was identified in crosses involving UFRGS17 and UFRGS93605 and two genes with epistasis in crosses with UFRGS 15, indicating that the tolerant genotypes included in this study have different genetic constitution.

KEY WORDS: Nutrient solution, Root regrowth, Dominance, Epistasis.

INTRODUCTION

Aluminum is one of the most abundant earth metals and is found in large amounts in tropical soils. The Brazilian tropical and subtropical areas are formed by acid soils, where the toxicity of this metal is one of the most important environmental stresses affecting cereal development (Delhaize *et al.*, 1993; Camargo, *et al*., 1992). Associated to this fact, the action of the lime application is limited to the zone of incorporation, and it certainly will not penetrate deeper than the plowing levels. Its application in sub superficial levels represents great cost to the farmer. Thus, the existence of cultivars with tolerance to toxic aluminum is of great importance for oat breeding programs.

Roots affected by Al^{+++} present a peculiar development at their tips. The meristematic region of the main and lateral roots, in the presence of Al^{+++}, gets darker, smaller and thicker, with fewer ramifications (Foy, 1974), resulting in low nutritional efficiency and water supply for the plant (Carver *et al*., 1988; Foy and Fleming, 1978). Such phenomenon allows the identification of genotypes that are sensitive to this cation.

According to Camargo (1984), Dornelles (1994) and Sànchez-Chacòn (1998), tolerance to Al^{+++} is easy to detect in tests under controlled conditions (in greenhouse or laboratories) and nutritive solution. This is, therefore, an easy method to identify plants that are tolerant to aluminum, saving space and time.

Several studies have investigated aluminum toxicity tolerance in cereals. However, few were carried out with oats. In an seminal study, Sànchez-Chacòn (1998) characterized the germoplasm from the UFRGS oat breeding program and identified one dominant gene for aluminum tolerance in nine populations from crosses between tolerant and sensitive genotypes. However, the presence of different sources of genes for aluminum tolerance among oat genotypes from the UFRGS that can be combined in a breeding program is unknown. The present work has been developed to study the inheritance of Al^{+++} tolerance in other source genotypes and to investigate the allelic relations between genes present in different oat genotypes.

MATERIALS AND METHODS

The assessment of the tolerance to aluminum toxicity was done with the method described by Camargo and Oliveira (1981) and adapted to oats

by Sánchez-Chacón (1998). Seedlings from P_1, P_2 and F_2 generations derived from the cross between the tolerant (UFRGS 15, UFRGS 17 and UFRGS 93605) and sensitive parents (UFRGS 911715 and UFRGS 93598) (Table 1) were evaluated for their response to Al^{+++} toxicity through the regrowth of the primary root. The F_2 segregating populations with their respective parents were submitted to nutritional solution in pots with 20 ppm of cation arranged in a completely randomized experimental design, considering that each pot constituted a population.

The seedlings assessed were divided in sensitive and tolerant in relation to their response to toxicity to Al^{+++}, having the class separation limit at 0.8 mm for all populations. The same class limit separation was used in the work done with oats by Sànchez-Chacòn (1998). A genetic hypothesis in relation to the number of segregating genes was proposed for each population, based on the frequency distribution obtained in the F_2 generation, and tested using the chi-square analysis (Steel and Torrie, 1960).

Crosses among genotypes within each tolerant and sensitive class were also performed. The F_2 populations evaluated for these crosses were UFRGS 17 x UFRGS 15 (number of individuals n = 76), UFRGS 17 x UFRGS 93605 (n= 58), UFRGS 93605 x UFRGS 15 (n=19), and UFRGS 911715 x UFRGS 93598 (n=70).

RESULTS AND DISCUSSION

We began testing the genetic hypothesis of just one gene segregating in crosses from populations between tolerant x sensitive genotypes since,

Table 1 - Genealogy, type of response to aluminum toxicity and mean± standard deviation (sd) of primary root regrowth (cm) of the oat genotypes included in this study. Janeiro, 1998.

Genotypes UFRGS	Genealogy	Aluminium response	Mean±sd[1/]
17	COR[2]/CTZ[3]/PENDEK/ME1563//76-29/76-23/75-28/CI833	Tolerant	2.32±1.0
93605	UFRGS 15/UFRGS 881920	Tolerant	1.57±0.7
15	COR[2]/CTZ[3]/PENDEK/ME 1563/c16crcpx/c7512/srcpx/74c8014	Tolerant	1.79±0.6
911715	UFRGS 86A 1194-2/UFRGS8	Sensitive	1.12±0.4
93598	UFRGS 15/UFRGS 881920	Sensitive	0.49±0.08

[1/] Mean±sd of each genotype grown in all pots of the experiment.

according to several studies, one to two genes for tolerance explain most of the variation for this character in cereal crops (Kerridge *et al.*, 1971; Camargo, 1984; Lagos *et al*., 1991; Camargo *et al*., 1992; Riede and Anderson, 1996; Johnson *et al*., 1997; Sànchez-Chacòn, 1998). This genetic hypothesis was confirmed for the populations from the crosses UFRGS 17 x UFRGS 911715, UFRGS 17 x UFRGS 93598 and UFRGS 93605 x UFRGS 911715 (Table 2) and is in agreement with that reported by Sànchez-Chacòn (1998).

A segregation ratio of 9 tolerant to 7 sensitive genotypes was observed for the F_2 populations derived from crosses involving the tolerant genotype UFRGS 15. This indicates that there are

two genes segregating in those populations and their interaction is shown through epistasis (Table 2).

Crosses between the tolerant UFRGS 17 x UFRGS 93605 and UFRGS 17 x UFRGS 15 showed continuous segregation and since the parental genotypes were both tolerant and overlapped for their response to Al^{+++}, it was not possible to classify the progeny accurately in two distinct phenotypic classes only (Wagner, 1999). The fact that crosses of sensitive genotypes with UFRGS 15 segregate as two instead of one gene, indicates that this source of tolerance to aluminum toxicity differs from those of UFRGS 17 and UFRGS 93605. It is possible, however, that one of these loci is common to all tolerant sources studied here. Unfortunately, due to the variation in the measurement of primary root regrowth from environmental effects and to the size of the F_2 populations examined, this hypothesis could not be accurately tested in this work. Thus, further studies using larger populations and genetic designs to control environmental effects may help to determine these allelic relationships.

Crosses between the sensitive genotypes UFRGS 911715 and UFRGS 93598 did not show discontinuous segregation and most F_2 progeny had from 0.3 to 0.8 mm of primary root regrowth, indicating that these sources have similar genetic constitution. Nevertheless, UFRGS 93598 is the most sensitive genotype that has been identified in our studies and is more affected by Al^{+++} than UFRGS 911715 as can be seen from the means and standard deviations of primary root regrowth of both genotypes in Table 1.

Variation within the fixed genotypes P_1 and P_2 for primary root regrowth and some overlapping of tolerant and sensitive classes were observed, indicating the influence of environmental effects on the expression of this trait. This result shows that, although the methodology used in this work was widely utilized in tolerance assessments in different species (Camargo, 1984; Ferreira *et al*., 1997; Sànchez-Chacòn, 1998), it still needs improvement in assessing oats. On the other hand, it is important to point out that the variation observed in this work was not bigger than that observed by Sànchez-Chacòn (1998). It was possible to distinguish genotypes that were tolerant and sensitive to aluminum. The parents from this study behaved like those described by Sànchez-Chacòn (1998).

Table 2 - Segregation of the F_2 generation from crosses between three tolerant and two sensitive oat genotypes to aluminum toxicity and the Chi-square test for each genetic hypothesis. Agosto, 1998.

Populations UFRGS	Number of Seedlings[1/]		Expected ratio (T:S)	c^2	P - value
	T	S			
17 x 911715	39	6	3:1	3.27	0.07
17 x 93598	22	3	3:1	2.25	0.13
93605 x 911715	46	18	3:1	0.33	0.56
15 x 911715	21	19	9:7	0.23	0.63
15 x 93598	36	33	9:7	0.46	0.49

[1/] S = Sensitive; T = tolerant.

CONCLUSIONS

Tolerance to aluminum toxicity is governed by either one dominant or two epistatic genes in the oat genotypes of different genetic constitution evaluated in this study.

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RESUMO

Herança Genética da Tolerância ao Alumínio em Aveia

A toxicidade do alumínio é um importante fator na limitação dos cultivos em solos ácidos. A melhor compreensão da genética dessa característica auxiliará os programas de melhoramento no desenvolvimento de genótipos tolerantes a esse metal. O presente trabalho foi desenvolvido para estudar a herança da tolerância ao alumínio e estabelecer as relações alélicas entre genes presentes em diferentes genótipos de aveia. Foram avaliados através do recrescimento das raízes primárias submetidas a 20 ppm de alumínio três genótipos tolerantes (UFRGS 15, UFRGS 17 e UFRGS 93605), dois sensíveis (UFRGS 911715 e UFRGS 93598) e as populações segregantes na geração F_2, proveniente do cruzamento entre esses genótipos. Foram identificados um a dois genes segregando para essa característica e as fontes de tolerância existentes possuem constituição genética distinta.

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