RESEARCH ARTICLES
J. Crop Sci. Biotech. 10 (2) : 106 ~ 111

Genetic Distances Among Rice Mutant Genotypes Assessed by AFLP and Aluminum Tolerance - Related Traits

Emilia Malone[1], Mauricio Marini Kopp[1], Gaspar Malone[2], Juliana Severo Castelo Branco[1], Fernando Irajá Félix Carvalho[1], Antonio Costa de Oliveira[1*]

[1]*Plant Genomics and Breeding Center, Eliseu Maciel School of Agronomy, Federal University of Pelotas, P.O. Box 354, 96010-900, Pelotas, RS, Brazil.*
[2]*DON MARIO SEMILLAS, Ruta Nac. 7, Km 208 - C.P 6740, Chacabuco, Provincia de Buenos Aires, Argentina.*

Abstract

Increasing genetic variability with mutagenic agents has been broadly employed in plant breeding because it has the potential to alter one or more desirable traits. In this study, a molecular analysis assessed by amplified fragment length polymorphisms (AFLPs) and a morphological analysis based on seedlings subjected to aluminum stress were compared. Also, an analysis of allelic frequencies was performed to observe unique alleles present in the pool. Genetic distances ranging from 0.448 to 0.953 were observed, suggesting that mutation inducing was effective in generating variability. The genetic distances based on morphological data ranged from 0 (genotypes 22 and 23) to 30.38 (genotypes 15 and 29). In the analysis of allelic frequency, 13 genotypes presented unique alleles, suggesting that mutation inducing was also targeting unique sites. Mutants with good performance under aluminum stress (9, 15, 18, and 27) did not form the same clusters when morphological and molecular analyses were compared, suggesting that different genomic regions may be responsible for their better performance.

Key words: variability, molecular markers, abiotic stress, aluminum to lerance

Introduction

Acid soils having high aluminum concentrations represent a serious problem for the adaptation of many important cereal crops (Echart and Molina 2001). The effects of aluminum in toxic concentrations are critical (Bennet and Breen 1991), becoming a limiting factor for plant growth (Foy 1974). Its damaging effect is expressed initially on roots, which become short and thick with a consequent reduction in root axis elongation (Foy 1976), causing a decrease in nutrient absorption (Freitas et al. 2006; Rheinheimer et al. 1994). Some of the mechanisms by which plants tolerate aluminum have recently been elucidated with the cloning of *ALMT1* (Sasaki et al. 2004). This aluminum activated malate transporter was more highly expressed in the root apices of tolerant genotypes when near-isogenic lines (NILs) were compared. Also, this mechanism seems to be conserved between monocots and dicots (Hoekenga et al. 2006; Magalhaes 2006). Rice has been considered a model for cereal breeding because of its small genome, the availability of genetic and physical maps, and the completion of its genome sequence (Gale and Devos 1998; IRGSP 2005; Izawa and Shimamoto 1996;). Therefore, rice can help to elucidate the understanding of abiotic stress tolerance, shedding light on the cloning of genes linked to aluminum tolerance. Evidence for a homolog in rice was found (Kopp et al. 2006) and its function was detected in rice chromosome 3 by QTL analysis (Nguyen et al. 2003).

Breeding programs have relied on many tools for the identification of genetic variability (Mohammadi and Prasanna 2003). The combination of molecular markers and multivariate analysis has the advantage of simultaneously evaluating many characters for better displaying the genetic dissimilarity (Corbellini et al. 2002; Manifesto et al. 2001; Roy et al. 2004).

Marker assessments of genotype collections have been described for sorghum (Oliveira et al. 1996), barley (Ellis et al. 1997), maize (Ajmone Marsan et al. 1998), wheat (Barret and Kidwell 1998; Vieira et al. 2007), ryegrass (Castro et al. 2003), and rice (Branco et al. 2007; Malone et al. 2006; Morais et al. 1999). Morphological markers, in association with multivariate techniques, are still employed for quantifying genetic distances

* **To whom correspondence should be addressed**
Antonio Costa de Oliveira
E-mail: acostol@terra.com.br
Tel: +055-5332757263

among genotypes in wheat (Roy et al. 2004) and oats (Benin et al. 2003; Marchioro et al. 2003).

The objective of the present work was to estimate the genetic dissimilarity among rice mutant genotypes assessed with AFLP markers and compare to the estimates obtained from differences in morphological traits under aluminum stress.

Materials and Methods

This work was carried out at the Plant Genomics and Breeding Center, Eliseu Maciel School of Agronomy, Federal University of Pelotas, Pelotas, RS, Brazil. Mutant genotypes were obtained from gamma irradiation of cultivar BRS 7 "Taim" seeds, as previously described (Zimmer et al. 2003).

Morphological analyses under aluminum stress were performed on 35 rice mutant genotypes at M_5 generation (Table 1). The original cultivar BRS 7 "Taim" (sensitive) and line 2-52-4 (tolerant) were used as controls, according to previous studies from our group (Freitas et al. 2006). The aluminum dosage used was 15 mg L^{-1}, indicated as the concentration that best discriminated rice genotypes under hydroponics (Freitas et al. 2006). The experimental design used was completely randomized blocks with three replications, and the experimental unit consisted of ten seeds. Treatments were 0 (control) and 15 mg L^{-1} Al^{3+}. The solution pH was adjusted daily (HCl 1N or NaOH 1N), in order to keep it within a 3.9-4.1 range. The variables analyzed were: coleoptile length (CL), primary root length (PRL), first leaf length (FLL), second leaf length (SLL), and first leaf insertion (FLI). Analysis of variance was performed considering a factorial model and Dunnett's test at 5% was used for the comparison of means, using the software SAS (Statistical Analysis System, 2000). The results obtained from morphological analyses were transformed to a phenotypic genetic distance matrix calculated using the distance of Mahalanobis (D^2) using the software GENES (Cruz 1997). A dendrogram was obtained, using the average linkage clustering procedure (UPGMA). The fitting between the distance matrix and the dendrogram was estimated by using a cophenetic correlation coefficient (r) (Sokal and Rohlf 1962) through the software NTSyS pc 2.1 (Rohlf 2000). The average genetic distance was used as a cutoff value for the description of clusters.

For the molecular analyses, ten seed bulks from each genotype were processed for DNA extraction using the CTAB method described by Saghai-Maroof (1984). DNA samples were gel quantified in 0.8% agarose gels stained with Ethidium bromide (5 μg mL^{-1}) under UV light (Sambrook et al. 1989) comparing with Low DNA Mass Ladder (Invitrogen - Life Technologies). AFLP reactions were performed according to the protocol described by the manufacturer (*Life technologies - GIBCO®*). Aproximately 100 ng DNA, from each genotype, was digested with restriction enzymes (*Eco*RI and *Mse*I) for three hours at 37 °C. Specific adaptors were added to the digestion product and incubated for two hours at 20 °C in the presence of T4 DNA Ligase. A 1/10 dilution in TE pH 8.0 was performed and used as a template for the pre-amplification reaction. A new dilution (1/50) was performed and used as stock for the selective amplification reactions. Restriction enzyme digestions, pre-amplifications, and selective amplifications were performed in PTC-100™ Termocycler (MJ Research). Pre-amplifications using primers with one selective base were performed according to the following PCR protocol: denaturation at 94 °C for 30 sec; annealing at 56 °C for 60 sec and extension at 72 °C for 60 sec, carried on for 20 cycles. The final amplification reaction using primers with three selective bases was performed as follows: denaturation at 94 °C for 30 sec; annealing at 65 °C (- 0.7°C /cycle) for 30 sec and extension at 72 °C for 60 sec for 11 cycles, followed by 30 cycles with denaturation at 94 °C for 30 sec; annealing at 56 °C for 30 sec and extension at 72 °C for 60 sec. A total of nine primer combinations were used: C1: *M*-CAA/*E*-AGG; C2: *M*-CTA/*E*-ACA; C3: *M*-CAC/*E*-AAC; C4: *M*-CAG/*E*-ACC; C5: *M*-CAT/*E*-AGC; C6: *M*-CTG/*E*-ACT; C7: *M*-CAA/*E*-AAC; C8: *M*-CAA/*E*-AGC; C9: *M*-CTG/*E*-AGG. The amplification products were separated on 6% polyacrylamide gels under denaturing conditions and silver stained according to Briard et al. (2000).

AFLP bands were scored as presence/absence and were used to estimate the genetic similarity among all genotype pairs. The calculations were performed using the NTSYS pc 2.1 software (Rohlf 2000) using Dice's coefficient (Dice 1945). Genetic similarity was transformed to genetic dissimilarity according to the following equation: $D_{ij}=1-S_{ij}$, where D_{ij}= genetic distance for each genotype pair (i and j) and S_{ij}= genetic similarity for each genotype pair (i and j). Based on the dissimilarity matrix generated, a dendrogram was obtained using the UPGMA (*Unweighted Pair-Group Method with arithmetic Averages*) clustering procedure. The adjustment between the dissimilarity matrix and the dendrogram was estimated by the cophenetic correlation coefficient (r), according to Sokal and Rohlf (1962). The average genetic distance was used as cutoff value to define genotype clusters. To estimate the correlation (association) between the distance matrices obtained from morphological and molecular data, Mantel's test with 1000 permutations was performed (Mantel 1967), using the NTSYS pc 2.1 software (Rohlf 2000). Allele frequencies were obtained from presence/absence of each marker on the evaluated genotypes.

Results and Discussion

Results from the analysis of variance are shown in Table 2. The F test (5%) detected significant effects for genotype, aluminum dose, and genotype vs. dose interaction, with an exception for the dose effect on variables coleoptile length (CL), first leaf length (FLL), and first leaf insertion (FLI). These results indicate that mutation inducing was effective in generating variability for the character aluminum tolerance, since the genotypes presented differential responses when subjected to the treatment levels used in this work.

The clustering analysis based on morphological data displayed genotypes with a range from very similar (22, 23, and 21) to very distinct (26, 29, and 15) responses (Fig. 1). The

cophenetic matrix (r) (Rohlf and Sokal 1962), obtained from comparing the dissimilarity matrix and the dendrogram was 0.79, reflecting the high accuracy by which the dendrogram is displaying the dissimilarity data. A determination coefficient (r^2) of 0.62 was obtained, indicating that 38% of the variation is at random. Based on the average genetic similarity (5.55) as a cutoff value, five clusters can be observed (Fig. 1): one major, two small, and three clusters formed by only one genotype. The major cluster included 27 genotypes (1, 8, 3, 30, 13, 31, 5, 19, 20, 21, 22, 23, 35, 11, 18, 2, 33, 12, 6, 10, 7, 17, 14, 25, 36, 34, and 24) which includes 72.97% of the genotypes (Group I). On the second group, families 9, 28, 27, 16, and 32 presented higher similarity (Group III), a smaller group was formed by families 26 and 37 (Group V). The remaining genotypes (4, 29, and 15) did not cluster with any other genotype, suggesting that mutations generated very dissimilar regions in their genomes. The control genotypes formed different clusters, I for 36 and V for 37, confirming their different morphological responses to aluminum. Based on genetic distance values calculated by the Mahalanobis (D^2) method, the closest genotypes were 22 and 23 with a distance value of 0 and the most distant genotypes were 29 and 15 with a value of 30.38. The genotypes used as control presented a distance value of 3.81.

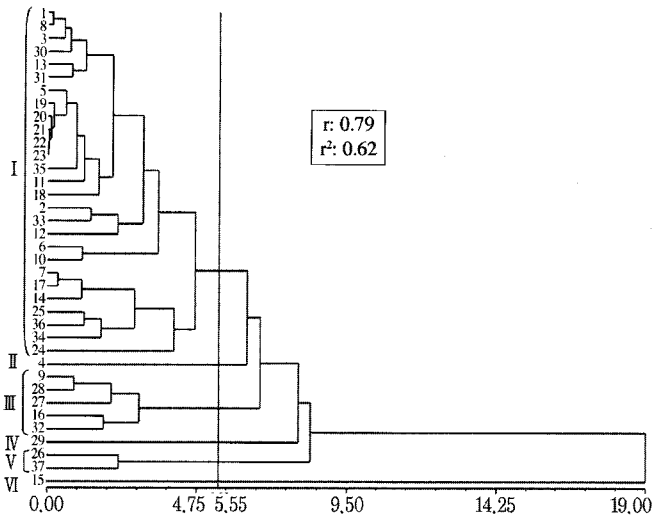

Fig. 1. Dendrogram of 35 mutant plus two control genotypes evaluated at 15 mg/L^{-1} aluminum, obtained from the analysis of morphological data using the Mahalanobis (D^2) genetic distance and UPGMA clustering procedure. The cutoff value used was 5.55. Plant Genomics and Breeding Center, UFPel, Pelotas-RS, 2006.

Table 1. List of rice mutant and control genotypes used for this study. Plant Genomics and Breeding Center, UFPel,Pelotas-RS, 2005.

Number	Composition
1	CGF-Z-M05-435
2	CGF-Z-M05-437
3	CGF-Z-M05-78 ARS1
4	CGF-Z-M05-243
5	CGF-Z-M05-45
6	CGF-Z-M05-188
7	CGF-Z-M05-42
8	CGF-Z-M05-44
9	CGF-Z-M05-79
10	CGF-Z-M05-121 ARS
11	CGF-Z-M05-303 CD
12	CGF-Z-M05-282
13	CGF-Z-M05-22 P
14	CGF-Z-M05-328
15	CGF-Z-M05-62 ARS2
16	CGF-Z-M05-440
17	CGF-Z-M05-436
18	CGF-Z-M05-79 ARS
19	CGF-Z-M05-280
20	CGF-Z-M05-205
21	CGF-Z-M05-260 P1
22	CGF-Z-M05-189
23	CGF-Z-M05-167
24	CGF-Z-M05-417 ARP
25	CGF-Z-M05-336
26	CGF-Z-M05-192
27	CGF-Z-M05-168
28	CGF-Z-M05-31 ARSP
29	CGF-Z-M05-65
30	CGF-Z-M05-59
31	CGF-Z-M05-444 P1
32	CGF-Z-M05-204
33	CGF-Z-M05-41
34	CGF-Z-M05-295
35	CGF-Z-M05-32
36	2-52-4*
37	TAIM*

*36: line 2-52-4; 37: cultivar BRS 7 "Taim"; tolerant and sensitive controls, respectively.

A second clustering analysis based on the morphological data was performed considering only the two variables that were significant in discriminating the genotypes (MRL and SLL). The analysis shows a better display of morphological responses of control and mutant genotypes. From the 35 mutant genotypes, again the most similar were 22 (CGF-Z-M05-167) and 23 (CGF-Z-M05-417 ARP). The cophenetic coefficient value (r) (Rohlf & Sokal 1962) obtained was 0.65. This value is considered moderate for the graphical display of distances. A determination coefficient (r^2) of 0.42 was obtained, indicating that 0.58% of the variation is at random. Based on the average genetic distance (2.53), one can observe the formation of five groups (Fig. 2). One major group, two small groups, and two groups formed by only one genotype were observed. The major group includes 26 genotypes (1, 5, 3, 29, 8, 4, 31, 6, 18, 11, 13, 15, 19, 35, 20, 22, 23, 30, 21, 2, 36, 14, 25, 34, 7, and 17) representing 70.27% (Group I). Group III was formed by genotypes 9, 10, 28, 12, 33, 16, and 32. A small group was formed by genotypes 26 and 37 (Group V). The remaining groups were formed by only one genotype: Group II formed by genotype 24 and Group VI formed by genotype 27. These two genotypes were isolated likely due to a higher dissimilarity caused by a higher number of detectable mutations. Based on the genetic distance values calculated by the method of Mahalanobis, the closest genotypes were 22 and 23 with a zero distance and the most distant genotypes were 26 and 27, showing a distance value of 16.86. The control genotypes clustered in different groups: group I, and group V for the sensitive (37) and tolerant (36) genotypes, respectively. The distance obtained for this genotype pair (sensitive/tolerant) was 2.76.

Based on the above results, it was possible to confirm the similarity among genotypes with good performance in the 15 mg L^{-1} aluminum stress (3, 15, and 18). These genotypes clustered

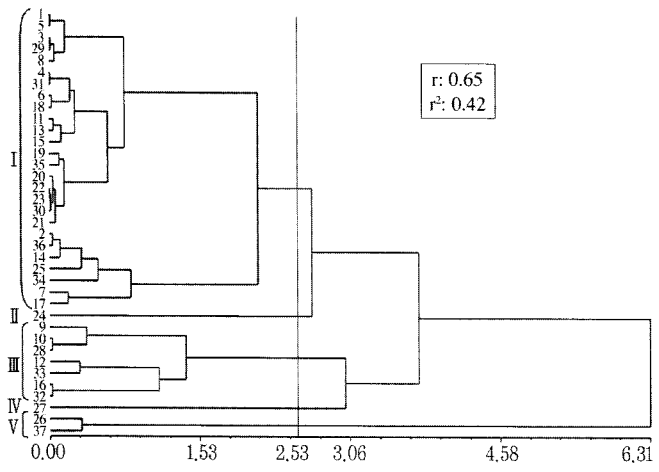

Fig. 2. Dendrogram of 35 mutant plus two control genotypes evaluated at 15 mg/L^{-1} aluminum, obtained from the analysis of morphological data considering only the most informative variables (MRL and SLL) using the Mahalanobis (D^2) genetic distance and UPGMA clustering procedure. The cutoff value used was 2.53. Plant Genomics and Breeding Center, UFPel, Pelotas-RS, 2006.

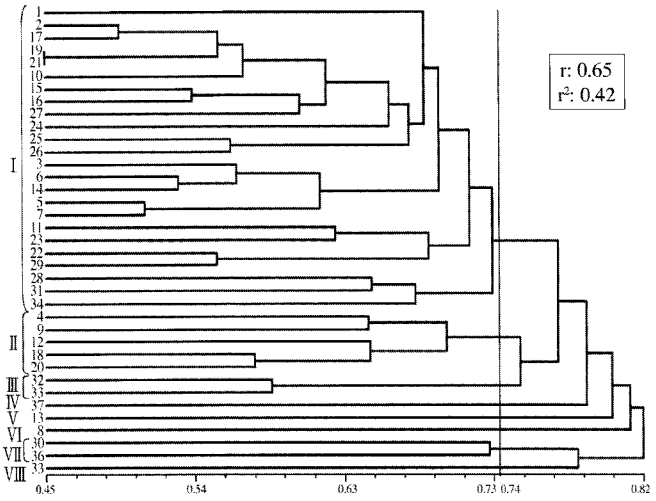

Fig. 3. Dendrogram of 35 mutant plus two control genotypes obtained form AFLP analysis using the DICE's similarity index (Dice, 1945) and UPGMA clustering procedure. The cutoff value used was 0.74. Plant Genomics and Breeding Center, UFPel, Pelotas-RS, 2006.

with the tolerant genotype, showing small dissimilarity values: 0.14 between genotypes 3 and 15; 1.12 between genotypes 3 and 18. The genotypes 15 and 18 showed a dissimilarity value of 0.46. From these three genotypes, two (15 and 18) present unique alleles in the allele frequency analysis, showing the potential of using these genotypes in marker-assisted breeding. The nature of the unique alleles are under investigation. The genotype 3 presented the highest relative performance under the stress for the five variables analyzed. The genotype 15 showed a mean value superior to the tolerant control under stress and together with genotype 18 was highly ranked in the relative performance analysis. The genotype 2 was the genotype that showed the closest distance to the tolerant control (0.026), suggesting a similar response to the stress. The genotype used as sensitive control clustered again with genotype 26, showing a distance of 0.31. Among the genotypes that formed single clusters, 24 was one of those showing unique alleles at the frequency analysis and performed better than the control genotype at 15 mg L^{-1} aluminum.

In the molecular analysis, nine primer combinations were used, amplifying a total of 206 markers, from which 184 (89.32%) were polymorphic among the 35 studied genotypes and 22 (10.68%) were monomorphic. The combinations producing more polymorphic bands were C3, C7, and C6 revealing 30, 26, and 25 polymorphic markers, respectively. The combination C9 was the one that evidenced the smallest number of polymorphic markers with only ten. The high number of polymorphic bands observed for some combinations indicates a great potential of AFLP markers to detect genetic variability present in this collection of genotypes and the high effect of mutation in creating variability. Among the 35 genotypes analyzed molecularly, the closest families were 19 and 21 (Fig. 3). The cophenetic matrix value, obtained from the comparison of similarity matrices and the dendrogram, was 0.65. This value is considered representative and reflects the accuracy of the dendrogram in graphically displaying the dissimilarity among the genotypes. The determination coefficient (r^2) was 42, indicating that 58% of the variation is at random. Based on the average value of genetic distances (0.74) one can observe eight clusters: two major, two small, and four clusters formed by only one family. The major cluster includes 24 genotypes (1, 2, 17, 19, 21, 10, 15, 16, 27, 24, 25, 26, 3, 6, 14, 5, 7, 11, 23, 22, 29, 28, 31, and 34) which represents 64.86% (Group I - Fig. 2). On the second cluster, genotypes 4, 9, 12, 18, and 20 were grouped (Group II - Figure 2). The remaining genotypes did not present a distinctive cluster. Four clusters are represented by only one family (IV, V, VI, and VIII) each, suggesting that the mutation inducing was higher in these genotypes. The cultivar BRS 7 "Taim" (37) which gave origin to the mutant genotypes was isolated in cluster IV, suggesting that all mutant genotypes studied had acquired molecular differences that were detected by the AFLP technique. In the molecular analysis, the control genotypes, as expected, did not cluster together, since genotype 2-52-4 (36) grouped with genotype 30 on cluster VII and the cultivar BRS 7 "Taim" (37) was the only genotype on cluster IV. These two clusters showed a genetic distance of 0.827, indicating that these two genotypes were more dissimilar than the average (0.74). Based on the genetic distance values calculated by DICE's method, the most

Table 2. Summary of analysis of variance, means and coefficient of variation (CV) for the traits coleoptile length (CL), main root length (MRL), first leaf length (FLL), second leaf length (SLL) and first leaf insertion (FLI) of 35 rice M5 genotypes, cultivar BRS 7 "Taim" and line 2-52-4, subjected to two levels of aluminum. Plant Genomics and Breeding Center, UFPel,Pelotas-RS, 2005.

S.V.	D.F.	Mean Squares				
		CL	MRL	FLL	SLL	FLI
Genotype	36	0.418*	16.516*	2.155*	9.180*	6.495*
Dose	1	0.754	1310.79*	0.508	3260*	0.535
Gen x Dose	36	0.253*	13.766*	1.064*	4.912*	3.804*
Error	144	0.079	2.588	0.267	1.647	1.257
Mean		2.073	8.536	4.208	15.108	6.560
C.V.		13.622	18.84	12.295	8.495	17.095

*Significant at 5% probability by the F test.

similar genotypes were 19 and 21 with a distance of 0.448, and the most distant were 33 and 9 showing a distance of 0.953.

Regarding the small association detected between molecular and morphological data (*r*: 0.046), Souza and Sorrells (1991) suggested that this small correlation could be caused by an insufficient representation of the genome when morphological data are used and a lack of association between the loci controlling the morphological traits and the molecular sequences studied. Another factor that contributes for the lack of association comes from the fact that most of the variation detected by molecular markers is non-adaptive and, therefore, not subjected to selection, as opposed to the morphological traits which are subjected to artificial and natural selection pressures, besides the great influence they suffer from environmental factors. In the analysis of allelic frequency, from the total number of markers evaluated (186), the highest frequencies (0.648) were observed for two markers, that were present in 24 out of 37 genotypes and the lowest frequencies (0.027) were observed for 13 markers that were present in one out of 37 genotypes. These unique markers were present in genotypes 9, 12, 13, 15, 16, 18, 17, 20, 22, 23, 25, 27, and 28. From the genotypes that presented unique markers, 27 and 15 had also mean values statistically superior to the tolerant control in the comparison of means test when evaluated at 15 mg L^{-1}aluminum. Although this is just a correlation, it could mean that these markers are potentially good for marker assisted selection for aluminum tolerance. Sequencing efforts are being performed in order to develop co-dominant markers from these sequences for further proof of their potential use.

References

Ajmone Marsan P, Castiglioni P, Fusari F, Kuiper M, Motto M. 1998. Genetic diversity and its relationship to hybrid performance in maize as revealed by RFLP and AFLP markers. Theor. Appl. Genet. 96: 219-227

Barrett BA, Kidwell KK. 1998. AFLP-based genetic diversity assessment among weath cultivars from the Pacific Northwest. Crop Sci. 38: 1261-1271

Benin G, Carvalho FIF, Oliveira AC, Marchioro VS, Lorencetti C, Kurek AJ, Silva JAG. Cruz PJ, Hartwig I, Schmidt DAM. 2003. Comparisons among dissimilarity measures and multivariate statistics as criteria for directing oat crosses (In Portuguese, with English abstract). Cienc. Rural. 33: 657-662

Bennet RJ, Breen CM. 1991. The aluminum signal: new dimensions to mechanisms of aluminum tolerance. Plant Soil 134: 153-166

Branco JSC, Vieira EA, Malone G, Kopp MM, Malone E, Bernardes A, Mistura CM, Carvalho FIF, Costa de Oliveira A. 2007. Irap and remap assessments of genetic similarity in rice. J. Appl. Genet. 48(2): 107-113

Corbellini M, Perenzin M, Accerbi M, Vaccino P, Borghi B. 2002. Genetic diversity in bread wheat, as revealed bi coefficient of parentage and molecular markers, and its relationship to hybrid performance. Euphytica. 123: 273-285

Cruz CD, Regazzi AJ. 1997. Biometrical models applied to plant breeding (In portuguese). UFV: Imprensa Universitária, Viçosa, p.390

Dice LR. 1945. Measures of the amount of ecological association between species. Ecology. 26: 297-307

Echart CL, Molina SC. 2001. Fitotoxicidade do alumínio: efeitos, mecanismo de tolerância e seu controle genético, Cienc. Rural. 31: 531-541

Efron B, Tibshirani RJ. 1993. An introduction to the bootstrap. Chapman and Hall, New York, p. 436

Ellis RP, Mcnicol JW, Baird E, Booth A, Lawrence P, Thomas B, Powell W. 1997. The use of AFLP to examine genetic relatedness in barley. Mol. Breeding. 3: 359-369

Foy CD. 1974. The plant root and its enviroment. Charlottesville: Carson EW: Effects of aluminium on plant growth, p.601-642

Foy CD. 1976. Differential aluminum and manganese tolerances of plant species and varieties in acid soils. Cienc. Cult. 28: 150-155

Freitas F, Kopp M, Sousa R, Dejalma P, Carvalho FIF, Oliveira AC. 2006. Absorption of P, Mg, Ca and P and tolerance of rice genotypes subjected to aluminum stress under hydroponics (In Portuguese, with English abstract). Cienc. Rural. 36: 72-79

Gale MD, Devos KM. 1998. Comparative genetics in the grasses. Proc. Natl. Acad. Sci. 95: 1971-1974

Hoekenga OA, Maron LG, Piñeros MA, Cançado GMA, Shaff J, Kobayashi Y, Ryan PR, Dong B, Delhaize E, Sasaki T, Matsumoto H, Yamamoto Y, Koyama H, Kochian LV. 2006. *AtALMT1*, which encodes a malate transporter, is identified as one of several genes critical for aluminum tolerance in *Arabidopsis*. Proc. Natl. Acad. Sci. USA. 103: 9738-9743

Hongtrakul V, Huestis GM, Knapp SJ. 1997. Amplified fragment length polymorphisms as a tool for DNA fingerprinting sunflower germplasm: genetic diversity among oilseed inbred lines. Theor. Appl. Genet. 95 : 400-407

IRGSP. 2005. The map-based sequence of the rice genome. Nature. 436: 793-800

Izawa T, Shimamoto K. 1996. Becoming a model plant: The importance of rice to plant science. Trends in Plant Sci. 1: 95-99

Magalhaes JV. 2006. Aluminium tolerance genes are conserved between monocots and dicots. Proc. Natl. Acad. Sci. USA 103: 9749-9750

Malone G, Marchioro VS, Zimmer PD, Malone E, Carvalho FIF, Oliveira AC. 2006. AFLP markers partially associated to panicle weight in oat. Magistra. 18: 55-61

Manifesto MM, Schlatter AR, Hopp HE, Dubcovsky J. 2001. Quantitative evaluation of genetic diversity in wheat germplasm using molecular markers. Crop Sci. 41: 682-690

Mantel N. 1967. The detection of disease clustering and a generalized regression approach. Cancer Res. 27: 209-220

Marchioro VS, Carvalho FIF, Oliveira AC, Cruz PJ, Lorencetti C, Benin G, Silva JAG, Schmidt DAM. 2003. Genetic dissimilarity between oat genotypes. (In Portuguese, with English abstract). Ciencia e Agrotecnol. 27: 285-294

Mohammadi SA, Prasanna BM. 2003. Analyses of genetic diversity in crop plants - Salient statistics tools and considerations. Crop Sci. 43: 1235-1248

Morais OP, Silva JC, Cruz CD, Regazzi AJ, Neves PCF. 1999. Genetic divergence among genotypes from the irrigated rice population CNA-IRAT 4. (In Portuguese, with English abstract) Pesq. Agropec. Bras. 34: 217-224

Nguyen BD, Brar DS, Bui BC, Nguyen TV, Pham LN, Nguyen HT. Identification and mapping of the QTLs for aluminum tolerance introgressed from the new source, *Oryza rufipogon* Griff., into indica rice (*Oryza sativa* L.). 2003. Theor. Appl. Genet. 106: 583-593

Oliveira AC, Richter T, Bennetzen JL. 1996. Regional and racial specificities in sorghum germoplasm assessed with DNA markers. Genome. 39: 579-587

Rheinheimer DS, Petry C, Kaminski J, Bartz HR. 1994. Influence of aluminum stress in tobacco plants: I. Effect on the root system, phosphorus and calcium absorption and dry matter yield (In Portuguese, with English abstract). Rev. Bras. Cienc. Solo. 18: 63-68

Rohlf FJ. 2000. NTSYS-pc: numerical taxonomy and multivariate analysis system, version 2.1. Exeter Software

Rohlf FJ, Sokal RR. 1962. Comparing numerical taxonomic studies. Syst. Zool. 30: 459-490

Roy JK, Lakshmikumaran MS, Balyan HS, Gupta PK. 2004. AFLP-based genetic diversity and its comparison with diversity based on SSR, SAMPL, and phenotypic traits in bread wheat. Biochem. Genet. 42: 43-59

Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW. 1984. Ribosomal DNA spacer length polymorphism in barley: Mendelian inheritance, chromosome location and population dynamics. Proc. Natl. Acad. Sci. USA. 89: 1477-1481

Sasaki T, Yamamoto Y, Ezaki B, Katsuhara M, Ju Ahn S, Ryan P, Delhaize E, Matsumoto H. 2004. A wheat gene encoding an aluminum-activated malate transporter. Plant J. 37: 645-653

Sambrook J, Maniatis T, Fritsch EF. 1989. A laboratory Manual. Second edition. Cold Spring Harbor Press, Cold Spring Harbor

Sokal RR, Rohlf FJ. 1962. The comparison of dendrograms by objective methods. Taxon. 11: 30-40

Souza E, Sorrells ME. 1991. Relationships among 70 North American oat germplasms. II. Cluster analysis using qualitative characters. Crop Sci. 31: 605-612

Statistical Analysis System. SAS: Statistical Analysis System-Getting Started with the SAS Learning Edition. 2002. Cary, NC: SAS Institute Inc. 2002. 86p

Vantoai TT, Peng J, St Martin SK. 1997. Using AFLP markers to determine the genomic contribution of parents to populations. Crop Sci. 37: 1370-1373

Vieira EA, Carvalho FIF, Chaves MS, Oliveira AC, Benin G, Hartwig I, Silva JAG, Bertan I, Martins AF, Martins LF. 2007. Virulence variability of *Puccinia coronata* f. sp. *avenae* Isolates Collected in Three Counties from Rio Grande do Sul State, Brazil. Plant Disease. 91: 66-70

Vos P, Hogres R, Bleeker M, Reijans M, Lee T, Hornes M, Fritjers A, Pot J, Peleman J, Kuiper M, Zabeu M. 1995. AFLP: a new technique for DNA fingerprinting. Nucl. Ac. Res. 23: 4407-4414

Zimmer PD, Mattos LAT, Oliveira AC, Carvalho FIF, Magalhães Jr AM, Kopp MM, Freitas FA. 2003. Identification of rice mutants (*Oryza sativa* L.) for agronomical and root system traits. Rev. Bras. Agrocienc. 9: 195-199