Crop Breeding and Applied Biotechnology 7: 59-66, 2007
Brazilian Society of Plant Breeding. Printed in Brazil

Organic acid tolerance in M_3 families of oat mutants

Mauricio Marini Kopp[1*], Jefferson Luís Meirelles Coimbra[2], Viviane Kopp da Luz[1], Rogério Oliveira de Sousa[3], Fernando Irajá Félix de Carvalho[1], and Antonio Costa de Oliveira[1]

Received 24 April 2006

Accepted 12 May 2006

ABSTRACT - *The objective of this study was to evaluate the response of 30 mutant oat lines to the phytotoxic action of organic acids produced in the soil. In a hydroponic system with three treatments (0,7 and 10 mM) the variables root (CR) and shoot (CPA) length; number of roots (NR) and root dry matter (MSR) were measured. Only CPA at a dose of 7 mM was not significant in the ANOVA. The clustering analysis by Tocher's method indicated the 7 mM dose as the most appropriate for stress evaluations. A relative yield analysis showed that the variable CR was most affected by the phytotoxic action followed by MSR. For all genotypes, the best adjustment to the regression was linear, presenting high R^2 values. Eight genotypes (23.33 %) were classified as tolerant, indicating that mutation induction can generate genetic variability for this character.*

Key words: phytotoxicity, *Avena sativa*, regression analysis, gamma-ray, mutation.

INTRODUCTION

Oat is an important alternative for crop rotation and plays a fundamental role in no tillage cropping systems owing to its capacities of recovering soil features (Menezes et al. 2001). Oat is therefore an excellent crop for predominantly monoculture regions, such as the southern part of Rio Grande do Sul state, Brazil, where irrigated rice followed by native pastures prevails (Porto 1997). The Southern Region as a whole covers an area of 6.8 million ha of hydromorphic (albaqualf) soils (Pinto et al. 2004). On such soils, the development and production of most cultivated species is impaired by the lack of natural soil drainage. Efficient drainage strategies with the goal of introducing crop rotation procedures have been developed. However, as long as no drainage procedure is applied, water excess in the soil provokes an anaerobic environment, favoring the formation of toxic substances (Camargo et al. 2001). The O_2 supply in the soil of completely flooded areas is about 10,000 times slower than under dry conditions (Ponnamperuma 1972), leading to an increase in anaerobic microorganisms which derive energy from organic matter for fermentative reactions (Lynch and Elliott 1983). During this process, intermediate products such as low molecular weight, short-chain alyphatic organic acids (acetic, propionic and butyric) are formed. These acids generally occur at concentrations between 0.1 and 14 mM at a ratio of 6:3:1 for acetic, propionic and butyric, respectively. A maximum peak is reached a few days after flooding, with a potentially toxic effect to the plants (Sousa and Bortolon 2002).

[1] Centro de Genômica e Fitomelhoramento, Departamento de Fitotecnia, Universidade Federal de Pelotas (UFPel), 96.010-900, Pelotas, RS, Brasil. *E-mail: marinikopp@gmail.com
[2] Departamento de Fitotecnia, Universidade do Estado de Santa Catarina (UDESC), 88.520-000, Lages, SC, Brasil
[3] Departamento de Solos, UFPel

Organic acid toxicity is expressed at the early stages of the crop in decreased germination, root growth and seedling weight and height and in more severe cases, the damage can still be sensed at more advanced stages, affecting tillering, nutrient absorption and grain yield (Camargo et al. 2001).

One condition that must be fulfilled to include oat in lowland crop rotations is the use of organic acid tolerant genotypes. Since no selection for lowland adaptation has been efficiently applied it is essential to increase the genetic variability, which can be achieved by mutation induction (Tulmann Neto et al. 1995 a, Tulmann Neto et al. 1995 b; Pandini et al. 1997). Mutation induction has contributed to increase the worldwide grain production and to produce mutation stocks for many traits (Coimbra et al. 2004). Currently, mutants are essential tools for studies involving variability, function, regulation and gene action, mainly due to the new technologies available (Maluszynski et al. 1998).

Mutation induction has been used to increase the frequency of spontaneous mutations and can be obtained by either chemical agents such as alkylating compounds or physical agents such as gamma rays (Ahloowalia and Maluszynski 2001). Independently of the method in use, the determination of variability is an important and decisive step which can be cumbersome when the traits are hard to measure, as in the case of root-related traits.

Genotype evaluation techniques were developed in three environments: i) under field conditions; ii), under semi-controlled greenhouse conditions and iii) with the use of nutrient solutions under controlled laboratory conditions (Duncan and Baligar 1990). Although the evaluation of genotypes under artificial environments does not take real field selection pressures into account (Duncan and Baligar 1990), selection based on field trials compiles data of a large number of uncontrolled variables, such as the differential responses to abiotic or biotic stresses (Wright 1989). In the case of cultivated grasses, studies show the significant correlations between parameters obtained in field assays and artificial environments, with soil or nutrient solution (Bilinski and Foy 1987).

The goal of this study was to evaluate the variability in the response of gamma ray-induced oat mutant genotypes to organic acids. Moreover, the appropriate acid concentration and response variable to evaluate mutant families was determined under hydroponic conditions aiming to simulate the phytotoxic action of the major organic acids formed during the anaerobic decomposition in the soil.

MATERIAL AND METHODS

This study was performed in the Laboratório de Di-haplóides e Hidroponia of the Centro de Genômica e Fitomelhoramento (CGF), Faculdade de Agronomia Eliseu Maciel, Universidade Federal de Pelotas (UFPel) and at the Centro Regional de Oncologia (UFPel), in Pelotas, state of Rio Grande do Sul, Brazil.

Oat seeds of cultivar UFRGS 14 were gamma ray-treated (^{60}Co) with an Eldorado 78 apparatus. The energy yield for a field of 30 x 30 cm at 25 cm from the source was 37.9 cGy (centigrays) min^{-1}. To determine the best dosage, a preliminary study with varying doses was performed to evaluate the effect of ^{60}Co ionizing radiation on seed germination. In this study, six treatments were used as follows: 0, 50, 100, 200, 400, and 600 Gy. Two hundred seeds were used in each treatment. They were immersed in distilled water for one hour right after irradiation and then rinsed under flowing tap water for an hour. A dosage of 400 Gy was established to induce mutation in the oat cultivar UFRGS 14.

A total of 3500 seeds pre-soaked as described above were subjected to a dosage of 400 Gy to form the M_1 population. These seeds, after being rinsed for an hour under tap water, were sown on the experimental field and bulk harvested. Seeds from the M_2 generation were placed on nets covering the surface of a hydroponic solution without any stress treatment for an evaluation of the root morphological traits and families formed from individual plants. The putative mutants were labeled and transferred to the soil for the production of M_3 seeds. In this generation, agronomic traits of shoots, yield and cycle were evaluated. Seeds from individual M_2 plants were harvested separately and the putative mutations identified. Table 1 presents some examples of morphological characterization of M_2 families.

To evaluate the response to organic acids, 30 M_3 oat families were picked from the CGF genepool based on phenotypes evaluated in the M_2 generation (Table 1). Seeds were germinated in growth chambers at 25 $\pm$ 1 ^{o}C for 72 hours on water-soaked filter paper. Ninety 90

Table 1. Morphological characterization of 30 M_3 oat families used to study tolerance to organic acid toxicity

Family	Description
1	CGF-M_3-Av-2 – Absence of seminal roots
2	CGF-M_3-Av-9 – Absence of seminal roots
3	CGF-M_3-Av-10 – Absence of seminal roots
4	CGF-M_3-Av-17 – High pilosity of the main root
5	CGF-M_3-Av-29 – Nodule presence on the roots
6	CGF-M_3-Av-31 – Excess of seminal roots
7	CGF-M_3-Av-40 – Main root with excess of secondary roots
8	CGF-M_3-Av-41 – Main root with excess of secondary roots
9	CGF-M_3-Av-46 – Main root with excess of secondary roots
10	CGF-M_3-Av-50 – Non-uniform root growth
11	CGF-M_3-Av-55 – Excess of seminal roots
12	CGF-M_3-Av-57 – Thick main root
13	CGF-M_3-Av-62 – Scarce roots
14	CGF-M_3-Av-73 – Excess of seminal roots
15	CGF-M_3-Av-130 – Excess of seminal roots
16	CGF-M_3-Av-133 – Excess of seminal roots
17	CGF-M_3-Av-139 – Absence of seminal roots and high pilosity on the main root
18	CGF-M_3-Av-178 – Absence of seminal roots
19	CGF-M_3-Av-183 – Excess of seminal roots
20	CGF-M_3-Av-192 – Absence of seminal roots
21	CGF-M_3-Av-232 – Excess of seminal roots
22	CGF-M_3-Av-244 – Excess of seminal roots
23	CGF-M_3-Av-245 – Excess of seminal roots
24	CGF-M_3-Av-286 – Excess of seminal roots
25	CGF-M_3-Av-307 – Excess of seminal roots
26	CGF-M_3-Av-308 – Excess of seminal roots
27	CGF-M_3-Av-309 – Excess of seminal roots
28	CGF-M_3-Av-700 – Broad flag-leaf sheath
29	CGF-M_3-Av-701 – Late cycle
30	CGF-M_3-Av-702 – Early cycle

UFRGS 14 = Homozygous Cultivar

similarly sized seeds of each family were sampled and husked before germination. After germination, 45 seeds with similar root length (~5mm) were transferred to a hydroponic culture. The experimental design consisted of complete randomized blocks with three replications and the experimental unit of five seeds per replication.

The hydroponic system consisted of 5.5 L plastic pots covered with nylon mesh. The net served as support for the seeds, allowing the roots to grow towards the nutrient solution. The pots were kept in a large water bath tank at 25 ± 1 °C, artificially lighted and aired for oxygen supply to promote root growth.

The nutrient solution contained: 4 mM Ca $(NO_3)_2$, 2 mM $MgSO_4$, 4 mM KNO_3, 0.435 mM $(NH4)_2SO_4$, 0.5 mM KH_2PO_4, 10 µM H_3BO_3, 0.10 µM $NaMoO_4$, 30 µM NaCl, 0.8 µM $ZnSO_4$, 0.3 µM $CuSO_4$, 2 mM $MnSO_4$, 10 µM Fe SO_4 + Na (Camargo and Oliveira 1981).

The treatments consisted of three organic acid concentrations. Each treatment received a mixture of the three major acids formed by the anaerobic fermentation in the soil: acetic, propionic and butyric acid, at a ratio of 6:3:1, respectively. The concentrations were 0 (control); 7 mM and 10 mM of acid mixture added to the nutrient solution. The pH was adjusted to 4.7 with either 1N HCl or 1N NaOH and monitored daily since the pH of nutrient solutions in experiments with organic acids is variable and interferes with acid toxicity (Rao and Mikkelsen 1977a).

The plantlets were grown in the hydroponic culture with treatments for 15 days. After this period, they were collected and the following traits evaluated: root (RL) and shoot length (SL) in cm, root number (RN) and root dry matter (RDM) in mg, weighed on a precision scale after drying to constant weight in an air circulation incubator at 60 °C. Results obtained by Sousa and Bortolon (2002) in rice indicate that the variables root and shoot growth were the most responsive to organic acid toxicity.

The data were subjected to variance and regression analyses (Steel and Torrie 1980) using SAS software (SAS 2002). To verify the dissimilarity among families, three dissimilarity matrices were obtained based on Mahalanobis' generalized distance. A clustering procedure for each concentration was performed according to Tocher's method, using software Genes (Cruz 2001).

RESULTS AND DISCUSSION

The results of the analysis of variance (Table 2) indicated significance (α = 5%) for the variables by the F test, except for the effects of family x treatment interaction (RDM) and treatment (RN).

The relative performances of the variables RL, RN, SL and RDM for each genotype and each dose as well as the average for each character are shown in Table 3. The lowest relative performances were observed for variable RL, suggesting that this variable was most affected by organic acid toxicity in the tested oat families. The results agree with those obtained by Rao and Mikkelsen (1977b) who observed more expressive reductions in the length of rice roots under organic acid stress. RDM also presented a lower relative performance as the acid concentrations increased. Physiological symptoms related to organic acid toxicity in rice are due to cell wall degradation, inhibition

Table 2. Summary of analysis of variance, means and coefficient of variation (C.V.) for the characters: root length (RL), root number (RN), shoot length (SL) and root dry matter (RDM) of 30 M_3 oat families and cultivar UFRGS 14, studied in nutrient solution with 3 organic acid concentrations

Sources of variation	df	Mean Squares			
		RL	RN	SL	RDM
Family	30	1.097*	1.028*	10.752*	4.431*
Treatment	2	533.531*	2.663	1.349.569*	622.075*
Family x Treatment	60	0.635*	0.849*	7.166*	1.566
Residue	180	0.193	0.234	3.087	1.167
Mean		4.126	3.520	16.489	5.494
C.V.		10.647	13.731	10.656	19.667

*Significant by the F test (α= 5%)

Table 3. Relative performance (%) of characters root length (RL), root number (RN), shoot length (SL) and root dry matter (RDM), for two levels of treatments compared to the control

Family	Relative performance (%)*							
	7 mM				10 mM			
	RL	RN	SL	RDM	RL	RN	SL	RDM
1	56.48	118.30	79.95	97.37	24.51	112.90	60.16	34.83
2	54.56	121.67	79.19	59.55	34.97	139.56	76.43	42.50
3	46.53	138.02	74.96	86.08	30.00	134.09	60.39	59.07
4	50.73	109.79	70.64	62.99	24.48	123.84	51.01	30.10
5	47.56	118.92	81.27	63.90	26.59	153.40	62.83	49.03
6	49.88	109.74	82.80	59.57	28.87	97.69	57.76	54.42
7	47.38	123.64	89.02	49.62	32.81	104.75	64.19	41.43
8	38.88	133.40	88.33	58.68	25.11	126.94	62.83	25.44
9	50.51	92.52	82.41	61.58	34.93	115.47	68.20	30.99
10	40.32	93.25	89.01	58.73	32.50	92.53	67.65	42.40
11	56.03	113.73	75.59	63.11	18.49	39.22	31.52	19.32
12	53.87	129.54	74.01	49.85	22.85	32.68	34.81	35.47
13	45.42	123.07	76.26	44.01	31.27	134.59	74.80	23.90
14	41.95	125.38	78.76	64.58	34.39	133.67	70.66	41.84
15	46.52	95.02	79.00	27.59	28.00	110.22	67.14	20.70
16	30.26	101.97	69.58	78.57	18.84	122.34	60.64	44.71
17	52.92	81.20	81.47	62.13	29.70	108.08	72.59	36.04
18	55.24	110.15	87.48	60.62	34.27	113.06	69.09	35.45
19	51.70	104.44	81.33	49.94	32.64	98.89	55.77	48.50
20	44.76	115.74	71.81	67.38	29.17	99.07	58.44	36.05
21	49.57	111.61	65.38	57.46	36.22	117.50	46.06	28.02
22	47.88	130.82	77.47	62.47	28.54	140.37	53.85	41.99
23	61.61	119.62	73.82	66.25	32.39	138.53	59.97	42.26
24	52.52	112.69	70.11	57.83	37.24	133.40	59.06	33.07
25	68.04	102.75	86.10	60.62	42.68	108.17	69.14	35.30
26	57.70	108.93	90.97	66.66	46.67	114.41	79.75	29.38
27	53.62	107.94	87.47	72.23	43.37	103.70	75.29	42.48
28	63.75	102.67	88.68	68.20	52.31	101.67	70.71	58.75
29	30.33	96.50	90.87	66.32	25.17	80.93	71.68	59.15
30	51.46	90.83	100.54	56.34	44.43	87.01	69.82	31.02
UFRGS-14	54.26	110.88	79.09	82.04	35.91	100.15	73.54	41.61
Mean	50.07	111.44	80.75	62.65	32.24	110.28	63.09	38.56

* Relative performance based on the absolute value of the control treatment (dose 0 mM)

of respiratory functions and a consequent decrease of cell division in roots that are in direct contact with the acids, explaining the decrease in root growth and dry matter accumulation (Armstrong and Armstrong 2001).

Root number (RN) increased its relative performance as described by Armstrong and Armstrong (2001), who reported decreased growth of adventitious roots in rice, promoting calli formation at the coleoptile basis and increasing the number of lateral roots under organic acid stress.

SL presented a high relative performance (63.09 %), in contradiction to results reported by Camargo et al. (1993), who stated a much lower relative performance (47%). This result may be an expression of the nutrient availability in early seedling stages when the roots developed less and storage compounds were accumulated in the shoot, leading to a decrease in the response to organic acid toxicity.

To determine the appropriate acid concentration for tolerance studies in oat mutant families, Tocher's clustering was performed, which is based on Mahalanobis' generalized distance (Table 4). The concentration that allows a genotype distribution across the highest possible number of groups is the most indicated for tolerance studies, because it roughly represents the genetic variability for the target character. The number of clusters observed at 7 mM was eight, while only five clusters were formed at 10mM. Intermediate concentrations to these two could enhance the results while concentrations above 10 mM would damage the seedlings considerably, decreasing the number of formed clusters.

Table 4. Grouping of 30 M_3 oat families and cultivar UFRGS 14 using Tocher's method based on Mahalanobis' generalized distance, considering the characters root length (RL), root number (RN), shoot length (SL), and root dry matter (RDM) evaluated in nutrient solution at three organic acid concentrations

Dose	Group	Family
0 mM	1	18-21-2-23-25-24-20-5-22-19-26-4-30
		7-1-6-17-27-16-3-31-15-12-8-13-29-14
	2	11-28-9
	3	10
7 mM	1	26-27-17-18-30-22-4-19-5-21-2-23-24
		6-20-7
	2	3-31-11-13-12-9-14-10
	3	16-29
	4	8
	5	15
	6	28
	7	1
	8	25
10 mM	1	20-22-19-7-23-24-5-18-2-17-4-1-21-25
		27-30-26-6-16-15-8
	2	11-12
	3	13-31-14-9-3-10
	4	29
	5	28

As observed above, the variable with the best response in the toxicity evaluation was RL. Based on this result, a regression was obtained for the families regarding this variable (Table 5), with the aim of observing the response of each family to the respective acid doses. The analysis of the family responses to different doses suggests that all families adjusted to a linear regression model. Besides, the significance ($P = 0.05$) of determination coefficients (R^2) for all regressions indicates that the root growth of all families was reduced as the doses increased. Growth reductions in the families 1, 2, 4, 7, 11, 23 and 25 were least expressive, according to the regression coefficients (b), since these were not significantly affected by the effects of doses when compared to the zero value by the t test. Camargo and Ferreira (1992) used a similar method for the study of wheat cultivar tolerance to Mn to discriminate tolerant and susceptible genotypes.

Some tolerant families presented morphological similarities in the roots (Table 1): an absence of seminal roots (1 and 2) and an increase in number of seminal roots (11, 23 and 25). Other tolerant families showed high pilosity (4) and excess of secondary roots (7). These different relations suggest that perhaps different mutations are associated with organic acid tolerance, which requires further investigations.

The root growth in the remaining families presented a profound reduction in function of the applied organic acid doses of, i.e., they presented significant values for the regression coefficient, indicating a higher sensitivity to phytotoxicity.

Comparing the data in Table 3 with the parameters obtained from the regression equations (Table 5), it can be concluded that the families presenting regression coefficients (b) with values significantly different from zero were the families with lowest relative performance, i.e., their roots were significantly shorter than in the control (0 mM dose). Some families had a low relative

Table 5. Regressions (y), regression coefficient (b), determination coefficient (R^2) and classification of 30 M_3 oat families and cultivar UFRGS 14, evaluated in nutrient solution at three organic acid concentrations for the variable root length (RL)

Families	Regression (y)	Coefficient		R^2	Classification*
		b	Prob > t		
1	6.28 – 0.19 x	- 0.19	0.5385	0.89	Tolerant
2	6.23 – 0.40 x	- 0.40	0.1234	0.91	Tolerant
3	7.26 – 0.66 x	- 0.66	0.0142	0.96	Sensitive
4	6.38 – 0.35 x	- 0.35	0.1762	0.93	Tolerant
5	6.60 – 0.52 x	- 0.52	0.0056	0.98	Sensitive
6	6.70 – 0.49 x	- 0.49	0.0080	0.98	Sensitive
7	6.72 – 0.64 x	- 0.64	0.0554	0.90	Tolerant
8	7.22 – 0.83 x	- 0.83	0.0055	0.96	Sensitive
9	7.56 – 0.62 x	- 0.62	0.0422	0.93	Sensitive
10	7.69 – 0.97 x	- 0.97	0.0002	0.99	Sensitive
11	7.38 – 0.14 x	- 0.14	0.2100	0.99	Tolerant
12	7.08 – 0.28 x	- 0.28	0.0416	0.99	Sensitive
13	7.43 – 0.74 x	- 0.74	0.0017	0.98	Sensitive
14	7.29 – 0.90 x	- 0.90	0.0006	0.98	Sensitive
15	7.19 – 0.62 x	- 0.62	0.0003	0.99	Sensitive
16	7.09 – 1.00 x	- 1.00	0.0001	0.99	Sensitive
17	6.34 – 0.38 x	- 0.38	0.0079	0.98	Sensitive
18	6.23 – 0.37 x	- 0.37	0.0052	0.98	Sensitive
19	6.62 – 0.48 x	- 0.48	<0.0001	0.99	Sensitive
20	6.53 – 0.64 x	- 0.64	<0.0001	0.99	Sensitive
21	6.16 – 0.56 x	- 0.56	0.0030	0.97	Sensitive
22	6.52 – 0.53 x	- 0.53	0.0017	0.98	Sensitive
23	6.32 – 0.15 x	- 0.15	0.3766	0.95	Tolerant
24	6.49 – 0.52 x	- 0.52	0.0102	0.96	Sensitive
25	6.17 – 0.11 x	- 0.11	0.6791	0.84	Tolerant
26	6.38 – 0.49 x	- 0.49	0.0005	0.98	Sensitive
27	6.43 – 0.57 x	- 0.57	0.0004	0.98	Sensitive
28	7.41 – 0.45 x	- 0.45	<0.0001	0.99	Sensitive
29	7.44 – 1.17 x	- 1.17	<0.0001	0.99	Sensitive
30	6.47 – 0.66 x	- 0.66	<0.0001	0.99	Sensitive
UFRGS 14	7.30 – 0.50 x	- 0.50	0.0002	0.99	Sensitive

* Based on the significance of regression coefficients (b) by the t test at 5 % error probability

performance and were scored as tolerant while others with high relative performance were scored as sensitive. This situation can be explained by the inclusion of a residue from regression coefficient values when compared to the zero value in the t test. The inclusion of residues in the estimates of statistical parameters is essential to obtain the real effects the experiment aims at.

Cultivar UFRGS 14 was considered sensitive to organic acid toxicity at all concentrations. On the other hand, seven of the 30 evaluated mutant families were tolerant, representing 23.33 % positive responses, suggesting that mutation induction with gamma rays (^{60}Co) at the dose 400 Gy provided high indices of establishment of mutant families tolerant to organic acid toxicity.

CONCLUSIONS

The variables root length (RL) and root dry matter (RDM) are the most responsive to organic acid toxicity and a 7 mM concentration of organic acid mixture of acetic, propionic and butyric acids at a 6:3:1 ratio, respectively, is suitable for the investigation of organic acid tolerance in oat mutant families.

Gamma-ray-induced mutation (^{60}Co) at 400 Gy is efficient to generate the necessary genetic variability for the selection of tolerant genotypes among oat mutant families, resulting in 23.33% positive mutants regarding tolerance to acetic, propionic and butyric acids in oat.

Tolerância a ácidos orgânicos em famílias mutantes M_3 de aveia

RESUMO - *O objetivo deste trabalho foi avaliar a resposta de 30 famílias mutantes de aveia a ação fitotóxica dos ácidos orgânicos produzidos no solo. O trabalho foi desenvolvido em sistema de hidroponia com 3 tratamentos (0, 7 e 10 mM). As variáveis mensuradas foram comprimento de raízes (RL) e parte aérea (SL), número de raízes (RN) e matéria seca de raízes (RDM). Foram procedidas análise de variância, onde apenas SL na dose de 7 mM não revelou significância; análise de agrupamento de Tocher, demonstrando que a dose de 7 mM se mostrou mais eficiente na avaliação do rendimento relativo, onde a variável RL foi a mais afetada pela ação fitotóxica seguida por RDM; e análise de regressão. Para todos os genótipos o melhor ajuste de regressão foi linear, apresentando valores de R^2 elevados. Oito genótipos (23,33 %) foram classificados como tolerantes, indicando que a indução de mutação pode gerar variabilidade genética para o caráter.*

Palavras-chave*:* fitotoxidez; *Avena sativa*; análise de regressão; raios-gama; mutação.

REFERENCES

Ahloowalia BS and Maluszynski M (2001) Induced mutations: a new paradigm plant breeding. **Euphytica 118**: 167-173.

Armstrong J and Armstrong W (2001) Rice and Phragmites: effects of organic acids on growth, root permeability, and radial oxygen loss to the rhizosphere. **American Journal of Botany 88**: 1359-1370.

Bilinski JJ and Foy CD (1987) Differential tolerances of oat cultivars to aluminum in nutrient solutions and in acid soils of plant. **Journal of Plant Nutrition 10**: 129-141.

Camargo CEO and Ferreira AWP (1992) Tolerância de cultivares de trigo a diferentes níveis de manganês em solução nutritiva. **Pesquisa Agropecuária Brasileira 27:** 417-422.

Camargo OCE and Oliveira OF (1981) Tolerância de cultivares de trigo a diferentes níveis de alumínio em solução nutritiva e no solo. **Bragantia 49**: 21-23.

Camargo FA, Santos GA and Rossielo ROP (1993) Efeito dos ácidos acético e butírico sobre o crescimento de plântulas de arroz. **Pesquisa Agropecuária Brasileira 28:** 1011-1018.

Camargo FA, Zonta E, Santos GA and Rossielo ROP (2001) Aspectos fisiológicos e caracterização de toxidez a ácidos orgânicos voláteis em plantas. **Ciência Rural 31**: 523-529.

Coimbra JLM, Carvalho FIF and Oliveira AC (2004) Genetic Variability induced by chemical and physical mutagenic agents in oat genotypes. **Crop Breeding and Applied Biotechnology 4**: 48-56.

Cruz CD (2001) **Genes: Aplicativo computacional em genética e estatística**. Editora UFV, Viçosa, 642p.

Duncan RR and Baligar VC (1990) Genetics, breeding, and physiological mechanisms of nutrient uptake and use efficiency: an overview. In: Baligar VC and Duncan RR (eds.) **Crops as Enhancers of Nutrient Use**. Academic Press, San Diego, p.3-35.

Lynch JM and Elliott LF (1983) Minimizing the potential phytotoxicity of wheat straw by microbial-degradation. **Soil Biology & Biochemistry 15**: 221-222.

Maluszynski M, Ahloowalia A and Ashri A (1998) Induced mutations in rice breeding and germplasm enhancement. In: IRRI (ed.) **Session of the International Rice Commission**. Cairo, p.7-9.

Menezes VG, Mariot CHP, Lopes MCB, Silva PRF and Teichmann LL (2001) Semeadura direta de genótipos de arroz irrigado em sucessão a espécies de cobertura de inverno. **Pesquisa Agropecuária Brasileira 36**: 1107-1115.

Pandini F, Carvalho FIF and Barbosa Neto JF (1997) Plant height reduction in populations of triticale (*X triticosecale* Wittmack) by induced mutations and artificial crosses. **Brazilian Journal of Genetics 20**: 483-488.

Pinto LFE, Laus JA and Pauletto EA (2004) Solos de várzea no sul do Brasil. In: Gomes AS and Magalhães Jr AM (eds.) **Arroz irrigado no sul do Brasil**. Embrapa-Informação Tecnológica, Brasília, p. 75-95.

Ponnamperuma FM (1972) The chemistry of submerged soils. **Advances in Agronomy 24**: 29-96.

Porto MP (1997) Método de seleção de plantas de milho para tolerância ao encharcamento do solo. **Pesquisa Agropecuária Gaúcha 3**: 187-190.

Rao DN and Mikkelsen DS (1977a) Effect of acetic, propionic, and butyric acids on young rice seedlings growth. **Agronomy Journal 69**: 923-928.

Rao DN and Mikkelsen DS (1977b) Effect of acetic, propionic, and butyric acids on rice seedlings growth and nutrition. **Plant and Soil 47**: 323-334.

Sousa RO and Bortolon L (2002) Crescimento radicular e da parte aérea do arroz (*Oryza sativa* L.) e absorção de nutrientes em solução nutritiva com diferentes concentrações de ácido acético. **Revista Brasileira de Agrociência 8**: 231-235.

SAS (2002) **SAS: Statistical Analysis System - getting started with the SAS learning edition**. SAS Institute Inc., Cary, 86p.

Steel RGD and Torrie JH (1980) **Principles and procedures of statistics**. McGraw-Hill, New York, 633p.

Tulmann Neto A, Camargo CEO, Alves MC, Santos RR and Freitas JG (1995a) Indução de mutação visando obtenção de resistência a doenças na cultivar de trigo IAC-24. **Pesquisa Agropecuária Brasileira 30**: 497-504.

Tulmann Neto A, Peixoto T, Alves MC, Oliveira JCV, Menten JOM and Athayde M (1995b) Indução de mutação na cultivar de soja IAC-8 visando à obtenção de precocidade. **Pesquisa Agropecuária Brasileira 30**: 237-244.

Wright RJ (1989) Soil aluminum toxicity and plant growth. **Communications in Soil Science and Plant Analysis 20**: 1479-1497.