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Effect of Propionic Acid in the Germination of Rice Genotypes

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Abstract

The objective of this work was to evaluate the germination of 12 rice genotypes under propionic acid stress, a phytotoxic compound produced in low drainage soils with high organic matter content. The tests were conducted with the first count of germination (PCG) and germination (G) of the genotypes subjected to 0, 3, 6, and 9 mM propionic acid concentrations. The seeds of each genotype were placed in germitest paper pre-soaked in treatment solutions forming individual bags. The germination was performed at 25 °C and the counts were carried out at 7 (PCG) and 14 days (G). A factorial random block design was performed with four replications of 50 seeds per genotype. Our study revealed that doses up to 9 mM propionic acid in the pre-soaking solution were efficient for genetic variability studies involving the character germination in rice; genetic variability for germination was detected in the collection of rice genotypes when subjected to propionic acid toxic effects. The genotypes Guichow, Dawn, and Toride-1 showed germination stability when subjected to increasing levels of propionic acid, and genotypes originated from irrigated system cultivation performed better when subjected to propionic acid stress. These three genotypes will be a good biological material to for enhance the resistance to phytotoxic compounds in rice.

Key words: abiotic stress, organic acids, Oryza sativa, phytotoxicity.

Introduction

Rice is one of the three major cereals in the world economy. Brazil is the major rice producer outside Asia reaching 1.86% of the world's production. Within Brazil, the Rio Grande do Sul State is responsible for over 50% of the rice produced in Brazil (Gomes and Magalhães Jr. 2004). The importance of rice goes beyond its social and economic importance having become a model species (Devos and Gale 2000; IRGSP 2005). Rice breeding has achieved a considerable increase in productivity. However, breeders are facing greater challenges every year

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hydromorphic soils, representing 20% of the State's total area (Pinto et al. 2004). In these soils, the majority of cultivated species have their development impaired due to low soil drainage and hypoxic conditions (Ponnamperuma 1972). Since Antonio Costa de Oliveira

in irrigated rice crops, a prolonged water layer is kept above the soil, the O2 present is consumed, and the anaerobic microorgan-

shortage of land. On the other hand, lower genetic gains are obtained every year due to a narrowing adapted gene pool used by breeders. Fortunately, the advances in molecular techniques allow geneticists and breeders to better discriminate genotypes regarding traits such as grain yield components, and biotic and abiotic stress resistance (Kahl and Lavi 2001; Sreenivasulu et al. 2007; Yang et al. 2000). The southern region of Brazil has over 6.8 million hectares of

caused by increases in water usage by urban populations and a

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ism's activity leads to the production of a series of toxic intermediates, including aliphatic short-chain organic acids (acetic, propionic, and butyric), that occur in the range of 0.1 to 14 mM (Angeles et al. 2005; Camargo et al. 2001; Gotoh and Onikura 1971) and a ratio of 6:3:1, respectively (Bohnen et al. 2005).

Direct tillage and minimal tillage seeding systems used for the irrigated rice crop tend to increase the amount of plant residues on the soil surface, forming higher levels of short-chain organic acids, which can limit growth and yield in the rice crop cultivated under these conditions (Johnson et al. 2006). The irrigated rice crop provokes anaerobic conditions, aggravating the effects of no tillage conditions. In these conditions, a low carbon conversion microbial metabolic efficiency is observed, generating higher levels of these intermediate compounds, irreversibly affecting the final yield of the crop established in this cropping system (Camargo et al. 1995; Johnson et al. 2006). The lower yields obtained in these situations may be the major factor retarding a large scale adoption of direct seeding in rice (only 5.4%) as opposed to winter cereals such as wheat, barley, and oats (over 90%) in the State (Pinto et al. 2004).

Since the majority of organic acid production occurs in the early seedling stages, the described effects could be more related to the seed germination stages (Lynch 1980; Neves et al. 2006, 2007; Neves and Moraes 2005). In the plantlet stage, there are a higher number of references regarding the negative effect of organic acids on the root elongation of rice (Camargo et al. 1993; Kopp et al. 2007a,d,e, 2008; Rao and Mikkelsen 1977a, b; Schmidt et al. 2007; Sousa and Bortolon 2002), barley, wheat, maize, oat (Kopp et al. 2007b), and clover (Lynch 1980). The majority of these reports indicate that these acids affect plant weight and height as well as dry mass. Other reports also mention reductions in tillering (Ansus and Reys 1979), plant height, panicle number (Gotoh and Onikura 1971), grain yield, and straw production in the rice crop (Camargo et al. 1995, 2001). There are also reports indicating the effect of organic acids on P, K, and Si (Takijima et al. 1960); K, P, NH₄, Mn, Mg, and Ca (Gotoh and Onikura 1971); P and K (Rao and Mikkelsen 1977b); N, P, K, Ca, and Mg (Schmidt et al. 2007; Sousa and Bortolon 2002) nutrient uptake.

The effect of organic acids is dependent on the solution pH in which the root development occurs. Low pH values increase the toxic potential of each organic acid because they increase the non-dissociated form of these molecules. A toxicity increase of up to 20% was observed when one pH unit was dropped in the nutrient solution in which the rice plants were growing (Kopp et al. 2007c). There is an apparent direct increase in toxicity with the carbon chain size (Angeles et al. 2005; Rao and Mikkelsen 1977a). A concentration of 10 mM for each acid caused root growth reductions of 44, 70, and 77% for acetic (CH₃COOH), propionic (CH₃CH₂COOH), and butyric (CH₃CH₂COOH) acids, respectively, confirming the higher toxicity of the acid with the longer chain (Kopp et al. 2007a).

The organic acid accumulation in the soil directly affects the crops causing respiration inhibition and cell membrane degradation of radicle meristematic tissue, leading to a halt in cell division (Angeles et al. 2005; Armstrong and Armstrong 2001;

Johnson et al. 2006). On the other hand, these toxic compounds affect mitochondrial functions, including oxidative phosphorilation uncoupling as well as metabolite transport and citosol soluble glicolytic enzyme inhibition. Also, endomembrane-associated enzymes such as those responsible for polysaccharide synthesis and ATPases are inhibited (Angeles et al. 2005). Organic acids have been reported to cause the efflux of inorganic ions and organic matter to the external medium, leading to damage in plasmalema integrity (Camargo et al. 2001). The combined effect of these inhibitory effects may lead to a marked reduction of vacuole solute accumulation, i.e., those substances needed for the water osmotic influx, keeping the cell turgescence and allowing the germination process.

Identifying and characterizing genetic variability for organic acid tolerance is the first step to study the inheritance and/or map the associated genomic regions. Genetic variability for the tolerance to organic acid in the vegetative period has been reported in oat (Kopp et al. 2007b) and rice (Kopp et al. 2007d,e; 2008). However, for the germination period, where the occurrence and potential damage of organic acids is higher, there is still a lack of information in the current literature. Genotypes with high germination performance under organic acid stress become important tools for the understanding of genetic variability, function, gene regulation, and gene action (Sreenivasulu et al. 2007). The cloned gene(s) can also be used to incorporate new traits into elite cultivars, through recombination or transformation techniques (Kahl and Lavi 2001; Yang et al. 2000). In the case of organic acid tolerance, the genes responsible for high germination rates will contribute to an improvement in genotype plant stands and consequently, improving the yield under no-tillage rice crops. This would create a trend towards no-tillage cropping in rice, leading to yield increases as well as lower environmental impact and production costs for this cereal.

The objectives of this work were to evaluate the germination of rice genotypes subjected to propionic acid and to identify genetic variability for the character propionic acid tolerance, as well as to provide breeders with information about genotypes that can produce better stands when germinated under no-tillage or minimal tillage conditions.

Materials and methods

The work was performed in the Double-Haploid and Hydroponics Laboratory located at the Plant Genomics and Breeding Center (CGF), Eliseu Maciel School of Agronomy, Federal University of Pelotas (UFPel), located at Pelotas County - RS. A total of 12 rice genotypes were subjected to four propionic acid concentration levels. The genotypes used belong to the working germplasm collection of CGF/UFPel. Genotypes from *indica* and *japonica* subspecies, originating from different cultivating (irrigated and upland) systems and locations were used (Table 1).

Effect of Propionic Acid in the Germination of Rice Genotypes

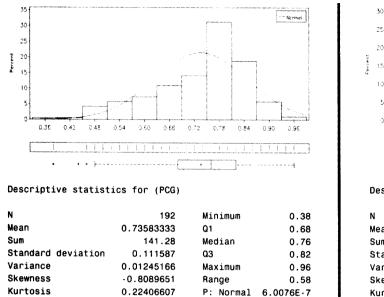


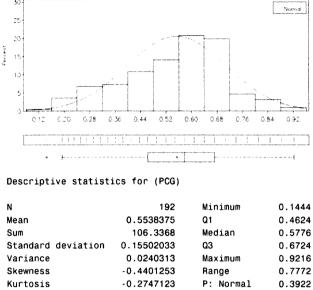
Fig. 1. Descriptive analysis of the variable first count germination (FCG), before and after data transformation (x'). Pelotas-RS, 2008.

The germination tests were conducted with 200 seeds, consisting of four replications of 50 seeds each. Seeds were placed in germitest paper rolls pre-soaked with the treatment solutions at an amount equivalent to 2.5 times the initial weight. The treatments consisted of propionic acid at 0, 3, 6, and 9 mM concentrations and the pH was adjusted to 4.7 with 1N HCl or 1N NaOH (Kopp et al. 2007c; Rao and Mikkelsen 1977a). The rolls were sealed with plastic bags to avoid cross contamination of treatments, since the propionic acid is quite volatile (Camargo et al. 2001). All the bags containing the genotypes and treatments were maintained in a germinator at constant temperature (25 °C) and the counting was performed in the seventh and fourteenth day after seeding (Brasil 1992).

The experimental design was a completely randomized factorial, and the variables evaluated were first count germination and germination. The data relative to the measured variables were subjected to a descriptive analysis and transformed. The analysis of variance was performed for a factorial model, considering the

Table 1. List of name, subspecies, and cultivating system for the genotypes used for the study of Rice germination under propionic acid stress. Pelotas-RS, 2008.

Number	Genotype	Subspecies	Cultivating system
1	BRS 7 - Taim	Indica	Irrigated
2	Fanny	Indica	Upland
3	Yonaochi	Japonica	Upland
4	Supremo - 1	Indica	Irrigated
5	Oryzica	Indica	Irrigated
6	Guichow	Japonica	Irrigated
7	Gbegbbete	Japonica	Upland
8	IAS-12 - Formosa	Indica	Upland
9	Gose Yonkoku	Japonica	Upland
10	Dawn	Japonica	Irrigated
11	Toride - 1	Indica	Irrigated
12	IAC - 47	Indica	Upland



dose and genotype as fixed. The interaction effects between these factors were tested by a linear regression analysis, using the significance of the distinct degrees of the polynomial as a function of different dose levels (quantitative). The data is presented in a graphic format for each individual genotype with the untransformed variables. All the analyses were performed with SAS software (Statistical Analysis System 2002).

Results and Discussion

The results of the variable descriptive analysis revealed an absence of variance normality and homogeneity for the variables first count germination (Fig. 1) and germination (Fig. 2). According to the results obtained, a x^2 data transformation was performed. For first count germination (Fig. 1) the distribution assimetry represented by the skewness value was highly influenced by the data transformation, changing from -0.81 to -0.44, making the data distribution more symmetric. The curtose value

Table 2. Summary of analysis of variance, means, and coefficient of variation (C.V.) for the variables first count germination and germination of 12 rice genotypes, subjected to four propionic acid concentrations. Pelotas-RS, 2008.

S.V.	D.F. –	Mean Squares			
	D.F. –	First count germination	Germination		
Genotype	11	0.0507*	0.0696*		
Dose	3	0.6356*	1.0233*		
Interaction	33	0.0263*	0.0338*		
Residue	141	0.00897	0.0130		
Mean		73.58%	81.16%		
C.V.		16.99	16.89		

^{*} Significant at 5% probability by the F test.

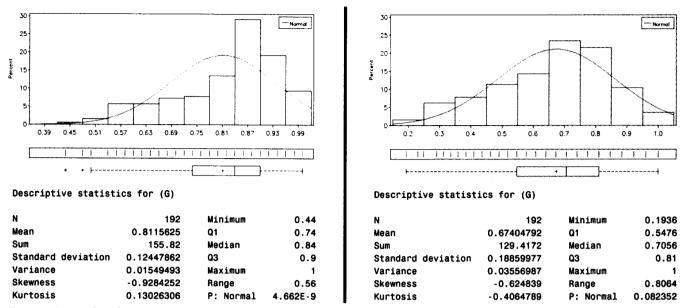


Fig. 2. Descriptive analysis of the variable germination (G), before and after data transformation (x²). Pelotas-RS, 2008.

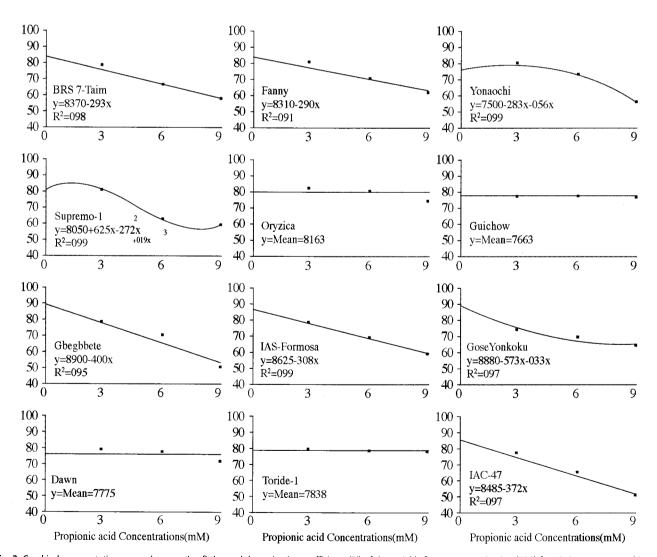


Fig. 3. Graphical representation, regression equation fitting and determination coefficients (R²) of the variable first count germination (FCG) for 12 rice genotypes subjected to four propionic acid concentrations. Pelotas-RS, 2008.

Table 3. Summary of analysis of variance of the regression model for the variables first count germination and germination of 12 rice genotypes, subjected to four propionic acid concentrations. Pelotas-RS. 2008.

	Mean Squares						
Genotype	First count germination		Germination				
	Degree of polynomium			Degree of polynomium			
	Linear	Quadractic	Cubic	Linear	Quadractic	Cubic	
BRS 7 - Taim	0.3050*	3.610 E-4	0.0043	0.4706*	0.0046	6.272 E-4	
Fanny	0.1568*	0.0046	0.01051	0.1552*	0.0020	0.0010	
Yonaochi	0.1450*	0.0722*	3.837 E-4	0.3539*	0.1094*	0.0079	
Supremo - 1	0.2789*	0.0019	0.0401*	0.4849*	0.0011	0.0248	
Oryzica	0.0196	0.0017	1.067 E-4	0.0634*	5.523 E-4	0.0014	
Guichow	0.0084	0.0015	0.0011	0.0094	0.0021	3.647 E-4	
Gbegbbete	0.5398*	0.0109	0.0020	0.8494*	0.0075	0.0060	
IAS - Formosa	0.3517*	8.100 E-7	3.4114 E-4	0.5703*	0.0012	9.194 E-4	
Gose Yonkoku	0.3066*	0.0454*	0.0102	0.2768*	0.0085	0.0037	
Dawn	0.0039	9.000 E-6	4.724 E-4	0.0476	0.0034	7.104 E-4	
Toride - 1	0.0054	5.153 E-4	5.984 E-4	0.0044	7.236 E-4	1.458 E-6	
IAC - 47	0.4393*	0.0053	0.0014	0.6815*	0.0111	3.819 E-4	
Residue		1.2499			1.8290		

^{*} Significant at 5% probability by the F test.

indicates that the data transformation had an effect increasing the frequency of classes of higher value because it changed from a positive (0.22) to a negative (-0.27) value. When the variable germination was analyzed (Fig. 2), the same conclusions can be obtained, i.e., the asymmetry was reduced and the frequency of high value classes was increased. In both cases, these changes on the variable distribution values were efficient in approximating the data to a normal distribution.

The results of the analysis of variance (Table 2) showed significant effects at P 0.05 for dose, genotype, and interaction (dose x genotype), for both variables. These results allow the conclusion that genotypes present differential responses to increase in propionic acid concentrations. This result implies in the partitioning of the effect into its simple effects. The differential response also indicates the presence of genetic variability for organic acid sensitivity. The significant effect due to the genotype also indicates that differences relating to the average magnitude of these variables for each genotype vary independently from their response. Therefore, the genotypes described as high germination ability under propionic acid stress in this work were those that showed no sensitivity to the acid toxicity. These genotypes were selected because there is a higher probability of having genes controlling the ability to germinate and keep cellular activity in the presence of the stress.

The analysis was followed by a check of the performance of the studied variables at different propionic acid levels, using a regression analysis, considering the genotypes factor as fixed. For both variables, with the application of linear regression analysis, parameters up to the third degree of the polynomial were obtained, represented by the values of medium squares and the corresponding significance in Table 3. The regression equations with the respective determination coefficients (R²) are pre-

sented in Figs. 3 and 4 for the variables first count of germination and germination, respectively. The doses used to perform the experiment were previously selected based on reports by Camargo et al. (1993); Kopp et al. (2007a, 2008); Neves et al. (2006, 2007); Neves and Moraes (2005); Rao and Mikkelsen (1977a,b); Sousa and Bortolon (2002) in order to obtain a relative reduction around 50% in the highest dose analyzing the different response variables. The results demonstrate that in none of the cases were observed reductions above 50%. These results suggest that the variables related to germination of studied genotypes (FCG and G) are less sensitive to propionic acid effects. However, the concentration levels used were efficient in discriminating the relative differences among the genotypes facing the germination character under aluminum toxic effects.

Analyzing the performance of genotypes regarding the variable first germination count (Fig. 3), it can be observed that the genotype Supremo-1, the higher degree of polynomium significance explaining the variable was cubic. In this case, it seems that an initial stability for the first germination count was observed, up to the 3 mM dose, followed by a significant reduction between the 3 and 6 mM doses, stabilizing again at high values of propionic acid between doses of 6 and 9 mM. The genotype Yonaochi presented a better adjustment of the quadractic regression and it can be observed that for reduced doses of propionic acid in the embedding solution there is a lower decrease in the variable first count of germination with a reduction at higher doses, especially above 6 mM of propionic acid. The genotype Gose Yonkoku also had a better fitting with a quadratic regression. However, in this case, the higher reduction observed in first count indices were between the doses of 0 and 3 mM. The genotypes that presented a significant variation and a linear regression fitting were BRS 7-Taim, Fanny, Gbegbbete, IAS-Formosa, and IAC-47. For these cases, it can be observed that reductions on the first count germination were constant for the dose ranges used in the experiment.

For the variable germination (Fig. 4), none of the genotypes presented cubic regression fitting and only the genotype Yonaochi showed a quadratic regression fitting, with an initial stability up to the 6 mM propionic acid and a large reduction on the germination at doses above this level. The genotype BRS 7-Taim, Fanny, Supremo-1, Oryzica, Gbegbbete, IAS-Formosa, Gose Yonkoku, and IAC-47 presented linear regression fitting, also with a significant variation, but in these cases with constant variations for the three doses (3, 6, and 9 mM) used in the experiment.

The genotype Oryzica that initially had demonstrated insensitive to propionic acid concentrations (FCG) showed significant reductions when the germination was analyzed after 14 days (G), i.e., in this case the genotype presents a good initial result but when it is subjected to a longer time under the stress it reacts negatively showing a reduction in germination. Fortunately, the advances in molecular techniques allow geneticists and breeders to better discriminate genotypes regarding traits such as grain yield components and biotic and abiotic stress resistance. Since there is no description in the literature of cut-off values for germination reduction, in order to consider a genotype as tolerant or

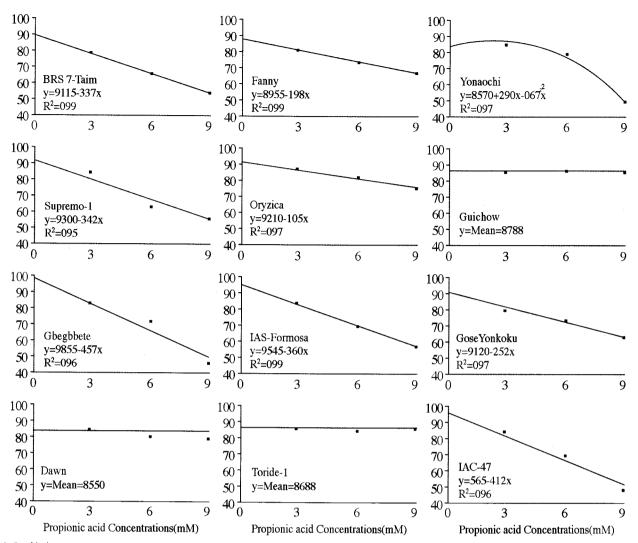


Fig. 4. Graphical representation, regression equation fitting, and determination coefficients (R²) of the variable germination (G) for 12 rice genotypes subjected to four propionic acid concentrations. Pelotas-RS, 2008.

sensitive, and there are no genotypes described as tolerant and susceptible controls, the genotypes were considered tolerant when they did not respond to increases in acid concentrations, i.e., those with means showing non-significant changes as a function of increases in the acid concentrations. The genotypes Oryzica, Guichow, Dawn, and Toride-1 did not show a significant variation regarding the different treatment levels for the variable first count germination (Fig. 3). The data demonstrate that for these genotypes the first count germination is kept constant when subjected to a concentration up to 9 mM propionic acid, reaching means of 81.63, 76.63, 77.75, and 78.38 % respectively. However, for the variable germination, the genotypes Guichow, Dawn, and Toride-1 maintained their means at constant levels when subjected to a dose up to 9 mM propionic acid, reaching 87.88, 85.50, and 86.88 %, respectively (Fig. 4).

The damaging effect of organic acids on rice plants is related to cell membrane degradation and loss of cell content to the external medium (Armstrong and Armstrong 2001). Also, the same report suggests that tolerant plants should possess genes

conferring a higher ability to form cell membranes that tolerate these acids. The analysis of Figs. 3 and 4 suggest that for both variables the tolerant genotypes are not the genotypes with higher germination potential. Genotypes that showed lower germination potential at the control dose (0 mM) should have the ability to maintain their cell viability through membrane maintenance when stressed with propionic acid. This is confirmed by the analysis of the intercept values for each genotype, where a high variability for first count germination in the absence of propionic acid (dose 0 mM) is observed. The first count germination values in the absence of acid ranges from 75 (genotype Yonaochi) to 89.5% (genotype Gose Yonkoku), both of which are propionic acid-insensitive genotypes that showed intermediate first-count germination values in the control dose. Considering the variable germination, a shorter range is observed for genotypes Yonaochi (86.5%) and Gbegbbete (96.5%) at the extremes of the distribution. Also, the insensitive genotypes show intermediate germination levels in the absence of the stress.

The results indicate an average value of 22.96% on the first germination count and 24.21% on the germination when all the genotypes are evaluated at the 9 mM dose. Neves et al. (2006, 2007) used acetic acid doses reducing up to 80% of first-count germination and germination values. However, these doses were not used to investigate the genetic variability for tolerance. Overall, the doses used in this work led to reductions in the germination characters that were large enough in order to discriminate among the studied genotypes revealing contrasting responses to the applied stress. Future studies may prove the correlation between the laboratory conditions and the real environmental conditions happening in the field.

By associating the results of tolerant genotypes with their descriptions listed on Table 1, one can observe that one *indica* and two japonica genotypes showed germination stability when subjected to the propionic acid stress. Also, all tolerant genotypes belong to the irrigated system. Kopp et al. (2008) discuss the likelihood of the breeding of genotypes under irrigated conditions provided an environment with high organic acid concentrations and therefore led to an indirect selection for tolerance to the character.

The japonica genotypes described as tolerant in this work belong to core germplasm collections showing high rusticity. In general, rustic genotypes are tolerant to a wide range of biotic and abiotic stresses as a function of their differential ability in forming the membranous system (Hincha and Hagemann 2004). This is in agreement with the proposition by Armstrong and Armstrong (2001), where tolerant plants should have genes capable of faster membrane formation after stress injuries. These *japonica* genotypes can be used by breeding programs in crosses to elite adapted cultivars. However, these crosses very often lead to negative impacts in the ideotype researched by breeders due to recombinations that bring unfavorable genes in a phenomenum also known as linkage drag. The most common feature that is damaged by such recombinations is grain quality, increasing the low amylose/amylopectin ratio characteristic of Brazilian cultivars (Magalhães Jr. et al. 2004).

The use of propionic acid-tolerant genotypes in plant breeding programs may contribute to increase germination rates of rice seeds under no-tillage or minimal tillage cultivation, causing reductions in production costs and yield increases as well as environmental benefits caused by better soil conservation practices when compared to conventional systems.

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