DIFFERENTIAL GERMINATION PATTERN OF RICE CULTIVARS RESISTANT TO IMIDAZOLINONE HERBICIDES

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INTRODUCTION

The different gene mutations associated with ALS-inhibiting herbicide resistance can result in variation in the germination rate of the mutated plants. In addition to the herbicide resistance obtained as a consequence of the ALS gene mutations, a decrease of the feedback inhibition of the Valine, Leucine and Isoleucine amino acids biosynthesis was identified in plasmids of *Arabidopsis thaliana* (SINGH et al., 1992). Furthermore, several studies indicated that both the differential inhibition of enzyme activity and the feedback regulation are differentially affected depending on the ALS mutation that occurs in the plant (YU et al., 2005). The same alteration of the ALS enzyme structure is also related to the modification of the binding site for the Valine, Leucine and Isoleucine amino acids (PRESTON et al., 2006). The alteration of the feedback regulation makes the ALS enzyme catalyze reactions constantly, resulting in the excessive synthesis of branched-chain amino acids and subsequent accumulation in the seeds, causing faster germination rate than the wild-type plants (DYER et al., 1993). The increased germination rate of herbicide-resistant biotypes can facilitate the evolution of herbicide resistance in plant communities. Although the imidazolinone herbicide-resistant rice is an important trait, there are few studies regarding the fitness of ALS-inhibiting herbicide-resistant rice. The rice cultivars IRGA 417, IRGA 422 CL and PUITÁ INTA CL have different ALS-inhibiting herbicide resistance alleles, and constitute appropriate models for these studies. The susceptible cultivar IRGA 417 is one of the genitors of the imidazolinone-resistant cultivars IRGA 422 CL and PUITÁ INTA CL.

Fitness refers to the ability of a population to occupy a given area under certain environmental conditions through the survival and reproduction and, in the case of agriculture, must take into account the effects of crop conditions (ORR, 2005). The gene flow of different cultivars promotes changes in the fitness of hybrids between cultivated and wild species. Considering that gene flow from imidazolinone herbicide-resistant rice cultivars can transfer ALS gene mutations to red rice, the fitness effects of these mutations can differentially affect the evolution of this weed. This knowledge is important because imidazolinone herbicide-resistant red rice populations may provide a fitness advantage related to an increased germination rate. The management of imidazolinone herbicide-resistant rice cultivars must prevent the evolution of red rice resistant to these herbicides, and this phenomenon can be differentially affected by the mutation of the ALS gene used in the development of the rice cultivars. Therefore, the objective of this study was to identify the fitness changes related to the increase in the germination rates in imidazolinone herbicide-resistant rice cultivars carrying three different ALS gene mutations.

MATERIALS AND METHODS

Five seed lots of the cultivars IRGA 422 CL, PUITÁ INTA CL, SATOR CL and IRGA 417 were evaluated. The experimental design was completely randomized disposed in a factorial scheme with five replications. Factor A was composed by the four cultivars, Factor B was composed of the five seed lots of each cultivar, and Factor C was composed by two temperatures 20ºC and 25ºC. Each experimental unit consisted of approximately 100 viable seeds. The seeds were placed in a 25x40 cm germination paper, which was moistened with

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distilled water and rolled. The paper rolls were positioned vertically within a glass container with a capacity of 2000 mL. The evaluation consisted of the germination counting at intervals of approximately 12 hours over eight days. The first evaluation occurred at 24 hours after the start of the experiment. The criterion adopted for defining seed germination was the emergence of the radicle ≥ 1 mm length. At each evaluation, the germinated seeds were removed to avoid erroneous recounting.

The cumulative percentage of seed germination over time was calculated for each treatment. The data were analyzed by the ANOVA mixed model, taking cultivars and temperature as fixed effects and the seed lots as the random effect. Subsequently, a non-linear regression analysis was performed by SAS 9.0 proc NLIN (SAS INSTITUTE, 2004). The variation in cumulative germination as a function of time was adjusted by the logistic model, as shown in Equation 1:

Eq. 1.
$$Y = \frac{100}{1+e^{(b_0(b_1-t))}}$$

where Y is the percentage germination at time t, b_0 is the slope of the tangent at the curve inflection point or germination rate at the inflection point (GRI), and b_1 is the time required to reach 50% germination (G_{50}). The rate or speed of germination at point b_1 is given by Equation 2:

Eq. 2.
$$GR = \sqrt{\left[\left(\frac{100*b_0}{4}\right)-1\right]},$$

where GR is the germination rate at b_1. The comparisons of the parameters among treatments were based on a confidence interval of 95%. Furthermore, the lag phase (LAG) was calculated for each treatment. The LAG is the time of the water absorption and metabolic activation phases of the germination process, also known as germination phases I and II, respectively (BEWLEY, 1997). The LAG was defined when the accumulated germination became statistically different from zero at a confidence interval of 95%.

RESULTS AND DISCUSSION

The ANOVA showed that the environmental effect characterized by seed lots was not significant (α=0.05). The ANOVA mixed model with the seed origin as a random effect was performed to avoid comparisons between seed lots of different cultivars and to consider them as a sample of a larger group of lots. Therefore, the absence of significance of the seed lot effect on germination indicates that the observed results are largely related to the cultivars and not to the environmental effect. At the temperature of 20ºC, the LAG the PUITÁ INTA CL cultivar was smaller than the other cultivars (Table 1). At 25ºC, the LAG of the PUITÁ INTA CL and SATOR CL cultivars was smaller than IRGA 422 CL and IRGA 417 (Table 1). The SATOR CL cultivar had a relatively short LAG compared to that observed at 20ºC (Table 1). The more intense decrease in the LAG in the SATOR CL cultivar than in the other cultivars can be related to the fact that this cultivar is a hybrid and has high heterosis. The hybrid vigor of the seeds of SATOR CL was manifested mainly in the temperature of 25ºC compared to LAG. At this temperature, the PUITÁ INTA CL cultivar had the LAG before 9 h, whereas IRGA 422 CL had a LAG equal to the susceptible cultivar (Table 1). Therefore, the hypothesis that imidazolinone herbicide-resistant rice cultivars germinate faster than the susceptible cultivars at low temperatures was confirmed.

Table 1. Germination and duration of lag phase in seeds of four rice cultivars at two temperatures, in average of five seed lots per cultivar.

Temperature	Cultivar	Germination (%)	[IC 95%]	Lag phase (h)
20ºC	IRGA 417	0,996	[0,436; 1,555]	57
	IRGA 422 CL	0,972	[0,255; 1,688]	48
	PUITÁ INTA CL	0,260	[0,027; 0,492]	33
	SATOR CL	1,000	[0,497; 1,502]	57
25ºC	IRGA 417	0,172	[0,003; 0,340]	33
	IRGA 422 CL	0,372	[0,022; 0,721]	33
	PUITÁ INTA CL	0,280	[0,058; 0,501]	24
	SATOR CL	2,996	[1,325; 4,666]	24

The germination pattern indicated a clear variation between cultivars at both temperatures, with PUITÁ INTA CL germinating before the other cultivars at 20ºC and SATOR CL at 25ºC (Figure 1). This result is verified by comparisons of the logistic regression parameters. At 20ºC, the IRGA 417 and IRGA 422 CL cultivars had similar GRI, whereas the PUITÁ INTA CL had a GRI higher than these cultivars and lower than the SATOR CL, which obtained the largest GRI (Table 2). On the other hand, the four cultivars had different G50 values from each other. The PUITÁ INTA CL cultivar had the lowest G50, whereas the IRGA 417 had the highest. However, the IRGA 417 and IRGA 422 CL had equal GR values, whereas the SATOR CL cultivar had the highest GR, followed by PUITÁ INTA CL (Table 2). At the temperature of 25ºC, the GRI was higher for the SATOR CL cultivar. PUITÁ INTA CL cultivar had an intermediate GRI between SATOR CL and the other two cultivars IRGA 422 CL and IRGA 417, whose GRI values were identical (Table 2). The G50 of the cultivars at 25ºC was lower than at 20ºC. This parameter at 25ºC was lower for SATOR CL followed by PUITÁ INTA CL, IRGA 422 CL and IRGA 417. The increase of the GR values for all cultivars at 25ºC compared with the GR values at 20ºC, and this increase in SATOR CL was more marked than in the other cultivars (Table 2). The GR of SATOR CL was higher than that of PUITÁ INTA CL, which in turn had a higher GR than those observed in IRGA 422 CL and IRGA 417. The latter two cultivars had similar GR values, as observed in the temperature of 20ºC (Table 2).

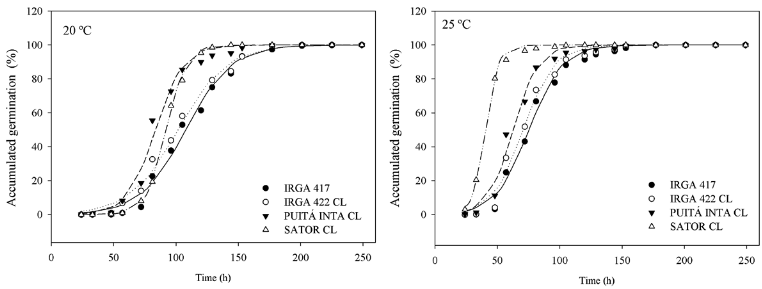

Figure 1. Germination pattern of four rice cultivars at 20 (left) and 25 ºC (right), in average of five seed lots per cultivar. The IRGA 422 CL, PUITÁ INTA CL and SATOR CL cultivars are resistant to imidazolinone herbicides. The IRGA 417 cultivar is susceptible to these herbicides.

Table 2. Logistic model parameters and germination rate of accumulated germination of four rice cultivars at two temperatures, in average of five seed lots per cultivar.

Temperature	Cultivar	b_0[1] ($\%.h^{-1}$)	[IC 95%]	b_1 (h)	[IC 95%]	GR ($\%.h^{-1}$)	[IC 95 %]
20ºC	IRGA 417	0,054	[0,050; 0,057]	107,8	[106,4; 109,1]	1,35	[1,26; 1,44]
	IRGA 422 CL	0,050	[0,046; 0,054]	101,7	[99,8; 103,6]	1,26	[1,15; 1,36]
	PUITÁ INTA CL	0,086	[0,080; 0,092]	83,5	[82,6; 84,4]	2,15	[2,00; 2,30]
	SATOR CL	0,119	[0,111; 0,125]	92,2	[91,6; 92,7]	2,97	[2,79; 3,14]
25ºC	IRGA 417	0,074	[0,066; 0,080]	75,4	[73,9; 76,7]	1,84	[1,67; 2,01]
	IRGA 422 CL	0,079	[0,072; 0,085]	70,7	[69,5; 71,9]	1,98	[1,81; 2,14]
	PUITÁ INTA CL	0,096	[0,088; 0,104]	62,7	[61,7; 63,7]	2,40	[2,20; 2,60]
	SATOR CL	0,178	[0,167; 0,188]	40,7	[40,1; 41,1]	4,45	[4,18; 4,71]

[1] b_0, GRI, germination rate at inflection point. $b_1 = G_{50}$, time to germination reaches 50%. GR, germination rate at b_1.

The results of the present study indicated that the imidazolinone herbicide-resistant rice cultivar PUITÁ INTA CL has a faster germination rate, indicated by the lower G_{50}, than the other rice cultivars at the temperature of 20ºC. The germination pattern of the IRGA 422 CL cultivar was similar to the susceptible IRGA 417 at both evaluated temperatures. The PUITÁ INTA CL cultivar carries the ALS gene mutation $Ala_{122}Thr$ (ROSO et al., 2010). This result indicated that these mutations probably alter the self-regulation of the ALS enzyme by the amino acids Leucine, Valine and Isoleucine, which can accumulate in the seeds. The $Ala_{122}Thr$ mutation confers a higher level of resistance to imidazolinone than the $Gly_{654}Glu$ and $Ser_{653}Asp$ mutations (ROSO et al., 2010). This may be related to the effect of this mutation on the amino acids' self-regulation of the ALS gene by affecting the Van der Waals interactions, hydrogen bonds or both, like the ALS-inhibiting herbicides (DUGGLEBY et al., 2008). As demonstrated in the present study, the same was observed in *Lactuca serriola* (ALCOCER-RUTHLING et al., 1992), *Kochia scoparia* (THOMPSON et al., 1994) and *Bromus tectorum* (PARK et al., 2004) whose ALS-inhibiting herbicide resistant biotypes germinated faster than the susceptible biotype mainly at low temperature.

The results obtained in the present study indicated that the imidazolinone herbicide-resistant cultivar PUITÁ INTA CL have pleiotropic effects of ALS gene mutations that affect the germination pattern. In the case of the hybrid cultivar SATOR CL, these effects are confounded with the heterosis effects, and the potential effect of the ALS gene mutation $Ser_{653}Asn$ could not be found in this experiment. Also, this study indicates a positive change in the fitness related to the germination pattern of the imidazolinone herbicide-resistant rice cultivars, which are herbicides whose mechanism of action is the inhibition of the ALS enzyme.

CONCLUSION

The PUITÁ INTA CL cultivar, which carries the ALS gene mutation $Ala_{122}Thr$, has faster seed germination than the other herbicide-resistant and the susceptible cultivars.

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