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Genetic tolerance to low temperatures in irrigated rice[1]

Tolerância genética de arroz irrigado a baixas temperaturas

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ABSTRACT - The aim of this study was to characterise genetic variability to low-temperature tolerance in the emergence stage of irrigated-rice genotypes under controlled and field conditions. Thirty-seven genotypes were evaluated, interspersed with controls of different levels of low-temperature tolerance. The design used was of randomised blocks with three replications. The genotypes were submitted to germination and emergence in controlled-temperature trials at 13 ºC (Trial I) and 17 ºC (Trial II), and in field environments with no cover (Trial III) and with a cover of polyethylene (Trial IV), where the soil temperature was monitored. The evaluations were carried out daily by counting the emerged seedlings and later inferring the speed of emergence index (SEI). The results showed (experiment I to IV) that the SEI varied from 0.61 to 4.23, increasing by 0.4259 for each 1 ºC increase in soil temperature. The genotypes Ostiglia, Diamante, Baldo, Carnaroli, Selenio, Loto e Amarelo, of subspecies japonica, and Ringo Miara-AC and Arelate, of subspecies indica, are promising sources of genetic tolerance to low temperature. Temperatures of less than 17 ºC reduce the number of emerged seedlings and delay development in each of the rice genotypes under test, with a greater or lesser negative effect on the initial plant stand per unit area.

Key words: *Oryza sativa* L.. Abiotic stress. Cold. Genetic variability.

RESUMO - Objetivou-se caracterizar a variabilidade genética para a tolerância a baixas temperaturas na fase de emergência de genótipos de arroz irrigado em condições de ambiente controlado e a campo. Foram avaliados 37 genótipos, com testemunhas intercaladas com diferentes níveis de tolerância à baixas temperaturas. O delineamento utilizado foi de blocos casualizados com 3 repetições. Os genótipos foram submetidos a germinação e emergência nos ensaios com temperatura controlada a 13 ºC (Ensaio I) e 17 ºC (Ensaio II) e em ambientes a campo, sem cobertura (Ensaio III) e com cobertura de polietileno (Ensaio IV), sendo monitorado a temperatura do solo. As avaliações foram realizadas diariamente por meio de contagem das plântulas emergidas e posterior inferência do índice de velocidade de emergência (IVE). Os resultados revelaram (experimento I a IV) que o IVE foi de 0,61 a 4,23, aumentando 0,4259 a cada 1 ºC de aumento de temperatura do solo. Os genótipos Ostiglia, Diamante, Baldo, Carnaroli, Selenio, Loto e Amarelo, da subespécie japonica e Ringo Miara-AC e Arelate, da subespécie indica, são fontes promissoras de tolerância genética à baixas temperaturas. Temperaturas abaixo de 17 ºC diminuem o número de plântulas emergidas e retardam o desenvolvimento de todos genótipos de arroz testados, afetando, em maior ou menor magnitude, negativamente no estande inicial de plantas por unidade de área.

Palavras-chave: *Oryza sativa* L.. Estresse abiótico. Frio. Variabilidade genética.

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INTRODUCTION

Rice (*Oryza sativa* L.) is the staple food of half the world's population (HAO; LIN, 2010). Due to the constant growth in population, there is a growing demand for the cereal, demonstrating the need for increases in production. In recent decades, there have been significant increases in the production potential of rice, which were achieved by advances in genetics (BRESEGHELLO *et al*., 2011; STRECK *et al*., 2018), as well as improvements in crop management. Among the successful changes in crop management, anticipating the sowing season has been suggested, so that the reproductive stage coincides with months with the smallest probability of low temperatures and the highest intensity of solar radiation (MERTZ *et al*., 2009; STEINMETZ *et al*., 2009; STEINMETZ; DEIBLER; SILVA, 2013).

Low temperatures are a common problem in rice cultivation, where sensitivity affects production of the cereal (ZHOU *et al*., 2012). Temperatures of less than 20 ºC can be harmful to the rice crop, and are common in temperate and subtropical areas (NANDA; SESHU, 1979), reducing the percentage and speed of seedling emergence (XU *et al*., 2008). The occurrence of such low temperatures during the early stages can impair the establishment and stand of the crop (CRUZ *et al*., 2013), affecting plant growth and development, which may lead to uneven grain maturation (BOSETTI *et al*., 2012). In addition, if the low temperatures occur later, they can cause a loss in grain yield due to spikelet sterility.

Low temperature is a factor which is unpredictable and abiotic in nature, as such, its negative effects on rice are difficult to control at the management level, which makes the genetic tolerance of cultivars the most viable alternative in the search for a solution to minimise damage and stabilise grain yield in areas subject to cold. Research aimed at obtaining genotypes with a greater tolerance to cold using the existing variability of available collections, is therefore a strategy of fundamental importance.

To develop rice cultivars with adequate tolerance to low temperature during the germination and seedling emergence stage, it is necessary to investigate the genetic resources available from various regions of the world that might serve as a genetic source for the subsequent transfer of genes involved in the capacity for low-temperature germination to elite strains or commercial cultivars.

The characterisation and subsequent selection of genotypes that are tolerant to low temperatures during the initial period of development is difficult due to the complex genetic basis of the trait and the lack of control of low-temperature stress under field conditions (CRUZ; MILACH, 2000). On account of the difficulties of field evaluations for cold tolerance, it is necessary to use strategies for conducting experiments under controlled conditions in order to characterise this trait.

In this context, the aim of this study was to characterise genetic variability to low-temperature tolerance in the emergence stage of irrigated rice genotypes under controlled and field conditions.

MATERIAL AND METHODS

The experiment was conducted at the Terras Baixas Station of Embrapa Clima Temperado, in the district of Capão do Leão, Rio Grande do Sul, Brazil (31°48'16" S, 52°24'46" W, at an altitude of 15 m). The Köppen-Geiger climate classification defines the climate as predominantly of type Cfa, simplified as humid subtropical.

Thirty-seven irrigated-rice genotypes were evaluated, considering genetic constitutions from several regions of origin (Table 1). The genotypes were arranged in a randomised block design, using control cultivars to compare the level of tolerance. The tolerant controls used were: Diamante, Norin Mochi and Tomoe Mochi (BOSETTI *et al*., 2012; ÑANCULAO *et al*., 2013; MOURA *et al*., 2018); the sensitive controls were: BRS Firmeza (FREITAS *et al*., 2011), BRS Querência (AMARAL *et al*., 2016) and BRS Pampa (VIGHI *et al*., 2016).

The seeds were tested for germination, employing three replications of 100 seeds for each cultivar, and left to germinate at a temperature of 25 ºC in rolls of paper towel (Germitest®) moistened with an amount of water equal to 2.5 times the weight of the substrate. Counts were taken of normal seedlings 7 and 14 days after sowing at the Seed Analysis Laboratory of Embrapa Clima Temperado, Pelotas, Rio Grande do Sul.

Four trials were carried out simultaneously: I) Trial in a controlled environment at a constant temperature of 13 ºC; II) Trial in a controlled environment at a constant temperature of 17 ºC; III) Field trial during a period with a high probability of low air and soil temperatures; and IV) Field trial in a protected environment employing a transparent polyethylene tarpaulin in a low tunnel. Each trial was set up in a solodic Haplic Eutrophic Planasol, with the aim of removing any effect from this factor in the trials.

Trials I and II were carried out in a temperature-controlled environment, so as to maintain the treatment factors constant at 13 ºC and 17 ºC. The seeds of each genotype were sown in polypropylene trays filled with soil, where one row of 50 seeds, 0.5 metre in length, represented one experimental unit. Soil moisture was constantly controlled by thermocouple sensors.

Table 1 - Irrigated-rice genotypes used to assess cold tolerance at emergence

Nº	Genotype	Origin	Subspecies	Nº	Genotype	Origin	Subspecies
1	Puitá Inta CL	Argentina	Indica	20	Jasmine	Thailand	Indica
2	BRS Pampa	Brazil	Indica	21	BRS AG 'Gigante'	Brazil	Japonica
3	BRS Querência	Brazil	Indica	22	BRS 358	Brazil	Japonica
4	BRS Firmeza	Brazil	Indica	23	Diamante	Chile	Japonica
5	BR-IRGA 409	Brazil	Indica	24	Originário Chinese	China	Japonica
6	BRS Pampeira	Brazil	Indica	25	Arroz Austral	Unknown	Japonica
7	IRGA 424	Brazil	Indica	26	Amarelo	Unknown	Japonica
8	BRS 7 'Taim'	Brazil	Indica	27	Ostiglia	France	Japonica
9	Aurelia	France	Indica	28	Ranghino	France	Japonica
10	Ringo Miara-AC	France	Indica	29	Selenio	Italy	Japonica
11	Arelate	France	Indica	30	Arborio	Italy	Japonica
12	Saturo	France	Indica	31	Baldo	Italy	Japonica
13	Soulanet	France	Indica	32	Balilla	Italy	Japonica
14	Sambuc	France	Indica	33	Carnaroli	Italy	Japonica
15	Sírio CL	France	Indica	34	Loto	Italy	Japonica
16	Gladio	Italy	Indica	35	Vialone Nano	Italy	Japonica
17	Artiglio	Italy	Indica	36	Norin Mochi	Japan	Japonica
18	Thaibonnet	Italy	Indica	37	Tomoe Mochi	Japan	Japonica
19	Jasmine 85	Thailand	Indica	-	-	-	-

Trials III and IV were set up to simulate field conditions; however, Trial IV was covered with a transparent polyethylene tarpaulin in a low tunnel, thereby giving greater control over the environmental conditions. The trials were sown in August 2016 (period with a high probability of low air and soil temperatures), in experimental units comprising one row, 0.5 metres in length, containing 50 seeds of each genotype. The soil temperature under field conditions was monitored by means of thermocouple sensors (thermometers) located at a depth of three centimetres, which read the soil temperature and moisture every 60 minutes, collecting 24 temperatures daily. The data were recorded by data logger and transferred to a computer.

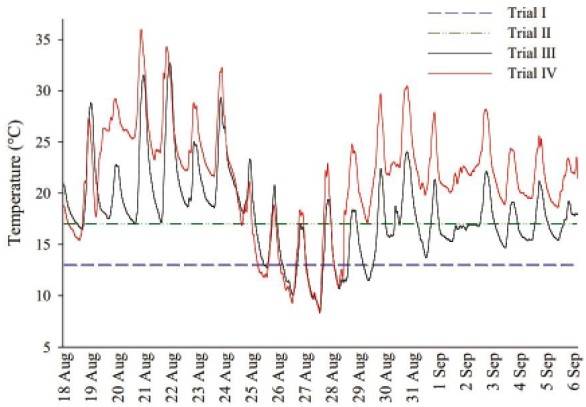
Each trial was monitored and evaluated daily, always at the same time (08:00), counting all the seedlings that emerged during that 24-hour period. The seedlings were counted when the coleoptile, developed from the embryo, broke the soil surface, initiating the process of emergence. Each emerged seedling was marked with a round wooden toothpick to avoid repeated counting. After evaluating each repetition, all the identified and marked seedlings were added to the daily-count spreadsheet. The counts ended once the emergence of each genotype had stabilised. From the daily count of emerged seedlings, the value of the desired response variable, the Speed of Emergence Index, was later inferred for each replication of the genotype under evaluation.

The analysis of cold tolerance was determined using the speed of emergence index (SEI) proposed by Maguire (1962), calculated with the formula SEI = (E1/N1) + (E2/N2) + (E3/N3) +... + (En/Nn), where: E1, E2, E3, ... En represent the number of seedlings included in the first, second, third and last count; and N1, N2, N3, ... Nn are the number of days between sowing and the first, second, third and last count respectively. The speed of emergence index was corrected using the germination power (PG%) of each genotype to configure the weighted speed of emergence. The speed of emergence index data were submitted to descriptive analysis, analysis of variance, and the Scott-Knott mean-value grouping test at 5% probability. The relationship of temperature (quantitative treatment factor) to the independent variable, speed of emergence index, was inferred by linear regression analysis. All analyses were carried out using the GENES genetics and statistics computer software (CRUZ, 2013).

RESULT AND DISCUSSION

In the experiments conducted in the field and under the low tunnel, a total of 480 soil temperatures were recorded up to the end of the experimental period; their intensity and fluctuation can be seen in Figure 1. It was found that between the seventh and eighth day after sowing, higher temperatures occurred due to variations in climate. In the afternoon the temperature reached 35.9 ºC in the environment covered with a polyethylene tarpaulin, and 32.6 ºC in the outdoor environment. The minimum temperature seen for both environments was 8.5 ºC. The mean temperature for the covered environment was 21.5 ºC, while for the outdoor environment it was 18 ºC. This demonstrates the great difficulty in inferring the tolerance of genotypes, especially under field conditions. Mittler (2006) states that crop improvement programs have achieved limited success in improving cold tolerance, mainly due to the polygenic control of the trait, unpredictable weather, and the interaction between the response to cold and to environmental factors (soil, nutrients and moisture, among others).

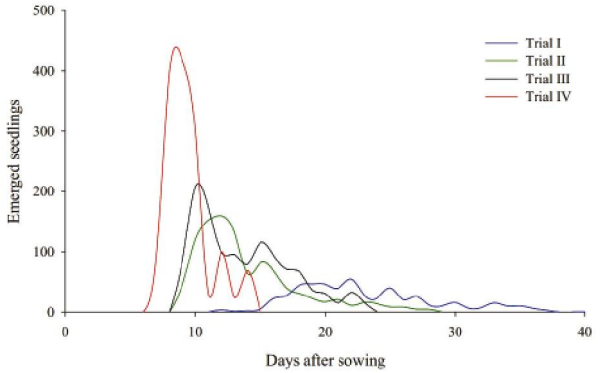
Figure 1 - Soil temperature measured every hour for twenty days, under outdoor conditions (Trial III) and covered with a low tunnel (Trial IV), as well as in a controlled environment (Trial I and Trial II)



It was found from Figure 2 that first emergence occurred in Trial IV (in the field in a low tunnel) from the seventh day after sowing, and continued until the fifteenth day. In the outdoor environment (Trial III), first emergence occurred only on the ninth day after sowing, continuing until 16 days after sowing.

For tests I and II under constant conditions of extreme low temperature for the crop, first emergence occurred later, from the twelfth and ninth day respectively, continuing for a much longer period, until day 35 (Trial I) and day 28 (Trial II). The daily frequency of emergence of each genotype was low, approximately 0.55 and 1.38 seedlings day^{-1}; in addition, at 13 ºC, the number of emerged seedlings was far lower than in the other experiments. According to Mertz *et al.* (2009), in an isoenzymatic analysis, a temperature of 13 °C causes a decrease in the activity of the enzyme esterase and an increase in the enzyme alcohol dehydrogenase. Furthermore, according to the authors, the start of esterase activity occurred only on the seventh day of the germination test, whereas, at the ideal temperature, it was already possible to see greater band intensity by the third day of germination.

Figure 2 - Emerged seedlings in relation to days after sowing the genotypes, in the four trials



For rice to achieve rapid and uniform emergence, the soil temperature should be equal to or higher than 20 ºC (STEINMETZ *et al.*, 2008). Therefore, at lower soil temperatures, there is a high risk of delay in emergence, with fewer emerged seedlings as a result. This delay and reduction in emerged seedlings directly affect the plant stand in the field, which in turn, implies a reduction in crop productivity. This happens due to the reduced number of fertile panicles, a result of the number of plants per unit area, this variable being one of the components that most affects grain production (RANI *et al.*, 2015).

The results also showed that as the temperature increased, the number of days to reach 50% of emerged seedlings decreased. In Trial I, half of the emerged seedlings was reached 11 days after emergence of the first seedling, this being the trial that took the most days to reach 50%. In trials II and III, this occurred five days after emergence of the first seedling, explained by the fact that for both trials the mean temperature was close, 17 ºC and

18 ºC respectively. Trial IV took the least time for 50% of the seedlings to emerge, requiring only three days.

The end of the counts and of the experiment was reached when there was no further seedling emergence in each of the genotypes in each trial.

An analysis of the statistical parameters in relation to evaluating tolerance in irrigated-rice genotypes at low temperatures in the emergence stage, determined from the speed of emergence index (SEI), showed differences in performance between the genotypes under study. The variation coefficient (CV) ranged from 5.07% to 31.50%.

The grouping based on the Scott-Knott test at 5% probability (Table 2) separated the genotypes into groups in the four trials, making it possible to characterise these genotypes in terms of tolerance to low-temperature abiotic stress on germination. In Trial I, with the lowest temperature, the analysis showed good accuracy for the control cultivars, since the Diamante and Norin Mochi genotypes presented as tolerant to cold, the Tomoe Mochi genotype as medium-tolerant to cold and the BRS Pampa, BRS Querência and BRS Firmeza genotypes as susceptible to cold.

In the same trial, Ostiglia, Baldo, Carnaroli, Selenio, Loto and Amarelo belonging to subspecies japonica stood out. However, the Ringo Miara-AC and Arelate genotypes of subspecies indica, proved to be similar to the genotypes of subspecies japonica, also being promising sources of cold tolerance. The results corroborate those of Mertz *et al*. (2009), who concluded that the Lemont and Oro cultivars of subspecies japonica, showed greater tolerance compared to the BRS Agrisul and BRS Chuí cultivars (subspecies indica), when submitted to a temperature of 13 °C during the germination-emergence stage. Among the 10 genotypes that showed the highest speed of emergence index,

Table 2 - Mean speed of emergence index (SEI) for the irrigated-rice genotypes under the conditions of a controlled environment at 13 ºC (Trial I), a controlled environment at 17 ºC (Trial II), field conditions with no cover (Trial III) and field conditions, covered with transparent polyethylene tarpaulin in a low tunnel (Trial IV)

Genotype	Trial			
	I	II	III	IV
Ostiglia	1.39 a	2.39 a	2,51 g	5,74 a
Ringo Miara-AC	1.27 a	2.90 a	3,29 e	3,70 e
Diamante	1.16 a	2.48 a	3,12 f	4,01 e
Arelate	1.17 a	2.63 a	2,57 g	1,28 i
Baldo	1.09 a	2.51 a	3,54 e	5,61 b
Carnaroli	1.05 a	2.65 a	5,02 b	5,32 c
Selenio	1.01 a	2.57 a	0,98 j	1,44 i
Loto	1.01 a	2.99 a	3,05 f	6,39 a
Amarelo	0.96 a	2.23 a	2,66 g	1,48 i
Norin Mochi	0.95 a	2.30 a	1,57 i	5,14 c
Originário chinese	0.88 b	1.77 b	1,51 i	3,91 e
Gladio	0.89 b	2.64 a	3,03 f	5,02 c
Saturo	0.87 b	2.35 a	1,99 h	3,31 f
Balilla	0.78 b	2.14 a	2,48 g	5,15 c
Thaibonnet	0.79 b	2.36 a	0,35 k	3,80 e
Tomoe Mochi	0.74 b	2.65 a	1,56 i	4,74 d
Sírio CL	0.68 b	2.39 a	3,81 d	5,12 c
Soulanet	0.64 b	2.44 a	4,43 c	5,52 b
Aurelia	0.61 b	1.98 a	3,28 e	4,19 e
Arborio	0.56 c	2.50 a	3,17 f	5,60 d
BRS Firmeza	0.52 c	2.39 a	2,17 h	4,54 d
Ranghino	0.53 c	2.32 a	3,92 d	6,16 a

Continued Table 2

BRS AG “Gigante”	0.49 c	2.15 a	2,35 h	2,48 h
BRS 7 “Taim”	0.30 c	1.70 b	3,82 d	4,23 e
Artiglio	0.28 c	1.59 b	3,05 f	4,83 d
IRGA 424	0.28 c	1.38 b	0,88 j	2,73 h
BRS 358	0.24 c	2.34 a	3,35 e	4,30 e
BR-IRGA 409	0.23 c	1.15 b	1,22 j	5,38 b
BRS Querência	0.24 c	1.43 b	1,54 j	3,44 f
Sambuc	0.20 c	0.54 c	2,91 f	5,95 a
BRS Pampeira	0.16 c	0.46 c	2,13 h	2,26 h
Vialone nano	0.17 c	1.81 b	5,54 a	6,12 a
Puitá Inta CL	0.15 c	1.35 b	0,94 j	0,93 j
Jasmine	0.11 c	1.33 b	1,04 j	2,93 g
BRS Pampa	0.10 c	0.51 c	1,50 i	4,16 e
Jasmine 85	0.03 c	1.20 b	2,67 g	3,90 e
Arroz Austral	0.00 c	1.28 b	1,41 i	5,57 b
Mean	0.61 D	2.00 C	2,55 B	4,23 A
CV (%)	31.50	17.20	7.94	5.07

*Mean values followed by the same letter do not differ by the Scott-Knott grouping test at 5% probability

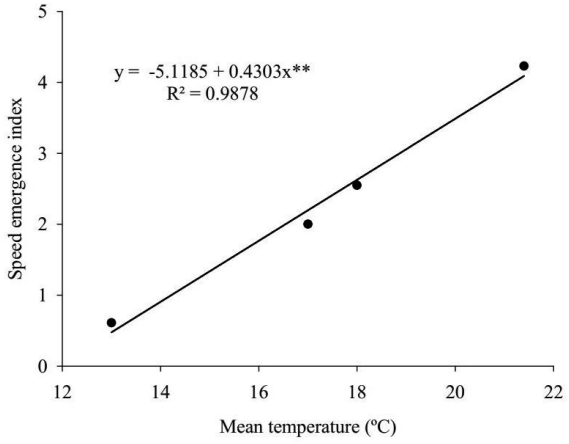
80% belong to subspecies japonica and only 20% are of subspecies indica, showing that subspecies japonica presented greater tolerance to cold. In Trial II, there was an increase of 227.87% in the SEI compared to the trial at 13ºC for the increase of 5 ºC. There was a large reduction in the proportion of genotypes of subspecies japonica among the highest values for the emergence speed index, which was represented by 60.8% of the total.

The trials conducted in the field, with and without a cover of polyethylene, showed great variation between the mean values for SEI, where genotypes of subspecies indica had a similar SEI with a greater number of emerged seedlings, compared to the trials at a lower temperature.

In Trial I, the minimum and maximum SEI found between genotypes were 0 and 1.39 respectively. For Trial II, the SEI varied from 0.46 to 2.99, a range of 2.53. In trials III and IV in the field, the minimum found was 0.35 and 0.93, and the maximum was 5.54 and 6.39, with a range of 5.19 and 5.46 respectively. This shows that the response of the genotypes under evaluation was affected by the trials.

In general, as the temperature decreased there was a significant reduction in the speed of emergence index. Comparing Trial I with the others trials, the mean speed of emergence index varied between 0.61 and 4.23. From the regression equation in Figure 3, the mean speed of emergence index increases linearly by 0.4259 for each 1 ºC increase in temperature. The coefficient of determination (R^2) of the first-order regression equation was 0.9878, showing that a large part of the variation in the speed of emergence index can be explained by the variation in soil temperature.

Figure 3 - Linear regression established between the mean temperature (ºC) and the mean speed of emergence index (SEI) for the 37 genotypes evaluated in the four trials



**Significant at 1% probability by t-test

CONCLUSIONS

1. The genotypes Ostiglia, Diamante, Baldo, Carnaroli, Selenio, Loto and Amarelo, belonging to subspecies japonica, and Ringo Miara-AC and Arelate, of subspecies indica, are promising sources of genetic tolerance to low temperature;
2. Temperatures of less than 17 ºC reduce the number of emerged seedlings and delay development in each of the rice genotypes under test, having a greater or lesser negative effect on the initial plant stand per unit area.

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