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SOAKING CURVE AND PHYSIOLOGICAL QUALITY OF RED RICE SEEDS UNDER SALT STRESS

CURVA DE EMBEBIÇÃO E QUALIDADE FISIOLÓGICA DAS SEMENTES DE ARROZ VERMELHO EM ESTRESSE SALINO

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ABSTRACT: Red rice stands out as a component of the diet of family farmers in the semi-arid Northeast. In this region, the accumulation of salts can occur on the soil surface causing saline stress to the plants. Studies related to salinity during the critical phases of plant development are recommended. Therefore, the objective was to evaluate the effect of salt stress on water uptake and germination of red rice seeds. The experimental design used was entirely randomized. The treatments were four levels of electrical conductivity of water: 1.0 dS/m, 2.0 dS/m, 3.0 dS/m and 4.0 dS/m. The following variables were evaluated: water absorption, humidity, germination percentage, germination speed index, and mean germination time. Salinity decreased the speed of water uptake and provided the lowest water accumulation required for germination of red rice seeds. Salinity increased the germination time of red rice seeds by improving germination performance.

Keywords: *Oryza sativaL.*, germination, vigor, salinity.

RESUMO: O arroz vermelho se destaca como componente da dieta dos agricultores familiares no semiárido Nordestino. Nessa região, o acúmulo de sais pode ocorrer na superfície do solo ocasionando estresse salino nas plantas. Estudos relacionada a salinidade durante as fases críticas de desenvolvimento das plantas são recomendados. Por isso, objetivou-se avaliar o efeito do estresse salino na absorção de água e germinação das sementes de arroz vermelho. O delineamento experimental usado foi inteiramente casualizado. Os tratamentos foram quatro níveis de condutividade elétrica de água: 1,0 dS/m, 2,0 dS/m, 3,0 dS/m e 4,0 dS/m. Foram avaliadas as seguintes variáveis: absorção de água, umidade, porcentagem de germinação, índice de velocidade de germinação e tempo médio de germinação. A salinidade diminui a velocidade de absorção de água e proporcionou o menor acúmulo de água necessário para germinação de sementes do arroz vermelho. A salinidade aumentou o tempo de germinação das sementes de arroz vermelho melhorando o desempenho germinativo.

Palavras-chave:*Oryza sativa L.*, germinação, vigor, salinidade.

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INTRODUCTION

Rice is an important ingredient in the daily diet of Brazilians, reaching all social classes, Barbosa (2007). The major rice production centers in the country are located in the South and Southeast representing more than half of all national production (CONAB, 2019). In Northeast Brazil, red rice stands out as a component of the diet of family farmers in the state of Paraíba, Rio Grande do Norte, Pernambuco, Ceará and Alagoas (PEREIRA; MORAIS, 2014).

In the Northeast, most red rice crops occur under dryland conditions that can eventually occur water deficit during the crop cycle. It is also possible that in these regions, the low rainfall associated with high evapotranspiration and the presence of the impermeable layer can provide the accumulation of salts on the soil surface (RIBEIRO et al., 2016). In drastic situations, excess soil salts have decreased the germination of agricultural crops (OLIVEIRA, 2009; FREIRE et al., 2016). Freire et al. (2018) recommend that salinity effect studies be focused on critical stages such as seed germination.

Salt stress can differentially affect germination and initial seedling growth of rice cultivars. Freire et al. (2018) found that the white criollo rice cultivar ligeirinho showed the best percentage of emergence up to the electrical conductivity of 3.0 dS/m, while the cultivar casadinho showed increasing emergence as the salinity of the solution increased. In another paper, the saline effect provided the best germination performance and growth in root dry mass for white rice cultivar BRS AG (CAVALCANTE et al., 2019). Although we observed these results for white rice, studies to evaluate the physiological potential and tolerance of red rice to salinity are scarce, especially in the critical phases of germination: water soaking and at radicle protrusion.

Studying the effect of salinity especially on the water soaking process by red rice seeds may provide important information on the behavior of germination speed and percentage. This may be related to the lower water availability and the toxic effects on plasma membranes of salinity (LIMA et al., 2005; SOUZA et al., 2010; LARRÉ et al., 2011). These factors resulting from salinity can delay and negatively affect germination. However, it is not elucidated how the lower water uptake positively affects seed germination as observed by Freire et al. (2018) Cavalcante et al. (2019).

In this context, evaluating the effect of salinity during water soaking by red rice seeds will provide important information on the relationship of soaking time with the amount of water absorbed. Thus, we will also verify how salinity influences from soaking to seed germination. Therefore, we aimed to evaluate the effect of salt stress on water uptake and germination of red rice seeds.

MATERIAL AND METHODS

The work was conducted in the Seed Technology Laboratory on the Auroras campus of the Universidade da Integração Internacional da Lusofonia Afro-Brasileira (UNILAB), located in Redenção-CE, at the geographical coordinates 4°13'05.1 "S and 38°42'50.3 "W. Seeds of a Creole variety of rice-red precedent from the Trairi-CE region were used. The germination test of the seeds was performed before installation of the experiment, verifying a germination percentage of 90%.

To prepare the electrical conductivity of the water to moisten the germ paper, sodium chloride, calcium and magnesium salts (NaCl, CaCl2.2H2O and MgCl.6H2O), in the proportion 7:2:1 respectively (RHOADES et al., 2000), were added to distilled water, obtaining concentrations of 2.0 dS/m, 3.0 dS/m and 4.0 dS/m.

The soaking curve was performed with 50 seeds between the germitest paper for each electrical conductivity mentioned above (BRASIL, 2009). The amount of each solution was established according to a ratio of 2.5 times the mass of the paper. The water absorption determinations were performed at the initial intervals of 2 and 6 hours. After this period, the determinations were performed every 6 hours of soaking. The total time was 48 hours. The water absorption was calculated using the expression:

$$\% \text{ Water Absorption} = ((m\_(ti+1)-m\_t0)/m\_t0)*100\%$$

where mti+1 corresponds to seed mass at the nth soaking period. The mt0 is the seeds mass at the zero time of water absorption. After water uptake was determined, the water uptake rate was calculated as the difference between the percentage of uptake at the last time minus uptake in the previous period. No regression analysis was performed for this variable.

In turn, for water uptake a non-linear regression analysis was performed for the model called rectangular hyperbola:

$$\hat{y}=ax/(b+x)$$

where ŷ corresponds to absorption estimated by the regression equation. The coefficient corresponds to the maximum water accumulation by rice seeds. The coefficient b corresponds to the period when half of the maximum water uptake by rice seeds occurs. The estimation of the coefficients was obtained automatically by the sigmplot program trial version 30 days.

The Durbin Watson and Kolmogorov-Smirnov test was applied to assess homoscedasticity and normality of the data. Spearman's test was calculated to assess the correlation between the absolute values of the residuals and the observed value of the dependent variable. The adjusted R, predicted error, and variance inflation factor were used, and the F test (5% probability) was used to select the best fit model of the rectangular hyperbola equations with 2 and 3 parameters. The program used was sigmplot version 14.

After the determination of the soaking, another experiment was performed to evaluate the physiological quality of the seeds in the above mentioned salt solutions.

The degree of seed moisture was evaluated according to the instructions of the RAS (BRASIL, 2009), using two samples with 5.0g of seeds each, placed in aluminum capsules sanitized and subjected to a forced air circulation oven at ±105 °C for 24 hours. Then the seeds were removed and placed for 10 min in a desiccator. The results were expressed as the mean percentage.

The germination test was performed according to the specifications of the RAS (BRASIL, 2009), using seeds from the 2019.2 crop. Seeds that presented radicles greater than or equal to two millimeters (mm) were considered germinated. The seeds were sown on germitest paper moistened with the amount of concentrations two and a half times the mass of the paper and placed to germinate in the germination chamber at a temperature of 25 °C. The results were presented in percent germination.

The experimental design was entirely randomized, with 4 treatments and 4 repetitions of 50 seeds. The counting of germinated seeds was performed daily, until the maximum germination expression of the lot. The germination velocity index (GVI) was calculated based on the formula described by Maguire (1962) and mean germination time (MGT) according to Labouriau (1983).

The results were submitted to variance and regression analysis using the ASSISTAT program. 7.7 Beta program. In the regression analysis, the equations were chosen based on the significance of the regression coefficients at the significance level of 1% (**) and 5% (*) by the F test, and the highest determination coefficient (R^2).

RESULTS AND DISCUSSION

The salt solutions influenced the water uptake of the seeds (Figure 1). These water uptake behaviors in the treatments were fitted to a rectangular hyperbola regression equation. This estimates in the numerator the maximum water accumulation (a) and in the denominator the period in which half of this maximum water accumulation occurs (b). The highest water uptake by seeds was approximately 224% in the 1.0 dS/m salt solution (Figure

1A). The shortest period to observe this water uptake was in the 1.0 dS/m solution. Half of this uptake was reached in 13 hours of soaking.

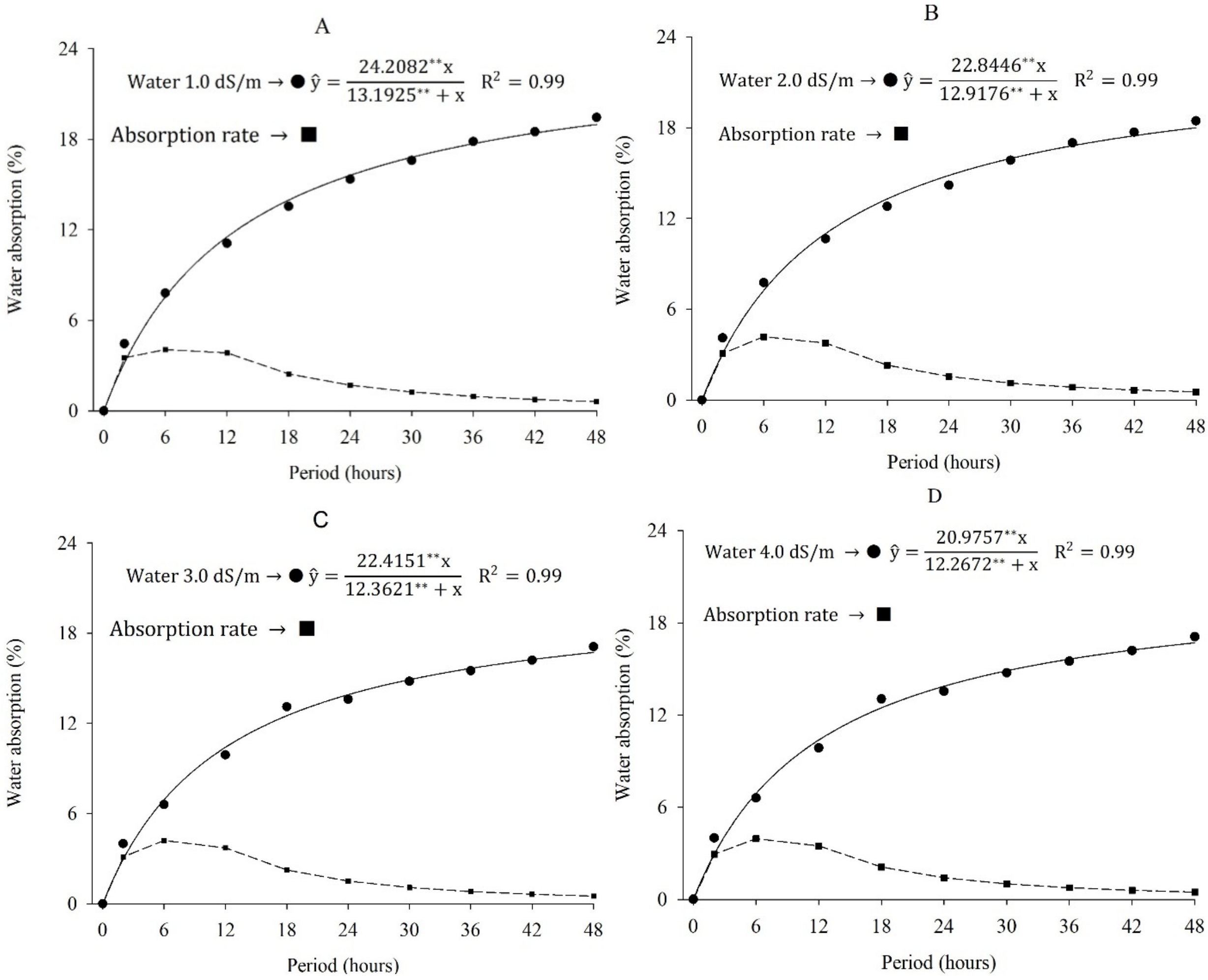


Figure 1.Percentage of water uptake of rice seeds in substrate moistened with water with electrical conductivities of 1.0 dS/min (A), 2.0 dS/m in (B), 3.0 dS/m in (C) and 4.0 dS/m in (D).

In the other soaking processes, especially in the substrate with saline water of 4.0 dS/m (Figure 1D), the maximum absorption was 4% lower when compared to that of 1.0 dS/m (Figure 1A). Half of this uptake occurred at approximately 12 hours for the salt solution. For this same period, the 1.0 dS/m salt solution gave the seeds a percentage of 1.6% more than the 4.0 dS/m treatment. This difference in water input in the later stages of soaking in the water with lower salinity and in the saline solutions may affect the structure of the plasma membrane of the seeds due to the rapid absorption of water (ZUCARELI et al., 2011).

The advancement of the soaking period resulted in the decrease in the rate of water absorption by rice seeds in all treatments after 24 hours (Figure 1). Such results are different from other works that observed an apparent stabilization of seed hydration in the early stages. Generally, this stabilization interval occurs between 9 and 20 hours after the beginning of the soaking process for white rice according to (YANG et al., 2007; BORTOLOTO et al., 2008). According to these authors it is common to see in germinated rice seeds a greater absorption of water and then after this a period of apparent stabilization of hydration, as described by Bewley and Black (1994).

Given the difference in water uptake of seeds between salinity treatments, we verified that the means of the variables related to physiological quality were influenced (Table 1). The higher speed of water uptake can alter the structure of the plasma membrane of the seeds' cells, hindering the enzymes with functions to repair the damage in the plasma membrane. Whereas, the lower speed of water absorption may favor the seeds due to the better performance of the enzymes that repair the plasma membranes. Zucareli et al. (2011) and Silva and Villela (2011), reported that the slow restructuring of plasma membranes may be related to seed vigor and water availability.

Table 1. Summary of analysis of variance (ANOVA) for germination percentage (G%), germination velocity index (GVI) and mean germination time (MGT) of red rice cultivar under different levels of salinity of irrigation water.

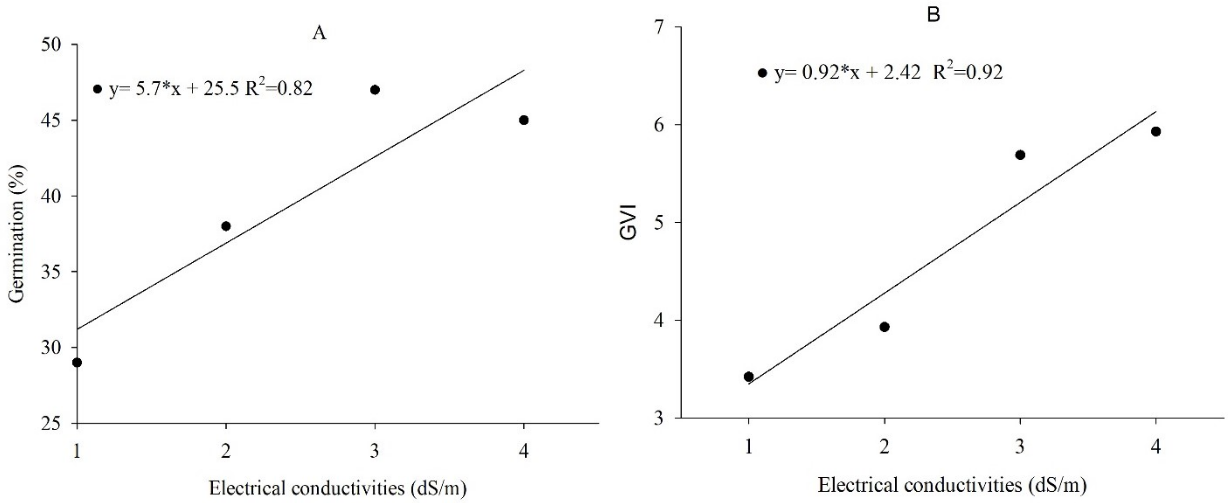
FV	GL	Mean square		
		G%	GVI	MGT
Tratament	3	358.20**	8.50**	0.81**
Residue	16	39.10	1.00	0.09
C.V. (%)		15.75	20.95	6.07

FV: Source of variation, GL: Degree of freedom, CV (%): Coefficient of variation, **: Significant at 1% probability level ($p < 0.01$), *: Significant at 5% probability level ($.01 =< p < .05$).

With regard to germination percentage, it is observed in Figure 2A, that the model that best fitted was quadratic polynomial.

The maximum estimated germination value was 46% for a water electrical conductivity of 3.6 dS/m. It is important to mention that rapid water absorption may have compromised membrane disruption of the seed cell compartments due to damage to the plasma membrane.

This reflected negative effects on the germination percentage of rice seeds, especially in the treatment with lower salinity. It is also important to mention that seeds with protrusion of the radicle were considered germinated



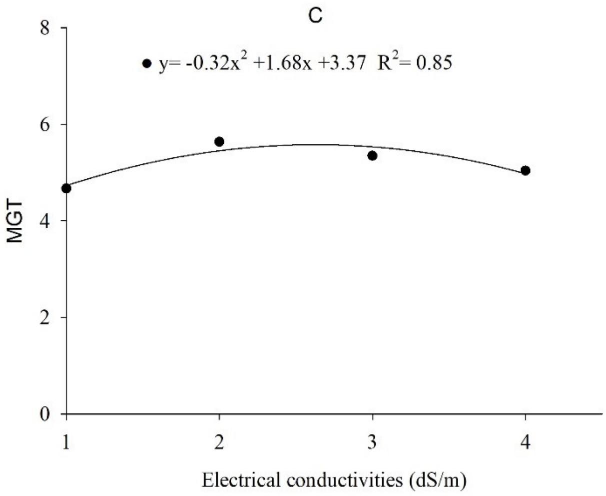


Figure 2.Germination percentagem in (A), germination speed index (GVI) in (B) and mean germination time (MGT) in (C) of rice soaked with water of different electrical conductivities.

The treatments with the highest salt contents obtained better germination results. These results differ from research developed with white rice tolerance to salinity. Benitez et al. (2010) compared the seeds of rice genotypes soaked only with solutions of 4 and 8 mg/L NaCl, these authors observed that the seeds showed lower performance in germination at the highest dose. In turn, Almeida et al. (2001), evaluating the behavior of four rice genotypes in ECs of 0.0; 2.5; 5.0; 7.5 and 10.0 dS/m, found that seedling emergence decreased as the salt concentrations increased. This difference between results may be related to the salt concentrations used.

The increase in EC provided an increase in the germination speed index (Figure 2B). In which the treatment with 4 dS/m reached a GVI of 5.92 germinated seeds per day while the treatment with 1.0 dS/m was 3.42 germinated seeds per day. This result diverges from the data obtained by Larré et al. (2011) that when evaluating the physiological quality of seeds of rice cultivars BRS Bojurú and BRS Querência subjected to salt stress obtained reduced vigor as a function of increasing salt concentration. Following a similar pattern, Neto et al. (2016) in work performed with salt stress in corn seeds, attested that the GVI is negatively affected when there is an increase in NaCl concentrations.

Differently from these results, in the present work, the reduction of osmotic potential with the increase of the electrical conductivity of the solution may have contributed with the restructuring of the membranes of red rice seeds and consequently, increasing the number of germinated plants per day. It is interesting to verify that in the more saline solution the maximum amount of water absorbed to allow germination was lower (Figure 1D). Consequently, the time to absorb this amount was also shorter when compared to the less saline treatment.

For the mean germination time, the model that best fitted the data was polynomial quadratic (Figure 2C). The estimated value of the electrical conductivity of the water of 2.62 dS/m provided the longest mean germination time of 5.57 days. This means that as the salts increase in the salt solution the seeds of this lot presented a longer period to germinate. These results are similar to those obtained by Rabbani et al. (2013) and Lewandoski et al. (2016). These authors found that sunflower seeds when subjected to increasing electrical conductivities increased the time to germinate.

These results are analogous to those of Coelho et al. (2017), who when analyzing the average germination time of four varieties of cowpea beans under salt stress, observed that salinity negatively affected all cultivars. As the electrical conductivities increased, more germination time was required, attributing this result to the reduction of osmotic potential in the seeds. Like Freire et al. (2018), they also observed that the average speed of rice emergence under salt stress suffered negative influence in the four cultivars tested with the ECs: 0.5; 1.0; 2.0; 3.0 and 4.0 dS/m.

The salinity of the solutions with higher electrical conductivity decreased the water absorption for the seeds of red rice during the soaking process.

This effect provided an increase in the germination percentage and higher germination speed index. Consequently, we observed a longer period for seed germination. It is worth noting that further studies of the effect of salinity on the germination of red rice seeds with higher saline concentrations are needed.

CONCLUSIONS

Salinity decreased the speed of water uptake and provided the lowest water accumulation required for germination of red rice seeds.

Salinity increased the germination time of red rice seeds by improving germination performance.

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