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EVALUATION OF THE RED RICE IMBIBITION TIME UNDER DIFFERENT FERTILIZING SOURCES

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ABSTRACT

Red rice is considered the predominant weed species in rice fields, causing significant economic losses to producers. The objective of this study was to evaluate the behavior of red rice seeds during the soaking of seeds in water, as well as the development of plants under two fertilization sources (NPK and complete nutrient solution). Seeds were subjected to different soaking durations in water: 0, 10, 20, 30, 40, 50, and 60 minutes. After four days of germination, the seedlings were divided into two treatment groups: (i) plants fertilized with NPK and (ii) plants fertilized with complete nutrient solution. The experiment was conducted in a completely randomized design, evaluating the variables: germination, number of leaves, plant height and dry matter accumulation. A significant increase in germination percentage, number of leaves, plant height and dry matter accumulation in both the aerial part and roots was observed with longer seed soaking durations. This effect was more expressive in red rice plants fertilized with the complete nutritive solution. Thus, we conclude that this fertilizer source enhances the competitive ability of this weed species against cultivated rice.

Palavras-chave:
Germinação
Planta invasora
Nutrição
Oryza sativa

AVALIAÇÃO DO TEMPO DE EMBEBIÇÃO DO ARROZ VERMELHO SOB DIFERENTES FONTES DE FERTILIZAÇÃO

RESUMO

O arroz vermelho é considerado a principal planta daninha na maioria das lavouras de arroz, causando sérios danos ao produtor. O objetivo do trabalho foi avaliar o comportamento de sementes de arroz vermelho durante a embebição das sementes em água, bem como o desenvolvimento das plantas sob duas fontes de fertilização (NPK e solução nutritiva completa). As sementes foram submetidas a diferentes tempos de embebição em água: 0, 10, 20, 30, 40, 50 e 60 minutos. Após 4 dias de germinação, as mudas foram divididas em dois grupos: (i) plantas fertilizadas com NPK e (ii) plantas fertilizadas com solução nutritiva completa. O experimento foi conduzido em delineamento inteiramente casualizado, avaliando-se as variáveis: germinação, número de folhas, altura da planta e produção de matéria seca. O aumento significativo nas variáveis % de germinação, número de folhas, altura de plantas de matéria seca da parte aérea e raízes, em função do tempo de aumento da escala de embebição das sementes em água foram mais expressivos nas plantas de arroz vermelho fertilizadas com solução nutritiva completa, concluindo que esta fonte de fertilizante confere a esta espécie daninha alta capacidade de competição com o arroz cultivado.

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INTRODUCTION

The estimated rice production for the 2023/2024 harvest was 10.59 million tons, representing a 5.5% increase compared to the volume produced in the previous harvest. Of this total, irrigated rice production accounted for 9.75 million tons, while dryland rice production reached 0.84 million tons (CONAB 2024).

Rice is the staple food for nearly half of the world's population and serves as primary source of starch (Yang *et al*., 2022, Zhang *et al*., 2023). Red rice has long been recognized as a healthy food in several Asian countries, including China, Japan and Korea, and has gained increasing attention due to its phytochemical content, particularly phenolic compounds with antioxidant properties. The concentration of these compounds varies significantly depending on edaphoclimatic conditions (Liu *et al*., 2017; Müller *et al*., 2021).

Seed germination involves the reactivation of embryo growth through a sequence of metabolic events, leading to the rupture of the tegument by the radicle. This process begins with water absorption by the seed and finishes with the elongation of the embryonic axis. Imbibition is fundamental for germination, as it initiates metabolic activity, facilitating the mobilization and assimilation of seed reserves for subsequent growth. The rate of imbibition depends on species-specific characteristics, such as chemical composition and tegument permeability (MALTA, 2011).

Thus, the objective of this study was to evaluate the behavior of the red rice seeds during imbibition, as well as the subsequent plant development under two fertilizing sources (NPK and complete nutrient solution), with the aim of improving its management and enhancing competitiveness of cultivated rice against this weed.

MATERIAL AND METHODS

The experiment was conducted under greenhouse conditions at the department of soil science, Institute of Agrarian Sciences, Federal Rural University of the Amazon (UFRA).

The red rice seeds used in the experiment were obtained from the municipality of Apodi, Rio Grande do Norte, with 15% of moisture content. The seeds were subjected to different imbibition duration (min.): 0, 1, 20, 30, 40, 50 and 60 minutes. For each imbibition period, 20 previously selected seeds were immersed in 0.150 L of distilled water. Upon completion of imbibition process, the seeds were sown in 0.30 x 0.30 m plastic bags filled with sand at field capacity, with 10 seedlings allocated per imbibition treatment. After four days of germination, seedlings were divided in two experimental groups: (i) plants receiving a single fertilization with 0.5 g of NPK 20-20-20 (containing 20% nitrogen, 20% phosphorus, 20% potassium), and (ii) plants fertilized with 0.050 L of a complete nutrient solution. The nutrient solution was composed of KH_2PO_4 (1M), KNO_3 (1M), $Ca(NO_3)_2.4H_2O$ (1M), $MgSO_4.7H_2O$ (1M) , a micronutrient solution(1M) and Fe-EDTA solution (1M). Fertilization was applied every four days for a total duration of 20 days.

The experiment was conducted using a completely randomized experimental design, in a 2x7 factorial scheme, consisting of two fertilization treatments and seven seed imbibition durations, with two repetitions per treatment. The data were subjected to analysis of variance and mean comparisons between fertilization treatments within each imbibition duration were performed independently using Tukey' s test ($p < 0.05$).

The germination percentage (GER) was calculated following the methodology of Chu *et al*. (2020), using the equation 01:

$$GER=(Ni \times 100)/Ns \qquad (01)$$

Where,
Ni represents the number of germinates seeds, defined as those that emerged from the substrate, and Ns denotes the total number of seeds sown.

A regression analysis of the germination percentage data was conducted as a function of seed imbibition time, using Microsoft Excel.

Throughout the 20-day experiment, physical analyses were performed every 5 days to monitor plant growth, including measurements of plant height and leaf number. At the end of the experiment dry mass of both the aerial part and the root systems was determined. Leaf number was assessed through the manual counting of the leaves with sheath, while plant height was measured using a 5 m metal measuring tape. Dry mass determination involved the collection and separation of plant material (roots and aerial part), followed by drying in a forced-air circulation oven at 70 °C until a constant weight was achieved.

The results of the plant physical analysis were evaluated using analysis of variance (ANOVA), followed by Tukey's test ($p < 0.05$) for mean comparisons. Statistical analyses were performed using the RBio software (Bhering, 2017).

RESULTS AND DISCUSSION

The results of the regression analysis of germination percentage (GER) in red rice as a function of seed imbibition time under two different fertilization treatments are presented in Figures 1a and 1b.

Upon analyzing Figure 1, the germination percentage of red rice seeds increase in relation to the imbibition time, reflecting the break in dormancy. For plants fertilized with NPK, the germination percentage at various imbibition times were as follows: 0 minutes: 68%; 10 minutes: 72%; 20 minutes: 75%; 30 minutes: 79%; 40 minutes: 81%; 50 minutes: 83% e 60 minutes: 85%). Both regression analyses of the germination percentage data as a function of seed imbibition time exhibited a linear relationship.

From a nutritional perspective , the macro- and micronutrients are essential elements that perform specific functions in plant metabolism. These nutrients are more effective when applied in an aqueous medium (complete nutrient solution), as this enhances their diffusivity to the root system compared to soil applications (Malavolta, 2018). Germination of red rice leads to the release and/or increase in the content of certain compounds, making it a promising alternative for incorporation into food products (Müller *et al*., 2021).

The results of the number of red rice leaves as a function of cultivation time under two fertilization treatments used in the study, NPK and complete nutrient solution, are presented in Figure 2.

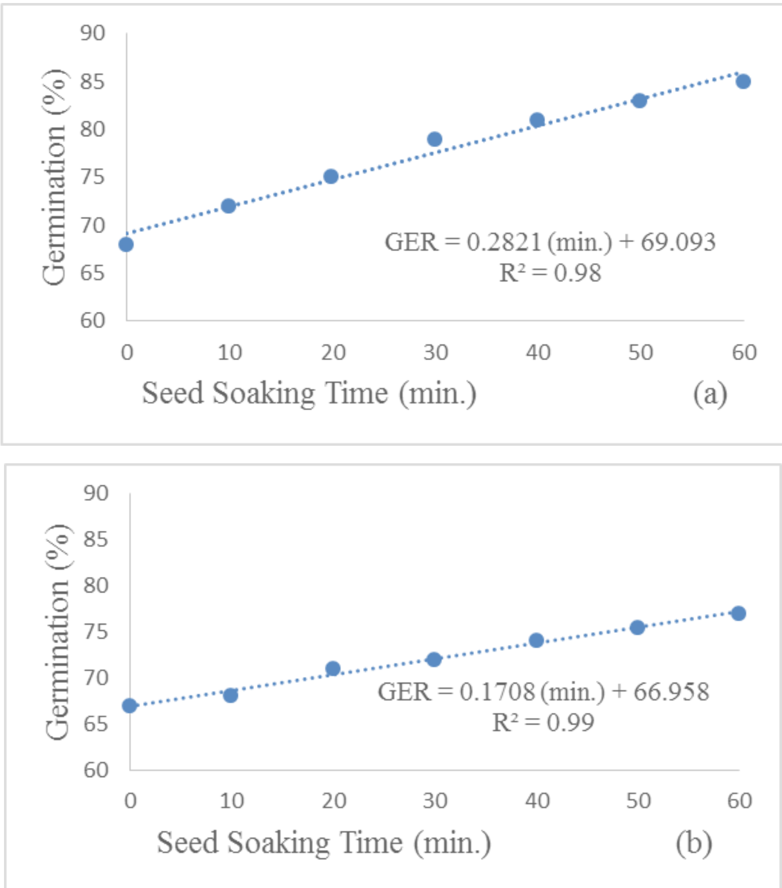

Figure 1. Percentage of germination of red rice as a function of seed imbibition time under two fertilization treatment (NPK (a) and complete nutrient solution (b)) is presented. The values represent the mean of ten repetitions ± standard deviation

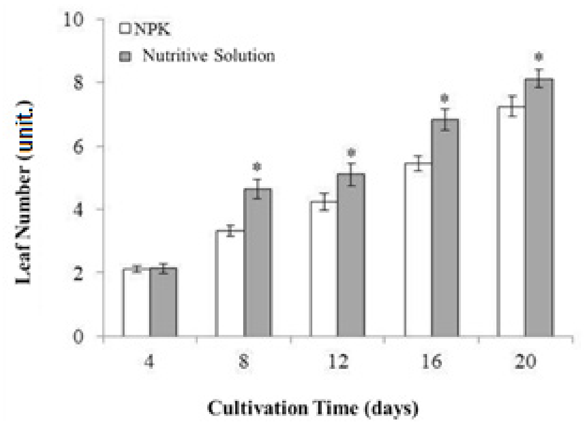

Figure 2. Number of leaves of red rice as a function of the cultivation time under two fertilization treatments (NPK and complete nutrient solution). The values represent the mean of seventy repetitions ± standard deviation. * Indicates significant differences between fertilization treatments on each day of evaluation

For the variable "number of leaves", no significant differences were observed by the Tukey test ($p > 0.05$) for the different imbibition times when compared within the same days of evaluation (4, 8, 12, 16 and 20 days) for each nutrient source. However, when comparing the global means of each nutrient source in the different days, significant differences were detected by the Tukey test ($p < 0.05$). From day 8 to the end of the experiment (day 20) a significant difference was observed in the number of leaves for plants cultivated with the complete nutrient solution.

The observed values for plants cultivated with nutrient solution were as follows "4 days: 2 leaves; 8 days: 3 leaves; 12 days: 4 leaves; 16 days: 5 leaves; and 20 days: 7 leaves per plant". In contrast, plants fertilized with only NPK presented the following values: "4 days: 2 leaves; 8 days: 4 leaves; 12 days: 5 leaves; 16 days: 6 leaves; and 20 days: 8 leaves per plant". Productive tillers are plants that consist of a stem, roots and leaves, and are capable of producing flowers [productive]. Rice tillers also produce panicle and may not reach a specific age. The number of productive tillers depends on the number of tillers produced by the plant. As the number of tillers increases, the number of productive tillers also increases (Swasti *et al*., 2020).

The results of plant height during the 20 days of red rice cultivation with an increasing seed imbibition time as a function of the cultivation time and fertilization with NPK or complete nutrient solution are shown in Tables 1 and 2.

Table 1. Plant height of red rice with increasing seed imbibition time (in minutes) as a function of the cultivation time (in days) and fertilization with NPK

Time (days)	Plant Height (cm)						
	0'	10'	20'	30'	40'	50'	60'
4	4.1±0.1^{D}	4.5±0.2^{C}	4.5 ±0.2^{C}	5.2 ±0.3^{B}	5.3 ±0.1^{B}	5.5 ±0.4^{A}	5.5 ±0.4^{A}
8	8.2±0.5^{G}	9.5±0.7^{F}	10.3±0.9^{E}	11.2±0.9^{D}	12.4±0.9^{C}	13.1±0.9^{B}	13.5±0.9^{A}
12	24.4±0.9^{G}	25.1±0.8^{F}	26.2±0.9^{E}	27.3±0.8^{D}	28.1±0.7^{C}	29.2±0.9^{B}	30.0±0.9^{A}
16	28.3±1.6^{G}	29.2±1.2^{F}	31.3±1.8^{E}	33.1±1.3^{D}	34.3±2.2^{C}	36.3±2.0^{B}	38.1±2.6^{A}
20	36.0±2.2^{G}	37.3±2.4^{F}	38.4±2.8^{E}	39.0±2.5^{D}	41.2±2.4^{C}	45.4±2.3^{B}	47.2±2.8^{A}

The values are the mean of ten repetitions ± standard deviation. Capital letters in the same row represents statistical difference between plants of different seed imbibition time (0, 10, 20, 30, 40, 50 e 60 minutes) within each evaluation day

Table 2. Height of the red rice with increasing seed imbibition time (in minutes) as a function of the cultivation time (in days) and fertilization with NPK

Time (days)	Plant Height (cm)						
	0'	10'	20'	30'	40'	50'	60'
4	3.5±0.1^{C}	4.1±0.4^{BC}	4.3±0.2^{B}	4.5±0.3^{B}	5.1±0.3^{AB}	5.3±0.5^{A}	5.5±0.4^{A}
8	10.1±0.8^{D}	10.5±0.9^{D}	11.1±0.9^{C}	12.3±0.9^{B}	13.3±0.8^{B}	14.4±0.9^{A}	14.5±0.9^{A}
12	28.2±1.2^{F}	29.3±1.8^{F}	31.3±1.5^{E}	34.1±1.6^{D}	36.4±1.1^{C}	38.1±1.9^{B}	39.0±1.6^{A}
16	31.3±2.0^{F}	32.1±2.1^{F}	34.1±2.2^{E}	36.2±2.3^{D}	38.2±2.09^{C}	40.2±2.5^{B}	42.3±2.3^{A}
20	38.4±2.5^{F}	39.2±2.1^{F}	40.2±2.4^{E}	43.4±2.6^{D}	45.1±2.7^{C}	48.2±2.8^{B}	50.1±2.9^{A}

The values are the mean of ten repetitions ± standard deviation. Capital letters in the same row represents statistical difference between plants of different seed imbibition time (0, 10, 20, 30, 40, 50 e 60 minutes) within each evaluation day

The parameter plant height of the red rice seedlings, based on increasing seed imbibition time in water (0, 10, 20, 30, 40, 50 and 60 minutes) in the different days of evaluation (4, 8, 12, 16 and 20 days), showed significant difference by the Tukey test ($p < 0.05$), for the different fertilization treatments (NPK and complete nutrient solution). Plants with longer imbibition times exhibited greater vegetative development, likely due to the break in dormancy. Furthermore, plants grown in the nutrient solution achieved higher values compared to those fertilized with NPK, which can be attributed to the greater diffusivity of nutrients in the aqueous medium (Malavolta, 2018).

As shown in Tables 1 and 2, plant height is an important evaluation parameter because, according to Rocha *et al*. (2020) greater plant height confers enhanced competitive ability. However, excessive plant height my result in lodging and potential productivity losses, which could be mitigated through crosses with white rice cultivars. The results of dry matter production in red rice as a function of seed soaking time, under two fertilization treatments used in the study, NPK and complete nutrient solution, are presented in Figure 3.

When comparing the average values of the dry matter production of the roots between the different fertilization treatments applied to red rice plants, a significant difference ($p < 0.05$) was observed both between the different seed imbibition times and between the fertilizer sources. The most notable variation occurred with the application of the complete nutrient solution, resulting in dry matter production ranging from 0.15 to 0.71 grams, compared to the NPK fertilization treatment (0.11 to 0.35 g). This variation is directly related to the deficiency of other nutrients in plants fertilized with NPK, leading to a reduction in the root system.

Figure 3 illustrates the dry matter production of both the aerial part and roots after 20 days of experiment, as a function of seed the imbibition time under two fertilizer sources (NPK and Complete Nutritive Solution). Similar to the physical parameters of the plants, dry matter production is also associated with the productivity of the crop. The average values for the aerial part dry matter ranged from 0.29 to 0.62 grams per plant fertilized with NPK and from 0.37 to 0.99 grams per plant fertilized with complete nutrient solution at 20 days cultivation, with significant differences observed between the different seed imbibition times and fertilizer sources. Comparatively higher values were reported by Malta, (2011), with an average of 2.09 grams per plant, when studying the growth

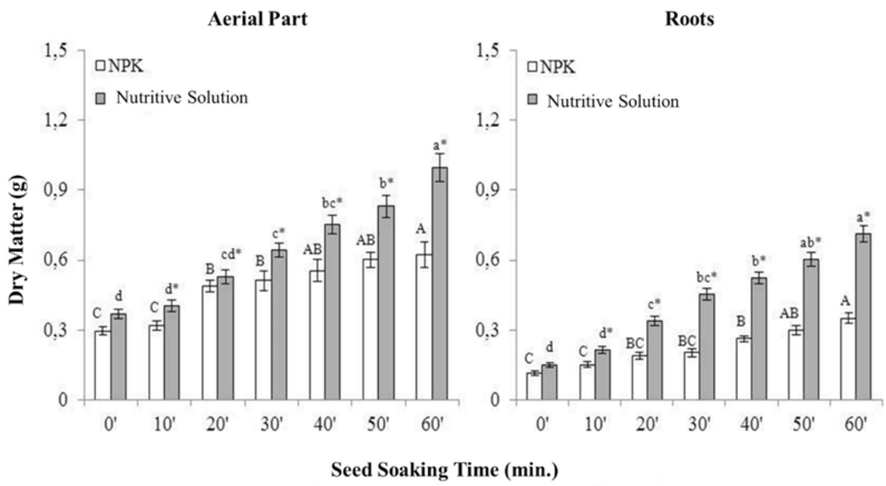

Figure 3. Dry matter production of red rice as a function of seed soaking time under two fertilization treatments (NPK and Complete Nutrient Solution).Values represent the average of ten repetitions ± standard deviation. Capital letters indicate significant differences in plant height at different seed imbibition times (0, 10, 20, 30, 40, 50 and 60 minutes) under NPK fertilization. Lowercase letters indicate differences between plants at different imbibition times under both NPK and complete nutrient solution fertilization. *Denotes significant differences between treatments with different fertilizers at each imbibition time

of red rice plants subjected to doses of charcoal with and without the application of organic matter (bovine manure). These results highlight the greater efficiency of using a nutrient solution and other fertilizer sources compared to NPK for red rice cultivation.

Red rice exposed to alkaline stress (such as 100 and 400 mM sodium bicarbonate) showed a significant reduction in the levels photosynthetic pigments, with chlorophyll decreasing by 80.0–84.1% and carotenoids by 84.1–92.6%. Additionally, overall growth was negatively impacted, as root length decreased by 68.6–92.3% and aerial part length by 57.6–82.8% (Sharma *et al*., 2023). The most abundant macronutrients found in red rice were carbohydrates (87.2 g 100 g^{-1} dw), followed by proteins (9.1 g 100 g^{-1} dw), fat (2.6 g 100 g^{-1} dw), ash (1.1 g 100 g^{-1} dw), and sucrose and raffinose were the only sugars detected, with sucrose exhibiting the highest concentration (0.74 g 100 g^{-1} dw) (Baptista *et al*., 2024).

Red rice and its by-products have also been recognized as potential food sources. In their study, Chen *et al*, (2023), demonstrated the potential use of red rice seed hulls as a functional food for the prevention and treatment of alcoholic liver disease. The consumption of red rice (*Oryza sativa* L.) has recently increased, partly due to its potential health benefits in the prevention of various diseases (Ontawong *et al*., 2024).

It is worth noting the precautions regarding citrinin, a mycotoxin typically produced after rice harvest due to contamination by filamentous fungi from the genera *Aspergillus*, *Penicillium*, and *Monascus* (Ponz-Perelló *et al*., 2024). These authors emphasize that red rice, commonly consumed in Asia, and dietary supplements derived from red rice are the primary sources of human exposure to citrinin.

CONCLUSIONS

- At 20 days of cultivation, red rice plants exhibited enhanced development when the seeds were soaked in water for longer durations (60 minutes), which facilitated a more effective break in seed dormancy, especially when combined with fertilization using a complete nutrient solution.
- The analysis of growth and development variables revealed that different fertilization sources endowed this weed species with a high competitive ability against cultivated rice. Consequently, genetic improvement programs should consider these variables when evaluating and selecting advanced rice lines.

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AUTHORSHIP CONTRIBUTION STATEMENT

NUNES, F. G. T.: Conceptualization, Data curation, Methodology, Supervision, Writing – review & editing; **BRITO, A. S.:** Data curation, Project administration, Writing – review & editing; **CARVALHO, F. I. M.:** Formal Analysis, Methodology, Software, Supervision, Writing – review & editing; **ROQUE, C. G.:** Funding acquisition, Methodology, Writing – review & editing; **CASTRO, T. R.:** Formal Analysis, Funding acquisition, Writing – review & editing; **OLIVEIRA, J. T.:** Data curation, Project administration, Supervision, Visualization, Writing – review & editing; **SILVA, P. A.:** Formal Analysis, Methodology, Software, Supervision, Writing – review & editing.

DECLARATION OF INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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