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Pre-drying periods and temperatures to overcome dormancy of rice seeds of the cultivar IRGA 431 CL

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ABSTRACT: Rice seeds have a dormancy which is understood as resistance to pre- and postharvest germination. Thus, the present study aims to evaluate periods and temperatures in the pre-drying of rice seeds (cultivar IRGA 431 CL) to overcome dormancy. An experiment was carried out in a completely randomized design at the Didactic and Seed Research Laboratory, at the Federal University of Santa Maria. Three lots of the cultivar IRGA 431 CL were used. For the present study, temperatures of 35, 40, 45, 50 and 55 °C in a forced-air circulation oven were used to overcome seed dormancy. At each temperature the seeds remained for periods of 24, 48, 72, 96, 120, 144 and 168 h. First count, germination, and seedling root and shoot lengths were evaluated in the experiment. The data obtained were subjected to analysis of variance and compared by response surface. Temperature between 41 and 44 °C for a period of 95 h is more indicated to overcome dormancy in seeds of the IRGA 431 CL cultivar in a forced-air circulation oven.

Index terms: exposure time, high temperatures, Oryza sativa L.

RESUMO: As sementes de arroz possuem uma dormência a qual é entendida como uma resistência à germinação pré e pós-colheita. Assim, o presente estudo tem como objetivo avaliar períodos e temperaturas na pré-secagem de sementes de arroz (cultivar IRGA 431 CL) para superação da dormência. Foi conduzido experimento em delineamento inteiramente casualizado no Laboratório Didático e de Pesquisa em Sementes, da Universidade Federal de Santa Maria. Foram utilizados três lotes da cultivar IRGA 431 CL. Para o presente estudo foram utilizadas as temperaturas de 35, 40, 45, 50 e 55 °C em estufa de circulação de ar forçado, para superar a dormência das sementes. Em cada temperatura as sementes permaneceram por um período de 24, 48, 72, 96, 120, 144, 168 h. No experimento foi avaliada a primeira contagem, germinação, comprimento de raiz e de parte aérea de plântulas. Os dados obtidos foram submetidos à análise de variância e comparados por superfície resposta. Para superação de dormência de sementes da cultivar IRGA 431 CL em estufa de circulação de ar forçado de ar forçado é mais indicada a temperatura entre 41° e 44 °C por um período de 95 h.

Termos para indexação: tempo de exposição, altas temperaturas, Oryza sativa L.

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INTRODUCTION

Rice seeds have dormancy that is understood as a resistance to pre- and post-harvest germination, which does not occur even when environmental conditions for emergence are provided. Rice dormancy is related to seed maturation levels and exposure to a set of environmental conditions established between the maturation stage and harvest (Menezes et al., 2009), i.e., rice seeds have a secondary dormancy, which is a response of the seed to a certain environmental condition, but the seed dormancy mechanism is not yet well defined. The induction of dormancy associated with rice seeds may be linked to several factors, such as the occurrence of high temperatures during maturation, the presence of inhibitory substances, and the accumulation of phenolic compounds (Menezes et al., 2009). In rice, maturation at 30 °C provides conditions to reduce the substances that inhibit germination, forming a smaller number of dormant seeds, which does not occur at 25 °C (Marcos-Filho, 2015). Rice seed dormancy varies between cultivars (Lima et al., 2019), and its intensity can vary according to genotype and environmental conditions during maturation and storage (Menezes et al., 2013).

Dormancy in the final stages of ripening is advantageous to rice seeds, as it represents a barrier to early germination of the mature or almost mature seed, when it is still attached to the parent plant (Menezes et al., 2009). However, this factor can be considered a problem when it continues after harvest, for periods longer than the off-season, which can occur in some cultivars and/or crop seasons, as the environment and the genetic basis of the seed influence germination. This factor will result in a low percentage of germination, with consequent unevenness in the initial plant stand, generating losses not only for producers, but also for the companies that sell these seeds.

Pre-drying has been widely used in seed analysis to overcome dormancy in rice seeds (Brasil, 2009; Menezes et al., 2009; Huang et al., 2020; Scariot et al., 2021). In this method, seeds are exposed to high temperatures for an extended period. To overcome dormancy in rice seeds, the Rules for Seed Testing (RAS) (Brasil, 2009) indicate pre-drying, in a relatively wide range, from 40 to 50 °C, for 96 hours, in an oven with air circulation. However, there may be a different behavior for each rice cultivar used in this temperature range proposed by RAS. In addition, excessive heating during drying can induce damage, including stress cracking, which reduces germination and destroys specific enzymes or even reduces the initial development potential (Gawrysiak-Witulska et al., 2019). As the cultivar IRGA 431 CL, released in 2018, has been among the three cultivars (IRGA 424 RI, Guri Intá CL and IRGA 431 CL) most sown in Brazil up to the moment of conducting the present study, there is a need to understand its germination behavior in order to better serve the market, as it has high production potential and excellent grain quality, with a low white-core rate, high amylose content, and high yield of whole grains in processing (IRGA, 2019).

The cultivar IRGA 431 CL was chosen for the present study because it has a low level of chalkiness in its structure when compared to the other cultivars most produced in Brazil, such as IRGA 424 RI and Guri INTÁ CL, in addition to having more vitreous grains, which are more accepted in the Brazilian market. Thus, significant changes in the formation of chalky grains can occur due to the movement of water inside the grain (Coradi et al., 2020; Srikaeo et al., 2016). Thus, there may be a different behavior for each rice cultivar used in this temperature range proposed by RAS. In addition, excessive heating during drying can cause damage to seeds, such as reduction in germination percentage, or even reduce their initial development potential. Thus, as the cultivar IRGA 431 CL is considered to have harder grains, the hypothesis is that, during the process of overcoming the dormancy of seeds of the rice cultivar IRGA 431 CL, the passage of oxygen and water to the husk is hindered, which reduces its germination power, so further tests are needed to contemplate the actual germination power of the cultivar IRGA 431 CL. Thus, the present study aimed to evaluate periods and temperatures in the pre-drying of rice seeds (cultivar IRGA 431 CL) to overcome dormancy.

MATERIAL AND METHODS

The experiment was conducted in a completely randomized design. Three lots of rice seeds of the IRGA 431 CL cultivar, harvested in the 2019/2020 season, were used. The lots were stored in the laboratory for a period of two

months after the seeds were harvested and processed.

Previously, analyses were carried out to characterize the seed lots, determining the physical and physiological quality. Thousand-seed weight was determined through eight replications of 100 seeds, weighed on a precision scale (0.001 g), with the result expressed in grams. Moisture content was determined by the oven method at 105 \pm 3 °C for 24 hours, using two replications for each lot, containing 4.5 \pm 0.5 g of seeds in each replication. The methodologies used are described in RAS (Brasil, 2009).

Freshly harvested seeds, before storage, were evaluated for germination (42%), first germination count (5%), root length (1.8 cm) and shoot length (1.1 cm), and when dormancy was overcome at 45 °C for 96 hours, as described in RAS (Brasil, 2009), the following results were obtained: germination (83%), first germination count (41%), root length (2.8 cm) and shoot length (1.2 cm). These data were obtained to evaluate the germination power of the seeds (data not tabulated).

After two months of seed storage, different temperatures were used to overcome dormancy: 35, 40, 45, 50 and 55 °C in a forced-air circulation oven. The seeds remained at each temperature for periods of 24, 48, 72, 96, 120, 144 and 168 h. The experiment was arranged with the average of the three lots of the cultivar IRGA 431 CL, forming a 5 × 7 factorial (five temperatures × seven periods).

The results were evaluated through the following tests:

Germination test (G): performed with four replications of 100 seeds on Germitest paper rolls moistened with distilled water in a proportion of 2.5 times the dry weight of the paper (Brasil, 2009). The rolls were placed in plastic bags, kept in a germinator with constant light at a temperature of 25 °C for 14 days, and then the seeds were evaluated. The results were expressed as percentage of normal seedlings.

First germination count (FC): performed along with the germination test, computing the averages of normal seedlings, five days after setting up the test. The results were expressed as percentage of normal seedlings.

Root length (RL) and shoot length (SL): on the fifth day after sowing, ten seedlings were randomly collected from the germination test, and their root and shoot lengths were measured using a millimetric ruler. The results were expressed in centimeters per seedling, as described by Krzyzanowski et al. (2020).

Analysis of variance was performed using SISVAR software, and the results were compared by response surface using Statistica software.

When analyzing the results of the analysis of variance for each trait evaluated in the study, it was decided to proceed with the analysis of the temperature × period variation factor, because there was significance at 5% probability of error for all variables (Table 1). In addition, this variation factor contemplates what was proposed in the objective of the present study. From this analysis, it is then reliable to perform the response surface analyses (Table 2 and Figure 1 to Figure 4).

<u> </u>	DE	Mean squares			
50	DF	FC	G	RL 27.21* 3.80* 1.48* 0.10 9.24	SL
Temperature (T)	4	4342.55*	29.99*	27.21*	0.20*
Period (P)	6	691.54 [*]	27.51 [*]	3.80*	0.08*
Τ×Ρ	24	1033.06*	6.42 [*]	1.48^{*}	0.06*
Residual	105	18.53	3.78	0.10	0.01
CV (%)		6.56	2.17	9.24	6.00

 Table 1.
 Summary of the analysis of variance for the traits first count (FC), germination (G), root length (RL) and shoot length (SL) of rice (*Oryza sativa* L.) seeds of the cultivar IRGA 431 CL.

SV: source of variation; DF: degrees of freedom; CV: coefficient of variation. *Significant at 5% probability of error by the F-test. Table 2. Equations and maximum points (x (period, in h) and y (temperature, in °C)) for the traits first count (FC, in %), germination (G, in %), root length (RL, in cm) and shoot length (SL, in cm) of rice (*Oryza sativa* L.) seeds of the cultivar IRGA 431 CL.

Equation	Maximum point	
Equation	х	У
FC=-0.0024x ² +0.0284y ² +0.0145xy-0.2329x-3.7182y+160.8599	84.25	43.95
G=-0.0005x ² -0.0096y ² -0.0003xy+0.1027x+0.8189y+69.983	90.33	41.24
RL=-0.000055081x ² -0.0091y ² -0.0005xy+0.0403x+0.8365y-16.2449	179.61	41.03
SL=-0.000019051x ² +0.0001y ² +0.000018432xy+0.0028x-0.0103y+1.4859	94.20	42.82



Figure 1. Response surface for first count (FC, in %) of rice (Oryza sativa L.) seeds of the cultivar IRGA 431 CL.



Figure 2. Response surface for germination (G, in %) of rice (Oryza sativa L.) seeds of the cultivar IRGA 431 CL.



Figure 3. Response surface for root length (RL, in cm) of rice (Oryza sativa L.) seedlings of the cultivar IRGA 431 CL.





RESULTS AND DISCUSSION

Initially, the moisture content of the seeds was determined to provide reliability in the tests of the present study, and the values obtained when the tests were set up were 12.2, 12.3 and 12.2%, for lots 1, 2 and 3, respectively. For rice seeds, 10 to 13% moisture content is considered a safe range for storage (Marcos-Filho, 2015). According to the Rules for Seed Analysis, the maximum tolerance range is 0.5% to 0.9% for differences between seed samples of straw species, such as rice and wheat, demonstrating even more reliability in the results, as the variation in the moisture content of rice seeds was 0.1%. For thousand-seed weight, uniformity was observed among the lots, with values of 24.9, 24.1 and 24.3 g for lots 1, 2 and 3, respectively.

The response surface results showed that, for the FC trait, the cultivar IRGA 431 CL requires a period of 84.25 h at a temperature of 43.95 °C to obtain the highest percentage (64.34%) of normal seedlings on the 5th day of evaluation (Figure 1). On the other hand, for the G trait, a period of 90.33 h at a temperature of 41.24 °C is required to obtain the highest percentage of seed germination (91.51%) (Figure 2). These results are consistent with the studies conducted by Menezes et al. (2009), who state that using temperature of 45 °C for pre-drying does not affect the physiological quality of the crop. Scariot et al. (2021), when comparing drying air temperatures of 55 and 65 °C, found that drying rice seeds with an air temperature of 65 °C did not negatively influence seed germination, but reduced vigor. Similar results were observed in the present study, in which higher temperatures (50 and 55 °C) of pre-drying positively affected the first seedling count more than the final germination percentage.

The maximum germination and vigor of rice seeds may correspond to the moment when dormancy is overcome (Marques et al., 2014). In addition to the need to obtain vigorous seeds, it is necessary to know the ideal conditions

for germination, because in some species dormancy brings difficulties to propagation. Thus, when analyzing the results of FC and G of freshly harvested seeds, compared with those obtained after 2 months of storage, it was observed that the cultivar has higher germination power and vigor when stored for a longer period, but still needs dormancy breaking for a period of up to 90.33 h at a high temperature of 43.95 °C to germinate. In two months of storage, field germination would occur with a greater number of plants and the plant stand would be initially larger, as the cultivar has greater vigor after this period.

Almeida et al. (2016), when testing the pre-drying at 50 °C for periods of 72 and 96 h in *Triticum aestivum* L., observed that the crop reaches the maximum germination value with the period of 72 h and, above this period (96 h), there is a reduction in germination. In addition, these authors observed that, despite affecting the germination capacity of wheat seeds, the method was efficient in differentiating the lots in relation to their quality. Similar results were found in the present study, in which the germination power (G) of the cultivar IRGA 431 CL is reduced for periods above or below 90.33 h. According to Marostega et al. (2015), the use of water and high temperature treatments increases the germination percentage of seeds, as they absorb water faster, accelerating the biochemical and metabolic reactions that determine the germination process.

Understanding the ideal temperature for dormancy breaking and the time for its application to the seed is of paramount importance for sowing in the field, so the seed will express its actual germination power, reducing competition with other invasive plants. This is because the structure of the seed can be compromised by excessive temperatures or periods, which will also mask the full germination power of the cultivar.

To obtain a higher RL (4.53 cm), a longer exposure period of 179.61 h at a temperature of 41.03 °C is required (Figure 3). This result may be linked to the fact that, when overcoming dormancy, the seed needs a period of heat, in which the seed coat shows cracks, making it permeable, which facilitates the entry of oxygen and water, necessary for the germination process, besides facilitating the drainage of germination-inhibiting substances (Marcos-Filho, 2015), as found in the cultivar IRGA 431 CL due to its seed dormancy. According to Dousseau et al. (2008), temperature is considered one of the main factors responsible for the final percentage of germination, which affects the speed of water absorption, the reactivation of metabolic reactions, which are fundamental to the processes of mobilization of reserves, and the resumption of root growth.

RL was higher when compared to the value obtained with freshly harvested seeds, and this is another key point to analyze the vigor of the cultivar; its roots were approximately 1.7 cm longer in plants with 2-month storage. However, in order to reach their greatest potential length (4.53 cm) the seeds required a very long period of exposure to a temperature of 41 °C, which becomes unfeasible and unprofitable in terms of production. However, as an equation was obtained and the various temperatures and periods analyzed can be used, replacing the unknown x with 96 h and keeping the unknown y at 41 °C yields a high RL value of 4.15 cm, so recommending a shorter period of exposure to heat remains feasible for the cultivar IRGA 431 CL.

For a higher SL (1.38 cm), a period of 94.20 h at a temperature of 42.82 °C is required (Figure 4). The data obtained in the present study are similar to those reported by Vergara et al. (2020), who studied the cultivar IRGA 424 RI and observed that the temperature of 40 °C became more promising to evaluate seedling length, germination percentage, and first count values.

The efficiency of dormancy breaking methods depends on the intensity of dormancy in the seeds (Garcia and Coelho, 2021). Imbibition may occur in rice seeds during germination when they are exposed to low temperatures, but embryo development does not occur, inducing damage to the embryo or seedlings, preventing the completion of the germination process. On the other hand, high temperatures allow seed imbibition, but do not allow embryo growth and seedling establishment (Matheus and Lopes, 2009). Differences in germination behavior, associated with temperature, are related to the physiological quality of the seed, which is its degree of physiological maturity at harvest or the progress of deterioration at membrane level.

Brown rice grains have large contours of linear bulges and depressions that extend longitudinally along their lipidrich surface. As found by Coradi et al. (2021), the IRGA 431 CL cultivar has a lower percentage of fat than IRGA 424 RI; however, the IRGA 431 CL cultivar has higher percentages of crude protein and starch in its whole grain. Thus, as there is less fat in the structure of this cultivar, it has a lower amount of lipids for the initial development of the embryo and, as rice proteins are located mainly in the endosperm, the germination reserves are lower compared to those of IRGA 424 RI, the most sown cultivar in Brazil. Also, as it has high percentages of starch and protein in its structure, in the endosperm, this seed will be harder and will have greater resistance to overcoming dormancy, making the entry of water and oxygen in its structure more difficult.

According to Menezes et al. (2009), due to the wide and complex interactions between its causes, dormancy in rice seeds varies between cultivars, lots, seeds, and from year to year, which makes it difficult to establish a single and efficient method to overcome it; however, these authors point out that pre-drying is an efficient method to overcome this dormancy.

CONCLUSIONS

Temperature between 41 and 44 °C for a period of 95 h is more indicated to overcome dormancy in seeds of the cultivar IRGA 431 CL in a forced-air circulation oven.

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