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Original article

Germination of rice seeds (*Oryza sativa* L.) at different temperatures

Germinación de semillas de arroz (*Oryza sativa* L.) a diferentes temperaturas

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ABSTRACT: The research was developed in the Base Scientific Science Unit, Los Palacios, Pinar del Río, belonging to the National Institute of Agricultural Sciences. The objective was to evaluate the influence of temperatures on the germination of four rice cultivars. Seeds of *Oryza sativa* L. (rice) were analyzed from the cultivars, INCA LP-5, Reforma, INCA LP-2 y Jucarito 104 (J-104). The germination temperatures considered in each trial were 10, 15, 20, 25, 30, 35 and 40 ºC. The experimental design used in all cases was completely randomized, each trial was evaluated individually and was repeated twice, we worked with the means of these repetitions. Germinated seeds were counted from their establishment until stabilization, with the data obtained was determined: Percentage of germination, seeds germinated per day and the Maguire Index. The results were subjected to an analysis of variance (ANOVA) with a confidence level of 95 %. The results of this work indicate that there is a sensitivity in the germination process of different rice cultivars to temperature effects. Each cultivar shows a greater or lesser germination resistance depending on the temperature. Therefore, temperature effect in the process is closely related to the genetic material with which it is working. In addition, with a germination speed increase in the increase in temperature, an optimal value is reached and then decreases, regardless of the cultivar.

Key words: cereal, vigor, reserve.

RESUMEN: La investigación se desarrolló en la Unidad Científico Tecnológica de Base, Los Palacios, Pinar del Río, perteneciente al Instituto Nacional de Ciencias Agrícolas. El objetivo fue evaluar la influencia de las temperaturas en la germinación de cuatro cultivares de arroz. Se analizaron semillas de *Oryza sativa* L. (arroz) de los cultivares INCA LP-5, Reforma, INCA LP-2 y Jucarito 104 (J-104). Las temperaturas de germinación consideradas en cada ensayo fueron 10, 15, 20, 25, 30, 35 y 40ºC. El diseño experimental utilizado en todos los casos fue completamente aleatorizado, cada ensayo fue evaluado individualmente y repetido dos veces, se trabajó con las medias de dichas repeticiones. Se contaron las semillas germinadas desde su establecimiento hasta la estabilización, con los datos obtenidos se determinó: porcentaje de germinación, semillas germinadas por día, Índice Maguire (IM) y número medio de días germinados (NMDG). Los resultados fueron sometidos a un análisis de varianza (ANOVA) con un nivel de confianza del 95%. Los resultados de este trabajo indican que existe sensibilidad en el proceso de germinación de diferentes cultivares de arroz ante los efectos de la temperatura. Cada cultivar muestra una mayor o menor resistencia de germinación en función de la temperatura. Por lo tanto, el efecto de la temperatura en el proceso está estrechamente relacionado con el material genético con que se esté trabajando. Además, con el incremento en la velocidad de germinación a partir del aumento de la temperatura, se alcanza un valor óptimo para después disminuir, independientemente del cultivar.

Key words: cereal, vigor, reserva.

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INTRODUCTION

Rice, wheat and maize are among the most important grains for human consumption worldwide.

Rice (*Oryza sativa*) is consumed by more than half of the population and provides 27 % of the food calories consumed by the population. Approximately 900 million of the world's poor depend on rice as consumers and/or producers (1).

In Cuba, rice occupies only 7.6 % of the 2,733.5 million hectares (ha) of land in the country of cultivated land (2). The agricultural yield in the country for the period 2013 to 2016 was 3.07 t ha^{-1} although yield potentials of between 6.4 to 7.7.5 t ha^{-1} have been between 6.4 to 7.4 t ha^{-1} for cold planting and 4.4 to 5.3 t ha^{-1} for cold planting 5.3 t ha$_{-1}$ for spring plantings. In the last five years, the country's production behavior in the country is similar to that of the aforementioned period. In order to meet the demands of the Cuban population, it has been necessary to import more than 50 % of the rice consumed in the country (3). All of the above is the reason why strategies are being followed to increase yields and reduce imports. The implementation of actions is strengthened where, genetic improvement of cultivars; seed production and the achievement of a correct phytotechnical with emphasis on achieving germination that guarantees an adequate plant density are among the most important (4).

Rice, wheat and and maize are among the most important cereals for human consumption worldwide. worldwide. Rice (*Oryza sativa*) is consumed by more than half of the world's population and provides 27 % of the calories provide 27 % of the calories of the food consumed by the population. Approximately 900 million of the world's poor depend on rice as consumers and/or producers (1) consumers and/or producers (1).

However, the response of rice cultivars to different environmental to different environmental conditions and to the great variability of climate is now a vital element in the is now a vitally important element in achieving high productivity in the face of climate change effects.

productivity in the face of the effects of climate change (5). In general, crop production is affected by climate variability and especially by the effect of temperatures. It is therefore important to know the most sensitive developmental stages and phenological stages, as well as the detrimental effect of temperatures on the processes (6). Of the phases and stages of cultivation, the germination process is considered one of the most affected by temperature due to its influence on the activity of enzymes regulating the speed of biochemical reactions occurring in the seed after rehydration (7).

At present, there is little information on temperature consequences on the germination of rice cultivars obtained in the country and a precise understanding of germination dynamics at different temperatures is required to ensure a number of seedlings in production practice. In in addition, this experiment is important to promote programs for the improvement of cultivars according to climate adaptation in the agricultural sector. Therefore, this work was carried out with the objective of evaluating the influence of temperatures on the germination of four rice cultivars.

MATERIALS AND METHODS

Experiments were carried out at the Base Scientific Science Unit, Los Palacios, Pinar del Río, belonging to the National Institute of Agricultural Sciences. Seeds of *Oryza sativa* L (rice) of four cultivars were analyzed: INCA LP-5, Reforma, INCA LP-2 and Jucarito 104 (J-104) (Table 1) (8, 9). These were supplied by the INCA seed group.

Once the crop was harvested, and with the purpose of lowering its humidity to 12 %, seeds were dried in an oven with forced air at 30 °C. Seed moisture was determined with a Japanese Kett J5 moisture meter. They were then stored in tightly covered plastic containers.

In the experiment, the germination temperatures considered in each trial were 10, 15, 20, 25, 30, 35 and 40 ºC. In each trial, the experimental unit corresponded to glass petri dishes 140 mm in diameter and 20 mm high, with 2 layers of filter paper moistened with distilled water on the bottom of the dishes and 40 seeds inside. Four

Table 1. Main biological characteristics of the rice (*Oryza sativa* L.) cultivars studied in the experiments

Characteristics	Cultivars			
	INCA LP-5	Reforma	INCA LP-2	Jucarito 104
Length of cycle (days)				
Low rainy season	128	122	134	147
Rainy" season	110	105	114	119
Classification according to cycle	Short	Short	Medium	Medium
Plant height (cm)	90	90-110	88	85
Panicle length (cm)	23	26	24	23
Grains per panicle	90	150	90	112
Mass of thousand grains (g)	29.5	26.6	29	28
Yield (t ha^{-1})				
Low rainy season	8.2	7.5	7.8	8.6
Rainy" season	5.7	5.7	6.7	5.9

replicates per treatment were established. Once seeds were placed on the plates, they were placed in a germination chamber regulated at the respective temperature and in dark conditions. The experimental design used in all cases was completely randomized; each trial (temperatures 10, 15, 20, 25, 30, 35 and 40 ºC) was evaluated individually and was repeated twice, working with the means of these replicates.

The germinated seeds were counted from establishment to stabilization, and the data obtained were used to determine:

- The germination percentage (GP): germinated seed was considered as that with a radicle length greater than or equal to 2 mm (10).

$$GP = \frac{Number\ of\ germinated\ seeds}{Number\ of\ sowed\ seeds} * 100 \quad (1)$$

- Germinated seeds per day (SGD): Germinated seeds were counted daily and the criterion used was the appearance of the radicle greater than or equal to 2 mm.
- Maguire index (IM): It represents the germination rate calculated through a weighted time (in days) of accumulated germination. Where G is the percentage of seedlings that germinate during the time interval t (11).

$$IM = \sum_{t=1}^{n} (G1/t1) \quad (2)$$

- Mean number of days germinated (NMDG) where: Ni is the number of seeds germinated within consecutive time intervals Ti is the time in days elapsed between the start of the test and the end of the interval, n is the total number of seeds germinated (12).

$$NMDG = \sum_{i=1}^{n} \frac{N_i * T_i}{n} \quad (3)$$

The assumptions of normality and homogeneity of variance (Bartlett's test and Akolmogorov-Smirnov, respectively) were checked for the data of each variable evaluated. The results (PG, MI) were subjected to an analysis of variance (ANOVA) with a confidence level of 95 %. Comparison of means was performed using Tukey's Multiple Range Test (P > 0.05) (13). For this purpose, the STATGRAPHICS Plus 5.0 Centurion program on Windows, version XV (14) was used.

RESULTS AND DISCUSSION

The final germination percentages are shown in Table 2. All the cultivars showed significant differences in the temperature range from 10 to 40 ºC. The highest values were observed in the range of 10 to 40 ºC. The highest values were observed between 20 and 30 ºC, while the lowest values were observed at 10 ºC. INCA LP-2 was the cultivar that best responded to effects of the different temperatures studied, with germination percentages ranging from 53 to 97 %. The cultivar INCA LP-5 was the most affected by the effects of temperatures of 10 and 15 ºC, since only 13 and 30 % germinated, respectively.

The influence of temperature on the germination percentage of seeds of crops of agricultural interest has been reported by several authors, who emphasize that the percentage of seed emergence guarantees 50 % of the success of production and point out that germination only occurs properly within a certain temperature range (15, 16). In studies carried out in peanut cultivation, it was demonstrated in all the genotypes studied that the germination response increased with the rise in temperature above 14 ºC. For the range between 16 and 32 ºC, germination percentages were obtained that meet the standard for commercialization of common seed for peanut (> 80 %); which was in all genotypes equal to or higher than 90 % (17). However, it should be noted that in the results of the present study with rice cultivars, there is a differential and particular response of the cultivars to germination temperature since there is variation in final percentages.

With the Maguire index (Figure 1), where the accumulated germination was weighted over time, it was possible to differentiate the role of temperature with respect to the germination process of the different cultivars. Figure 1 shows that there are statistically significant differences between the different temperatures for each cultivar.

In general, an increase in the values of the index is observed with increasing temperature, and then decreases, so that a well-defined maximum of 30 ºC was observed for all the cultivars under study. However, the cultivars at 10 and 15 ºC had a low index (between 0.53 and 6.4) and showed in this sense a considerable affectation in germination speed as a response to the presence of low temperatures. Although the values in INCA LP-2 are higher

Table 2. Rice seed germination percentages at different temperatures

	Cultivars			
Temperature (ºC)	INCA LP-5	Reforma	INCA LP-2	J-104
10	13.13 e	28.13 de	53.13 d	53.13 c
15	30.63 d	40.00 cd	71.25 c	40.00 b
20	98.13 a	90.00 a	81.88 b	91.88 a
25	100.00 a	98.75 a	96.88 a	99.38 a
30	84.40 b	63.13 b	96.88 a	93.13 a
35	72.50 c	49.38 bc	93.75 a	48.13 b
40	39.38 d	14.38 e	68.75 b	20.63 c
SE x	0.06***	0.05***	0.03***	0.06***

Different letters in the same column indicate significant differences (Tukey's test, p≤0.05)

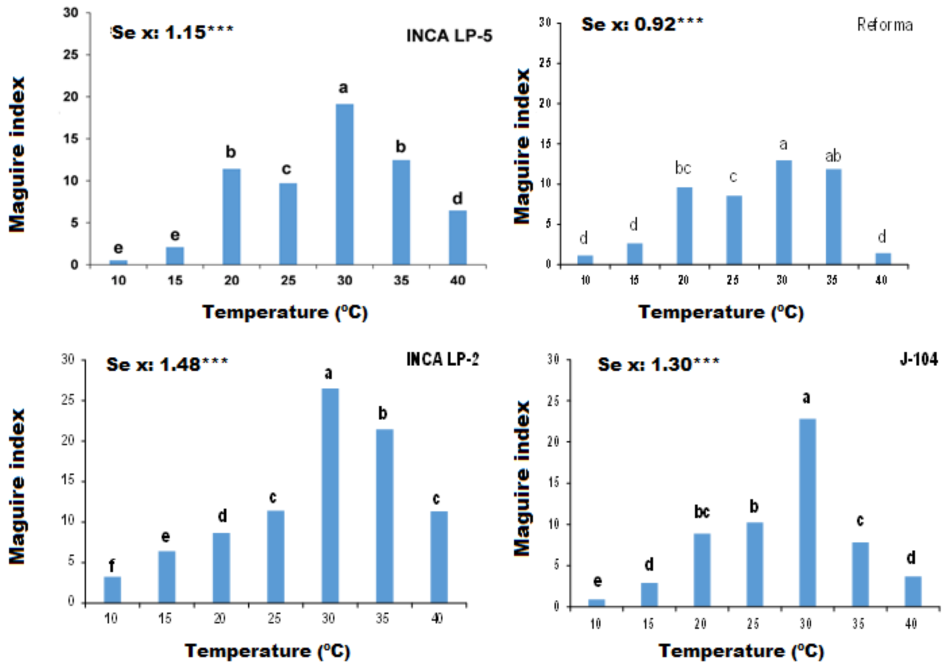

Figure 1. Maguire index of rice seeds of four cultivars (INCA LP-5, Reforma, INCA LP-2 and Jucarito 104) at different temperatures (10, 15, 20, 25, 30, 35 and 40 ºC)

than those ones of the rest. It may be related to the genetic characteristics of this cultivar. This cultivar could be taken into account for further studies in which tolerance of cultivars to relatively low temperatures is sought and for the selection of progenitors in the rice-breeding program seeking tolerance to abiotic stress. In this regard, some authors state that even when it is impossible to eliminate all stress factors to which crops are subjected, it is of vital importance the knowledge of the way in which these cause their effects in the physiological processes of plants. The identification of crops and/or cultivars that survive and tolerate damages that can act more severely to others of the same species, since it is possible to modify them genetically or minimize the effects that they are capable of producing (18).

Another aspect to highlight is that in the cultivar J-104 the values of this index decrease considerably at temperatures of 35 and 40 ºC (values between 3 and 7 approximately), aspect that can also be related to the genetic characteristics of this cultivar and demonstrates its susceptibility to high temperatures during the germination process. In the climatic conditions of Cuba, it is of great interest the problem that exists regarding the decrease of germination in the different existing cultivars at temperatures lower than 15 ºC and above 30ºC. This is because the sowing of the crop is done in two seasons, "cold" and "spring", so that the existing climatic conditions during the germination stage could increase or decrease the days for a given cultivar, especially for its greater or lesser susceptibility to temperatures during the germination process. There are also studies where seed germination indexes are used for the early selection of genotypes tolerant to certain stresses (16, 19). Several authors highlight the importance of this index (IM), since it weights and combines the parameters germination capacity, speed and uniformity to evaluate the quality of germination; the higher the result, the higher the quality of the seed and the response of the cultivars to the conditions in the germination process (20).

In aspects pointed out by some authors, it is highlighted that the poor germination of seeds at high temperatures is related to the synthesis of proteins in the embryo, which influences its germination speed. In this regard, it is reported that several processes occur in the seed that depend solely on the reserves, the gibberellic acid of the embryo acts on the aleurone layer where the enzyme amylase is activated, which initiates the degradation of reserve substances contained in the endosperm and cotyledon. From the digestion of reserve tissues, various compounds are released to be reused in multiple synthesis processes. Complex molecules such as celluloses, hemicelluloses, starches, amylopectins, lipids, lignins, proteins, nucleic acids, vitamins and hormones are degraded to simple molecules by specific enzymes (21). Other authors emphasize the role of the pericarp in the thermo inhibition of seeds, pointing out that this is partly due to the presence of inhibitors in the tissue or also due to the fact that in the presence of high temperatures the oxygen requirements are relatively high and do not allow the pericarp to maintain these demands (22).

The results of the present work coincide with studies carried out by other authors, who report that in most cases the germination speed increases with increasing temperature although very high temperatures also tend to decrease it. With studies using various temperatures within a certain range (10-40 ºC), the authors state that it is possible to find the optimum germination temperature. They emphasize in their results that temperatures above 32 ºC can decrease the germination speed index of seeds. However, regardless of the genotype, increasing the temperature up to 38 ºC significantly decreases the germination speed (22, 23). Other authors (24) also exposed the negative effect of high temperatures on germination speed. They pointed out that once the optimum temperature level is reached, where germination speed is higher, a decrease occurs as temperatures approach their maximum limit where irreversible damage to seeds occurs.

The average number of days to germination in the rice cultivars studied varied considerably according to the germination temperature (Table 3). In general, a different behavior to that observed for germination speed (Maguire index) was observed, since as temperature increased, the mean number of days to germination decreased for all the cultivars under study. The cultivar INCA LP-2 showed a shorter time (approximately 7 days) for the germination process at low temperatures (10 ºC) compared to the rest of the cultivars studied, which required 10 days. However, at high temperatures (40 ºC) the result was different.

The NMDG has been used by several authors to highlight differences in the seed germination process in certain studies. When comparing the germination of sorghum seeds of different caliber, it was observed that seeds of larger caliber presented the lowest values of this variable (10). On the contrary, in a study carried out in the cultivation of corn where three cultivars were used and their seeds were submitted before the germination test to different immersion times in water at 100 °C (0, 2, 4, 6 and 8 seconds). As a result, there was no significant response between treatments in terms of NMDG, since the values ranged between four and five days (25). However, the results of this study clearly show the resistance to germination of each cultivar at the different temperatures to which seeds were subjected, due to the significant differences between treatments. This aspect is highlighted by other authors (26) who have determined the NMDG for this purpose.

CONCLUSIONS

The results of this work indicate that there is sensitivity in the germination process of different rice cultivars to the effects of temperature. Each cultivar shows greater or lesser germination resistance as a function of temperature. Therefore, the action of temperature on the process is closely related to the genetic material being worked with. In addition, with an increase in germination speed as the temperature increases, an optimum value is reached and then decreases, regardless of the cultivar.

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Table 3. Mean number of days to germination (NMDG) of rice seeds at different temperatures

	Cultivars			
Temperature (ºC)	**INCA LP-5**	**Reforma**	**INCA LP-2**	**J-104**
10	10.00 a	10.00 a	6.70 a	10.00 a
15	5.90 b	6.00 b	4.65 b	5.58 b
20	3.54 d	3.87 e	3.91 c	4.28 c
25	4.24 c	4.62 c	3.46 d	4.01 d
30	2.09 f	2.20 f	1.68 g	1.91 g
35	2.53 e	1.94 g	1.95 f	2.57 e
40	2.44 e	4.00 d	2.79 e	2.33 f
SE x	0.50***	0.48***	0.31***	0.50***

Different letters in the same column indicate significant differences (Tukey's test, p≤0.05)

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