



Germination tests in four new rice lines obtained by hybridizations in Los Palacios

Ensayos de germinación en cuatro nuevas líneas de arroz obtenidas por hibridaciones en Los Palacios

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ABSTRACT: The quality of the rice seed is a fundamental factor that must be considered in any agricultural production program. The objective was to evaluate the physiological quality of the seed of four new rice lines by descriptive and analytical methods, for which a completely randomized design with three repetitions was used and two trials were carried out, the first consisted of a standard germination test where the number of normal and abnormal seedlings, coleoptile and radicle length, and vigor index were determined. In the second trial, seed emergence counts were carried out for ten days and the emergence speed index, the total emergence percentage and the germination speed coefficient were determined. The results showed that Lines 3, 4 and INCA LP-5 were the genotypes with the best performance in the germination percentage; Line 2 and INCA LP-7 with the highest percentage of normal seedlings and the control INCA LP-5, followed by Line 2, with significant differences between them, the best in vigor index and radicle length. The control INCA LP-5 achieved 50 % of emerged seeds at 7 days and the rest of the genotypes accomplish to exceed that value between days 9 and 11. INCA LP-5 statistically surpassed all genotypes in the characters speed index emergence, emergence percentage and germination speed coefficient.

Keywords: *Oryza sativa*, emergency speed, seed, vigor.

RESUMEN: La calidad de la semilla de arroz es un factor fundamental que debe ser considerado en cualquier programa de producción agrícola. El objetivo fue evaluar la calidad fisiológica de la semilla de cuatro nuevas líneas de arroz mediante métodos descriptivos y analíticos, para lo cual se empleó un diseño completamente aleatorizado con tres repeticiones y se realizaron dos ensayos, el primero consistió en un test de germinación estándar donde se determinó el número de plántulas normales y anormales, longitud del coleóptilo y la radícula y el índice de vigor. En el segundo ensayo se realizaron conteos de emergencia de la semilla durante diez días y se determinaron el índice de velocidad de emergencia, el porcentaje total de emergencia y el coeficiente de velocidad de germinación. Los resultados mostraron que las Líneas 3, 4 e INCA LP-5 fueron los genotipos de mejor comportamiento en el porcentaje de germinación; Línea 2 e INCA LP-7 con el porcentaje más alto de plántulas normales y el testigo INCA LP-5, seguido de la Línea 2, con diferencias significativas entre ellos, los mejores en el índice de vigor y la longitud de la radícula. El testigo INCA LP-5 logró el 50 % de semillas emergidas a los siete días y el resto de los genotipos sobrepasa esta cifra entre los días 9 y 11. INCA LP-5 superó estadísticamente a todos los genotipos en los caracteres índice de velocidad de emergencia, porcentaje de emergencia y coeficiente de velocidad de germinación.

Palabras clave: *Oryza sativa*, emergencia, semilla, vigor.

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INTRODUCTION

Rice is the cereal that constitutes the basis for feeding more than half of the world's population. Global paddy rice production in 2020 was set at 493,790 million tons (1), while Cuba is one of the countries that records high rice consumption values with 72 kg per capita per year, so the country has decided to revive the production of this cereal through a development program that allows, gradually, to achieve national self-sufficiency.

However, in the country's production conditions, in the last 20 years, the average production has not exceeded 3.5 tha⁻¹ and among the most common causes of this problem are identified technological indiscipline and non-compliance with good agricultural practices, soil and nutritional problems and the availability of water resources (2), in addition to the insufficient number of short and medium cycle genotypes to establish an adequate varietal structure and the limited availability of quality seed to meet the production demand.

The quality of rice seed is a fundamental factor that should be considered in any agricultural production program, taking into account that the agronomic characteristics of the cultivars obtained through research reach farmers through good seed (3). This could affect the yield of rice grains, the differences in this aspect can cause the reduction and non-uniformity of the emergence in the field, causing lack of homogeneity in the initial growth of the plants, and consequently affecting the crop and its yield.

The choice of seed is one of the first decisions the farmer has to make each year, when deciding which cultivar to plant and the quality of seed to use. In the last fifty years there have been important advances in the world of seeds that have contributed to the evolution towards an increasingly more technified agriculture, with a substantial reduction in labor, together with an increasingly important use of machinery, fertilizers, phytosanitary products and new cultivars (4).

The physiological quality of seeds has been characterized by germination and vigor, and this is generally evaluated by the germination test that confers to seeds favorable conditions of humidity and temperature, allowing to express the maximum potential to produce normal seedlings (3). Seed analysis provides information and establishes a standard to determine the level of quality, a good management of which directly influences its commercial value. Several tests are used to evaluate the efficiency of rice seed, such as: first germination count, accelerated aging, seedling vigor classification, speed of emergence, among others (5).

Taking into account the above mentioned antecedents, the objective of this work was to carry out germination tests to evaluate the physiological quality of seeds of four new rice lines by means of descriptive and analytical methods.

MATERIALS AND METHODS

Location and general conditions of the tests

The work was carried out at the Genetic Improvement laboratory of the Scientific and Technological Unit of Los Palacios Base, belonging to the National Institute of Agricultural Sciences (INCA) in Los Palacios municipality, Pinar del Río province, Cuba. Seeds of six indica rice genotypes were used, four new lines obtained by hybridization in the institution itself and the INCA LP-5 and INCA LP-7 controls, of short and medium cycles, respectively.

TRIAL 1

The standard germination test was carried out under laboratory conditions, using Petri dishes of 9 cm in diameter and 1.5 cm in height, with 25 seeds per dish, with a completely randomized design and three replications, where the genotypes constituted the treatments. To maintain humidity conditions in the plates, filter paper was placed at the bottom of the plates and humidity conditions were maintained throughout the experiment by periodically adding distilled water. Germination was counted seven days after the initiation of the test and the seedlings obtained were evaluated, identifying the number of normal and abnormal seedlings. The length of the coleoptile and radicle of the normal seedlings per plate was also determined on the fifth day after sowing, these data are expressed in cm, and are considered an indicator of vigor.

The vigor index (Iv) was calculated with the values obtained in each evaluation using the formula:

$$Iv = \frac{(\text{Coleoptile growth} + \text{Radicle growth})}{x\% \text{ germination}}$$

TRIAL 2

Sowing was carried out in 46 x 70 cm root ball trays at a rate of one seed per site with a 1:1 soil-organic matter substrate. The size of the alveoli is 2.5 x 2.5 cm, depth of 7.0 cm and the distance between seedlings was approximately 3 cm. Thirty seeds of each genotype were sown and a completely randomized design with three replications was used, the genotypes constituted the treatments. Seed emergence counts were performed for ten days and the following variables were evaluated:

- Emergence velocity index (EVI). It was obtained through the daily count of seedlings emerged from the soil after sowing, taking as emerged seedlings those that protruded from the substrate and was calculated by the expression:

$$IVE = \sum_{i=1}^n \frac{x_i}{n_i}$$

x_i : number of seedlings emerged per day.

n_i : number of days after sowing.

n: number of counts.

- Total percentage of emergence (% E), seedlings emerged up to the last day of the evaluation were counted and the result was obtained by dividing the total number of seedlings emerged by the total number of seeds sown, multiplied by 100.

$$\%E = \frac{\text{Number of emerged plants at the last count}}{\text{Number of seeds sown}} * 100$$

- Germination speed coefficient (Vg), defined by integrating the germination times of each seed and calculated by the following formula:

$$Vg = \frac{1}{Tg} * 100 \quad Tg = \frac{\sum (Ni * Di)}{\sum Ni}$$

Tg: Seed germination time.

Ni: Number of seeds germinated on day Di.

Di: Time elapsed since sowing.

Information analysis

For both tests, the methodology adapted by González and Orozco, 1996 (6) was used.

Variables expressed in percent were transformed ($\arcsin\sqrt{(\%)}$), because they did not comply with normality tests. The data obtained were processed by means of a simple rank analysis of variance (ANOVA), and the means were determined with the Duncan's Multiple Range Test at 5 %, using the STATGRAPHICS Plus v.5 statistical program.

RESULTS AND DISCUSSION

TRIAL 1

The analysis of variance for germination percentages, normal and abnormal plants and vigor index showed significant differences among genotypes at a 95 % significance level (Table 1).

Germination analysis methods can be classified into descriptive and analytical, the former allowing a preliminary evaluation of the results, while the analytical methods consist of the application of mathematical functions that describe the germination behavior of the seeds. Descriptive methods are useful as a first step in the analysis; however, to objectively obtain average times and germination rates,

the application of analytical methods is required, which has been studied by several authors in different crops (7-11).

The germination percentage achieved its highest values in the cultivar INCA LP-5 and Lines 3 and 4, without statistical differences between them, although only Line 2, together with INCA LP-7, showed the highest percentage of normal seedlings and, correspondingly, the lowest numbers of abnormal plants.

It is known that the physiological quality of the seed depends on multiple factors that can affect it, such as delays in harvesting, a common situation in our tropical conditions, deficiencies in crop development, and delays in seed drying, mechanical damage during harvesting and threshing or in processing, storage under unfavorable conditions, pathogenic affectations, among others (3).

Germination is the first step in the development of seeds into new plants when favorable environmental conditions trigger growth (12, 13). In research carried out with the cultivar INCA LP-5, where the effect of extracts of humic substances on germination and seedling growth was evaluated, the desired physiological effect on germination was observed for all treatments (more than 90 % of seeds germinated), however, it was proved that the dilutions used of the humic extracts did not affect the germination percentage of rice seeds (8).

In works carried out in Brazil, where germination was evaluated, the records at 15 days are shown, and the results showed that the percentage of normal plants, abnormal and non-germinated seeds, as well as the germination energy and plants were affected by the specific weight of the seeds (14).

Other authors, in the search for the reduction of productivity losses caused by pathogenic organisms, conducted a trial with the objective of evaluating the cytotoxic potential of aqueous extracts of *Syzygium aromaticum* as a possible alternative treatment of quinoa seeds and found that variables such as germination speed index, emergence, emergence speed index and root length did not show significant differences in comparison with the control treatment (15).

Likewise, when studying the drying type factor, a higher germination percentage (normal seedlings) was obtained with oven drying (56.8 %), which statistically surpassed sun drying (37.6 %). It was also confirmed that the cultivars

Table 1. Results of the Simple Ranked Analysis of Variance for germination percentages, normal and abnormal seedlings and vigor index

Genotypes	% Germination	% Normal Plants	% Abnormal Plants	Iv
INCA LP-7	95.0 c	95.0 a	0 c	361.0 c
INCA LP-5	97.5 a	89.6 b	8.0 a	507.0 a
Line 1	94.0 c	90.0 b	4.0 b	347.8 c
Line 2	96.0 b	96.0 a	0 c	460.8 b
Line 3	97.0 ab	89.0 b	8.0 a	368.6 c
Line 4	98.0 a	90.0 b	8.0 a	352.8 c
$\bar{X}$	96.25*	91.60*	4.67*	399.67*
CV (%)	3.77	5.79	8.56	12.57

Means with equal letters do not differ from each other (Duncan's Multiple Range Test, $p \leq 0.05$)

increased the percentage of normal seedlings as a function of the increase in the days of drying (16).

In other works, on the subject, it has been found that grain weight and germination percentage (together with dry weight and shoot length) were the main characteristics responsible for the differences in germination speed among 50 aromatic rice varieties (17).

The vigor index excels in INCA LP-5, followed by Line 2, with statistical differences among them, and lines 1, 3 and 4, together with the cultivar INCA LP-7, reached the lowest indexes.

In this sense, several authors state that seed vigor can be understood as the sum of attributes that confer to the seed the potential to germinate, emerge and quickly establish an adequate population of normal seedlings under a wide diversity of environmental conditions. Generally, very vigorous seeds have many advantages in agricultural production, such as resistance to adverse stresses, rapid emergence, and improved yield (18).

In studies on seed longevity of rice genotypes, when analyzing parameters such as seedling length, seedling dry weight, and seedling vigor index, it is observed that these parameters gradually decrease among genotypes during storage time, which may be due to inherent genotypic difference (19).

Figure 1 shows the results of the analysis of variance for coleoptile and radicle length. For the first variable there were no significant differences between genotypes, while for radicle length INCA LP-5 showed the highest value followed by Line 2, both exceeding 4 cm.

The International Seed Testing Association considers the germination process of a seed as the establishment of a metabolically active state, physiologically manifested by cell division and differentiation. The first expression of this process is the emergence of the radicle, and a seed is usually considered to have germinated when the radicle reaches a length greater than 3 mm (4).

It is also suggested that rice is unique among cereals in its ability to germinate not only when submerged but also under anaerobic conditions. Germination under submerged or anoxic conditions is characterized by a longer coleoptile and delayed radicle emergence. In researches where temperate and tropical japonica rice accessions were analyzed, a great variability in coleoptile length was shown (20).

In work conducted in Thailand, when the germination response of nine rice cultivars was evaluated under simulated acid rain, the results showed that rice seed germination decreased after exposure to this factor. Acid rain significantly reduced seedling root length, root-to-shoot ratio, and crown roots per seedling (21).

TRIAL 2

The percentage of emergence over time is plotted in Figure 2, INCA LP-5 and INCA LP-7 controls reached 50 % of emerged seeds at 7 and 10 days, respectively, while lines 1, 3 and 4 exceeded this figure at day 9 and line 2 at 11 days.

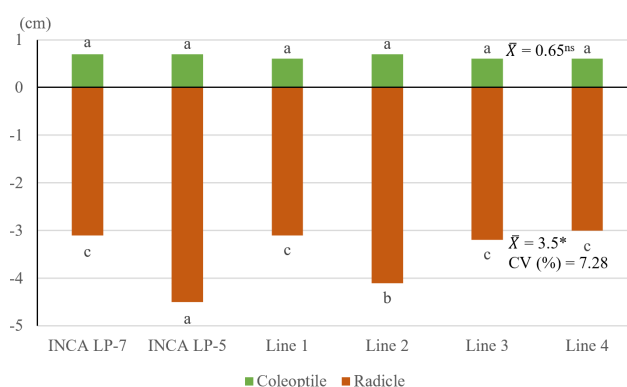


Figure 1. Simple Ranked Analysis of Variance (ANOVA) results for coleoptile and radicle length

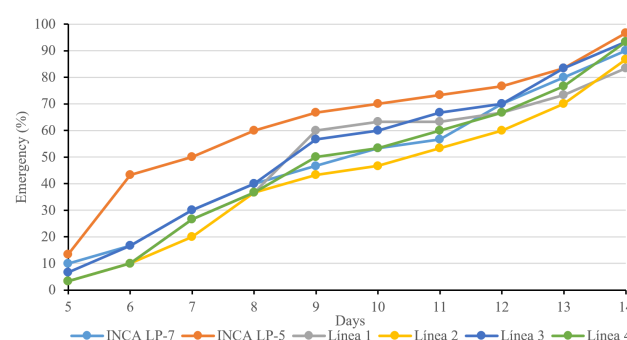


Figure 2. Seed emergence curve for each genotype

To increase production it is necessary to use high quality seeds, associated with correct agronomic practices, however, currently the rate of use of certified seeds does not reach the demanded levels, a similar situation occurs in rice production in Cuba. Deteriorated seeds have low germination and vigor, therefore, they tend to produce weak plants with reduced yield potential. It is presumed that batches of rice seeds with different levels of physiological quality show differing performance in field emergence and in the initial phase of seedling growth (3).

For all the traits shown in Table 2, there are significant differences between treatments and the INCA LP-5 control statistically outperforms the rest of the genotypes in all of them.

Line 3 and INCA LP-7, with no significant difference between them, were superior to the remaining lines in the emergence speed index variable. In other studies on the influence of harvest time on the emergence speed index, it was found that seeds harvested 25 days after flowering presented the highest values, decreasing with the delay in rice harvest (3).

In the emergence percentage variable, except for Line 1, the others had no differences with the INCA LP-7 control and all were surpassed by INCA LP-5. In research conducted with red rice, although there are no differences in the percentage of emergence between this and the cultivars, the emergence rate index shows that the red rice emerged before the varieties. These results suggest that the interference capacity of red rice is mainly due to the

Table 2. Results of the Simple Ranked Analysis of Variance for the Index of Emergence Velocity (IVE), the Total Emergence Percentage (% E) and the Germination Velocity Coefficient (Vg)

Genotypes	IVE	%E	Vg
INCA LP-7	3,17 b	90 b	10,51 c
INCA LP-5	3,90 a	97 a	11,84 a
Line 1	2,95 c	83 c	10,92 b
Line 2	2,85 c	87 b	9,96 d
Line 3	3,29 b	93 b	10,65 c
Line 4	3,10 c	93 b	10,11 d
$\bar{X}$	3,21*	91*	10,66*
CV (%)	1,54	5,28	2,47

Means with equal letters do not differ from each other (Duncan's Multiple Range Test, $p \leq 0.05$)

high speed at which it germinates, emerges and grows in the early stages of plant development (22).

The germination speed coefficient showed a significant difference among most genotypes. Line 1 was superior to Line 3 and INCA LP-7 and these in turn achieved statistically different values above Lines 2 and 4.

The literature consulted suggests that the ranking of genotypes, in terms of germination speed, was constant over the years and the conditions of water availability and barriers to water absorption, in the peel and pericarp, were important determinants of germination speed (23).

Other work indicates that genotypic differences in germination speed under favorable conditions are likely to have a large effect on the time required for seedlings to emerge in the field, which in turn would have a significant implication on early vigor and grain yield under drier conditions (24).

On the other hand, some authors suggest that varieties with rapid germination are required for early vigor, particularly when early drought can affect grain yield and also in weedy fields (25, 18). Others suggest that germination percentage and speed of germination are slightly reduced when rice was grown under water deficit (26).

CONCLUSIONES

- Lines 3, 4 and INCA LP-5 have the best performance in germination percentage; Line 2 and INCA LP-7 have the highest percentage of normal seedlings and the control INCA LP-5, followed by Line 2, with significant differences between them, are the best genotypes in vigor index and radicle length.
- The INCA LP-5 control achieved 50 % of emerged seeds at 7 days and the rest of the genotypes exceeded this figure between days 9 and 11.
- INCA LP-5 statistically outperforms all genotypes in the characters emergence speed index, emergence percentage and germination speed coefficient, seconded by Line 3 and INCA LP-7 in the first, by Lines 2, 3, 4 and INCA LP-7 in the second and by Line 1 in the third.

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