



SELECTING RICE MUTANTS WITH GOOD AGRONOMIC PERFORMANCE UNDER CONDITIONS OF LOW WATER SUPPLIES

Selección de mutantes de arroz de buen comportamiento agronómico en condiciones de bajo suministro de agua

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ABSTRACT. The present work is part of the researches that are carried out in the Regional Project of the International Organization of Atomic Energy (IAEA) "Mutation Breeding of Alimentary Cultivations in Latin America" where Cuba participates. The aim of this project is to obtain new rice varieties tolerant to drought using nuclear techniques, for that which is necessary to determine indicators for early selection of tolerant genotypes and to identify somaclones and/or mutants of good behavior under low water supply. For this study were used, 13 mutants obtained in the National Institute of Agricultural Sciences (INCA) as well as the rice varieties Amistad-82 and J-104. The response to the hydric stress under field conditions was determined, using irrigation during the first 45 days, interrupting later for the plant cycle, were determined: I) the height of the plant, II) weigh of 1000 grains, III) length of panicle, IV) number of full grains, V) vain grains, VI) number of panicle for lineal meter and VII) yield for square meter. Likewise *in vitro* the answers to the drought with a concentration of 5 g L⁻¹ of PEG-6000 to simulate the hydric stress and the Relative Tolerance Index of root and of height were evaluated. Some indicators for early selection of tolerant genotypes starting from the existent correlation among the characters evaluated in the field *in vivo* and *in vitro* were also determined. The INCA genotypes LP-10 and 8552 showed a better behavior under conditions of low supplies of water and INCA LP 16 genotypes and mutant 8553 were the most susceptible because they could not panicular under the same conditions.

RESUMEN. El presente trabajo formó parte de las investigaciones realizadas en el Proyecto Regional de la Organización Internacional de Energía Atómica (OIEA) "Fortalecimiento de Cultivos Alimentarios en América Latina" y que tuvo como objetivo obtener nuevas variedades de arroz tolerantes a la sequía, mediante el empleo de técnicas nucleares, para lo cual se hizo necesario determinar indicadores para la selección temprana de genotipos tolerantes e identificar somaclones y mutantes de arroz de buen comportamiento en condiciones de bajo suministro de agua. Para el estudio se emplearon 13 mutantes obtenidos en el Instituto Nacional de Ciencias Agrícolas (INCA), así como las variedades Amistad 82 y J-104. Se determinó la respuesta al estrés hídrico en condiciones de campo, utilizándose riego durante los primeros 45 días, suspendiéndose posteriormente durante todo el ciclo de la planta, determinando: I) la altura de la planta; II) peso de 1000 granos; III) longitud de la panícula; IV) granos llenos; V) granos vanos; VI) número de panícula por metro lineal y VII) rendimiento por metro cuadrado. Asimismo *in vitro*, se evaluaron las respuestas a la sequía con una concentración de 5 g L⁻¹ de PEG-6000 para simular el estrés hídrico y se determinó el Índice de Tolerancia Relativo de la raíz y de la altura de la plántula. También se determinaron los indicadores para la selección temprana de genotipos tolerantes, a partir de la correlación existente entre los caracteres evaluados *in vivo* e *in vitro*. Los genotipos INCA LP-10 y 8552 fueron los de mejor comportamiento en condiciones de bajo suministro de agua y los genotipos INCA LP 16 y el mutante 8553 resultaron ser los más susceptibles, pues no lograron panicular bajo las mismas condiciones.

Key words: rice, evaluation, genotypes, drought

Palabras clave: arroz, evaluación, genotipos, sequía

INTRODUCTION

Rice (*Oryza sativa* L.) is the second staple of importance to men after wheat, it is among the most important food crops in the world, taking into account

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the percentage of the world population that use that grain as the main source calories. Currently, it stands out as the primary food for more than half of the world population and it is estimated that by 2025 the human population will be 8,3 billion of which 50 % waste them. These figures indicate that global production should increase by 70 % to satisfy the population demand^A.

In Cuba, rice is one of the main crops, due to the large consumption habit of it, reporting consumption per capita annual estimated at around 70 kg, well above almost all countries of the American continent (1). However, the average crop yield has remained around 3 t ha⁻¹ lower than the world average and fails to satisfy domestic demand. Low yields of this crop are associated with the shortage of irrigation, high temperatures, soil salinity, inadequate management in production and damages caused by pests and diseases^B.

In recent years, global changes in climatic conditions have led to the intensification and drought prolongation (2). Water deficiency causes induction of water deficit in plants due to decreased availability in the soil (3). The water deficit is the highest incidence abiotic stress in plant growth (4, 5, 6) and one of the limiting factors especially in rice production (7, 8, 9).

The use of adapted varieties with a certain degree of tolerance to water deficit conditions and improving water management technologies are important alternatives to minimize the effects of water deficiency in the soil. In Cuba, different breeding programs have developed aimed at obtaining rice varieties with adaptability to the conditions of low water supplies, through conventional breeding methods and the use of nuclear and biotechnological techniques^{C, D}.

Of particular interest to the breeder, have methods of rapid and early screenings and indicators tolerance group that will increase efficiency in the germplasm selection with water stress tolerance.

^A Aguilar, M. P. "Funciones del agua". En: *Cultivo del arroz en el Sur de España*, edit. Las Torres-Tomejil, España, 2006, p. 189.

^B González, T. A. Conferencia "Retos y perspectivas de la producción de granos". En: ECOARROZ, Los Palacios, Cuba, 2015.

^C Instituto de Investigaciones del Arroz. *Instructivos Técnicos para el Cultivo del Arroz*. 2005, 112 p.

^D González, M. C. "Resultados obtenidos en el programa de mejoramiento genético para la tolerancia al estrés hídrico y salino en el cultivo del arroz (*Oryza sativa* L.) a partir del empleo de técnicas biotecnológicas". En: *Encuentro Internacional del Arroz*, La Habana, Cuba, 2008, p. 3.

MATERIALS AND METHODS

BEHAVIOR EVALUATION OF RICE GENOTYPES GROUPS UNDER LOW WATER SUPPLY IN THE FIELD

In the experimental area of the National Institute of Agricultural Sciences (INCA), a group of genotypes obtained in this institution were planted by the use of nuclear and biotechnology techniques (Table I). Sowing was done directly on a Ferralitic red soil and applied 0,4 t ha⁻¹ using fertilization^C. At 15 and 45 days after the 15 genotypes germinated was made using an application of urea (0,2 t ha⁻¹). Drip irrigation was used during the first 45 days, and then suspended throughout the cycle.

Thirty plants per variety were evaluated at the time of harvest. Standard Evaluation System for Rice (10) was used to evaluate the characters listed below:

- ◆ Grains filled by panicles (GF/panicle)
- ◆ Vain grains per panicles (VG/panicle)
- ◆ Yield per square meter (Y/m²)
- ◆ Panicle length (cm)
- ◆ 1000 grain weight (grams)
- ◆ Plant height (cm)
- ◆ Panicles per linear meter

With the obtained data, simple variance analysis was performed for each character and where significant differences that the multiple range test of Duncan was performed using statistical software (SPSS) (11).

IN VITRO EVALUATION OF DROUGHT TOLERANCE ON A GROUP OF SOMACLONS AND RICE SEEDS

Seeds of the genotypes evaluated under field conditions were placed on Petri dishes with filter paper as a support, which were wetted with a concentration of 5 g L⁻¹ of polyethylene glycol 6000 (PEG.6000). Fifty seeds per plate and two replicates per treatment were used. Only distilled water was applied to control.

At 15 days four plants per treatment were assessed, the plant height (cm) and length of the root system (cm).

With values obtained concerning the tolerance index it was determined according to the following formula:

$$ITR_{\text{height}} = \frac{\text{Height plant (control)} - \text{Plant height (PEG)}}{\text{Plant height (control)}} \cdot 100$$

$$ITR_{\text{root}} = \frac{\text{Length of root system (control)} - \text{Length of root system (PEG)}}{\text{Length of root system (control)}} \cdot 100$$

Table I. Rice genotypes used in the experiments and their origin

No	Genotypes	Origin	Place
1	Lp7	Mutants Amistad 82	Cuba (INCA)
2	Lp8	Mutants Amistad 82	Cuba (INCA)
3	Lp9	Mutants Amistad 82	Cuba (INCA)
4	Lp10	Mutants Amistad 82	Cuba (INCA)
5	Lp12	Mutants Amistad 82	Cuba (INCA)
6	Lp13	Mutants Amistad 82	Cuba (INCA)
7	Lp16	Mutants Amistad 82	Cuba (INCA)
8	Lp17	Mutants Amistad 82	Cuba (INCA)
9	J-104	IR480-5-9-2/IR-930-16-1	Cuba (IIA)
10	A-82	IR-1529ECICA/UN11R3223	Cuba (IIA)
11	Gines	Mutant J-104(20Gy)	Cuba (INCA)
12	8551	Mutant J-104(20Gy)	Cuba (INCA)
13	8552	Mutant J-104(20Gy)	Cuba (INCA)
14	8553	Mutant J-104 (20 Gy)	Cuba (INCA)
15	8555	Mutant J-104 (20 Gy)	Cuba (INCA)

(IIA) Grain Research Institute

DETERMINATION OF INDICATORS FOR SELECTION UNDER LOW WATER SUPPLY AND TOLERANT GENOTYPES

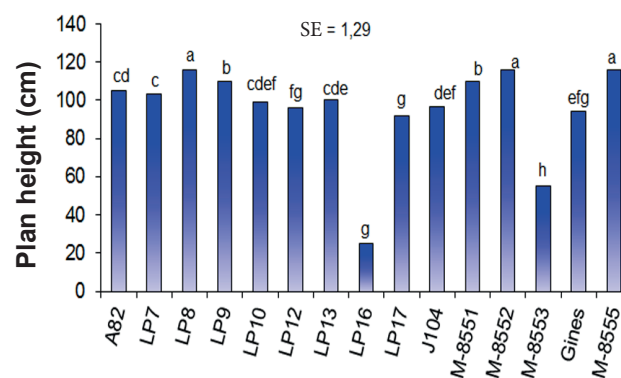
For identifying possible indicators to be used in early selection of tolerant genotypes under water, analysis simple correlations between traits evaluated in vivo and in vitro were performed also principal component analysis (PCA) was performed to determine genotypes of better behavior under water stress, using the statistical software (SPSS) (11).

RESULTS AND DISCUSSION

BEHAVIOR EVALUATION OF A GROUP OF RICE GENOTYPES UNDER LOW WATER SUPPLY CONDITIONS IN THE FIELD

In analyzing the results of the effect of low water in plant height (Figure 1) was observed that the LP-16 genotype, followed by the mutant 8553 were the most affected under low water supply. This could be attributed, among other causes, mechanisms that plants have to adapt to drought, as in the case of stomatal closure is simply the ability to close the stomata completely before the cell is injured by drying, also reduced plant growth during water deficiencies, which may cause damages in photosynthesis (12). In turn, others have noted that the water stress can cause cell elongation, so the genotype plays an important role considering that the most resistant suffer fewer damages (12, 13).

This criterion corroborates the results of other studies that reflect the susceptibility or tolerance of rice varieties and somaclons under these conditions of low water supply (14, 15). Some researchers have suggested that stress resistance is not a simple phenomenon that can occur in two ways: the first is when the plants develop internal mechanisms so that the cells are not under stress; the second is when there is stress tolerance, which is the ability to survive and function properly even under internal conditions and extreme drought^E. Besides water stress is, as to the amount of plant material concerned, the most important plants may suffer (16). Similarly it is categorized as the most depressive factor of rice productivity, affecting the metabolism of carbon and nitrogen, so that productivity and yield decrease (17, 18).



SE= standard error

Figure 1. Effects of low water on plant height with different varieties

^ELima, H. "Resistencia a factores adversos". En: *La resistencia genética de las plantas cultivadas*, La Habana, Cuba, 2006, p. 100.

When analyzing the number of panicles per linear meter (Figure 2) it showed that there were significant differences among the genotypes studied. The cultivar with the greater number of panicles was the LP-8 with 134 panicles per square meter. It is noted that increasing soil temperature the concentration of the N-N ammonia enzyme under stress increases, stimulating the number of panicles per square meter and the number of filled grains per panicle (3). Genotypes of the fewer panicles were LP-17, J-104, Gines and 8555.

Regarding the panicle length (Figure 3) it showed no significant differences among genotypes except LP-16 and the line 8553, who failed to develop, as they died before the panicleation. In vain grains per panicle (Figure 4) significant differences were observed, appreciating that 8552 and Gines varieties had high values of empty grains and other genotypes were maintained with acceptable values.

In the filled grains per panicle (Figure 5) significant differences were observed. Mutants 8552 and 8555 were the best values of this indicator, keeping the other genotypes with acceptable values. In some cases and in certain weather conditions, the percentage of filled grains may be more limiting for yield than the number of panicles; therefore, for any given situation, the causes of yield variation and their components must be examined^F.

An outstanding feature of rice tolerant to low water supplies is their ability to produce consistently, fully fertile panicles, contributing to the stability of yields, although these are relatively low^A.

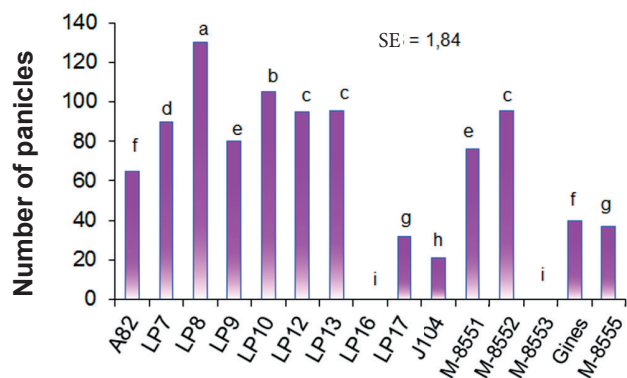


Figure 2. Effects of low water supply on the number of panicles per linear meter with different varieties

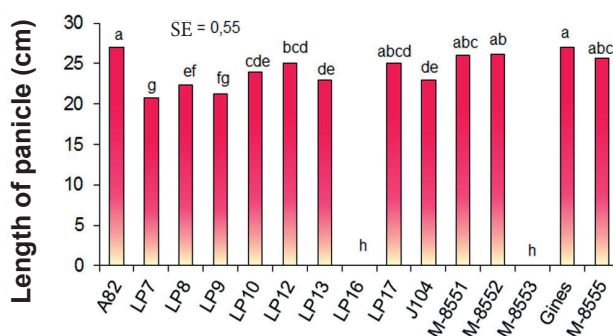


Figure 3. Effects of low water on the panicle length with different varieties

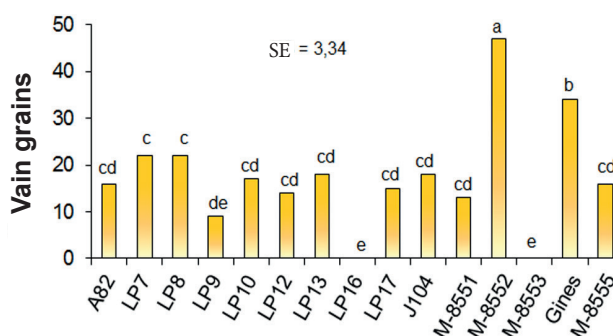


Figure 4. Effects of low water on vain grains with different varieties

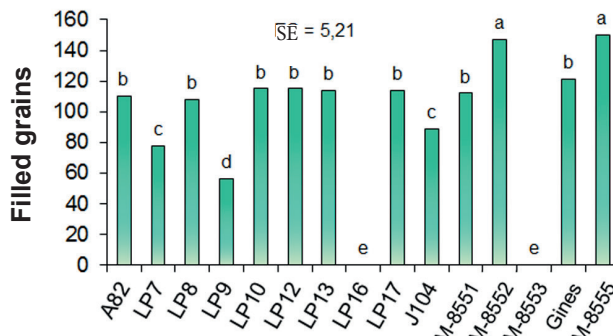


Figure 5. Effects of low water on the grains filled with different varieties

From the analyzes carried out (Figure 6) it was found that the genotype of greater weight of grains per panicle, under conditions of low water supplies, under field conditions, was INCA LP-9. The remaining genotypes showed adequate performance under these same conditions.

In analyzing the results of the agricultural yield (Figure 7), significant differences between genotypes studied were found, highlighting the genotype 8552 with the highest performance in conditions field. The LP-8, LP-10 and LP-12 genotypes also showed adequate returns, under the same conditions. The genotype with lower agricultural behavior was J-104 variety.

^F Socorro, M.; Alemán, L. y Sánchez, S. "El cultivo del arroz en Cuba". En: Taller Nacional FAO/MINVEC/IIA, La Habana, Cuba, 2000, p. 8.

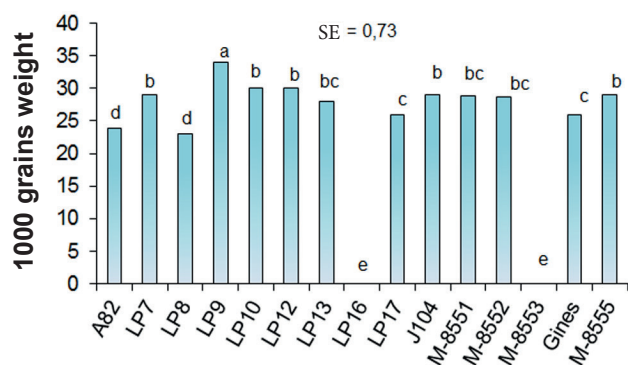


Figure 6. Effects of low water on the weight of 1000 grains with different varieties

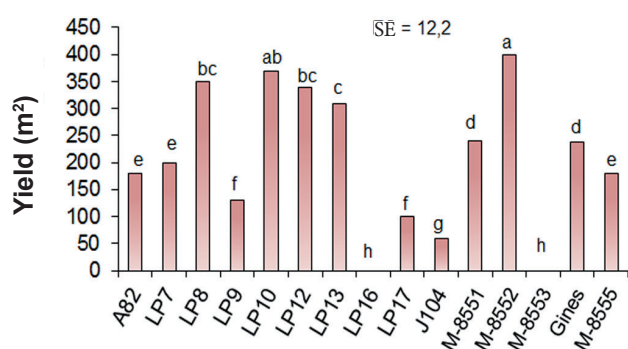


Figure 7. Effects of low water supply on yield per square meter with the different varieties

The performance is of great importance for the weight as criterion when selecting varieties to introduce them to production. Several authors have found that the water deficit induced in the vegetative phase decreases yield rice plants (18).

It should be noted that temperatures during the development of the experiments were high (32 to 34 °C). Usually, the critical temperature values below 20 °C are above 30 °C so, these genotypes in addition to tolerate water stress, ensure adequate filling of the grains in high temperature conditions.

It is stated that as the temperature increases the respiration rate increases and translocation of photosynthetic products to the grains is reduced, thus decreasing the mass of the grains; increases the number of sterile or partially filled grains and the quantity and quality of the grain (19, 20) is reduced.

RESULTS OF SCREENINGS, CONDUCTED UNDER LABORATORY CONDITIONS

The tolerance index, determined from the evaluation of the root system length and plant height under controlled conditions, showed the existence of differences in the tolerance of the genotypes in the

early stages of development, under laboratory with a concentration of 5 g L⁻¹ PEG 6000. The LP-10 genotype showed the lowest relative tolerance index, in relation to plant height (Figure 8). However, 8553 genotype had the highest relative tolerance index under these same conditions.

Growth is a process associated with both the increase in cell number (cell division), and in size (cell elongation) (21). Decreased plant height exposed to water deficit, could be interpreted as an inhibition in cell elongation, as cell elongation is more sensitive to reducing turgor than cell division (22, 23). Also it noted a reduction in the extent of cell wall tissues in leaves of rice plants, resulting in growth (24). Regarding the Index Relative Tolerance root, the LP16 and LP17 genotypes were the most susceptible ones (Figure 9) with values below 60 %, by the action of water deficit; however, the LP-8 and 8552 genotypes (Figure 9) had an adequate growth of the root system under water stress caused by PEG 6000

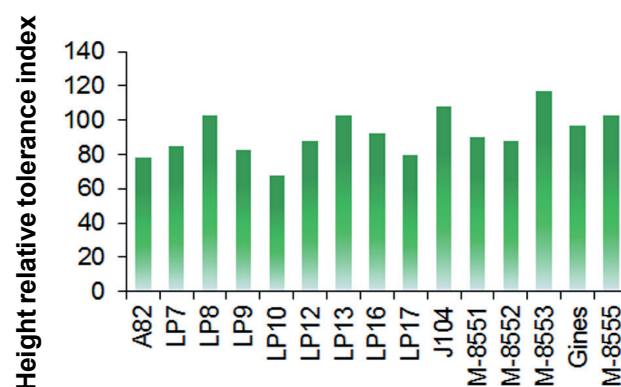


Figure 8. Height relative tolerance index of different varieties

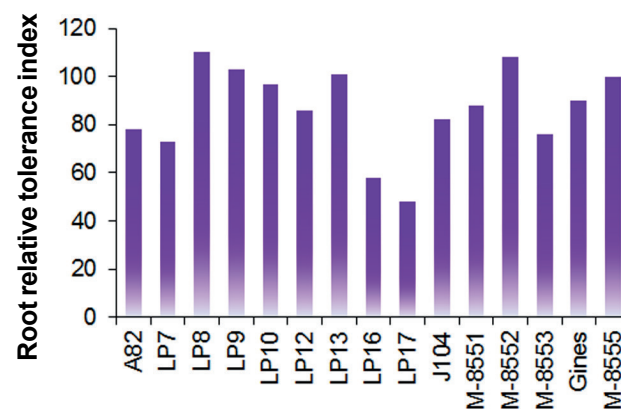


Figure 9. Root relative tolerance index of different varieties

The development of the root system is a desirable characteristic in rice varieties of good resistance to water deficit (9) and contributes to increase water absorption by the plant (25, 26). This adaptive response is closely related to the genotype characteristics.

Differences in root growth among rice varieties exposed to water deficit have been found where the most adaptability varieties to rainfed conditions, presented a greater depth of the root system and fewer roots (8).

In rice plants, grown in drought conditions, a greater length of the root system in drought tolerant varieties have been detected and have been linked to an evasion mechanism of water deficit, which contributes to greater absorption of water accumulated in the layers deeper soil (27, 28). It has been suggested that the length of the root is one of the most related characters to drought evasion in rice cultivation (29, 30).

Generally, it is important to notice that the root system length is one of the fundamental characteristics of the species resistant to drought so, have a deep root system that allows the plant to low water potentials, continue its development through moisture absorption of this to a greater depth in the soil (29, 30); is an indicator to be considered for the selection of varieties with adaptability to these conditions (31, 32).

DETERMINATION OF INDICATORS FOR THE SELECTION

The performance under low water supplies showed significant positive correlations with plant height, the relative tolerance index of the root system, the number of panicles per meter, the panicle length, number of filled grains and 1000 grain weight.

However, it showed no significant correlations with the index relative tolerance of plant height (Table II), which is consistent with what was found in performance tests, using originated varieties through biotechnological techniques⁶.

The performance is set according to its components: panicles per plant, filled grains, 1000 grain weight⁶. This analyzes show a direct positive effect of panicle and filled grains on performance; however, these indicators are assessed at harvest time, the tolerance index of the root system could be used for the early selection of drought tolerant genotypes.

GENOTYPE SELECTION OF GOOD BEHAVIOR UNDER LOW WATER SUPPLY CONDITIONS

From Principal component analysis performed, it was shown that with the use of the first two components (C1 and C2) could explain 77,45 % of the variability and characters greater correlation with the first component were root relative tolerance index, plant height, panicle number, panicle length, full grains, 1000 grain weight and performance. The second component was explained by the relative tolerance index of plant height (Table III).

As for the distribution of the studied genotypes from the evaluated indicators, it was observed wide dispersion, which shows the high variability in their behavior under conditions of low water supply (Figure 10). Genotypes with the best overall performance under low water supplies were the LP-10 and 8552. The LP-16 and 8553 genotypes were the most susceptible because not withstand stress and died before the panicleation, followed by LP-17 and J-104.

⁶ Madruga, A. "Cuba por aumentar sus rendimientos arroceros". *Granma*, La Habana, Cuba, 2009.

Table II. Phenotypic correlations between different traits evaluated *in vivo* and *in vitro*

Characters	Yield	ITR height	ITR root	Height	Number of panicle/m	Length panicle	Filled grains	Vain Grains	1000 grain weight
Yield	1	-0,330	0,603*	0,633*	0,908**	0,636*	0,691**	0,412	0,582*
ITR height	-0,330	1	0,227	-0,165	-0,300	-0,350	-0,274	-0,281	-0,356
ITR root	0,603*	0,227	1	0,620*	0,634*	0,428	0,395	0,558*	0,431
Height	0,633*	-0,165	0,620*	1	0,664**	0,917**	0,800**	0,710**	0,909**
Number panicle/m	0,908**	-0,300	0,634*	0,664**	1	0,612*	0,525*	0,411	0,636*
Length panicle	0,636*	-0,350	0,428	0,917**	0,612*	1	0,908**	0,660**	0,934**
Filled grains	0,691**	-0,274	0,395	0,800**	0,525*	0,908**	1	0,553*	0,746**
Vain Grains	0,412	-0,281	0,558*	0,710**	0,411	0,660**	0,553*	1	0,677**
1000 grain weight	0,582*	-0,356	0,431	0,909**	0,636*	0,934**	0,746**	0,677**	1

* The correlation is significant at the 0,05 level (bilateral)

** The correlation is significant at the 0,01 level (bilateral)

Table III. Contribution Values of the components to the total variation in rice plants. Correlation of components with the studied variables

Variables	Components	
	C1	C2
ITA	-0,342	0,831
ITR	0,648	0,686
Height	0,936	-0,8377
# panicle	0,804	0,143
Panicle length	0,932	-0,178
Filled grains	0,855	-0,133
Vain grains	0,743	-0,0457
1000 grain weight	0,905	-0,164
Yield	0,818	-0,8695
% variance	63,316	14,134
% cumulative	63,316	77,450

ITA: index of relative height tolerance, ITR: root tolerance index, P.1000 grains: 1000 grain weight

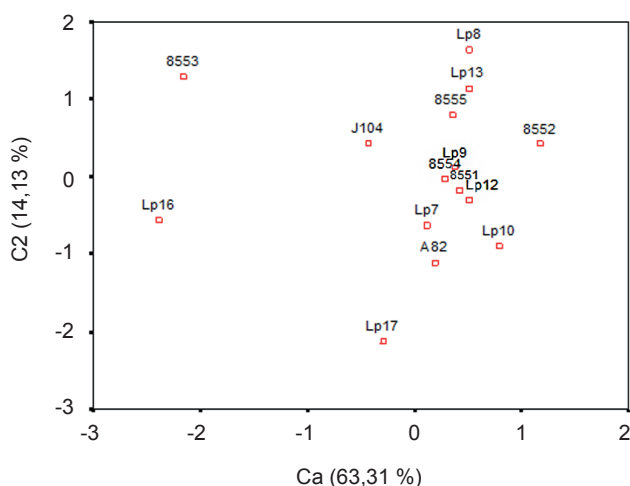


Figure 10. Distribution of indicators for the evaluated variables

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