

EFFECT OF SALINITY IN THE INITIAL STAGES OF THE DEVELOPMENT OF THREE RICE CULTIVARS (*Oryza sativa* L.)

Efecto de la salinidad en estadios iniciales del desarrollo de tres cultivares de arroz (*Oryza sativa* L.)

Elizabeth Cristo Valdés^{1✉}, María C. González², Elsa Ventura³ and Aida T. Rodríguez¹

ABSTRACT. The work was carried out in the biotechnology laboratory at the Scientific Technological Base Unit "Los Palacios" (UCTB), which belongs to the National Institute of Agricultural Sciences (INCA) in Los Palacios municipality, in province of Pinar del Río in the March to May period. One hundred (100) seeds of three rice cultivars obtained by different methods of improvement, INCA LP-10, J-104 and INCA LP-7, this last salinity tolerant control were selected and were germinated in petri dishes with paper of Filter and wetted with 15 mL the different saline concentrations of sodium chloride (NaCl) (4000, 7000 and 10000 ppm), distilled water was used as the control. The number of germinated seeds was evaluated at 7 days, determining the germination percentage and at 15 days the height of the seedlings, root length and accumulation of fresh and dry biomass were evaluated in 10 seedlings per replicate, being determined the index of Tolerance to salinity. Varietal differences were observed regarding the live response of the cultivars under study with saline concentrations, where the cultivar INCA LP-7 and INCA LP-10 showed the best tolerance indices for these abiotic stress conditions.

Key words: NaCl, abiotic stress, plant breeding

RESUMEN. La salinización de los suelos es uno de los procesos que provoca el cambio climático que provoca serias afectaciones en los rendimientos agrícolas de los cultivos. El trabajo se desarrolló en el laboratorio de biotecnología de la Unidad Científico Tecnológica de Base "Los Palacios" (UCTB), perteneciente al Instituto Nacional de Ciencias Agrícolas (INCA) en el municipio Los Palacios, provincia Pinar del Río en los meses de marzo hasta mayo. Se seleccionaron 100 semillas de tres cultivares de arroz obtenidos por diferentes métodos de mejora, INCA LP-10, J-104 e INCA LP-7, este último cultivar es el control tolerante a la salinidad, las cuales se colocaron a germinar en placas de Petri con papel de filtro y se humedecieron con 15 mL de concentraciones salinas de cloruro de sodio (NaCl) (4000, 7000 y 10000 ppm), como control se usó agua destilada. Se evaluó a los siete días el número de semillas germinadas, determinándose el porcentaje de germinación y a los 15 días se evaluaron la altura de las plántulas, longitud de la raíz y acumulado de biomasa fresca y seca en diez plántulas por réplica, así como el índice de tolerancia a la salinidad. Se observaron diferencias varietales en cuanto a la respuesta en vivo de los cultivares en estudio con las concentraciones salinas, donde el cultivar INCA LP-7 e INCA LP-10 mostraron los mejores índices de tolerancia para estas condiciones de estrés abiótico.

Palabras clave: NaCl, estrés abiótico, mejoramiento genético

¹Unidad Científico Tecnológica de Base "Los Palacios", Instituto Nacional de Ciencias Agrícola, Gaveta Postal 1, San José de las Lajas, La Habana, Cuba, CP 32700

²Instituto Nacional de Ciencias Agrícolas (INCA), carretera San José-Tapaste, km 3½, Gaveta Postal 1, San José de las Lajas, Mayabeque, Cuba. CP 32700

³Centro de Desarrollo de Productos Bióticos del Instituto Politécnico Nacional, carretera Yautepec-Jojutla km 8, Calle CEPROBI, No. 8, Col. San Isidro, Yautepec, Morelos, México. C.P. 62731

✉ ecristo@inca.edu.cu

INTRODUCTION

Rice is the second most important food for man, after wheat, it constitutes 80 % of the diet and provides 20 % of the calories to more than half of the world's population. If the current rate of population growth is taken into account, it can be estimated that the global demand for rice increases to more than 170 million tons in a period of 25 years ⁽¹⁾.

In Cuba, rice is one of the main foods for the population, due to the great habit of consumption of the same, reporting an annual per capita of 60 kg, well above almost all the countries of the American continent and close to the patterns of consumption of some Asian countries, but so far, the national production satisfies a little more than 50% of the needs, so the country is forced to complete them with imports ⁽²⁻⁴⁾.

Although the Cuban varietal policy is made up of more than 10 rice cultivars, these have presented difficulties to express their maximum productive potential and the average agricultural yield is 3.2 t ha⁻¹, lower than the world average ⁽⁵⁾, motivated by different causes, effects due to the climate, technological indiscipline, affectations by pests, little availability of water and problems with salinization, the latter being one of the most important, since more than one million hectares are affected by the salinity and one million one hundred thousand with a tendency to salinize ⁽⁶⁾.

Salinity is one of the most damaging stresses today. Inadequate irrigation of soils, as well as climate change, cause this phenomenon to reach a global level. There are reports that show that the area of the planet affected by salinization ranges between 40-50 % ⁽⁷⁾.

This situation makes it necessary to study the incidence of salinity in order to increase production; therefore, research is carried out aimed at obtaining and evaluating cultivars with adaptability to these conditions ⁽⁶⁾.

Within this context, it is of particular interest for the breeder to have early and rapid selection methods and indicators of the degree of tolerance, which allow increasing the efficiency in the selection of germplasm with tolerance to salinity ⁽⁸⁾.

However, it is necessary to increase the number of indicators in rice that may be useful in the discrimination of genotypes tolerant to such abiotic stress. Based on this goal, in recent years Cuban researchers have released a group of rice varieties, which has enabled higher yields per hectare ⁽⁶⁾.

In this work, the objective was to evaluate the effect of different salt concentrations (NaCl) on the germination and initial growth of seedlings of three genetically grown rice cultivars.

MATERIALS AND METHODS

The work was developed in the Biotechnology laboratory of the Scientific Technological Unit of Base "Los Palacios", belonging to the National Institute of

Agricultural Sciences (INCA). In February 2017, under controlled conditions, 100 seeds were selected of three rice cultivars obtained by different improvement methods, INCA LP-10, J-104 and INCA LP-7, this last cultivar is the control tolerant to salinity (Table 1), which were placed to germinate in Petri dishes with filter paper and moistened with 15 mL of saline solution, using as a stress agent sodium chloride (NaCl) to (4000, 7000 and 10,000 ppm), to simulate salinity stress. As a control, distilled water was used. The plates were placed in a growth chamber, at a temperature of 28 ± 2 °C.

Table 1. Origin of the cultivars evaluated in the study

Cultivars	Progenitors	Breed
INCA LP - 10	Somaclón Amistad - 82	INCA
INCA LP -7	Somaclón Amistad - 82	INCA
J- 104	IR 480-5-2/ IR30-16-1	Perú

EVALUATIONS

The number of germinated seeds was evaluated seven days later, determining the percentage of germination and after 15 days the height of the seedlings, the length of the root and the accumulated of fresh and dry biomass in 10 seedlings per replica were evaluated.

From these data the tolerance to salinity (TS) was calculated, according to the formula described (9).

$$TS = 100 (IE/IC)$$

where

TS: tolerance to salinity

IE and IC: represent the average of the indicators evaluated in the stressful solution and control, respectively

STATISTICAL PROCESSING

A completely randomized design with three repetitions was used and the data were processed through the statistical program STAGRAPHICS Plus v 5.0, where a two-factor analysis of variance was performed; the obtained means were certified by the Tukey multiple range test at a 5 % error probability.

RESULTS AND DISCUSSION

When analyzing the results of germination (Figure 1), it was found that there are differences in all cultivars evaluated, as salt concentrations increase, highlighting cultivars INCA LP-7 and INCA LP-10 with the highest tolerance index in germination at concentrations of 7,000 ppm of salt; however, the cultivar J-104 presented in this concentration the lowest values of

tolerance index. In addition, it was evidenced, in the concentrations of 10,000 ppm of salt, that the cultivar J-104 presented susceptibility to this abiotic factor. This could be attributed, among other causes, to the loss of the ability to absorb water or a toxic effect of the ions, which is manifested by the decrease in the germination tolerance index. In studies carried out by other authors in other cultivars, it has been shown that as saline concentrations increase, there is a decrease in salinity tolerance rates in germination, which may be due to difficulties in the processes of imbibition of the seed and the damages to this are attributed to metabolic imbalances ^(8,10).

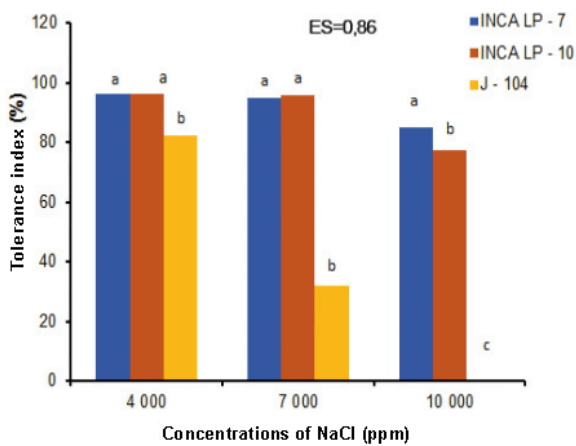


Figure 1. Behavior of the salinity tolerance index in germination in three rice cultivars

Regarding the height of the plants (Figure 2 and Photo 1), it was possible to appreciate that there are differences among all the cultivars under study. As the salt concentrations increase, a reduction in the height of the plants is observed, highlighting the cultivar INCA LP-7 with the highest index of tolerance to salinity, classifying it as tolerant in the concentration of 10,000 ppm of salt and for the cultivar J-104 the affectation was so drastic that there was no germination of the seeds.

Similar results were obtained by other researchers in other cultivars, where it is argued that high salt concentrations induce a substantial reduction in growth, caused by the imbalance of specific ions and toxic effects caused by the accumulation of ions (6,11,12). It is valid to point out that the plants germinated in the salt concentration (4000 ppm) had a higher leaf texture and an intense green coloration.

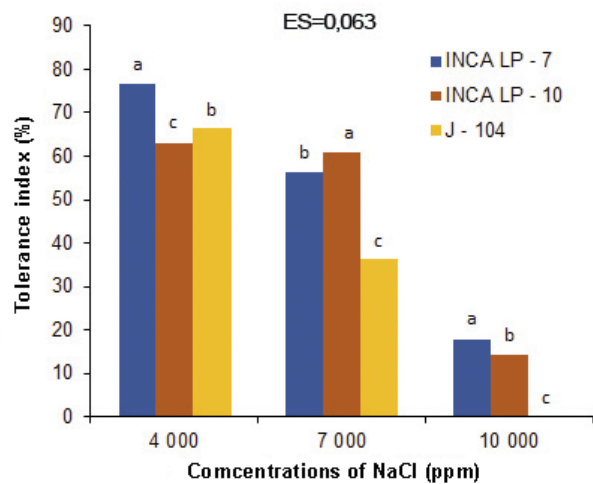


Figure 2. Behavior of the index of tolerance to salinity in the height of the seedlings in three rice cultivars



Photo 1. Evaluation of INCA LP-7 cultivar in different salt concentrations

When evaluating the length of the root system (Figure 3, Photos 2 and 3), it was found that there were differences, as saline concentrations increased in all the evaluated cultivars, highlighting the cultivar INCA LP-10 with the highest index of tolerance in the concentration of 7,000 ppm of salt. In the concentration of 10,000 ppm of salt, the cultivars INCA LP-7 and INCA LP-10 stood out as those with the best performance in terms of the tolerance index, while the cultivar J-104 showed general susceptibility, causing death to these such high concentrations of salts.

Studies carried out on different rice cultivars have indicated that the root system of plants is severely affected as saline concentrations increase, being one of the indicators that contributes most to tolerance to this abiotic factor.

The capacity of the plants to grow in saline solutions reflects a complex of physiobiochemical particularities, especially at a general level in the metabolic processes, which constitute the basis for their later development and the formation of a strong root system; which, in turn, has an impact on greater tolerance to adult plants ⁽¹⁰⁻¹⁴⁾.

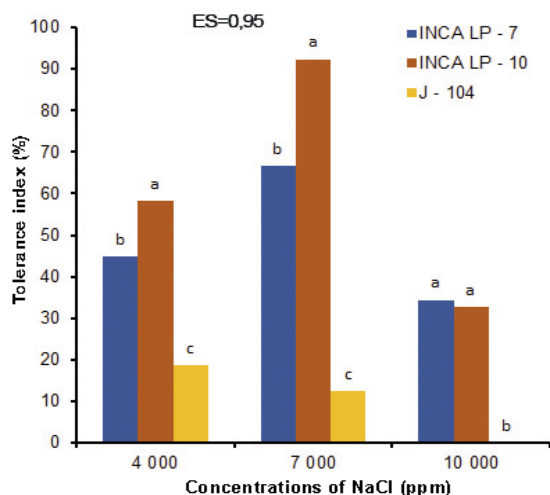
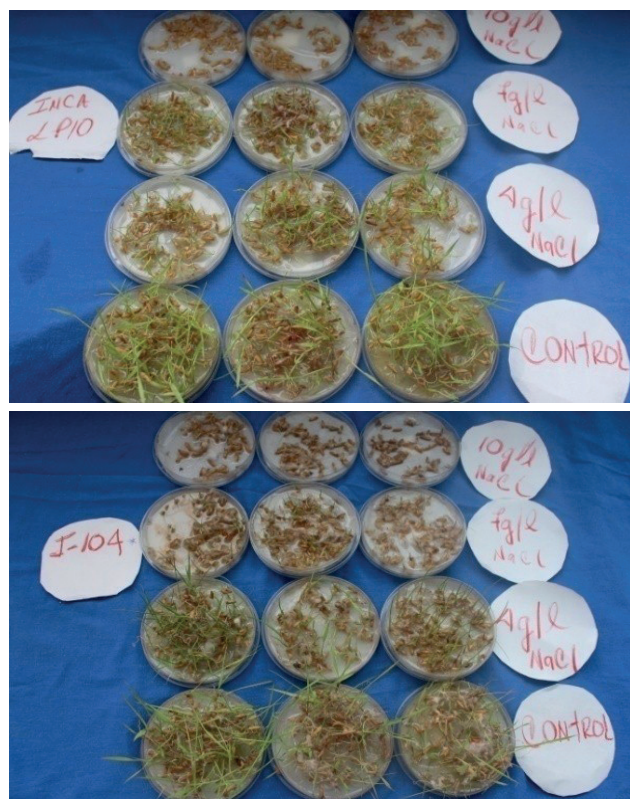


Figure 3. Behavior of the salinity tolerance index on the length of the root in three rice cultivars



Photos 2 and 3. Evaluation of cultivars INCA LP-10 and J-104 in different salt concentrations

The production of fresh matter (Figure 4), was also affected by the salinity of the medium, observing a differentiated behavior in the tolerance index in the different cultivars, exhibiting the best results INCA LP-10 cultivars, followed by the INCA LP-7, the latter was released to production for its tolerance to salinity and the worst performance was obtained by J-104, being very susceptible to the salt concentration of 10,000 ppm.

This could be due, among other causes, to the damages caused by the salinity stress by producing a strong reduction of the cellular osmotic potential and the toxicity of the sodium and chloride ions. In studies carried out by other researchers ⁽¹¹⁾, where the growth of rice seedlings at different concentrations of NaCl was evaluated, the great affectations caused by salt stress in fresh dough, where osmotic factors influence directly were proved, as well as the toxicity of the ions throughout the development stage.

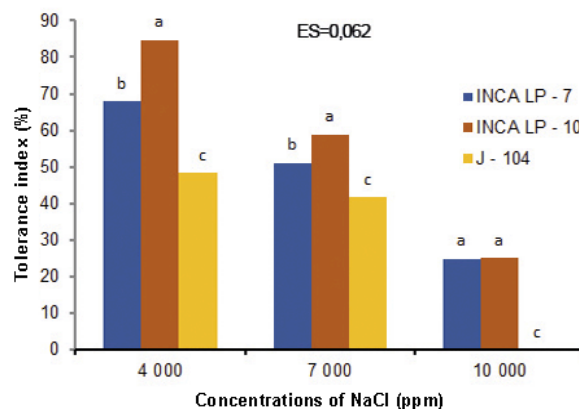


Figure 4. Behavior of the index of tolerance to salinity in the fresh mass in three rice cultivars

In the production of dry matter (Figure 5), differences were found in the tolerance indexes of all cultivars studied, as saline concentrations were increased, standing out in the concentrations of 4000 and 7000 ppm of salt the cultivar INCA LP- 10, followed by INCA LP-7, which demonstrates the tolerance of these cultivars, obtained by somatic cultivation, to the strong abiotic stress; However, in the concentration of 10,000 ppm of salt, the cultivar INCA LP-7 accumulated the largest amount of dry biomass, which is closely related to agricultural yield. A reduction in cultivar J-104 at all concentrations was also observed and became drastic in that of (10,000 ppm) salt.

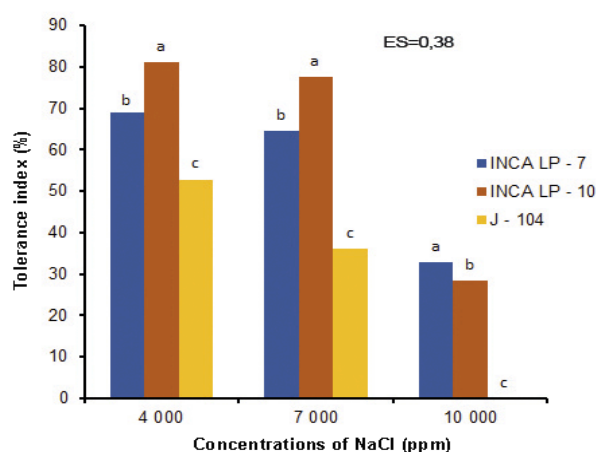


Figure 5. Behavior of the salinity tolerance index in the dry mass in three rice cultivars

This increase in cultivar INCALP-7 in the values of dry mass in the concentrations of 10,000 ppm of salt, can be justified, among other causes, by an increase in the production of organic solutes (sugars, proline, amino acids), to counteract the osmotic effects of salinity and the retention of the ions in the vacuoles of the roots in the growth of the different organs, which allow the plants to tolerate very high cellular or extracellular concentrations of salt^(8,10-12).

Different authors have pointed out that plants subjected to salinity conditions, adjust osmotically and reduce their internal osmotic potential, for which they use a part of their photosynthates. In addition, it has been found that salinity affects the dry mass of rice seedlings^(8,10,13,14).

According to research carried out by some authors^(2,13,15), the dry mass is the most appropriate criterion to measure the growth and the magnitude of the capacity of the assimilation system of the plant; the yield of the grain of rice depends largely on the total production of dry mass and its distribution towards them.

In studies carried out by other authors, where the behavior of these cultivars was evaluated in soils affected by salts, similar results were found in which the accumulated increase in dry mass was related to agricultural yield^(3,15).

CONCLUSIONS

Varietal differences were observed in the initial stages of development, in terms of the *in vivo* response of the cultivars under study, with the saline concentrations of sodium chloride, where the cultivars INCALP-7 and INCALP-10 showed the best indices of tolerance for these conditions of abiotic stress.

BIBLIOGRAPHY

1. Khush GS. Strategies for increasing the yield potential of cereals: case of rice as an example. Gupta P, editor. *Plant Breeding*. 2013;132(5):433–6. doi:10.1111/pbr.1991
2. González T. Informe anual, Arroz. La Habana, Cuba: Instituto de Investigaciones del Arroz, MINAG; 2011.
3. Polón R, Castro R, Ruiz M, Maqueira L. Práctica de diferentes alturas de corte en el rebrote y su influencia en el rendimiento del arroz (*Oryza sativa* L.) en una variedad de ciclo medio. *Cultivos Tropicales*. 2012;33(4):59–62.
4. Rivero LLE, Suárez CE. Instructivo Técnico Cultivo de Arroz. La Habana, Cuba: Asociación Cubana de Técnicos Agrícolas y Forestales; 2015. 77 p.
5. ONEI. Oficina Nacional de Estadísticas. Cuba [Internet]. 2014 [cited 2018 Jul 4]. (Anuarios Estadísticos Agropecuarios.). Available from: <http://www.one.cu/aec2016.htm>
6. Cristo E, Pérez N, González M. Comportamiento de genotipos de arroz (*Oryza sativa* L.) promisorios para suelo salino. *Cultivos Tropicales*. 2012;33(3):42–6.
7. Planos E, Rivero R, Guevara V. Impacto del cambio climático y medidas de adaptación en Cuba. Instituto de Meteorología; Proyecto GEF/PNUD de la República de Cuba, La Habana; 2013. 427 p.
8. García A, Dorado M, Pérez I, Montilla E. Efecto del déficit hídrico sobre la distribución de fotoasimilados en plantas de arroz (*Oryza sativa* L.). *Interciencia*. 2010;35(1):46–54.
9. González LM, Prieto F, Aguilera RM, Guardia L, Pérez A. Desarrollo de metodología de evaluación para resistencia a la salinidad en arroz. In: Memoria del Coloquio Cubano-Francés sobre mejoramiento y manejo de vertisuelos. In Montpelier: Editorial EMSAMLINRA; 1991. p. 329–36.
10. Suárez RS, Reyes RR, Bodes OG. Evaluación de ocho variedades de arroz (*Oryza sativa* L.) en el municipio Manatí, provincia Las Tunas. *Innovación Tecnológica*. 2013;19(4):49.
11. Martínez L, Reyes Y, Falcón A, Núñez M. Efecto del tratamiento a las semillas con quitosana en el crecimiento de plántulas de arroz (*Oryza sativa* L.) cultivar INCALP-5 en medio salino. *Cultivos Tropicales*. 2015;36(1):143–50.
12. Alam MM, Tanaka T, Nakamura H, Ichikawa H, Kobayashi K, Yaeno T, *et al.* Overexpression of a rice gene (OsHAP2E) confers resistance to pathogens, salinity and drought, and increases photosynthesis and tiller number. *Plant Biotechnology Journal*. 2015;13(1):85–96. doi:10.1111/pbi.12239

13. Nounjan N, Theerakulpisut P. Effects of exogenous proline and trehalose on physiological responses in rice seedlings during salt-stress and after recovery. *Plant, Soil and Environment*. 2012;58(7):309–15. doi:10.17221/762/2011-PSE
14. Longping Y, Xiaojin W, Fuming L, Guogui M, Qiusheng X. *Hybrid Rice Technology*. Philippines, China: Sponsored by the Romon Magsaysay Foundation; 2014. 131 p.
15. Campo S, Baldrich P, Messeguer J, Lalanne E, Coca M, San Segundo B. Overexpression of a Calcium-Dependent Protein Kinase Confers Salt and Drought Tolerance in Rice by Preventing Membrane Lipid Peroxidation. *Plant Physiology*. 2014;165(2):688–704. doi:10.1104/pp.113.230268

Received: October 12th, 2017

Accepted: June 20th, 2018

