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INDICATORS OF INITIAL GROWTH AND NUTRITIONAL STATUS FOR EARLY SELECTION GENOTYPES OF RICE (*Oryza sativa* L.) SALT TOLERANT

Indicadores del crecimiento inicial y del estado nutricional para la selección temprana de genotipos de arroz (*Oryza sativa* L.) tolerantes a la salinidad

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ABSTRACT. In order to identify possible indicators of initial growth and nutrition for early selection of rice genotypes tolerant to salinity, tests with two cultivars with different degree of salinity tolerance ('Pokkali' and 'Amistad-82') were developed under controlled conditions, saline solutions tailored to electrical conductivities (EC) of 8 and 12 dSm^{-1} by the addition of NaCl and a control with distilled water. The percentage of germination at 15 days after sowing (DAS) and the growth parameters: plant height, root length, dry mass of the aerial part (MSPA), dry mass of the root system (MSSR) to 21 DAS were evaluates. Meanwhile, in leaves and roots of both cultivars under controlled conditions and using nutrient solutions for the simulation of salt stress (CE 8 and 12 dSm^{-1}), was determined at seedling stage, the content of nitrogen (N), phosphorus (P) , potassium (K), sodium (Na) , and sodium / potassium ratio. The results show a differential behavior of the cultivars in their degree of tolerance to salinity, allowing use some of the variables evaluated as indicators for discrimination of rice genotypes tolerant to salinity.

Key words: rice, salinity, growth, indicators

RESUMEN. Con el objetivo de determinar posibles indicadores del crecimiento inicial y nutricionales para la selección temprana de genotipos de arroz tolerantes a la salinidad, se desarrollaron ensayos con dos cultivares con diferente grado de tolerancia a la salinidad ('Pokkali' y 'Amistad- 82') en condiciones controladas, con soluciones salinas ajustadas a conductividades eléctricas (CE) de 8 y 12 dSm^{-1} mediante la adición de NaCl y un control con agua destilada. Se evaluó el porcentaje de germinación a los 15 días después de la siembra (DDS) y los parámetros del crecimiento altura de la planta, longitud de la raíz, masa seca de la parte aérea (MSPA), masa seca del sistema radical (MSSR) a los 21 DDS. En tanto, en hojas y raíces de ambos cultivares, en condiciones semicontroladas y utilizando soluciones nutritivas para la simulación del estrés salino, con CE de 8 y 12 dSm^{-1}, se determinó en estado de plántula, el contenido de nitrógeno (N), fósforo (P), potasio (K), sodio (Na), así como la relación sodio/potasio. Los resultados evidencian un comportamiento diferencial de los cultivares en cuanto a su grado de tolerancia a la salinidad, lo que permite usar algunas de las variables evaluadas, como indicadores para la discriminación de genotipos de arroz tolerantes a salinidad.

Palabras clave: arroz, salinidad, crecimiento, indicadores

INTRODUCTION

The our planet area, affected by salinization, is around 800 million hectares, making it one of the most serious problems facing agriculture worldwide (1, 2, 3), of which Cuba is not exempt, presenting around 7,08 million hectares as agricultural area, of which one million hectares are affected and 1,5 million have potential salinity problems. This represents for our country, about 14 % of affected areas with different salinization degrees (4). Excess salt ions in the soil solution, causes damage involving the growth and crop development and therefore a decrease in yield potential (5).

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These data are the result of disorders caused in the plant metabolism mainly by changes in soil osmotic potential, nutritional imbalance disorders by the interaction between the toxic ions and essential nutrients for growth and development as well as the oxidative stress, induced from different effects by increased reactive oxygen species (ROS) in cell components (5).

In the recovery and management politics of these soils, the use of species and varieties tolerant to salt stress is considered as the most pragmatic event to obtain good yields under saline conditions (6) and it has received special attention by different researchers in Cuba and world regions (5, 7). Although this subject has been the subject of multiple investigations, particularly in the last 30 years, expectations are not all fulfilled yet and we will have to continue working on the genetic improvement of different crops to confront this problem.

Rice is one of the most economically important crops worldwide. It is the most consumed grain for more than half of the population, after wheat (8). However, its productivity is seriously affected by salinity at each development stage.

For the above stated, improvements for salinity tolerance in rice cultivation requires technical evaluation of different materials, which must be fast to keep a gradient evaluation corresponding with the large amount of improved material. It has been suggested that selection in early stages of growth for the development of new rice varieties tolerant to salinity, provides a rapid assessment that it is difficult in advanced vegetative states or reproductive conditions (9) and helps to reduce the obtaining period of new cultivars, to reducing the number of materials for the next stages of improvement, which would be troublesome to work with genotype large volumes and reduce other environmental effects, because it works in controlled or semi-controlled conditions.

The success of each selection and evaluation methods of new materials in genetic improvement program is related to objective definition[A] as well as the determination of viable indicators for genotype selection with appropriate behavior in these unfavorable conditions, are very important tools (9).

Therefore, there has been studied the biochemical parameter variation, because, to some extent, the biochemical basis of tolerance are known and which of these would constitute adaptative mechanisms which are induced in plants and at the same time, having a close relationship with stress (10). The need for indicators for genotype early selection to salinity tolerant rice requires researchers, plant breeders of rice cultivation, new challenges which have to be developed. In this way we would be contributing to the use of affected areas with salinity problems in the country.

This study aimed to determine indicators of early growth and nutritional status in the vegetative stage of seedlings for early selection of salt tolerant rice genotypes.

MATERIALS AND METHODS

In the study were used seeds of two rice cultivars with different responses to salinity: Pokkali international witness tolerant to salinity (7) and Amistad 82 (A-82) witness susceptible to salinity (9). Seeds were disinfested with sodium hypochlorite 3 % for five minutes; then they washed three times with distilled water.

For initial growth indicators, 50 seeds in plates were placed (10,5 x 0,8 cm) having filter paper as a support to which received 10 mL of saline solutions using NaCl to obtain 8 and 12 dSm^{-1} EC as treatments. The control treatment was applied only distilled water. Three plates were planted per treatment, which were placed in the dark for 48 hours to promote germination. Subsequently, they were kept at a temperature of 25 ± 2 °C and a photoperiod of 16 h light and 8 h dark, in a completely randomized design. The experiment was repeated twice in the same time working conditions.

Fifteen days after sowing (DAS) germinated seeds were counted and the germination percentage and 21 (DAS) plant height (PH) was evaluated, the root length (RL), the dry mass was determined of the aerial part (DMAP) and root system (DMRS) and the relationship between the shoot and root system (AP/RS) was determined.

Nutritional indicators for three pre-germinated seeds were transplanted into plastic containers (200 mL) containing ground Hydromorphic Ferruginous Nodular Gley (11) and were placed in boxes containing distilled water. The experiment was conducted under greenhouse conditions. Fifteen days after planting (APL) they were induced by salt stress applying Murashige and Skoog nutrient solution 50 % (½ DM) (12) adjusted to 8 and 12 dSm^{-1} EC obtained by adding NaCl and a control nutrient solution with ½ DM.

[A] IIArroz. Manejo de semillas y producción de semillas de arroz. En: Conferencias del curso pre-congreso IV Encuentro Internacional de Arroz. 2008, 2 de junio: Palacio de conversiones. de la Habana, Cuba, 111 pp.

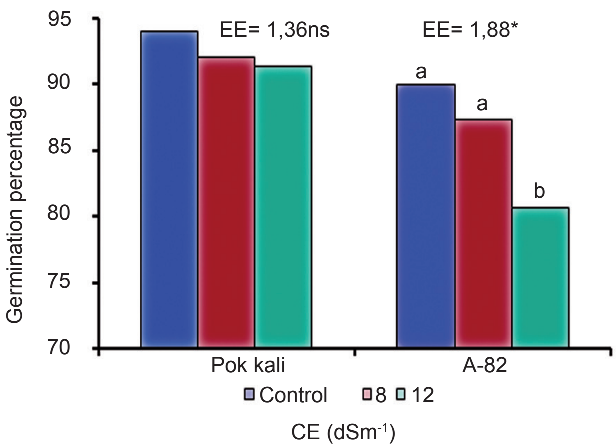
Fifteen days after tax salt stress, leaf and root system of three containers of each cultivar were sampled to determine the percentage of N by colorimetric determination with Nessler reagent, the percentage of P by colorimetric determination of color formation blue molybdate phosphoric complex and K^+ and Na^+ percentage flame photometry (13). With the values of K^+ and Na^+ ratio Na^+/K^+ was determined. Completely randomized design with six replicates and the experiment was repeated twice in the time in the same working conditions.

The data are processed by simple variance analysis, based on a linear fixed effects model using SPSS version 11.5 and significant differences among treatments were verified by multiple range test of Tukey for a significance level of 5 %.

RESULTS AND DISCUSSION

Determination of morphophysiological indicators during germination in growth early stages

Results showed different behavior of studied phenotypes for seed germination at different salt concentrations tested (Figure 1). It was observed a decrease in seed germination in the A-82 growing as increased EC growth medium, showing significant difference (p≤0,05) compared to control 12 dSm^{-1} EC, not so for EC 8 dSm^{-1}.



* (P≤0,05)
Common letters do not differ according to Tukey test (p≤0,05)

Figure 1. Seed Germination percentage of two rice cultivars in saline conditions

Pokkali cultivar was not significantly different (p≤0,05) in terms of seed germination for any of the EC studied. In this case, the good performance of seed germination in Pokkali, offers advantages for better establishment, growth and effective development their seedlings in salt stress conditions.

Pokkali cultivar behavior can be associated that under these conditions, could maintain the water absorption through the integument, so that the physical processes such as seed imbibition and hydrolysis of booking polysaccharides not affected, which provides good germination.

The decrease in seed germination of the cultivar A-82 in saline conditions, can be attributed to lower absorption of water could affect imbibition of them because it is one of the earliest events that occurs during seed germination[B].

The different behavior observed between both cultivars in 12 dSm^{-1} EC, discriminates among genotypes, making seed germination in an efficient indicator for early selection. In general, the initial growth parameters evaluated were influenced by salt stress in both cultivars (Table). In spite of the stress influence, different behavior of the cultivars was detected in root length and dry mass accumulation of root system to EC 8 dSm^{-1}.

In cultivar Pokkali, significant stimulation (p≤0,05) for control of RL and DMRS in dsm^{-1} 8 EC, was evidenced while the A-82 cultivar, showed a significant reduction of LR and DSRS. The dry mass of the aerial part showed no significant changes in the Pokkali cultivar with respect to its control reducing 8 dsm^{-1} EC to 12 dsm^{-1} one; however, in A-82, the dry mass of the aerial part was most affected since it decreased with the increase of EC in the growth medium. These parameters can be indicators for selecting tolerant genotypes to salt stress in the growth initial stage in the rice cultivation

RL stimulation detected in the tolerant crop (Pokkali) is an important feature of this cultivar. This response usually is attributed to the need of plants absorbing water under high salinity and drought, causing an root system elongation A more elongated in osmotic stress conditions, which can be caused both by lack of water as a high salt concentration in the growth medium, could contribute to the search for water and to better development in these growing conditions. Furthermore, the shorter length of A-82 root may be the result of cell turgor loss (5).

[B] García, A. Efectos fisiológicos del déficit hídrico inducido en fases tempranas del crecimiento de plantas de arroz (*Oryza sativa* L.) y su aplicación en la selección de variedades tolerantes. [Tesis de Doctorado]. Instituto de Investigaciones Fundamentales en la Agricultura Tropical, Instituto de Investigaciones de Riego y Drenaje. 2009. 99 pp.

The decreases in biomass accumulation under saline conditions have been observed in a wide range of crops (14, 15, 16). DMRS increment shown in Pokkali cultivar may be related to the length increasing of the root system (Table) and, with this cultivar tolerance to salt stress, because a more developed root system in this condition can improve water absorption. The increase in water absorption when there is a greater development of root system could help maintain the photosynthetic activity expressed in dry mass[B].

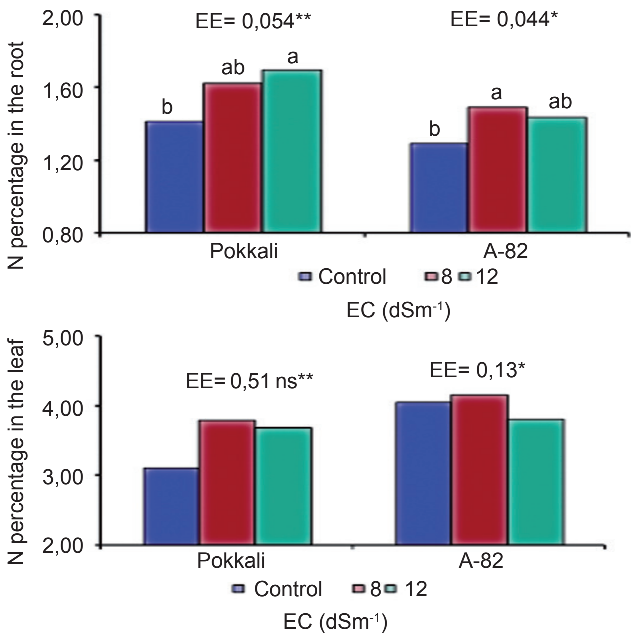
It was detected that the salt stress had an important influence on reducing the plant height of both cultivars (Table). The salt stress influence on plant height, unable to detect different behavior of evaluated cultivars hence, is not considered an efficient indicator for the selection of genotypes tolerant rice to salinity in early growth stages.

Decreases in seedling height are the result of a turgor loss to cellular level, caused by the osmotic potential decrease in the cultivar growth medium. Turgor pressure in plants is essential for cell growth in expansion and stomatic opening. The salt presence in the soil decreases the osmotic potential, causing water stress in plants leading to a turgor loss that with its considerable effect on growth[C].

Nutritional indicators (N, P, K^+, Na^+ and Na^+/K^+)

A different behavior of crops to analyze their nutritional status according to their tolerance degree to salt stress was appreciated. Figure 2 shows the nitrogen percentage in the root of both rice cultivars. It was interesting the result found in connection with no influence salinity on nitrogen in leaves, which may indicate that salt concentration increase, not greatly affect this macro-nutrient translocation in the aerial part rice cultivation. However, for the root system, a significant increase of N content is seen in the Pokkali cultivar in EC 12 dSm^{-1}, while A-82 cultivar the increase was 8 dSm^{-1} EC. This indicates a different answer depending on cultivar tolerance to salinity.

[C] Pesqueira, J. Cambios bioquímicos, morfológicos y ecofisiológicos en plantas del género Lotus bajo estrés salino. [Tesis de Doctorado]. Universidad Politécnica de Valencia, Departamento de Biología Vegetal. 2008. 173 pp.



** (P≤0,01) * (P≤0,05)
Common letters do not differ according to Tukey test (p≤0,05)

Figure 2. N content in roots and leaves of Pokkali and rice A-82 cultivars in salt stress conditions and control during seedling stage, 15 days after the stress imposed

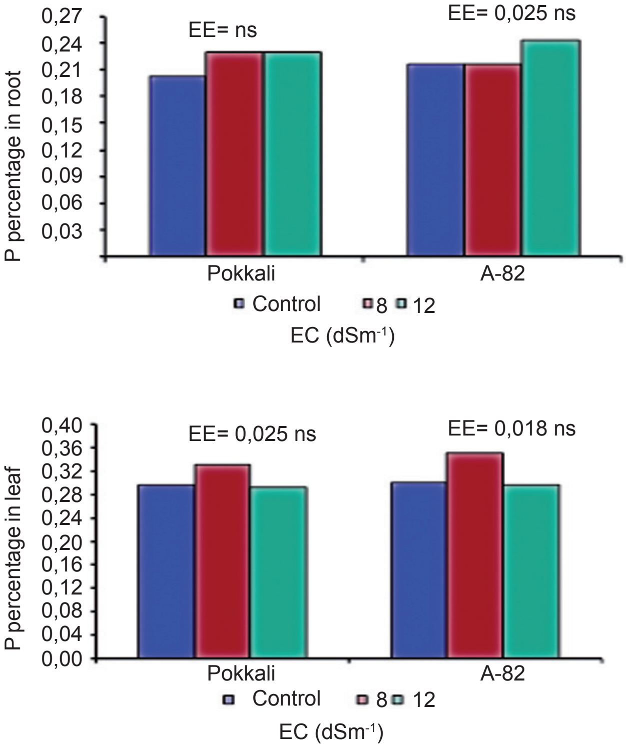
Significant differences (p≤0,05) of N content observed in the EC 12 dSm^{-1} at the root of Pokkali cultivate respect for their control, show that this cultivar maintains a better nutrient absorption for tested conditions, which reaffirms its best behavior to salt stress compared to cultivate A-82 for N content at the root t in EC 12 dSm^{-1} could be an efficient indicator for selecting rice genotypes tolerant to salinity in vegetative state of seedlings

The phosphorus content (Figure 3) does not show significant changes with increasing salt concentrations (such as those used in the experiment), for studied cultivars. This suggests that this nutrient is not affected in saline conditions for growing rice. Detected results are not consistent with those reported in other crops of economic interest.

Seedling height (SH), longitude of the root system (RL), dry mass of the area part (DMAP) and dry mass of the root system (DMRS) in two rice cultivars at 21 days of culture in different salt concentrations

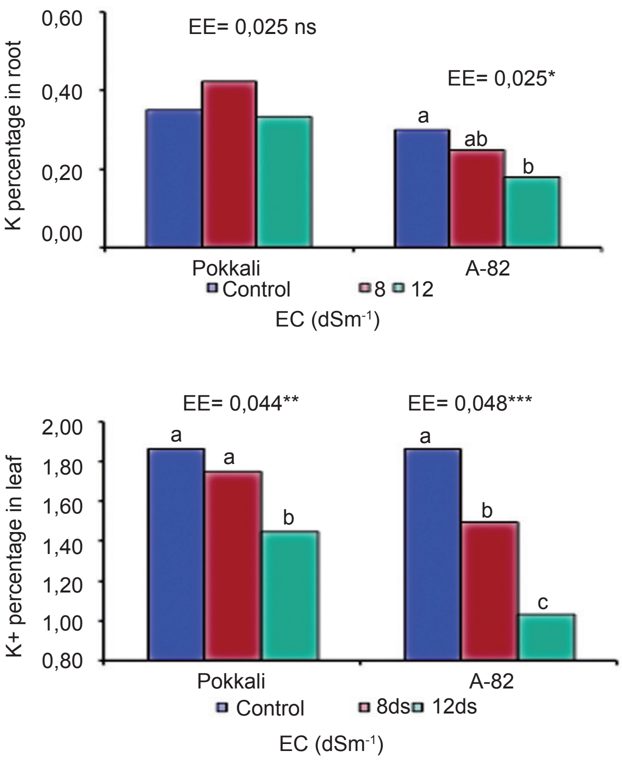
Varieties	EC (dSm^{-1})	Variables			
		SH (cm)	RL (cm)	DMAP (mg)	DMRS (mg)
Pokkali	Control	7,47a	7,97b	7,62a	5,0b
	8	5,57b	12,16a	8,25a	7,75a
	12	3,86c	3,75c	5,37b	1,12c
	EEx	0,28***	0,26***	0,21***	0,65***
A-82	Control	6,46a	5,80a	7,0a	2,87a
	8	2,58b	2,41b	6,12b	1,62b
	12	1,15c	1,42 c	3,25c	0,5c
	EEx	0,27***	0,25***	0,17***	0,18***

Common letters do not differ according to Tukey test (p≤0, 05) *** (P≤0,001)



ns (not significant)

Figure 3. P content in roots and leaves of Pokkali and A-82 rice cultivars in salt stress conditions and control during seedling stage, 15 days after the stress imposed



*** (P≤0,001) ns (not significant)
Common letters do not differ according to Tukey test (p≤0,05)

Figure 4. K content in roots and leaves of Pokkali and A-82 rice cultivars in salt stress conditions and control during seedling stage, 15 days after the stress imposed

Here, in the tomato crop, P content decreases were observed with increasing salt concentrations in the growth medium[D].

On the other hand, a similar behavior was detected to that found in this work in the rice cultivation, by stating that P content in leaves unchanged with increasing salt concentrations (17). Given cultivar behavior in the P content, we can assume that this is not a discriminating indicator between rice tolerant cultivars and susceptible to salinity.

In both leaves and roots the potassium content decrease was visible with increasing salt concentrations in the growth medium on growing A-82. However, Pokkali cultivar did not show significant reductions (p≤0,05) of K^+ content in the root (Figure 4).

The K^+ content was more affected in A-82, showing statistical differences of 8 sdm^{-1} EC leaves and 12 dSm^{-1} EC root. In Pokkali cultivar only one effect of salt stress was detected in leaves to 12 dS m^{-1} EC being this, a relevant result due to the potassium importance in the salt tolerance, showing a differential behavior between the two cultivars.

Potassium translocation to the aerial part also showed reductions with EC increase in growth medium. Nevertheless, Pokkali cultivar presented less reduction of this element in leaves than A-82 cultivar with increasing the growth medium EC.

As solute in vacuoles, the K^+ has an important role in controlling water relations helping cell turgor (14). It is well known the importance of maintaining turgidity in cell growth therefore plant growth can be inhibited by reducing the K^+ concentration which reduces the osmotic adjustment capacity and cell turgor maintenance.

[D] Medina, L. R. Respuesta del tomate (*Solanum lycopersicum* L.) bajo estrés salino a la inoculación con hongos micorrízicos arbusculares adaptados a esta condición (Tesis de Maestría). Instituto Nacional de Ciencias Agrícolas. La Habana. 2010. 76 pp.

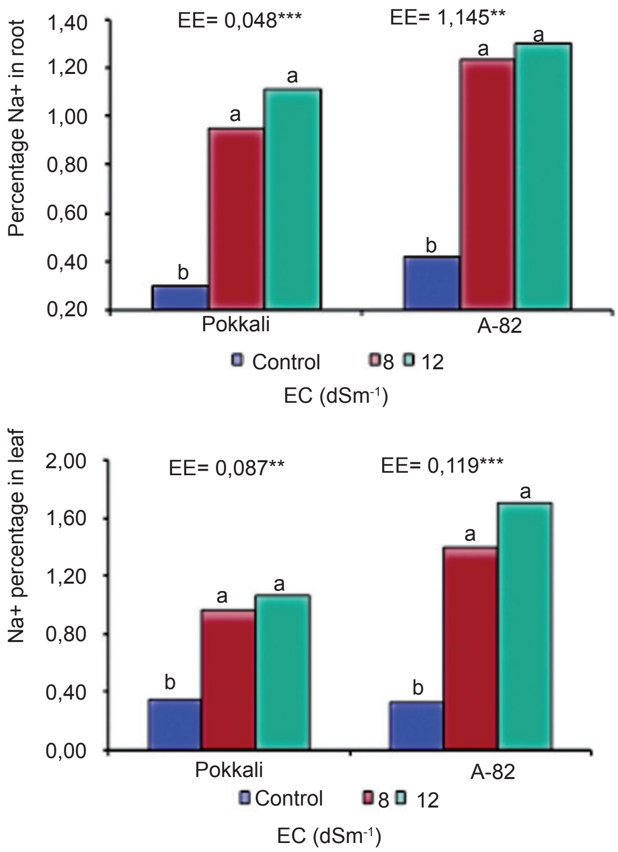
In this case, the best maintenance of normal potassium content in Pokkali, both the root and the aerial part, may constitute a tolerance mechanism to salt stress, which contributes to cell turgor maintaining and growth in saline conditions. In this sense it is claimed that the plant tolerance to salinity is attributed to their ability to avoid toxicity by Na^+ and maintenance of K^+ concentration (14). As previously stated, the potassium content in leaf and root of rice cultivars in a similar study design is an efficient indicator for the selection of genotype salinity tolerant

Regarding sodium (Figure 5), a significant difference ($p \leq 0,05$) of its content in both leaves and root systems of both cultivars as increased the EC in the growth medium was observed. Pokkali cultivar accumulated lower Na^+ content in roots and leaves in these conditions and translocated less content of this toxic ion at the leaf area to the A-82 growing. However, no different cultivars behavior was detected because both Pokkali and A-82 differ significantly from the corresponding control in 8 and 12 dsm^{-1} EC.

The Na^+ accumulation in plant tissue in saline medium growth is attributed to a decrease in cell membrane integrity, due to substitution of Ca^{2+} for Na^+ that directly affects their biological functions (5). In this paper, the content of Na^+ in plant tissue, does not allow discrimination among rice cultivars sensitive to salt stress tolerant, which is not an efficient indicator for the selection of salt tolerant genotypes.

The Na^+/K^+ relation is affected with increasing the EC in the growth medium for both cultivars, being more marked in the root system compared to leaf area for both, showing a significant increase ($p \leq 0,05$) of it (Figure 6). The differential behavior between cultivars was significant, since the Pokkali cultivating kept lower Na^+/K^+ to A-82 one with EC medium increase, which is given by a lower Na^+ absorption and better maintenance of K^+ absorption. This suggests that Pokkali is able to maintain adequate absorption of K^+ in saline medium.

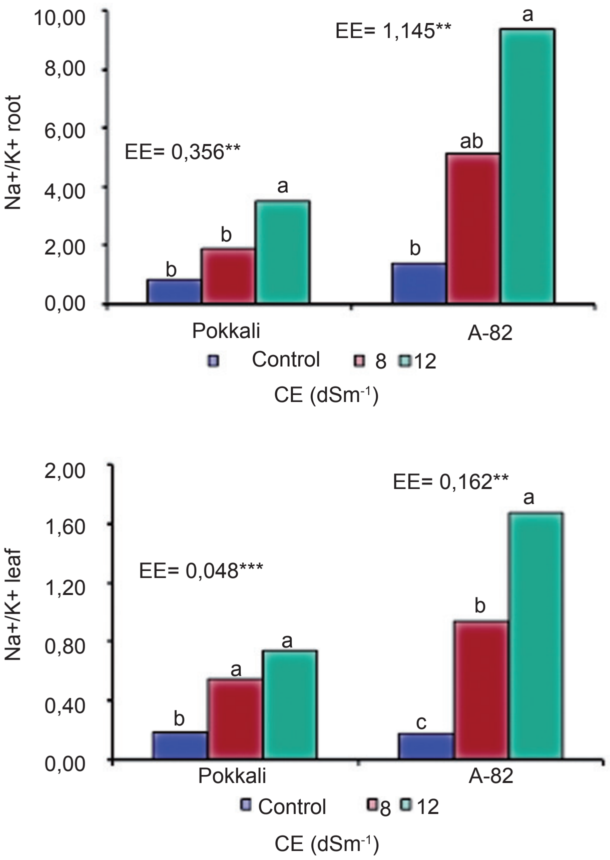
In this case, in Pokkali cultivar, significant differences ($p \leq 0,05$) of the Na^+/K^+ relation in leaves 8 dSm^{-1} EC were observed, but this does not differ from the EC 12 dSm^{-1}. Though, A-82 leaves, statistical differences were observed in 8 and 12 dSm^{-1} regarding its control. At root, the Na^+/K^+ ratio increased to a lesser extent in Pokkali cultivar and statistically significant differences were detected in 12dSm^{-1} EC, while A-82, the Na^+/K^+ relation showed increasing trend from EC 8 dSm^{-1} differs from the control EC 12 dSm^{-1}.



*** ($P \leq 0,001$)
** ($P \leq 0,01$)
Common letters do not differ according to Tukey test ($p \leq 0, 05$)

Figure 5. Na^+ content in roots and leaves of Pokkali and A-82 rice cultivars in salt stress conditions and control during seedling stage, 15 days after the stress imposed

Similar results have been observed for the Na^+/K^+ relation in other crops of economic interest (14). The relation increase Na^+/K^+ often it attributed to the competition effect between Na^+ and K^+ ions in the medium of root development (18). The Na^+/K^+ lower ratio in Pokkali is highly related to its tolerance to salt stress. The salinity stress tolerance in plants is attributed to its ability of avoiding the Na^+ toxicity and the maintenance of Ca^{2+} and K^+ concentration (14).



Common letters do not differ according to Tukey test (p≤0, 05)

Figura 6. Na^+/K^+ relation in roots and leaves of Pokkali and A-82 rice cultivars in salt stress conditions and control during the seedling stage, 15 days after the stress imposed

CONCLUSIONS

It is concluded that changes caused by salt stress in the growth initial phase and nutritional status at the seedling stage allowed to determine indicators discriminating among genotypes. Such is the case in germination percentage, RL, DMPA, DMRS, the N content in root, the K^+ content in roots and leaves and the Na^+/K^+ relation. These indicators can be efficient for the early selection of rice genotype salt tolerant, which can be incorporated in genetic improvement national programs of this crop.

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