International Journal of Agriculture and Crop Sciences.
Available online at www.ijagcs.com
IJACS/2015/8-3/346-349
ISSN 2227-670X ©2015 IJACS Journal

Effect of different levels of salinity on germination and early seedling growth of three rice varieties cultivated in Mauritania

Soukeina Mint El Mokhtar[1,2], Abdoulaye Samb[2], Abdallahi Ould Moufid[3], Ali Ould Mohamed Salem Boukhary[1] , Taleb Khyar Ould Djeh[1,3,*]

1. UR-Génomes et milieux, Faculté des Sciences et Techniques, Université des Sciences, de Technologie et de Médecine, nouveau campus universitaire, BP 5026, Nouakchott, Mauritania
2. Laboratoire des produits naturels, Faculté des Sciences et Techniques, Université Cheikh Anta Diop, Dakar, Sénégal.
3. Département de production et protection végétale, Institut Supérieur de l'Enseignement Technologique (ISET), Rosso, Mauritania.

Corresponding author email: taleb_2001@yahoo.fr

ABSTRACT: Rice (*Oryza sativa* L.) is one of the major crops in Mauritania and the productivity of currently used cultivars is strongly reduced at high soil salt levels. We compared the response of three rice cultivars to different levels of salinity stress (0, 3 and 100 mM). Germination and early seedling growth were evaluated through the percentage of germination, shoot and root length, salt stress index, leaf and root proline contents. The results obtained at the early stages of plant growth showed variable physiological and biochemical responses of the tested varieties *vis-à-vis* of the applied salinity levels The Sahel 108 showed better germination response under salinity stress conditions. At four-leaf stage, Sahel 201 and IR28 showed the highest salt stress index values suggesting their susceptibility to salinity. Leaf proline content of rice varieties have been significantly increased by increasing the salinity level, so that the most increasing was happened at 100 mM NaCl treatment in leaves of Sahel 108 veriety. Sahel 108 was the superior variety under all tested salt levels.
Key words: rice; varieties; salt stress; germination; NaCl; Mauritania.

INTRODUCTION

Global food production will need to increase by 38% by 2025 and by 57% by 2050 (Wild, 2003) if food supply to the growing world population is to be maintained at current levels. Rice (*Oryza sativa* L. and *O. glaberrima* Strudl) is one of the most important cereal crops in the developing world that provides food for more than half of the world population. In Asia and Africa, rice is the main source of calories for rural and urban populations (Sasaki and Burr, 2000). This crop is regarded as a salt sensitive especially at young seedling stage, where varying degree of mortality occurs at 50mM NaCl (Flowers and Yeo, 1981). Salinity is the major constraint affecting growth parameters and metabolism in plants which leads most often to a decrease in crop yields, making this phenomenon a threat to global food security. Moreover it is estimated that 20 million hectares of arable lands worldwide are affected by salinity (Cheverry, 1995). In Mauritania, a Sahel-Saharan country, rice is the staple food for a large part of the inhabitants. It contributes nearly to 45 % of the total cereal production with 81,000 tons annually (wintering and hot season campaigns). The rice production is dominated by the irrigated system. The Trarza and Gorgol regions located along the Senegal River Valley accounted for 87% of the average production. Rice also contributes to over 14% of the cereal needs nationwide, and nearly 10% of the income of rural farmers (www.fao.org/fileadmin/use upload/SDIP/./EtatAvancementMR.ppt). The abandoned rice lands due to salinity are great challenge that recently takes importance in Mauritania. Germination and early seedling are important stages in the life cycle of crop plants particularly in saline soils as it determines the degree of crop establishment. To produce satisfactorily under saline conditions seeds must germinate and seedlings must vigorously, pass through the salty layer of the soil and survive (Huang et al., 2003). One of the strategies to cope with the extent of land and water salinization and therefore improve crop productivity is the selection of naturally salts tolerant varieties. Thus, the objective of this study was to explore the effect of increasing salinity levels on germination and early seedling growth of three rice varieties commonly grown in Mauritania in order to better predict rice plants survival when established onto saline sites.

MATERIALS AND METHODS

PLANT MATERIAL

In this experiment the following varieties of rice (Oryza sativa L.) IR 28, Sahel 108 and Sahel 201 representing the most used in rice plantations in Mauritania were investigated. The seeds of tested varieties were kindly provided by the crops laboratory of the Higher Institute of Education and Technologies (ISET), Rosso, Mauritania .

Germination assay

Seeds of the tested rice varieties were surface-sterilised with 5% sodium hypochlorite for 3 min, rinsed 3 times with sterile distilled water and briefly blotted onto sterile paper towels. They were then allowed to germinate at 25±0.5°C in the dark, in Petri dishes containing two sheets of filter paper, saturated with distilled water (control) or NaCl solutions at 50 and 100 mM. There were 20 seeds in a Petri dish and three replications by each treatment. A seed was considered to have germinated when the emerging root had elongated to 3 mm. germinated seeds was recorded each 24 hours over a period of 3 days.

Hydroponic culture

five day old seedling were transplanted into plastic containers filled with nutrient solution consisted of 1.5 mM $Ca(NO_3)_2.4H_2O$, 0.5 mM $MgSO_4.7H_2O$, 1 mM KNO_3 , 1 mM KH_2PO_4 , 1 µM $CaSO_4.7H_2O$, 30 µM H_3BO_4, 50 µM Fe-EDTA , 10 µM $MnSO_4$, $ZnSO_4.7H_2O$ 1 (Jemal et al., 2005). Seedlings were then incubated in a culture room (BINDER, Germany) under the following conditions: 12/12 hours photoperiod with a temperature of 30°C day and 25°C night and average relative humidity of 60 %. The culture media were regularly aerated and refreshed every two days. On the fifth day, plantlets were transferred into a new nutrient solution containing one of the following NaCl concentrations: 0 (distilled water), 50 and 100 mM NaCl. After 5 days of salt treatments aerial and root part of the plants were harvested, measured and analyzed.

Germination rate

Germination rate is the percentage of seeds that germinate over the 1-, 2- and 3-days periods. It was calculated using the following formula:

Germination rate (%) = (number of seeds that germinated/number of seeds on the dish) x 100

Growth parameters

Growth parameters were estimated by measuring the following parameters on the rice plantlets subjected to 5 days salt stress: shoot length (SL), root length (RL) and salt stress index (SSI) using three replicates for each treatment and each variety. The salt stress index was estimated using the following formula :

SSI = RL/SL

Where, RL is the root length and SL, the shoot length

Proline content

Proline content in leaves and roots of rice plantlets subjected to 5 days salt stressed and unstressed plantlets was assessed using the ninhydrine colorimetric method (Bates et al., 1973).

Statistical analysis

The data obtained were subjected to a two-way analysis of variance to determine the differences between treatments and varieties, and when significance occurred, means were compared using Duncan's multiple range test at 5% significance level. Data were processed using XLSTAT v.4.04 software.

RESULTS AND DISCUSSION

Seed germination and growth parameters

Two-way analysis of variance (ANOVA) related to seed germination and growth parameters showed both a significant varietal and salt treatment effects for all tested variables (data not shown). Germination percentage of the seeds decreased with an increase of salinity level (Figure 1). When exposed to 0, 50 and 100 mM NaCl, germination of seeds of the tested varieties was started after 24 hours of setting except the IR28 seeds were no germination occurred at the concentration of 100 mM NaCl. However, after 48 hours of incubation, seeds germination reached 80% for Sahel 108 and Sahel 201 and 73% for IR 28. Delayed in germination under higher salinity level have been reported by Begum et al 2000 in maize (Zea mays L.), Sultana et al 1999 in Barley (Hordeum vulgare L.) and Benderraj et al 2010 in Wheat (Triticum aestivum L.). It

has been suggested that high concentrations of NaCl reduce the imbibitions of water because of lower osmotic potential of the medium which negatively affects seed germination (Jamil et al., 2006; Munns et al., 2008). Moreover, Shereen et *al* 2011 reported that sodium chloride reduced α-amylase activity in germinating seeds of some inbred line of rice even at low NaCl concentrations with tolerant lines exhibited higher enzymatic activity than the sensitive ones.

Duncan's multiple range test distributed rice varieties into various classes according to the considered NaCl concentration (Table 1) with a maximum of three classes in the 100 mM NaCl application. In general, results showed the negative effects of salinity on shoots and roots length of the tested rice varieties. For instance, Sahel 201 and IR28 shoots length (SL) decreased by 13.79% and 24.13% respectively for the treatments 50 and 100 mM NaCl compared to the control (distilled water), whereas the decrease in shoot length of the Sahel 108 variety was slightly lower under salt stress. According to Werner and Finkelstein (1995) salinity decreases water absorption and growth of roots and shoots. Moreover, the evaluation of salt stress index showed that, at 6 mM NaCl, this ratio increases by 68.17%, 27% and 24.67% respectively for Sahel 201, IR28 and Sahel 108 when compared to their respective controls. Similar results were reported by Benderradji (2010) in wheat (Triticum aestivum L.).

Proline content

The results demonstrated that the varieties and salinity levels effects were significant for proline contents in leaves and root of stressed rice plantlets (Table 2). Interaction effect between variety and salinity level was also significant. In general, rice plantlets subjected to salt stress, accumulate significantly higher amounts of proline in their leaves compared to their roots (Figure 2). The Sahel 108 plantlets accumulated in their leaves significantly higher amount of proline at 100 mM NaCl compared to Sahel 201 and IR28 plantlets. The proline increase ratios of salt applications in relation with the control application were determined to be between 92 and 76.5% for Sahel 108 and Sahel 201 respectively. It should be noted that, when exposed to 100 mM NaCl, IR28 seedlings showed a decrease in the amounts of proline in their leaves and roots compared to those exposed to 50 mM NaCl. Numerous studies have shown that the proline content in higher plants increases under different environmental stresses including high salinity conditions (Yoshiba et al., 1995). However, the correlation between proline accumulation and abiotic stress tolerance in plants is not always apparent. For example, high proline levels can be characteristic of salt- and cold-hypersensitive Arabidopsis (Arabidopsis thaliana) mutants (Liu and Zhu, 1997). Proline content is also high in drought-tolerant rice varieties (Choudary et al., 2005), but is not correlated with salt tolerance in barley (Hordeum vulgare) (widodo et al., 2009). Nevertheless, it can be stated that plants which synthesize large amounts of proline are more tolerant to stress conditions (de Lacerda, et al., 2005). It can be concluded that in the absence of NaCl, Sahel 108 has good germination rate compared to Sahel 201 and IR 28. However, salt stress differently affects seedling growth properties of the tested rice varieties where Sahel 108 variety was the most tolerant. Physiological response to salinity and performance of the tested rice varieties in a field trial environment are currently conducted to confirm their tolerance or susceptibility of the salinity field.

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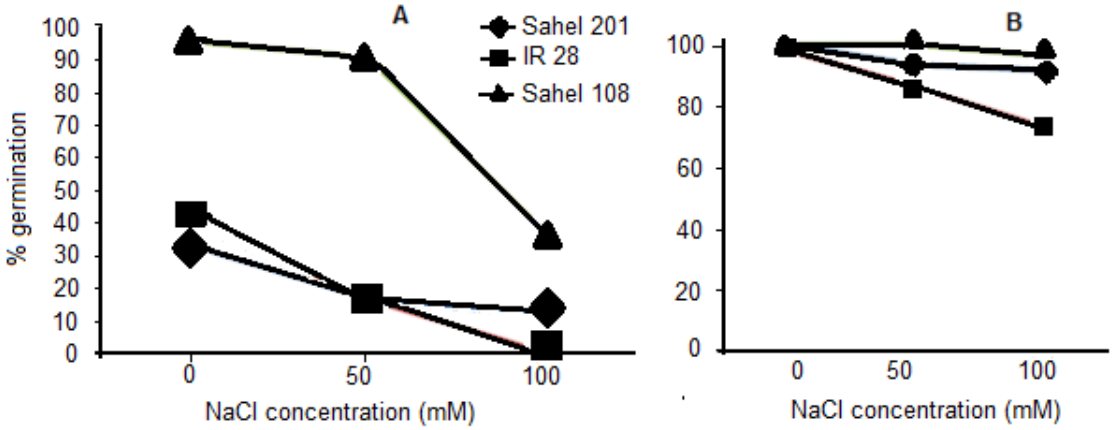

Figure 1. Effect of different levels of salinity on germination percentage of seeds of rice varieties. (A) after 24h and (B) after 48 of incubation.

Table 1. Comparison of main effect of salinity levels on growth parameters. Values followed by the same letter for the same NaCl concentration and the same variable are not significantly different at 5% level according to Duncan's test.

Variety	NaCl (mM)	SL(cm)	RL(cm)	SSI
Sahel 201	0	29,3 a	13 a	0.44a
	50	25 a	10,3 a	0.41a
	100	22 a	16,2 a	0.74a
IR28	0	30,3 a,b	12,3 a	0.41a
	50	26 a	9,8 a	0.38b
	100	23,5 a	12,3 b	0.52b
Sahel 108	0	31 b	9,6 b	0.31b
	50	30 b	11 b	0.37b
	100	25.6 b	9,8 c	0.38c

SL : shoot length ; RL : root length ; SSI: salt stress index.

Table 2. Analysis of variance on proline content in leaves and roots of three rice varieties plantlets under salinity stress.

Variance	d.f	Leaf proline	Root proline
Variety (V)	2	10829.03**	12.82**
Salinity (S)	2	45330.2**	46.35**
V x S	4	45428.1**	25.63**

**: Significant at 0.01 probability levels.

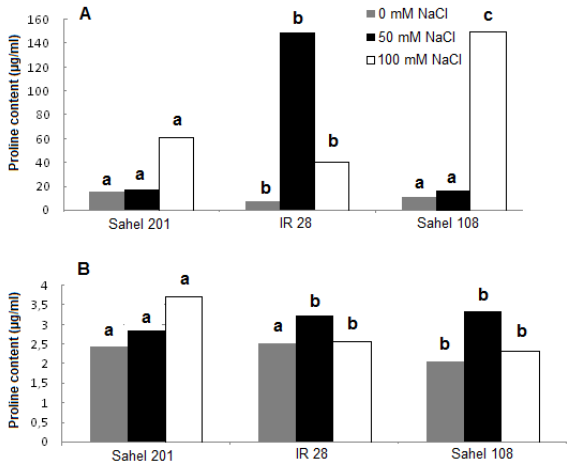

Figure 2. proline content in leaves (A) and roots (B) of three rice plantlets under salinity stress. Means were compred at 5% level according to Duncan's test.