https://doi.org/10.1590/1678-4499.20210172

PLANT BREEDING
Article

Mapping phenotypic parameters linked to salt stress tolerance strategies in rice lines

Daniel Amorim Vieira[1], Mayra Alejandra Toro-Herrera[1], João Paulo Pennacchi[1], Ane Marcela das Chagas Mendonça[2], Paulo Eduardo Ribeiro Marchiori[1], Flávia Barbosa Silva Botelho[3], João Paulo Rodrigues Alves Delfino Barbosa[1,*]

1. Universidade Federal de Lavras – Departamento de Biologia – Lavras (MG), Brazil.
2. Universidade Federal do Sergipe – Programa de Pós-Graduação em Desenvolvimento e Meio Ambiente – São Cristovão (SE), Brazil.
3. Universidade Federal de Lavras – Departamento de Agricultura – Lavras (MG), Brazil.

Received: June 10, 2021 | Accepted: Nov. 25, 2021

Section Editor: Rafael Vasconcelos Ribeiro

***Corresponding author:** jp.barbosa@dbi.ufla.br

How to cite: Vieira, D. A., Toro-Herrera, M. A., Pennacchi, J. P., Mendonça, A. M. C., Marchiori, P. E. R., Botelho, F. B. S. and Barbosa, J. P. R. A. D. (2022). Mapping phenotypic parameters linked to salt stress tolerance strategies in rice lines. Bragantia, 81, e1122. https://doi.org/10.1590/1678-4499.20210172

ABSTRACT: Salinity is recognized as one of the main abiotic stresses, causing declines in the productivity of many crops, such as rice. Considering the importance of rice as a staple food crop, the generalized pattern of salinization in soils around the globe and the sensitivity of the crop to saline soils, the aim of this study was to evaluate the sensitivity / tolerance of upland rice lines. The upland rice lines used in this work were obtained from the Genetic Improvement Program of the Universidade Federal de Lavras. The experiment was carried out in a greenhouse, with a block design and factorial scheme, with 10 lines and four NaCl treatments (0, 3, 15 and 45 mmol·L^{-1}) on the substrate. Parameters related to the emergence rate, seedling survival rate, growth and architecture, biomass allocation, gas exchange and productivity were evaluated. Salinity levels influenced the emergence and survival rates, especially at the highest levels of NaCl, with lines L1, L4 and L5 showing survival below 60%. These lines were excluded from the experiment, and the other seven lines were carried out until the end of the crop cycle. Grain weight per plant (GWP) was used as an indicator of greater tolerance to salinity, and lines L6, L7 and L8 showed outstanding performance under treatments with increased NaCl. L6 had the highest GWP, while L7 and L8 had less pronounced decreases with increasing NaCl doses. These three lines may be recommended for further studies about the impacts of NaCl on rice production.

Key words: abiotic stress, climate change, *Oryza sativa* L., sustainable agriculture, plant physiology.

INTRODUCTION

The rice crop (*Oryza sativa* L.) has great socioeconomic importance, being the staple food crop of more than half of the world population (Kazemi et al. 2018). Demand for rice is expected to increase by 60% with the observed pattern of world population growth by 2050 (Kromdijk and Long 2016; FAO 2019). This required increase in production is challenging, as the increase in demand is not in line with the reduction in crop productivity, mainly due to the negative impacts of adverse abiotic conditions and the limited opening of new agricultural areas (Aggarwal et al. 2019; Loboguerrero et al. 2019). Thus, the search for solutions to meet the demand for sustainable and environmentally friendly food production is necessary and urgent (Chou et al. 2019).

In the context of climate change, salinity is one of the most common abiotic stresses generated by the combined action of human activity and environmental modifications. Salinity reaches about 20% of all agricultural land around the globe, being even more impactful in irrigated fields, where it reaches 33% of areas (FAO 2018). The mineral elements that most contribute to salinity in agricultural fields are sodium (Na^+) and chloride (Cl^-), so that Na is mainly present in upland rice growing areas (Maathuis 2014; Reddy et al. 2017).

Palliative techniques to correct saline soils are often expensive, and the use of salt-tolerant genotypes is the most common strategy for planting in these areas (Shahbaz and Ashraf 2013; Hairmansis et al. 2014; Rasel et al. 2021; Tin et al. 2021), making salinity tolerance an important trait for achieving higher grain yields (Momayezi et al. 2009). In this context, the lack of reliable genotype screening techniques is one of the biggest limitations in the development of varieties with salinity tolerance, at the current moment (Gregorio et al. 2002).

Plants that are classified as tolerant to salinity generally have three main mechanisms to avoid or mitigate the impacts of stress: stem ions independent tolerance or osmotic tolerance, ion exclusion and tissue tolerance (Pires et al. 2015). Despite the importance of rice and the perspective of increasing in areas affected by salinity in the future, the study of the responses of this crop to this abiotic stress is not fully understood (Pires et al. 2015).

The aim of this work was to evaluate the effects of fertilization in substrate with NaCl on the physiological aspects, growth and development, and production of upland rice lines of the Upland Rice Genetic Improvement Program at Universidade Federal de Lavras (UFLA). More specifically, it aimed to select lines with greater tolerance to salinity and explain their strategies to overcome the limitations imposed by different levels of salt stress.

MATERIAL AND METHODS

Plant material, experimental design, and conditions

The experiment was carried out from November 2018 to March 2019 in a glasshouse at UFLA, in Lavras (MG), Brazil (21°13’40”S and 44°57’50”W GRW). The experiment was in a block design, in a factorial scheme with two factors: 10 rice lines, and four NaCl doses. Each treatment had five replicates with one plant per pot, totalizing 200 experimental units. The 10 lines were selected from Upland Rice Breeding Program of the UFLA and were identified as: L1 (CMGFG LAM 20-2), L2 (CMG ERF 85-14), L3 (BRS ESMERALDA), L4 (BRS MG CAÇULA), L5 (CMG ERF 85-6), L6 (CMG 2085), L7 (CMG F6 LAV1-7), L8 (CMG 1896), L9 (CMG ERF 221-29), and L10 (CMG ERF 85-13). The Na doses were: 0, 3, 15, and 45 mmol of NaCl, representing 0, 0.18, 0.87, and 2.61 g of $NaCl \cdot Kg^{-1}$ of substrate, respectively. The doses of NaCl were chosen based on the literature, going from a dose that could be beneficial (ranging from 3 to 15 $mmol \cdot L^{-1}$ of NaCl) up to a dose with potential to cause damage to the rice crop (around 45 $mmol \cdot L^{-1}$) (Cui et al. 1995; Reddy et al. 2017).

Ten seeds were sown in 4 L pots, filled with a substrate composed by clay (red oxisol) and sand (washed sand) in a 1:2 ratio. Fertilization was performed fortnightly, following recommendation for upland rice (Alvarez et al. 1999): 10 g of ammonium sulphate ($(NH_4)_2SO_4$), 4 g of monoammonium phosphate (MAP), and 3.63 g of potassium nitrate (KNO_3) per plant (corresponding to 120 $kg \cdot ha^{-1}$ P_2O_5, 100 $kg \cdot ha^{-1}$ N, and 80 $kg \cdot ha^{-1}$ K_20, respectively).

Irrigation was performed daily at 5 p.m., through weighing lysimetry, using a digital scale. The maximum retention capacity of the substrate was defined, and water level was completed to 100% of the evapotranspiratory demand, in every irrigation. The imposition of the NaCl (Synth, model PA-ACS, molecular weight 53.44 $g \cdot mol^{-1}$) treatments was made by incorporating the specific quantities for each treatment in the substrate, when the pots were filled. A plastic recipient was placed on the bottom of each pot to capture the drained water. This water was reincorporated to the substrate on the following day irrigation, aiming to maintain constant NaCl levels, avoiding leaching of the salt.

Seedling emergence and plant survival analysis

The evaluation of seedling emergence was performed 10 days after sowing, in the V1 stage (collar formation on first expanded leaf on the main stem), according to the used growth and development scale (Counce et al. 2000). The emergence rate (ER, in %) was calculated as the number of emerged seedlings divided by the 10 sown seeds in each pot. After emergence evaluation, thinning was performed leaving one plant per pot, with each pot containing one plant being considered an experimental unit. At 30 days after emergence, in the V6 stage (collar formation on sixth leaf on the main stem) (Counce et al. 2000), the survival rate was evaluated for all the lines and NaCl treatments, in five replicates.

A minimum of 80% of survival rate was set as the threshold for selecting lines according to their capacity to tolerate salinity. Thus, the lines L1, L4 and L5, which presented survival rates of 60, 20, and 40%, respectively, were sensitive to salinity and not evaluated on the further steps of the experiment. However, the pots were kept in the experiment to avoid unbalance of experimental units.

Gas-exchange and chlorophyll index

Gas-exchange and chlorophyll index evaluations were performed at 30 and 60 days after emergence, on the respective V6 and V13 stages (Counce et al. 2000). The gas-exchange evaluations were carried out between 9 and 11 a.m., using an infra-red gas analyser (IRGA LI-6400XT, LI-COR). The light level, measured in photosynthetic photon flux density (PPFD), was of 1,000 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The following parameters were measured: net photosynthesis (A, in $\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$), stomatal conductance (gs, in $mol\ H_2O \cdot m^{-2} \cdot s^{-1}$), and transpiration (E, in $mmol\ H_2O \cdot m^{-2} \cdot s^{-1}$). The instantaneous water use efficiency was calculated as the ratio A/E (WUE, in $\mu mol\ CO_2 \cdot mmol^{-1}\ H_2O$), according to Esmaeilpour et al. (2016). Chlorophyll index (SPAD) was measured using a chlorophyll meter (atLEAF+, FT Green LTC). Gas-exchange and chlorophyll analyses were performed in the central area of the youngest and fully expanded leaf of the main tiller.

Leaf analysis, plant growth, and yield components

At the end of the experimental period, 120 days after emergence, several developmental and biomass allocation traits parameters were evaluated. For the developmental traits, the following parameters were measured: flag leaf length (FLL, in cm), total number of panicles (TNP, unitless), panicle length (PL, in cm) and weight of 100 grains (WHG, in g) (at 13% moisture), according to Alvarez et al. (2012). The total grain weight per plant (GPW, in g) was also measured, at the same moisture as WHG. For the biomass allocation traits, the following parameters were measured: leaf dry matter (LDM, in g), root dry matter (RDM, in g), shoot dry matter (SDM, in g, as the sum of stem and leaf dry matter), total dry matter (TDM, in g, as the sum of root and shoot dry matter), root/shoot ratio (RSR, unitless, as RDM/SDM), and harvest index (HI, in $g \cdot g^{-1}$, as the ratio between grain and shoot dry matters). Samples for dry matter were placed in a forced air oven, at 65°C, until constant weight. Leaf samples were also collected for the evaluation of sodium content, in five replicates, for each combination of lines and NaCl doses. The replicates were mixed in a single sample, which was analysed through the method of emission flame spectrometry to obtain leaf Na content (Malavolta et al. 1997) (Table 1).

Statistical analysis

The normality of data was evaluated through the analysis of the residuals' distribution by Kruskal-Wallis test. Initially, analyses of variance were used to evaluate the effect of single factors and the possible interaction, for each analysed parameter. After that, all variables were subjected to regression analysis. For the regression, first- and second-degree polynomial models

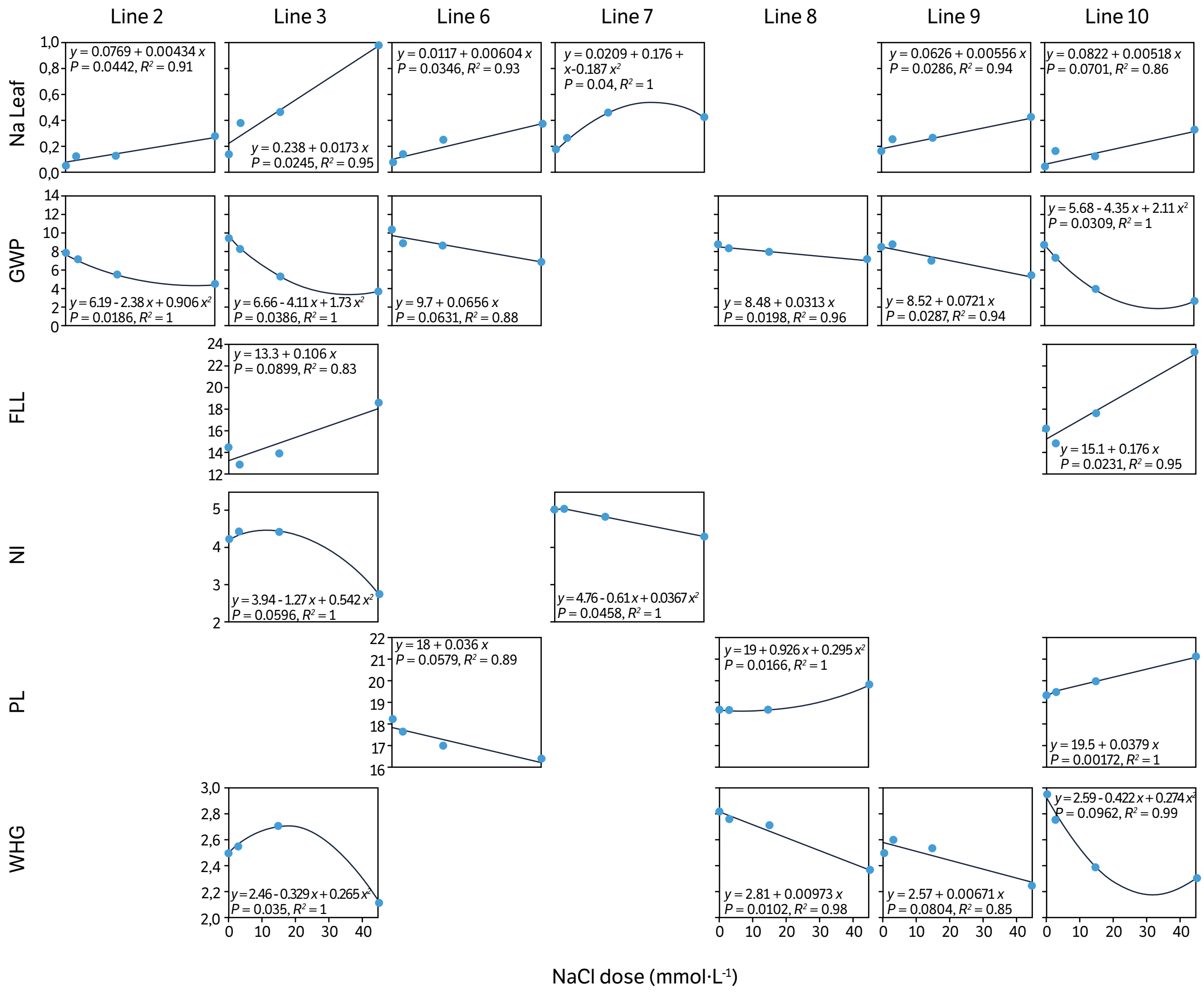
Table 1. Analysis of leaf sodium (Na) content in the harvest phase in the lines (L) and salinity (S).

Treatment	Na	Treatment	Na	Treatment	Na	Treatment	Na
L2S1	0.049	L6S1	0.080	L8S1	0.139	L10S1	0.048
L2S2	0.127	L6S2	0.144	L8S2	0.129	L10S2	0.155
L2S3	0.132	L6S3	0.249	L8S3	0.395	L10S3	0.131
L2S4	0.273	L6S4	0.374	L8S4	0.367	L10S4	0.321
L3S1	0.148	L7S1	0.039	L9S1	0.030	–	–
L3S2	0.395	L7S2	0.135	L9S2	0.115	–	–
L3S3	0.486	L7S3	0.360	L9S3	0.145	–	–
L3S4	1.014	L7S4	0.303	L9S4	0.311	–	–

were fitted to describe the mathematical relationships between the measured traits and the NaCl doses. The percentage of change for parameters between 0 and 45 mmol·L^{-1} of NaCl doses were calculated as the difference of the parameters divided by the value at the parameter at the 0 NaCl dose. For the data of foliar sodium content, additionally to the regression to soil Na levels, a Pearson correlation analysis to GWP was performed. Only statistically significant regressions ($p < 0.1$) are presented in this manuscript. Statistical analyses were conducted using the RStudio statistical software (v. 1.2.5033, RStudio). The main packages used were agricolae, ggplot2, multcomp, ggpubr and Rmisc.

RESULTS

The Na doses applied to soil were differently managed by the cultivars in terms of transportation to the leaves (Fig. 1), showing contrasting patterns. For instance, L3 presented a prominent linear increase in leaf Na levels with increases in Na in the soil. Other genotypes also presented the same pattern, but with much smaller slope, potentially showing a mechanism to control the transport of Na from soil to leaf, as L2, L6, L9, and L10. On the other hand, L7 and L8 (although



Na Leaf: sodium leaf; GWP: grain weight per plant; FLL: flag leg length; NI: number of inflorescences; PL: panicle length; WHG: weight of 100 grains; *p*: significance level of the linear regression; R^2: coefficient of determination of the linear regression.

Figure 1. Linear regression of developmental parameters *vs.* NaCl doses, for seven rice lines. Only the linear regressions with p<0.1 are shown in the figure.

the regressions were not significant) showed a quadratic pattern were the leaf Na content increased until the level of 15 mmol·L^{-1} of NaCl in the soil, but showed limited increase of Na in the leaf when the levels increased to 45 mmol·L^{-1} of NaCl in the soil (Fig. 1).

Regarding the emergence rate (Table 2), there was no significant interaction ($p > 0.05$) between the factors, but there was a significant difference ($p < 0.05$) for the lines and salt doses when analysed separately. In this context, L10 had a lower emergence rate than L3, L5, L7, and L9. The highest emergence rate was observed at the 3 mmol·L^{-1} NaCl dose, followed by the 0 and 15 mmol·L^{-1} NaCl doses. On the other hand, the lowest emergence rate was observed at the 45 mmol·L^{-1} NaCl dose (Table 2).

The same pattern of no interaction ($p > 0.05$) between lines and salinity was observed for developmental and biomass allocation parameters. In gas-exchange parameters, the interactions were analysed for lines, salinity, and time with non-significant results. By this reason, the analysis was focused on the linear regression for each parameter *versus* the salinity level, for each single line.

The GWP was used as an indicator of the level of tolerance / sensitivity to salt stress. The lines showed higher GWP at 0 and 3 mmol·L^{-1} NaCl doses with significant reductions at 15 and 45 mmol·L^{-1} NaCl doses (Table 2). In all salinity levels, L6 presented higher GWP than L2 and L10 (Table 2). In general, plants showed a decrease of GWP as the soil NaCl level increased. The linear regressions among GWP and NaCl levels were significant for all the lines, with exception of L7 (Fig. 1). The Pearson correlation analysis between GWP and leaf Na content did not show significant results for the lines ($p = 0.1096$), However, the doses of salt in the soil were negatively correlated to GWP ($p = 0.0029$, correlation; $r = -0.54$).

L8 presented the lowest loss in GWP (-18%) from 0 to 45 mmol·L^{-1} NaCl followed by intermediate losses in L6, L9, and L2 (-34, -36 and -42%, respectively), and highest losses in L3 and L10 (-60 and -69%, respectively). These different patterns can be explained based on the behaviour of other parameters related to yield-components, biomass allocation and gas-exchange parameters, as highlighted in the sequence (Fig. 1, Table 3).

L8 presented an increase in PL (+6%) and a decrease in WHG (-16%), although for the last it was not as sharp as the decrease for L3 (-56%), and L10 (-93%) (Fig. 1, Table 3). L8 presented a decrease in TDM (-39%). However, the main

Table 2. Tukey *post-hoc* test for emergence rate (ER), survival rate (SR) at the highest NaCl doses and grain weight per plant (GWP) for two factors (lines and salinity)*.

Parameter			
Factor	**ER (%)**	**SR (%)**	**GWP (g)**
Lines (L)			
L1	69.8 abc	60 -	-
L2	72.4 abc	80 -	6.28 bc
L3	75.2 ab	80 -	6.81 abc
L4	62.2 bc	20 -	-
L5	73.9 ab	40 -	-
L6	68.0 abc	100 -	8.67 a
L7	79.7	80 -	7.90 ab
L8	72.1 abc	80 -	8.03 ab
L9	77.0 a	100 -	7.38 abc
L10	60.5 c	100 -	5.68 c
Salinity(S)			
0	68.6 b	-	8.85 a
3	83.1 a	-	8.24 a
15	73.2 b	-	6.32 b
45	59.5 c	-	5.37 b

*Equal lowercase letters do not differ significantly between treatments; different lowercase letters between treatments are significant by the Tukey's test ($p<0.05$).

Table 3. Differences (in %) between parameters at 0 and 45 mmol·L^{-1} of NaCl for seven rice lines*.

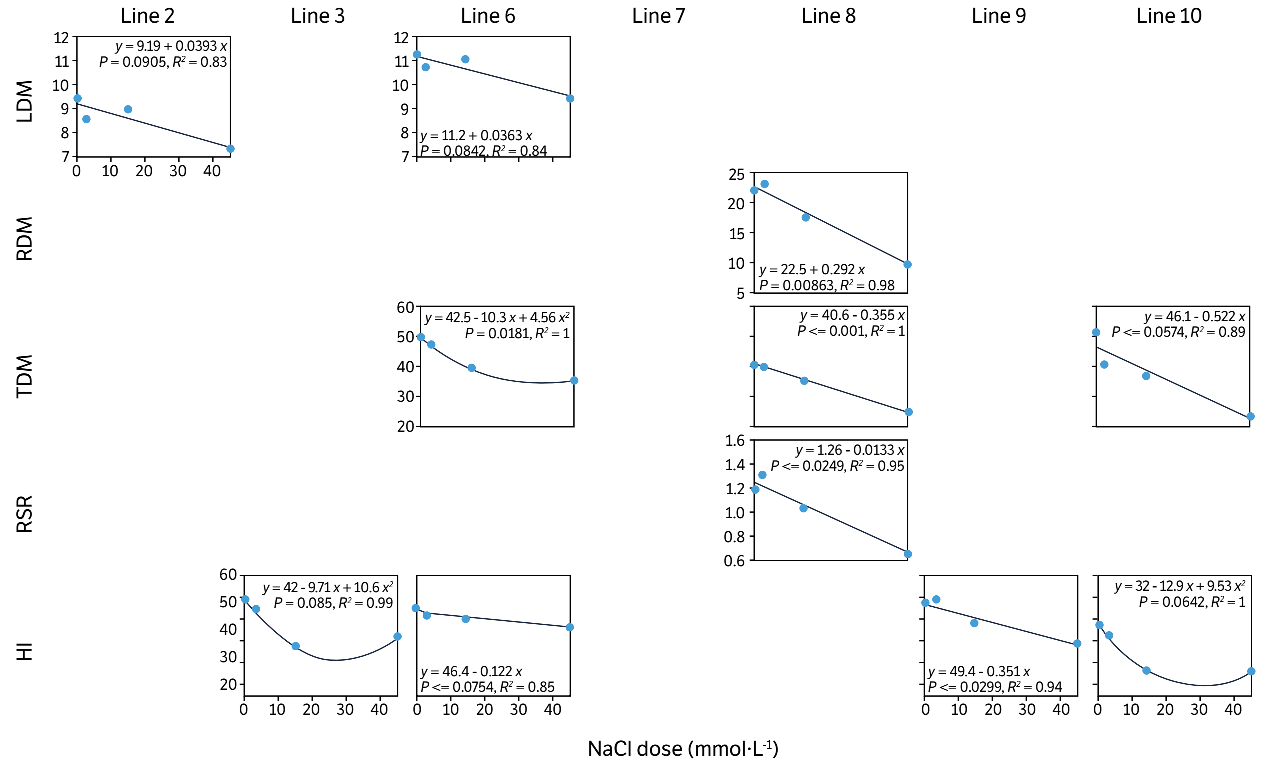
		Lines						
Group	**Parameter**	**L2**	**L3**	**L6**	**L7**	**L8**	**L9**	**L10**
Morphological	GWP	-42	-60	-34	-13	-18	-36	-69
	FLL	-4	27	-5	28	13	20	44
	NI	-22	-35	4	-15	-8	12	-25
	PL	-2	-4	-10	-9	6	5	9
	TNP	-5	2	7	-4	-8	9	-4
	WHG	-6	-56	-1	-7	-16	-10	-93
	LDM	-22	-27	-16	-10	-18	3	-27
	RDM	-23	-69	-31	-9	-57	-14	-60
	SDM	-42	-60	-34	-13	-18	-36	-69
	TDM	-27	-60	-28	-10	-39	-15	-54
	RSR	7	-52	-14	-1	-45	4	-31
	HI	-13	-27	-14	-5	0	-30	-42
	A (V6)	16	-3	13	-1	-8	-8	-4
	gs (V6)	-9	-26	-2	-33	-10	-18	-16
	E (V6)	4	1	-10	-21	-3	-14	-9
	WUE (V6)	-10	-1	-21	-20	4	-6	4
	A (V13)	0	34	-13	6	14	-2	16
	gs (V13)	-28	20	-29	-15	24	-2	27
	E (V13)	-18	23	-15	-5	21	2	22
	WUE (V13)	-19	-11	1	-10	5	3	4

*Negative and positive black numbers: respectively, decreases and increases in the percentage of change between 0 and 45 mmol·L^{-1} of NaCl for non-significant (p>0.1) linear regressions; negative (red) and positive (green) numbers: respectively, decreases and increases in the percentage of change between 0 and 45 mmol·L^{-1} of NaCl for significant (p<0.1) linear regressions; GWP: grain weight per plant; FLL: flag leg length; NI: number of inflorescences; PL: panicle length; TNP: total number of panicles; WHG: weight of 100 grains; LDM: leaf dry matter; RDM: root dry matter; SDM: shoot dry matter; TDM: total dry matter; RSR: root to shoot ratio; HI: harvest index; A: net photosynthesis; gs: stomatal conductance; E: transpiration; WUE: water use efficiency; V6 and V13: collar formation on sixth and thirteenth leaf on the main stem, respectively (Counce et al. 2000).

reduction was related to the root biomass instead of the shoot biomass, what can be noticed by a pronounced decrease in RSR (-45%) (Fig. 2, Table 3). Also, L8 presented an increase in A (+14%) at the V13 stage (Fig. 3, Table 3).

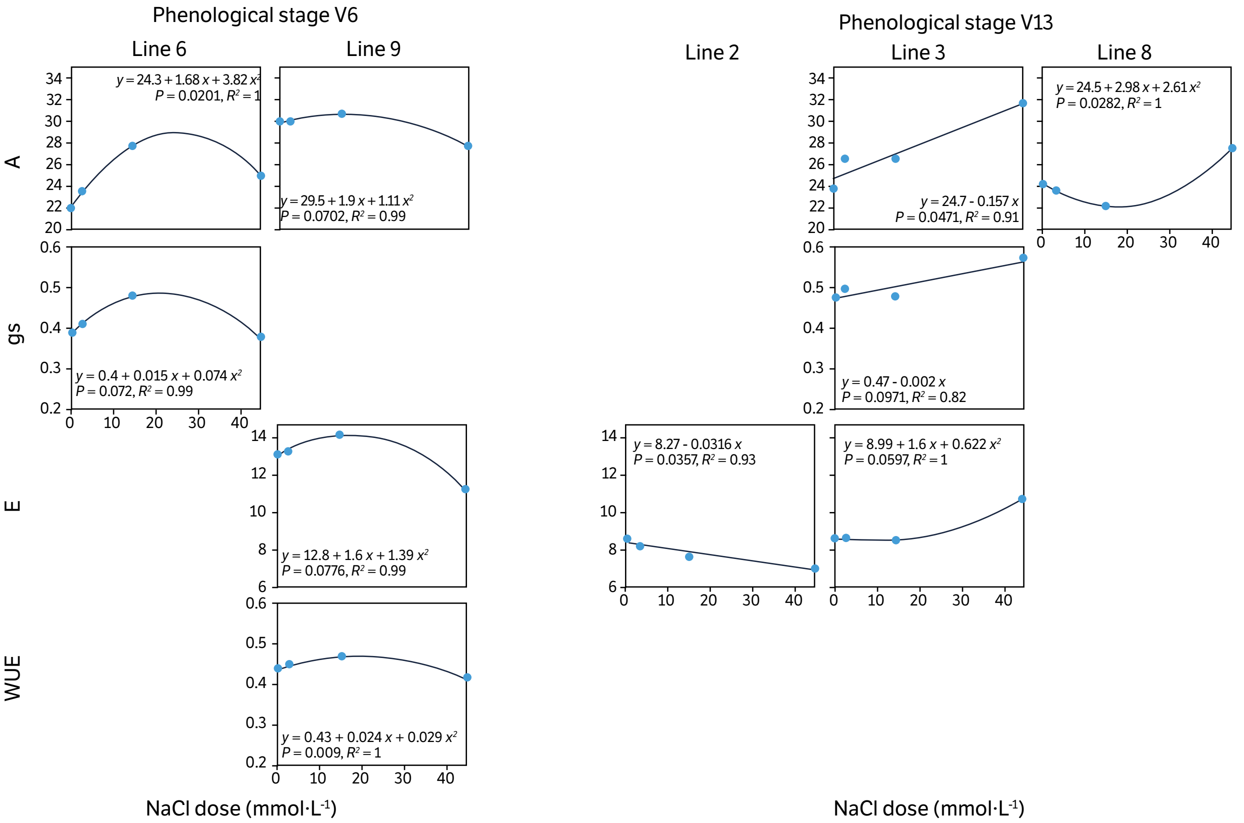
From the group of intermediate losses in GWP, L6 decreased in PL (-10%), but it maintained GWP in opposition to the other lines (Fig. 1, Table 3). It presented a decrease in LDM and TDM (-16 and -28%, respectively), but it was not highly impacted in the allocation of biomass to the grain, what can be observed by the slope of HI (-14%) (Fig. 2, Table 3). Also, it presented an increase in A (+16%) without major changes in gs (-2%) at V6 stage (Fig. 3, Table 3). For L9, if compared to L6, it did not present decrease in PL, but had higher losses in WHG (-10%) and HI (-30%) (Figs. 1 and 2, Table 3). It has a decrease in A (-8%) and E (-14%), with WUE also decreasing (-6%) (Fig. 3, Table 3). L2 decrease in GWP may be related to the decrease in LDM (-22%) (Fig. 2, Table 3).

Considering the lines with the higher losses, L3 presented an increase in FLL (+27%), A (+34%), and gs (+20%) at V13 stage (Figs. 1 and 3, Table 3). However, this higher potential of carbon uptake may have not been related to lower GWP losses, since it was observed decreases in NI (-35%), WHG (-56%), and HI (-27%) (Figs. 1 and 2, Table 3). In addition to that, the combination of higher FLL and E may be related to higher water loss. L10, which presented the higher losses in GWP, presented increases in FLL (+44%) and PL (+10%) (Fig. 1, Table 3). However, this was not enough to compensate the losses in WHG (-93%), TDM (-54%), and HI (-42%) (Fig. 1 and 2, Table 3). No differences were observed for leaf chlorophyll among lines, salinity levels and their interaction.



p: significance level of the linear regression; R²: coefficient of determination of the linear regression; LDM: leaf dry matter; RDM: root dry matter; TDM: total dry matter; RSR: root to shoot ratio; HI: harvest index. Only the linear regressions with *p*<0.1 are shown in the figure.

Figure 2. Linear regression of biomass allocation parameters *vs*. NaCl doses, for seven rice lines.



p: significance level of the linear regression; R²: coefficient of determination of the linear regression; A: net photosynthesis; gs: stomatal conductance; E: transpiration; WUE: water use efficiency; V6 and V13: collar formation on sixth and thirteenth leaf on the main stem, respectively (Counce et al. 2000)..

Figure 3. Linear regression of gas-exchange parameters *vs*. NaCl doses, for seven rice lines. Only the linear regressions with p<0.1 are shown in the figure.

DISCUSSION

Our first results showed that NaCl influenced the emergence rate, and the lines presented different tolerance to this condition. Soil salinity affects seed germination and emergence by creating an osmotic potential on the outer part of the seed, preventing or limiting water intake to the inner part of the seed, or by the toxic effect of Na^+ and Cl^- ions to the seed (Khajeh-Hosseini et al. 2003). There is a report of no influence of salinity in emergence rate of rice seeds, showing a potential tolerance, even in doses of 160.3 $mmol \cdot L^{-1}$ NaCl (Khan et al. 2014). However, we noticed that L10 presented lower emergence rate than L3, L5, L7, and L9 (Table 2).

For seedling survival, L1, L4 and L5 presented lower rates than 60%, at the highest NaCl levels, after 30 days of emergence (Table 2). This behaviour of higher seedling death could reveal the sensitivity of the mentioned lines to salinity, at the early stages of plant development, and a possible tolerance of the other lines. However, survival and emergence tolerances to salinity may not be linked, once L5 presented high emergence rate, but lower survival, while L10 presented the opposite behaviour, with lower emergence, but higher survival rate (Table 2). This indicates that the strategies to alleviate salinity effects may be different at each developmental stage. One of the plant strategies to mitigate salt stress at early stages in rice is related to the capacity to accumulate Na^+ and Cl^- ions in structural tissues and in the bigger vacuoles of older leaves, decreasing their accumulation in younger leaves (Wang et al. 2012). This may raise the carbon fixation by younger leaves and increase the chances of plant survival, at the seedling and early development stages (Reddy et al. 2017).

Under salinity conditions, the nitrogen metabolism is rearranged towards amino acids synthesis, aiming to alleviate the impact of the stress conditions, what can decrease chlorophyll content (Xu et al. 2016), as observed in rice by Amirjani (2012). However, our results showed that salinity did not influence significantly ($p > 0.05$) the chlorophyll content of leaves at V6 and V13 stages. No differences were also observed among the lines. This result is corroborated by Kazemi et al. (2018), working with rice varieties in salinity conditions of 0, 30, 60, and 90 $mmol \cdot L^{-1}$ NaCl and also by Chiconato et al. (2019) for sugarcane plants in salinity levels of 0, 40, 80, and 160 mM NaCl.

The ionic unbalance due to the excess of Na^+ and Cl^- ions on the soil and inside the plant cells causes the reduction of water absorption leading to the changes in the physiological process, causing the so-called physiological drought (James et al. 2011; Nishimura et al. 2011). Some of these changes include the membrane disruption, reduction in photosynthetic capacity, and the lack of capacity of detoxification of the reactive oxygen species (ROS) due to the damage occasioned to the antioxidant enzymes (Rahnama et al. 2010; James et al. 2011). These processes may cause cell death and lead to initial senescence of old leaves, progress to young leaves and, latest, collapse of the whole plant (Quintero et al. 2007; Siringam et al. 2011).

Considering carbon fixation and the processes related to it, the photosynthetic capacity of plants grown under salinity is reduced, depending on the level and duration of the stressful condition (Munns 2002), as well as the genotypic differences of the varieties/lines and the plant development stage (Dadkhah 2013). In this study, the different salinity levels did not significantly change the gas-exchange parameters, although the factor time and lines were significant ($p < 0.001$). However, the regression analysis showed significant differences in gas-exchange parameters as a function of the increase in NaCl levels (Fig. 3).

A general pattern of response to salinity, at the whole plant level, is the reduction in growth and development (Munns and Tester 2008), linked to lack of carbon assimilation and lower source capacity (Morales et al. 2012). This is related to the above-mentioned limitations in gas-exchange, impacting in availability of intercellular carbon for photosynthesis due to stomatal closure, motivated by lower leaf water potential. By this reason, the capacity to maintain or increase transpiration rates and stomatal conductance, under saline conditions, is an indicator of tolerance to salinity (Harris et al. 2010; Hernández et al. 2018; Razzaque et al. 2019).

In our study, L3 presented this increase of gs and E under higher salinity, what also increased A (Fig. 3). However, this was not enough to increase or maintain GWP at higher NaCl doses, what is because other traits, as WHG, were drastically decreased (Fig. 1). This may be related to the trade-offs between photosynthesis and allocation from leaves to the grain, represented by lower HI (Fig. 2), usually observed in the late crop development stages (Sultana et al. 1999). Other trade-off between gas-exchange parameters is the possible change in WUE, mainly when A and E changes in different scales with NaCl treatments. L9 presented a slow decrease in WUE (-6%, Table 2), what is in accordance with Singh and Sasahara's (1981) results, which reported no changes in WUE under salt treatments.

In cereals, and consequently in rice, the grain production is highly dependent on the photosynthetic capacity and the source-sink relations. Variations in the assimilation capacity and in the carbohydrate synthesis, accumulation and partition directly influence grain production (Biswal and Kohli 2013). In rice, the three upper leaves, including the flag leaf, are the main sources of carbohydrates for the grain filling process, available from leaf photosynthetic assimilation and driven to the panicle (Yoshida 1972). The importance of the flag leaf is even higher under abiotic stress conditions, as salinity (Slewinski 2012). However, in our study, the two lines which showed significant increases in FLL with NaCl treatment intensification, L3 and L10, were the most penalized in GWP losses (Fig. 1, Table 3). This may be related to the fact that an increased leaf area may have led to higher transpiration levels, increasing the water flow from root to shoot, and consequently increasing the Na^+ and Cl^- ions concentration on leaf tissues.

Through the foliar analysis of Na, which showed a negative correlation between GWP and the doses of salt, it was possible to notice the direct effects of salinity on the production of grains in rice, also corroborated by Wang et al. (2020). The higher decrease in GWP for L3 may be related to the incapacity to block Na transportation from soil to leaf, at higher soil Na levels. On the other hand, L7 and L8 may show a strategy of blocking Na transportation to the leaf, at the same higher levels, reducing its impact and helping in the maintenance of GWP.

In terms of biomass allocation, the shoot dry matter is intrinsically related to the rice crop performance (Yeo et al. 1990; Razzaque et al. 2019). The line L8, the one with the lowest significant loss in GWP, presented a sharp decrease in RDM, but a less pronounced decrease in SDM, what can be observed by the pattern of RSR (Fig. 2 and Table 3). Changing the balance between shoot and root proved to be a successful strategy to keep GWP, under salinity conditions. The reduction in RDM may have allowed a lower assimilation of NaCl from the soil, but without causing a pronounced reduction in SDM. In this way, the plant can have enough photosynthetic active structure to maintain source capacity to feed the sinks (grains) (Sultana et al. 1999). However, the pattern of reduction in root mass goes against the findings of Razzaque et al. (2019), which reported the increased in root dry matter as a trait strongly linked to salt tolerance.

The potential benefits of Na^+ ions in plant metabolism have been a matter of discussion recently (Kronzucker et al. 2013; Maathuis 2014). Some studies suggest that in low concentrations Na^+ may be beneficial to the plant due to its common availability in soils, the osmotic control of plants and turgor maintenance (Pardo e Quintero 2002; Subbarao et al. 2003; Wu 2018), potentially improving cell expansion and promoting rapid growth as a competitive advantage (Kronzucker et al. 2013). It may also be a functional substitute to K^+, as both ions share similar functions in plant metabolism, reducing the dependence of potassium fertilizers, which are normally more expensive (Maathuis 2014; Wakeel et al. 2011).

Further advantages of the presence of NaCl in soils, in determined concentrations, are related to the ion exchange on the vacuole. When Na^+ is transported into the vacuole, K^+ ions may be transferred to the cytosol, through the H^+ proton pump. This may increase the availability of K^+ ions to crucial metabolic processes (Wakeel et al. 2011). Furthermore, Na^+ may act in the control of stomata movement, in substitution to K^+, although this process is not completely explained (Robinson et al. 1997; Roelfsema and Hedrich 2005). There are reports of improved stomata control (Marschner 1995) and water use efficiency (Gattward et al. 2012) in plants grown under a condition of Na^+/K^+ availability, instead of only potassium fertilization. Also, Pires et al. (2015) reported that the capacity to keep a low Na^+/K^+ ratio is not always the main trait for salt tolerance in rice.

It is important to highlight that this study did not aim to compare the benefits of Na^+ or its potential to substitute K^+, as the potassium fertilization was performed as recommended for the rice crop (Alvarez et al. 1999). However, we observed that some parameters did not show difference or even a beneficial change at the lower NaCl doses, 3 or 15 $mmol{\cdot}L^{-1}$. This can be exemplified by the behaviour of WHG for L3 or gas-exchange parameters for L6 and L9 at V6 stage or L3 at V13 stage. Of course, as this experiment it was not planned to test this specific role of NaCl. The statistical methods and the NaCl concentrations were not the most appropriate to infer about those results.

Finally, the lines L6, L7 and L8 are recommended as potential genotypes to the studies of salt tolerance strategies in rice. Together with the possible Na^+ benefits at lower levels, other approaches may boost research in salt tolerance in rice: the potential map of jasmonate-related genes, which are recognized to play a major role in the tolerance to osmotic stress in rice (Tang et al. 2020) and the use of molecular approaches for gene identification (Liu et al. 2019).

CONCLUSION

In this study, different patterns of behaviour of rice lines from the Upland Rice Genetic Breeding Program of UFLA were mapped, for many parameters linked to crop production. L1, L4 and L5 lines showed increased death rate under the highest NaCl doses and showed to be saline-sensitive at the early developmental stages. At the end of the cycle, using GWP as an indicator of plant performance under salinity, L6, L7 and L8 lines presented the best results. L6 presented the highest GWP, while L7 and L8 presented the less pronounced decrease in GWP with NaCl increases. Those lines are recommended for further tests for evaluation of salt stress impact on the rice crop.

AUTHORS' CONTRIBUTION

Conceptualization: Vieira, D. A., Toro-Herrera, M. A., Pennacchi, J. P., Mendonça, A. M. C., Botelho, F. B. S., Marchiori, P. E. R., Barbosa, J. P. R. A. D.; **Data Acquisition:** Vieira, D. A., Toro-Herrera, M. A.; **Data Analysis:** Vieira, D. A., Toro-Herrera, M. A., Pennacchi, J. P., Barbosa, J. P. R. A. D.; **Methodology:** Vieira, D. A., Barbosa, J. P. R. A. D., Botelho, F. B. S., Marchiori, P. E. R.; **Writing – Review and Editing:** Vieira, D. A., Toro-Herrera, M. A., Pennacchi, J. P., Mendonça, A. M. C., Botelho, F. B. S., Marchiori, P. E. R., Barbosa, J. P. R. A. D.

DATA AVAILABILITY STATEMENT

All dataset were generated or analyzed in the current study.

FUNDING

Conselho Nacional de Desenvolvimento Científico e Tecnológico
[https://doi.org/10.13039/501100003593]
Grant nº: 141662/2017-4

Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
[https://doi.org/10.13039/501100002322]
Grant nº: 001

Upland Rice Genetic Breeding Program of the Universidade Federal de Lavras.

ACKNOWLEDGEMENTS

All authors thank Joyce Pereira Alvarenga and Layane Silva for having contributed positively to the data collection and analysis.

REFERENCES

Aggarwal, P., Vyas, S., Thornton, P. and Campbell, B. M. (2019). How much does climate change add to the challenge of feeding the planet this century? Environmental Research Letters, 14, 043001. http://doi.org/10.1088/1748-9326/aafa3e

Alvarez, R. C. F., Crusciol, C. A. C., Nascente, A. S., Rodrigues, J. D. and Habermann, G. (2012). Gas exchange rates, plant height, yield components, and productivity of upland rice as affected by plant regulators. Pesquisa Agropecuária Brasileira, 47, 1455-1461. https://doi.org/10.1590/S0100-204X2012001000007

Alvarez, V. V. H., Novaes, R. F., Barros, N. F., Cantarutti, R. B. and Lopes, A. S. (1999). Interpretação dos resultados das análises de solos. In: Ribeiro, A. C.; Guimarães, P. T. G. (Eds.). Recomendação para o uso de corretivos e fertilizantes em Minas Gerais: 5° aproximação. Viçosa: Comissão de Fertilidade do Solo do Estado de Minas Gerais. p. 25-32.

Amirjani, M. R. (2012). Effect of NaCl stress on rice physiological properties. Archives of Phytopathology and Plant Protection, 45, 228-243. https://doi.org/10.1080/03235408.2011.559034

Biswal, A. K. and Kohli, A. (2013). Cereal flag leaf adaptations for grain yield under drought: knowledge status and gaps. Molecular Breeding, 31, 749-766. https://doi.org/10.1007/s11032-013-9847-7

Chiconato, D. A., Sousa Junior, G. S., Santos, D. M. M. and Munns, R. (2019). Adaptation of sugarcane plants to saline soil, Environmental and Experimental Botany, 162, 201-211. https://doi.org/10.1016/j.envexpbot.2019.02.021

Chou, J., Xu, Y., Dong, W., Xian, T., Xu, H. and Wang, Z. (2019). Comprehensive climate factor characteristics and quantitative analysis of their impacts on grain yields in China's grain-producing areas. Heliyon, 5, e02846. https://doi.org/10.1016/j.heliyon.2019.e02846

Counce, P. A., Keisling, T. C. and Mitchell, A. L (2000). Uniform and adaptative system for expressing rice development. Crop Science, 40, 436-443. https://doi.org/10.2135/cropsci2000.402436x

Cui, H. C., Takeoka, Y. and Wada, T. (1995). Effect of sodium chloride on the panicle and spiketet morphogenesis in rice. Japanese Journal Crop Science, 64, 587-592. https://doi.org/10.1626/jcs.64.587

Dadkhah, A. (2013). Effect of salinity on carbon isotope discrimination of shoot and root of four sugar beet (Beta vulgaris L.) cultivars. Journal of Agricultural Science and Technology, 15, 901-910.

Esmaeilpour, A., Van Labeke, M. C., Samson, R., Boeckx, P. and Van Damme, P. (2016). Variation in biochemical characteristics, water status, stomata features, leaf carbon isotope composition and its relationship to water use efficiency in pistachio (Pistacia vera L.) cultivars under drought stress condition. Scientia Horticulturae, 211, 158-166. https://doi.org/10.1016/j.scienta.2016.08.026

[FAO] Food and Agriculture Organization (FAO). (2018). FAO and Lomonosov Moscow State University Handbook for saline soil management. Rome: FAO.

[FAO] Food and Agriculture Organization (FAO). (2019). Food Outlook: Biannual Report on Global Food Markets. Rome: FAO. Licence: CC BY-NC-SA 3.0 IGO.

Gattward, J. N., Almeida, A. A. F., Souza Jr., J. O., Gomes, F. P. and Kronzucker, H. J. (2012). Sodium-potassium synergism in Theobroma cacao: stimulation of photosynthesis, water-use efficiency and mineral nutrition. Physiologia Plantarum, 146, 350-362. http://doi.org/10.1111/j.1399-3054.2012.01621.x

Gregorio, G. B., Senadhira, D., Mendoza, R. D., Manigbas, N. L., Roxas, J. P. and Guerta, C. Q. (2002). Progress in breeding for salinity tolerance and associated abiotic stresses in rice. Field Crops Research, 76, 91-101. https://doi.org/10.1016/S0378-4290(02)00031-X

Hairmansis, A., Berger, B., Tester, M. and Roy, S. J. (2014). Image-based phenotyping for non-destructive screening of different salinity tolerance traits in rice. Rice, 7, 16. https://doi.org/10.1186/s12284-014-0016-3

Harris, B. N., Sadras, V. O. and Tester, M. A. (2010). water-centred framework to assess the effects of salinity on the growth and yield of wheat and barley. Plant Soil, 336, 377-389. https://doi.org/10.1007/s11104-010-0489-9

Hernández, M. A. L., Alvarez-Bernal, D., Cárdenas, G. C. and Estrada, S. O. (2018). Improvement of saline soils with Vicia sativa L. from a semiarid region. Bragantia, 77, 501-509. https://doi.org/10.1590/1678-4499.2017275

James, R. A., Blake, C., Byrt, C. S. and Munns, R. (2011). Major genes for Na+ exclusion, Nax1 and Nax2 (wheat HKT1; 4 and HKT1; 5), decrease Na+ accumulation in bread wheat leaves under saline and waterlogged conditions. Journal Experimental Botany, 62, 2939-2947. https://doi.org/10.1093/jxb/err003

Liu, C., Chen, K., Zhao, X., Wang, X., Shen, C., Zhu, Y., Dai, M., Qiu, X., Yang, R., Xing, D., Pang, Y. and Xu, J. (2019). Identification of genes for salt tolerance and yield-related traits in rice plants grown hydroponically and under saline field conditions by genome-wide association study. Rice, 12, 88. https://doi.org/10.1186/s12284-019-0349-z

Loboguerrero, A. M., Campbell, B. M., Cooper, P. J. M., Hansen, J. W., Rosenstock, T. and Wollenberg, E. (2019). Food and Earth systems: priorities for climate change adaptation and mitigation for agriculture and food systems. Sustainability, 11, 1372. https://doi.org/10.3390/su11051372

Kazemi, S., Eshghizadeh, H. R. and Zahedi, M. (2018). Responses of four rice varieties to elevated CO_2 and different salinity levels. Rice Science, 25, 142-151. https://doi.org/10.1016/j.rsci.2018.04.002

Khajeh-Hosseini, M., Powell, A. A., and Bingham, I. J. (2003). The interaction between salinity stress and seed vigour during germination of soyabean seeds. Seed Science and Technology, 31 715-725. https://doi.org/10.15258/sst.2003.31.3.20

Khan, M. H. U., Malook, I., Atlas, I. M. A., Jan, M., Jan, S. U. and Shah, G. (2014). The effect of sodium chloride (NaCl) stress on seed germination and seedling growth of rice (Oryza sativa L.). Journal of Bio-Molecular Sciences, 2, 100-107.

Kromdijk, J. and Long, S. P. (2016). One crop breeding cycle from starvation? How engineering crop photosynthesis for rising CO_2 and temperature could be one important route to alleviation. Proceedings of Royal Society B, 283, 20152578. http://doi.org/10.1098/rspb.2015.2578

Kronzucker, H. J., Coskun, D., Schulze, L. M., Wong, J. R. and Britto, D. T. (2013). Sodium as nutrient and toxicant. Plant Soil, 369, 1-23. https://doi.org/10.1007/s11104-013-1801-2

Maathuis, F. J. (2014). Sodium in plants: perception, signalling, and regulation of sodium fluxes. Journal Experimental Botany, 65, 849-858. https://doi.org/10.1093/jxb/ert326

Malavolta, E., Vitti, G. C. and Oliveira, S. A. (1997). Avaliação do estado nutricional das plantas: princípios e aplicações. 2. ed. Piracicaba: POTAFOS.

Marschner, H. (1995). Mineral nutrition of higher plants. 2. ed. New York: Academic Press.

Momayezi, M. R., Zaharah, A. R., Hanafi, M. and Mohd Razi, I. (2009). Agronomic characteristics and proline accumulation of Iranian rice genotypes at early seedling stage under sodium salts stress. Malaysian Journal of Soil Science, 13, 59-75.

Morales, S. G., Trejo-Téllez, L. I., Gómez Merino, F. C., Caldana, C., Espinosa-Victoria, D. and Herrera Cabrera, B. E. (2012). Growth, photosynthetic activity, and potassium and sodium concentration in rice plants under salt stress. Acta Scientiarum-Agronomy, 34, 317-324. https://doi.org/10.1590/S1807-86212012000300012

Munns, R. (2002). Comparative physiology of salt and water stress. Plant Cell Environment, 25, 239-250. http://doi.org/10.1046/j.0016-8025.2001.00808.x

Munns, R. and Tester, M. (2008). Mechanisms of salinity tolerance. Annual Review of Plant Biology, 59, 651-681. https://doi.org/10.1146/annurev.arplant.59.032607.092911

Nishimura, T., Cha-Um, S., Takagaki, M., Ohyama, K. and Kirdmanee, C. (2011). Survival percentage, photosynthetic abilities and growth characters of two indica rice (Oryza sativa L. spp. indica) cultivars in response to isosmotic stress. Spanish Journal of Agriculture Research, 9, 262-270. https://doi.org/10.5424/sjar/20110901-162-10

Pardo, J. M. and Quintero, F. J. (2002). Plants and sodium ions: keeping company with the enemy, Genome Biology, 3, reviews1017.1. https://doi.org/10.1186/gb-2002-3-6-reviews1017

Pires, I. S., Negrão, S., Oliveira, M. M. and Purugganan, M. D. (2015). Comprehensive phenotypic analysis of rice (Oryza sativa) response to salinity stress. Physiologia Plantarum, 155, 43-54. https://doi.org/10.1111/ppl.12356

Quintero, J. M., Fournier, J. M. and Benlloch, M. (2007). Na^+ accumulation in shoot is related to water transport in K^+ starved sunflower plants but not in plants with a normal K+ status. Journal of Plant Physiology, 164, 60-67. https://doi.org/10.1016/j.jplph.2005.10.010

Rahnama, A., James, R. A., Poustini, K. and Munns, R. (2010). Stomatal conductance as a screen for osmotic stress tolerance in durum wheat growing in saline soil. Functional Plant Biology, 37, 255-263. https://doi.org/10.1071/FP09148

Rasel, M., Tahjib-Ul-Arif, M., Hossain, M. A., Hassan, L., Farzana, S. and Brestic, M. (2021). Screening of salt-tolerant rice landraces by seedling stage phenotyping and dissecting biochemical determinants of tolerance mechanism. Journal of Plant Growth Regulation, 40, 1853-1868. https://doi.org/10.1007/s00344-020-10235-9

Razzaque, S., Elias, S. M., Haque, T., Biswas, S., Jewel, G. M. N. A., Rahman, S., Weng, X., Ismail, A. M., Walia, H., Juenger, T. E. and Seraj, Z. I. (2019). Gene expression analysis associated with salt stress in a reciprocally crossed rice population. Scientific Reports, 9, 8249. https://doi.org/10.1038/s41598-019-44757-4

Reddy, I. N. B. L., Kim, B. K., Yoon, I. S., Kim, K. H. and Kwon, T. R. (2017). Salt tolerance in rice: focus on mechanisms and approaches. Rice Science, 24, 123-144. https://doi.org/10.1016/j.rsci.2016.09.004

Robinson, M. F., Véry, A. A., Sanders, D. and Mansfield, T. A. (1997). How can stomata contribute to salt tolerance? Annals of Botany, 80, 387-393. https://doi.org/10.1006/anbo.1996.0435

Roelfsema, M. R. G. and Hedrich, R. (2005). In the light of stomatal opening: new insights into “the Watergate”. New Phytologist, 167, 665-691. https://doi.org/10.1111/j.1469-8137.2005.01460.x

Shahbaz, M. and Ashraf, M. (2013). Improving salinity tolerance in cereals. Critical Reviews in Plant Sciences, 32, 237-249. https://doi.org/10.1080/07352689.2013.758544

Singh, M. K. and Sasahara, T. (1981). Photosynthesis and transpiration in rice as influenced by soil moisture and air humidity. Annals of Botany, 48, 513-518. https://doi.org/10.1093/oxfordjournals.aob.a086155

Siringam, K., Juntawong, N., Cha-Um, S. and Kirdmanee, C. (2011). Salt stress induced ion accumulation, ion homeostasis, membrane injury and sugar contents in salt-sensitive rice (Oryza sativa L. spp. indica) roots under isosmotic conditions. African Journal of Biotechnology, 10, 1340-1346.

Slewinski, T. L. (2012). Non-structural carbohydrate partitioning in grass stems: a target to increase. Journal of Experimental Botany, 63, 4647-4670. https://doi.org/10.1093/jxb/ers124

Subbarao, G. V., Ito, O., Berry, W. L. and Wheeler, R. M. (2003). Sodium: a functional plant nutrient. Critical Reviews in Plant Sciences, 22, 391-416. https://doi.org/10.1080/07352680390243495

Sultana, N., Ikeda, T. and Itoh, R. (1999). Effect of NaCl salinity on photosynthesis and dry matter accumulation in developing rice grains. Environmental and Experimental Botany, 42, 211-220. https://doi.org/10.1016/S0098-8472(99)00035-0

Tang, G., Ma, J., Hause, B., Nick, P. and Riemann, M. (2020). Jasmonate is required for the response to osmotic stress in rice. Environmental and Experimental Botany, 175, 104047. https://doi.org/10.1016/j.envexpbot.2020.104047

Tin, H. Q., Loi, N. H., Labarosa, S. J. E., McNally, K. L., McCouch, S. and Kilian, B. (2021). Phenotypic response of farmer-selected CWR-derived rice lines to salt stress in the Mekong Delta. Crop Science, 61, 201-218. https://doi.org/10.1002/csc2.20354

Wakeel, A., Farooq, M., Qadir, M. and Schubert, S. (2011). Potassium substitution by sodium in plants. Critical Reviews in Plant Sciences, 30, 401-413. https://doi.org/10.1080/07352689.2011.587728

Wang, H., Zhang, M. S., Guo, R., Shi, D. C., Liu, B., Lin, X. Y. and Yang, C. W. (2012). Effects of salt stress on ion balance and nitrogen metabolism of old and young leaves in rice (Oryza sativa L.). BMC Plant Biology, 12, 194. https://doi.org/10.1186/1471-2229-12-194

Wang, W., Cai, C., He, J., Gu, J., Zhu, G., Zhang, W., Zhu, J. and Liu, G. (2020). Yield, dry matter distribution and photosynthetic characteristics of rice under elevated CO2 and increased temperature conditions. Field Crops Research, 248, 107605. https://doi.org/10.1016/j.fcr.2019.107605

Wu, H. (2018). Plant salt tolerance and Na^+ sensing and transport. The Crop Journal, 6, 215-225. https://doi.org/10.1016/j.cj.2018.01.003

Xu, J. W., Huang, X., Lan, H. X., Zhang, H. S. and Huang, J. (2016). Rearrangement of nitrogen metabolism in rice (Oryza sativa L.) under salt stress. Plant Signal Behavior, 11, e1138194.

Yeo, A. R., Yeo, M. E., Flowers, S. A. and Flowers, T. J. (1990). Screening of rice (Oryza sativa L.) genotypes for physiological characters contributing to salinity resistance, and their relationship to overall performance. Theoretical and Applied Genetics, 79, 377-384. https://doi.org/10.1007/BF01186082

Yoshida, S. (1972). Physiological aspects of grain yield. Annual Review of Plant Physiology, 23, 437-464. https://doi.org/10.1146/annurev.pp.23.060172.002253