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Root Architecture of Rice as Affected by Phosphorus Starvation and Salt Stress

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Introduction

Soil salinity is an important problem for plant growth and development and therefore for productivity, especially in arid and semiarid regions (Hussain et al., 2019). In Cuba, 14.9% of the agricultural area and 9.1% of the surface of the country are affected by salts (ONEI, 2017). Salt stress has antagonistic effects on some macronutrients like phosphorus (P). Phosphorus is one of the most important macronutrient for plant growth and its availability has a strong effect on root morphology (Gruber et al., 2013). Low P availability reduces the growth of the main root and increases the formation and elongation of lateral roots in Arabidopsis (Al-Ghazi et al., 2003). The root system architecture (RSA) also can be changed by P availability and salt stress interaction as shown by Kawa et al. (2016) in Arabidopsis. Rice is one of the most important crops in the world and its production and yield is affected by salinity globally (Ghosh et al. 2016). However, the effects of P deficiency under saline stress on rice is barely studied, especially on the rice RSA. In our study, we quantified the effect of salt level and P availability on RSA of two rice genotypes (INCA LP-5 and Perla de Cuba) in mini-rhizotrons

Material and Methods

The two *Oryza sativa* genotypes INCA LP-5 of the National Institute of Agricultural Sciences (Pérez and Castro, 2000) and Perla de Cuba, produced by the Grain Research Institute, were grown from October to November 2019 in a greenhouse at the Faculty of Agronomy, University of Rostock. The temperature was 26/21 °C (day/night) and the light intensity was ~7500 Lux. After imbibition, four seeds of each genotype were put in mini-rhizotrons for germination. A fine mesh (20 µm) divided the seeds and roots from the filled in sand (as in Parra-Londono et al. 2018). Eight mini-rhizotrons were settled randomly inside plastic boxes with an angle of ~70°.

The boxes were covered with aluminum foil during the first two days for germination and filled with one of four different nutrient solutions: Yoshida solution with 10 ppm P and no NaCl (Yoshida et al., 1976), Yoshida solution with a low concentration of P (1 ppm), Yoshida solution with NaCl (50 mM) and Yoshida solution with low concentration of P (1 ppm) and NaCl (50 mM). The solutions were changed every week. Three of the four seedlings were removed after three days to grow only one plant per each mini-rhizotron. Roots were protected from light by covering the boxes with aluminum foil. The experiment was conducted with five replications per treatment and genotype for 21 days. At harvest dry weight was measured and root images acquired. Image processing and phenotyping were carried out with the free software package for RSA phenotyping GiA Roots (Galkovskyi et al., 2012) to analyze the parameters describing the size, extent, and distribution of the root network. Plant P concentrations were measured after dry ashing and extraction with HCl using a Perkin Elmer Optima 8300 DV ICP-OES spectrometer (Perkin Elmer, Waltham, MA, USA)

Results and Discussion

Both rice genotypes, INCA LP-5 and Perla de Cuba, had a significant decrease in dry weight with salt stress but not with P starvation (Figure 1A) and an even higher reduction was with the combination of P starvation and salt stress. This significant biomass reduction with P starvation and saline stress has been reported in two barley genotypes (Khosh Kholgh Sima et al., 2012) and *Catapdium rigidum* (Zeibi et al. 2018). Also Tang et al. (2019) showed that the lowest biomass values in maize were obtained with the combination of salt stress and P starvation.

P uptake into the plant showed a significant reduction in presence of high salt concentration in both rice genotype, especially with P starvation and salt stress combined (Figure 1B). This reduction in P uptake has also been reported before in sorghum with the application of salt stress (Kausar and Gull, 2019) and in barley in presence of both stresses (Sima et al., 2012).

The root system architecture was also more affected by high salt concentration than by P starvation. The size traits network perimeter (NP), network surface area (NSA) and network length (NL) were reduce in both INCA LP-5 and Perla de Cuba rice genotypes by P starvation and salt stress (Table 1) with an interaction effect of both stresses in Perla de Cuba for NL and NSA traits. Extent traits: network depth (ND), network width (NW) and network convex Area (NCA) and distribution traits: network length distribution (NLD), maximum number of roots (MNR) and specific root length (SRL) showed a higher reduction under saline stress condition than under low P availability. In general, the root system architecture traits were more affected in Perla de Cuba genotype that has a smaller root system even under control conditions. Kawa et al. (2016), had shown that both stresses, salt and low P availability, can change the root architecture

in Arabidopsis but discover that individual RSA components integrate salt stress and P starvation in a different way, with a dominant effect of salinity as our results showed. Also Tang et al. (2019) had shown that low P availability and salt stress decrease total root length and root surface in maize.

Table 1. Means, standard errors and significance for root system architecture traits of two rice genotypes harvested at 21 days of growth in mini-rhizotrons in normal and low concentration of P, 0 and 50 mM of NaCl. Different letters meaning significant differences between treatments within one genotype. Two-way ANOVA results P < 0.05; P < 0.01; P < 0.01; P < 0.001; ns, not significant.

Traits	Genotype	NaCl (0 mM)		NaCl (50 mM)		ANOVA		
		P (10 ppm)	P (1 ppm)	P (10 ppm)	P (1 ppm)	Р	NaCl	P x NaCl
NP (cm)	INCA LP-5	1084 ± 154 a	327 ± 90.8 bc	$665\pm79.9~b$	224 ± 44.9 c	**	***	ns
	Perla de Cuba	1085 ± 25.7 a	$194 \pm 14.7 c$	$412~\pm~35.3~b$	$110 \pm 40.4 \mathrm{c}$	***	***	ns
NL (cm)	INCA LP-5	595 ± 107 a	$465\pm60.0~b$	223 ± 60.5 bc	149 ± 30.0 c	**	**	ns
	Perla de Cuba	419 ± 15.3 a	$286\pm22.8\ b$	$128\pm8.30\ c$	74 ± 27.0 c	**	***	***
NSA (cm ²)	INCA LP-5	43.3 ± 6.10 a	$26.8\pm3.52~b$	12.9 ± 3.27 bc	$8.80 \pm 1.79 \ c$	**	***	ns
	Perla de Cuba	23.9± 0.92 a	16.5 ± 1.38 b	$7.59\pm0.53~c$	4.32 ± 1.58 c	**	***	***
ND (cm)	INCA LP-5	15.9 ± 0.66 a	16.0 ± 1.07 a	13.7 ± 1.32 ab	11.1 ± 1.21 b	ns	***	ns
	Perla de Cuba	11.6 ± 0.92 a	10.5 ± 1.04 a	$6.25\pm0.33~b$	$4.59\pm0.95~b$	ns	***	***
NW (cm)	INCA LP-5	10.6 ± 1.02 a	5.31 ± 1.38 ab	$8.40 \pm 1.01 \; b$	$3.83\pm0.62~b$	ns	***	ns
	Perla de Cuba	$8.27\pm0.49~a$	6.23 ± 0.49 ab	$5.65\pm0.49~b$	$2.42\pm0.75~c$	***	***	ns
NCA (cm ²)	INCA LP-5	111 ± 15.7 a	$82.3 \pm 12.4 \text{ ab}$	$43.2\pm10.0\ b$	$28.2\pm6.04~b$	ns	***	ns
	Perla de Cuba	54.1 ± 2.48 a	41.1 ± 6.06 a	$17.1\pm1.09~b$	9.63 ± 4.24 b	**	***	**
NLD	INCA LP-5	$0.90\pm0.20\ ns$	1.06 ± 0.20 ns	1.38 ± 0.24 ns	$0.89\pm0.14~ns$	ns	ns	ns
	Perla de Cuba	$1.14 \pm 0.40 \text{ a}$	1.21± 0.26 a	$0.10\pm0.06~b$	0.66 ± 0.19 a	ns	***	ns
MNR	INCA LP-5	49.6 ± 6.02 a	$31.2\pm4.36~b$	$18.4\pm4.35~b$	15.2 ± 2.13 b	*	***	ns
	Perla de Cuba	46.6 ± 2.31 a	$37.2 \pm 3.69 \text{ ab}$	$28.4\pm3.18\ bc$	$16.4\pm4.10\ c$	***	***	ns
SRL (cm cm ^{-3})	INCA LP-5	3669 ± 64.3 ns	$3595 \pm 79.2 \text{ ns}$	3581 ± 68.5 ns	3480 ± 75.2 ns	ns	ns	ns
	Perla de Cuba	3716 ± 25.8 a	3585 ± 40.3 ab	$3405 \pm 103 \text{ ab}$	$3597 \pm 66.1 \text{ b}$	ns	***	*

Conclusions and Outlook

Results demonstrated that for two rice Cuban genotype salt stress has a stronger effect than P starvation for plant dry weight, P uptake and RSA traits in both INCA LP-5 and Perla de Cuba genotype within three weeks of growth. Perla de Cuba genotype with a smaller root system than INCA LP-5 was more susceptible to P starvation. This results suggesting that for the experimental conditions the P concentration used could be to be considered as P starvation. In the future, we suggest to use bigger rhizotrons system to grow the rice for a longer time period and also conduct field experiments.



Figure 1. Dry weight (A) and P uptake per plant (B) of two rice genotypes, INCA LP-5 (black) and Perla de Cuba (grey) in four growth conditions: Yoshida solution, Yoshida solution with a low concentration of P (1 ppm), Yoshida solution with NaCl (50 mM) and Yoshida solution with low concentration of P (1 ppm) and NaCl (50 mM). The graphs show the results of a Two-way ANOVA, different letters between mean values according to Tukey's test mean significant differences ($p \le 0.05$) separately for each genotype.

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