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RESPONSE OF RICE (*Oryza sativa* L.) TO SALINITY STRESS AT GERMINATION AND EARLY SEEDLING STAGES

Alpay Balkan[1,*], Temel Gençtan[1], Oğuz Bilgin[1] and Hakan Ulukan[2]

[1]University of Namık Kemal, Faculty of Agriculture, Department of Field Crops, 59030, Tekirdağ, Turkey;
[2]University of Ankara, Faculty of Agriculture, Department of Field Crops, 06100, Ankara, Turkey.
[*]Corresponding author's e-mail: abalkan@nku.edu.tr

Response of rice (*Oryza sativa* L. cv. Osmancık-97) to eight salinity (NaCl) levels (0, 2, 4, 8, 12, 16, 20 and 24 dS m^{-1}) was studied at germination and early seedling stages during 2013. Surface sterilized seeds were germinated at 25 ± 1°C for 14 days in a growth chamber and measurements were taken at 3, 6, 12, 24, 36, 48 and 72 h for water uptake of seeds. Germination was completely arrested at 20 and 24 dS m^{-1} salinity levels. While mean germination time increased with increasing salinity, water uptake, germination rate, coleoptile length and shoot length were significantly decreased. Root number, root length, shoot length, root fresh weight, root dry weight, shoot fresh weight and shoot dry weight significantly increased up to 4 to 8 dS m^{-1}, and then these characters were significantly decreased by increasing salinity level. It was concluded that rice cultivar viz. Osmancık-97 has tolerance up to a salinity level of 8 dS m^{-1} at the germination and early seedling stages.
Keywords: Rice (*Oryza sativa* L.), NaCl, water uptake, germination, seedling.

INTRODUCTION

Crop plants are usually exposed to a multitude of natural biotic and abiotic stresses that limit their growth and productivity (Kazemi and Eskandari, 2011). Salinity stress is a major limiting factor affecting crop production in addition to other environmental stresses such as high temperature, drought and cold stresses. This is particularly the problem of agriculture in many arid and semi-arid parts of the world (Ekmekci *et al.*, 2005). The United Nations Environment Program (UNEP) estimates that approximately 20% of agricultural land and 50% of crop land in the world is salt-stressed (Sairam and Tiyagi, 2004; Yan, 2008). Therefore, the total worldwide area of land affected by salinity is about 190 million ha (FAO, 2010). Salinity is a serious constraint to obtaining increased crop yields under irrigated agriculture throughout the world (Aslam *et al.*, 1993). Particularly, chlorides (NaCl, $CaCl_2$ and $MgCl_2$), sulfates (Na_2SO_4), nitrates ($NaNO_3$ and KNO_3), carbonates and bicarbonates (Na_2CO_3 and $NaHCO_3$) are the main contributors to salinity (Gursoy *et al.*, 2012).

Rice (*Oryza sativa* L.) is the only crop that grows under water and is adapted to flooded conditions. It is one of the most important warm season cereals commonly grown in the world and Turkey. The area planted with rice was about 164 million ha worldwide; its production was about 722 million tonnes with an average yield of 440 kg/ha in 2011 (FAO, 2011). Rice is described as a salt-sensitive crop, and soils are naturally saline in areas where rice is grown due to border irrigation (Maas and Hoffman, 1977). Rice is particularly sensitive to salt stress at post germination stages (Shobbar *et al.*, 2012). Seed germination is usually the most critical stage in seedling establishment, determining successful crop production (Almansouri *et al.,* 2001). Salinity affects seed germination by creating osmotic stress due to reduced water uptake or through ionic imbalance due to toxic effects of sodium (Na^+) and chloride (Cl^-) ions (Hosseini *et al.*, 2003). In salt-affected rice growing areas, substantial loss in seedling establishment, leaf chlorosis, and final yield reduction are observed (Zeng and Shannon, 2000). At low concentrations, salt suppresses rice plant growth and at higher concentrations can cause death (Hakim *et al.*, 2010). According to the classification of crop tolerance to salinity, rice is within the sensitive division from 0 dS m^{-1} to 8 dS m^{-1} (Maas, 1986). It has been reported that the threshold and slope for rice are 3.0 dS m^{-1} and 12% per dS m^{-1} of saturated soil extract (EC_e), respectively (Maas and Hoffman, 1977). In addition, many studies have reported and emphasized that crop plants including rice are significantly affected by salinity stress, particularly during germination and early seedling growth (Heenan *et al.*, 1988; Bohra and Doerffling, 1993; Chuan and Ching, 1995; Lutts *et al.*, 1996; Zeng *et al.*, 2003; Alam *et al.*, 2004; Chinnusamy *et al.*, 2005; Walia *et al.*, 2005; Parker *et al.*, 2006; Moradi and Ismail, 2007; Negrao *et al.*, 2011; Amirjani, 2012; Shobbar *et al.*, 2012). Therefore, this study was conducted to determine the response of rice (*Oryza sativa* L. cv. Osmancık 97) to eight salinity levels (0, 2, 4, 8, 12, 16, 20 and 24 dS m^{-1}) at germination and early seedling growth stages.

MATERIALS AND METHODS

This research was carried out in the seed laboratory of the Department of Field Crops, Faculty of Agriculture, University of Namık Kemal, Tekirdag, Turkey, in 2013. Seeds of rice cultivar viz. Osmancık-97, used as the experimental material in this study, were obtained from the Thrace Agricultural Research Institute, Edirne, Turkey. This cultivar is widely cultivated in neighbor countries (Bulgaria and Greece) as well as Turkey. It has also been registered in Bulgaria.

The experiment was arranged in a randomized plot design with four replications. Solutions with eight different salt levels (0, 2, 4, 8, 12, 16, 20 and 24 dS m^{-1} electrical conductivity) for salinity stress were adjusted using NaCl (SIGMA-ALDRICH Co., USA) (Maas, 1993). Distilled water was used as the control solution (0 dS m^{-1}). Healthy and uniform seeds were initially treated with a 1.5% solution of sodium hypochlorite for 3 min for surface sterilization (Chun *et al.*, 1997). Residual chlorine was eliminated by washing the seeds with distilled water. Twenty seeds for each NaCl concentration were quickly dried with a paper tissue after surface sterilization (Jamil and Rha, 2007). Seeds were weighed by electronic balance (Sartorius, d=0.001) and were placed on sterile filter papers (Whatman's No.1) in 9 cm-diameter sterile Petri dishes which contained 10 ml of salinity treatment. The filter papers were changed once after every 2 days to prevent accumulation of salts (Rehman *et al.*, 1996). In order to prevent evaporation, Petri dishes were put into locked transparent plastic bags (Kaya *et al.*, 2005). Seeds were allowed to germinate at 25 ± 1 ^{o}C for 14 days in a growth chamber (Şehirali, 1997). For determination of water uptake, seeds were removed from the Petri dishes at regular intervals, quickly surface-dried with a paper tissue, weighed and returned to the original conditions. Measurements for water uptake were taken at 3, 6, 12, 24, 36, 48 and 72 h (Alam *et al.*, 2003). The water uptake (WU) was calculated according to a formula presented in Rahman *et al.* (2008):

$$WU, \% = (W_2\text{-}W_1/W_1) \times 100$$

Where, W_1(g) = initial weight of seed; W_2(g) = weight of seed after absorbing water for a particular time.

At 14 days after sowing, germination rate (GR) was calculated with the following formula (Aslam *et al.*, 1993; ISTA, 2009):

$$GR \% = (\text{No. of germinated seeds/No. of total seeds}) \times 100$$

A seed was considered to be germinated when the radicle protruded 1 mm. Germinated seeds were counted daily, and the mean germination time (MGT) was computed each day for every salinity level by the following formula (Ellis and Roberts, 1980):

$$MGT = \sum(fx) / \sum f$$

Where, f is the number of germinated seeds in the day of counting; x is the counting days.

The number of roots (RN-no/plant), root length (RL-cm), root fresh and dry weight (RFW and RDW-mg), coleoptile length (CL-cm), shoot length (SL-cm), shoot fresh and dry weight (SFW and SDW-mg) were measured at 14 days after sowing. Dry weights were determined after drying samples at 70°C for 48 h in an oven (Bohm,1979).

All data obtained from this study were subjected to statistical analysis using MSTAT-C software (Version 1.42, Michigan State University, East Lansing, MI, USA). The parameters given as percentage were subjected to arcsine transformation before statistical analysis. Water uptake was statistically analyzed according to randomized split-plot design and other characteristics were statistically analyzed according to randomized plot design. LSD (Least Significant Difference) test (P≤0.05) was used to determine differences among means (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Germination was completely inhibited at 20 and 24 dS m^{-1} salinity levels; therefore, data from these treatments were not evaluated in the study.

Table 1. Water uptake (%) of rice seeds at different salinity levels.

Time (hour)	Salinity levels (dS m^{-1})						Mean
	0	2	4	8	12	16	
3	11.09 u	11.33 tu	10.47 uv	9.86 v	9.92 v	9.64 v	10.38 g
6	14.99 r	15.31 r	13.28 s	13.14 s	12.82 s	12.39 st	13.65 f
12	20.85 o	20.86 o	20.16 op	19.30 pq	19.13 pq	18.29 q	19.76 e
24	26.11 kl	25.75 lm	24.80 m	24.77 m	23.54 n	22.66 n	24.60 d
36	29.53 gh	28.69 hı	27.62 ıj	26.93 jk	25.69 lm	25.28 lm	27.29 c
48	33.20 e	32.26 ef	30.29 g	29.43 gh	27.84 ıj	26.97 jk	30.00 b
72	45.80 a	43.81 b	38.44 c	37.04 d	31.86 f	29.75 gh	37.78 a
Mean	25.94 a	25.43 b	23.58 c	22.92 d	21.54 e	20.71 f	23.35
$LSD_{(P\leq0.05)}$	Time: 0.572	Salinity levels: 0.442	Time x Salinity levels: 1.169				
F values	Time: 2398.976**	Salinity levels: 172.780**	Time x Salinity levels: 22.561**				

**: Significant at 1 %. Distinct letters indicate significant differences according to LSD test (P≤0.05).

Germination characteristics:

Water uptake (WU-%): WU of seeds was significantly (P≤0.01) affected by time, salinity levels and their interaction (Table 1). Increases in WU of seeds were observed at the measurement times, and seeds of rice continued WU during the 72 h. About 50% of the water absorbed by seeds was imbibed in the first 12 h. The highest WU (37.78%) was obtained at 72 h; the lowest WU (10.38%) was determined at 3 h. WU of seeds significantly decreased with increasing salinity levels. Highest WU (25.94%) was observed at 0 dS m^{-1} (control), and decreased with each increase in salinity level (Table 1).

WU of seeds varied between 9.64-45.80% in time x salinity level interaction. WU decreased as salinity levels increased at measurement times. WU reached a maximum at 0 dS m^{-1} salinity (control) at 72 h. The lowest WU was at 16 dS m^{-1} at 3 h (Table 1).

WU is the fundamental requirement for the initiation of seed germination. It was observed in our study that salinity stress caused lower WU by rice seeds. This may be a result of osmotic stress created by salinity in the germination medium. Hosseini *et al.* (2003) also explained that salinity affects the WU of seeds by creating osmotic stress. Our results are in agreement with the results of Prakash and Prathapasenan (1988), Ahmad *et al.* (2000), Munns (2002), Alam *et al.* (2003), Kaydan and Yagmur (2008), Nizam (2011) and Shereen *et al.* (2011) that salinity stress inhibits water uptake of seeds.

Germination rate (GR-%): GR was significantly (P≤0.01) affected by salinity levels, where increases in severity of salinity stress reduced GR (Table 2). While no significant differences were observed under salinity levels 0 (control), 2, 4 and 8 dS m^{-1}, increasing salinity to higher levels did affect GR. The highest GRs were 98.80, 97.50, 97.50 and 95.00% for 0, 2, 4 and 8 dS m^{-1} salinity levels, respectively; the lowest GR (87.50%) was determined at the highest salinity level (16 dS m^{-1}) (Table 2). In general, GR declined with increasing salinity levels. Presumably, the main factor for reduced GR is the osmotic effect due to salinity level (Hakim *et al.*, 2010). In addition, salinity affects seed germination through ionic imbalance due to toxic effects of sodium (Na^+) and chloride (Cl^-) ions (Hosseini *et al.*,2003). Salinity also upsets plant hormone levels and reduces the utilization of seed reserves (Afzal *et al.*, 2012). Our results are in accord with those of Heenan *et al.* (1988), Khan *et al.* (1997), Ahmad *et al.* (2000), Rahman *et al.* (2001), Alam *et al.* (2003), Jamil and Rha (2007), Hakim *et al.* (2010), Shereen *et al.* (2011) and Afzal *et al.* (2012).

Mean germination time (MGT-day): Salinity levels had a significant effect (P≤0.01) on MGT (Table 2). MGT increased with increasing salinity levels. The shortest MGT (3.12 days) was found at a salinity level of 0 dS m^{-1} (control), followed by 2 dS m^{-1} (3.28 days). A salinity level of 16 dS m^{-1} had the longest MGT (4.72 days). Increase of MGT indicates that germination is comparatively delayed by salinity stress. Thus, MGT was 1.5 days longer at 16 dS m^{-1} as compared to control. This may result from seeds of rice having a lower WU at high salinity levels due to osmotic stress (Table 1). Therefore, seeds of rice germinated in a shorter time at low salinity levels in our study. The increase in MGT due to salinity (osmotic stress) was also supported by Rahman *et al.* (2001), Alam *et al.* (2003), Jamil and Rha (2007), Hakim *et al.* (2010), Nizam (2011), Shereen *et al.* (2011), and Afzal *et al.* (2012).

Root characteristics:

Root number (RN-no./plant): The effect of salinity level on RN was statistically significant (P≤0.01) (Table 3). Mean RN varied between 3.68 and 7.03. RN increased significantly up to 8 dS m^{-1} as compared to control, and then decreased with further increases of salinity. Thus, the salinity level of 2 dS m^{-1} had the highest RN, followed by 4 dS m^{-1} (6.95 no.) and 8 dS m^{-1} (6.48 no.). As expected, the lowest RN was determined at 16 dS m^{-1} salinity. Osmotic pressure in the germination medium increased with increasing salinity levels. Therefore, RN of rice seedlings increased to provide enough water uptake under osmotic pressure. However, RN decreased at higher salinity levels. Presumably, higher salinity reduced the utilization of seed reserves (Afzal *et al.*, 2012), and seedling growth was negatively affected. Findings similar to our results were reported by Nizam (2011) in perennial ryegrass, who found that RN increased up to 8 dS m^{-1} salinity; beyond 8 dS m^{-1} RN decreased.

Root length (RL-cm): RL was significantly (P≤0.01) affected by salinity level (Table 3). RL increased at salinity levels of 2 and 4 dS m^{-1} as compared with control (0 dS m^{-1}). This might be due to physiological drought stress (or osmotic stress) created by the salinity. Similarly, Yagmur and Kaydan (2008) reported in triticale that PEG and NaCl increased root length at low concentrations (-0.45 MPa), but

Table 2. Germination rate and mean germination time of rice seeds at different salinity levels.

Parameters	Salinity levels (dS m^{-1})					
	0	**2**	**4**	**8**	**12**	**16**
Germination rate (%)	98.80 a	97.50 a	97.50 a	95.00 ab	92.50 b	87.50 c
Mean germination time (day)	3.12 e	3.28 de	3.44 cd	3.53 c	4.15 b	4.72 a
$LSD_{(P\leq0.05)}$	Germination rate: 3.816			Mean germination time: 0.201		
F values	Germination rate: 10.768**			Mean germination time: 81.519**		

**: Significant at 1 %. Distinct letters indicate significant differences according to LSD test (P≤0.05).

this increase in root length stopped at osmotic potential of -0.77 MPa in NaCl. Shobbar *et al.* (2012) also observed that longer roots may help plants to absorb more water under drought conditions. In this study, RL decreased with increasing salinity above 4 dS m^{-1}. Among six different salinity levels, the longest RL (9.25 cm) was determined at 2 dS m^{-1}, followed by 4 dS m^{-1} (8.62 cm) in the same statistical group. RL was the lowest at 16 dS m^{-1} (1.40 cm). In this study, the gradual decrease in RL with increase in salinity (especially, over 4 dS m^{-1}) might be due to greater inhibitory effects of NaCl to root growth (Rahman *et al.*, 2001). Our result agrees with the work of Nizam (2011) in perennial ryegrass, who reported that RL increased to a salinity level of 2 dS m^{-1}, and then decreased with increase in salinity level. Our results are also in line with the findings of Jamil and Rha (2007) and Hakim *et al.* (2010).

***Root fresh weight (RFW-mg)*:** There were significant differences ($P \leq 0.01$) among salinity levels for RFW (Table 3). Mean RFW varied between 13.40 (16 dS m^{-1}) and 50.55 mg (2 dS m^{-1}). Our results showed that RFW increased up to 4 dS m^{-1}. This might be due to increase of RL and RN up to 4 dS m^{-1}. Rahman *et al.* (2001) also reported that lower levels of salinity were not very inhibitory to fresh weight of root in rice seedlings. However, the effect of higher salinity levels on RFW was negative in this study. The reduction in RFW may be due to toxic effects of NaCl (Kazemi and Eskandari, 2011). Findings similar to our results were reported by Aslam *et al.* (1993), Ahmad *et al.* (2000), Alam *et al.* (2004), Jamil and Rha (2007), Kazemi and Eskandari (2011), and Nizam (2011), who found that RFW decreased under high salinity stress.

***Root dry weight (RDW-mg)*:** The mean values of RDW at different levels of salinity are presented in Table 3. RDW was not affected by salinity stress from 0 to 8 dS m^{-1}. Similar findings were reported by Nizam (2011) in perennial ryegrass. However, the highest RDW (7.48 mg) was determined at 2 dS m^{-1}, followed by 4 dS m^{-1} with 7.15 mg and 8 dS m^{-1} with 6.13 mg in the same statistical group. This might be due to increasing RFW at low salinity levels (Table 3). This might also be due to accumulation of soluble carbohydrates in the roots in order to increase osmotic pressure of roots for the uptake of water. Above 8 dS m^{-1}, RDW significantly decreased with increasing salinity levels. Therefore, a salinity of 16 dS m^{-1} had the lowest RDW (2.75 mg). This result indicates that root growth of rice seedlings is significantly decreased under severe salinity stress. Our results are also in agreement with the findings of Ahmad *et al.* (2000), Rahman *et al.* (2001), Lee *et al.* (2003), Alam *et al.* (2004), Jamil and Rha (2007), Hakim *et al.* (2010), Kazemi and Eskandari (2011), who found that RDW was inhibited by high salinity levels.

Shoot characteristics:

***Coleoptile and shoot length (CL and SL-cm)*:** Differences among salinity levels were statistically significant ($P \leq 0.01$) for CL and SL (Table 4). While CL and SL did not significantly change from 0 to 4 dS m^{-1} salinity levels, they

Table 3. Some root characteristics of rice seedlings at different salinity levels.

Salinity levels (dS m^{-1})	Root number (no./plant)	Root length (cm)	Root fresh weight (mg)	Root dry weight (mg)
0	6.08 ab	7.88 bc	38.33 ab	5.98 a
2	7.03 a	9.25 a	50.55 a	7.48 a
4	6.95 a	8.62 ab	42.90 ab	7.15 a
8	6.48 ab	7.12 c	37.73 b	6.13 a
12	5.48 b	3.91 d	22.80 c	4.15 b
16	3.68 c	1.40 e	13.40 c	2.75 b
$LSD_{(P \leq 0.05)}$	1.231	1.064	12.501	1.604
F values	9.127**	73.231**	10.565**	11.359**

**: Significant at 1%. Distinct letters indicate significant differences according to LSD test ($P \leq 0.05$).

Table 4. Some shoot characteristics of rice seedlings at different salinity levels.

Salinity levels (dS m^{-1})	Coleoptile length (cm)	Shoot length (cm)	Shoot fresh weight (mg)	Shoot dry weight (mg)
0	1.96 a	7.52 a	36.98 b	5.35 ab
2	1.88 ab	7.36 a	42.30 a	5.83 a
4	1.85 ab	7.28 a	37.83 ab	6.15 a
8	1.71 bc	6.62 b	33.95 b	5.80 a
12	1.59 c	5.04 c	25.95 c	4.68 bc
16	1.38 d	3.86 d	19.80 d	3.98 c
$LSD_{(P \leq 0.05)}$	0.203	0.609	5.077	0.836
F values	9.794**	53.123**	23.990**	8.578**

**: Significant at 1 %. Distinct letters indicate significant differences according to LSD test ($P \leq 0.05$).

significantly decreased above 4 dS m^{-1}. This means that shoot growth of rice can tolerate up to 4 dS m^{-1} salinity. The longest CL (1.96 cm) and SL (7.52 cm) were measured at 0 dS m^{-1} (control); the shortest CL (1.38 cm) and SL (3.86 cm) were observed at 16 dS m^{-1}. The decrease in SL with increasing salinity might be due to reduction in physiological availability of water with increase in solute suction or accumulation of toxic ions (Na^+, Cl^-, etc.) within the seedlings (Ahmad *et al.*, 2000). Reduction of SL is a common phenomenon of rice and other crop plants grown under high saline conditions (Khan *et al.*, 1997; Ahmad *et al.*, 2000; Rahman *et al.*, 2001; Lee *et al.*, 2003; Atak *et al.* 2006; Jamil and Rha, 2007; Kaydan and Yagmur, 2008; Yağmur and Kaydan, 2008; Hakim *et al.*, 2010; Nizam, 2011; Afzal *et al.*, 2012; Amirjani, 2012).

***Shoot fresh weight (SFW-mg)*:** Results of this study showed that SFW was significantly ($P\leq0.01$) influenced by salinity level (Table 4). The highest SFW (42.30 mg) was determined at 2 dS m^{-1}, followed by 4 dS m^{-1} (37.83 mg) in the same statistical group. Similar results were obtained for RFW (Table 3). These results show that seedling growth of rice was not significantly affected by low salinity levels. However, SFW decreased with increasing salinity levels (especially over 4 dS m^{-1}); a salinity level of 16 dS m^{-1} had the lowest SFW (19.80 mg). Reduction in SFW was attributed to decreased water potential of the rooting medium and growth inhibition related to osmotic effects under salt stress (Munns, 2002). The findings of Aslam *et al.* (1993) in rice, Ahmad *et al.* (2000) in rice, Prado *et al.* (2000) in quinoa, Rahman *et al.* (2001) in rice, Alam *et al.* (2004) in rice, Kaya *et al.* (2005) in rapeseed, Jamil and Rha (2007) in rice, Yağmur and Kaydan (2008) in triticale, Haq *et al.* (2009) in rice, Kazemi and Eskandari (2011) in rice, Nizam (2011) in perennial ryegrass, Amirjani (2012) in rice confirm our results, reporting that high salinity levels caused a significant reduction in SFW.

***Shoot dry weight (SDW-mg)*:** The influence of salinity level on SDW was statistically significant ($P\leq0.01$) (Tabel 4). Above 8 dS m^{-1}, SDW decreased with increasing salinity levels. The highest SDW (6.15 mg) was determined at 4 dS m^{-1}, followed by 2 dS m^{-1} (5.83 mg), 8 dS m^{-1} (5.80 mg) and 0 dS m^{-1} (5.35 mg), which were statistically in the same significance group. This result indicated that dry matter production of rice seedlings can be maintained up to 8 dS m^{-1} salinity. Similarly, it has been reported by Rahman *et al.* (2001) that lower levels of salinity were not very inhibitory to SDW. As expected, in this study the lowest SDW (3.98 mg) was determined at 16 dS m^{-1}. Reduced SDW of rice seedlings has also been reported by Heenan *et al.* (1988), Fageria (1985), Khan *et al.* (1997), Ahmad *et al.* (2000), Zeng and Shannon (2000), Rahman *et al.* (2001), Alam *et al.* (2004), Moradi and Ismail (2007), Haq *et al.* (2009), Hakim *et al.* (2010), Kazemi and Eskandari (2011), Afzal *et al.* (2012), and Amirjani (2012).

***Conclusion*:** It was concluded that rice cultivar (Osmancık-97) used in this study showed differential response to salinity stress. Germination and early seedling growth were inhibited by increasing salinity levels in the germination medium. Although water uptake of rice seeds was reduced with increasing salinity, the seeds continued to take up water through 72 h. However, germination was delayed and mean germination time elongated due to reduced water uptake. No seed germinated at salinity levels of 20 and 24 dS m^{-1}. On the other hand, root and shoot growth of rice were not significantly affected by salinity levels up to 8 dS m^{-1} during the early seedling stage. Therefore, rice cultivar Osmancık-97 can be recommended for cultivation under moderate salt stress conditions. Further investigations are needed, however, to enhance our understanding of the salt stress effects during the whole growing cycle of rice.

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