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EFFECTIVENESS OF NUTRIENT MANAGEMENT IN MANAGING SALINE AGRO-ECOSYSTEMS: A CASE STUDY OF *LENS CULINARIS* MEDIK

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Abstract

Lentil is one of the main pulse crops of south west Asia including Iran that may experience some degree of salinity in many areas of production. Many studies denote the ameliorative effects of elevated Ca^{2+} and K^+ concentrations in the growth medium of salinized plants. The interactive effects of salinity, calcium and potassium on physiomorphological traits of a local Lentil cultivar (Robbat) were studied in a green-house experiment. Treatments included 4 levels of NaCl (0, 15, 30, and 45 m*M* NaCl), 2 levels of $CaCl_2$ (0 and 10 m*M*), and 2 levels of KCl (0 and 10 m*M*) that was added to the nutrient solution. Salinity higher than 15 m*M* substantially reduced the plant growth as reflected by a decrease in the plant height, leaf number, dry matter accumulation and seed yield. Application of supplemental calcium and potassium resulted in partially restoring the adverse effects of high salinity (not more than 30 m*M*) on plant growth, whilst simultaneous application of both of them imposed a negative effect on growth parameters. In many traits, additional calcium showed higher ameliorative effects than potassium on plant growth characteristics. Potassium could control the sodium accumulation at highest level of salinity, but Ca and combination of potassium and calcium could not reduce Na accumulation in leaves of lentil. By adding any type of cation to the growth medium, the leaf total cation concentration increased significantly but the average cation concentrations in different levels of NaCl and application of Ca or K or their combination were not significantly different. Based on our results, salinity higher than 30 m*M* is not recommendable for production of lentil even by application of Ca^{2+} and K^+.

Introduction

Many crop species suffer a decline in growth while exposed to salinity stress. Many studies denote the interaction between NaCl and the elevated Ca^{2+} and K^+ concentrations on plants (Reid & Smith, 2000; Naz *et al.*, 2010a; 2010b). Calcium has many roles in plants and is required in different levels, depending on the process involved from micro amounts in regulating cytosolic metabolism to macro amounts in cell wall structure, and moreover, its interaction with other elements under NaCl stress is proven (Ashraf *et al.,* 2008). Potassium is an essential activator for some enzymes in cytosol, and Na^+ can rarely substitute for these biochemical functions. It is known that Ca^{2+} sustains K^+ transport and K^+/Na^+ selectivity at the plasma membrane in Na^+- challenged plants (Shabala *et al*., 2006; Zahoor *et al*., 2012).

Lentils are excellent source of protein and also rich in important vitamins, minerals, soluble and insoluble dietary fiber. Lentil (*Lens culinaris* Medik.) is one of the traditional pulses of arid and semi-arid regions which is cultivated mainly under rain-fed conditions (Aniat-ul-Haq *et al.*, 2010). Although, lentil is sensitive to some environmental stresses specially salinity, but it is mainly cultivated in arid and semiarid regions of Iran which is considered as relatively tolerant to drought (Kafi *et al.,* 2005). Kafi *et al.,* (2005) examined germination characteristics of 12 Iranian lentil genotypes under five levels of negative water potential (0, -4, -8, -12, -16 bar) and reported that lower water potential significantly reduced germination percentage, germination rate, radicle and plumule length and dry weight. Lentil has high water-use efficiency, nearly 2 kg m^{-3} under normal conditions, that is much higher than other legumes such as broad bean and soybean. This crop, however, is much more salt sensitive and produces reliable yield only in non-saline soils. At EC_e of 2 dS/m, the yield reduction is about 20% and at EC_e of 3 dS/m it is 90–100% (Katerji *et al.,* 2001).

Turan *et al*., (2007) reported that adding NaCl resulted in decrease of the dry weight, stomatal conductance, proline concentrations, K^+ content, total chlorophyll concentrations of lentil. Tewari & Singh (1991) found a continuous decrease in chlorophyll *a* and *b* content, nitrate and nitrite reductase enzymes activities and DNA and RNA content of lentil with increasing exchangeable sodium percentage. Sidari *et al.,* (2008) have identified two lentil cultivars, Ustica and Pantelleria, that could be utilized not only in breeding programs to improve the salinity resistance of lentil, but also to be cultivated in environments where soil salinity is a frequent constraint. Ashraf & Waheed (1990) reported that improvement in NaCl tolerance in this species is possible, due to the existence of the great variability of tolerance to NaCl among lentil varieties.

Considering the fact that no published research have got through studying the interaction of Ca^{2+} and K^+ with salinity stress on lentil plant, this study has been carried out to understand the interactive effects of these two macro nutrients with salt stress on lentil, a crop planted mostly in arid saline soils.

Materials and Methods

Plant growth and treatments: The seeds of Robbat cultivar of lentil (*Lens culinaris* Medik) were grown in sand filled pots with 10 cm diameter and 30 cm depth at the Research Greenhouse of Ferdowsi University of Mashhad, Iran in winter and spring of 2011. Seeds were directly planted in pots and were irrigated using a circulated system in which the nutrient solution (Hoagland nutrient solution (Hoagland & Arnon, 1950) as well as the salt, calcium and potassium treatments for any particular treatment (standing for 3 replications of each treatment) were recycled to the pots. The nutrient solutions were renewed every other week to maintain the nutrients and salts in proper level. The plants were supplied by natural illumination, average maximum and minimum temperature of 28 and 22°C, respectively and average relative humidity of 60±10%.

Salt as well as calcium and potassium treatments started at the third leaf stage. Sodium chloride was added in 4 concentrations of 0, 15, 30 and 45 m*M* to the nutrient solution. To avoid osmotic shock of salinity, saline treatment was imposed incrementally by increasing the concentration of 15 m*M* every other day until the final concentration reached. Calcium treatment in two levels (0 and 10 m*M* $CaCl_2$) and potassium treatment in two levels (0 and 10 m*M* KCl) were also imposed along with the NaCl treatment.

Measurement of plant growth: Average height and number of expanded leaves monitored weekly in two plants in each treatment just after salinity application up to ripening. After measuring the fresh weight of shoots, they were separated into leaf blades, stems and inflorescences precisely and number of pod per plant, number of kernel per pod, number of branches per plant were counted and the samples were oven dried at 75°C for 48 hours, and then vegetative and reproductive organs were weighed. Plants were harvested at physiological ripening (91 days after planting (DAP)) and all analytical measurements including dry weight of leaves, stems, potassium, sodium and calcium were conducted at this stage. The concentrations of K, Na and Ca in the leaves were measured using the flame photometry method.

Statistical analysis: The experiment was designed and analyzed as factorial based on a completely randomized design with salinity treatment (in 4 levels), supplemental calcium (in 2 levels), and supplemental potassium (in 2 levels), with 3 replications. Statistical significance, where indicated, is at 5% level as determined by analysis of variance and Fisher's least significant difference (LSD) test.

Results

Morphological traits: There was no significant difference in main stem height of all treatments up to 36 DAP, but afterward, salinity significantly reduced the plant height as shown in Fig. 1, but at 91 DAP salinized plants showed statistically the same height of control plants. This reduction in height was also observed in supplemental Ca^{2+} and K^{+} at 45 m*M* NaCl (Fig. 1). Application of additional Ca^{2+} or K^{+} in combination caused a decrease in lentil height (Fig. 1). Therefore, application of 10 m*M* $CaCl_2$ as well as 10 m*M* KCl to the root medium not only did not ameliorate salinity effects on lentil height, but also caused a reduction in plant height, especially at high NaCl stress.

There was no significant difference in plant leaf number of all treatments up to 49 DAP, but after then, it was significantly decreased by NaCl , while elevated calcium promoted total leaf number, particularly at high levels of NaCl (Fig. 2). Leaf production in all treatments were sharply increased from 49 to 63 DAP so that, more than 50% of leaves were emerged during this two weeks interval. Application of Ca^{2+} and $K^{+,}$ solely or together, did not make up the adverse effects of salinity, in comparison to control (Fig. 2). Supplemental Ca^{2+} led to delay in cessation of leaf production but K^{+} did not impose the same effect (Fig. 2).

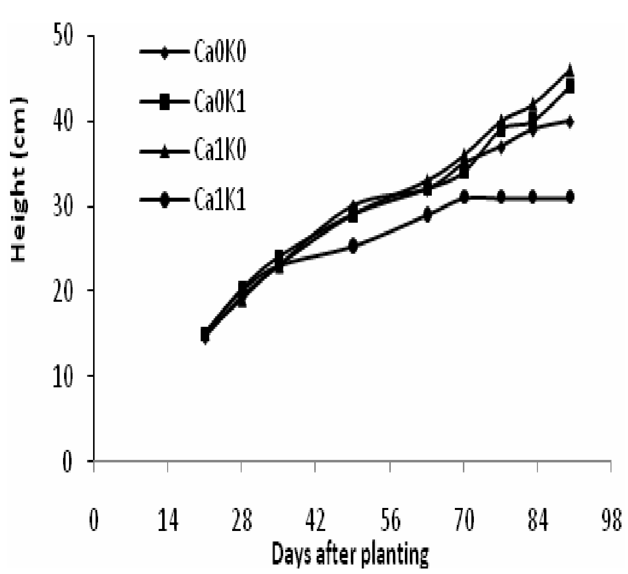

Fig. 1. Interactive effects of salinity, potassium and calcium on height of lentil in different sampling dates from 21 to 91 days after planting. Ca_0K_0 refers to no $CaCl_2$ and no KCl, Ca_0K_1 is refers to no $CaCl_2$ and 10 m*M* KCl, Ca_1K_0 refers to 10 m*M* $CaCl_2$ and no KCl and Ca_1K_1 refers to 10 m*M* $CaCl_2$ and 10 m*M* KCl application.

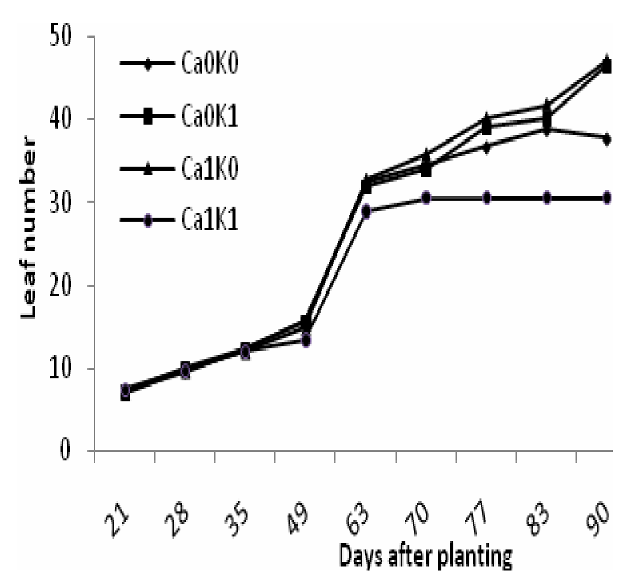

Fig. 2. Interactive effects of salinity, potassium and calcium on leaf number of lentil in different sampling dates from 21 to 91 days after planting. Ca_0K_0 refers to no $CaCl_2$ and no KCl, Ca_0K_1 is refers to no $CaCl_2$ and 10 m*M* KCl, Ca_1K_0 refers to 10 m*M* $CaCl_2$ and no KCl and Ca_1K_1 refers to 10 m*M* $CaCl_2$ and 10 m*M* KCl application.

Plant growth: Exposure of lentil plants to NaCl stress significantly reduced growth and accumulation of dry matter. Average total dry weight declined from 1.97 g plant^{-1} in control to 0.99 g plant^{-1} i 45 m*M* NaCl. Application of 10 m*M* $CaCl_2$ caused an increase in total dry weight from 1.42 g plant^{-1} to 3.56 g plant^{-1}, and application of 10 m*M* KCl increased average dry weight from 1.42 to 2.62 g plant^{-1} (Table 1). In contrast, combination of $CaCl_2$ and KCl caused a significant decrease in dry matter accumulation (from 1.42 g plant^{-1} in no Ca and K to 1.26 g plant^{-1} in combination of $CaCl_2$ and KCl application). Leaf dry weight as well as vegetative and reproductive dry weight showed the same trend of total dry weight (Table 1). Application of Ca^{2+} or K^+ ameliorated partially the adverse effects of high levels of salinity on leaf, stem and reproductive dry weight (Table 1).

Table 1. Interactive effects of salt stress, calcium and potassium on leaf, stem, reproductive and total dry weight of lentil.

	Leaf dry weight (g plant^{-1})				**Stem dry weight (g plant^{-1})**			
$CaCl_2$ (m*M*)	**0**	**0**	**10**	**10**	**0**	**0**	**10**	**10**
KCl (m*M*)	**0**	**10**	**0**	**10**	**0**	**10**	**0**	**10**
NaCl (m*M*)								
0	0.80	0.78	0.84	0.24	0.37	1.71	1.20	0.32
15	0.63	0.90	1.85	0.73	0.23	0.30	2.30	0.76
30	0.21	0.36	0.53	0.27	0.31	0.34	0.28	0.52
45	0.29	0.60	0.36	0.21	0.23	0.88	0.84	0.29
	LSD=0.22				LSD=0.15			
Average	0.48	0.66	0.90	0.36	0.29	0.81	1.15	0.48
NaCl (m*M*)	**Total dry weight (g plant^{-1})**				**Reproductive dry weight (g plant^{-1})**			
0	1.97	4.28	4.41	1.00	0.80	1.79	2.37	0.48
15	1.50	3.31	6.81	1.81	0.94	2.11	2.66	0.56
30	1.23	0.88	1.62	1.31	0.41	0.13	0.81	0.62
45	0.99	2.16	1.40	0.91	0.47	0.53	0.20	0.03
	LSD=1.59				LSD=0.63			
Average	1.42	2.62	3.56	1.26	0.66	1.14	1.51	0.42

LSD = Least significant differences at 5%

Pod number per plant, as one of the main yield components, was significantly affected by adding NaCl higher than 15 m*M*. This parameter reduced from 15 in control to 2 at 45 m*M* NaCl (Table 2). Adding Ca^{2+} and K^+ significantly increased pod number with or without NaCl application. Application of 10 m*M* KCl or $CaCl_2$ increased pod number from 15 in control plants to 26 and 30, respectively, but at salinity higher than 30 m*M* NaCl, adding these salts did not result any positive effect on lentil pod number (Table 2). Combination of Ca^{2+} plus K^+ caused a significant reduction in pod number even in absent of NaCl (Table 2). Calcium always showed a better performance than potassium and both of them better than NaCl application in pod number of lentil in this experiment, e.g. from average of 9.42 in NaCl application to 16.75 in Ca^{2+} and to 14.50 in K^+ application, respectively (Table 2).

Seed number per plant was also affected by NaCl stress and decreased from 12.33 in salt free to 6.66 in 45 m*M* NaCl. Combination of Ca^{2+} or K^+ mostly reduced this parameter in all sodium chloride levels. Average seed number per plant also followed the same pattern as pod number per plant followed (Table 2).

Harvest index (HI) did not show a clear trend in all treatments but adding NaCl to the growth medium caused an increase in HI, from 0.32 in control to 0.37 in 45 m*M* NaCl, but these results were not obtained for $CaCl_2$ and KCl treatments and both of them caused a decrease in HI compared to control and NaCl treatments (Table 2).

Seed yield, as the main economic parameter, also decreased to less than 50% at 45 m*M* NaCl salinity compared to control. On the other side, both Ca and K caused a higher seed yield in the presence of 15 *mM* salinity as well as control. Seed yield increased from 0.70 in control to 1.46 g plant^{-1} in K application and to 1.28 g plant^{-1} in Ca application. At higher levels of NaCl, K and Ca could not ameliorate negative effects of NaCl on seed yield and even in high salt concentration application of Ca and K salts magnified the negative effects of NaCl. Combination of $CaCl_2$ and KCl caused a significant decrease in seed yield (from 0.70 g plant^{-1} in Ca and K free to 0.46 g plant^{-1} in no NaCl application and from 0.33 in highest level of NaCl to almost Zero in Ca and K combined application) (Table 2).

Table 2. Interactive effects of salt stress, calcium and potassium on pod number, seed number per plant, harvest index and seed yield of lentil.

	Pod no.plant^{-1}				Seed no.plant^{-1}			
$CaCl_2$ (m*M*)	**0**	**0**	**10**	**10**	**0**	**0**	**10**	**10**
KCl (m*M*)	**0**	**10**	**0**	**10**	**0**	**10**	**0**	**10**
NaCl (m*M*)								
0	15.00	26.00	30.00	9.00	12.33	22.33	29.66	8.00
15	12.67	25.33	26.33	12.33	12.00	26.00	23.33	10.00
30	8.00	3.00	9.66	11.33	8.33	3.00	14.66	11.33
45	2.00	3.66	1.00	0.50	6.66	2.33	0.00	0.50
	LSD= 4.66				LSD= 3.33			
Average	9.42	14.50	16.75	8.29	9.83	13.42	16.91	7.46
NaCl (m*M*)	**Harvest index**				**Seed yield (gplant^{-1})**			
0	0.32	0.33	0.42	0.35	0.70	1.27	1.69	0.46
15	0.38	0.48	0.31	0.15	0.59	1.46	1.28	0.55
30	0.36	0.12	0.40	0.32	0.51	0.16	0.78	0.60
45	0.37	0.20	0.11	0.36	0.33	0.12	0.00	0.01
	LSD= 0.06				LSD= 0.23			
Average	0.36	0.28	0.31	0.30	0.53	0.75	0.94	0.41

LSD = Least significant differences at 5%

Cation accumulation: Leaf sodium content in control plants was 0.65% of dry matter and increased by increasing NaCl concentration in the growth medium to 2.08% of dry matter. In control conditions, K and Ca application did not affect sodium content but at highest level of salinity potassium could ameliorate sodium accumulation, but calcium and combination of potassium and calcium treatments could not reduce Na accumulation in leaves of lentil (Table 3).

Table 3. Interactive effects of salt stress, calcium and potassium on leaf sodium, potassium, calcium and total cations concentration based on percentage of dry matter of lentil.

	Sodium (%)				Potassium (%)			
$CaCl_2$ (m*M*)	**0**	**0**	**10**	**10**	**0**	**0**	**10**	**10**
KCl (m*M*)	**0**	**10**	**0**	**10**	**0**	**10**	**0**	**10**
NaCl (m*M*)								
0	0.65	0.66	0.58	0.60	1.15	1.44	0.59	1.88
15	1.00	1.46	0.69	1.26	1.64	1.95	1.22	1.67
30	1.31	1.53	1.91	1.16	1.46	2.05	1.12	1.51
45	2.08	1.37	2.35	1.29	2.16	1.51	0.93	0.71
	LSD=0.38				LSD=0.38			
Average	1.26	1.26	1.38	1.07	1.60	1.74	0.97	1.44
NaCl (m*M*)	**Calcium (%)**				**Total cations**			
0	1.47	1.62	1.25	2.23	3.27	3.72	2.42	4.71
15	1.56	1.35	2.44	2.41	4.20	4.76	4.35	5.34
30	1.43	1.71	2.21	2.48	4.20	5.29	5.24	5.15
45	1.46	1.71	2.39	2.31	5.70	4.59	5.67	4.31
	LSD=0.41				LSD=0.99			
Average	1.48	1.60	2.07	2.36	4.34	4.60	4.42	4.87

LSD = Least significant differences at 5%

Leaf potassium content in control plants was 1.15% of dry matter and increased by increasing NaCl concentration in the growth medium to 2.16% of dry matter. In control conditions, application of 15 m*M* KCl increased K content from 1.15 to 1.44% but it reduced to 0.59% by addition of 10 m*M* $CaCl_2$. Combination of potassium and calcium reduced K accumulation in leaves of lentil (Table 3).

Leaf Ca content in control plants was 1.47% of dry matter and did not change significantly by increasing NaCl concentration in the growth medium. In control conditions K and Ca application did not affect Ca content but at highest level of salinity application of Ca or K and their combination increased Ca accumulation in leaves of lentil e.g., from 1.47% in control to 2.39 and 2.31% in Ca and combination of Ca and K application (Table 3).

Total cations in control plants was 3.27% of dry matter and did not change significantly, by increasing K or Ca in the growth medium. By adding any type of cation to the growth medium, the total cation concentration increased significantly. The average cation concentration in different levels of NaCl and application of Ca, K or their combination was not significantly different (Table 3).

Discussion

Grain legumes generally are considered as salt sensitive crops (Katerji *et al*., 2001). The salinity effect on bacterial activity with respect to nitrogen fixation might explain their salt sensitivity. Lentil under saline conditions systematically shows lower values of nitrogen fixation during the whole growing season (Katerji *et al*., 2001).

Lentil is a short legume and its maximum height was less than 35 cm in this experiment. NaCl salinity did not impose a significant effect on plant height up to 77 DAP but the differences were significant at last two weeks. Application of 10 m*M* $CaCl_2$ as well as 10 *mM* KCl to the root medium not only did not ameliorate salinity effects on lentil height, but also caused a reduction in plant height. Both Ca^{2+} and K^+ increased the stem weight of plant in control as well as 45 m*M* NaCl, therefore the number of branches and probably stem diameter are the main cause of higher stem weight. It seems that main stem and branches in lentil are not strong sinks and its short life span and interference of reproductive with vegetative growth of this plant, caused a lower dry matter allocation to stems. Combination of Ca^{2+} and K^+ in control and in the presence of salinity may further reduce osmotic potential of the root medium. The other possible reason may be addition of more chloride to the medium and imposition of its negative effects on height and dry matter accumulation. Badeoglu *et al.,* (2004) reported that salt stress caused a significant decrease in length and wet-dry weight of both shoot and root tissues of lentil.

Leaf number remained unchanged by the application of NaCl but application of 10 m*M* $CaCl_2$ as well as 10 m*M* KCl and their combination to the root medium reduced leaf number per plant after 63 DAP. The main cause of leaf number reduction in the last three weeks of lentil might be due to accumulation of more cations, mainly Na^+, or anions particularly Cl in the leaves that need energy for plant to manage them. Elevated Ca^{2+} concentration in the root medium result higher leaf Ca^{2+} concentration, and higher growth speed, especially in young leaves of sunflower (Mutlu & Bozcuk, 2007).

Dry matter production of lentil plant was strongly reduced by NaCl salinity higher than 15 m*M*. Reduction in dry weight reflects the increased metabolic energy cost and reduced carbon gain due to salinity (Ashraf, 2004). Our findings showed that K^+ and Ca^{2+} application not only stimulated the negative effects of salinity on growth, but also increased dry matter accumulation particularly at control and low NaCl stress. This is in agreement with the data of some authors who have reported ameliorative effect of K^+ on salinity tolerance (Shirazi *et al*., 2005; Mutlu & Bozcuk, 2007). In plants, K^+ plays an essential role as an osmoticum and charge carrier (Ashraf *et al*., 2008).

The ameliorative effect of Ca^{2+} was always better than K+, in this experiment. The protective effect of Ca^{2+} in salinized plants is probably due to its role in maintaining membrane integrity, because one of the primary effects of salinity is a disruption of membrane integrity caused by displacement of Ca^{2+} from the cell surface by Na^+ (Cramer *et al*., 1990; Ashraf, 2004; Ahmad *et al*., 2007; Ahmad *et al*., 2009). Interactive effects of Ca^{2+} and K^+ caused lower vegetative and reproductive dry weight, denoting higher investment in management of increasing cations and anions for better performance under saline conditions (Ahmad *et al*., 2006; Ashraf *et al*., 2008; Ahmad *et al*., 2010). In an experiment on sorghum, Shariat Jafari *et al*., (2009) also found that hat K^+ application not only stimulated the negative effects of salinity on growth, but also reduced dry matter accumulation particularly at low and medium stress, whilst, application of supplemental calcium resulted in partially restoring the adverse effects of high salinity on sorghum growth.

The positive effects of supplemental Ca^{2+} and K^+ on grain yield of lentil might be due to lowering exchangeable sodium percentage (ESP) of the growth medium, because as much as the rate of other cations in the gowth medium increases the rate of sodium decrease. Singh *et al.,* (1993) also pointed out that increasing level of exchangeable sodium percentage decreased the plant height, leaf area, leaf dry weight, total biomass production and finally the grain yield of lentil genotypes.

In general, according to our findings, lentil plant is salt-sensitive and irrigation water should contain less than 30 m*M* for growing lentil with minimum yield loss. It is also seems that Ca^{2+} and K^+ content of Hoagland nutrient solution is not on optimum levels for lentil growth, therefore additional Ca^{2+} and K^+ even in non saline conditions caused an increase in seed yield of lentil. Addition of supplemental Ca^{2+} and K^+ to the slightly salinized (less than 30 m*M*) growth medium of lentil could improve productivity of this plant in the presence of salinity.

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