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THE EFFECTS OF HIGH SALINITY (NaCl) AND SUPPLEMENTARY PHOSPHORUS AND POTASSIUM ON PHYSIOLOGY AND NUTRITION DEVELOPMENT OF SPINACH

Cengiz Kaya[1]*, David Higgs[2] and Halil Kirnak[3]

[1]University of Harran, Agriculture Faculty, Horticulture Department, 63200 Sanliurfa-Turkey
[2]University of Hertfordshire, Environmental Sciences, College Lane, Hatfield, Herts AL10 9AB, UK
[3]University of Harran, Agriculture Faculty, Irrigation Department, 63200 Sanliurfa-Turkey

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Summary. An outdoor pot experiment was carried out in sand culture to investigate the response of spinach (*Spinacia oleracea*) cv. "Matador" grown at high salinity to supplementary phosphorus and potassium. Plants were tested during a period from germination to vegetative growth stage. Treatments initiated for seedling and more matured vegetative growth stages were (1) complete nutrient solution alone (C), (2) C+supplementary 5 mM KH_2PO_4 supplied via leaves (C+FoKP), (3) C+60 mM NaCl (C+S) and (4) C+S+supplementary 5 mM KH_2PO_4 supplied via leaves (C+S+FoKP). Seedling growth, vegetative growth, relative water content (RWC) chlorophyll concentration and water use of spinach were reduced significantly by high salinity. The C+S+FoKP treatment resulted in increases in fresh weight, RWC, water use and chlorophyll concentrations. Membrane permeability was impaired in the plants grown at high salinity. Foliar application of 5 mM KH_2PO_4 solution maintained membrane permeability by decreasing electrolyte leakage from leaves of plants grown at high salinity. High (60 mmol.L^{-1}) NaCl in nutrient solution resulted in plants with very leaky root systems as measured by high K efflux; this leakiness was ameliorated by foliar application of 5 mM KH_2PO_4. Cumulative potassium release from intact roots was higher in plants at high salinity. These data clearly show that NaCl status affects root mem-

* Corresponding author, e-mail: c_kaya70@yahoo.com

brane integrity. Sodium (Na) concentration in plant tissues increased for both species, especially in lettuce, in the elevated NaCl level. High salinity lowered the concentrations of P and K in leaves, but supplementary potassium (K) and phosphorus (P) enhanced concentrations of these two elements in the leaves. The results suggest that supplementary P and K can reduce the adverse effects of high salinity on plant growth and physiological development.

Key Words: Salinity, Spinach, Lettuce

Introduction

Salinity is an environmental stress that limits growth and development in plants. The response of plants to excess NaCl is complex and involves changes in their morphology, physiology and metabolism (Hilal et al., 1998).

Translocation of salt into roots and to shoots is a outcome of the transpirational flux required to maintain the water status of the plant and unregulated transpiration may cause toxic levels of ion accumulation in the shoot (Yeo, 1998). The supply of mineral ions to the leaf growing region may decline. Lower transpiration rate, coupled with reduced ion uptake by the roots, or reduced xylem loading, may cause poor supply via the xylem. So it is possible that an inadequate supply of ions to the expanding region may restrict cell division and/or expansion when plants are grown at high levels of NaCl (Berstein et al., 1995). In expanding leaves, salinity has disturbed concentrations of K (Jeschke and Wolf, 1985) and P (Martinez and Lauchli, 1991). Xylem concentration of K declined to about half control values in plants grown at high salt (Wolf et al., 1990). An immediate response to salinity is stomatal closure. However, due to water potential difference between the atmosphere and leaf cells, and the need for carbon fixation, this is an untenable long-term strategy of tolerance (Hasegawa and Bressan, 2000). One way of controlling salt flux to the shoot is the entry of ions into xylem stream (Flowers and Yeo, 1992). The large quantities of ions in mature or older leaves accumulated under salt stress (Munns, 1993). Older leaves may restrict ion deposition in meristematic and actively growing cells and meristematic cells are less exposed to ions delivered through the transpiration stream and their small vacuolar space is not conducive to ion storage (Wyn Jones, 1981).

The responses of plants to high salinity may be expected to vary with different growth stages. This has been shown by Chartzoulakis and Klapaki (2000) in pepper; Chartzoulakis and Loupassaki (1997) in eggplant; Dumbroff and Copper (1974) in tomato. Young seedlings and plants at the flowering stage seem to be more sensitive than mature stages (Lutts et al., 1995).

One of the major factors inducing leaf senescence is the decrease of chlorophyll content under saline conditions (Chen et al., 1991). Leaf senescence is also correlated with increased membrane permeability at high salt concentration (Dhindsa et al.,

1981). It was reported that excess NaCl in the growth medium induces structural changes in bean roots, as well as leakage of ions correlated with alterations of the cell membranes (Cachorro et al., 1995).

Salt tolerance of plants can be grouped in three categories: (1) exclusion of salt followed by transport and compartmentation of salt, (2) morphological features and biomass distribution of plant shoots and roots and (3) physiological and metabolic events that counteract the presence of salt at cellular level (Winicov, 1998). One of the most effective ways to overcome salinity problems is the introduction of salt tolerance to crops. However, breeding for tolerance to salinity in crops has usually been limited by lack of reliable traits for selection (Noble and Rogers, 1992 and Shannon, 1985). Investigations on tolerance to saline environments frequently point to restricted ion accumulation and organic solutes synthesis as major adaptations leading to salt resistance in glycophytes (Greenway and Munns, 1980). Moreover, there are multiple genes that seem to act in concert to increase salinity tolerance and certain proteins involved in salinity stress protection have also been recognised (Bohnert and Jensen, 1996). Other workers have linked NaCl stress with macro-nutrient deficiencies, for example high NaCl concentration has been shown to induce phosphorus and potassium deficiencies in tomato (Adams, 1988, 1991) and in cucumber (Sonneveld and Kreij, 1999).

An alternative strategy for coping with salinity could, therefore, be to attempt to supplement P and/or K where the growth medium is known to be or may become saline at some time during the crop growth cycle. In this paper we report an experiment with spinach. The experiment was conducted to investigate the effects of root zone salinity on P and K nutrition and other parameters and also to study the effects of supplementary potassium and phosphorus on salt stressed spinach during growth period.

Material And Methods

Plant culture and treatments

A pot experiment was conducted with spinach (*Spinacia oleracea*) cv. ‘Matador’ at the research field station of the Agriculture Faculty of University of Harran . To assess seedling growth, 10 seedlings per treatment replicate (i.e. 40 seedlings per treatment) were planted in a complete randomised block design of spinach at 10 cm depth in small plastic pots filled with fine sand. The pots were placed out-doors at average daily temperatures of 10°C (minimum) and 20 °C (maximum) and in an ambient sunlight for 3 weeks. During this time, supplementary 5 mM KH_2PO_4 was sprayed as a foliar spray to the whole shoot of seedlings grown at high (60 mM) salinity twice a week one week after planting. The volume of spray solution was 5 and 10 ml in first

and second week for each time. Therefore in total, 30 ml of 5 mM KH_2PO_4 was sprayed to each seedling. Treatments initiated for seedling and more matured vegetative growth stages were (1) complete nutrient solution alone (C), (2) C+supplementary 5 mM KH_2PO_4 supplied via leaves (C+FoKP), (3) C+60 mM NaCl (C+S) and (4) C+S+supplementary 5 mM KH_2PO_4 supplied via leaves (C+S+FoKP).

Twenty one days after planting, seedling growth was assessed. Four plants per replicate were harvested for chemical analysis and fresh weight determination and 1 plants per replicate for the determination of chlorophyll and membrane permeability and 1 plants for determination of cumulative K release.

The remaining 4 plants per treatment replicate were transplanted into containers containing 5 kg of washed sand (one plant per container) to assess vegetative growth. Pots were covered with black plastic covers to minimise evaporation, to avoid surface contamination due to foliar spray and to prevent algal growth. Composition of nutrient solution was ($mg.L^{-1}$): 270 N, 31 P, 234 K, 200 Ca, 48 Mg, 2.8 Fe, 0.5 Mn, 0.5 B, 0.02 Cu, 0.05 Zn and 0.01 Mo.

Plants were grown on for a further 7 weeks in the above three treatments. The volume of nutrient solution applied to the root zone of plants ranged from 30ml to 200ml per container each day depending on plant size, temperature and solar radiation. Foliar KH_2PO_4 application was repeated one week before harvest. During this time, the total volume of spray solution applied was 330ml of 5mM KH_2PO_4 for each plant. Additional water ranging from 100–300 ml per pot per day was also applied to the root zone depending on plant growth and treatment. Excess water drained through holes in the container bases. The pH was adjusted each time to 5.5 with a minimum volume of 0.1mM KOH. Seventy days after planting (49 days after transplanting) harvesting to asses vegetative growth was initiated. Two plants per replicate were used for chlorophyll analysis and membrane permeability determinations and the remaining two plants were used for fresh weight determination and chemical analysis.

Leaf relative water content

Leaf relative water content (LRWC) was calculated based on the methods from Yamasaki and Dillenburg (1999). Leaves were always collected from mid section of plant in order to minimise age effects. Individual leaves were first removed from stem and then weighed to obtain fresh mass (FM). In order to determine the turgid mass (TM), leaves were floated in distilled water inside a closed petri dish. During the imbibition period, leaf samples were weighed periodically, after gently wiping the water from the leaf surface with tissue paper. At the end of the imbibition period, leaf samples were placed in a pre-heated oven at 80 °C for 48 h, in order to obtain dry mass (DM). All mass measurements were made using an analytical scale, with precision of 0.0001 g. Values of FM, TM and DM were used to calculate LRWC using the equation below:

$$\text{LRWC (\%)} = [(\text{FM}-\text{DM})/(\text{TM}-\text{DM})]\times 100$$

Determination of potassium release

Cumulative K release from the intact root systems was determined using the method from Parker et al. (1992) to monitor membrane integrity. Seedlings were removed intact from the containers and the whole root systems soaked in 4 mM $CaCl_2$ for 15 min. The seedlings were then transferred to 500 mL plastic containers containing aerated nutrient solution which contained 2.0 mM $CaCl_2$, 0.2 mM Na_2HPO_4 3 µM H_3PO_4 and 113 µM Na_3-DTPA at pH 5.5. Seedlings were grown on in this solution in the dark at 25 °C and exudates collected after 6, 12, 24 and 48 h. After each sampling nutrient solutions were replaced. Sample solutions were evaporated to dryness on a hot plate, redissolved in 25 mL of a 0.05 M HCl–0.22 M LiCl solution. Cumulative K released by the root systems was analysed by flame photometer (Corning 410, UK).

Chlorophyll determination

Two plants per replicate at each stage were used for chlorophyll determination. Fresh leaf samples taken from the youngest fully expanded leaf were extracted with 90% acetone and absorption values were obtained read using a UV/Vis Spectrophotometer (Bausch & Lomb, Belgium). Chlorophyll concentration was calculated using the formulae from Strain and Svec (1966).

Electrolyte leakage

Electrolyte leakage was used to assess membrane permeability according to Lutts et al. (1995). Electrolyte leakage was measured using an Electrical Conductivity Meter (EC). Leaf samples of two plant per replicate were taken and cut into 1 cm segments. The samples were then placed in individual stoppered vials containing 10 ml of distilled water after three washes with distilled water to remove surface contamination and incubated at room temperature (ca. 25 ºC) on a shaker (100 rpm) for 24 h. Electrical conductivity (EC) of bathing solution (EC1) was recorded after incubation. The same samples were then placed in an autoclave at 120 ºC for 20 min and second reading (EC2) was taken after cooling the solution to room temperature. The electrolyte leakage was calculated as EC1/EC2 and expressed as percentage.

Water use

Water use was calculated on a water balance approach since volumes of water applied to the root zone and drained from the pots were recorded. Water loss by evaporation was considered negligible and unlikely to differ between treatments. Since plant containers were covered in plastic. Amounts of water applied to each plant were calculated for three and seven weeks in seedling and vegetative growth stages respectively. A tensiometer was placed at a depth of 10 cm in the pots and irrigation was started

when the soil moisture tension was –10 kPa. Amount of water applied was based on full container capacity.

Chemical analysis

Six mature leaves per pot were sampled and pooled into one sample. The leaves were washed in detergent solution to remove any dust on leaf surfaces, soaked in 0.5 M HCl for 20 seconds, followed by three to four rinses with distilled water and dried at 70 °C for 48 h to constant weight. The dried leaves were ground to powder using a pestle and mortar and stored in polyethylene bottles.

For chemical analyses, ground samples were ashed at 550 °C for six hours. The white ash was taken up in 2 M hot HCl, filtered into a 50-ml volumetric flask and made up to 50 ml with distilled water. Na, K and P were determined in these sample solutions. P was analysed by a vanadate-molybdate method using a UV/visible spectrophotometer (Bausch & Lomb, Belgium) and Na and K in the sample solution were analysed using a flame photometer (Corning 410, UK) (Chapman and Pratt, 1982).

Data were analysed using a Statview ANOVA computer programme. Means were separated by LSD test ($P < 0.01$).

Results and discussion

Plant growth and visible symptoms

Fresh weight and chlorophyll content were used to assess seedling and vegetative growth at high salinity and to test the effect of supplementary KH_2PO_4 on plant growth. Both fresh weight and chlorophyll content were significantly reduced at 60 mM salinity at both growth stages (Table 1). The reduction was greater in vegetative growth stage than in seedling stage. These results are in agreement with Bar-Tal et al. (1991) for corn, Adams (1988) and Satti and Al-Yahyi (1995) for tomato, Kaya et al. (2001) in cucumber and pepper and Leidi and Saiz (1997) for cotton.

Visible symptoms of salinity on plants are stunting shoot and root growth, smaller leaves in size. Foliar sprays of KH_2PO_4 mitigated the detrimental effect of high salt; plants receiving this produced almost the same fresh weight and chlorophyll values as those for unstressed plants. These results are in full agreement with our previous work on cucumber and pepper (Kaya et al., 2001a).

Electrolyte Leakage

Electrolyte leakage was used to assess membrane permeability. Addition of 60 mM NaCl into nutrient solution induced significant increases in electrolyte leakage (Table 1). Similar results were obtained in rice by Lutts et al. (1996), in tomato by Kaya et al. (2001b) and in cucumber and pepper by Kaya et al. (2001a).

Table 1. Fresh weight (g.plant^{-1}), total chlorophyll (mg.kg^{-1}) and electrolyte leakage (%) of spinach grown at high salinity with or without supplementary P and K.

Treatments	Fresh Weight[1]		Total Chlorophyll[2]		Electrolyte Leakage[2]	
	Seedling growth	Vegetative growth	Seedling growth	Vegetative growth	Seedling growth	Vegetative growth
C	23 a[3]	165 a	1646 a	1749 a	8.6 b	9.8 b
C+S	13 b	116 c	1454 b	1525 b	20.3 a	35.6 a
C+S+PK	19 a	148 b	1579 a	1657 a	11.5 b	13.7 b

[1] Means of four replicates and each replicate includes four and two plants in seedling and vegetative stage respectively.
[2] Each replicate includes two plants in both stages
[3] Within each column, same letter indicates no significant difference between treatments ($P < 0.01$)
C: Plants receiving normal nutrient solution; S: 60 mM sodium chloride; PK: 5 mM KH_2PO_4 supplemented via leaves

Increases in membrane permeability at vegetative stage were greater than at seedling stage at high salinity and this also shows a strong link between time of exposure to high salinity and membrane permeability.

Foliar sprays of KH_2PO_4 resulted in a significant decrease in membrane permeability, and all cases electrolyte leakage values were restored to values comparable with unstressed plants (C). These results indicate that deleterious effects of salinity on membrane permeability can be largely mitigated by supplementary KH_2PO_4. This finding is in agreement with Kaya et al. (20001a and 2001b) who showed similar effects in cucumber, pepper and tomato.

Water Use and Relative Water Content

Plant water use was decreased by adding 60 mM NaCl into nutrient solution (Table 2). Sonneveld and Voogt (1990) have reported that water use in tomato was reduced with application of high NaCl. Bingham and Garber (1970) found that water uptake declined in corn with increasing NaCl concentration. Kaya et al. (2001b) in tomato and Kaya et al. (2001a) in cucumber and pepper have also reported that high salinity in root medium reduced water use. The data presented here show that differences in water use between stressed and unstressed plants were getting greater during the stress period; this indicates a strong link between the adverse effects of NaCl and time.

Foliar sprays of KH_2PO_4 resulted in increased values for daily water use. Values for water use of plants receiving additional KH_2PO_4 were very close to those for unstressed plants indicating that this treatment is restoring normal plant cell water relations, negating the effects of salinity.

Leaf relative water content was lower in plants grown at high salinity compared to cotrol treatment and this was increased by foliar KH_2PO_4 sprays (Table 2). This

Table 2. Water use (ml.plant^{-1}·day^{-1})[1] and relative water content (%) of spinach grown at high salinity with or without supplementary P and K.

Treatments	Water Use		Relative Water Content	
	Seedling growth	Vegetative growth	Seedling growth	Vegetative growth
C	109 a	224 a	95 a	91 a
C+S	89 b	155 c	87 b	78 b
C+S+PK	101 a	199 b	92 a	88 a

[1] Means of four replicates and each replicate includes six and four plants for seedling and vegetative growth stages respectively.
Within each column, same letter indicates no significant difference between treatments ($P < 0.01$)
C: Plants receiving normal nutrient solution; S: 60 mM sodium chloride; PK: 5 mM KH_2PO_4 supplemented via leaves

clearly shows that supplemetary KH_2PO_4 mitigated adverse effect of water stress in the plant due to high salinity and this helped relative water content increase.

K release from roots

Plants grown at high salinity had very leaky root systems as evidenced by high K efflux; this leakiness was ameliorated by foliar spray of KH_2PO_4 (Table 3). These results show that high NaCl in nutrient solution has a detrimental effect on root membrane integrity. Our data indicate that high salt causes damage to the root membranes as evidenced by high K efflux. Generally, the root permeability of plants was decreased significantly under salt stress. This could be an explanation for the reduction in water absorption rate and may contribute to a similar reduction in nutrient uptake under salinity condition (Pessarakli and Tucker, 1988).

Table 3. Cumulative potassium (µmol.g^{-1} root DM)[1] release from the roots of spinach grown at high salinity with or without supplementary P and K.

Treatments	Time (h)			
	6	12	24	48
C	15 b	21 b	27 b	39 b
C+S	36 a	68 a	126 a	198 a
C+S+PK	19 b	29 b	38 b	46 b

[1] Means of four replicates and each replicate includes two and four plants for seedling and vegetative growth stages respectively.
Within each column, same letter indicates no significant difference between treatments ($P < 0.01$)
C: Plants receiving normal nutrient solution; S: 60 mM sodium chloride; PK: 5 mM KH_2PO_4 supplemented via leaves

Mineral ion concentrations

As expected sodium (Na) concentration increased in leaves and roots of plants in the presence of NaCl in the nutrient solution (Table 4). Salt tolerance in glycophytes is associated with the ability to limit uptake and/or transport of saline ions from the root zone to shoot (Greenway and Munns, 1980). Our results suggest this occur in spinach. The accumulation of Na in roots provides a mechanism for spinach to cope with salinity in the rooting medium and/or may indicate the existence of an inhibition mechanism of Na transport to leaves. Sodium is absorbed from the medium while subsequent upward movement through shoot is restricted by reabsorbtion of the Na from the xylem stream, probably in exchange for K (Yeo et al., 1977) and in the bassal stem and root region (Jeschke, 1984).

Table 4. Sodium concentration (% DW)[1] of spinach grown at high salinity with or without supplementary P and K.

Treatments	Seedling growth		Vegetative growth	
	Leaves	Roots	Leaves	Roots
C	0.28 b[2]	0.46 c	0.37 c	0.49 c
C+S	0.64 a	0.94 a	0.79 a	1.67 a
C+S+PK	0.54 a	0.68 b	0.58 b	1.39 b

[1] Means of four replicates and each replicate includes four and two plants for seedling and vegetative growth stages respectively.
[2] Within each column, same letter indicates no significant difference between treatments ($P<0.01$)
C: Plants receiving normal nutrient solution; S: 60 mM sodium chloride; PK: 5 mM KH_2PO_4 supplemented via leaves

Sodium concentration was significantly higher in the foliar spray of KH_2PO_4 treatments compared to the control but not as high as the high salt treatment . The decrease in leaf Na may partially be explained by a “dilution effect” (i.e. increase in biomass production). These results are closely in agreement with the findings obtained by others, for example, Satti and Al-Yahyai (1995); Kaya et al. (2001b) for tomato, Kaya et al. (2001a) for cucumber and pepper and Asch et al. (1999) for rice.

Concentrations of both P and K decreased in in leaves of spinach in the presence of NaCl in the nutrient solution, but increased in the roots (Tables 5 and 6). Our results suggested that translocations of both P and K were restricted from roots to shoots and so P and K accumulated in roots in high salt treatment. It has previously been reported that leaf K concentration is lowered by increasing NaCl concentration in nutrient solution or in the soil e.g. in cucumber (Sonneveld and Kreij, 1999), in tomato (Adams, 1991) in maize and barley (Benes et al., 1996). Adams (1988 and 1991) also noted that leaf P concentration decreased in tomato plants with increasing NaCl concentration in nutrient solution.

Table 5. Potassium concentration (% Dw)[1] of spinach grown at high salinity with or without supplementary P and K.

Treatments	Seedling growth		Vegetative growth	
	Leaves	Roots	Leaves	Roots
C	4.3 a[2]	5.5 c	4.7 a	5.9 c
C+S	3.4 c	6.9 b	3.1 c	7.6 b
C+S+PK	3.8 b	7.4 a	4.2 b	8.5 a

[1] Means of four replicates and each replicate includes four and two plants for seedling and vegetative growth stages respectively.
[2] Within each column, same letter indicates no significant difference between treatments ($P<0.01$)
C: Plants receiving normal nutrient solution; S: 60 mM sodium chloride; PK: 5 mM KH_2PO_4 supplemented via leaves

Table 6. Phosphorus concentration (% Dw)[1] of spinach grown at high salinity with or without supplementary P and K.

Treatments	Seedling growth		Vegetative growth	
	Leaves	Roots	Leaves	Roots
C	0.39 a[2]	1.59 c	0.37 a	1.68 c
C+S	0.29 b	1.77 b	0.29 b	1.88 b
C+S+PK	0.36 a	2.16 a	0.36 a	2.36 a

[1] Means of four replicates and each replicate includes four and two plants for seedling and vegetative growth stages respectively.
[2] Within each column, same letter indicates no significant difference between treatments ($P<0.01$)
C: Plants receiving normal nutrient solution; S: 60 mM sodium chloride; PK: 5 mM KH_2PO_4 supplemented via leaves

Foliar spray of KH_2PO_4 corrected the deficiencies of both P and K within the plant. This finding is in agreement with Satti and Al-Yahyai (1995) who showed that additional P and K in nutrient solution corrected P and K deficiencies in tomato grown in a saline medium.

The marked accumulation of Na in roots of P/K supplemented plants (especially in the vegetative phase) may provide a mechanism whereby spinach copes with root zone salinity and/or may indicate the existence of an inhibition mechanism in root cells which limits Na transport to the leaves.

Conclusion

In the light of this experiment, it can be concluded that:

1) Plants grown at high salinity exhibited reduced fresh weight, chlorophyll

content, water use, relative water content, and increased electrolyte leakage and K release compared to control plants

2) High NaCl induced P and K deficiencies in the leaves.

3) Foliar spray of KH_2PO_4 alleviated the adverse effects of high salinity on plants and improved all parameters mentioned above.

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