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COUNTERACTING EFFECT OF FOLIAR APPLICATION OF MACRONUTRIENTS ON SPINACH BEET (*Beta vulgaris var. cycla*) GROWN UNDER NaCl-SALINITY STRESS

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ABSTRACT

A pot experiment with spinach beet (*Beta vulgaris var. cycla*) was conducted in the green house of Micronutrients Project, Plant Nutrition Department, National Research Centre, Egypt. The objective of the study was to investigate the positive effects of macronutrients foliar application on spinach beet grown under NaCl-salinity stress conditions. Plants were irrigated with three qualities of water (fresh water, 50mM NaCl and 100mM NaCl). Foliar spray of nutrients (10% N, 4% P_2O_5, 7% K_2O and 0.02% MgO) and foliar spray of water (control) were applied three times with 10 day intervals from the beginning of NaCl-salinity treatments. The results showed that NaCl-salinity treatments had a negative effect on spinach beet dry weights of both shoots and roots. Foliar application of nutrients could ameliorate the negative effects of NaCl-salinity in this connection. Root growth appears to be less affected by NaCl-salinity than shoot as shown by the marked increase in root/shoot ratio at the highest NaCl-salinity treatment (100mM). Macronutrient foliar application treatments showed a marked effect in increasing this ratio. Increasing NaCl-salinity reduced the content of shoot N, P, K, Ca and Na. Foliar application of macronutrients had a marked positive effect in increasing the uptake of these nutrients. The negative residual effects on soil properties in relation to NaCl-salinity were reduced. A specific protein fraction of 90 KDa was detected in the leaf extract of 100Mm NaCl-salinity plus macronutrient foliar application. Generally, it could be concluded that spraying with macronutrient solution could counteract the negative effect of NaCl-salinity on growth and nutrient contents of spinach beet.

***Key words**: foliar spray, macronutrients, nacl-salinity, spinach beet.*

1. INTRODUCTION

World resources of fresh water are exhausted through the increasing demand to satisfy the different needs of the increasing world population. Salts in soil and /or irrigation water are becoming very serious problems facing the production of food in many countries, especially, in arid and semi-arid regions of the Mediterranean basin. Brackish or even saline water is used for agriculture purposes (Hamdy *et al.*, 2002). The use of irrigation water with high soluble salts content greatly decreases growth, nutrients uptake, crop yield and crop quality (Salem, 1992, Elmorsy *et al.*, 1993 and Hepaksoy *et al.*, 1999) and has a negative effect on soil fertility as well, as a result of salt accumulation in the soil. There are differences in tolerance to salinity among plant species and even among cultivars within the same species. Hill and Koenig (1999) reported that crops are classified into four major groups concerning their response to water of different salinities; *i.e.* tolerant, moderately tolerant, moderately sensitive and sensitive. Spinach beet is an annual crop grown for its leaves that are used in the same way as spinach .It is a potential salt-removal crop, found in several countries; e.g. Spain and Turkey. This crop was found to be adapted up to 100-150 mM NaCl. Sodium concentration measured in its shoots reached 5.63% and it could remove 346 kg NaCl/ ha. Within a growth period of 76 days, the total growth period for this crop is 120 days (Salt Control Project ,2002). Attempts have been made to improve the salt tolerance of crops. One of them is using nutrient foliar feeding to counteract Na^+ and Cl^-effects to plants (El-Fouly and Salama, 1999, Schmidhalter *et al.*, 1999 and El-Fouly *et al.*,2002). Therefore, this work was designed to investigate the positive effects of macronutrients foliar application on spinach grown under NaCl-salinity stress conditions.

2. MATERIALS AND METHODS

A pot experiment was conducted in the greenhouse of the Micronutrients Project, National Research Centre. Black plastic pots of 13 cm internal diameter and 12 cm depth were used. Pots were filled with 2 kg sandy soil. Each pot received 0.28g calcium superphosphate (15.5% P_2O_5), 0.7g ammonium sulphate (20.6% N) and 0.2g potassium sulphate (48% K_2O) at sowing. Six seeds were sown in each pot on the 10^{th} of December 2002. Twenty days after sowing, the plants were thinned to 3 plants in each pot. Fresh water was used in irrigation from sowing date till thinning (20days of age). Thereafter, pots were irrigated with three qualities of water: fresh water, water containing 50 mM NaCl and 100mM NaCl. Six pots were used for each treatment, half of them were sprayed with a compound containing 10% N, 4% P_2O_5, 7% K_2O and 0.02% MgO at a rate of 5 ml/l, and the others were sprayed with fresh tap water. Foliar spray was repeated 3 times, at 10 day intervals from the beginning of NaCl-salinity stress.

Treatments were arranged as spilt plots in a randomized complete block design (RCBD) where macronutrient foliar applications occupied the main blocks and NaCl-salinity treatments were allocated at random in the sub-blocks (Snedecor and Cochran, 1967). The total volume of irrigation water from thinning till harvest was 1050 ml/pot. Plants of each pot were gently removed from the soil on the 5^{th} of March 2003. They were divided into roots and shoots, dried and weighed. Soil and shoots of each pot were prepared for analysis (Chapman and Pratt, 1978).

2.1. Chemical analysis

2.1.1. Plant

One gram taken from the ground shoots/pot was dry ashed in a muffle furnace at 500 C° for 6 hours, then extracted by 2N HCl (ADAS,1981). Total P was determined using Perkin-Elmer Lambda 2 spectrophotometer through molybdate-vanadate method (ADAS,1981). Total K, Na and Ca were determined using Dr. Lang-M8D flamephotometer. Total nitrogen was determined in the dried plant shoots based on Micro-Kjeldahl method (Ma and Zauzage, 1942) using Buechi-320 steam distillation unit. Macronutrients uptake

were calculated according to the equation:
mg/pot = nutrient concentration (%) X shoots dry weight X 10

2.1.2. Soil

Soil reaction (pH) and electrical conductivity (E.C.) were determined in 1: 2.5 soil /water suspension (Jackson, 1973). Sodium was determined after extraction using ammonium acetate at pH 7 (Jackson, 1973). Chloride was determined by silver nitrate titration method (Chapman and Pratt, 1978) .

2.1.3. Protein extraction

Plant leaves were excised and homogenized using a mortar and pestle (leaves/buffer ratio 1:4 w/v) extraction buffer at pH 7.2 containing 250 mM sucrose, 0.2 mM DTT, 2% polyvinyl poly-pyrolidine (w/v). The homogenate was filtered and centrifuged at 15000 r.p.m. for 30 min. The resulting supernatant was used for protein electrophoresis (Rothe, 1997).

2.1.4. Protein electrophoresis

Protein extract of leaf was identified by sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis ; i.e.SDS-PAGE, according to Laemmli (1970) using (w/v) 12% polyacrylamide and 0.1% SDS. Each sample (30-µg protein) was loaded onto the slots. The gel was stained with coomassie blue R 250, and destained with an aqueous acetic acid and methanol solution. Molecular weight marker ranging from 20 to 180 KDa was used to estimate the molecular weight of the purified protein.

2.1.5. Data recorded

- Dry weights of both shoots and roots/pot.
- Macronutrients uptake of shoot/pot.
- Residual effect of NaCl-salinity on soil.
- Protein electrophoresis.

Data obtained were subjected to the statistical analysis using a Costat 2.00 computer program, copyright Cohort Software (1986).

3.RESULTS AND DISCUSSION

3.1. Effect of macronutrients foliar spray and NaCl-saline water irrigation on plant growth and macronutrients uptake

3.1.1. Effect of NaCl-salinity

3.1.1.1. Plant dry weight

Increasing NaCl of irrigation water significantly reduced the dry biomass accumulation of spinach beet plants. Shoot dry weight decreased by about 22 and 54%, while root dry weight decreased by 21 and 42% due to watering plants with 50mM and 100mM NaCl, respectively, as compared with control treatment (Table 1).

3.1.1.2. Root/shoot ratio

Watering the plants with saline water increased the root/shoot ratio of spinach beet plants. However, only a significant increase in the ratio was found under the highest salinity level; *i.e.*, 100mM NaCl (Table1). This is due to the fact that shoot dry weight was more affected than root dry weight.

3.1.1.3. Macronutrients uptake

Increasing salinity in irrigation water resulted in marked decreases of nitrogen, phosphorus, potassium, calcium as well as sodium uptake by spinach beet plants (Table 2). The decrease in sodium uptake might be due to the decrease in dry weight rather than to the decrease in it's concentration.

3.1.2. Effect of macronutrients foliar application

3.1.2.1. Plant dry weight

Spraying plants with macronutrients had a marked positive effect on the dry weight of spinach beet plants. Foliar nutrient spray resulted in 24 and 34% increase in the dry weight of both shoot and root, respectively (Table 1).

3.1.2.2. Root/shoot ratio

Macronutrients foliar spray resulted in a significant increment in root/shoot ratio of about 5% (Table 1).

Table (1): Effect of macronutrients foliar application under NaCl-saline water irrigation on spinach beet plant growth.

Growth Characteristic	Shoot dry weight (g/pot/3 plants)			Root dry weight (g/pot/3 plants)			Root/shoot ratio		
Treatment	**NF**	**WF**	**Mean**	**NF**	**WF**	**Mean**	**NF**	**WF**	**Mean**
Control	4.79	4.01	4.40	1.77	1.39	1.58	0.37	0.34	0.36
50 mM NaCl	3.65	3.04	3.35	1.42	1.07	1.25	0.39	0.35	0.37
100 mM NaCl	2.38	1.70	2.04	1.07	0.74	0.91	0.45	0.44	0.45
Mean	3.61	2.91		1.42	1.07		0.41	0.38	
LSD 5%									
Salinity (S)	0.21			0.07			0.03		
Foliar spray (FS)	0.17			0.06			0.02		
(S) X (FS)	NS			NS			NS		

- NF = Macronurrients foliar application
- WF = Water foliar application

3.1.2.3. Macronutrients uptake

Spraying spinach beet plants with macronutrients solution resulted in raising the uptake of N, P, K, Ca as well as Na (Table 2).

3.1.3. The interaction between NaCl-salinity and macronutrients foliar application

3.1.3.1. Plant dry weight

No marked effect for the interaction between NaCl-treatments and macronutrients foliar application was noticed. Macronutrients foliar spray in combination with fresh water showed the highest dry weight values for both shoots and roots. On the other hand, the lowest values were obtained due to combination between the highest saline treatment (100mM NaCl) and water foliar spray (Table 1).

3.1.3.2. Root/shoot ratio

The interaction between NaCl-salinity and macronutrients foliar feeding showed a non-significant effect (Table 1).

3.1.3.3. Macronutrients uptake

The interaction effect between spraying spinach beet plants with macronutrients solution under irrigation with NaCl-salinized water showed marked effect only on calcium uptake. The highest uptake recorded was due to irrigation with fresh water in combination with spraying plants with macronutrients. However, the lowest uptake was due to the highest NaCl level with tapwater foliar application.

On the other hand, no marked interaction effect on nitrogen, phosphorus and potassium uptake by spinach beet plants was recorded (Table 2).

From the obtained results of this study, it could be concluded that watering spinach beet plants with saline water had a marked negative effect on growth. This negative effect may be due to the high concentrations of soluble salts in soil solution of the root zone. These concentrations of soluble salts through their high osmotic pressures affect negatively plant growth by restricting the water uptake by the roots (Sliman and Ghandorah, 1988) or through upsetting the ionic balance in plant tissues, where the accumulation of some essential cations required for normal growth e.g. Ca, K and Mg in plant were greatly reduced under saline conditions. Besides, plants grown under salinity stress revealed absorption and accumulation of higher amounts of other cations; e.g. Na and Cl. Such imbalance among essential and/ or other non-essential ions greatly disturbed the metabolic and physiological activities of the plants (Salem, 1992). It is also well known that increasing sodium chloride in the root zone decreases the photosynthesis process through reducing stomatal aperture which interferes with CO_2 diffusion (Awada *et al.*, 1995 and Ashraf *et al.*, 1998). Salinity can affect plant growth in an indirect way, where increasing ions such as Na^+ and Cl^- in root medium besides their toxic effect, are responsible for raising soil pH less resulting in availability of some nutrients; e.g. P, Fe, Zn and Mn for plant uptake. In spite of the negative effect of salinity on plant growth, root growth appears to be less affected than shoot growth. This conclusion is explained by increasing root/shoot ratio of salinity stressed plants. Cuartero and Fernandez-Munoz (1999) and Tozlu *et al.* (2002) confirmed such results. Salinity had a marked negative effect on plant nutrients uptake. The negative effect may be due to the shortage of water uptake where salts attract water (*i.e.* competing with plants for it) so, reducing plant's ability to extract water from soil. It is also found that increasing Na^+ reduces K^+ and Ca^{2+} uptake (Song and Fujiyama, 1996 and Grattan and Grieve, 1999) and increasing Cl^- reduces NO_3^- uptake (Al-Rawahy *et al.*, 1992).

It is also important to note that foliar spray of nutrients could increase root/shoot ratio and ameliorate the negative effect of salinity on the dry weight accumulation through promoting nutrients uptake by plant roots (Abdalla and Mobarak,1992 and El-Fouly *et al.*, 1997).

It is quite evident that foliar feeding showed a positive effect in increasing the ability of plant to take up nutrients. Oosterhuis (1998) and Soepardi (1998) reported that foliar feeding of a nutrient might promote root absorption of the same nutrient or other nutrients through increasing root growth.

3.2. Residual effects of saline water irrigation and macronutrients foliar spray on some soil properties related to salinity

3.2.1. Chloride

Results in Table (3) show that using NaCl-saline water in irrigation resulted in a marked increase in soil chloride concentration. Using saline water containing 50mM and 100 mM NaCl resulted in

Table (2): Effect of macronutrients foliar application under NaCl-saline water irrigation on shoot nutrients uptake (mg/pot/3 plant shoots).

Macronutrients	Nitrogen (N)			Phosphorus (P)			Potassium (K)			Calcium (Ca)			Sodium (Na)		
Treatment	NF	WF	Mean	NF	WF	Mean	NF	WF	Mean	NF	WF	Mean	NF	WF	Mean
Control	206.7	175.0	190.9	13.4	13.1	13.3	65.5	59.1	62.3	47.9	34.8	41.4	159.4	129.4	144.4
50 mM NaCl	129.0	88.0	108.5	10.1	9.6	9.9	50.5	44.9	47.7	29.2	28.3	28.8	146.0	135.0	140.5
100 mM NaCl	79.3	58.0	68.7	6.8	5.0	5.9	37.2	27.8	32.5	18.2	11.9	15.1	113.6	90.8	102.2
Mean	138.3	107.0		10.1	9.2		51.1	43.9		31.8	25.0		139.7	118.4	
LSD 5%															
Salinity (S)	14.2			0.71			4.4			4.0			7.4		
Foliar spray (FS)	11.6			0.58			3.6			3.2			6.1		
(S) X (FS)	NS			NS			NS			5.6			NS		

Table (3): Effect of macronutrients foliar application under NaCl-saline water irrigation on some soil properties.

Property	Cl^- (ppm)			Na^+ (mg/100g)			EC (dS/m)			pH		
Treatment	NF	WF	Mean	NF	WF	Mean	NF	WF	Mean	NF	WF	Mean
Control	202	155	178.5	11.82	12.23	12.03	0.47	0.61	0.54	8.61	8.59	8.60
50 mM NaCl l	350	397	373.5	33.82	48.46	41.14	0.97	1.28	1.13	8.88	8.93	8.91
100 mM NaCl	430	471	450.5	67.62	64.23	65.93	1.16	1.36	1.26	9.11	9.03	9.07
Mean	327.3	341.0		37.75	41.67		0.87	1.08		8.87	8.85	
LSD 5%												
Salinity (S)	22.2			1.60			0.09			0.10		
Foliar spray (FS)	NS			1.31			0.08			NS		
(S) X (FS)	31.4			2.27			NS			NS		

110 and 153%; respectively, increase in soil chloride concentration as compared with using fresh water in irrigation. On the other hand, macronutrients foliar spray showed no marked effect on soil chloride concentration. As for the interaction between nutrient foliar spray and NaCl, it is worthy to mention that 100mM NaCl in combination with fresh water foliar spray showed the highest soil chloride concentration. However, applying fresh water in combination with water foliar spray showed the lowest value.

3.2.2. Sodium concentration

Soil sodium concentration markedly increased by increasing NaCl-salinity in irrigation water. As compared with using fresh water, using 50 and 100 mM NaCl in irrigation water resulted in 242 and 449% increments in soil sodium concentration; respectively (Table 3). However, macronutrients foliar spray could decrease soil sodium concentration by about 9%. Macronutrients foliar spray under NaCl-salinity showed significant effect; where, 100 mM NaCl with macronutrients foliar spray gave the highest soil sodium concentration and the lowest value was obtained from using fresh water foliar spray.

3.2.3. Soil electrical conductivity (E.C.)

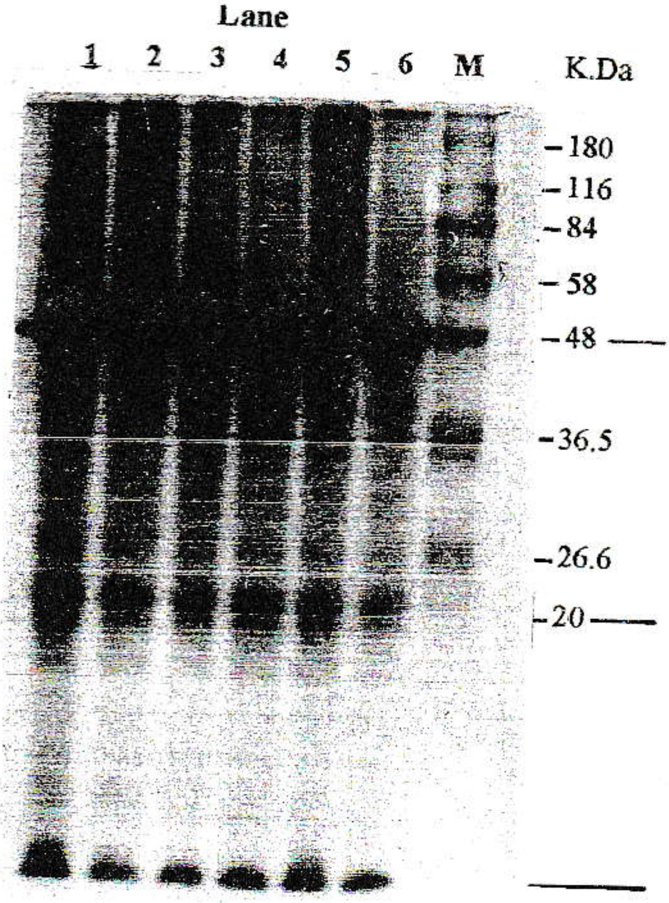
Soil electrical conductivity increased by increasing NaCl in irrigation water. However, macronutrients foliar spray showed reduction in soil electrical conductivity (Table 3). No marked effect due to the interaction between macronutrients foliar spray and NaCl-salinity was obtained.

3.2.4. Soil reaction (pH)

Irrigation with solution containing 100 mM NaCl showed marked increase in soil reaction as compared to the other two salinity treatments. Soil reaction did not significantly differ due to macronutrients foliar spray treatments. No marked effect due to the interaction between macronutrients foliar spray and salinity was calculated.

3.3. Protein pattern

In general, it could be seen in Fig. (1) that all treatments showed three major distinct bands with different molecular weights as



Lane 1 = Control + macronutrients foliar application
Lane 2 = Control + water foliar application
Lane 3 = 100 mM NaCl + macronutrients foliar application
Lane 4 = 100 mM NaCl + water foliar application
Lane 5 = 50 mM NaCl + macronutrients foliar application
Lane 6 = 50 mM NaCl + water foliar application
M = Marker

Fig. (1): Protein pattern of extracted soluble protein from spinach beet leaves induced under different NaCl stress conditions.

follows:

- Around 48 KDa (high molecular weight).
- Around 20 KDa (medium molecular weight).
- Less than 20 KDa (low molecular weight).

In addition, the number of protein bands and the density of each band showed much variation among treatments.

For example, looking at the first treatment (lane No. 1), when the plants were grown under control treatment in combination with nutrient foliar spray, four bands with molecular weights of around 116, 65, 26 and 18 KDa could be distinguished. The aforementioned bands are not present in lane 2 under control treatment combined with water foliar spray. It could be concluded that the appearance of such bands might be related to macronutrients spray. In the case of lane No. 3 (100mM NaCl plus nutrient foliar spray), one new band was exhibited with molecular weight of around 90 KDa, which was not evident in lane 4 (i.e.with 100 mM NaCl in combination with water foliar spray). Therefore, this new band supports the finding in lane 1, that nutrient foliar spray would create new protein bands, which might be correlated with increased plant resistance to salinity stress conditions. The banding pattern of the third treatment (i.e. 50 mM NaCl with the presence or absence of nutrient foliar application) was similar in the number of bands and also, in the relative mobility of each band except in lane 5 which showed high density in all bands. Gonzalez *et al*. (2001) found similar results on four bean cultivars.

Conclusion

From the aforementioned results, spinach beet showed halophytic behaviour of absorbing salts from salt-affected soils. It is recommended to use such crop coupled with macronutrients foliar application to naturally reduce remaining salts in the soils; especially, in the case of using saline water in irrigation.

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تأثير الرش الورقي بالعناصر الكبرى على نمو نبات بنجر السبانخ النامي تحت ظروف الإجهاد الملحي لكلوريد الصوديوم

محمد مصطفى الفولي- الزناتي عبد المطلب على أبو النور- عبد الوهاب عبد المقصود عبد المجيد

قسم تغذية النبات- المركز القومي للبحوث- الدقي- القاهرة

ملخص

أجريت تجربة أصص بصوبة مشروع العناصر المغذية الصغرى- بالمركز القومي للبحوث لدراسة تأثير الرش بالعناصر الكبرى على نبات بنجر السبانخ النامي تحت ظروف الإجهاد الملحي الناشئ عن استخدام كلوريد الصوديوم. رويت النباتات بثلاث أنواع من الماء (الماء العذب- ماء يحتوى على ٥٠ مللمول كلوريد الصوديوم- ماء يحتوى على ١٠٠ مللمول كلوريد الصوديوم). وتم رش النباتات بمركب يحتوى على ٠% نتروجين ، ٤% خامس أكسيد الفوسفور، ٧% أكسيد البوتاسيوم، ٠,٠٢% أكسيد الماغنسيوم وذلك بمعدل ٥ مللي لتر/لتر وكذلك رُشت النباتات بالماء العذب (للمقارنة). وأوضحت النتائج أنه:-

كان لمعاملات الملوحة تأثيرا سلبيا على النمو متمثلا في الوزن الجاف لكلا من المجموع الخضري والجذري. وكان لرش النباتات بالعناصر أثر إيجابي في تخفيف حدة هذه الآثار السلبية. كان نمو المجموع الجذري أقل تأثرا من المجموع الخضري حيث أوضحت النتائج ارتفاع نسبة المجموع الجذري للمجموع الخضري تحت ظروف الري بماء يحتوى على ١٠٠ مللمول كلوريد الصوديوم. وأدى الرش بالعناصر إلي زيادة هذه النسبة. أدى الري بالماء المالح إلي انخفاض محتوى المجموع الخضري للنبات من العناصر (ن ، فو ، بو ، كا ، ص)، وأدى الرش بالعناصر إلى زيادة محتوى المجموع الخضري من هذه العناصر. كان للري بالماء المالح أثرا سلبيا على خواص التربة الطبيعية المتعلقة بالملوحة. أدى الرش الورقي بالعناصر مع الري بماء يحتوى على ١٠٠ مللمول كلوريد الصوديوم إلى تكوين نوع من بروتين الأوراق عند٩٠ كيلو دالتون.

المجلة العلمية لكلية الزراعة – جامعة القاهرة – المجلد (٥٥) العدد الرابع (أكتوبر ٢٠٠٤):٥٨٧ – ٦٠٢.