Journal of Applied Botany and Food Quality 79, 24 - 32 (2005)

Lehrstuhl für Gemüsebau, Wissenschaftszentrum Weihenstephan der TU München, Freising

Alleviation of salinity effect on artichoke productivity by *Bacillus subtilis* FZB24, supplemental Ca and micronutrients

Saleh, S.A., Heuberger, H., Schnitzler, W.H.

(Received December 10, 2004)

Summary

Salinity is one of the most severe factors limiting bud yield and quality in globe artichoke (*Cynara scolymus* L.) production. In this study, three strategies to alleviate the negative effect of salinity on artichoke productivity and quality were investigated. In sand culture, artichoke plants were exposed to NaCl-saline nutrient solution (6.5 dS m^{-1}). The effect of inoculation of salt-stressed plants with *Bacillus subtilis* FZB24, supplemental Ca into saline nutrient solution and foliar application of Fe, Mn and Zn were evaluated under greenhouse conditions in 2002. Salinity reduced vegetative growth and bud yield of artichoke plants, and even more lowered product quality. There were sharp increases of both Cl and Na contents in all plant parts by application of NaCl into the nutrient solution compared to the non-saline control. The productivity was ameliorated by inoculation of salt-stressed plants with *Bacillus subtilis* FZB24 and nutrient additives as anti-salinity treatments. Application of *Bacillus subtilis* FZB24 ranked first to alleviate the adverse effects of salinity, followed by supplemental calcium into saline nutrient solution, while foliar application of a mixture of Fe, Mn and Zn showed almost no effect.

Introduction

Water deficit and increasing salinity are major factors threatening food production in the world. Salt stress is known to be a limiting factor for plant growth, yield and quality of harvest product. It is a serious problem for commercial agriculture in many arid and semi-arid regions, where rainfall is normally lower than evapotranspiration. In many cases, salt-stress is caused by irrigation with saline water. In coastal and sub-coastal areas in the Mediterranean basin (artichoke's cultivation areas), the salinization of irrigation water is an increasingly concerning issue. Although artichoke is placed in the moderately salt-tolerant category, especially during the vegetative stage (FRANCOIS, 1995), yield and bud quality are highly negatively affected by salinity (FRANCOIS et al., 1991; GRAIFENBERG et al., 1993 and 1995; VINCENZO et al., 2000). The most important challenge for increasing artichoke yield and quality is to alleviate the adverse effects of salinity. While water of better quality is difficult to obtain, a series of practices can be sought to lessen the adverse effect of salinity on the plants. There are special strategies to decrease the negative effects of salinity on plant physiology and on agronomical traits, for example by additional nutrients and applications of plant beneficial microorganisms. The beneficial effect of supplemental Ca on growth of salt-stressed plants has been widely recognized (LOPEZ and SATTI, 1996; CAINES and SHENNAN, 1999; NAVARRO et al., 2000; BIE et al., 2004; KAYA et al., 2002). The level of Ca in the external solution needed for optimal growth in saline conditions is usually between 5 and 10 mmol l^{-1} depending on the salinity level (CRAMER, 2002). Likewise, the optimal Na/Ca ratio is somewhere between 10 and 20 for most plants.

Beside Ca and K, positively charged micronutrients are less competitive for uptake and, due to altered pH, less soluble under saline conditions (GRATTAN and GRIEVE, 1994). Hence, the foliar application of micronutrients seems to be an appropriate tool to circumvent this barriere at the soil and root level in order to maintain adequate nutrient levels in the shoot.

Recently, the application of bacteria strains of *Bacillus subtilis* FZB24 has been assumed to reduce the negative effects of salinity and provide enhancement effects as biocontrol agent for several vegetable crops (BOCHOW et al., 2001; SCHMIEDEKNECHT et al., 2001).

Up till now, no studies have been undertaken concerning any of these ameliorative factors to overcome salt problems for artichoke. There is an obvious need for research on such strategies for artichoke. Thus, artichoke plants (cv. Green Globe) were evaluated under saline conditions and the effectiveness of anti-salinity treatments such as nutrient supply (Ca, Fe, Mn, Zn) and biocontrol agent (*Bacillus subtilis* FZB24) was investigated under controlled greenhouse conditions in a hydroponic system.

Materials and methods

The experiment was conducted in 2002 at the Research Station Dürnast, Chair of Vegetable Science, Life Science Center Weihenstephan, Technische Universität München, in Freising (southern Germany). The investigation was carried out in an environmentally controlled greenhouse. Maximum greenhouse air temperature ranged from 16 to 20°C, with a minimum night temperature of 14°C. Relative humidity ranged from 60 to 75%.

Eight-week old artichoke seedlings (cv. Green Globe) of good quality were transferred into sand-filled plastic pots (10 l). The sand with particle-size < 0.8 mm (93.0% sand, 6.2% silt, and 0.8% clay) was free of Na and Cl ions. For a good plant establishment, all seedlings were first fertigated with a non-saline full nutrient solution adjusted to pH 5.5-6.5 and EC value of approximately 1.5 dS m^{-1} in a closed system with recirculating nutrient solution. This solution contained the macronutrients NO_3, NH_4, P, K, Ca, Mg, and SO_4 and micronutrients Fe, Mn, Zn, B, Cu, and Mo at 14.0, 2.0, 2.0, 6.5, 3.75, 1.0, and 1.0 mmol l^{-1} and 15, 10, 5, 25, 0.75, and 0.50 µmol l^{-1}, respectively. Four weeks later a solution of 50 mmol l^{-1} NaCl was added to the nutrient solution of the saline treatments, resulting in a final EC value of 6.5 dS m^{-1}.

Three strategies of anti-salinity additives were compared to saline and non-saline controls. Under saline conditions, plants were treated with Ca supplement, *Bacillus subtilis* FZB24 or foliar application of a mixture of micronutrients (Fe, Mn, and Zn).

1. Ca supplement was added at 5 mmol l^{-1} to the saline nutrient solution using $CaCl_2$.
2. *Bacillus subtilis,* strain FZB 24 WG® (FZB Biotechnik GmbH, Berlin) was inoculated into the root zone of plants exposed to saline nutrient solution. This registered water-soluble granulate formulated on cornstarch as carrier contained 10^{11} spores g^{-1}. A pure bacterial spore suspension for application was prepared by dissolving 0.2 g l^{-1} of the granulate resulting in 2 10^{10} spores l^{-1}. For root bacterization, seedling substrate was watered with

1.0 l m^{-2} bacterial spore-suspension at two true leaves stage and a second time with 0.5 l per pot directly after transplanting to the 10-L pots. At last, *Bacillus subtilis* FZB24 was added once to the nutrient solution at 2 10^{-8} spores l^{-1}.

3. The foliar application of the micronutrients Fe, Mn, and Zn was given as a mixture of 60, 320, and 220 mg l^{-1}, respectively, as Flory 72 (6% EDDHA-chelated Fe), Manganese sulfate (32% Mn) and Zinc sulfate (22% Zn). The solution was sprayed four times in 15 days intervals (200 ml per plant) with the control of pure water sprays in all other treatments.

The experiment was arranged in a complete block design with 3 replications, each plot containing 8 plants.

The nutrient solution was applied by dripper nozzles four times (from 9^{00} to 15^{00}) daily for 15 minutes regulated by a timer resulting in the application of 4 l per plant and 30-50% drainage rate. Two months after transplanting, irrigation frequency was increased to 8 times (from 9^{00} to 16^{00}) per day resulting in 8 l per plant corresponding to plant development, increased temperature and radiation as well as leaching requirement in order to maintain the drainage rate. Fresh water was refilled automatically according to consumption. To maintain the adequate EC and pH of the nutrient solution, EC and pH were measured daily and adjusted by adding stock-nutrient solution and sulfuric acid, respectively.

NaCl was added at 50 mmol l^{-1} to the nutrient solution to obtain an EC of 6.5 dS m^{-1}. Every two weeks, the content of the nutrient solution and the recipe of the nutrient-stock solution were adjusted according to the complete nutrient and Na and Cl ions analysis of the drainage water. One month after treatments start, the entire nutrient solution was renewed after having flushed the system with deionized water. The experiment ended when the plants had stopped to produce marketable buds.

Evaluated parameters

1. Growth characters: Plant height, number of leaves per plant and dry weight of the 4th-youngest leaf were determined 15, 30 and 45 days after treatment start. Total dry weight of shoots and roots were evaluated at the end of the experiment.
2. Photosynthetic activity: The net photosynthetic rate of fully expanded and well light exposed leaves was measured with a portable porometer (Lci4, ADC BioScientific Ltd. Hoddesdon, Herts, England). The measurement was done during sunny days, approximately at 2 weeks intervals.
3. Yield: Buds were harvested at one-week intervals. The marketable yield per plant and bud weight was determined after exclusion of buds that had black spots (non-marketable) by Ca disorder.
4. Water: The supplied and drained quantities of water were recorded daily to calculate consumption during the growing season. Water use efficiency was calculated as g marketable bud yield per l of consumed water.
5. Nutrient status: Cl, Na, K, Ca, Mg, Fe, Mn, and Zn content were determined fortnightly in the 4th-youngest leaf, at harvest in the edible part of main and secondary buds, and at the end of experiment in shoots and roots. For chemical analyses the material was dried for 3 days in an oven at 70°C. Afterwards, the samples were ground with a Culatti MFC grinder equipped with a 1-mm sieve. Na, K, Ca, Mg, Fe, Mn, and Zn were determined by the flame AAS (VARIAN Spectra AA 100) after 6 hours ashing at 550°C and digested with conc. HCl. Cl was determined by $Hg(NO_3)_2$ titration according to the method of VDLUFA (1983) after extraction with distilled water.

Statistical analysis

The treatment effects were evaluated by analysis of variances, and differences among treatment means were determined by least significance difference (LSD) at $P < 5\%$ as reported by GOMEZ and GOMEZ (1984).

Results

Vegetative growth and physiological characters

Vegetative growth of artichoke plants represented by plant height, number of leaves per plant, dry weight and area of the 4th-youngest leaf was depressed by the salinity treatment compared to the non-saline control 15, 30, and 45 days after treatment start (Tab. 1). Moreover, the same effect was obtained for net photosynthesis rate at all measurement dates.

Inoculation of salt-stressed plants with *Bacillus subtilis* FZB24, addition of supplemental Ca to the saline nutrient solution or foliar application of macronutrients more or less decreased the adverse effect of salinity on vegetative growth characters and improved net photosynthesis. *Bacillus subtilis* FZB24 was most effective, followed by supplemental Ca, then the foliar spraying of micronutrients, which did not always improve plant growth. Compared to the saline control, *Bacillus subtilis* FZB24 increased plant height, area and dry weight of the 4th-youngest leaf at all measurement times, number of leaves 15 days and net photosynthesis rate 15 and 45 days after treatments start. With regard to the effect of additional Ca into saline nutrient solution, plant height at all measurement times, number of leaves 15 days, dry weight of the 4th-youngest leaf 15 and 30 days and its area 30 days and net photosynthesis rate 15 and 45 days after treatments start increased compared to saline control. Foliar spraying of micronutrients (Fe-Mn-Zn) only slightly enhanced plant height at all measurement times, as well as number of leaves and area of the 4th-youngest leaf 15 days after treatments start.

The dry weights of total shoots and roots at the end of the generative phase, 90 days after treatment start, are documented in Tab. 3. Shoot dry weight decreased due to salinity (6.5 dS m^{-1}). Only inoculation with *Bacillus subtilis* FZB24 treatment proved good effectiveness on shoot dry weight, but no positive effect was observed for additional Ca or foliar spraying of micronutrients. While dry weight of roots was not altered by any of the studied treatments.

Bud yield

Saline nutrient solution reduced total yield of buds per plant to 50% and yield of marketable buds to 40% compared to the non-saline control (Tab. 2). Inoculation with *Bacillus subtilis* FZB24 or extra Ca enhanced both total and marketable yield under salinity conditions, without differences between both additives. For instance, inoculation of salt-stressed plants with *Bacillus subtilis* FZB24 and additional Ca into saline nutrient solution increased the marketable yield to 145 and 135%, respectively, compared to saline control. On the other hand, no significant increase in bud yield of salt-stressed plants was noticed by foliar application of micronutrients (Fe-Mn-Zn).

The weight of main and secondary buds was reduced by the salinity treatment (Tab. 2). Inoculation with *Bacillus subtilis* FZB24 increased and additional Ca tended to raise the weight of main buds of salt-stressed plants compared to salinity. Foliar application of micronutrients (Fe-Mn-Zn) was not effective to improve artichoke bud weight under saline conditions.

Tab. 1: Effect of nutrient and *Bacillus subtilis* FZB24 additive on vegetative growth characters and net photosynthesis (µmol m^{-2} s^{-1}) of salt-stressed artichoke plants during the vegetative stage. Leaf area and dry weight were measured at the 4th-youngest leaf.

Treatments	Plant height [cm]	Number of leaves [plant^{-1}]	Leaf area [cm^2]	Leaf dry weight [g]	Net photosynthesis [µmol m^{-2} s^{-1}]
15 days after treatment start					
No salinity	80 a	11.0 a	557 a	8.7 a	12.8 a
Salinity only	67 d	9.7 c	351 c	6.1 c	5.8 c
Salinity + Ca	70 c	9.8 bc	397 c	7.1 bc	9.2 b
Salinity + *Bacillus*	73 b	10.3 b	445 b	7.5 b	12.6 a
Salinity + Micronutrients	71 c	9.9 bc	403 bc	6.7 c	6.3 c
30 days after treatment start					
No salinity	89 a	14.1 a	629 a	10.3 a	7.9 a
Salinity only	72 d	11.5 c	464 c	8.0 b	3.3 b
Salinity + Ca	77 c	12.8 b	500 bc	9.3 ab	3.9 b
Salinity + *Bacillus*	81 b	12.7 b	545 b	10.0 a	4.8 b
Salinity + Micronutrients	76 c	12.5 b	481 c	8.6 b	3.5 b
45 days after treatmens start					
No salinity	100 a	15.6 a	573 a	9.7 a	8.6 a
Salinity only	79 d	13.7 b	449 b	8.2 b	4.1 c
Salinity + Ca	83 c	14.4 b	441 b	8.6 b	5.8 b
Salinity + *Bacillus*	90 b	14.5 b	530 a	9.3 ab	6.3 b
Salinity + Micronutrients	82 cd	14.1 b	456 b	8.2 b	4.3 c

Means within each column and sampling date followed by the same letter are not significantly different at $P < 5\%$ (n=3).

Tab. 2: Effect of nutrient and *Bacillus subtilis* FZB24 additive on total and marketable bud yield (g plant^{-1}) and bud weight (g bud^{-1}) of salt-stressed artichoke plants.

Treatments	Total yield [g plant^{-1}]	Marketable yield [g plant^{-1}]	Bud weight	
			main [g bud^{-1}]	secondary [g bud^{-1}]
No salinity	1707 a	1413 a	167 a	166 a
Salinity only	845 c	569 c	95 c	114 b
Salinity + Ca	1060 b	768 b	100 bc	124 b
Salinity + *Bacillus*	1134 b	828 b	114 b	121 b
Salinity + Micronutrients	871 c	575 c	101 bc	108 b

Means within each column followed by the same letter are not significantly different at $P < 5\%$ (n=3).

Water consumption

The water-related parameters were not statistically evaluated due to the irrigation setup without replications. Consumption of water per plant was reduced in the saline treatments, irrespective of the application of *Bacillus subtilis* FZB24, extra Ca or micronutrients spraying (Tab. 3). WUE as g yield of marketable buds per l consumed water decreased to 3.2 due to salinity, but reached about the level of the non-saline control with *Bacillus* inoculation or supplementary Ca. Foliar application of micronutrients did not improve WUE under saline conditions.

Chemical composition

Data presented in Tab. 4 exhibit the effect of salinity, supplemental nutrients and *Bacillus subtilis* FZB24 on the content of Cl and Na in the different parts of salt-stressed plants. The obtained results

Tab. 3: Effect of nutrient and *Bacillus subtilis* FZB24 additive on total root and shoot dry weight (g plant^{-1}) and water use characteristics of salt-stressed artichoke plants.

Treatments	Shoot dry weight [g plant^{-1}]	Root dry weight [g plant^{-1}]	Net consumption [l plant^{-1}]	Water use efficiency [g l^{-1}]
No salinity	486 a	117 a	318	2.27
Salinity only	378 b	109 a	176	1.19
Salinity + Ca	425 b	118 a	180	1.39
Salinity + *Bacillus*	449 ab	112 a	181	1.51
Salinity + Micronutrients	400 b	111 a	176	1.22

Means within each column and bud kind followed by the same letter are not significantly different at $P < 5\%$ (n=3; water characteristics n=1).

Tab. 4: Effect of nutrient and *Bacillus subtilis* FZB24 additive on Cl and Na concentrations (%, dry weight based) in different plant parts of salt-stressed artichoke compared to the non-saline control

Treatments	4th-youngest leaf at weeks after start of treatment			Bud, edible part		Total shoot	Roots
	2 weeks	4 weeks	6 weeks	Main	Secondary	12 weeks	
Cl concentration (%)							
No salinity	1.02 c	0.86 c	0.90 c	0.49 b	0.53 c	0.86 d	0.45 b
Salinity only	4.19 a	4.17 a	3.12 a	1.35 a	1.65 ab	5.06 b	0.61 a
Salinity + Ca	3.33 b	4.19 a	2.79 b	1.38 a	1.77 a	5.23 a	0.65 a
Salinity + Bacillus	3.27 b	3.65 b	2.69 b	1.38 a	1.45 b	4.03 c	0.56 a
Salinity + Micronutrients	3.57 b	4.26 a	3.13 a	1.31 a	1.41 b	5.73 a	0.68 a
Na concentration (%)							
No salinity	0.97 d	0.73 c	0.29 c	0.36 a	0.19 d	0.96 d	0.36 c
Salinity only	3.48 a	3.29 ab	2.13 a	0.57 a	0.87 a	4.44 b	0.50 ab
Salinity + Ca	2.58 c	3.03 b	1.64 b	0.29 a	0.70 b	3.74 c	0.45 b
Salinity + Bacillus	3.04 b	2.76 b	1.51 b	0.35 a	0.56 c	3.18 c	0.45 b
Salinity + Micronutrients	2.70 bc	3.78 a	2.13 a	0.55 a	0.69 b	5.16 a	0.63 a

Means within each column and element followed by the same letter are not significantly different at $P < 5\%$ (n=3).

demonstrate sharp increases of both Cl and Na contents in all plant parts by application of NaCl to the nutrient solution compared to the non-saline control. The differences in Na content in the edible part of main buds among all studied treatments were not large enough to be significant. Inoculation of stressed plants with *Bacillus subtilis* FZB24 reduced Cl and Na in the 4th-youngest leaf 2, 4 and 6 weeks after treatments start and in shoots and in edible part of secondary buds, and Na content in roots. Also, application of extra Ca reduced the content of Cl in the 4th-youngest leaf 2 and 6 weeks after treatments start, while the content of Cl in shoots at the end of the experiment increased with extra Ca. The addition of Ca to the nutrient solution reduced Na content in the 4th-youngest leaf in week 2 and 6, in the edible part of secondary buds and in shoots 12 weeks after treatments start. With regard to the effect of foliar application of the Fe, Mn and Zn mixture on Cl content, a decrease in the 4th-youngest leaf 2 weeks after treatments start and an increase in the shoots were detected. Na content in the 4th-youngest leaf 2 weeks after treatments start and in edible part of secondary buds decreased by foliar application of micronutrients, while Na content tended to increase in the 4th-youngest leaf 4 weeks after treatments start and in shoots and roots. No changes were measured in the content of Cl in the edible part of main buds and in roots from saline conditions with or without any anti-salinity treatment.

The contents of K, Ca and Mg of the 4th-youngest leaves during the vegetative stage were not affected by salinity and anti-salinity treatments (Tab. 5). Saline nutrient solution (6.5 dS m^{-1}) decreased K content in the edible part of main buds, Ca content in the edible part of main buds and roots and Mg content in the edible part of secondary buds and shoots. The content of K and Mg was not increased by any anti-salinity treatment. While Ca content increased in the edible part of main buds and roots by supplemental Ca in the saline nutrient solution. In addition, foliar application of micronutrients enhanced Ca content in roots.

Only minor differences in the content of the micronutrients Fe, Mn, and Zn in different parts of artichoke appeared among treatments (Tab. 6). The analyses revealed increases in the content of Mn in

Tab. 5: Effect of nutrient and *Bacillus subtilis* FZB24 additive on K, Ca and Mg concentrations (%, dry weight based) in different plant parts of salt-stressed artichoke compared to the non-saline control.

Treatments	4th-youngest leaf at weeks after start of treatment			Bud, edible part		Total shoot	Roots
	2 weeks	4 weeks	6 weeks	Main	Secondary	12 weeks	
K concentration (%)							
No salinity	3.08 a	3.57 a	3.47 a	4.86 a	3.39 a	3.29 a	0.79 a
Salinity only	3.39 a	2.91 a	2.68 a	3.05 b	3.41 a	3.18 a	0.81 a
Salinity + Ca	3.52 a	2.96 a	3.98 a	3.46 b	3.59 a	4.37 a	0.88 a
Salinity + Bacillus	3.31 a	3.52 a	3.70 a	3.71 b	3.78 a	4.12 a	0.92 a
Salinity + Micronutrients	3.56 a	3.02 a	3.05 a	3.54 b	3.08 a	3.92 a	0.83 a
Ca concentration (%)							
No salinity	1.47 a	1.27 a	0.80 a	0.31 a	0.20 a	1.60 a	0.35 a
Salinity only	0.92 a	0.92 a	0.69 a	0.26 b	0.16 a	1.20 a	0.25 b
Salinity + Ca	1.38 a	1.21 a	0.65 a	0.33 a	0.17 a	1.83 a	0.29 ab
Salinity + Bacillus	1.22 a	1.15 a	0.66 a	0.24 b	0.15 a	1.34 a	0.19 b
Salinity + Micronutrients	1.08 a	1.13 a	0.65 a	0.26 b	0.16 a	1.24 a	0.27 ab
Mg concentration (%)							
No salinity	0.30 a	0.28 a	0.31 a	0.26 a	0.21 a	0.39 a	0.11 a
Salinity only	0.22 a	0.16 a	0.18 a	0.17 a	0.15 b	0.25 b	0.09 a
Salinity + Ca	0.25 a	0.20 a	0.16 a	0.13 a	0.14 b	0.26 b	0.11 a
Salinity + Bacillus	0.26 a	0.19 a	0.18 a	0.15 a	0.14 b	0.25 b	0.11 a
Salinity + Micronutrients	0.21 a	0.18 a	0.17 a	0.19 a	0.13 b	0.30 b	0.11 a

Means within each column and element followed by the same letter are not significantly different at $P < 5\%$ (n=3).

most plant tissues and the content of Zn in the 4th-youngest leaf 2, 4, and 6 weeks after treatment start by foliar spraying of micronutrients compared to the other treatments. Also, inoculation of salt-stressed plants with *Bacillus subtilis* FZB24 increased the content of Mn in edible part of main buds. Likewise, the content of Zn in the 4th-youngest leaf 2 weeks after treatments start increased with application of *Bacillus subtilis* FZB24 and extra Ca. The content of Fe in any plant tissue was not altered by the studied treatments.

Discussion

Response of artichoke to salinity

The clarity of morphological effects and physiological processes, which are induced by salinity, can lead to a better management of plants even under saline conditions. The three main physiological effects of NaCl salinity on plants are an increased osmotic potential in the soil solution leading to water-uptake restrictions, as well as imbalance in nutrient uptake and NaCl ion toxicity.

The observed reduced vegetative growth and yield was most likely the result of all three main effects. Water status of the plants was not directly measured. However, a certain water deficit in the plants was indicated by a 45% lower water consumption of the plants, while total leaf area and leaf number was only reduced by 22 and 13%, respectively. Artichoke, like other plants, reacted to water deficit with increased stomatal resistance resulting in a lower photosynthetic rate (BRUGNOLI and LAUTERI, 1991; VINCENCO et al., 2000), which was reduced in this study by more than 50%. This was possibly also caused by malfunction of the photosystem and related processes due to nutrient imbalance or ion toxicity. Water deficit of plants also reduces cell turgor pressure, cell enlargement and cell wall expansion, which should also have contributed to the smaller artichoke leaves. In the end, the reduction in the net photosynthetic rate from reduced total leaf area finally depressed plant growth (MUNNS, 1993). The actual reduction in plant dry weight compared to the non-saline control was 23%, which was only half of the reduction predicted by the model of GRAIFENBERG et al. (1993). Also, VINCENZO et al. (2000) noticed that the net photosynthesis and transpiration rates were reduced progressively as EC increased.

In contrast to other parts, salinity did not much affect the root dry weight, suggesting higher tolerance for this plant part to salinity compared to the other plant parts. This is in line with GRAIFENBERG et al. (1993; 1995) and VINCENZO et al. (2000). GRAIFENBERG et al. (1995) assumed that this response was due to the high exclusion potential of external cortical root layers of established plants to presence of ion excess in the soil solution. In our study, root dry weight was assessed at the end of the generative period when taproots and the rhizome have become fleshy to serve as the storage organ for production of the new offshoots (RYDER et al., 1983). Such kind of non-transpiring storage organs are mainly supplied by phloem sap (STRASBURGER et al., 2002). Like in barley, where the phloem has a lower Na concentration than the xylem because of strong Na retention in leaves (WOLF et al., 1991), the low Na content in roots

Tab. 6: Effect of nutrient and *Bacillus subtilis* FZB24 additive on Fe, Mn and Zn concentrations (mg kg^{-1} dry weight based) in different plant parts of salt-stressed artichoke compared to the non-saline control

Treatments	4th-youngest leaf at weeks after start of treatment			Bud, edible part		Total shoot	Roots
	2 weeks	4 weeks	6 weeks	Main	Secondary	12 weeks	
Fe concentration (mg kg^{-1})							
No salinity	38 a	42 a	89 a	50 a	44 a	83 a	117 a
Salinity only	39 a	45 a	43 a	39 a	40 a	92 a	80 a
Salinity + Ca	52 a	47 a	50 a	31 a	39 a	122 a	98 a
Salinity + Bacillus	44 a	38 a	57 a	31 a	41 a	99 a	95 a
Salinity + Micronutrients	64 a	52 a	84 a	38 a	36 a	144 a	131 a
Mn concentration (mg kg^{-1})							
No salinity	32 b	26 b	36 b	24 b	15 a	129 b	35 a
Salinity only	36 b	40 b	53 b	26 b	207 a	79 b	19 b
Salinity + Ca	36 b	36 b	34 b	29 b	19 a	71 b	16 b
Salinity + Bacillus	31 b	38 b	46 b	39 a	19 a	79 b	20 b
Salinity + Micronutrients	319 a	228 a	335 a	36 a	22 a	437 a	32 a
Zn concentration (mg kg^{-1})							
No salinity	40 c	73 b	98 b	39 a	95 a	156 b	49 a
Salinity only	44 c	67 b	116 b	46 a	68 a	124 b	46 a
Salinity + Ca	68 bc	77 b	128 b	49 a	69 a	125 b	54 a
Salinity + Bacillus	91 b	82 b	85 b	37 a	61 a	121 b	66 a
Salinity + Micronutrients	175 a	160 a	310 a	49 a	89 a	391 a	60 a

Means within each column and element followed by the same letter are not significantly different at $P < 5\%$ (n=3).

of this study and of GRAIFENBERG et al. (1995) suggest the maintenance of sublethal Na concentrations in the below-ground plant part of artichokes sustaining its sink potential. In contrast, roots of 9-week old seedlings showed similarly high Na concentrations like the shoots after 4 weeks irrigation with 50 to 150 mM NaCl (data not shown). This deviating response could be due to not yet developed external cortical root layers, following the argument of GRAIFENBERG et al. (1995) or/and to the presence of exclusively non-fleshy roots of the seedlings.

Bud yield per plant was more deteriorated by saline nutrient solution than vegetative growth. Total and marketable yield of buds per plant were 50 and 40%, respectively, compared to the non-saline control. A similar magnitude for the decrease of bud yield under saline conditions was obtained by FRANCOIS et al. (1991), FRANCOIS (1995) and GRAIFENBERG et al. (1993; 1995). Yield reduction was mainly attributed to the negative effect of salinity on the weight of buds rather than to the number of buds per plant (data not shown). This may be explained as a main result of adverse effects on plant growth and assimilation rate, accordingly, decreasing dry matter accumulation. TARANTINO et al. (2000) also found that the bud size was reduced, while the percentage of dry matter and fiber content increased in the buds. The even stronger reduction in marketable bud yield was due to marginal leaf necrosis, which was also experienced by FRANCOIS et al. (1991) and GRAIFENBERG et al. (1995) at similar EC-levels.

The concentrations of Cl and Na in the 4th-youngest leaf, shoots and the edible part of buds were 3-5 times higher in the salinity treatment compared to the non-saline control. In contrast, in roots the concentrations were only increased by about 37%. Similar results were obtained by FRANCOIS (1995) and GRAIFENBERG et al. (1995), who reported that Cl and Na were increased in artichoke organs, especially in old and middle leaves. The Cl and Na concentration in the 4th-youngest leaf 6 weeks after treatment start was lower than in the young leaves sampled 2 and 4 weeks before. This is possibly due to a higher number of old leaves, which are including and accumulating these ions. Similarly, the increase in Na and Cl concentration in the edible part of the buds due to salinity was less pronounced than in the total shoot. Salt inclusion mechanism in the old vegetative tissues (in cell vacuoles) resulting in low phloem salt concentrations seems to help the plant attenuate salt stress effects, because it permits the young, low-transpiring tissues to remain at lower salt concentrations and to better maintain their development and productivity (YEO, 1983; YEO and FLOWERS, 1982; WOLF et al., 1991; MUNNS, 1993).

The concentration of K, Ca and Mg tended to be reduced by salinity in most plant tissues (except K and Mg in roots and K in shoots), where the lower concentrations were significant for K in the edible part of main buds, Ca in the edible part of main buds and roots and Mg in edible part of secondary buds and shoots. This common effect deriving from uptake competition between Na and nutrient cations at the roots was also found for artichokes by GRAIFENBERG et al. (1995). The missing effect on K in roots can be explained by redistribution of K via phloem to non-/low-transpiring tissues like roots or young leaves which was reported for several plants, e.g.

barley under saline conditions (DELANE et al., 1982; WOLF et al., 1991). On the other side, the inner, edible part of the artichoke buds are also low transpiring but showed reduced K concentrations. For Ca, retranslocation via phloem is not as relevant due to its low phloem mobility; hence all tissues were more or less similarly affected with Ca reduction. This is in contrast to GRAIFENBERG et al. (1995) who reported smaller reduction in Ca contents in stems, buds and particularly roots.

The striking result from our study is that the content of micronutrients, e.g., Fe, Mn and Zn in plant tissues was not changed by salinity compared to the control treatment. Although the nutrient solution contained appropriate rates of micronutrients, deficiency symptoms appeared even under non-saline conditions. Mn and Fe concentrations range around and below the lower boundary concentrations to be sufficient, whereas Zn levels are within the sufficiency range according to BERGMANN (1986). The suboptimal uptake of these mirconutrients was probably due to the intermittent high pH of the nutrient solution (>7.0), which led to decreased availability of micronutrients. Accordingly, micronutrients uptake was limited by high pH rather than EC of the nutrient solution under the conditions of the presented study.

Alleviation of salt stress by *Bacillus subtilis* FZB24

Inoculation of stressed artichoke plants with *Bacillus subtilis* FZB24 proved most effective to decrease the adverse effect of salinity on vegetative growth characters and improved gas exchange leading to the increase of the net photosynthesis rate. The remarkable improvement effect of bacterization on the growth of plants under saline conditions can be attributed to the stress tolerance-inducer of *Bacillus subtilis* FZB24, which acts as plant growth and health promoter and antistressor agent (SCHMIEDEKNECHT et al., 1998; BÖHME, 1999; GROSCH et al., 1999; BOCHOW et al., 2001). BOCHOW et al. (2001) specifically investigated the effect of *Bacillus subtilis* FZB24 on salt-stressed vegetables. They reported that fruit yield of eggplant and bell pepper, which was reduced by irrigation with saline water (EC 6.5 dS m^{-1}) to 15-20 and 30-55 % of the control, respectively, was increased by *Bacillus subtilis* FZB24 inoculation to 50-60 and 55-85 % of the control, respectively.

It is difficult to explain the interactions of how *Bacillus subtilis* FZB24 improves salt tolerance of artichoke plants since little scientific work has been published on the physiological processes related to the rather more often reported positive agronomic effects of this microorganism. The key is possibly lying in the exudation of various metabolites like hormones (mainly IAA and zeatin, also gibberelic acid, abscisic acid), precursors of hormones, proteins and other active substances (KILIAN et al., 2000; IDRIS et al., 2004). It has been hypothesized for the mode of action of *Bacillus subtilis* FZB24 under salt-stress conditions by BOCHOW et al. (2001) that the given bacterial production of auxin and auxin precursors during root colonization induces a flush in the plant auxin synthesis, which changes the regulation of the appropriate mechanisms. The described exudates can directly or indirectly affect salt tolerance by altering the hormonal balance in the plant. In salt stressed plants the cytokinine level is lower and the abscisic acid level is higher than in unstressed plants (POLJAKOFF-MAYBER and LERNER, 1994). Thus, growth-stimulating hormones and precursors directly provided by *Bacillus subtilis* FZB24 or an increased level of cytokinines produced by the larger root system (more root tips) (KILIAN et al., 2000) alter the hormonal balance towards promotion of plant growth and leaf longevity. This is in line with a higher leaf number and higher area of newly formed leaves in *Bacillus*-treated artichoke plants, where towards the end of vegetative stage the leaf area approached that of non-saline control plants. As a consequence, bud yield was significantly increased. In addition, the more vigorous leaves represent a larger storage pool for Na and Cl, which relieves the developing and photosynthetically active leaves from increasing salt concentrations and improves their productivity. This hypothesis is supported by the significantly lower Na and Cl concentrations in the 4th youngest leaves of plants inoculated with *Bacillus subtilis* FZB24 and net photosynthetic rates equal to the non-saline control.

Most recently, ethylene is often discussed to play a role in stress release under stressing conditions, especially salt stress (MAYAK et al., 2004). It has already been shown that some bacteria and even another Bacillus strain (*B. amyloliquefaciens* IN937) produces the enzyme ACC-deaminase which prevents endogenous ethylene production by the plant thus releasing the stress signaling transduction pathway (GLICK et al., 1998). In summary, the inoculation of salt-stressed plants with *Bacillus subtilis* FZB24 stimulated the vegetative growth, supported the compartmentalization mechanism of harmful elements (Na and Cl) and improved assimilation even under salinity conditions. However, further research should be undertaken in order to attain a better understanding of the induction mechanisms of salt-stress tolerance by *Bacillus subtilis* FZB24.

Alleviation of salt stress by supplying Ca

Additional Ca improved vegetative plant growth, total and marketable yield, reduced Na and Cl concentration in leaves and Na in the shoot and buds, brought back Ca concentration in the edible part of the buds to the control level (non-saline) and tended to increase Ca in the 4th youngest leaf, entire shoot and root. On the other side, Ca supplements did neither affect root biomass measured at the end of the generative phase, nor K and Mg concentrations which were slightly reduced by salinity in some organs. The Na/Ca ratio of 13.3 applied in our study in the saline control was already quite low, since it was within the frame of 10 to 20 which was considered optimal for most plants by Cramer (2002). However, lowering Na/Ca ratio to 5.7 improved plant growth and Ca concentrations in tissues as described above. Beneficial effects of supplemental Ca on shoot and root growth of salinity stressed plants also occur in other but not all studied species and even varies between cultivars (GRATTAN and GRIEVE, 1999; CRAMER, 2002) and organs, where roots reacted more than shoots did (CRAMER, 2002). KAYA et al. (2002) reported that additional Ca improved strawberry productivity and water use efficiency under saline conditions. The same trend was reported by LOPEZ and SATTI (1996) for tomato productivity.

For improved tolerance of plants against salinity there are two main mechanisms: First restricting entry of salt into the plant and second minimizing salt concentration in the cytoplasm and cell wall (MUNNS, 2002). Supplemental Ca contributes to the restriction of Na movement into the plant through the decrease of Na/Ca ratio in the root rhizosphere and favour Ca versus Na uptake. This results in lower Na concentrations and improved functionality and growth of the tissues. It also induces higher Ca concentration in the root tissue, which is essential for membrane and cell-wall integrity, regulation of ion transport and selectivity including Na exclusion mechanisms in the rhizodermis (EPSTEIN, 1972, CRAMER et al., 1985, CRAMER, 2002). The reduced Na content in almost all analyzed organs indicates that Ca was effective in the salinity stressed artichoke plants of the study. The content of K and Mg, which was or tended to be reduced by salinity, could not be restored by additional Ca. This suggests that the Ca effect could be rather based on a higher Ca/Na ratio than on enhanced membrane selectivity in the root tissue. On the other side, since Ca is in direct competition with K and Mg, increasing Ca by 5 mmol l^{-1} alters the cation ratio at the cost of K and Mg uptake and may mask the positive Ca effects on membrane integrity. In addition, Cl was added at 10 mmol l^{-1} together with Ca but did not or only slightly increase Cl concentrations in the tissues, suggesting func-

tioning membranes, which could be functioning even without additional Ca. Finally, a third negative side effect of Ca addition is the increase of EC in the solution: 1 dSm^{-1} by 5 mmol l^{-1}. This further decreased soil water potential and potentially reduced bud yield by additional 10% applying the function of GRAIFENBERG et al. (1993).

Apart from the roots, higher Ca concentrations in the shoot may have improved membrane functionality of tonoplasts, which play an important role in compartmentalization and potential maximum concentration of salt into the vacuoles (second tolerance mechanism). However, the data from our study do not contribute to this aspect of tolerance.

Alleviation of salt stress by foliar application of micronutrients

Foliar application is used to alleviate micronutrient deficiencies at the time of translocation and uptake. The foliar application of micronutrients by means of foliar sprays offers a method supplying nutrients to plants more rapidly than methods involving root application, especially under stress conditions (MARSCHNER, 1995). Our results show that foliar application of a mixture of the micronutrients (Fe-Mn-Zn) resulted in tremendously increased concentrations of Mn and Zn in leaves, but there was neither an effect on bud yield nor interactions with other nutrients under investigation. Higher micronutrient concentrations were expected, but the extreme levels measured in the leaves surely derived from the remnants of the spraying film on the leaf surface. It is not clear to which extend the nutrients were absorbed by the leaf. Buds, which were not touched by the spraying, showed no significant increase in micronutrient concentration, except for Mn compared to any other treatment. The higher Mn concentrations in the roots (fleshy rhizome) are possibly a result of translocation within the plant and to a lesser extent the effect from spraying solution, which has dripped off from the treated plants. The fact that only Mn was translocated can be attributed to its higher mobility within the plant (BERGMANN, 1986). Our findings do not support the recommendation to use foliar application of micronutrients for artichokes under stress conditions like increased EC or pH levels of soil nutrient solution.

Acknowledgements

This work was partly supported by the International Centre for Scientific Culture (ICSC) – World Laboratory (Lausanne, Switzerland).

References

BERGMANN, W. (ed.), 1986: Farbatlas Ernährungsstörungen bei Kulturpflanzen. VEB Gustav Fischer, Jena.

BIE, Z., ITO, T., SHINOHARA, Y., 2004: Sodium sulfate, sodium bicarbonate and supplemental calcium on the growth of lettuce. Acta Hort. 644, 433-440.

BOCHOW, H., EL-SAYED, S.F., JUNGE, H., STAVROPOULOU, A., SCHMIEDEKNECHT, G., 2001: Use of *Bacillus subtilis* FZB24 as biocontrol agent. IV. Salt-stress tolerance induction by *Bacillus subtilis* FZB24 FZB24 seed treatment in tropical vegetable field crops, and its mode of action. J. Plant Diseases Protection 108, 21-30.

BÖHME, M., 1999: Effects of lactate, humate and *Bacillus subtilis* FZB24 on the growth of tomato plants in hydroponic systems. Acta Hort. 481, 231-239.

BRUGNOLI, E., LAUTERI, M., 1991: Effects of salinity on stomatal conductance, photosynthetic capacity, and carbon isotope discrimination of salt-tolerant (*Gssypium hirsutum* L.) and salt-sensitive (*Phaseolus vulgaris* L.) C_3 nonhalophytes. Plant Physiol. 95, 628-635.

CAINES, A., SHENNAN, C., 1999: Interactive effects of Ca^{2+} and NaCl salinity on the growth of two tomato genotypes differing in Ca^{2+} use efficiency. Plant Physiol. Biochem. 37, 569-576.

CRAMER, G.R., 2002: Sodium-Calcium interactions under salinity stress. In: Läuchli, A., Lüttge, U. (eds.), Salinity: Environment-plants-Molecules, 205-227. Kluwer Academic Publishers, Netherlands.

CRAMER, G.R., LÄUCHLI, A., POLITO, V.S., 1985: Displacement of Ca^{2+} by Na^+ from the plasmalemma of root cells. A primary response to salt stress. Plant Physiol. 79, 207-211.

DELANE, R., GREENWAY, H., MUNNS, R., GIBBS, J., 1982: Ion concentration and carbohydrate status of the elongation leaf tissue of *Hordeum vulgare* L. growing at high NaCl. J. Exp. Bot. 33, 557-563.

EPSTEIN, E., 1972: Mineral nutrition of plants: Principles and perspectives. John Wiley & Sons, New York.

FRANCOIS, L.E., DONOVAN, T.J., MASS, E.V., 1991: Calcium deficiency of artichoke buds in relation to salinity. HortScience 26, 549-552.

FRANCOIS, L.E., 1995: Salinity effects on bud yield and vegetative growth of artichoke *Cynara scolymus* L. HortScience 30, 69-71.

GLICK, B.R., PENROSE, D.M., LI, J., 1998: A model for the lowering of plant ethylene concentrations by plant growth-promoting bacteria. J. Theoretical Biol. 190, 63-68.

GOMEZ, K.A., GOMEZ, A.A., 1984: Statistical Procedures for Agricultural Research. John Wiley & Sons, Inc., New York.

GRAIFENBERG, A., PAOLA, M.L., GIUSTINIANI, L., 1993: Yield and growth of globe artichoke under saline-sodic conditions. HortScience 28, 791-793.

GRAIFENBERG, A., GIUSTINIANI, L., TEMPERINI, O., PAOLA, M.L., 1995: Allocation of Na, Cl, K and Ca within plant tissues in globe artichoke *Cynara scolymus* L. under saline-sodic conditions. Scientia Horticulturae 63, 1-10.

GRATTAN, S.R., GRIEVE, G.M., 1994: Mineral nutrient acquisition and response by plants grown in saline environments. In: Pessarakli, M. (ed.), Handbook of plant and crop stress, 203-226. Marcel Dekker, New York.

GRATTAN, S.R., GRIEVE, G.M., 1999: Salinity-mineral nutrient relations in horticultural crops. Sci. Hort. 78, 127-157.

GROSCH, R., JUNGE, H., KREBS, B., BOCHOW, H., 1999: Use of *Bacillus subtilis* FZB24 as biocontrol agent. III. Influence of *Bacillus subtilis* FZB24 on yield in soilless culture. J. Plant Diseases Protection 106, 568-580.

KAYA, C., KIRNAK, H., HIGGS, D., SALTALI, K., 2002: Supplementary calcium enhances plant growth and fruit yield in strawberry cultivars grown at high (NaCl) salinity. Sci. Hort. 93, 65-74.

IDRIS, E.E., BOCHOW, H., ROSS, H., BORRISS, R., 2004: Use of *Bacillus subtilis* as biocontrol agent. VI. Phytohormone-like action of culture filtrates prepared from plant growth-promoting *Bacillus amyloliquefaciens* FZB24, FZB42, FZB45 and *Bacillus subtilis* FZB37. J. Plant Diseases Protection 111, 583-597.

KILIAN, M., STEINER, U., KREBS, B., JUNGE, H., SCHMIEDEKNECHT, G., HAIN, R., 2000: FZB24 Bacillus subtilis FZB24 – mode of action of a microbial agent enhancing plant vitality. Pflanzenschutz-Nachrichten Bayer 53 (1), 72-93.

LOPEZ, M.V., SATTI, S.M.E., 1996: Calcium and potassium-enhanced growth and yield of tomato under sodium chloride stress. Plant Science 114, 19-27.

MARSCHNER, H., 1995: Mineral nutrition of higher plants. Academic Press, London.

MAYAK, S., TIROSH, T., GLICK, B.R., 2004: Plant growth-promoting bacteria confer resistance in tomato plants to salt stress. Plant Physiol. Biochem. 42, 565-572.

MUNNS, R., 1993: Physiological processes limiting plant growth in saline soils: some dogmas and hypotheses. Plant, Cell Environ. 16, 15-24.

MUNNS, R., 2002: Comparative physiology of salt and water stress. Plant Cell Environ. 25, 239-250.

NAVARRO, J.M., MARTINEZ, V., CARVAJAL, M., 2000: Ammonium, bicarbonate and calcium effects on tomato plants grown under saline conditions. Plant Science 157, 89-96.

POLJAKOFF-MAYBER, A., LERNER, H.R., 1994: Plants in saline environments. In: Pessarakli, M. (ed.), Handbook of plant and crop stress, 65-96. Marcel Dekker, New York.

RYDER, E.J., DE VOS, N.E., BARI, M.A., 1983: The globe artichoke *Cynara scolymus* L. HortScience 18, 646-653.

SCHMIEDEKNECHT, G., BOCHOW, H., JUNGE, H., 1998: Use of *Bacillus subtilis* FZB24 as biocontrol agent. II. Biological control of potato diseases. J. Plant Diseases Protection 105, 376-386.

SCHMIEDEKNECHT, G., ISSOUFOU, I., JUNGE, H., BOCHOW, H., 2001: Use of *Bacillus subtilis* FZB24 as biocontrol agent. V. Biological control of diseases on maize and sunflowers. J. Plant Diseases Protection 108, 500-512.

STRASBURGER, E., NOLL, F., SCHENCK, H., SCHIMPER, A.F.W., 2002: Lehrbuch der Botanik für Hochschulen. 35. publication, major revision by Sitte, P., Weiler, E.W., Kadereit, J.W., Bresinsky, A., Körner, C. Spektrum Akad. Verl., Heidelberg.

TARANTINO, E., FLAGELLA, Z., VOLPE, D., DE CARO, A., 2000: Effect of different irrigation volumes of saline water on artichoke yield and soil salinity. IV International Congress on Artichoke, October 17-21, Valenzano-Bari, Italy.

VDLUFA, 1983: Methodenbuch des VDLUFA III, Futtermitteluntersuchung. Darmstadt, Deutschland.

VINCENZO, B., VITO, C., VINCENZO, B.V., FRANCESCA, B., 2000: Response of artichoke to water salinity levels. IV International Congress on Artichoke, October 17-21, Valenzano-Bari, Italy.

WOLF, O., MUNNS, R., TONNET, M.L., JESCHKE, W.D., 1991: The role of the stem in the partitioning of Na^+ and K^+ in salt-treated barley. J. Exp. Bot. 42, 697-704.

YEO, A.R., 1983: Salinity resistance: physiologies and prices. Physiol. Plant 58, 214-222.

YEO, A.R., FLOWERS, T.J., 1982: Accumulation and localisation of sodium ions within the shoots of rice (*Oryza sativa*) varieties differing in salinity resistance. Physiol. Plant 56, 343-348.

Addresses of the authors:

Said A. Saleh, Vegetable Research Department, National Research Centre, El Behoos St., Dokki, Cairo, Egypt

Heidi Heuberger, Institut für Pflanzenbau und Pflanzenzüchtung, Bayerische Landesanstalt für Landwirtschaft, Vöttinger Straße 38, D-85354 Freising

W.H. Schnitzler, Lehrstuhl für Gemüsebau, Technische Universität München, Dürnast 2, D-85350 Freising