Salt tolerance of spinach as related to seasonal climate

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Abstract


Three sets of experiments with spinach (Spinacia oleracea L., cv. Racoon) were conducted under saline water irrigation during different time periods between December 7, 2012–June 15, 2013 to understand the impact of increased temperature on salt tolerance in cool season crops. The first experiment consisted of 4 different salinity levels: 0, 4, 7, 9 dS/m and the two subsequent experiments each had 6 different levels of saline water: 0, 4, 7, 9, 12, and 15 dS/m. Irrigation water salinity up to 9 dS/m did not cause any yield loss in spinach during the first set of experiments, indicating that this cultivar is considerably more salt tolerant than spinach varieties reported in the literature. Severe salinity caused yield loss and decreased all gas exchange and vegetative parameters. It was found that spinach was considerably more salt tolerant under cool season late winter conditions than under warmer climatic conditions. The increase in temperature between experiment I and II was 12.5°C while the relative yields decreased by 31% at the same salinity treatment (9 dS/m).

Keywords: salt stress; leafy vegetables gas exchange; temperature; evapotranspiration

High quality fresh water is in increasing demand throughout the world but especially in arid and semiarid regions where agriculture depends on irrigation and fresh water use exceeds sustainable supply. Nonetheless, these regions often have abundant supplies of marginal quality water (Irshad et al. 2009). In addition, the faster-than-predicted change in global climate and various scenarios for climate change suggest that the semi-arid regions of the globe including the Mediterranean region face increasing aridity in the near future (Chaves et al. 2009). An additional challenge to crop production in semi-arid regions is increased temperature associated with climate changes. Projected increases in temperature will result in a shorter time period in which spinach and other cool season crops may be grown in semi-arid irrigated regions. Important environmental factors that have been shown to have a significant interaction with salinity include temperature, wind, humidity, light and air pollution (Shannon et al. 1994). Specific physiological needs may be also associated with different aspects of irrigation with saline water. Plants have to mainly cope with seasonal fluctuations of soil water potentials (e.g. spring–summer rains that leach out the salt brought about by irrigation), which may require rapid stomatal responses to minimize water stress during the hottest hours of the day (Horchani et al. 2010). The salt tolerance of spinach is considered intermediate among herbaceous crops (Long, Baker 1986). Spinach is defined as a cool climate vegetable, the minimum temperature for seed germination is 2°C, and optimum range reported as 7 to 24°C. Young plants can
withstand temperatures as low as –9°C and as high as 32°C (FES 2005).

The effect of temperature on salt tolerance is not clear, with some researchers reporting enhanced salt tolerance and other decreased salt tolerance with increased temperature. The objective of our study was to evaluate the seasonal (temperature) differences in spinach growth under different salinity levels. The irrigation water was evaluated as ground waters and drainage waters in Mediterranean and other coastal regions are generally chloride-dominated.

**MATERIAL AND METHODS**

Three sets of experiments were conducted outdoors with spinach (*Spinacia oleracea* L., cv. Ra-coon) on different dates (December 2012–March 2013, April–May 2013 and late April–June 2013) at Riverside, California, USA (lat. 33°58’24”, long. 117°58’12”). The first and second set of the experiment seeds were planted on December 7 and April 1, respectively, in outside large sand tanks. Seeds were sown directly in the sand culture tanks, 10 cm apart and with 40 cm between rows in three rows per tank. The seedlings were later thinned to 25 plants per row. The sand culture tanks ($1.5 \times 3 \times 2$ m deep) were filled with sand mixed with 10% peat moss (on volume basis) with an average bulk density of $1.38 \text{ g/cm}^3$. At saturation, the sand had an average volumetric water content of $0.30 \text{ m}^3/\text{m}^3$. Each plot was irrigated with solutions prepared in an individual reservoir ($1.5 \text{ m diameter} \times 2.2 \text{ m depth}$) having a volume of 4,500 l. A third set of experiments was conducted in smaller outdoor tanks ($82 \times 202 \times 84 \text{ cm deep}$), filled with sand having an average bulk density of $1.4 \text{ g/cm}^3$. The seeds for the third experiment were sown at April 9, 10 cm apart and 30 cm between rows. The water reservoir volume was 1,750 l/tank. Both small and large tanks utilized similar irrigation systems where irrigation solutions were pumped from the reservoirs to the tanks completely saturating and leaching the sand with drainage water returning to the reservoir through a subsurface drainage system at the bottom of each tank, thus maintaining an essentially uniform and constant salinity in the root zone. The nutrient solution utilized a modified half Hoagland’s solution with (in mM): $2.5 \text{ Ca(NO}_3)_2$, 3.0 $\text{ KNO}_3$, 0.17 $\text{ KH}_2\text{PO}_4$, 1.5 $\text{ MgSO}_4$, 0.05 Fe as sodium ferric diethylenetriamine pentaacetate (NaFe-EDTA), 0.023 $\text{ H}_3\text{BO}_3$, 0.005 $\text{ MnSO}_4$, 0.0004 $\text{ ZnSO}_4$, 0.0002 $\text{ CuSO}_4$, and 0.0001 $\text{ H}_3\text{MoO}_4$. The base nutrient solution without added salts served as the non-saline control ($0.85 \text{ dS/m}$) in all experiments. The target electrical conductivities of the irrigation waters ($\text{EC}_{iw}$) of 4, 7, 9, 12, 15 $\text{ dS/m}$ were achieved by adding $\text{CaCl}_2$, $\text{MgCl}_2$, $\text{NaCl}$, $\text{Na}_2\text{SO}_4$ to the base tap water-nutrient solution (Table 1) by using a model delv-

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$\text{EC}_{iw}$ – electrical conductivity of irrigation water
Measurement of shoot and fresh weight was taken immediately after harvesting. Shoots were oven dried at 65°C to constant weight after washing with deionized water, for determination of dry matter.

Chlorophyll contents of leaves were measured by a handheld chlorophyll meter (SPAD-502; Konica Minolta Sensing, Inc., Osaka, Japan) on the fifth fully expanded leaves and then averaged (Khan et al. 2003). Net photosynthetic rate (NP), leaf stomatal conductance ($g_s$), and leaf transpiration ($Tr$) of plants were measured on the fifth fully expanded leaves using a portable Li-Cor 6400 Photosynthesis System (Li-Cor, Nebraska, USA) two weeks before the harvest. The measurement conditions were leaf chamber photosynthetically active radiation (PAR), 1,100 µmol/m²·s; leaf to air vapour deficit pressure, 1.7 to 2.6 kPa; leaf temperature 26–28°C and chamber CO$_2$ 380 µmol/mol.

**RESULTS AND DISCUSSION**

The climate data were obtained from the CIMIS (2012) weather station (California Irrigation Management Information System (weather station 44 = UC Riverside CIMIS, 2012–2013).
Daily mean reference evapotranspiration (ET₀) values of each experiment and growing periods as number of days are shown in Fig. 1. ET₀ values show the seasonal differences in water demand among the experiments. Depending on that seasonal difference and related climatic parameters (primarily temperature) the growing period is longest for the first experiment, shorter for the second experiment and shortest in the third experiment, consistent with the increasing temperature during the growing period of experiments I-III. During the first experiment, ET₀ was very low during the first ten days, averaging 1.96 mm. In contrast the minimum ET₀ values were 4.43 and 6.36 for experiments II and III, respectively. The mean daily ET₀ values for the first, second and third experiments were 2.73, 5.29 and 5.95 mm respectively. The seedling stage of experiments II and III are different regarding ET₀ values (Fig. 1). These climate differences during the vegetation period make possible to evaluate seasonal effects on spinach growth and salt tolerance and the differences on the different vegetation stages. It is reported that spinach grows optimally at 15–20°C, and spinach growth is affected by climatic conditions (FES 2005). Average temperatures in the growing periods of experiment I, II and III were 11.9°C, 17.9°C, 20.15°C, respectively. In our climatic variations, the best temperature range was in experiment II. Contrary to expectations, the potential evapotranspiration (PET) during the course of the experiments decreased with increasing temperature; PET was 276.3, 240.6 and 202.1 mm for experiment I, II and III, respectively. This result is explained by the shorter growing period of the experiments with increasing temperature, as shown in Table 2.

Relative humidity was similar for the three experiments, while solar radiation was much greater for experiments II and III as compared to Experiment I (Table 2).

It is indicated that increased temperature results in decreased salt tolerance (SHANNON et al. 1994). Among others, AHI and POWERS (1938) were the first to report on salt tolerance and temperature. They studied the effect of two temperatures (13°C and 21°C) on the salt tolerance of salt grass and alfalfa. They concluded that increased temperature decreased salt tolerance and stated that in the “cold house the total weight of dry matter obtained at the highest concentration of sea water was more than three times as much as that in the warm house”.

### Growth response

The experimental design had three separate planting dates. Differences in yield of the controls and salinity treatments varied depending on the climatic factors. As salinity increased above the control, yield more than doubled for experiment I, almost doubled for experiment II and increased only by 15% for experiment III (Fig. 2). This response cannot be attributed to the increase in daily ET₀ from experiments I–III. As discussed above the cumulative ET₀ was greatest during the cool season experiment. Spinach is a cool season crop but it appears that the increased (daily) water demand (and salt uptake) in warmer climatic conditions was more than compensated by increased growth rate and subsequent dilution of salt in the plant tissue.

The results from the three experiments indicate that the “salt tolerance” of spinach is highly dependent on the climatic conditions. The salt tol-
erance is considerably greater specifically under cooler temperatures. Experiment I, under seasonal cooler conditions not only had no yield loss at EC\textsubscript{i} 9 dS/m, but the yield at this EC\textsubscript{i} was the highest of all treatments and approximately twice that of the control (Fig. 2). In contrast, in experiment III at EC 9 dS/m the yield declined by 27% from that at EC\textsubscript{i} and EC 4, and was less than the yield of the control. In experiment II the yield decline also began above EC\textsubscript{i} 4 and the decline was significant between EC 7 dS/m and all subsequent EC levels as shown in Table 2.

For all three experiments, moderate salinity levels had higher yield compared to the control treatments (EC\textsubscript{i} less than 1 dS/m). Some earlier studies showed that spinach had higher yield under moderate salinity conditions (YOUSIF et al. 2010; MAZLOOMI, RONAGHI 2012). SPEER and KAISER (1991) reported that spinach showed little growth impairment within a 17 day period after addition of 100 mM NaCl to hydroponic cultures and TOMEMORI et al. (1996) found that sea water diluted to 1,000 mg/l salt improved spinach growth in sandy soil. However, spinach salt tolerance threshold was reported as 2.0 dS/m, and the slope as 7.6% (LANGDALE et al. 1971). According to the statements of SHANNON et al. (2000), based on the spinach values in MAAS and HOFFMAN (1977), EC\textsubscript{i} for C\textsubscript{50}, the value at which the yield is reduced by 50% would be 8.6 dS/m. The C\textsubscript{50}, EC value at which yield is reduced by 50% relative to the control, calculated from our experiments is approximately 15 dS/m, (expressed in terms of irrigation water EC) indicating either much greater salt tolerance for the cv. Racoon, as compared to their cultivars of Spinacia oleracea ( cvs Space and New Zealand), or evaluation of salt tolerance during warm conditions (SHANNON et al. 2000; WILSON et al. 2000; SULEIMAN et al. 2002). Using the Maas-Hoffman salt tolerance model and the conversion factor EC\textsubscript{50} = 0.472 × EC\textsubscript{sw} determined for our sand tank soil media, the calculated threshold EC\textsubscript{i} is greater than 4.2 dS/m (highest salinity treatment) during cool season growth, between 3.3–4.2 dS/m during the intermediate climate condition and between 1.9–3.3 dS/m for the experiment with the warmest climate. Since EC 9 dS/m water did not cause any yield loss in the first experiment, irrigation water treatments of EC 12 and 15 dS/m were added to experiments II and III. In these experiments, relative yields increased at low salinity levels and then decreased at the higher salinity levels. In contrast to experiment I, experiments II and III had yield loss at EC\textsubscript{i} of 9 dS/m. However fresh weights of plants were still higher at EC 4 and 7 dS/m treatments compared to the controls in experiments II and III, as was also observed for experiment I.

**Vegetative parameters**

The general effect of salinity in crops is reduced growth rate resulting in smaller leaves, shorter stature, and sometimes fewer leaves (SHANNON, GRIEVE 1999). Under cooler conditions (Experiment I) there was no significant difference in leaf number with increasing salinity, despite the large increase in yield with increasing salinity for this experiment (Fig. 2). Experiments II and III had significantly more leaves than experiment I. This may be related to the higher temperatures at the beginning of the experiments II and III. Also, there was a significant increase in leaf number with increasing salinity in experiment II. Leaf number increased under low salinity levels and decreased only slightly with increased salinity in experiments II and III. Yield loss associated with salinity was thus not associated with leaf number, indicating that salinity stress reduces leaf size rather than leaf number.

Data for the leaf area per plant is presented in Fig. 3. These data show first an increase in leaf area then a decrease with increasing salinity, with experiment II having the largest leaf area. These data are similar to the yield data presented in Fig. 2, confirming that the adverse impact of salinity on spinach yield is associated with smaller leaves rather than a lesser number of leaves. Shoot heights of spinach in experiments I and II increased at EC 4 dS/m relative to control and were similar at
EC 7 dS/m. In experiment I shoot height increased up to EC 9 dS/m even though it is not statistically significant above EC 4 dS/m. In experiment II, above EC 7 dS/m, shoot height started to decrease and continued to decrease until the highest level of salinity (15 dS/m). Experiment III had the longest shoot height at control and shoot height gradually decreased with increased salinity. The salinity level at which shoot height starts to decrease also decreased with warmer growing periods. This may be explained by the seasonal ET difference in the experiments. Although salt stressed plants had generally lower shoot growth, it was observed that higher evapotranspiration also causes lower shoot growth under salt stress. There was a large reduction in growth in experiments II and III above 4 and 7 dS/m respectively. The trends in dry weight with salinity are very similar in all three experiments except for one treatment in experiment I where the values increased at EC 9 dS/m. Seasonal differences can be seen in Fig. 3 as the critical salinity level for yield is 4 dS/m in experiment III, 7 dS/m in experiment II and above 9 dS/m in experiment I (no decrease in dry weight at 9 dS/m).

**Gas exchange measurements**

Gas exchange measurements were conducted in experiments I and II. Salinity levels of irrigation waters strongly influenced leaf gas exchange parameters. In both experiments, photosynthesis first increased with salinity and then decreased with subsequent increases in salinity, as shown in Fig. 4. The decrease in photosynthesis occurred above EC 7 dS/m in experiment I and above 4 dS/m in experiment II. It is reported that photosynthesis, together with cell growth, is among the primary processes affected by salinity (Munns et al. 2006). In our study, reductions in photosynthesis were observed before there was yield loss, consistently with the concept that this is a primary process af-
In experiment II, the reduction in photosynthesis started at lower EC (4 dS/m) than in experiment I, consistently with the yield response where yield loss occurred at lower salinity in experiment II. Reduction in photosynthesis was suggested as responsible for at least part of the growth and yield reduction caused by salt stress (Prior et al. 1992; Munns 2002). In our results, reduction in photosynthesis ($P_{n}$) did not cause yield loss in experiment I, as the highest EC of 9 dS/m was apparently not sufficiently high, but in experiment II it can be concluded that reduction in $P_{n}$ adversely affected yield. It is unclear if this reduction is caused by inhibition of photosynthesis or by nutrient deficiency in growing tissues, as previously discussed by Munns (1993). Along with the decreasing $P_{n}$, the stomatal conductance ($g_{s}$) decreased with increased salinity stress; however, in experiment II stomatal conductance increased up to 4 dS/m and then started to decrease after subsequent increases in salinity. Stomatal closure, considered to relate to the osmotic component of salinity, was reported to be primarily responsible for photosynthesis inhibition in some studies (Bañuls 1995; Paranychianakis et al. 2004). In our results, the trends in stomatal conductance and photosynthesis with salinity were in good agreement in experiment II. However, in experiment I where ET$_{0}$ was lower than in experiment II, irrigation water with 4 dS/m increased photosynthesis but decreased stomatal conductance. It is now widely accepted that stomatal closure is not due to turgor loss, but it is a highly regulated response to salinity (Munns 1993). All salinity levels had higher stomatal conductance in experiment II as compared to experiment I, again consistent with the yield data. Transpiration rate of spinach decreased significantly in salt-stressed spinach leaves with respect to controls. Experiment I transpiration values are lower than those in experiment II at comparable salinity levels; this is explained by the temperature differences between the growing conditions.
seasons, with experiment II experiencing higher ET₀ than experiment I.

Flanagan and Jefferyes (1989) suggested that the severe reductions in stomatal conductance and transpiration rate under salt stress represent adaptive mechanisms to cope with excess salt, rather than merely a negative consequence of it. The same conclusion was made for our spinach study since moderate salinity levels resulted in decreased transpiration and stomatal conductance, before yield decreases are observed. Higher chlorophyll values in experiment I as compared to experiment II are evident even in the control. Also, the chlorophyll values in experiment I increased with increasing salinity, consistently with increasing yield. In contrast, in experiment II, the chlorophyll values increased as salinity increased and yield decreased. The SPAD values are lower in experiment II which had a warmer growing season as compared to the experiment I. Gitelson et al. (2003) indicated that leaf chlorophyll content is strongly dependent on plant stress. However, some studies have reported that chlorophyll content increases under conditions of salinity such as in Amaranthus (Wang, Nil 2000). A sharp increase in chlorophyll was measured at moderate salinity relative to the control, along with a corresponding yield increase. The chlorophyll decrease at higher salinity was not statistically different even with the large decrease in yield. These results show that spinach chlorophyll content is not significantly affected by salinity.

CONCLUSION

In this study, spinach (Spinacia oleracea L., cv. Racoon) was exposed to salt stress at increasing EC levels (0, 4, 7, 9, 12, 15 dS/m) of irrigation water under different seasons. According to our results, plant fresh and dry weight initially increased as the salinity of the irrigation water increased, and the yield decreased only under high salinity levels. This cultivar was considerably more salt tolerant than other cultivars reported in the literature. Our yield did not decrease until ECₑ of > 4.2 dS/m under cool climate conditions and ECₑ of 1.9–3.3 dS/m under warmer conditions, and yield did not depend on water composition. Recycled drainage water or low quality saline water from other sources can be reused to irrigate spinach after germination and seedling establishment under non-saline conditions. This enables production under the climate of Mediterranean type where winter rains reduce soil salinity and subsequent irrigation with saline water is possible. Moderate salinity concentrations were beneficial to yield. It is also evident that characterization of salt tolerance must consider climatic conditions, as under cool season spinach was considerably more salt tolerant than under warmer climatic spring conditions. Decreased salt tolerance occurs when increased temperature is not sufficient to cause heat stress. The salt tolerance decrease with increased temperature is also expected for other cool season crops.

References


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