

Spinach biomass yield and physiological response to interactive salinity and water stress



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ABSTRACT

Critical shortages of fresh water throughout arid regions are forcing growers to decide among the following options, applying insufficient fresh water, causing water stress, applying saline water causing salt stress or applying some combination minimizing saline water application, causing combined water and salt stress. A comprehensive approach to manage drought and salinity is to evaluate the impact of water stress and salt stress individually and then examine their interactions on plant production. To analyze salinity and water stress responses and their interaction together on spinach growth, an experiment was conducted from April 1 to May 21, 2013, using 6 different irrigation waters at electrical conductivity (EC): 0.85, 4, 7, 9, 12, 15 dS m⁻¹. Soil moisture was recorded by sensors and stress treatments had the following soil water matric pressure control (−45 kPa), −200 to −300 kPa, and −400 to −500 kPa. We evaluated three replicates per treatment for yield, vegetative parameters, ion composition, and physiological parameters. The results showed that the spinach yield response to salt and water stress was very different. Spinach yield initially increased with salinity and subsequently decreased only when the irrigation water was EC 9 dS m⁻¹ and above (osmotic pressure of −310 kPa). In contrast, yield decreased at the first water stress level (−230 kPa) relative to control. The additional presence of salinity stress decreased the relative yield response due to water stress. Similarly under water stress the relative yield response to increasing salinity was reduced. Although no model provided good prediction of stress response, the best predictive model (relative error) was one that considered the response to multiple stresses as the product of the response to the individual stresses.

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1. Introduction

Drought and salinity are the two major abiotic stresses dramatically limiting crop growth and productivity worldwide and the area affected by these two stresses is still increasing (Wang et al., 2003). The optimal approach to counter drought and salinity stress is development of tolerant crop varieties. Thus, it is important to understand the mechanisms of drought and salinity tolerance in plants, both to develop new varieties and to develop management practices to minimize the adverse effects. Drought is considered the primary destructive, crop yield-limiting factor, and detailed knowledge of its impact on plant growth regulation is crucial (Avramova et al., 2015). The adverse impact on crop production may increase as climate change is predicted to increase the frequency and severity of crop water stress, causing significant yield loss (Trenberth et al.,

2014; Obidiegwu et al., 2015; Zhan et al., 2015). Salinity, another important abiotic stress limiting crop production is also increasing in extent worldwide at an estimated rate of 1.5 million ha per year (Eynard et al., 2006) and is estimated to affect 23% of cultivated lands (Tanji and Wallender, 2012).

Drought, salinity, extreme temperatures and oxidative stress are often interconnected, and may induce similar cellular damage. For example, drought and/or salinity are considered to be manifested primarily as osmotic stress, resulting in the disruption of homeostasis and ion distribution in the cell (Serrano et al., 1999; Zhu, 2001). The apparent similarity of the effects of salinity and drought has raised the question as to whether the same change in the plant water status caused either by salinity or by drought leads to the same yield reduction (Katerji et al., 2004).

Several studies have separately evaluated the effects of salinity stress and drought stress expressed as osmotic and matric potential (De Pascale et al., 2007; Xu and Leskovar, 2015). A few studies have evaluated the interaction of salinity and water application as related to yield (Shani and Dudley, 2001; Shani et al., 2007), interac-

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tions of salinity and leaf water potential (Katerji et al., 2009; Katerji et al., 2011), consecutive salinity and non-saline (PEG) osmotic treatments as a proxy for water (matric) stress (Nagy and Galiba, 1995). However, there is very little information on plant response where the matric stress was measured and controlled. Ahmed et al. (2013), examined salt and water stress interactions by withholding irrigation, allowing the water content to decrease to a soil moisture content of 4% where it remained for 10 more days. Previous studies thus either made periodic irrigations to saturate the soil and then delayed subsequent irrigation, applied less quantities of water to induce drought without measuring matric potential or induced drought at the end of the experiment. In these instances matric potentials were either unknown or fluctuated widely during the experiments.

Our objective in this study was to determine and then compare the separate and interactive effects of water and salinity stress, conducting experiments under defined and essentially constant matric and osmotic stress over almost the entire life cycle of the plant (after seedling establishment). We also tested the hypothesis that the effect of combined stress on yield can be represented by multiplying the response to the individual stresses.

2. Materials and methods

The experiment was conducted outdoors with spinach (*Spinacia oleracea* L., cv. Ragoon) during the interval between 1 April–21 May 2013 at Riverside, Calif., (lat.33E58'24', long. 117E58'12'). Seeds were sown directly in sand culture tanks, 10 cm apart and 40 cm between rows. We planted three rows per tank in the outside large tanks at 1 April. The seedlings were later thinned to 25 plants per row. Sand culture tanks (1.5 × 3 × 2 m deep) were filled with sand mixed with 10% peat moss (at volume basis) with an average bulk density of 1.38 g cm⁻³. Peat moss was added to increase the water holding capacity of the sand. The sand mix had an average volumetric water content of 0.30 m³ m⁻³.

Six different irrigation waters (mixed salt composition) at EC; 0.85 (control), 4, 7, 9, 12, 15 dS m⁻¹ were used in the experiment (Table 1). Each plot was irrigated with solutions prepared in individual reservoirs (1.5 m diameter × 2.2 m deep) having a volume of 4500 L. Irrigation solutions were pumped from the reservoirs to the tanks and then returned to the reservoirs through a subsurface drainage system at the bottom of each tank, maintaining a uniform and constant salinity profile. Initial irrigations consisted of nutrient solution made up in Riverside California U.S.A. tap water with nutrients added as (in mM): 2.5 Ca (NO₃)₂, 3.0 KNO₃, 0.17 KH₂PO₄, 1.5 MgSO₄, 0.05 Fe as sodium ferric diethylenetriamine pentaacetate (NaFe-EDTA), 0.023 H₃BO₃, 0.005 MnSO₄, 0.0004 ZnSO₄, 0.0002 CuSO₄, and 0.0001 H₃MoO₄. This solution served as the base nutrient solution. The base nutrient solution without added salts served as a non-saline control (<1.0 dS m⁻¹) in all experiments. As the water from each of the sand tanks drained back into its own irrigation reservoir we were able to measure water use in each tank by measuring water volumes in the irrigation reservoirs.

Final electrical conductivities of the saline irrigation waters (EC_{iw}) of 4, 7, 9, 12, 15 dS m⁻¹ were achieved by adding CaCl₂, MgCl₂, NaCl, Na₂SO₄ and base nutrients to tap water (Table 1). For calculation of the treatment salt concentrations we used the EXTRACT CHEM model (Suarez and Taber, 2012) that predicts the EC and osmotic pressure of input solution compositions. Salinization was initiated after the first pair of true leaves was fully expanded on all the plants. Salts were added in 4 equal increments over a period of 4 days to avoid osmotic shock to the seedlings.

Measurements of the water content (θ) of the substrate were accomplished using calibrated (ln(θ) = -6.99 + 16V - 9.9V², R² = 0.91) dielectric soil moisture sensors (ECH₂O-10 probes,

Decagon, Pullman, WA, USA¹) inserted at 10 cm depth. A total of 16 ECH₂O moisture sensors were used in the study. The ECH₂O moisture sensors were connected to a multiplexer (AM25T, Campbell Sci., Logan, UT, USA), which in turn was connected to a data logger (CR10X, Campbell Sci.) to record the sensor output. The water retention curve was determined using the pressure plate method (Klute, 1986). The measured water contents from the sensors were then converted to matric potential using the water retention curve. Drought treatments were designed with soil water matric pressure targets of D1 (-200 to -300 kPa), treatment D2 (-400 to -500 kPa) and control D0 (no water stress, >-45 kPa).

Plant photosynthetic rate (Pn), stomatal conductance (g_s), transpiration rate (T_r) and concentration of intercellular CO₂ (C_i), were measured on the third fully expanded upper leaves along the right abaxial side of the leaf lamina between 10:00–11:00 am one week before harvest using a portable Li-Cor 6400 Photosynthesis System. The measurement conditions were leaf chamber PAR (photosynthetically active radiation), 1100 μmol m⁻² s⁻¹; leaf to air vapor deficit pressure, 1.7–2.6 kPa, leaf temperature 20–22 °C and chamber CO₂ concentration 400 μmol mol⁻¹. The leaf greenness of the spinach plants was determined by a portable chlorophyll meter (SPAD-502; Konica Minolta Sensing, Inc., Japan) at the time of the gas exchange measurements and given as leaf chlorophyll values. SPAD measurements were made on the youngest, fully expanded leaves, then averaged (Khan et al., 2003).¹

We measured the fresh weight of the above ground parts of all plants (three rows) for each replication (three). A plant from each of three rows and for each replication (9 plants per treatment) was also measured for root length, root weight, shoot height, number of leaves, and leaf area. We measured the water consumption from each of the reservoirs below the tank and combined these data with the fresh weight yield to obtain the water use efficiency (WUE). WUE (g mm⁻¹) was calculated by dividing the total plant fresh weight (g) by the actual evapotranspiration (ET_a in mm) as described by De Pascale et al. (2011). ET_a of spinach grown in tanks was calculated using a water balance equation where

$$ET_a = \Delta V / A \quad (1)$$

and ET_a is the actual evapotranspiration (mm), ΔV is the water consumed by the crop (mm³) and A is the area of the experimental tank (mm²). The ΔV is calculated from,

$$\Delta V = V_i - V_f \pm \Delta S - D \quad (2)$$

where V_i and V_f and are the initial and final volumes (mm³) in the reservoir system, respectively, D is the water volume discharged out of the system and ΔS is the change in sand tank moisture content (mm³). Since we have a closed system (tank plus reservoir) with no discharge, D is zero. The experimental design was a randomized complete block design with three replications for yield, vegetative parameters, ion composition, and physiological parameters. All of the data obtained from the measurements were evaluated statistically by analysis of variance to compare the effects of drought levels and irrigation waters using SPSS package software (SPSS, 2004). The differences among the means were compared using the Duncan multiple tests. General Linear Model analysis was performed to determine the relationship between selected parameters.

In order to evaluate the abiotic stress models with an additional data set, we included data from a preliminary experiment conducted during December 2012– March 2013. The general details of the salt tolerance aspect of the experiment are provided in Ors and

¹ Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

Table 1
Irrigation water composition of the salinity treatments.

Salinity level EC _i (dS m ⁻¹)	OP (kPa)	Ca ²⁺	Mg ²⁺	Na ⁺	SO ₄ ²⁻	Cl ⁻	K ⁺	NO ₃ ⁻	K ⁺ /Na ⁺
		(mmol _c L ⁻¹)							
0.85	-40	3.44	0.81	1.75	1.45	0.92	3.2	5.2	1.82
4.0	-160	8	2	29	19.5	19.5	3.2	5.2	0.11
7.0	-250	17	8	48	36.5	36.5	3.2	5.2	0.07
9.0	-310	21	12	63	48	48	3.2	5.2	0.05
12.0	-450	30.2	15	87.7	64.5	65.3	3.2	5.2	0.036
15.0	-590	28.5	20	118	82.1	84.3	3.2	5.2	0.027

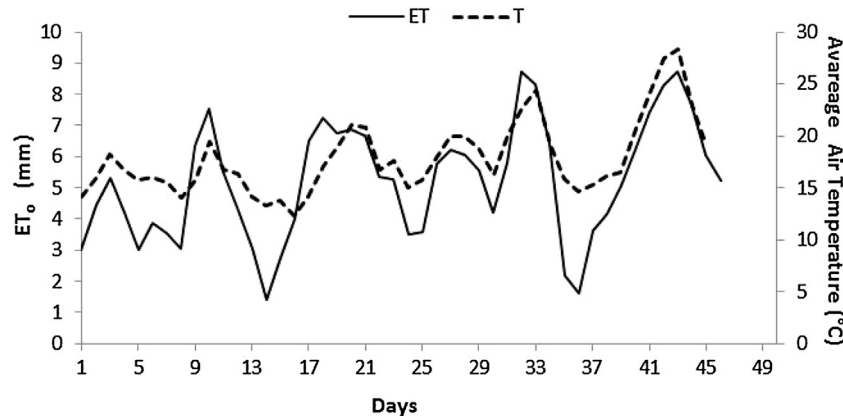


Fig. 1. ET₀ and average daily temperature (obtained from local CIMIS station) as related to days from planting to harvest (Ors and Suarez, 2016).

Suarez, (2016). In addition to the data reported earlier on salinity response, we also had treatments that combined salinity and drought stress. The salinity levels for this experiment were 0.85, 4.0, 7.0, and 9.0 dS m⁻¹ (Ors and Suarez, 2016). The drought levels were similar to those of the main experiment described above, D0, D1 and D2 levels correspond to soil water matric pressure of >−45 kPa, −200 to −300 kPa, and −400 to −500 kPa, respectively.

3. Results and discussion

The climate data were obtained from CIMIS weather station (California Irrigation management Information System, Weather station no. 44, UC Riverside) that provides hourly calculations. Daily mean ET₀ values and growing periods as number of days are presented in Fig. 1 (Ors and Suarez, 2016). The ET₀ values show the daily fluctuation and the general trend of increasing ET₀ with time, which relates primarily with increasing air temperature, as shown in Fig. 1.

As shown in Fig. 2 we were able to maintain relatively constant water content for each of the three drought treatments. The mean matric potentials in the root zone were calculated from the water content (Fig. 2) and the water retention curve (Fig. 3). The mean water contents were 0.16 (m³ m⁻³), 0.11 (m³ m⁻³) and 0.09 (m³ m⁻³), and the mean matric potentials were −44.6 kPa, −231 kPa, and −446 kPa for the D0, D1 and D2 drought (matric) stress treatments respectively.

The changes in water content and resultant salinity fluctuations between the wetting and drying periods were relatively minor. The volumetric water fluctuation in the non drought treatments of between 0.17 after irrigation and 0.15 just before irrigation represents a change of 12%, in both water content and in EC soil water (EC_{sw}), thus a mean EC_{sw} of 6% greater than the EC_{iw}. In a similar manner for the drought treatments D1 and D2, the volumetric water content fluctuation was between 0.10–0.13 and between 0.08–0.10 respectively, corresponding to mean EC_{sw} values being 6.5% and 5% respectively greater than EC_{iw} values.

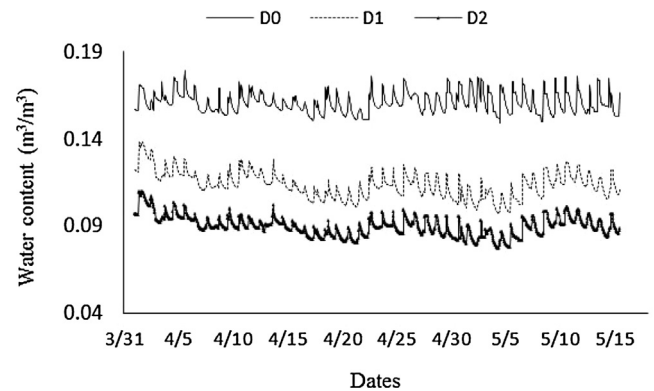


Fig. 2. Water content for water stress treatments (D0, D1, and D2) of the tanks measured by sensors.

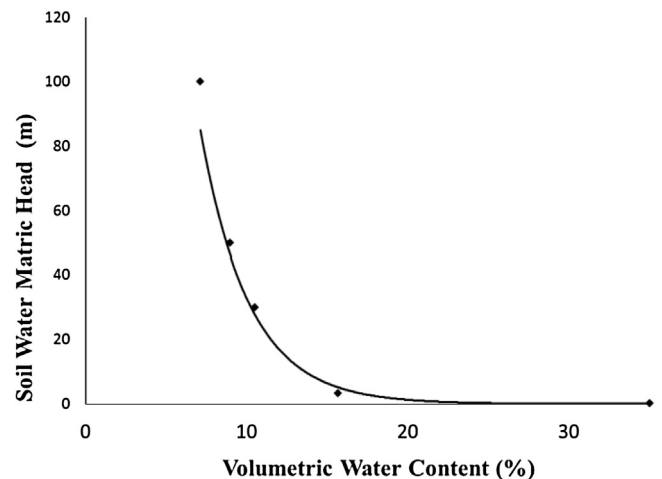


Fig. 3. Soil water retention curve determined with pressure plate apparatus.

Table 2
Two-way ANOVA of effects of drought (D) and salinity (S) and their interactions (DxS) on spinach plant.

Dependent variables	D	S	DxS
Fresh weight	365.14***	114.11***	27.63***
Dry weight	185.37***	70.71***	15.08***
Shoot height	602.29***	203.10***	23.01***
Root length	48.39***	14.16***	6.73***
Root weight	129.84***	70.00***	13.16***
Leaf number	40.28***	32.11***	16.43***
Leaf area	602.19***	204.00***	23.01***
Photosynthetic rate	21.29***	27.09***	3.98***
Stomatal conductance	15.61***	81.79***	10.04***
Chlorophyll content	2.56 ns	12.45***	1.57 ns

F-values. NS: nonsignificant, *** $\alpha = 0.001$.

3.1. Growth response to stress

There is extensive information on the reduction in growth related to salt stress for a large number of crops (Maas and Hoffman, 1977; Greive et al., 2012). Reduction in fresh weight due to drought stress has also been reported earlier for a number of plant species (Taylor and Ashcroft, 1972; Petropoulos et al., 2008; Álvarez et al., 2011; Čerešević et al., 2014). Both salt (Greive et al., 2012) and drought stress (Taylor and Ashcroft, 1972) vary widely among plants, thus requiring crop specific information for modeling and predictions.

The two-way ANOVA presented in Table 2 showed that there were highly significant differences in fresh weight related to salinity and to drought. The interaction of salinity and drought was also highly significant. Thus subsequent statistical analysis compared data at either the same drought stress or the same salinity. As shown in Fig. 4, under, well watered conditions (D0), the plant fresh weight initially increased at moderate salinity levels and then started to decrease. The yield decrease became significant at EC 9 dS m⁻¹ and was not significantly lower than the control until EC 15 dS m⁻¹. The highest fresh yield in D0 treatments was obtained at EC 4.0 dS m⁻¹ and the lowest was at 15 dS m⁻¹. The observation that initial increases in salinity result in increased yield has also been noted by others. For example, Yamada et al. (2016) found that sodium application increased yield of amaranthaceous plants including Swiss chard, beet and spinach, with spinach yield increasing with NaCl application of up to 80 mmol L⁻¹.

The significant yield loss in our experiment below control yield occurred above EC_{iw} 9 dS m⁻¹. Using the relationship determined for our soil media, where volumetric water content of the saturation extract = 0.35 and the average volumetric water content of the non water stressed treatments after irrigation was 0.17 (see Fig. 2) then for the control and salinity treatments EC_e = 0.486x EC_{sw}, where EC_{sw} is the EC of the soil water in situ. The EC of the irrigation water increased slightly over time as related to concentration of salts during the experiment. Measured volume losses from the reservoirs resulted in mean salinity increases ranging from 7% for control to 2% for D2. Correcting for concentration increases over time in the reservoirs and changes in EC between irrigation cycles (EC_{sw} 6% greater than EC_{iw}), the non drought EC_{sw} values exceed the initial EC_{iw} values by 13%. Thus the irrigation water of 9 dS m⁻¹ EC corresponds to an EC_e of 4.9 dS m⁻¹. This value is greater than the salinity threshold value of EC = 2.0 dS m⁻¹ cited in Greive et al. (2012) but less than the value of 6 dS m⁻¹ calculated from the hydroponic data of Yamada et al. (2016). However Greive et al. (2012) and Yamada et al. (2016) did not specify the variety used and varietal differences may be large. Also, Ors and Suarez (2016) determined that spinach salt tolerance was seasonally dependent.

As shown in Fig. 4 in the absence of salt stress, water stress decreased yield. The D2 treatment was highly significantly lower

than the control. Yield loss at water stress of -230 kPa suggests that spinach is less sensitive to water stress than other vegetable crops, as they generally experience yield loss in the range of -20 to -60 kPa (Taylor and Ashcroft, 1972).

Under the first level of water stress (D1), yields decreased relative to the no water stress treatments (D0), at all salinity levels as shown in Fig. 4. However, the D1 treatments had no sharp yield loss due to salinity until EC 12 dS m⁻¹ (Fig. 4). The highest fresh yield in D1 treatments were obtained at 7 dS m⁻¹ and a large decrease occurred in yield at EC 12 dS m⁻¹ and a further decrease at EC 15 dS m⁻¹.

Under more severe water stress (D2) the yields were lower under low salinity (relative to D0 and D1), but at high salinity the yields were similar in the D1 and D2 treatments (Fig. 4). Additionally under D2 water stress conditions, the highest salinity yields were not significantly different than those of the control (Fig. 4). Thus, for spinach, moderate water stress resulted in reduced yield but also reduced impact of salt stress as compared to non-drought conditions.

The data showed that some salt stress improved yield, but that with increasing water stress the optimal salinity level was shifted to higher levels of salinity (osmotic stress). Additionally, yield response to salinity and water stress were very different. At moderate salinity stress (-250 kPa) yield increased relative to the control, while at comparable water stress yield was decreased. At higher salinity stress (-450 kPa) yield was only slightly below the control (in the absence of water stress) while comparable water potential stress (in the absence of salt stress) were 65% lower than the control. Thus spinach fresh weight was more adversely affected by water stress than salt stress at comparable pressure potentials.

Additionally we conclude from the results of this experiment that moderate salinity even in the presence of moderate water stress increases yield of spinach raccoon cv. These results compliment earlier reports in the absence of drought that show that spinach has higher yield under mild salinity conditions (Downton et al., 1985; Mazloomi and Ronaghi, 2012; Osawa, 1963; Speer and Kaiser, 1991; Yousif et al., 2010; Yamada et al., 2016; Ors and Suarez, 2016)

3.2. Vegetative parameters

The vegetative parameters, dry weight, shoot height, root length, root weight, leaf number and leaf area of each treatment are shown in Fig. 5. Dry weight of the spinach plants (Fig. 5a) had the same trends with salinity and drought as fresh yield results (Fig. 4). All vegetative parameters showed highly significant differences with salinity and drought treatments and all salinity x drought interactions were also highly significant (Table 2). We thus separate the salinity and drought treatments and analyze salinity effects at the three drought levels and drought effects at the individual salinity levels. Increasing salinity resulted in first significantly increasing and then significantly decreasing shoot height (Fig. 5b), similar to plant fresh weight relations, with the effect being greater under non-drought conditions. As shown in Fig. 5b shoot height was significantly greater under non-water stress treatment (D0) than water stress treatments (D1, D2) for all salinity levels and D1 treatments shoot heights were significantly greater than those of D2 treatments for all but two salinity levels. Under severe water stress, intermediate salinity concentrations still had a favorable effect on shoot height but most differences were not significant (Fig. 5b).

Water stress caused significantly longer root length (Fig. 5c) across all salinity treatments, as compared to the non-stressed control treatment (D0). Root weight significantly decreased with increasing water stress for all salinity levels (Fig. 5d), however it first increased then decreased significantly with increasing salinity, for all water stress treatments. These length and weight results

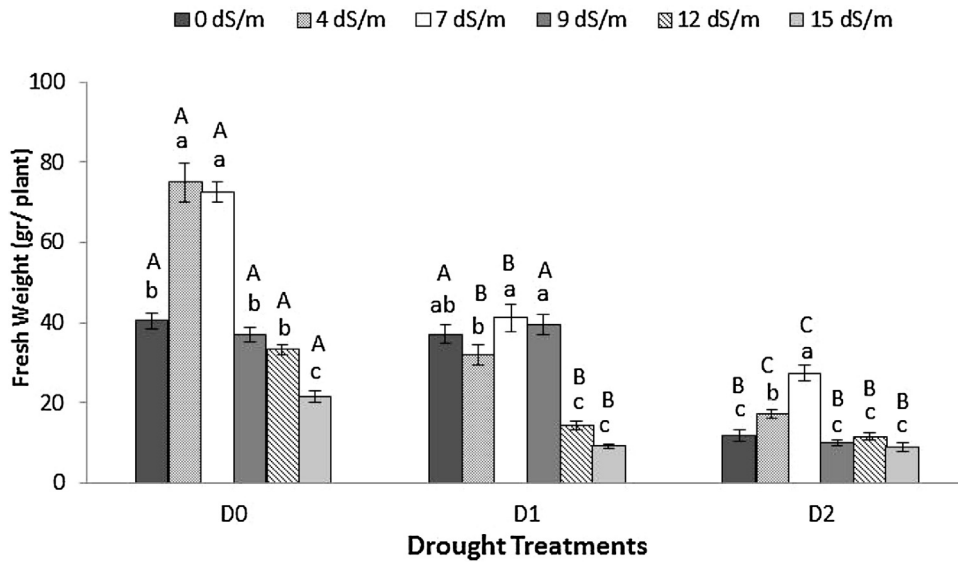


Fig. 4. Spinach fresh weight yield as related to irrigation water salinity under different water stress conditions. Yield data from the D0 treatments is taken from Ors and Suarez (2016). The D0, D1 and D2 treatments correspond to water potentials of -44.7 kPa, -231 kPa, and -446 kPa, respectively. Bars with different letters significantly differed at $P < 0.001$. Different lower case letters indicate significant differences in fresh weight related to salinity at the same drought level. Different capital letters indicate significant differences related to water stress at the same salinity level.

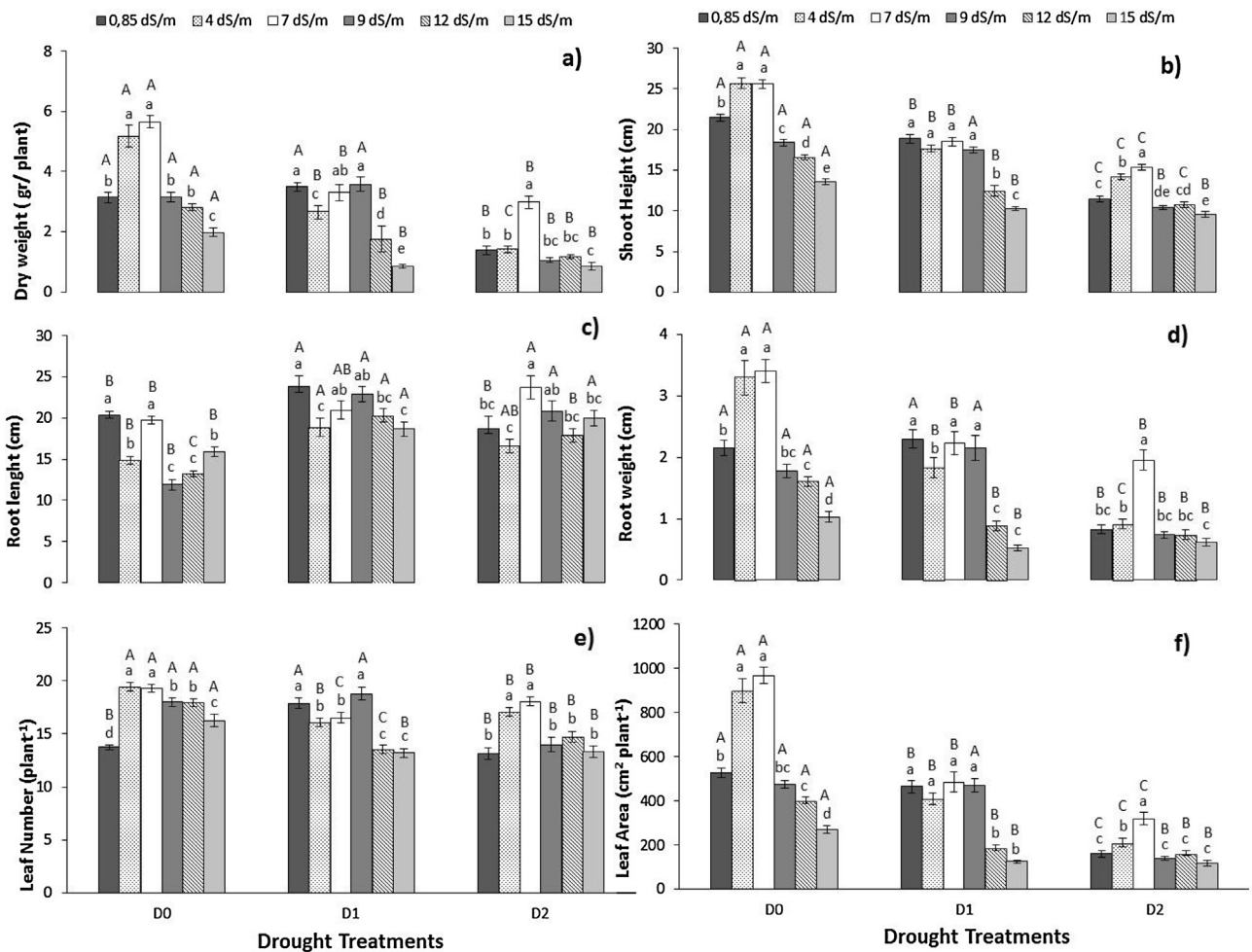


Fig. 5. Effects of increasing irrigation water salinity on dry weight, shoot height, root length, root weight, leaf number, leaf area of spinach under different drought stress levels. The D0, D1 and D2 treatments correspond to water potentials of -44.7 kPa, -231 kPa, and -446 kPa, respectively. Values represent means \pm SE. Bars with different letters significantly differed at $P < 0.001$. Different lower case letters indicate differences related to salinity at the same drought level. Different capital letters indicate differences related to water stress at the same salinity level.

indicate that there were changes in root structure to adapt to drought stress. Deep rooting was found to be a critical factor for drought resistance, influencing the ability of the plant to absorb water from the deeper layers of the soil (Franco et al., 2006, 2011). Under water stress, roots develop a capillary structure and elongate to obtain water from depth. Thus it seems reasonable that under non water stress conditions root structure would be shorter and thicker. In agreement with this, thinner roots under drought stress were reported earlier for *Silene vulgaris* by Franco et al. (2008).

Leaf number first significantly increased then significantly decreased with increasing salinity, but the differences were relatively small (Fig. 5e). Leaf number also decreased slightly (but significantly) with increasing water stress across all salinity levels (Fig. 5e). Plant leaf area increased significantly with initial increase in salinity level under non-water stress conditions and then decreased significantly and rapidly further increase in salinity (Fig. 5f). Water stress decreased the leaf area as compared to non-water stress treatment but even under severe water stress conditions (D2) intermediate salinity levels significantly increased plant leaf area. Reduced plant size, leaf area, and leaf area index (LAI) are all plant mechanisms for moderating water use and reducing injury under water stress (Blum, 2004; Mitchell et al., 1998).

3.3. Ion concentrations

The content of all major ions in spinach leaves was significantly affected by salinity and water stress treatments (Table 3), but concentrations did not decrease to values that indicate deficiency (except for K and Mg under non-saline, high drought treatment). Under well-watered conditions, Na and Cl increased with increased salinity treatments and K decreased. The irrigation water had a constant K level thus decreased leaf K was likely related to the decreased K/Na ratio in the irrigation water (Table 1) and thus plant ion competition. Spinach has been reported to utilize Na for osmotic adjustment as well as K (Sugiyama and Okada, 1988). Water stress in the absence of salt stress resulted in a small decrease in Ca concentration and decreased Mg, Na and K. Under saline

conditions Na, K, and Cl were reduced with water stress relative to the non-stressed condition. Similarly, both salinity and water stress significantly affected the Mg content of spinach leaves (Table 3), with Mg increasing with increasing salinity, consistent with increasing Mg in the irrigation water. The intermediate water stress treatments (D1) with irrigation waters of EC 12 and 15 dS m⁻¹ resulted in the highest plant Mg contents.

3.4. Gas exchange measurements

Gas exchange measurements conducted two weeks before harvest are shown in Fig. 6. Irrigation water salinity and water stress strongly affected leaf gas exchange parameters. Pn, Tr and g_s were highly significant, as was the interaction (Table 2). Chlorophyll content was highly significant for salinity but not for water stress (Table 2). As shown in Fig. 6a, photosynthesis first increased slightly (non-significant) then subsequently decreased significantly in the non water stressed treatments. It is reported that photosynthesis is among the primary processes affected by salinity (Munns et al., 2006), and may be responsible for at least part of the yield reduction caused by salinity (Prior et al., 1992; Munns, 2002). Our photosynthesis data are consistent with our yield data, in that there was no reduction in both yield and photosynthesis until EC above 7 dS m⁻¹.

However, in our results under water stress there was a reduction in yield but generally no reduction observed in photosynthesis (expressed per leaf area) under both saline and non-saline conditions (Fig. 6a). We conclude that for spinach, the yield loss that occurs with water stress is not attributed or associated with decreases in leaf photosynthesis rate. This is in contrast to earlier findings with chard (Delfine et al., 2003). They considered that horticultural crops in Mediterranean climates have high growth rates sustained by high gas exchanges and thus, they are particularly vulnerable to drought stress. In their experiment the reduction in growth rate occurred at the same time as the reduction in gas exchange.

Changes in transpiration rate in each treatment and experiment are presented in Fig. 6b; as expected salt stress significantly

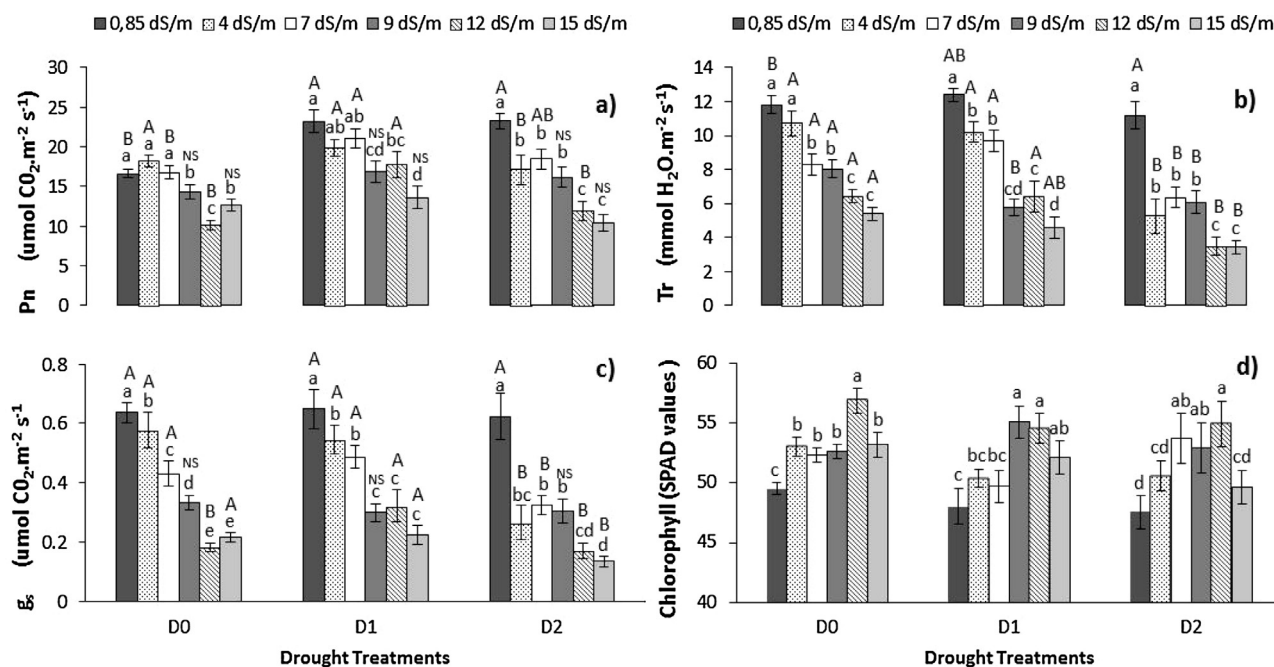


Fig. 6. Effects of increasing irrigation water salinity on Pn, Tr, g_s, and chlorophyll content of spinach under different water stress levels, The D0, D1 and D2 treatments correspond to water potentials of -44.7 kPa, -231 kPa, and -446 kPa, respectively. Bars with different letters significantly differed at $P < 0.001$. Different lower case letters indicate differences related to salinity at the same drought level. Different capital letters indicate differences related to drought at the same salinity level.

Table 3
Mineral composition of spinach leaves.

Drought Treatments	EC	Ca	Mg	Na	K	Cl	S
	dSm ⁻¹	mmol kg ⁻¹					
D0	0.85	248a	628b	216e	3021a	269d	176c
	4.0	188d	709a	632d	2937a	88317c	179c
	7.0	204bcd	731a	903c	2998a	999bc	189b
	9.0	220abc	768a	1278b	2346b	942bc	188b
	12.0	223ab	737a	1386b	2329b	1114b	198a
	15.0	192cd	733a	1752a	1994c	2300a	197a
D1	0.85	202bc	393d	117e	2430ab	433d	151d
	4.0	208bc	612c	537d	2491ab	2387b	190cd
	7.0	242ab	764ab	1043c	2674a	3197a	214bc
	9.0	173c	705bc	13690b	2357b	1388c	235a
	12.0	232ab	882a	10221c	2222b	1229c	228ab
	15.0	255a	832a	1524a	1831c	1345c	218bc
D2	0.85	203bc	289c	113d	1912c	299b	88d
	4.0	231ab	668b	784c	2497a	734ab	165bc
	7.0	195c	623b	78079c	2219b	1382a	249a
	9.0	227ab	643b	976b	1861c	1178a	204b
	12.0	244a	794a	1371a	1971c	1338a	224ab
	15.0	198c	816a	1477a	1822c	1346a	253a

Different letters indicate differences related to salinity at the same drought level. Values followed by the same letter are not significantly different at $P \leq 0.001$.

decreased transpiration, and at all drought stress levels. The effect of salinity under severe water stress (D2) was more evident compared to effect of salinity under well-watered conditions (D0). Differences related to drought under non-saline conditions were not significant (Fig. 6b). The stomatal conductance (g_s) data (Fig. 6c) was very similar to the Tr data, except that the g_s decrease with increasing salinity was more pronounced. The severe reduction in Tr and g_s in response to salinity might represent an adaptive mechanism to cope with excess salt rather than merely a negative consequence of it (Flanagan and Jefferies, 1989; Tattini et al., 2002). Water stress did not reduce Tr and g_s under non saline conditions (Fig. 6b and 6c), however salinity stress caused a significant decrease for each drought level. These results indicate that the spinach plant response to water and osmotic stress is indeed different, in agreement with the yield data discussed above. The sharp decrease in stomatal conductance with increased saline water irrigation even under severe water stress (D2) indicated that under water stress, salinity was still the more limiting factor on g_s . Decreased stomatal conductance under salinity has been reported on spinach earlier (Delfine et al., 1998; Yousif et al., 2010).

SPAD values are proportional to the amount of chlorophyll present in the leaf (Ling et al., 2011). We observed a general increase in chlorophyll with increasing salinity for all three water stress treatments, as shown in Fig. 6d. Increasing water stress had no statistically significant effect on chlorophyll content (Fig. 6d). Chlorophyll content is expected to decrease under stress conditions. However, an increase in chlorophyll content of the plant under water stress was observed earlier with purslane (Rahdari et al., 2012) and spinach (Xu and Leskovar, 2015). Our results are also consistent with the almost constant chlorophyll content under salt stress observed with cucumber (Yildirim et al., 2008) and sunflower (Liu and Shi, 2010).

3.5. Water use efficiency

As shown in Fig. 7 there were large differences in WUE related to salinity. In all instances an initial increase in salinity increased the WUE to approximately double the value of the control (Fig. 7a). Subsequent increases in salinity resulted in decreased WUE. The WUE did not change in a simple manner with increasing salinity but it can be explained if we compare WUE (Fig. 7) to the yield data (Fig. 4). At each water stress level the WUE was positively and well related to the yield, moderate salinity increased both yield and

WUE. This means that for spinach there can be not only significant yield increase but also significant water savings when irrigating with moderately saline water.

Under conditions of high salinity WUE is reduced, thus more water is required than assumed by the relation

$$\frac{ET_a}{ET_c} = \frac{Y_a}{Y_{max}} \quad (3)$$

Where ET_a is measured ET, ET_c is the crop ET at maximum yield and Y_a and Y_{max} are the actual and maximum yield, respectively.

In contrast, there was no consistent trend in WUE related to water stress, and significant effects occurred mostly at EC 7 and 9 dSm⁻¹ where stress increased WUE. Thus water stress does not appear to be a viable general strategy for saving water when growing spinach. This is in contrast to the findings of Franco et al. (2006) and Fernández et al. (2006) who reported higher WUE for drought stressed plants. A likely explanation of this difference is that their results are expressed in terms of yield and water application while we measured water consumption under stress.

3.6. Modeling plant yield response to combined salinity and water stress

One model developed for predicting plant response to salinity and drought stress is that of additive stress (Childs and Hanks, 1975) where the response is related to the combined matric and osmotic potential; in this instance the following equations can be utilized,

$$\alpha_s = \frac{1}{1 + \left(\frac{h}{h_{50}}\right)^b} \quad (4)$$

$$S(h) = (\alpha_s(h))S_p \quad (5)$$

Where α_s is the dimensionless stress response function (relative yield) h is the sum of osmotic and matric stress and h_{50} is the stress value at which there is a 50% yield loss, and b is an empirical fitting parameter generally set to 3.0.

An alternative model considers the response to stress to be additive (van Genuchten, 1987). Thus

$$\alpha_{\psi_s} = \frac{1}{1 + \left(\frac{h}{h_{\psi_{50}}}\right)^b} \quad (6)$$

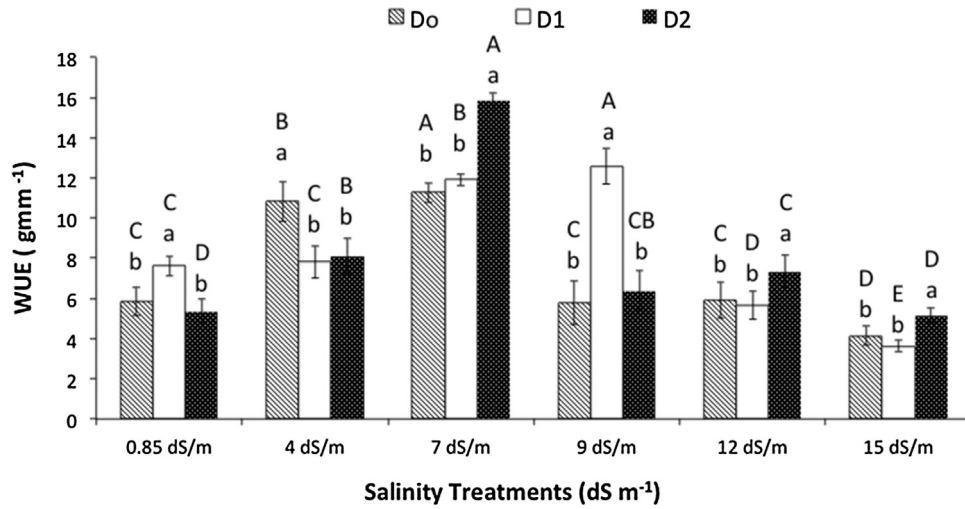


Fig. 7. Water use efficiency as related to salinity and water stress. Bars with different letters significantly differed at $P < 0.001$. Different lower case letters indicate differences related to salinity at the same drought level. Different capital letters indicate differences related to drought at the same salinity level.

$$\alpha_{\phi s} = \frac{1}{1 + \left(\frac{h}{h_{\phi 50}}\right)^b} \quad (7)$$

And in this case,

$$S(h_{\psi}, h_{\phi}) = [1 - ((\alpha_{\psi}(h_{\psi}) - 1) + (1 - \alpha_{\phi}(h_{\phi})))S_p] \quad (8)$$

where S_p is the potential water uptake rate and S is the predicted uptake rate. A third model, used in the UNSATCHEM model (Suarez and Simunek, 1996) uses a response function where the product of the response to water and salt stress is calculated, using different response functions for matric and osmotic stress ($h_{\psi 50}$, and $h_{\phi 50}$ respectively).

$$S(h_{\psi}, h_{\phi}) = (\alpha_{\psi}(h_{\psi}))(\alpha_{\phi}(h_{\phi}))S_p \quad (9)$$

Further, we can utilize the following yield function (Steward and Hagan, 1973; Suarez and Simunek, 1996) instead of Eq. (3)

$$\frac{Y_a}{Y_{max}} = 1 - B_0 \left(1 - \frac{ET_a}{ET_p}\right) \quad (10)$$

where B_0 is the slope of the relative yield versus $ET_a - ET_c$

Using Eq. (10) we can correct for non-constant WUE. That WUE is not constant for spinach yield under salinity is demonstrated above in Section 3.5. Although it is convenient to characterize salt stress in terms of the E_c , comparison among experiments is feasible only in well watered experiments of comparable soil texture. As described by the relationships above, plants respond to in situ salt and matric stress, hence modeling response is in units of osmotic or matric potential.

Dudley and Shani (2003) examined the relationship between water application, salt stress and yield for a field melon experiment. They modeled the yield by optimizing the values of $h_{\psi 50}$, and $h_{\phi 50}$, assuming water uptake to be directly proportional to biomass yield. The soil $h_{\psi 50}$ was thus calculated based on the water consumption estimated from the yield, and was not measured. The UNSATCHEM formulation (Eq. (9)) provided a satisfactory representation of their data. Another important difference between our experiments and those of Dudley and Shani (2003) is that they varied the quantities of water applied, independent of salinity stress while we controlled water content (and thus water stress) rather than irrigation quantities.

There is considerable data including that for corn, alfalfa, sorghum (James et al., 1982) that indicate that yield and ET are linearly related when insufficient rain or irrigation water is applied. In

this case dY/dT is constant but the WUE decreases with decreasing yield (because of the intercept of a finite ET at zero yield).

There is also considerable data that indicates that in a similar manner, relative yield and relative transpiration are also linearly related (Nimah and Hanks, 1973; Bresler and Hoffman, 1986), however this need not indicate that Y/ET_a is constant. Unlukara et al. (2010) fit a linear relation ($r^2 = 0.97$) between $(ET_{max} - ET_a)/ET_{max}$ and $Y_{max} - Y_a/Y_{max}$, with a decrease in WUE from 4.8 g/L under non-saline conditions to a WUE of only 2.5 at $EC = 7 \text{ dS m}^{-1}$ for eggplant (fruit yield). A similar increase in WUE with increasing salinity can be calculated from the yield-ET data given in Skaggs et al. (2006) for alfalfa and wheatgrass; they also obtain a linear relation between yield and ET ($r^2 = 0.85$).

In addition, other factors have been shown to greatly impact WUE, such as nutritional status. Ritchie (1983) showed that increasing N fertilization from 90 to 134 kg ha⁻¹ of N caused a change in Y/ET of 15%; increases in P fertilization on wheat caused comparable changes as well.

The results of our study (Fig. 7) for spinach as well as studies with other crops (Semiz et al., 2014) indicate that WUE is not constant and can vary considerably in relation to salinity. The differences between measured water consumption and calculated values using Eq. (3) may be important if one uses yield to calculate water consumption and then in turn matric stress, as most researchers have done.

The response of yield to salt stress in the absence of matric stress is shown in Fig. 8a. The yield increases from -40 kPa to -200 kPa and then decreases, with $h_{\psi 50}$ at -465 kPa (from the fitted line). We used the fitted equation,

$$Y_a/Y_{max} = 176.436e^{-\left(\frac{-x-216.234}{255.371}\right)^2} \quad (11)$$

to predict relative yield from osmotic pressure. The response to matric stress in the absence of salt stress shows a maximum yield under no stress and a $h_{\psi 50}$ at -400 kPa. Preliminary analysis suggests that matric potential stress is more detrimental than osmotic salt stress. However, spinach is relatively unique in that low salinity does not produce the optimal yield and some increase in salinity is beneficial, thus the matric and osmotic stress functions are very different.

Using the relationships in Fig. 8a and b as stress response functions we next compare results from the combined stress treatments with the various model predictions. We examine only the data with both osmotic and matric stress, meaning we did not use any of

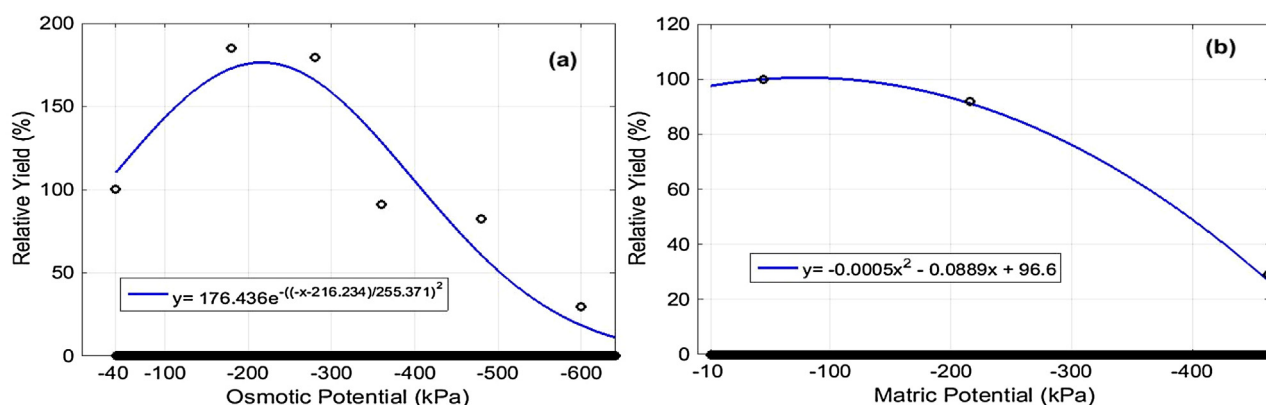


Fig. 8. The response of yield to salt stress in the absence of matric stress (a) and the response of yield to water stress in the absence of osmotic stress (b).

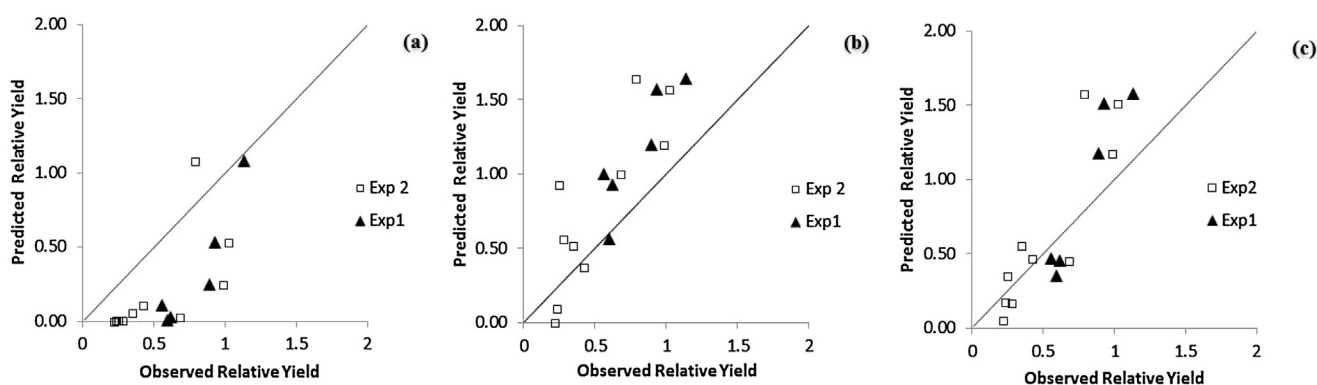


Fig. 9. Predicted and observed relative yield where predictions are based on a) adding the measured matric and osmotic potential and predicting yield from the osmotic potential relationship, Eq. (5), b) adding the osmotic potential response and the matric potential response using the regression relationships from Fig. 8 using Eq. (8) and c) multiplying the individual matric and osmotic response functions (Fig. 8) and using Eq. (9).

Table 4

Fresh weight per plant (g) of the supplementary spinach experiment (given as Exp1) for combined water and salt stress.

Drought treatments kPa	Salinity treatments (dS m ⁻¹)		
	4	7	9
	Fresh weight (g)		
D1 (-200) to (-300)	113.2	93.3	89.7
D2 (-400) to (-500)	55.9	62.4	59.

the data shown in Fig. 8a and b, data where only one stress was imposed. Shown in Fig. 9a is the predicted and observed results from the additive water and matric potential model (Eq. (5)), Fig. 9b shows the results where the stresses are added (Eq. (8)), and Fig. 9c shows the results where the individual response functions are multiplied (Eq. (9)).

We present spinach fresh yield data from this experiment (Fig. 4), as well as results from a preliminary spinach experiment under similar conditions (data shown in Table 4), along with the predictions based on the three models.

Using Duncan test we determined that for the combined data sets from both experiments, there were no significant differences between model a (added potentials) and model b (added response) but model c (multiplicative response) was significantly different ($P < 0.05$) than model a and b. Also, model c had the smallest mean% error as compared to the data. Similar results were obtained using data only from the main experiment (labeled Exp 2 in Fig. 9). In all instances (combining all data or analyzing the experiments separately) when ranking the fit model c had the smallest mean error followed by model b and then model a. Adding the individ-

ual responses from the stresses (Fig. 9b) results in over prediction of relative yield. Using the multiplicative model improved yield predictions but there was still over prediction for the high relative yield values (where the salinity response provides yield above the control values). We attribute this relatively marginal prediction capability to the complex spinach yield response at low salinity (Fig. 8a). Predictions can be greatly improved by regression of the predictions and observations in Fig. 9a, b and c, but we attempted to independently predict relative yield. Additional experiments with a crop that has a less complex salinity response would enable further evaluation and development of a combined abiotic stress response model.

4. Conclusion

Yield and physiological responses of Spinach plants (*Spinacia oleracea* L., cv. Ragoon) to osmotic and matric stress were very different. Yield did not correlate in a simple manner with salinity. At moderate salt stress (-250 kPa) yield increased while at comparable water stress yield decreased relative to the control. At high salt stress (-450 kPa) relative yields were only slightly below the control while at comparable water stress relative yields were 65% lower than the control. Plants grown under water stress also experienced less sensitivity to salt stress than plants grown under non drought conditions, consistent with salinity response data observed earlier for other abiotic stresses. WUE initially increased with increasing salinity, then decreased in a manner mirroring the yield response to salinity. There was little effect of water stress on WUE. Increases in WUE under water stress were observed only at intermediate salin-

ity, thus water stress is not likely viable for saving water unless salinity is in the ECE range of 3–4.5 dS m⁻¹.

Since WUE varied greatly as related to salinity, determining crop response to combined salt and water stress requires direct measurement of matric potential rather than indirect calculation assuming water consumption is proportional to relative yield. Spinach relative yield under combined water and salinity stress was best predicted by the multiplicative stress response model. Nonetheless, perhaps due to the complex nature of the spinach yield response to salinity, model predictions exhibited considerable error when compared to measured yields.

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