Uptake and distribution of ions reveal contrasting tolerance mechanisms for soil and water salinity in okra (*Abelmoschus esculentus*) and tomatoes (*Solanum esculentum*)

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Running title: Salinity tolerance mechanisms in tomato and okra

Abstract

Okra and tomatoes are major vegetable crops commonly grown under irrigation, and understanding whether they respond to salinity by withstanding (*tissue tolerance*) or avoiding (*salt exclusion*) accumulation of salt in the shoots will assist with management for optimising yield under declining soil and water resources. Both crops were grown in non-saline (0.0 dS/m) and saline (3.0 dS/m) loamy sand and drip irrigated with water of 0.0, 1.2 or 2.4 dS/m. Differences in the growth and yields of the two crops under saline conditions were associated with uptake and distribution of cations, especially Na. The tomato employed *tissue tolerance* mechanism in response to salinity and produced fruits even when shoot/root Na concentration was >3.0; concentrations of Na in tomato tissues was in the order shoots > roots ≈ fruits. Okra

was sensitive to shoot Na such that a shoot/root Na concentration as low as 0.13 reduced yield by as much as 35%; this crop thus employed *salt exclusion* mechanism and minimised shoot accumulation of Na, which was distributed in the order fruits > roots > shoots. Root and shoot concentrations of Na, P and S were correlated with flower abortion and negatively correlated with yield and yield components in both crops. Fresh fruit produced on the saline soil were reduced by 19% in tomato compared with 59% in okra, relative to yields on non-saline soil. Water salinity reduced fresh fruit yields by as much as 36% with every unit (dS/m) rise in water salinity compared with 27 % in okra. Soil salinity significantly reduced water-use by 6% in tomatoes and 29% in okra, but had no impact on water use efficiency (WUE) that averaged 3.9 g of fresh fruits/L for tomatoes and 1.75 g/L for okra. Every 1.0 dS/m rise in water salinity reduced water-use by 0.33 L in okra and 3.31 L in tomatoes, and reduced WUE by 2.61 g/L in tomatoes and 0.53 g/L in okra. Soil salinity explained <5% of the variance in yields in tomatoes and 10–20% in okra, while water salinity explained 48–68 % of the variance in tomatoes and about 40% in okra. We conclude that (1) water salinity was more injurious to yield in both crops than soil salinity, and (2) yield losses due to salinity can be minimised through frequent leaching of soil salt under okra and increased irrigation intervals in tomatoes.

Keywords: flower abortion, fruit yield, root growth, shoot/root Na, salinity, water-use, water-use efficiency

1.0 Introduction

Crop species differ in their responses to saline conditions as a result of their relative tolerance to ionic phytotoxicity. Two basic mechanisms that define crop tolerance of salinity involve 'salt exclusion' or 'tissue tolerance', each of which is implemented to a varying degree by different

species with halophytes being adept almost equally at both (Munns et al, 2006). *Salt exclusion* mechanism involves prevention of ions from getting into the transpiration stream by either minimising their uptake from the growth media or if taken up expelling the ions into the bathing/rooting medium, and/or restrained rates of root to shoot transfer. In *tissue tolerance*, on the other hand, salts are sequestered in vacuoles of cells, especially in root tissue, thereby restricting their transport into the cytoplasm of shoot tissues that are generally more sensitive to salinity stress than roots, and where more physiological and enzymatic processes occur (Rogers and West, 1993; Maas and Hoffman, 1977). Either or both of these mechanisms can be overwhelmed resulting in phytoxicity and death under extreme salinity.

Severity of impact of salinity on the plant also varies with the source of salinity, i.e. from water or soil. Maas and Hoffman (1977) argued that plant response is primarily determined by the salinity of the irrigation water rather than of the soil**.** This is because availability and uptake of salt is governed by the availability of water and irrigation and/or rainfall reduces concentration of salts especially in the top layer of soil where most plant roots reside; furthermore, the salts are not available to the plant when the topsoil dries. They explained how salinity of the topsoil will approximate that of the irrigation water, but will be more severe at the bottom of the root zone (Maas and Hoffman, 1977). Such a situation should be particularly beneficial to plants that exclude salts as the predominant mechanism for salinity tolerance.

Several ions have been associated with causing phytoxicity under saline conditions and differ in their adverse impact on plants (Shannon and Grieve, 1999). Amongst these, Na and Cl are the most commonly associated with saline injury in plants, because they are easily accumulated in shoot where they interfere with enzymatic, developmental and physiological processes (Flowers, 2004; Ghanem et al., 2009; Munns et al. 2006; Shannon and Grieve, 1999). Stunted plant growth

and reduced yields have often been associated with excessive Na and Cl concentrations in the leaf that cause 'scorching' and 'firing' of leaves (Shannon and Grieve, 1999) and/or impairment of CO_2 assimilation and photosynthetic capacity (Yunusa et al., 2009). Low yields, however, could also result from late onset of reproductive phase and disruption of the processes involved. In tomatoes, poor flower viability was associated with accumulation of Na at the expense of K in the flower tissues and resulted in low fruit numbers, i.e. low sink capacity, and consequently reductions in the overall fruit yield (Ghanem et al., 2009). Accumulation of Na in the leaves can interfere with uptake of several other cations such as Ca, K and Mg. This can impair tolerance of salinity, which is generally enhanced when plants selectively accumulate K relative to other cations especially Na (Ashraf, 2004; Maksimović et al., 2010).

Tomato (*Lycopersicon esculentum* Mill.) and okra (*Abelmoschus esculentus* (L.) Moench) are important vegetable crops commonly grown under irrigation. Extensive assessments of growth, physiologic and biochemical responses to salinity have been undertaken for tomatoes (e.g. Ghanem et al., 2009; Barbagallo et al., 2012; del Amor et al., 2001; Perez-Alfocea *et al.*, 2010), but okra has received limited investigation in understanding its growth and yield responses to ionic stress arising from media and/or water salinity. In this study, we compared ionic uptake and partitioning, and their influence on the growth and yield of okra and tomatoes grown on saline soil and irrigated with water of different salinities. The aims were to (1) quantify relative tolerance to soil and water salinity, and (2) identify which of the two mechanisms of salinity tolerance is dominant in the two crops.

2.0 Materials and Methods

2.1 The Crops

This study was undertaken in a glasshouse at the School of Environmental and Rural Sciences, the University of New England, Armidale , Australia, over a 5-month period between March and July in 2012. Tomato (*Solanum esculentum* 'Rouge de Marmande') and okra (*Abelmoschus esculentus* 'Clemson's spineless') were raised from seeds obtained from a commercial supplier (Mr Fothergill's Seeds of Australia©). The seeds were sown into vermiculite (0.0 dS/m) and watered with tap water (EC of 0.025 dS/m) and they germinated within 6 days. The seedlings were allowed to grow for 2 weeks (heights of 8–12 cm for okra and 10–18 cm for tomato), before being transplanted into potted soils having different salinity. Three seedlings were transplanted per pot then thinned down to two after 10 days and finally to one after 20 days.

2.2 Salinity treatments

A loamy sand soil (83% sand and 10% clay) having base salinity of 0.018 dS/m, pH of 6.27, and water content at field capacity of 22% was collected from the nearby university research farm (30^o 29' 16" S, 151^o 38' 29" E). Of this soil, 6 kg was weighed into each of 48 thick plastic bags. Each bag was prepared to receive any one of the six treatments arising from factorial combinations of the following:

- 2 levels of soil salinity: Control (0.018 dS/m) and saline (3.0 dS/m)
- 3 levels of water salinity: 0.025 dS/m (control), 1.2 dS/m (medium salinity) and 2.4 dS/m (high salinity)

The soil salinity treatment of 3 dS/m was generated by adding 1% (w/w) table salt (NaCl) to half the number of the bagged soil samples; the other half of the bagged soil samples received no salt. The salinity and pH of the soil were determined using a bench top meter (Labchem-CP©Benchtop Conductivity/TDS -pH/mV meter, TPS Pty Ltd., Brisbane, Australia).

All 48 bags received additional 2 kg soil that was pre-mixed with 2.5 g compound (12.2% N, 5.1% P, 13.7% K, 4.5% Ca and 1.1% Mg) fertiliser (Muriate of Potash, CSBP Ltd, Australia). The bags were thoroughly shaken to achieve a homogeneous mixture. The bagged soil was then transferred into separate, numbered plastic pots each having a diameter of 25 cm at the top, 19 cm at the base and a depth of 24 cm. The three levels of irrigation water salinity (denoted as 0, 1.2 and 2.4 dS/m) were obtained using tap water (EC, 0.025 dS/m) and dissolving 0, 88 or 225 g NaCl/L, respectively. The tap water was considered as the control treatment. These solutions were then stored in separate 220 L PVC tanks.

2.3 Experimental layout and glasshouse weather

The experimental units (pots) were laid out on benches in a glasshouse in a randomized design. There were 24 pots per species, made up of two soil and three water salinity treatments in four replicates. The glasshouse was maintained at a diurnal temperature range of 24–28ºC and relative humidity of 30–50%.

2.4 Irrigation and nutrient management

Each pot was supplied with a dripper that ran from a hose from the respective tank containing the three saline solutions treatments. Each pot was irrigated at a rate of 100 mL for 5 min every day, and was brought to field capacity every week to avoid water stress. Leachate was collected separately from each pot every week and its volume determined. A 25 mL sub-sample of

leachate was stored in a dark cool room and later analysed for pH and EC, and the rest of the leachate returned to their respective pots to maintain prescribed salinity for the pots. The salinity of the water in the reservoirs was checked weekly to ensure that the prescribed salinity was maintained.

All the pots were each supplied with 200 mL nutrient solution (16 g/L of Aquasol Hortico containing NPK in 23:4:18) at 20 days after transplanting (DAT) and repeated when the plants in the control treatments (non-saline soil and non-saline water) showed symptoms of nutrient deficiency such as yellowing along the edges, curled leaves or early senescence of the older leaves. Ten grams of dolomite (9% Mg and 14.5% Ca) was added to each pot to correct a Mg deficiency for both crops evident by darkening of the fruit at the base in the control plants.

2.5 Measurements

2.5.1 Plant growth

The height and leaf number for each plant was assessed every ten days, while leaf area was determined on the last thinned plant at 20 DAT. Leaf area was measured with a scanning device (CID Portable Leaf Area Meter CI-202, CID Bioscience Inc., Camas, WA, USA). The relative chlorophyll concentration in the leaves was determined at 95 and 117 DAT using an optical device (SPAD 502 Plus Chlorophyll Meter, Minolta, Japan); the SPAD readings were converted to chlorophyll content according to Coste et al. (2010). Dates of appearance of first flower and fruit were recorded, while numbers of flowers and fruits were counted daily. Flower abortion was taken as the total number of fruits by the plant divided by the total number of flowers counted for the same plant during its lifetime.

151 **2.5.2 Fruit yield and quality**

152 The fruits were carefully picked as they matured and weighed fresh. Weight of fruits harvested
153 from individual plants were collated and summed after picking the last fruit to determine total
154 yield. Sugar content of tomato fruit was determined on 1.0 ml squeezed juice using a hand-held
155 device (Cobras© Accutrend© Plus instrument, Roche Ltd, Schweiz, Switzerland).

156 The fruits along with the shoots were dried at 60^{o} C for 72 h to determined dry weights. The
157 roots were recovered from the pots, thoroughly washed and also dried at 60^{o} C for 72 h. Total dry
158 weight per plant was determined as the sum of dry weights of fruits, roots and shoots.

159 **2.5.3 Water use**

160 Amounts of water supplied to, and drained from, each pot was recorded and water-use was
161 obtained as: water-use (WU) = water applied (L) - water drained (L). The weekly values for WU
162 were summed at the end of the trial to obtain total amount of water used by the plant in each pot.
163 Water-use efficiency (WUE) was determined as: total weight of fresh fruit (kg)/WU (L).

164 **2.5.4 Elemental uptake and distribution**

165 Dried samples of the fruit, root and shoot tissues were ground separately using a mortar and
166 pestle to pass a 2 mm screen. Subsamples of the ground tissues (~0.5 g) were digested in
167 concentrated HNO_3 (70%) and H_2O_2 (30%) in a microwave digester. The digests were brought to
168 final volumes of 100 mL with double-deionized water, and the elemental contents determined
169 using ICP-MS (ICP-MS Agilent 7500CE, Agilent Technologies, Inc. Santa Clara, USA).

170 **2.6 Statistical analyses**

171 All data collected were subjected to analysis of variance (ANOVA) using SPSS Statistics for

Windows v17.0 (SPSS Inc., Chicago, USA). The data were first tested for normality; Levene's test was used to determine equality of variances among the treatment groups. Statistical significance was determined when $p \leq 0.05$. Tukey's highest significant difference (HSD) was used for mean separation when a treatment effect was significant; data presented here are means of at least four replicates. One aim of this work was to examine inter-relationships between plant growth and yield variables, root and shoot nutrient concentrations vis-à-vis the salinity treatments. The number of variables, however, was large (>30), therefore principal component analysis (PCA) was used to reduce the dimensionality of the data by extracting and summarising most of the variance in the multivariate data into a few dimensions. The variables analysed here had different units (mass, area, number, etc.), so the PCA analyses used a correlation matrix as input.

3.0 Results

3.1 Growing conditions

The temperature in the glasshouse fluctuated within 15% of their set values during the course of the study. There was a spike in temperature in mid-July that caused the humidity to deviate by up to 25% from the set range of 30–50%, otherwise the humidity remained within 10% of the desired range throughout the study period. The photosynthetically active radiation (PAR) within the glasshouse ranged between 260 and 900 µmol $m^{-2}s^{-1}$ during daylight hours.

3.2 Plant growth characteristics

Responses of vegetative and reproductive growth traits to salinity are summarised in Table 1. Leaf production, leaf area and height of tomato plants were reduced on the saline soil and by saline irrigation. On the saline soil, tomato plants were 12% shorter, had 25% fewer leaves that

had 73% smaller total area, compared with those on the non-saline soil. Relative chlorophyll concentration, flower numbers and flower abortion in the tomato were insensitive to soil salinity. Irrigation water salinity significantly reduced leaf area, numbers of leaves and flowers and plant height, but increased relative chlorophyll concentration and flower abortion in this crop.

All growth variables in okra were reduced on the saline soil and by saline irrigation, while flower abortion increased in response to the salinity treatments (Table 1). In okra, flower abortion increased under salinity treatments, and more so than in tomato. Of all the traits examined in both species, leaf area was the most sensitive to salinity irrespective of its source. Only weak interactions were observed between soil and water salinity in their effects on the measured variables in both crops, but were strong on chlorophyll concentrations in tomato.

3.3 Fruit yield and quality

Saline irrigation severely reduced the yield and yield components of tomato (Table 2). When compared with the control, the 2.4 dS/m water salinity, reduced fruit yield by 88%, fruit number by 77% and fruit size by 54%. Soil salinity also negatively affected tomato yield and yield components, except the average fruit size. Water and soil salinity, however, increased sugar concentration in tomato fruits, and for plants on the non-saline soil, irrigation with saline water increased fruit sugar concentration by up to 34%, whereas on the saline soil, the increase was 74% (Table 2).

The yield and yield components of okra were significantly reduced by water and soil salinity; the exception was fruit size (Table 2). Irrespective of soil salinity, increasing water salinity from 0.0 to 2.4 dS/m reduced fruit yield and number by more than 50%, but fruit size was comparatively less sensitive. Okra lost more fruits on saline soil (19%) than the tomato (7%). Total fruit weight

per plant was the most responsive yield variable to both water and soil salinity in okra as in the tomato. The yield response to irrigation water salinity was driven primarily via fruit number whereas the response to soil salinity was almost equally driven both yield components.

3.4 Total biomass production and its partitioning

The dry weight of tomato plants fell significantly with water salinity on both saline and non-saline soils (Table3). The weights of the plant components (roots, shoots and fruits) followed similar trend in their response to water salinity. On both soils, water salinity reduced root/shoot and fruit/shoot (putative harvest index). In contrast to water salinity, soil salinity had no significant effect on plant dry weight or on its partitioning in the tomato.

The severity of adverse impact of salinity on plant dry weight and its components (roots, shoots and fruits) in okra increased with water salinity, especially on the saline soil. Water salinity also reduced root/shoot ratio but fruit/shoot ratios were unaffected. Soil salinity affected okra total biomass, its components and partitioning (Table 3).

3.5. Water use and water-use efficiency

Water used by tomato was reduced on saline soil and by salinity of the irrigation water (Table 4), and more so with water salinity (~17%) than soil salinity (6%). While water-use efficiency (WUE) or the amount of fresh fruits produced for tomato per unit volume of water was not affected by soil salinity, it fell with each increase in the salinity of irrigation water. The deterioration in WUE with increasing salinity of the irrigation water was more severe on saline soil than on non-saline soil. There were significant correlations between either the water-use or WUE with water salinity:

Water-use: $y = -3.31x + 45.35$, $r^2 = 0.81$, $n = 24$ 1a

WUE: $y = -2.61x + 7.69$ $r^2 = 0.59$, n = 24 1b

Water use for okra was reduced by water and soil salinity (Table 4). On the non-saline soil, water-use was only reduced when water salinity was raised to 2.4 dS/m, but on the saline soil water-use was reduced with every increase in water salinity. On average, okra used about 11 L of water less when grown on saline soil than on non-saline soil. The WUE for okra fell with every increase in water salinity on the non-saline soil, dropping by 52% at the highest water salinity treatment, while it declined by 43% with saline irrigation on the saline soil. There was, however, no significant difference between the two soils in their mean WUE. The water-use and WUE were related with water salinity as:

Water-use: $y = -0.334x + 36.8$, $r^2 = 0.45$, n= 23 2a

WUE: $y = -0.53x + 2.22$, $r^2 = 0.33$, n = 23 2b

3.6 Elemental uptake and distribution

Soil salinity did not alter nutrient concentrations in tomato tissues, but in the okra it increased concentrations of Na and P in the roots and fruits, in addition to S in the roots (Fig. 1). Concentration of nutrients in the root of tomatoes was in the order Na > Ca ≈ Mg > K > S > P, while in the shoot the order was Na > K > Ca > Mg > P ≈ S. Elemental concentrations in the fruit was dominated by Na on both saline and non-saline soils.

Soil salinity significantly increasing concentrations of Na, P and S in the root, P in the shoot and Na and P in fruit in okra; Na was the dominant nutrient in both root and fruit, while Ca and K dominated in the shoot (Fig. 1). Concentrations of Na and K in the roots, and of Ca, K and Mg in the shoots, were higher for okra than found in tomatoes. Saline irrigation increased concentrations of Na in all the three tissues of the plant, in addition to those of S in the root and

fruit, and of K, P and S in the shoot, in the tomato (Fig. 2a – c). Saline irrigation reduced concentration of Ca, but increased that of K, in the shoot. In okra, saline irrigation increased concentrations of Na in all the plant parts, and reduced those of Ca and K in the shoots (Fig. 2d – f). Shoot concentrations of Na in okra was not more than a third that found in the tomato, while those of Ca, K and Mg in okra were twice those in the tomato. In both crops, soil and water salinity generally increased shoot/root Na concentrations, more so in the tomato in which the ratio was 0.84 – 3.06 in saline conditions compared with 0.06–0.38 in okra (Table 3).

3.7 Relationships between ionic concentrations and plant growth and yield variables

Inter-relationships between root and shoot mineral nutrient concentrations, plant growth and yield variables for each species are displayed along the first two orthogonal dimensions from PCA for the two crops (Fig. 3). For tomato, the inter-relationships between the nutritional status and plant traits are shown along the first two PCA dimensions, which jointly extracted about 60% of the total variance (Fig 3a). The first dimension (40% of the variance) reflects impact of water salinity and shows that there were positive associations among the shoot P, K, S, Na, Cu, Zn, Mn, root Na concentrations, and floret abortion (all with moderate to high positive loadings), and all these were negatively correlated with fruit yield, water-use, WUE, fruit number per plant as well as shoot Ca level (all with high negative loadings). The second dimension (20% variance) revealed the impact of soil salinity. It contrasted root nutrient status (positive loadings) with leaf number and area, plant height and floret abortion (all with negative loadings) to show a generally inverse association between the two sets of variables. The impacts of the three water salinity levels were distinctly separated, with hardly any overlaps amongst the symbols, along the first principal dimension (Fig. 3b). The influences of the soil salinity treatments were apparent along the second dimension albeit less distinctly, with some overlaps between blue and red symbols,

283 than observed with water salinity treatments.

284 For okra, the first dimension extracted 45% of the total variance as a measure of the impact of
285 water salinity on tissue nutrient concentrations, yield and growth variables (Fig. 3c). This
286 dimension reveals a negative correlation between root and shoot Na and P status (high negative
287 loadings and closely associated), on the one hand, and the plant growth and yield variables as
288 well as shoot concentrations of Mn, Mg, Ca, S and K status (high positive loadings), on the
289 other. There was thus a dichotomous association amongst these variables. In one group were Na
290 and P either in root or shoot that had negative associations with WU, WUE, fruit yield and
291 growth variables (chlorophyll on the 26th, leaf number and area, fruit number, and plant height).
292 In the other group were shoot concentrations of Ca, Mg, Mn, S, and K and root concentration of
293 K, all which had positive associations with the physiological, growth and yield variables. The
294 second dimension of portraying impact of soil salinity accounted for about a further 17% of the
295 variance; this had high loadings on root concentrations of Ca, Cu, Mg, Mn and Zn (Fig. 3c). The
296 variation represented by the second dimension was however only weakly associated with the
297 plant growth and yield variables. Overall impacts of soil and water salinity are clearly displayed
298 in figure 3c. It shows that the control and high (2.4 dS/m) irrigation were well separated, with
299 those of medium salinity overlapping with the other two, along dimension 1; there were
300 significant overlaps in the responses between the two soil salinity levels, especially with saline
301 irrigation, along dimension 2.

302 As would be expected, there were also strong associations among the physiological, plant growth
303 and yield variables. For example, the amount of water used per plant was closely related with the
304 number of leaves per plant, leaf area and functional state as indicated by the late season
305 chlorophyll concentrations. Similarly, a tight clustering was evident among fruit number and

yield per plant, plant height and water use efficiency. Differential impacts of water- and soil-salinity were further illustrated in terms of their relative contributions to total variance, e.g., in yield and yield components for both crops (Fig. 4). Overall, not more than 5% of the variance in fruit yield and the main yield components for tomatoes were due to soil salinity compared to 10–28% in okra. In contrast, water salinity accounted for at least 50% of the variance in yield and associated components in tomatoes, much higher than a maximum of 40% variance accounted for in okra.

4.0 Discussion

Both crops were adversely impacted by salinity, but they differed in their relative sensitivity to the source of salinity. Soil salinity was less injurious to tomato, which experienced a yield reduction of just 13% compared with 48% in okra on the saline soil relative to non-saline soil (Table 2). The two crops also differed in their attributes that were more sensitive to soil salinity. Vegetative attributes (height, leaf number and area) were adversely affected, while the physiological and reproductive attributes (chlorophyll contents and number of flowers produced and their survival) remained unaffected in the tomato on saline soil. This was contrary to reductions in all the three categories of plant attributes in okra grown on the saline soil (Table 1). The two crops, however, were affected by water salinity with both crops experiencing significant reductions in yield with every step increase in salinity on both soils. Regression analyses (data not presented) using pooled data for all treatments showed that fruit yield in tomato fell by almost 124 g/plant (36% of yield under non-saline conditions) with every unit increase in water salinity. Every unit increase in water salinity reduced yield in okra relative to non-saline irrigation by 17–31 g/plant with an average of 28% fall. Thus, tomato was more sensitive to saline irrigation.

329 The tomato showed a large tolerance to shoot concentration of Na. An increase in shoot/root Na
330 to 1.05 caused a loss of only 14% in fruit yield, on saline soil (Table 3). It was likely that the Na
331 in the shoot was sequestered in the vacuoles and away from the cytoplasm of the leaf, where
332 most biochemical processes occur, consistent with *tissue tolerance* mechanism of salinity
333 (Munns et al., 2006). Saline irrigation, however, increased tissue concentrations of Na
334 throughout the tomato plant, with shoot concentration doubling with every step up in the water
335 salinity treatment (Fig. 2) and raising shoot/root Na to as high as 3.06 (Table 3). It was probable
336 that such a high Na load would have overwhelmed the vacuolar capacity to sequester Na which
337 must have then 'leaked' into the cytoplasm of the leaf to impair growth processes. This appeared
338 to have occurred in the current study when shoot/root Na concentration exceeded the mean value
339 of 0.8 found on non-saline soil. The tissue tolerance in tomato could be associated with its large
340 capacity for osmotic adjustment that maintained osmotic potential of the leaf constant above -1.0
341 MPa even with saline irrigation of up to 7.4 dS/m (Pasternak et al., 1986).

342 In contrast to tomato, okra was more sensitive to shoot Na and so minimised partitioning this
343 nutrient to the shoot. The shoot/root Na concentration in okra did not exceed 0.35 in plants on
344 saline soil irrigated with saline irrigation, which was desirable since even the low shoot/root Na
345 concentration of 0.16 with 1.2 dS/m irrigation on non-saline soil reduced fresh fruit yields by
346 36%. Minimising the transfer of Na to the shoot (mainly leaves) by the okra was consistent with
347 *salt exclusion* mechanism for tolerating saline conditions. In this crop the fruits become a Na
348 sink almost as large as the roots when the crop was exposed to saline environments (Fig. 1 and
349 2).

350 The other factor in salinity responses in both crops is the role of other cations in either being
351 detrimental to yield or buffering the phytotoxic effects of Na. For instance, P concentration in

either shoot or roots was negatively, while Ca and Mg were positively correlated with fruit yields and several other yield attributes in both crops (Fig. 3). Excessive tissue concentration of P in okra was reported to induce deficiency of several micronutrients such as Zn and Mn (Loneragan et al., 1981) that play key roles in enzyme systems and chlorophyll synthesis. Shoot P concentration of 0.25% (2500 mg/kg) far exceeded the upper limits of 40 mg/kg found in several studies (Akande et al., 2006).

Preferential accumulation of K over Na in the shoot (mostly leaves) is another mechanism commonly associated with salinity tolerance in plants (Gorham et al., 1990). The biplots of our data show the shoot concentration of K and yields for okra being on the same side of the reference line on dimension one in the plot of vector loadings (Fig. 3). The shoot K/Na values found here were much larger than K/Na values published for okra of not more than 2.0 even under non-saline conditions (Saleem et al., 2011), possibly a result of high nutrient management in the current study. Tissue K and yield and growth variables for the tomato were on the opposite sides of the reference line on the first dimension suggesting an inverse relationship. It was possible that K might have been antagonistic to uptake of other cations such as Ca and Mg in the tomato since both ions had low shoot concentrations that were just fractions of those found in okra (Fig. 2 and 3), or even when compared to 4% reported in several vegetable crops (Maksimović and Ilin, 2012).

Increases in shoot Na in the two crops adversely affected growth and yield variables, including developing flowers and fruits. Increased incident of flower abortion under saline conditions has been widely reported for many plant species, including crops as varied as tomatoes (Ghanem et al., 2009), chickpea (Krishnamurthy et al., 2011), sunflower (Francois, 1995) and jojoba (Benzioni et al., 1992). The mechanism of flower abortion due to salinity is not fully understood,

but the results presented here reveal it could be the result of high concentrations of macro (K, P and S) and micro-nutrients (Na, Cu and Zn) in the shoot of tomato (Fig. 3a).

Reductions in growth and associated processes due to salinity (Table 3), including water-use and water-use efficiency (Table 4), are consistent with many previous studies on tomatoes (Barbagallo et al. 2012) and okra (Adewoye et al., 2010; ul-Haq et al., 2012). Reductions in root growth are often associated with low water and osmotic potential in the rhizosphere that then impedes uptake of nutrient and water (Munns and Tester, 2008), thereby restricting root and shoot growth that would have constrained water-use in both crops (Table 4). Soil and water salinity both increased glucose content of tomato fruit as found in several earlier studies and was associated with increased K concentration in the fruits (Machado et al., 2003; Yurtseven et al., 2005) as we present here.

For both crops, the impact of soil salinity was much smaller than of saline irrigation, especially for tomato. Under field conditions, preferential ion uptake from the less saline topsoil has been invoked to explain differential plant growth responses to soil *vs* water salinity (Maas and Hoffman, 1977). The extent to which such preferential water extraction explained the lower phytoxicity of the soil salinity in the current study is not clear since the roots proliferated the whole of the 24 cm deep soil. Although it was possible that the frequent irrigation from the top could have created a concentration gradient in the soil profile over time, it was more likely that the dissolved salt in the irrigation water was more readily available since its addition coincided with irrigation that increased water availability, which promoted absorption of dissolved salt by the plant (Maksimović et al., 2010) in preference to the salt sourced from the soil.

These results suggest that contrasting irrigation strategies are needed to optimise productivity for the two crops under saline conditions. The high sensitivity of tomato to irrigation salinity

suggests that reducing irrigation events, i.e. longer irrigation intervals, would minimise the potential for the uptake and accumulation of salts dissolved in the irrigation water by plants. For okra, frequent and regular over irrigation will leach out the salt and prevent its accumulation in the root zone. Frequent irrigation with saline water of up to 4.9 dS/m, twice the maximum used in the current study, maintained the matric potential in the root zone of silty clay above the threshold of -30 kPa to maintain crop water-use (Wan et al., 2007).

3 Summary and conclusions

Tomato and okra differed in their responses to soil or water salinity. The tomato due to its apparent inability to divert Na away from the shoot (mainly leaves), showed *tissue tolerance* in maintaining reasonable yields even as shoot/root Na concentration rose to 0.8. This crop must have sequestered the Na in the vacuoles of leaf tissues allowing maintenance of growth processes, but the storage capacity of vacuoles would have been overwhelmed with increased salt load due to water salinity. Okra was quite sensitive to shoot Na with yield significantly reduced with shoot/root Na as low as 0.15. In okra, we found most tissue Na in fruits and little in leaves, functioning as a *salt exclusion* mechanism. The yield penalty due to saline irrigation was therefore more severe in the tomato that lost about 85% of its fresh fruits than in the okra that lost an average of 64% of its fresh fruits. Saline irrigation was more injurious to plants than water salinity in both crops, accounting for the overwhelming majority of variance, probably due to greater availability to the plants of dissolved salt in irrigation water than in the soil.

These results suggest that contrasting irrigation strategies are needed to optimise productivity for the two crops under saline conditions. The high sensitivity of tomato to irrigation salinity can be managed by extending irrigation intervals to minimise opportunities for salt uptake and accumulation. By contrast, frequent and regular over irrigation will leach out the salt and

421 prevents its accumulation in, the root zone to ensure high yields in okra.

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428

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511 levels on yield, fruit quality and water consumption of native centre Anatolian tomato
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513 Table 1. Impact of soil and water salinity on plant growth variables for glasshouse grown tomatoes and okra.

Soil salinity (dS/m)	Water salinity (dS/m)	Leaf area/ plant (cm^2)	Leaves/ plant[1]	Final plant height (cm)	Chlorophyll content ($\mu g/cm^2$) at		Flowers/ Plant[2]	Flower abortion (%)
					95 DAT	117 DAT		
				Tomato				
0.0	0.0	406.6a	84.0a	145.8a	65.1a	31.9a	42.8a	79.3c
	1.2	350.0b	70.3b	137.0ab	67.1ab	38.7b	45.0ab	75.7bc
	2.4	272.5c	67.3bc	133.0bc	86.4c	47.3c	40.8c	93.8a
	Mean	*343.0A*	*73.8A*	*138.6A*	*72.3A*	*39.0A*	*42.8A*	*82.8A*
3.0	0.0	126.7a	59.8a	136.0a	65.7a	40.2a	41.0a	68.3c
	1.2	84.0b	59.3a	124.0b	60.3b	44.4ab	43.8ab	84.1b
	2.4	68.5c	48.5b	110.0c	60.9b	46.5b	40.5bc	92.9a
	Mean	*93.0B*	*55.8B*	*123.3B*	*62.4A*	*43.8A*	*41.7A*	*81.9A*
				Okra				
0.0	0.0	108.7a	14.8 a	116.5a	68.2a	68.6a	11.8a	15.5c
	1.2	95.3b	11.0 b	101.8b	65.7ab	63.8b	6.8b	7.3bc
	2.4	73.1c	10.0 bc	76.8c	53.0c	52.7c	6.5b	22.8a
	Mean	*92.3A*	*11.9A*	*97.9A*	*62.0A*	*61.4A*	*8.3A*	*15.2A*
3.0	0.0	12.0a	14.5a	78.8a	51.4a	57.3a	8.3a	24.1c
	1.2	4.0b	7.0b	48.3b	54.9b	44.1b	9.3a	33.9b
	2.4	1.0c	5.3bc	31.3c	41.9c	42.5bc	6.0c	45.6ba
	Mean	*5.3B*	*8.9B*	*52.8B*	*49.2B*	*47.6B*	*7.8B*	*32.6B*

514 [1] measured at 20 days after transplanting (DAT); for each crop, means in the same columns followed with the same letter(s) for a given soil salinity are statistically
515 similar at $p \leq 0.05$; the lowercase letters compare means for water salinity levels, and uppercase letters compare means for soil salinity

516

517 Table 2. Impacts of soil and water salinities on fresh fruit yields and yield components for glasshouse grown tomato and okra.

Soil salinity (dS/m)	Water salinity (dS/m)	Fruits/plant	Total fruit yield/plant (g)	Weight/fruit (g)	Glucose content in tomato fruit (mmol/L)
			Tomato		
0.0	0.0	9.0a	341.8a	37.7a	42.5a
	1.2	11.2ab	213.4b	19.7b	53.2b
	2.4	2.2c	49.9c	14.4c	56.9c
	Mean	*7.4A*	*201.7A*	*23.9A*	*50.8A*
3.0	0.0	11.7a	366.3a	30.9 a	50.7a
	1.2	6.5b	119.6b	18.1b	71.7b
	2.4	2.5c	38.1c	13.2c	88.0c
	Mean	*6.9B*	*174.6B*	*20.7A*	*70.1B*
			Okra		
0.0	0.0	10.0a	107.9 a	11.5a	*nd*
	1.2	6.3b	69.8 b	10.7a	*nd*
	2.4	3.0c	45.5c	9.0b	*nd*
	Mean	*6.4A*	*74.4A*	*10.4A*	*nd*
3.0	0.0	6.3a	61.4a	9.1a	*nd*
	1.2	6.3a	27.3b	8.3b	*nd*
	2.4	3.0b	26.7c	8.9ab	*nd*
	Mean	*5.2B*	*38.5B*	*8.7A*	*nd*

518 *nd*, not determined; for each crop, means in the same columns followed with the same letter(s) for a given soil salinity are statistically similar at $p \leq 0.05$;
519 the lowercase letters compare means for water salinity levels, and uppercase letters compare means for soil salinity.

520 Table 3: Impacts of soil salinity and water salinity on the dry weights of plant tissues, and shoot/root ratio in Na concentrations for glasshouse grown
521 tomato and okra.

Soil salinity (dS/m)	Water salinity (dS/m)	Root (g)	Shoot (g)	Fruit (g)	Total[1] (g)	Root/shoot	Fruit/shoot	Shoot/root Na
				Tomatoes				
0.0	0.0	12.9a	64.9a	1.3 a	79.1a	0.19 a	0.02 a	0.32b
	1.2	4.2b	62.9ab	1.1 ab	68.2b	0.06 b	0.017 ab	0.84a
	2.4	3.4bc	49.6c	0.7 bc	53.7c	0.06 b	0.014 bc	1.21a
	Mean	*6.8A*	*59.1A*	*1.0A*	*67.0A*	*0.10A*	*0.017A*	*0.71B*
3.0	0.0	4.9a	62.8a	1.3a	69.0a	0.07 a	0.020 a	1.05b
	1.2	3.2b	55.7ab	1.2b	60.1b	0.05 ab	0.021 b	1.28b
	2.4	2.6bc	39.8c	0.6bc	43.0c	0.06 bc	0.015 c	3.06a
	Mean	*3.5A*	*52.7A*	*1.0A*	*57.3A*	*0.06A*	*0.018A*	*1.54A*
				Okra				
0.0	0.0	4.6a	17.4a	1.6a	23.6a	0.26a	0.09a	0.12a
	1.2	2.8b	12.5b	1.0b	16.3b	0.20b	0.08b	0.06b
	2.4	1.9c	8.6c	0.8c	11.3c	0.20b	0.09a	0.16a
	Mean	*3.1A*	*12.8A*	*1.1A*	*17.0A*	*0.22A*	*0.08A*	*0.12B*
3.0	0.0	3.1a	12.7a	1.3a	17.1a	0.20a	0.10a	0.20b
	1.2	0.7b	4.7b	0.8b	5.9b	0.10b	0.10a	0.20b
	2.4	0.5bc	4.2bc	0.5c	5.5c	0.10b	0.10a	0.38a
	Mean	*1.4B*	*7.2B*	*0.8B*	*9.5B*	*0.13B*	*0.1B*	*0.24A*

522 [1]sums of root, shoot and fruit at harvest; for each crop, means in the same columns followed with the same letter(s) at a given soil salinity are statistically similar at $p \leq$
523 0.05; the lowercase letters compare means for water salinity levels, and uppercase letters compare means for soil salinity.

524 Table 4: Impacts of soil salinity and water salinity on water-use and water use efficiency for fruit yield

525 (WUE) for glasshouse grown tomatoes and okra.

Soil salinity (dS/m)	Water salinity (dS/m)	Water use (L/plant)	W UE (g/L)
		Tomato	
0.0	0.0	47.4a	7.1 a
	1.2	40.6b	4.5b
	2.4	38.1c	1.0c
	Mean	*43.3A*	*4.2A*
3.0	0.0	44.4a	7.6a
	1.2	39.9b	2.5b
	2.4	37.8c	0.8c
	Mean	*40.7B*	*3.6A*
		Okra	
0.0	0.0	37.1a	2.9a
	1.2	36.6ab	1.9b
	2.4	32.2c	1.4c
	Mean	*35.3A*	*2.0A*
3.0	0.0	29.0a	2.1a
	1.2	25.0b	1.2b
	2.4	21.0c	1.2b
	Mean	*25.0B*	*1.5A*

526 For each crop, means in the same columns followed with the same letter(s) at a given soil salinity are statistically
527 similar at $p \leq 0.05$; the lowercase letters compare means for water salinity levels, and uppercase letters compare
528 means for soil salinity.

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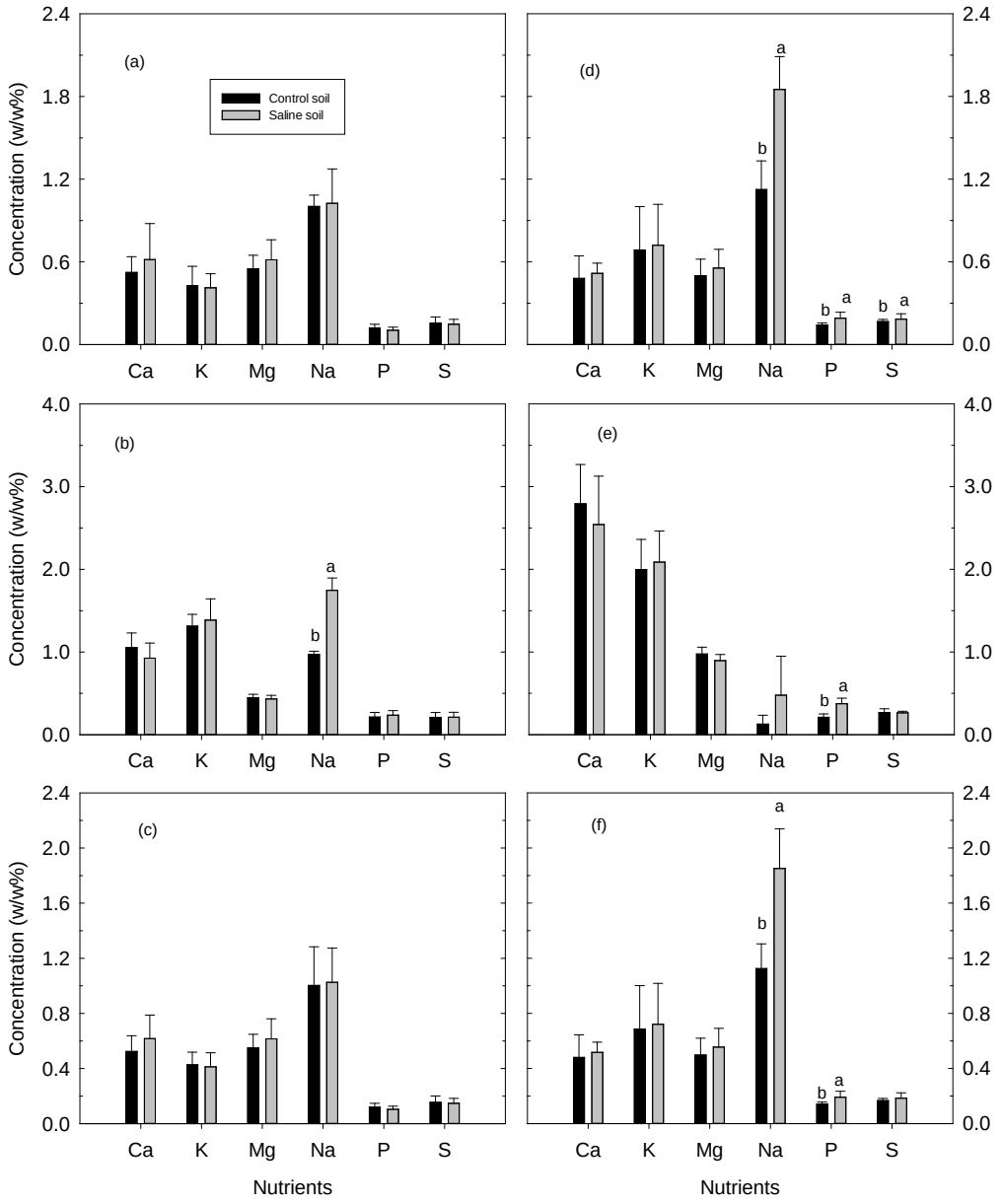
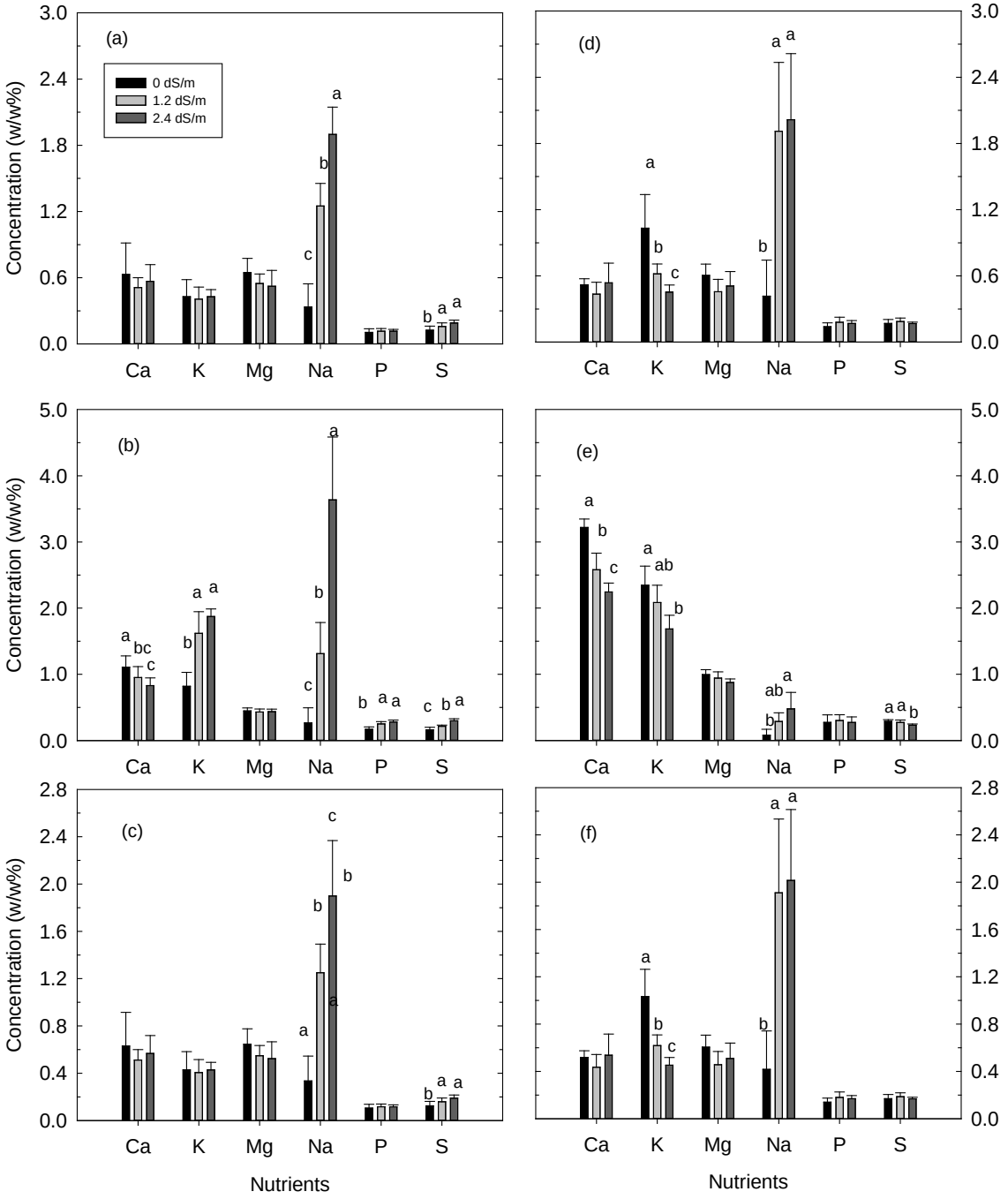


Figure 1. Impact of soil salinity on nutrient concentrations in the root (a, d), shoot (b, e) and fruit (c, f) at harvest for tomato (a – c) and okra (d – f). Where treatment means are significantly different ($p < 0.05$) are indicated by different letters.



538

539 Figure 2. Impact of saline irrigation on nutrient concentrations in the root (a, d), shoot (b, e) and
540 fruit (c, f) at harvest for tomato (a–c) and okra (d–f). Where treatment means are significantly
541 different ($p < 0.05$) are indicated by different letters.

542

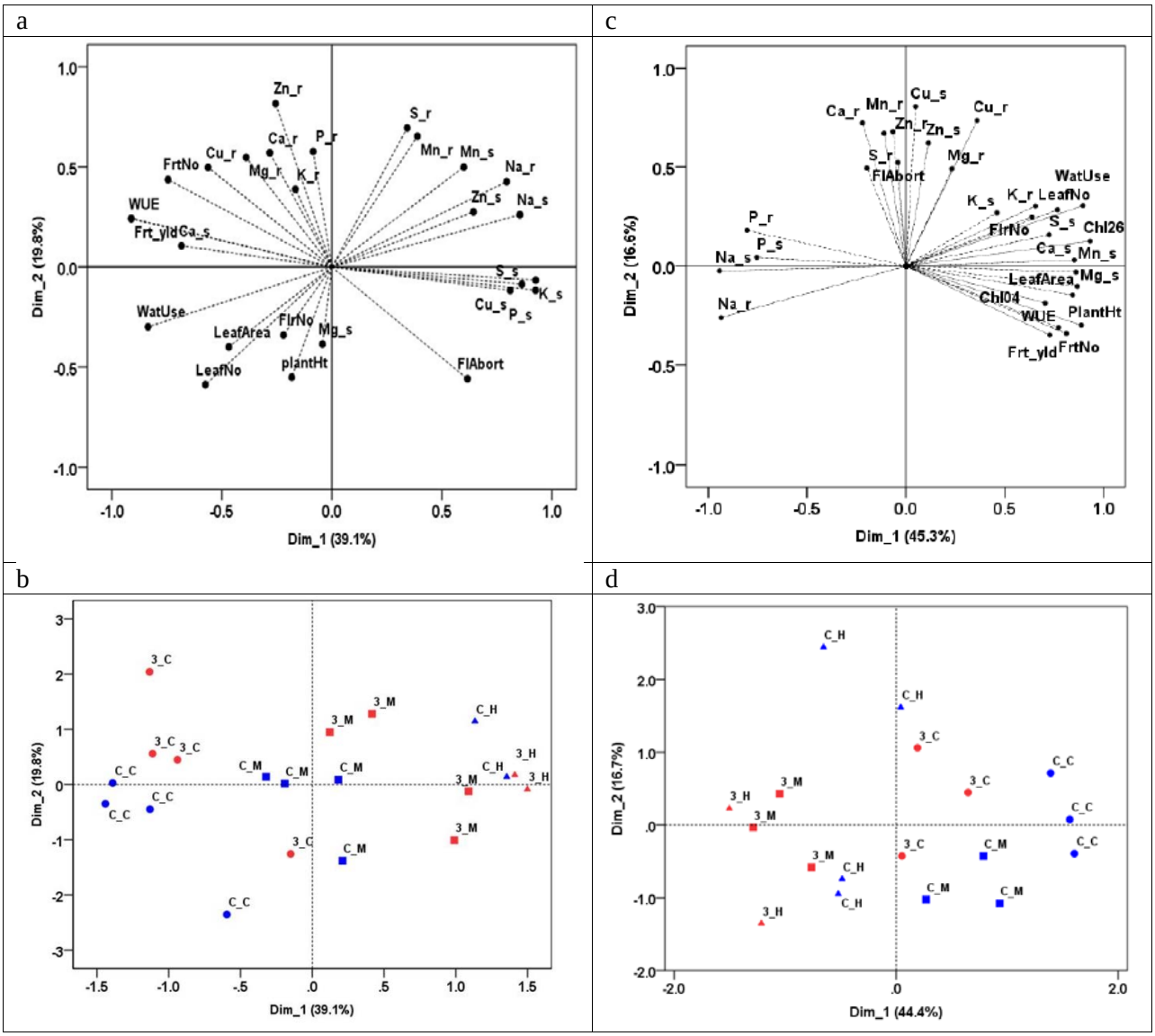
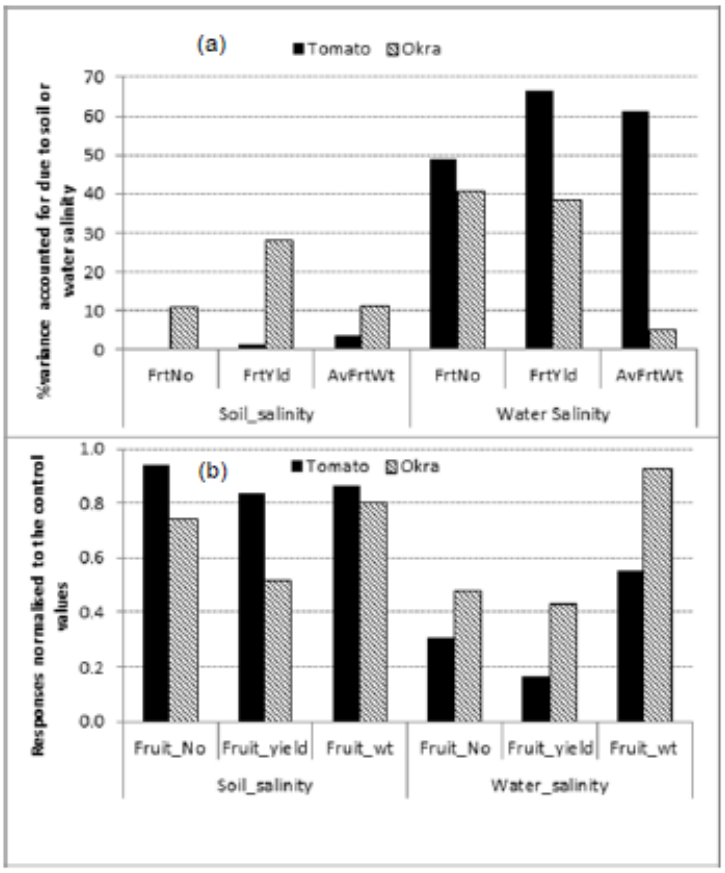


Figure 3. Interrelationships amongst plant response variables generated by principal component analyses (PCA) showing vector loadings (a, c) and biplots for salinity treatments (b, d) for tomatoes (a, b) and okra (bc, d). Codes in b and d are: C (control, 0 dS/m, circles) M (medium, 1.2 dS/m, squares) and H (high, 2.4 dS/m, triangles) irrigation water salinity, and C (control, 0 dS/m, blue) and 3 dS/m (red) soil salinity. The variables plotted are water-use (WU), leaf area (LA), leaf number (leafNo), chlorophyll concentrations (ch) on two dates, flower number (FlwrNo) and flower abortion (FlAbrt), plant height (PlantHt), fruit yield (FrtYld) and fruit number (FrtNo), and ionic concentrations in the shoot (_s) or root (_r).



551

552 Fig. 4. Relative impacts of soil salinity and water salinity on selected yield variables for okra and
553 tomato: (a) proportions of variance due to the respective salinity source, and (b) plant response
554 variables normalized over control values.

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556