Plasticity in stomatal density and morphology in okra and tomatoes in response to soil and water salinity

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Abstract

Okra (*Abelmoschus esculentus*) and tomatoes (*Lycopersicum esculentum*) were grown in saline (3.0 dS m^{-1} NaCl) and non-saline soil and irrigated with saline (2.4 dS m^{-1} NaCl) or non-saline water to determine the response of stomatal density and morphology to salinity. Stomata density (stomata number per unit leaf area) for tomato grown on saline soil was reduced by 33% (12 mm^{-2}) compared with those on non-saline soils (18 mm^{-2}); this reduction was more severe on the adaxial leaf surface where stomatal density was low. Similar reductions in stomatal density were observed in tomato irrigated with saline water. Stomata size in tomato was significantly reduced by about 20% with both types of salinity, thus the proportion of leaf surface area occupied by the stomata in salt-stressed plants, i.e., stomata area index (SAI), averaged 4.4% in salt-stressed plants compared with 5.5% in plants grown in non-saline conditions. Okra, on the other hand, maintained a similar stomatal density (average 22 mm^{-2}) on both saline and non-saline soils, but saline irrigation marginally increased the density. In okra, the abaxial leaf surface accounted for about 68% of the total stomata under both saline and non-saline conditions. Individual stoma size in okra was increased by up to 15% on both leaf surfaces due to salinity, hence, the SAI increased from an average of 9.0% under non-saline conditions to 11.7% under saline stress. Notwithstanding the increase in SAI for okra, salinity reduced stomatal conductance by more than 50% in both crops. The stomatal conductance was generally much larger in okra than in tomato, and was as large in okra exposed to salinity as for tomato in the absence of salinity.

Keywords: fruit yield, leaf area, stomata size, stomatal conductance, tissue ion content

INTRODUCTION

Stomata are the primary site for the exchange of water vapour and CO_2 on plant leaves. They are highly sensitive to environmental stresses and alter their numbers and distribution on the leaf surface (Maggio et al., 2007; Omamt et al., 2006; Shabala et al., 2013). Under saline conditions a general reduction in stomatal density (number/unit leaf area) has been reported in a wide number of species such as quinoa, *Chenopodium quinoa* (Shabala et al., 2013), amaranth, *Amaranthus* spp., (Omamt et al., 2006), okra, *Abelmoschus esculentus*, (Shahid et al., 2011), tomato, *Solanum esculentum*, and cucumber, *Cucumis sativus* (Adams and Ho, 1995). Reductions in stomatal density and size are considered strategies to minimise uptake of water and the dissolved salt so as to protect leaves from toxic accumulation of ions and eventual dehydration (Orsini et al., 2012).

According to the United Nations Food and Agriculture Organisation (FAO), 20% of irrigated land, or 8 million ha, is under threat of rising soil salinity and salt accumulation in the uppermost layers of the soil (Maggio et al., 2011). Furthermore, agriculture uses more than 60% of the global freshwater supply, which is declining, and there is increasing need to recycle/reuse drainage water or other poor quality water that are often saline to achieve increases in agricultural production as a result of increasing global population (Dolnicar and Schäfer, 2009).

Tomatoes and okra are important vegetable crops widely grown under irrigation. Irrigated soils in arid and semi-arid regions of the world face the most serious salinity

problem, while these are the areas that rely heavily on irrigation. These two crops possess contrasting mechanisms in their response to salinity with tomato showing a tissue tolerance mechanism in allowing substantial accumulation of sodium in its shoot, while okra minimises such accumulation through a salt exclusion mechanism (Kamaluldeen et al., 2014). It is not certain, however, how these contrasting mechanisms impact stomatal characteristics. This paper extends and presents data on the stomata characteristics for the two crops used in the study of Kamaluldeen et al. (2014) with the aim of addressing the question of whether tomato and okra differ in their stomatal characteristics (density and size) when exposed to saline conditions.

MATERIALS AND METHODS

This is a glasshouse study undertaken between March and July of 2012 at the University of New England, Armidale (30°29'16"S; 151°38'29"E), Australia, full details of which are described in Kamaluldeen et al. (2014). Briefly, tomato (*Solanum esculentum* 'Rouge de Marmande') and okra (*Abelmoschus esculentus* 'Clemson's spineless') were raised from seeds sown in vermiculite (0.0 dS m^{-1}) and watered with tap water (EC of 0.025 dS m^{-1}). The seeds germinated within 6 days and were allowed to establish for 2 weeks (8-12 cm high for okra and 10-18 cm for tomato) before they were transplanted on March 31, 2012 into plastic pots filled with 6 kg of loamy soil (83% sand and 10% clay) having a salinity of 0.02 dS m^{-1} and pH of 6.3 The soil in each pot had its salinity set at approximately 0 dS m^{-1} or raised to 3.0 dS m^{-1} by mixing it with table salt (NaCl) at 1% (w/w). The pots were watered with drippers using tap water having a salinity of 0 or 2.4 dS m^{-1}, achieved by dissolving table salt at 0 or 225 g L^{-1}, and the solutions kept in separate tanks. The amount of water applied was recorded and drainage water was collected every week and returned to their respective pots. The plant were supplied with a compound fertiliser (Muriate of Potash: 12.2% N, 5.1% P, 13.7% K, 4.5% Ca and 1.1% Mg, CSBP, Australia) at transplanting, and repeated several times during the study.

Stomatal conductance was measured using the Delta-T Porometer© AP4-UM-3 (Delta-T Devices Ltd., Cambridge, UK) at 2-hourly interval between 0600 and 1800 h on 91 days after transplanting (DAT). The measurement was made on the topmost fully expanded leaf around midday and repeated every seven days. Stomatal number and size were determined by carefully peeling off the epidermal tissue on both the adaxial and abaxial surfaces from the two topmost fully expanded leaves on each plant at 70 DAT (10 weeks after transplanting). Stomata were counted and their length and width measured under a light microscope displayed on a computer monitor. These measurements were limited to all plants in three replicates.

RESULTS AND DISCUSSION

Stomatal density and size

Lower stomatal density (numbers per unit leaf area) was observed on both tomato and okra leaves, the effect being significant in the former, by salinity of soil and water (Figure 1). The crops however differed in the extent and spatial nature of their stomatal responses to salinity. Tomato had significantly lower stomatal density on both surfaces of the leaf when grown on saline soil; this was particularly evident on the adaxial leaf surface where stomatal density was 33% of that found on plants grown on non-saline soil. By contrast, okra maintained similar stomatal density on both leaf surfaces irrespective of soil salinity. The impact of water salinity on the stomatal density for both crops was similar to that observed with soil salinity. However, while tomato generally reduced stomatal density on both leaf surfaces, okra nominally increased stomata numbers when irrigated with saline water.
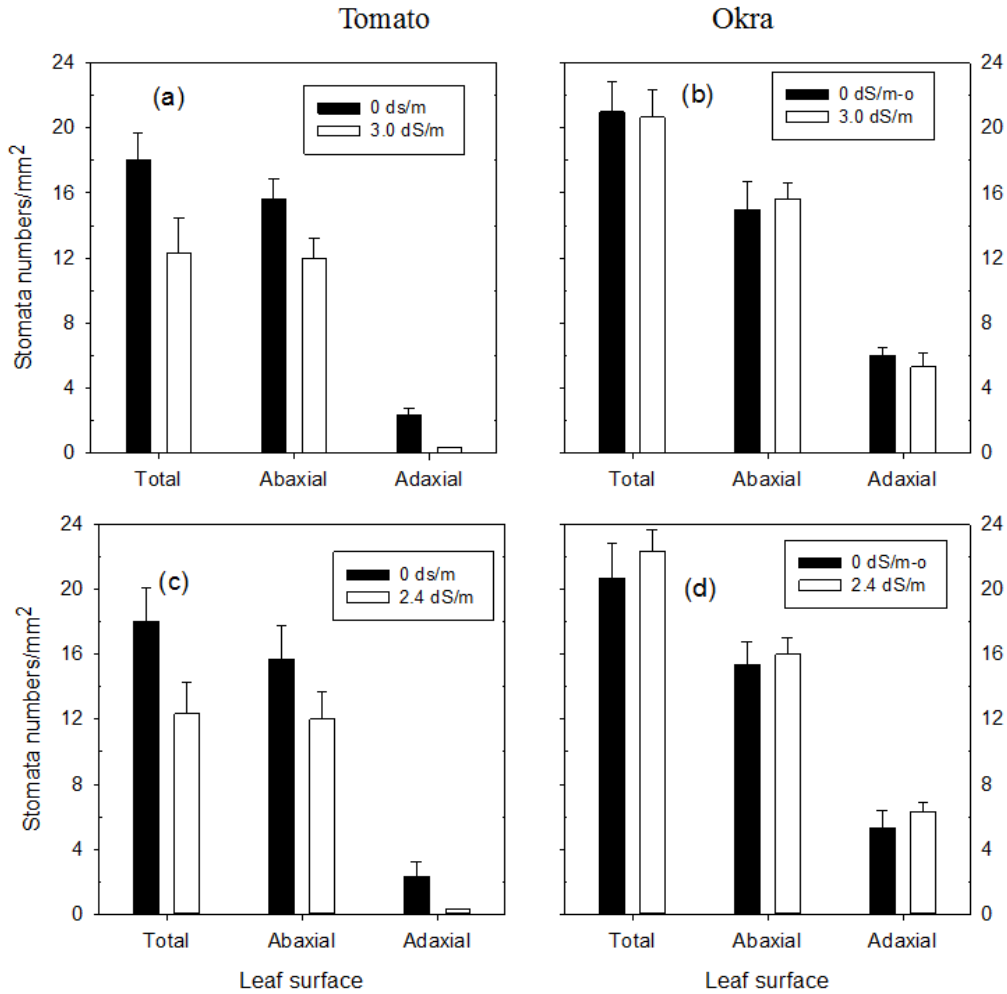
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Figure 1. Mean (±s.e.) for stomatal density measured on the abaxial and adaxial leaf surfaces of tomatoes and okra grown on soil of 0 dS m^{-1} (non-saline) or 3.0 dS m^{-1} (saline) soil (a, b) and irrigated with water of 0 or 2.4 dS m^{-1} (c, d). The measurements were made at 10 weeks after transplanting.

Striking differences were observed in the size of stomata between the two crops when subjected to saline conditions (Figure 2). While tomato reduced it stomatal size by as much as 25% on the abaxial leaf surface, okra increased stomatal size by up to 30% on both leaf surfaces and especially on the adaxial leaf surface, when grown on saline soil (Figure 2a, b). Saline water application reduced stomatal size only on the adaxial leaf surface in tomato, but increased it on the abaxial surface of okra leaf (Figure 2c, d).
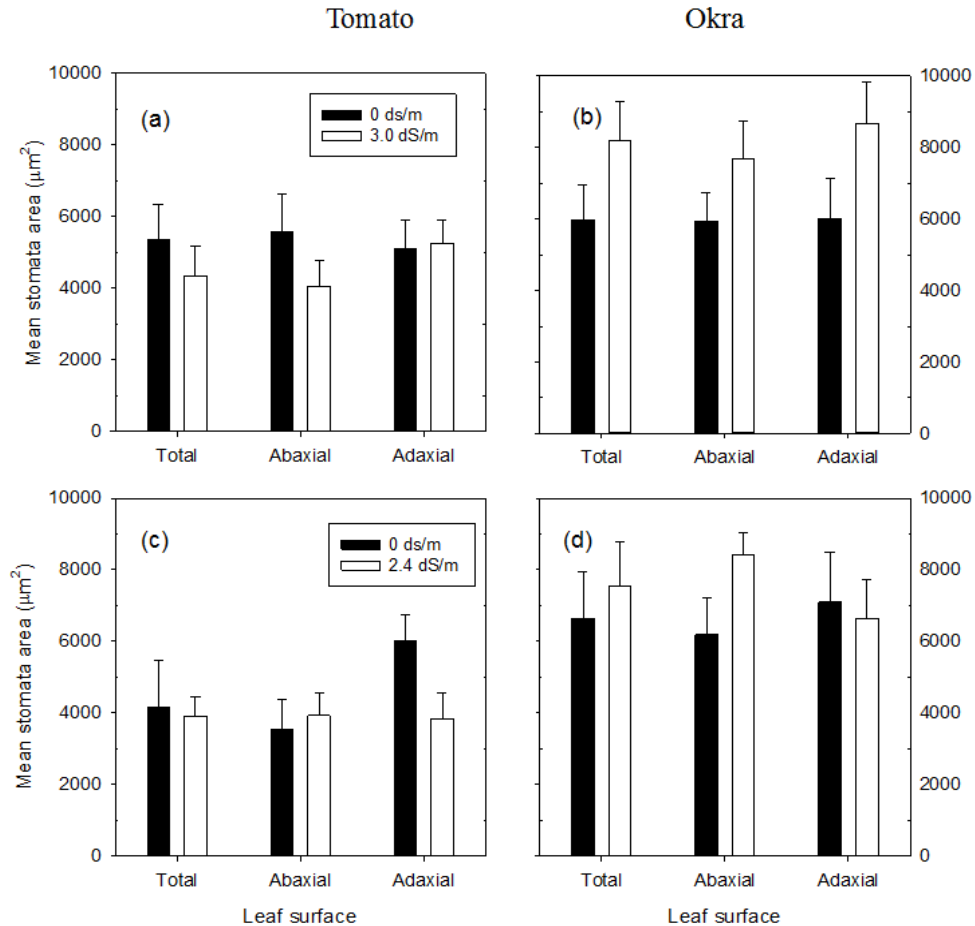
Figure 2. Mean (±s.e) for stomatal area measured on the abaxial and adaxial leaf surfaces of tomatoes and okra grown on soil of 0 dS m^{-1} (non-saline) or 3.0 dS m^{-1} (saline) soil (a, b) and irrigated with water of 0 or 2.4 dS m^{-1} (c, d). The measurements were made at 10 weeks after transplanting.

It is thus apparent that tomato reduced both the density and size of size of stomata in response to salinity, while okra increased stomatal size while maintaining density. Alterations in the distribution and size of stomata between leaf surfaces in plants exposed to saline conditions have been associated with elemental distribution (Shahid et al., 2011). Sodium accumulation in the leaf has been shown to reduce stomatal density in a wide range of plant species, including the halophyte quinoa (Shabala et al., 2013), okra and tomatoes (Orsini et al., 2012). In the current study, stomata density on both leaf surfaces (StN_a, StN_b) and their sums (StN_tot) in okra were negatively correlated with shoot sodium concentration (Na_s) as can be seen along PC_1 axis from principal component analysis (Figure 3b). Furthermore, the stomatal numbers were negatively correlated with Fe, Mg and Al (Figure 3a). In tomato especially, low stomata density in the tomato was associated with conditions of low salinity, such as control soil/control water (CC); whereas stomata area (StA), especially on the abaxial surface of okra, was associated with moderate saline conditions of non-saline soil/saline water (CH) or saline soil/non-saline water (HC).
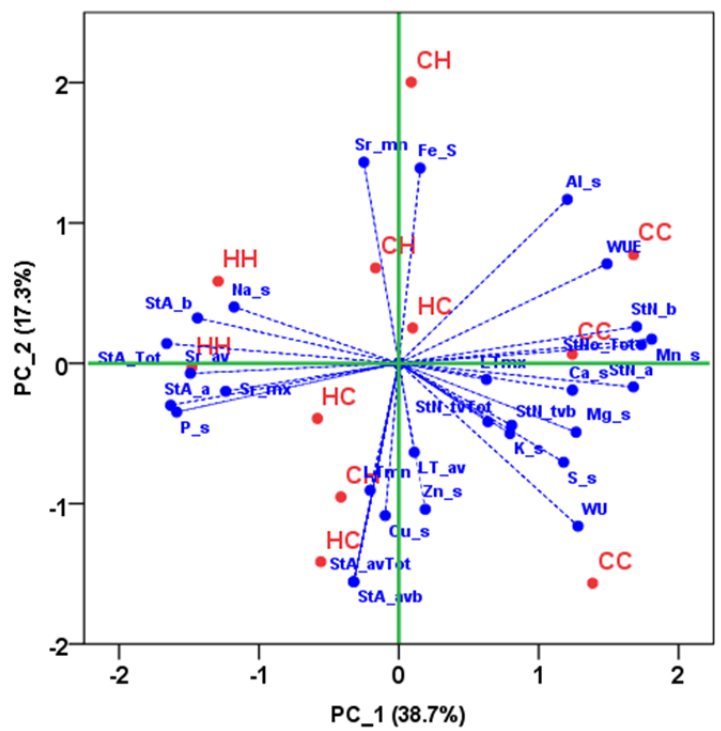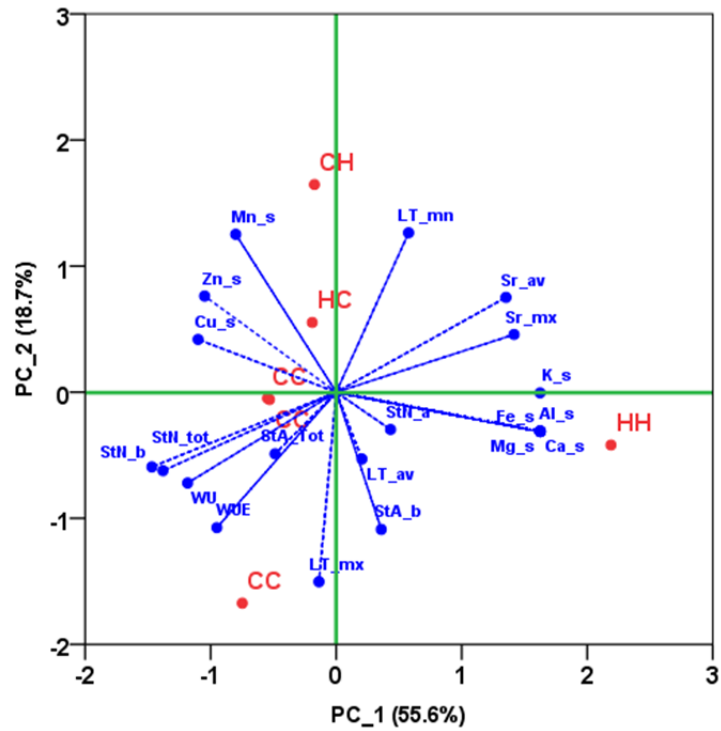
Figure 3. Principal component analysis for displaying correlations amongst stomatal characteristics measured at 10 week after transplanting and ionic concentrations in the shoot measured at harvest for (a, top panel)) tomato and (b, bottom panel) okra. Variables represented are stomatal density (StN) and area (StA) on abaxial (_b) or adaxial (_a), water use (WU), water use efficiency (WUE), mean leaf temperature (LT_av) or maximum leaf temperature (LT_mx).

In both tomato and okra stomata density was positively associated with conditions of low salinity, such as control soil/control water (CC) (Figure 3). By contrast, stomatal size was strongly and positively associated with conditions of high salinity (HH), especially in okra. However, there appeared to be a size vs. density trade-off or compensatory response (Figure 3b). Stomatal area on okra leaves was generally associated with high saline conditions of saline soil/saline water (HH) or HC. This was because the okra in this study restricted Na accumulation in its shoot far more effectively than did the tomato (average 0.5 vs. 1.8%), but concentrated more of this element in the roots than the tomato (1.0 vs. 1.8%) (Kamaluldeen et al., 2014).

The reduction in stomatal size in the tomato was more pronounced with soil salinity than it was with water salinity (Figure 2a, c), because Na accumulation in the shoot was far larger with water salinity than it was with soil salinity (Kamaluldeen et al., 2014). This suggests that the salt added through irrigation water was more readily available for plant uptake than dry salt added to the soil. This is because water uptake is generally greater from near the surface layer, to which water is directly applied, and declines exponentially with depth (Javaux et al., 2008; Prasad, 1988). Furthermore, irrigation leaches out some pre-existing soil salt from the top layers, and the frequent irrigation used in the present study minimises the impact of soil salinity than irrigation salinity, although the leachate was returned to pots.

Plasticity in stomata characteristics is demonstrated by the data in Table 1. It can be seen that while only 14% of the stomata were found on the adaxial leaf surface of tomato in non-saline conditions, this percentage was reduced to not more than 6% under saline conditions. The stomata on the adaxial leaf surface were about 24% the size of those on the abaxial surface, and were further reduced to 4-10% depending on soil and/or water salinity conditions. In contrast, okra had almost 40% of its stomata on the adaxial leaf surface and remained largely so even under saline conditions, except on saline soil with saline irrigation in which stomata on the adaxial surface were reduced to one third of those on the abaxial surface. Thus, salinity had a milder impact on okra's stomata than those of tomatoes. Responses of okra stomatal sizes were similar to stomatal density.

Table 1. Mean (± s.e.) for selected leaf characteristics for tomato and okra grown on soil of either 0 or 3.0 dS m^{-1} and irrigated with water of 0 or 2.4 dS m^{-1} in the glasshouse: leaf temperature, stomatal area index (total area of stomata/total area of leaf) and above/below ratios in the magnitudes of selected stomata characteristics.

Salinity		Leaf temp[1] (°C)	Stomatal area index	Adaxial/abaxial ratios in stomatal		
Soil	Water			Density	Area	Conductance
Tomato						
0.0	0.0	31.5±0.5	0.08±0.02	0.14	0.24	0.26
	2.4	31.2±0.2	0.04±0.01	0.06	0.10	na
3.0	0.0	31.9±0.9	0.05±0.02	0.04	0.06	0.30
	2.4	31.2±0.2	0.05±0.01	0.06	0.04	0.14
Okra						
0.0	0.0	32.1±0.4	0.12±0.01	0.37	0.38	0.36
	2.4	31.2±0.2	0.13±0.05	0.35	0.36	na
3.0	0.0	31.6±0.4	0.17±0.04	0.40	0.37	0.37
	2.4	31.1±0.3	0.18±0.05	0.34	0.31	0.15

[1]Measured at 60 days after transplanting; *na*, data not available.

Stomatal conductance and water use

The impact of the reductions in stomatal characteristics was apparent in the stomatal conductance in both crops (Figure 4). The reduction in the stomatal conductance in tomato due to soil salinity was generally smaller than that induced by water salinity, while the

difference in stomatal conductance between plants under the control or saline conditions was narrower in okra. Generally, stomatal conductance was higher in okra than in the tomato. The low stomatal conductance in the tomato may translate into lower transpiration that would have constrained transport of excess ions from the root and accumulating them in the leaves as a tolerance mechanism to salinity (Maggio et al., 2007), while the trend was opposite in okra.
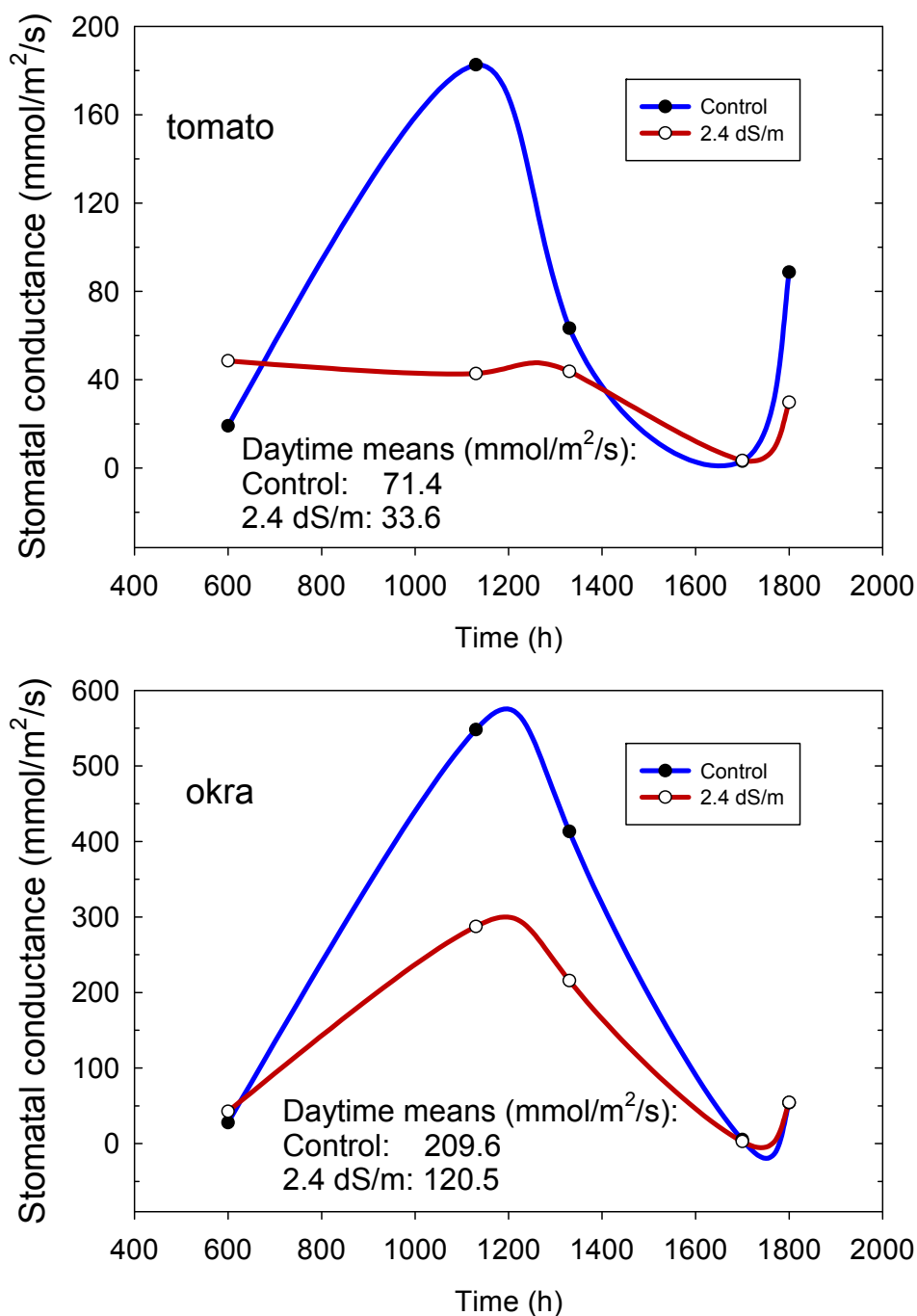

Figure 4.   Diurnal trends in stomatal conductance for tomato and okra in response salinity of soil (a, top panel) or water (b, bottom panel) measured at 91 days after transplanting.

Differences in stomatal conductance were also revealed in the water use during the growth of the two crops (Table 2). The water use in response to soil salinity was different for both crops. While there was no significant difference in water-use by tomato when grown on non-saline and saline soil, okra used almost 30% less water on the saline soil compared with non-saline soil. Saline irrigation, however, reduced water-use in both crops, and in tomato was 17% on non-soil and 24% on saline; whereas in okra reductions in water-use due to saline irrigation was 15% on non-saline and 38% on saline soil. Thus, okra suffered a greater decline in water use under the combined effect of soil and water salinity than did tomato. The differences in the water-use were also partly due to alterations in the leaf area produced by the plants. Reductions in the leaf area due to soil salinity in both crops were up to a factor of 2.5 in tomato and almost 14 in okra (Table 2). Saline irrigation also reduced leaf area in tomato by 33% on non-saline soil and 54% on saline soil; the respective reductions in leaf area in okra were 33 and 92%. These reductions in leaf area due to saline irrigation were far larger than those in the water-use and even in stomatal conductance, suggesting that non-stomatal conductance could be a significant contributor to transpiration in these two crops. The intrinsic impact of salinity on non-stomatal vapour conductance is poorly understood, but was observed at night in grapevines under saline irrigation (Yunusa et al., 2000) and commonly associated with high vapour pressure deficits (Maier-Maercker, 1999; Zeppel et al., 2010).

Table 2. Summary of growth and yield variables for tomato and okra grown on soil of either 0 or 3.0 dS m^{-1} and irrigated with water of 0 or 2.4 dS m^{-1} in the glasshouse.

Soil salinity	Water salinity	Leaf area plant^{-1} (cm^2)	Fruit yield (g plant^{-1})	Water-use (L plant^{-1})	Water-use efficiency (g L^{-1})
			Tomato		
0.0	0.0	407±6	342±24	47.4±1.5	7.1±1.7
	2.4	273±10	50±14	38.1±0.3	1.0±0.4
3.0	0.0	127±3	366±34	44.4±1.7	7.6±0.8
	2.4	69±3	38±5	37.8±0.2	0.8±2
			Okra		
0.0	0.0	109±2	108±12	37.1±0.2	2.9±0.2
	2.4	73±5	46±6	32.2±0.4	1.4±0.2
3.0	0.0	12±1	61±6	29.0±0.2	2.1±0.1
	2.4	1±1	27±3	21.0±0.2	1.2±0.1

Assuming the differences in the leaf area early in growth (Table 2) was maintained for much of the season, the mean specific water use (water use per unit leaf area) on non-saline soil was 3.91 kL m^{-2} in okra which was thrice the magnitude found in the tomato (1.28 kL m^{-2}); the differences in magnitude were even larger on the saline soil, being 4.49 kL m^{-2} in tomato and 117.1 kL m^{-2} in okra. Thus okra in addition to having larger SAI (Table 1), its stomata were also more transmissive of water vapour out of the leaf than in the tomato. As such water requirements by okra under saline conditions would be larger than for tomato, which was consistent with our observations of wilting being more severe during the day in okra than in the tomato.

Fruit yield and water-use efficiency

Fruit yield was not significantly impacted by soil salinity in tomato, but was reduced by 64% in okra (Table 2). Saline irrigation, however, reduced fruit yield by 85-90% in tomato and 38-64% in okra. Water-use efficiency (WUE) for tomato was unaffected by saline soil, but was reduced by 23% in okra, whereas it was reduced by up to 89% in the tomato, much larger than the 50% in okra, with irrigated with saline water. Tomato was therefore more tolerant of soil salinity than it was of water salinity, while the reverse was true for okra. This difference in tolerance mechanism between the two crops was associated with the

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accumulation and distribution of ions, especially Na, in the plant. The tomato exhibited tissue tolerance mechanism in accumulating Na mostly in its shoot, where its interference with the physiological processes within the canopy would be more detrimental; whereas, okra employed salt exclusion mechanism in minimising Na accumulation in the shoot (Kamaluldeen et al., 2014).

CONCLUSIONS

Salinity stress affected stomatal density and/or stomal size in tomato and okra. The stomatal density and size in tomato were reduced which may play an important role in water relations and overall transpiration. On the other hand, salinity did not reduce stomatal density and even increased stomatal size in okra. This, in addition to okra, exhibiting higher stomatal conductance suggests that the water requirements of salt-stressed okra might be higher than that of tomato under the same conditions.

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