

A water-centred framework to assess the effects of salinity on the growth and yield of wheat and barley

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Abstract We used a water-centred framework (yield = transpiration × transpiration efficiency × harvest index) to investigate the effect of soil salinity on growth and yield of wheat and barley. Our working hypothesis is that salinity reduces transpiration proportionally more than transpiration efficiency. We established a glass-house experiment with the factorial combination of four varieties (wheat: Janz, Krichauff; barley: Mundah, Keel) and three soil treatments: a control with no NaCl added, and NaCl added to achieve soil $EC_{1.5}$ 0.75 dS m⁻¹ and 1.5 dS m⁻¹. Pot-grown plants were watered to weight to determine transpiration and shoot dry matter was determined using a non-destructive image analysis system. Consistent with our hypothesis, salinity reduced transpiration (30–60%) proportionally more than transpiration efficiency (0–35%); transpiration

accounted for 90% of the variation in shoot growth across varieties and treatments. Against this pattern, there were time- and variety-dependent responses. The rate of leaf appearance and the transpiration efficiency of Janz, Krichauff and Keel showed a two-stage response to salinity. In stage 1, salt-stressed plants maintained rate of leaf appearance and transpiration efficiency close to or slightly below those of the controls. After a clear break point where the slope changed, stage 2 was characterised by a substantial reduction in both traits. Stage 2 was not evident in salt-stressed Mundah, which maintained a relatively high rate of leaf appearance and transpiration efficiency. Across species, harvest index increased from 0.40 in controls to 0.47 at 0.75 dS m⁻¹. Harvest index of plants grown at 1.5 dS m⁻¹ was unaffected in wheat, and was reduced in barley. We propose that an understanding of the effect of salinity on crop development, growth and yield requires integration of low-level traits in a framework of resource capture, resource-use efficiency and plant allocation. Osmotic stress tolerance, Na⁺ exclusion, and tissue tolerance to accumulated Na⁺ would improve yield of salt-stressed crops to the extent that these traits contribute to the maintenance of water uptake and harvest index.

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Introduction

Sodium chloride is the most important component of soil salinity affecting more than 800 M ha worldwide, hence the selective pressure and emergence of plant adaptations to NaCl (Turkan and Demiral 2009). Plant adaptations to salinity include three non-exclusive mechanisms: osmotic stress tolerance, exclusion of toxic ions such as Na⁺ or Cl⁻ and tissue tolerance to accumulated Na⁺ or Cl⁻ (Munns and Tester 2008). This framework is particularly useful for the analysis of genetic, metabolic and cellular processes.

With a focus on whole-plant and crop levels, the conceptual approach of Passioura (1977) analyses biomass production as a function of transpiration and transpiration efficiency (biomass per unit transpiration); shoot biomass is then scaled to grain yield using harvest index. Passioura's model is widely used to dissect adaptations to water deficit (Reynolds and Tuberosa 2008; Richards 2006) but it has rarely been applied in salinity studies. Indeed, we have a fragmented understanding of the relative importance of transpiration and transpiration efficiency in reducing whole-plant growth and yield of salt-stressed plants.

Salinity reduced transpiration proportionally more than transpiration efficiency in a number of experiments under controlled and field conditions (Grewal 2010; Halvorson and Reule 1976; Holloway and Alston 1992; Katerji et al. 1997; Rawson 1986; Shani and Ben-Gal 2005). The main deviation from this pattern is from studies where salinity *increased* transpiration efficiency of water stressed plants (McCree and Richardson 1987; Richards 1992). However, neither Richards (1992) nor McCree and Richardson (1987) established the factorial combination of salinity and water deficit necessary to quantify the effects of individual stresses and their interaction. In a factorial field experiment with two water and three salinity regimes, salinity did not affect the ratio between grain yield and evapotranspiration in well-watered durum wheat and barley, but did slightly increase the ratio under water deficit (Katerji et al. 2009).

Many studies have focused on the effects of salinity on traits related to the water and carbon economies of plants, including stomatal conductance, transpiration and stomatal and non-stomatal control of photosynthesis (Chow et al. 1990; James et al. 2006; Lu et al. 2003; Zhu and Meinzer 1999). Scaling up from short term measurements of stomatal conduc-

tance and gas exchange to long-term plant water use and growth is, however, not straightforward (Denison 2009; Jarvis 1995; Jarvis and McNaughton 1986; Sinclair et al. 2004). Long-term, whole-plant transpiration efficiency is often unrelated to photosynthesis-transpiration ratio derived from short term gas exchange measurements (Kramer and Boyer 1995). Direct assessment of plant growth in terms of transpiration and transpiration efficiency is therefore not trivial, and provides a mechanistic account of salinity effects at the whole-plant level.

The aims of this study were to analyse the effects of salinity on the growth and yield of cereals using a water-centred framework, and to explore the links between the traits in the model of Munns and Tester (2008) and resource-based models of crop growth and yield (Sadras 2009; Sadras et al. 2005). Our core working hypothesis is that salinity reduces the biomass of well-watered wheat and barley by reducing transpiration, rather than transpiration efficiency. In contrast to the majority of studies where whole-plant transpiration efficiency was derived from measurements at a single time, here we have used non-destructive imaging technology to characterise the dynamics of growth (Rajendran et al. 2009). In combination with gravimetric determination of transpiration in pot-grown plants, we aimed to capture the dynamics of development, growth, transpiration and transpiration efficiency as salinity stress intensified with plant ontogeny.

Materials and methods

Treatments

We combined four varieties and three salinities in a factorial experiment with ten replicates per treatment. Two wheat varieties, Krichauff and Janz, and two barley varieties, Mundah and Keel were selected; as parents of mapping populations, these varieties allow for future genetic studies. The salt treatments were a control (no NaCl added), and NaCl added to achieve soil EC_{1:5} 0.75 dS m⁻¹ and 1.5 dS m⁻¹.

Growing conditions

Sterilised, germinated seeds were sown in pots in a naturally lit greenhouse maintained at 21°C and 50% relative humidity between 17th March and 20th

August 2009 in Adelaide (34° 55' S). A single plant per pot was grown to allow for image analysis (Rajendran et al. 2009). Pots (15 cm diameter × 15 cm depth) were lined with clear plastic bags to prevent water leakage and filled with 2.4 kg of substrate comprising a 1:1 mix of Waikerie sand and air-dried, sieved, and steam-sterilised top soil from Roseworthy campus, University of Adelaide. Pots were half-filled with fully-fertilised soil mixture treated with the appropriate amount of NaCl: nil (control), 2.5 g NaCl to achieve 0.75 dS m⁻¹ (≈ 24 mM NaCl in the soil solution) and 5 g NaCl to achieve 1.5 dS m⁻¹ (≈ 40 mM NaCl). The amounts of NaCl required to achieve the nominal treatments were determined in an assay using 25 to 2,000 mM NaCl and the actual soil mixture. Pots were then filled with the remaining untreated soil mixture. The stratification of salt in the pots sought to generate a relatively gradual development of stress, rather than reproducing the subsoil conditions in the field, which would have required deeper pots (Holloway and Alston 1992).

Measurements

Rate of leaf appearance was derived from weekly leaf counts as in Calderini et al. (1996). Shoot dry matter was estimated non-destructively using a LemnaTec Scanalyzer 3D (LemnaTec GmbH, Wuerselen, Germany) as described in Rajendran et al. (2009). Briefly, a top image and two side images at 0° and 90° were taken to provide three 2-D images of the plant. A single quadratic calibration function was used to transform pixels to shoot dry matter: dry matter (g) = 6.81×10^{-6} Pixel + 2.16×10^{-11} Pixel². This function applied to all four varieties ($R^2=0.97$; $n=146$). Measurements were discontinued at 48 days after sowing, when changes in plant structure rendered the technique inapplicable. Plant transpiration was determined gravimetrically and pots were watered to field capacity with a 1 to 3 d frequency. To minimise soil evaporation, the soil was covered with a layer of plastic pellets and residual soil evaporation was measured gravimetrically in pots with no plants.

To complement the dynamic characterisation of development, growth, transpiration and transpiration efficiency, we took a series of snapshot single-leaf measurements including sodium content, leaf chlorophyll (47 and 83 days after sowing) and gas exchange (33 days after sowing). Sodium was measured on the

fully expanded 4th leaf and chlorophyll and gas exchange in the youngest fully expanded leaf. For sodium analysis, leaves were dried to constant weight and digested in 1% nitric acid overnight in a hot block at 85°C (Thermoline Scientific, Northgate, Australia). The solution was diluted as necessary and analysed with a flame photometer (Model 420 Sherwood Scientific, Cambridge, UK). Leaf chlorophyll was measured with SPAD-502 Chlorophyll Meter (Konica Minolta, Australia). Leaf gas exchange was measured at noon (PAR ~ 800 mmol m⁻² s⁻¹) with an LI-6400XT Portable Photosynthesis System (LiCor, Lincoln, Nebraska, USA) and chamber settings consistent with glasshouse growing conditions (temperature = 25.2°C, relative humidity = 50.1%).

At maturity (157 days after sowing), shoots were harvested and oven-dried to constant weight to determine total shoot and grain dry matter. Harvest index was calculated as the ratio of grain and shoot dry matter. Grains per plant were counted and average weight of individual grain was determined from total grain weight and number.

Data analysis

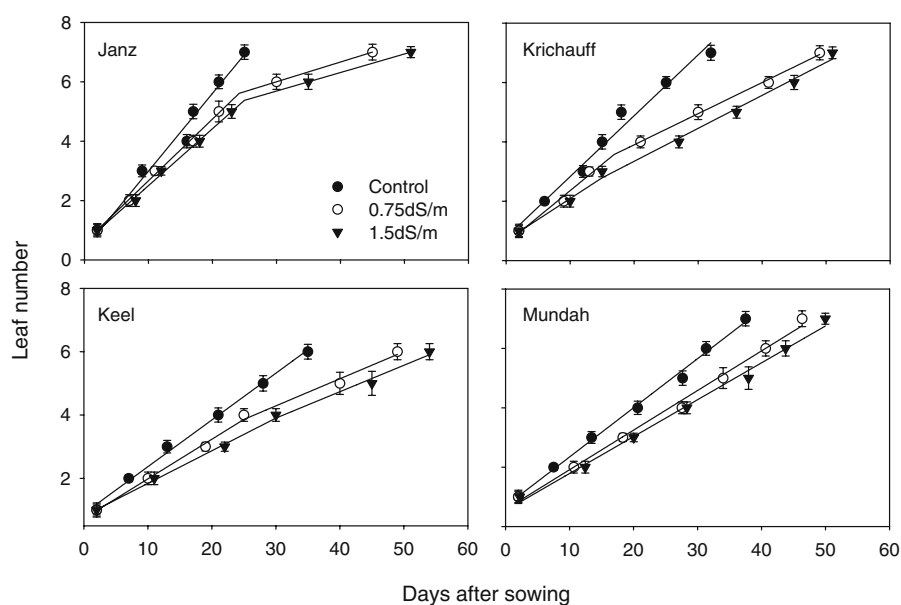
ANOVA was used to test the effects on variety, salinity and variety × salinity interactions. Linear and piece-wise regression of leaf number *versus* time was used to calculate leaf appearance rate. Transpiration efficiency was calculated in two ways: as the ratio between shoot biomass and transpiration for each measurement date, and as the slope of linear regressions between shoot dry matter and cumulative transpiration. Where departures from linearity were apparent in the relationship between shoot dry matter and transpiration, we used piece-wise regression rather than more complex models with more parameters. This allows for approximate estimates of transpiration efficiency at distinct phases of plant responses to salinity at the expense of the more realistic gradual change captured by non-linear (e.g. logistic) models.

Results

Rate of leaf appearance

Salinity reduced the rate of leaf appearance in all four varieties (Fig. 1, Table 1). Janz, Krichauff and Keel

Fig. 1 Dynamics of leaf appearance in wheat (Janz, Krichauff) and barley (Keel, Mundah) as affected by soil salinity. Error bars are two standard errors of the mean. Parameters of the fitted functions are in Table 1



had a two-stage response to salinity. In stage 1, the rate of leaf appearance was reduced by 14 to 29% in relation to the controls. In stage 2, the rate declined by ~70% in Janz and 40–50% in Krichauff and Keel. The onset of stage 2, i.e. the break point in the leaf number *versus* time function, was cultivar and salinity specific and ranged from 15 to 30 days after sowing. Mundah's response to salinity was quantitatively and qualitatively different; rather than a two-stage response, it had a single reduction in the rate of leaf appearance from 0.17 leaf d⁻¹ in controls to 0.13 leaf d⁻¹ at 0.75 dS m⁻¹ and 0.12 leaf d⁻¹ at 1.5 dS m⁻¹.

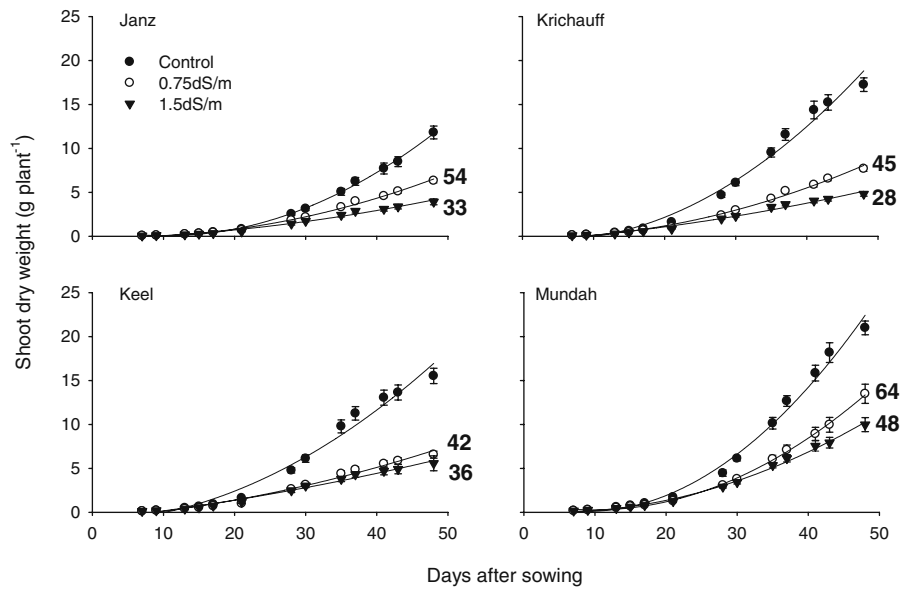
Dynamics of shoot growth and transpiration

Figure 2 shows the dynamics of shoot growth. A statistically significant effect of salinity was first detected on the third date of sampling and all three sources of variation, i.e. salinity, variety and interaction, became highly significant as stress progressed with ontogeny (all $P < 0.0001$). In relation to controls at the end of the measurement period, the shoot dry matter of Janz, Krichauff and Keel was reduced by 54–65% at 0.75 dS m⁻¹ and by 72–79% at 1.5 dS m⁻¹. Salinity caused smaller growth reductions

Table 1 Rate of leaf appearance in wheat (Janz, Krichauff) and barley (Keel, Mundah) as affected by soil salinity. Salt-stressed Janz, Krichauff and Keel showed a two-stage response with an initial reduction in leaf appearance rate (Stage 1) followed by a second reduction (Stage 2) after a cultivar- and salinity-specific break point. Controls of all four cultivars and Mundah under all treatments maintained a steady rate, which is reported as Stage 1

Variety	Salinity	Rate of leaf appearance (leaf d ⁻¹)		Break point (d)
		Stage 1	Stage 2	
Janz	Control	0.262±0.016		
	0.75 dS m ⁻¹	0.208±0.007	0.082±0.012	24.2±1.31
	1.5 dS m ⁻¹	0.192±0.006	0.071±0.006	25.0±1.49
Krichauff	Control	0.205±0.013		
	0.75 dS m ⁻¹	0.177±0.028	0.105±0.004	16.8±3.26
	1.5 dS m ⁻¹	0.151±0.020	0.122±0.008	15.0±2.89
Keel	Control	0.148±0.005		
	0.75 dS m ⁻¹	0.127±0.007	0.082±0.012	30.0±3.89
	1.5 dS m ⁻¹	0.105±0.004	0.082±0.012	25.0±9.82
Mundah	Control	0.166±0.005		
	0.75 dS m ⁻¹	0.133±0.005		
	1.5 dS m ⁻¹	0.121±0.004		

Fig. 2 Dynamics of shoot growth in wheat (Janz, Krichauff) and barley (Keel, Mundah) as affected by soil salinity. Shoot dry weight was estimated non-destructively using an image analysis system. Error bars are two standard errors of the mean. Numbers are shoot weight in the last measurement date as percentage of controls



in Mundah, i.e. 44% at 0.75 dS m⁻¹ and 62% at 1.5 dS m⁻¹.

Figure 3 shows the dynamics of cumulative plant transpiration. A statistically significant effect of salinity was already detected on the first date of sampling and all three sources of variation became highly significant as stress progressed with ontogeny (all $P < 0.0001$). At 48 days after sowing, when

the last sample of dry matter was taken, cumulative transpiration ranged from 71 to 49% of controls.

Transpiration efficiency

Table 2 summarises transpiration efficiency calculated as the ratio between shoot biomass and transpiration across measurement dates up to 48 days after sowing.

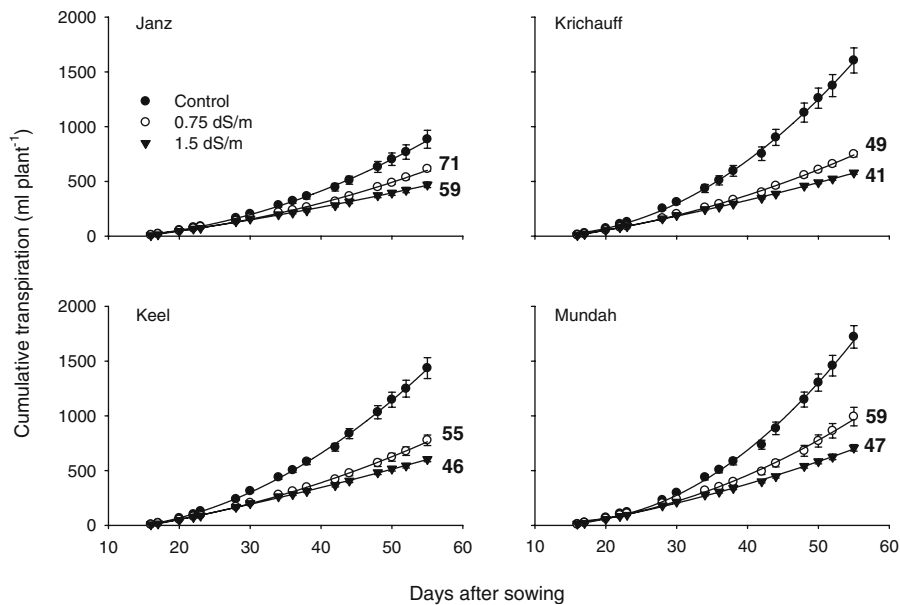


Fig. 3 Dynamics of cumulative plant transpiration in wheat (Janz, Krichauff) and barley (Keel, Mundah) as affected by soil salinity. Error bars are two standard errors of the mean.

Numbers are transpiration at 48 days after sowing as percentage of controls; this date corresponds to the last measurement of shoot dry matter

Table 2 Transpiration efficiency (g l^{-1}) of wheat and barley as affected by salinity

Treatment	Wheat		Barley	
	Janz	Krichauff	Keel	Mundah
Control	3.4±0.27 a	2.9±0.34 a	2.8±0.33 a	3.7±0.37 a
0.75 dS m^{-1}	3.5±0.18 a	2.4±0.16 ab	2.1±0.14 b	3.2±0.30 a
1.5 dS m^{-1}	3.0±0.21 a	1.9±0.13 b	2.1±0.12 b	3.2±0.28 a

Values are means \pm s.e. calculated as ratios between shoot biomass and transpiration across sampling dates. Different letters indicate significant salinity effect for a given variety ($P < 0.05$)

There was a minor, largely non-significant ($P > 0.05$) effect of salinity at 0.75 dS m^{-1} whereas reductions in transpiration efficiency at 1.5 dS m^{-1} ranged from 13 to 35% with respect to controls. A dynamic analysis of biomass as a function of transpiration revealed time and variety-dependent responses to salinity (Fig. 4). Transpiration efficiency of Janz, Krichauff and Keel showed a two-stage response to salinity. In stage 1, reductions with respect to controls were 12–27% at 0.75 dS m^{-1} and 26–39% at 1.5 dS m^{-1} (inset of Fig. 4, open bars). In stage 2, transpiration efficiency declined substantially, particularly at high salinity (inset of Fig. 4, closed bars). Transpiration efficiency of salt-treated Mundah was steady over the measurement period; it was unaffected at 0.75 dS m^{-1} ($P > 0.05$) and was reduced by 16% at 1.5 dS m^{-1} .

Relative response of transpiration and transpiration efficiency to salinity

To address our working hypothesis, we compared the effect of salinity on transpiration and transpiration efficiency on a normalised scale (Fig. 5). Considering the whole period to 48 days after sowing, salinity stress that reduced transpiration by 30–60% reduced transpiration efficiency by 0–35%; these magnitudes were comparable to those reported by Holloway and Alston (1992) and Grewal (2010) (Fig. 5a). During stage 1, transpiration was more responsive to salinity than transpiration efficiency (open symbols in Fig. 5b). During Stage 2, quantitative effects of salinity were similar for transpiration and transpiration efficiency (closed symbols in Fig. 5b). Stage 1

Fig. 4 Shoot dry weight as a function of cumulative plant transpiration in wheat (Janz, Krichauff) and barley (Keel, Mundah) as affected by soil salinity. Insets show transpiration efficiency, i.e. the slope of the regressions; for salt-treated Janz, Krichauff and Keel piecewise regression returned an initial slope (open bar) and a second slope after a break point (closed bar). Error bars are one standard error of the regression slopes

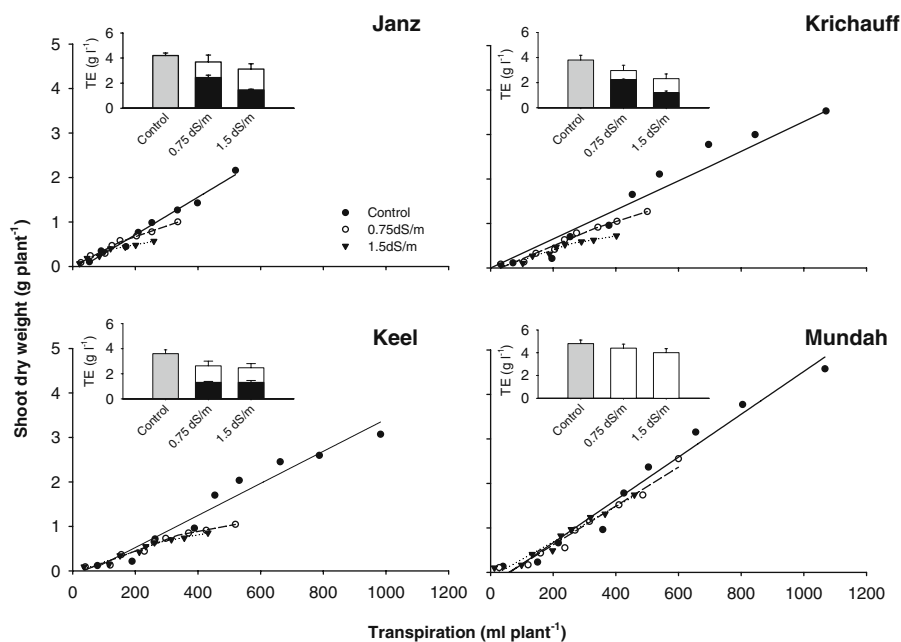
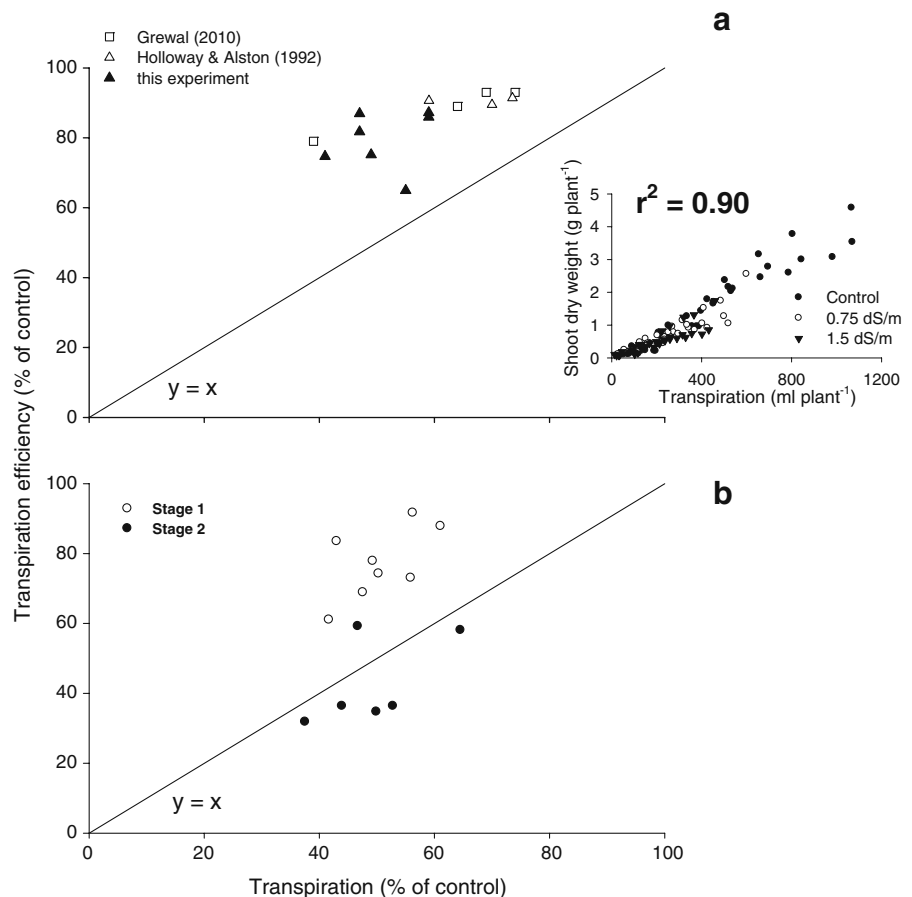


Fig. 5 Relative effects of salinity on transpiration and transpiration efficiency. **a** Transpiration efficiency and water use for the whole period up to 48 days after sowing. Data from Grewal (2010) and Holloway and Alston (1992) are included for comparison. **b** Transpiration efficiency and water use split in Stages 1 and 2. Inset shows the relationship between shoot biomass and transpiration for the data pooled across varieties and treatments



accounted, however, for most of the measurement period in Janz, Krichauff and Keel and Stage 2 was not evident in Mundah. Consistently, transpiration accounted for 90% of the variation in shoot growth for the pooled data (inset Fig. 5).

Leaf chlorophyll and gas exchange

Table 3 summarises snapshot measurements of leaf chlorophyll and leaf gas exchange. ANOVA indicated a significant effect of variety and salinity on SPAD measured 47 days after sowing; barley leaves were greener than their wheat counterparts, and salinity consistently increased leaf greenness in all varieties. In Keel for example, SPAD increased from 46 in controls to 54 under high salinity. Significant variety and interaction effects were recorded 83 days after sowing; higher SPAD with increasing salinity was still evident in wheat but the trend started to revert in

barley, particularly in Keel where control plants gave readings of 45 and high salinity plants read 34.

ANOVA indicated significant effects of variety, salinity and their interaction on leaf stomatal conductance, photosynthesis and transpiration but no effect on the assimilation-to-transpiration ratio. Salinity consistently reduced stomatal conductance, photosynthesis and transpiration. Barley was more responsive than wheat to salinity: the more marked reductions in stomatal conductance, photosynthesis and transpiration were recorded for Keel at 0.75 dS m⁻¹ and for Mundah under high salinity. The short-term, leaf based ratio of assimilation and transpiration and longer-term biomass-to-transpiration ratio were unrelated (data not shown).

Leaf sodium concentration

Leaf sodium concentration was significantly affected by variety, salinity and their interaction (all $P <$

Table 3 Leaf chlorophyll (SPAD measurements at 47 and 83 days after sowing) and gas exchange variables (33 days after sowing) in wheat (Janz, Krichauff) and barley (Keel, Mundah) as affected by soil salinity

Variety	Salinity	Leaf chlorophyll (SPAD units)		Conductance (mol H ₂ O m ⁻² s ⁻¹)	Photosynthesis (μmol CO ₂ m ⁻² s ⁻¹)	Transpiration (mmol H ₂ O m ⁻² s ⁻¹)	Photosynthesis/Transpiration (μmol CO ₂ / mmol H ₂ O)
		47 DAS	83 DAS				
Janz	Control	37.8±0.59 ^a	41.3±0.87	0.28±0.026	10.6±0.63	3.0±0.20	3.5±0.11
	0.75 dS m ⁻¹	41.6±0.51	43.5±1.18	0.12±0.010	8.5±0.34	1.6±0.10	5.4±0.30
	1.5 dS m ⁻¹	43.2±0.55	46.7±0.62	0.08±0.008	5.4±0.64	1.2±0.11	4.6±0.54
Krichauff	Control	44.5±0.86	42.6±0.90	0.22±0.035	11.7±0.72	2.6±0.31	5.2±0.61
	0.75 dS m ⁻¹	46.6±0.52	47.3±1.93	0.15±0.020	9.8±0.79	2.0±0.21	5.0±0.28
	1.5 dS m ⁻¹	47.6±0.94	46.9±1.45	0.11±0.016	7.8±1.03	1.6±0.20	5.9±1.23
Keel	Control	46.4±1.16	45.2±1.05	0.24±0.028	12.1±1.23	2.7±0.23	4.4±0.35
	0.75 dS m ⁻¹	53.1±0.82	39.0±1.69	0.08±0.011	7.5±0.69	1.2±0.13	6.3±0.33
	1.5 dS m ⁻¹	54.0±0.92	33.9±2.91	0.08±0.009	6.6±0.98	1.3±0.12	5.6±1.03
Mundah	Control	43.9±1.07	40.4±0.87	0.17±0.016	12.5±0.81	2.2±0.16	5.7±0.18
	0.75 dS m ⁻¹	50.9±0.65	36.7±1.92	0.11±0.011	7.7±1.03	1.6±0.14	5.6±1.20
	1.5 dS m ⁻¹	51.5±1.72	48.3±2.06	0.04±0.011	2.5±0.43	0.7±0.15	4.7±1.16
	Variety	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.254
	Salinity	<0.0001	0.113	0.002	0.001	0.002	0.344
	Interaction	0.070	< 0.0001	0.003	0.008	0.017	0.429

P values at the bottom of table are from analysis of variance

^aStandard error of the mean

0.0001). Control wheat plants had less than 2 mM Na kg⁻¹ DW compared with their barley counterparts which had 5–10 mM Na kg⁻¹ DW (Fig. 6). In response to intermediate salinity, leaf sodium concentration increased 2-fold in Krichauff, 4-fold in Keel and 7.6-fold in Mundah; this salinity treatment did not change sodium concentration of Janz in relation to controls. In response to high salinity, leaf sodium

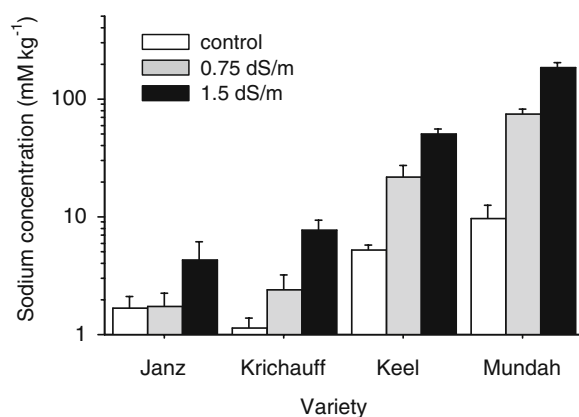


Fig. 6 Sodium concentration in the fourth leaf of wheat (Janz, Krichauff) and barley (Keel, Mundah) as affected by soil salinity. Error bars are one standard error of the mean

concentration increased 2.5- to 6.7-fold in wheat and 9.6- to 18.8-fold in barley.

Yield and its components

Table 4 summarises yield and yield components at maturity. Salinity, variety and their interaction affected all yield components, except for the lack of variety effect on grain size. The best relative yields were 84% of controls for Keel at 0.75 dS m⁻¹ and 32% of controls for Mundah at 1.5 dS m⁻¹.

Yield was closely associated with shoot dry matter (yield=0.55+0.38 shoot DW, $r^2=0.88$, $P<0.0001$). The range of shoot dry matter under salinity was 36 to 69% of controls for barley and 18 to 61% of controls for wheat.

Yield was strongly associated with grain number (yield=-0.20+0.038 grain number, $r^2=0.94$, $P<0.0001$) and weakly associated with grain size (yield=-7.3+0.37 grain size, $r^2=0.35$, $P<0.05$). The range of grain number under salinity was 31 to 85% of controls for barley and 22 to 70% of controls for wheat. Under salinity, Janz and Mundah maintained grain size close to that of controls, whereas grain size was reduced in Krichauff and Keel.

Table 4 Yield components of wheat (Janz, Krichauff) and barley (Keel, Mundah) as affected by soil salinity

Variety	Salinity	Yield (g plant ⁻¹)	Shoot dry weight (g plant ⁻¹)	Harvest Index	Grain number (plant ⁻¹)	Grain size (mg grain ⁻¹)
Janz	Control	8.2±0.75	19.2±1.60	0.42±0.012	264±22.0	31.1±0.68
	0.75 dS m ⁻¹	6.3±0.43	11.8±0.81	0.53±0.007	162±12.5	38.5±1.35
	1.5 dS m ⁻¹	2.0±0.16	4.4±0.43	0.45±0.017	59±5.0	33.0±1.75
Krichauff	Control	11.6±0.46	25.1±0.76	0.46±0.011	270±11.9	42.9±2.00
	0.75 dS m ⁻¹	6.8±0.30	14.7±0.50	0.46±0.011	189±8.3	35.8±0.63
	1.5 dS m ⁻¹	2.2±0.20	4.6±0.47	0.49±0.018	76±5.3	29.3±1.26
Keel	Control	6.9±0.28	17.7±0.97	0.39±0.012	163±13.7	42.3±2.50
	0.75 dS m ⁻¹	5.8±0.57	12.3±0.72	0.46±0.026	138±10.6	42.4±2.83
	1.5 dS m ⁻¹	1.4±0.14	6.4±0.41	0.22±0.012	54±2.7	26.5±1.52
Mundah	Control	10.7±0.46	32.2±0.57	0.33±0.012	296±11.0	36.1±0.88
	0.75 dS m ⁻¹	6.9±0.28	15.1±0.53	0.45±0.011	189±7.7	36.4±0.02
	1.5 dS m ⁻¹	3.4±0.24	11.5±0.42	0.29±0.020	92±6.6	36.9±0.74
	Variety	<0.0001	<0.0001	<0.0001	<0.0001	0.120
	Salinity	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	Interaction	<0.0001	<0.0001	<0.0001	0.0001	<0.0001

P values at the bottom of table are from analysis of variance

Yield was unrelated to harvest index ($P>0.43$). However, harvest index responded strongly to variety by salinity interaction. In relation to controls, 0.75 dS m⁻¹ had no effect on the harvest index of Krichauff, and increased harvest index in Keel, Janz and Mundah. Under high salinity, wheat cultivars maintained a slightly greater harvest index than the corresponding controls. In barley, high salinity caused a moderate reduction in the harvest index of Mundah and a larger reduction in Keel.

Discussion

Growing conditions

The limitations of experiments using pot-grown plants are well established (Ben-Porath and Baker 1990; Passioura 2006; Sadras et al. 1993; Wise et al. 1990). Our study used pot-grown plants in a glasshouse to achieve accuracy in measuring transpiration and transpiration efficiency at the expense of realism in relation to field-grown plants. We therefore briefly compare the development and growth of our plants in relation to plants grown in larger soil volumes in both field and controlled environments. The break point in the rate of leaf appearance detected in our study is

typical but not universal in field-grown cereals (Abeledo et al. 2004; Calderini et al. 1996; Jamieson et al. 1995). Quantitatively, the rate of leaf appearance in our control plants ranged from 0.15 to 0.26 leaf d⁻¹, which corresponds to phyllochron (i.e. inverse of the rate) between 80 and 142°Cd leaf⁻¹. This compares with reported phyllochron of field grown crops in temperate environments, e.g. 89 to 123°Cd leaf⁻¹ for wheat (Jamieson et al. 1995), 63 to 133°Cd leaf⁻¹ for barley (Abeledo et al. 2004). Stomatal conductance, transpiration rate and SPAD readings in our control plants were similar, and photosynthetic rates slightly lower than typical values of cereals under field conditions (e.g. Cabrera-Bosquet et al. 2009). Transpiration efficiency of control plants in our experiment ranged from 2.8 to 3.7 g l⁻¹, in comparison to 2.7 g l⁻¹ for control wheat plants in experiments using 1.2 m long pots (Holloway and Alston 1992), and a range from 2.0 to 3.4 g l⁻¹ for control wheat and barley in experiments using 0.5 m long pots (Richards 1992). Control wheat in the experiment of Holloway and Alston (1992) achieved shoot biomass (24.1 g pot⁻¹) and grain yield (9.1 g pot⁻¹) which are within the ranges for the control plants in our experiment (Table 4). Altogether, these comparisons indicate that the plants in our experimental conditions developed at rates that were

comparable to those of field grown crops in temperate environments, and dynamics of growth estimated using image analysis, yield and transpiration efficiency were comparable to those reported for plants grown in larger pots.

Salinity in the light of a resource-centred model

Salinity reduced grain yield primarily by reducing shoot biomass (Table 4). Consistent with our working hypothesis, biomass decreased as a result of a large reduction in transpiration, with a secondary contribution from a reduction in transpiration efficiency (Fig. 5). This agrees with both the general observation that abiotic stresses reduce capture rather than efficiency in the use of resources (Monteith 1977; Sadras et al. 2005; Steduto and Albrizio 2005; Valle et al. 2009) and with the particular observation of transpiration-driven effects of salinity on plant growth (Grewal 2010; Halvorson and Reule 1976; Holloway

and Alston 1992; Katerji et al. 1997; Shani and Ben-Gal 2005).

Here we propose a framework that links the Munns and Tester (2008) two-phase model of plant responses to salinity and the resource-driven model of Sadras and colleagues (Sadras et al. 2005, Sadras 2009), as illustrated in Fig. 7. Pathway [1] is the initial osmotic phase of plant response to salinity; it begins immediately after the salt concentration around the roots reaches a threshold, and leads to a reduction in shoot growth mediated by reduced leaf expansion, reduced rate of leaf appearance, and reduced stomatal conductance. The relative importance of these mechanisms is dependent on both the genotype and the intensity of the stress, as illustrated in Fig. 1 for leaf appearance rate. Architectural root traits are likely to be important in defining the onset of the osmotic phase in environments of south-eastern Australia where salinity increases with soil depth (Rodriguez et al. 2006; Sadras et al. 2002). Indirect selection for yield of

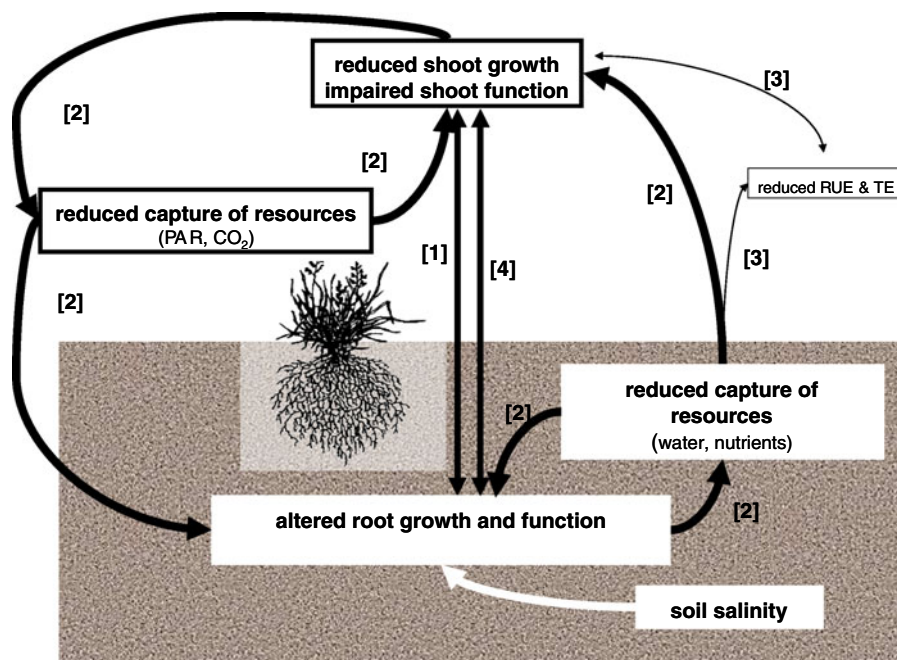


Fig. 7 Mechanisms of crop growth responses to soil salinity. Pathway [1] is the osmotic phase involving direct root perception of soil salinity, and root signals inducing reduction in shoot growth mediated by reduced stomatal conductance, reduced leaf expansion and reduced rate of leaf appearance; the two-way arrow allows for hypothetical shoot-to-root signalling. Pathway [2] involves a strong, reinforcing loop of reduced shoot and root growth, which is mediated by impairment of the ability of root systems and canopies to capture resources.

Pathway [3] involves reductions in the efficiency in the use of resources, as exemplified by radiation use efficiency (RUE) and transpiration efficiency (TE). Pathway [4] is the ion-specific phase of plant response to salinity, which starts when salt accumulates to toxic concentrations in the old, fully-expanded leaves unable to dilute the salt arriving in them, contributing to faster senescence. Pathways [1] and [4] are based on Munns and Tester (2008) and Pathways [2] and [3] on Sadras et al. (2005) and Sadras (2009)

wheat in eastern Australia has favoured shallower root systems associated with wider growth angle and lower number of seminal axes in southern environments compared to their counterparts adapted to deep clay soils in the northern cropping region (Manschadi et al. 2006). Shallower root systems may be adaptive in terms of capture of water and nutrients in southern environments, but they might also be advantageous in delaying the onset of the osmotic phase in saline soils.

Pathway [2] involves a strong feedback process whereby the initial reduction in growth of the shoot, root or both closes a loop that may eventually override other processes. Pathway [2] highlights the importance of interactions between salinity and availability of resources, mainly water (Katerji et al. 2009; Richards 1992) and nitrogen (Abdolzadeh et al. 2008; Esmaili et al. 2008).

Pathway [3] involves changes in radiation use efficiency and transpiration efficiency; these efficiencies are stable except under conditions of severe stress (Sinclair and Muchow 1999; Steduto et al. 2007). In our study, transpiration efficiency determined from biomass and transpiration showed stability or relatively small reductions in response to salinity, in agreement with previous studies (Grewal 2010; Halvorson and Reule 1976; Holloway and Alston 1992; Katerji et al. 1997; Shani and Ben-Gal 2005). Reductions in transpiration efficiency of salt-stressed plants may be partially related to the diversion of photosynthate for controlling accumulation of Na^+ and minimising deleterious effects of accumulated Na^+ , as these processes might consume a large fraction of the plant's energy budget (Malagoli et al. 2008). In agreement with Rawson (1986), we verified the relative stability of transpiration efficiency derived from short-term gas exchange measurements in salt-stressed plants (Table 3). The lack of association between short-term leaf gas exchange and long-term plant water use efficiency is not uncommon and highlights the importance of suitable scaling up approaches (Kramer and Boyer 1995; Sinclair et al. 2004). The salt-induced increase in chlorophyll concentration may have contributed to the maintenance of photosynthesis and transpiration efficiency of young expanded leaves. Our data support a two-stage effect of salinity on leaf nitrogen concentration (Table 3). First, in young plants, salinity accelerates senescence of basal leaves and favours translocation of nitrogen to young expanding leaves, hence in-

creasing their nitrogen concentration, chlorophyll, and potentially their photosynthesis and transpiration efficiency (Brueck 2008). Second, in older plants with no expanding leaves, salinity eventually accelerates senescence of upper leaves, leading to a reversal of the initial salt effect, i.e. lower leaf chlorophyll content in salt-stressed plants. Furthermore, we hypothesise that the ability to maintain the rate of leaf appearance in salt-stressed Mundah may be an important aspect of the overall adaptation of this cultivar to salinity. The superior performance of Mundah in soils with high levels of boron normally associated with salinity and alkalinity in South Australia has been documented in field studies (Long et al. 2003). In the limited set of varieties compared in our study, Mundah was the worst in terms of sodium exclusion (Fig. 6) and the best in terms of maintenance of rate of leaf appearance, transpiration, transpiration efficiency, biomass and yield in response to salinity. In the framework of Munns and Tester (2008), it follows that Mundah must be tissue tolerant. An alternative hypothesis is that Mundah is neither a salt excluder nor tissue tolerant. Instead, Mundah's strategy might involve maintenance of a high rate of leaf appearance and a rapid turnover of leaf material, mainly nitrogen, so ensuring some degree of compensatory performance by young expanding leaves. The vertical distribution of nitrogen in the canopy has important implications for crop-level photosynthesis and resource-use efficiency (Lemaire and Gastal 2009) and deserves closer attention in terms of crop responses to salinity.

Pathway [4] is the ion-specific phase of plant response to salinity; it begins when salt accumulates to toxic concentrations in the mature, fully-expanded leaves unable to absorb further salt. This effect leads, eventually, to accelerated senescence and further reductions in capture of resources. For Janz, Krichauff and Keel, but not for Mundah, our dynamic analysis showed a step change in both leaf appearance rate (Fig. 1) and transpiration efficiency (Fig. 4) which might be related to the onset of this ion-specific phase. The break points of these two traits need to be tested under field conditions, where progression of salt stress is likely to be slower than in our pot-grown plants. Interestingly, the break point for leaf appearance rate (~15–30 days after sowing) occurred earlier than the change in slope for transpiration efficiency (~36–40 days after sowing) as expected from the

known sensitivity of leaf expansion in response to salinity (Taleisnik et al. 2009).

The step from plant growth (Fig. 7) to grain yield requires consideration of harvest index in response to salinity (Table 4). Intermediate salinity increased harvest index in all four varieties; this is consistent with field studies with durum wheat and barley (Katerji et al. 2009). Under high salinity, harvest index was maintained in wheat but was reduced in barley, particularly in Keel. It is not possible to conclude from our data, however, that this is a species-specific response. Harvest index is a direct, non-linear function of the fraction of seasonal water transpired after anthesis (Sadras and Connor 1991). Elucidating the effects of stress on harvest index requires consideration of the dynamics of water use and assimilation during the growing cycle. Further insight can be obtained by closer focus on the critical window of determination of grain number and size (Ghiglione et al. 2008; Sadras and Denison 2009). The plasticity of grain number and stability of grain size in response to salinity found in our study can be interpreted in evolutionary terms (Gambín and Borrás 2009; Sadras 2007) as an expansion of the early model of Smith and Fretwell (1974).

In conclusion, an understanding of the effect of salinity on crop development, growth and yield requires integration of low-level traits in a framework of resource capture, resource-use efficiency and plant allocation. We propose that osmotic stress tolerance, Na^+ exclusion, and tissue tolerance to accumulated Na^+ would improve yield of salt-stressed crops to the extent that these traits contribute to the maintenance of water uptake and harvest index.

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