Plant Soil (2010) 330:103–113
DOI 10.1007/s11104-009-0179-7

REGULAR ARTICLE

Response of wheat to subsoil salinity and temporary water stress at different stages of the reproductive phase

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Received: 26 April 2009 / Accepted: 18 September 2009 / Published online: 21 October 2009

Abstract To examine the effects of subsoil NaCl salinity in relation to water stress imposed at different growth stages, wheat was grown in a heavy texture clay soil (vertosol) under glasshouse conditions in polythene lined cylindrical PVC pots (100 cm long with 10.5 cm diameter) with very low salinity level (EC_e 1.0 dS/m; ESP 1.0 and Cl 30 mg/kg soil) in top 10 cm soil (10–20 cm pot zone) and low salinity level (EC_e 2.5 dS/m, ESP 5, and Cl 100 mg/kg soil) in top 10–20 cm soil (20–30 cm pot zone). The plants were exposed to three subsoil salinity levels in the 20–90 cm subsoil (30–100 cm pot zone) namely low salinity (EC_e: 2.5 dS/m, ESP: 5, Cl: 100 mg/kg soil), medium salinity (EC_e: 4.0 dS/m, ESP: 10, Cl: 400 mg/kg) and high salinity (EC_e: 11.5 dS/m, ESP: 20, Cl: 1950 mg/kg) in the subsoil (20–90 cm soil layer: 30–100 cm pot zone). Watering of plants was withheld for 20 days commencing at either early booting or anthesis or mid grain filling, and then resumed until maturity, and these treatments were compared with no water stress. Water stress commencing at anthesis stage had the most depressing effect on grain yield and water use efficiency of wheat followed by water stress at grain filling stage and early booting stage. High subsoil salinity reduced grain yield by 39.1, 24.3%, and 13.4% respectively in plants water-stressed around anthesis, early booting, and mid grain filling compared with 36.6% in well-watered plants. There was a significant reduction in root biomass, rooting depth, water uptake and water use efficiency of wheat with increasing subsoil salinity irrespective of water regimes. Plants at high subsoil salinity had 64% of their root biomass in the top 0–30 cm soil and there was a marked reduction in subsoil water uptake. Roots also penetrated below the non-saline surface into salinised subsoil and led to attain high concentration of Na and Cl and reduced Ca/Na and K/Na ratio of flag leaf at anthesis stage. Results suggest that high subsoil salinity affects root growth and water uptake, grain yield and water use efficiency even in well water plants. Water stress at anthesis stage had the most depressing effect on wheat.

Keywords Exchangeable sodium percentage · Grain yield · Ionic balance · Root function · Water uptake · Water use efficiency

Responsible Editor: Timothy J. Flowers.

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Introduction

Soil salinity is a major limiting factor to crop production in many countries (Munns 2005; Rengasamy 2006; Ashraf et al. 2008; Katerji et al. 2009). Impacts of salt on the growth, development and productivity of crops may vary considerably depending upon the level of stress and growth stage of the crop (Grieve et al. 1994; Mass et al. 1994; Munns 2002). The majority of past research on salinity/sodicity has been based either on topsoils or solution culture/sand culture studies. There is a dearth of information on salinity/sodicity in subsoils, which for many crops are an important potential source of water and nutrients (Rengasamy 2002; Dang et al. 2006, Wong and Asseng 2007). The limitation to productivity imposed by subsoil salinity/sodicity in Australian rainfed cropping systems has only recently been realised. These effects need to be quantified and understood in order to improve the productivity of these difficult soils (Rengasamy 2002; Rengasamy et al. 2003). This need is particularly great in the northern grains region of Australia, where winter crops depend heavily on subsoil water conserved through a preceding summer fallow. For these crops, subsoil water potentially comprises a much bigger component of total crop water use than in southern Australia, and therefore the effects of subsoil salinity/sodicity may be proportionally greater. Well developed, functioning root systems within subsoils are presumably required to access subsoil water and nutrients.

Subsoil salinity may affect plants directly via several mechanisms including sodium or chloride toxicity, competition for uptake of other cations, or osmotic effects on water uptake (Naidu and Rengasamy 1993; Rengasamy et al. 2003; Dang et al 2008; Katerji et al. 2009). Many soils in Australia also have high exchangeable sodium percentage (ESP) besides salinity problem in subsoils which have marked detrimental effect on soil structure and consequently on root growth (Rengasamy et al. 2003). High ESP also causes a build up of Na in plant tissues, which interferes with the uptake of nutrients and affect the plant growth (Naidu and Rengasamy 1993). Alternatively, subsoil salinity may simply favour root growth in the less saline parts of the heterogeneous soil profile, possibly leading to reduced root growth in subsoils whilst ever the surface soil remains moist, resulting in a less well developed root system for later subsoil water uptake.

The primary objective of the work described here was to determine if subsoil salinity amplifies plant responses to water shortage by restricting subsoil root growth and water uptake, and effects of subsoil salinity may be escalated when there is water stress during early booting or anthesis or grain filling stage.

Materials and methods

The effects of variable subsoil salinity in relation to water stress imposed at different growth stages were examined on wheat grown in polythene lined cylindrical PVC pots (100 cm long, 10.5 cm diameter) under glasshouse conditions. Temperature in the glasshouse during growth period varied from 10 to 27˚C during the day and 5 to 15˚C during the night. The top 0–10 cm zone of pots was kept unfilled in all the pots for watering the plants. A heavy texture soil (vertosol) with very low salinity (EC_e 1.0 dS/m; ESP 1.0 and Cl 30 mg/kg soil) in top 10 cm soil (10–20 cm pot zone) and low salinity level (EC_e 2.5 dS/m, ESP 5, and Cl 100 mg/kg soil) in top 10–20 cm soil (20–30 cm pot zone). The experiment involved combinations of three salinity levels namely low salinity (EC_e: 2.5 dS/m, ESP: 5, Cl: 100 mg/kg soil), medium salinity (EC_e: 4.0 dS/m, ESP: 10, Cl: 400 mg/kg) and high salinity (EC_e: 11.5 dS/m, ESP: 20, Cl: 1950 mg/kg) in the subsoil (20–90 cm soil layer: 30–100 cm pot zone) and four water regimes designed to impose stress at different stages of development. These three subsoil salinity levels were created by adding 0, 500, 3,000 mg NaCl/kg soil respectively for low, medium and high salinity level. Watering was maintained throughout in a control treatment, but suspended at the beginning of either booting, anthesis, or mid grain filling for 20 days, and then resumed until maturity. The experiment was conducted in completely randomised block design with 3 replications for each treatment combination. The different ECe, ESP and Cl values in topsoil and subsoil reflected the soil conditions almost similar observed in most soils with subsoil constraints in the North-western NSW, Australia (Grewal et al. 2004).

Soils with variable salinity levels were watered to approximate field capacity (0.32 g/g) with double deionised water and allowed to equilibrate at 25°C in a glasshouse for six weeks followed by oven drying of this soil at 45°C. The exact weight of dried soil (6 kg/pot) as per treatments (low, medium and high subsoil salinity) was added into bottom of the pots followed by tapping of pots and watering to field capacity. The soil in all these pots was allowed to settle for about hour. Another layer (0.9 kg soil/pot) of low salinity soil (EC_e 2.5 dS/m, ESP 5, and Cl 100 mg/kg soil) was added in all the pots irrespective of treatments followed by tapping of pots and watering of this layer to field capacity. Finally another layer (0.9 kg soil/pot) of very low salinity soil (EC_e 1.0 dS/m; ESP 1.0 and Cl 30 mg/kg soil) was added in all the pots followed by watering to field capacity of this layer. A polypropylene tube (30 cm long, 3 cm internal diameter) was inserted into the upper 30 cm zone of each pot for watering the subsoil.

Wheat (cv. Sunvale) was sown 2 cm deep in each pot (5 seeds/pot). After emergence the soil surface was covered with a 5 cm deep layer of white polystyrene beads to check the soil evaporation. Two uniform plants were kept in each pot 3 weeks after sowing. The plants were watered with double deionised water on alternate days to return the soil water content to the approximate field capacity. Watering treatments were commenced at the early boot stage (65 days after sowing), anthesis (appearance of anthers on more than 50% of spikes, at 95, 98 and 101 days in the high, medium and low subsoil salt, respectively) and mid grain filling (120, 123 and 125 day, respectively in treatments with high, medium and low subsoil salt). Pots were weighed every two days to determine water-use. On the assumption that the mulch of polystyrene beads had prevented soil evaporation, water-use efficiency was determined based both on grain yield (g grain/L water transpired) as well as total above-ground dry matter (g above-ground dry matter/L water transpired) at harvest.

Harvest index was calculated by dividing grain yield dry matter of each treatment with their respective total above-ground dry matter (grain and shoot dry matter) at harvest.

Relative leaf water content (RWC) of the third fully opened leaf from the top was measured 16 days after suspending watering at the early boot stage. Relative leaf water content of the flag leaf was also determined 16 days after suspending watering at anthesis. RWC was determined by the equation (Barrs and Weatherley 1962):

$$RWC = \frac{(FW - DW)}{(TW - DW)} \times 100$$

FW, DW and TW are fresh weight, dry weight and turgid weight. Leaf discs (20) were collected into weighed sealed vials and weighed for fresh weight, then floated on double deionised water for 8 hours under light near the compensation point to attain maximum turgidity. These discs were blotted dry then weighed for turgid weight and oven dried for their dry weight.

Water soluble carbohydrate (WSC) content of leaves and stems was determined at 113 days after sowing, which was 18 days after suspending watering at anthesis. The Water soluble carbohydrates were extracted twice in boiling water for 30 minutes on each occasion, then measured using the anthrone method (Yemm and Willis 1954).

Plants were harvested at maturity and separated into leaves, stems and grain, oven dried at 65°C for 48 h, then weighed for dry matter. Roots were washed with tap water followed by a dip in deionised water. Roots from each pot were sectioned into 0–10, 10–20, 20–50, 50–70 and 70–90 depths. These roots were then oven dried to determine root distribution. Prior to washing out the roots, soil was sub sampled to determine water content gravimetrically, which led to determine the residual water in soil at harvest.

The oven dried samples of roots, leaves, stems, and grains were digested in concentrated nitric acid at 130°C for elemental analysis by inductively coupled plasma spectrometry (Zarcinas et al. 1987).

Results

Early growth, plant symptoms and phenology

Germination, emergence and initial growth of plants were not influenced by subsoil salinity treatments. Symptoms of salt stress in plants grown at high subsoil salt first appeared six weeks after sowing. These symptoms included smaller size leaves, reduced tiller number, necrotic spots on leaf tips and necrosis of leaf margins of older leaves. Cessation of watering for 20 days at the early boot stage led to severe wilting of plants, which recovered quickly after watering. New tillers that formed in these plants were mostly unproductive and resulted in delayed maturity and non-uniform ripening of spikes. Salt stress accelerated development, regardless of water regime, advancing heading by 3–4 days, anthesis by 5–6 days, and maturity by 4–9 days. Cessation of watering for 20 days at booting delayed heading,

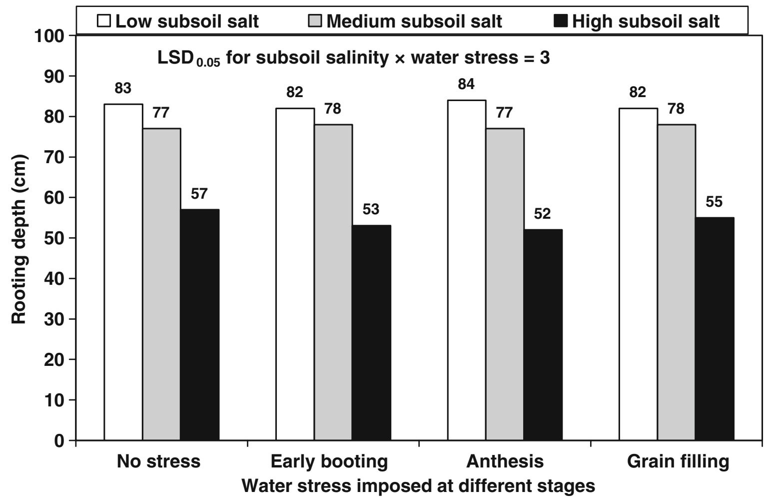

Fig.1 Effects of subsoil salinity and water stress imposed at different growth stages on rooting depth of wheat

anthesis and maturity by 3, 5 and 7 days, respectively. Conversely, cessation of watering for 20 days following anthesis or from the middle of grain filling advanced maturity by 6, 4 and 3 days in the low, medium and high salt treatments.

Root growth

Increasing subsoil salinity significantly ($P<0.05$) reduced the maximum rooting depth from 82 cm in low salt treatments to 77 and 54 cm in the medium and high salt treatments (Fig. 1). Imposing water stress either at the early boot stage or anthesis had very little effect on the rooting depth of plants grown.

Root dry weight was also significantly ($P<0.05$) reduced by increasing subsoil salinity, being almost halved between the low and high subsoil salt treatments (Table 1). There was a smaller but still significant effect of water stress, but there was no interaction between the treatments. That is, subsoil salinity did not amplify the root biomass response to water shortage imposed at three different times.

Table 1 Root dry weight (g/plant) of wheat plants influenced by subsoil salinity levels (Low, Medium and High) and water stress imposed at different growth stages

Water stress	Subsoil salt treatment			Mean
	Low	Medium	High	
No stress	9.30	8.00	5.15	7.50
Early booting	8.35	6.85	4.45	6.55
Anthesis	8.45	6.90	4.75	6.70
Grain-filling	8.60	7.35	4.85	6.95
Mean	8.70	7.30	4.80	

$LSD_{0.05}$: Subsoil salt,0.25; Water stress, 0.30; Interaction, NS

With respect to root distribution down the pot in treatments with high subsoil salt, most roots (64% of root biomass) were concentrated in the top 0–30 cm soil, and there were no roots below 60 cm (Fig. 2). With low subsoil salinity, only 37–42% of roots were in the top 0–30 cm (the range reflecting water regime), and roots were almost evenly distributed up to 90 cm depth (20% in 30–50 cm, 19% in 50–70 cm and 24% roots in bottom 70–90 cm depth). The root distribution responses to water regime were significant ($P<0.05$) but small.

Relative leaf water content (RWC) and water soluble carbohydrates (WSC)

RWC was measured 16 days after suspending watering at two stages of plant development, 'early booting' and 'anthesis' (Table 2). At both stages, the interaction between subsoil salt and water stress was significant ($P<0.05$). Whilst water stress *per se* had the greatest effect on RWC, the effect of increasing subsoil salinity was marked when plants were also water stressed (low RWC), especially when the water stress was imposed following anthesis.

The concentration of WSC in both leaves and stems decreased by an average 25% when watering was suspended after anthesis (Table 3). High salinity in the subsoil further slightly reduced the WSC concentration in leaves but increased it in stems ($P<0.05$).

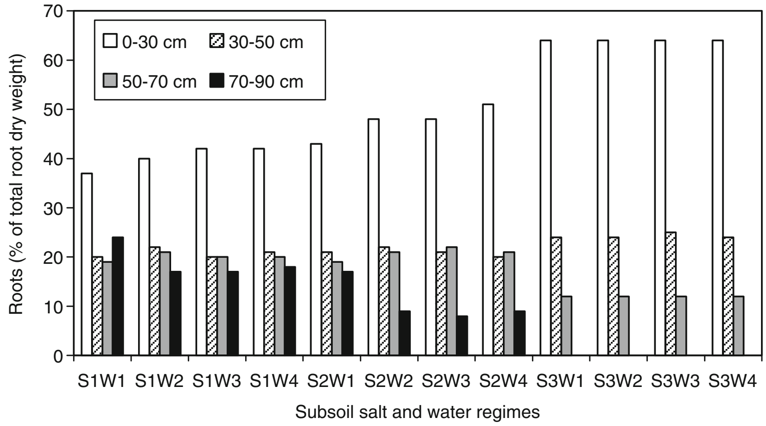

Fig. 2 Effects of subsoil salinity and water stress imposed at different growth stages on distribution of roots (% of total root dry matter) at different depths were significant. The LSD0.5 for 0–30 cm, 30–50 cm, 50–70 and 70–90 cm depth roots were 5, 2, 2 and 3 respectively. S1, S2 and S3 represent low, medium and high salinity respectively in subsoil. W1, W2, W3 and W4 represent no water stress, early booting stage, anthesis and grain filling stage water stress respectively

Na and Cl concentration and ionic balance (Ca/Na and K/Na ratios) of leaves

Both Na and Cl concentrations in flag leaf at anthesis stage increased significantly with increases in subsoil salinity (Table 4). Imposing water stress further increased Na and Cl concentration, but only in medium and high subsoil salinity and not in low subsoil salinity. The well-watered plants at high subsoil salinity contained 0.365 mmol/g Na and 0.404 mmol/g Cl compared with only 0.058 mmol/g Na and 0.189 mmol Cl/g in plants at low subsoil salts. Water stress following anthesis increased the concentrations of Na and Cl in flag leaf to 0.430 mmol/g and 0.432 mmol/g, respectively, in plants at high subsoil salt.

The Ca/Na and K/Na ratios of flag leaf at anthesis were depressed markedly by increasing subsoil salinity, irrespective of water regimes (Data not given). The Ca/Na ratio of flag leaf fell from 10.36 at low subsoil salt, to 1.58 at high subsoil salt. Like the Ca/Na ratio, the K/Na ratio of flag leaf was depressed from 36.4 to 5 by increasing subsoil salt from low to high, which was further reduced to 4.3 when water stress was imposed at anthesis.

Water uptake (transpired water), water use efficiency and residual water

As discussed in methodology that the mulch of polystyrene beads used in pots prevented soil evap-

Table 2 Effect of subsoil salinity levels on relative leaf water content (RWC) of no stress and water stressed plants at booting and anthesis stages

Subsoil salt	RWC booting		RWC Anthesis	
	No stress	Water stressed	No stress	Water stressed
Low	95	65	90	73
Medium	95	63	89	69
High	93	59	86	60
$LSD_{0.05}$: Subsoil salt×water stress				
	5		7	

Table 3 Effect of subsoil salinity levels and water stress imposed at anthesis stage on water soluble carbohydrate (WSC) content of leaves and stems determined 16 days after imposing water stress

Subsoil salt	WSC in leaves (%)		WSC in stems(%)	
	No stress	Water stressed	No stress	Water stressed
Low	32.0	24.0	42.2	30.0
Medium	31.5	23.2	42.7	30.4
High	30.0	21.5	43.3	33.3
$LSD_{0.05}$:Subsoil salt×water stress				
	2.1		1.5	

Table 4 Effect of subsoil salinity (Low, Medium, High) and water stress on Na and Cl concentrations (mmol/g DM) of flag leaf at anthesis stage

Water stress	Na concentration(mmol/g DM)			Mean	Cl concentration (mmol/g DM)			Mean
	Low	Medium	High		Low	Medium	High	
No stress	0.058	0.088	0.365	0.170	0.189	0.316	0.404	0.303
Early booting	0.060	0.092	0.372	0.175	0.191	0.319	0.412	0.307
Anthesis	0.063	0.098	0.430	0.197	0.223	0.332	0.432	0.329
Grain-filling	0.058	0.087	0.364	0.170	0.190	0.315	0.405	0.303
Mean	0.060	0.091	0.383		0.198	0.321	0.413	
$LSD_{0.05}$:								
Subsoil salt	0.017				0.011			
Water regime	0.018				0.012			
Subsoil salt×water stress	0.034				0.022			

oration, so water uptake actually refer to here as transpired water by wheat plants. Increasing subsoil salinity significantly reduced total transpired water under all water stress treatments, whilst suspending water application for 20 days also reduced transpired water (Table 5). Also, high subsoil salt and suspending watering reduced water use efficiency (WUE) based both on grain yield basis and above-ground dry matter basis, except there was no effect of salt on water use efficiency based on grain yield basis when watering ceased in mid grain filling (Table 5). WUE based on grain yield basis in high subsoil salt plants was 29%, 21%, 35% and 1% less in no stress, water stress at early-booting, anthesis and mid-grain filling stage respectively. Similarly the WUE based on above-ground dry matter under high subsoil salt reduced by 23%, 19%, 15% and 7% respectively in no stress, water stress at early-booting, anthesis and mid-grain filling stage.

There was significantly greater residual water left in the subsoil after harvest in high subsoil salinity compared with low subsoil NaCl salinity, in all water stress treatments (data not given). These differences in residual water were particularly marked where watering ceased at mid grain filling, with the high salt treatment leaving >600 mL more water than the low salt treatment.

Yield attributes and harvest index

High salts in the subsoil reduced tiller numbers per plant in all water regimes but had little effect on kernel number per spike or kernel weight (Table 6). Whilst water stress following anthesis severely re-

Table 5 Effect of subsoil salinity and water stress on transpired water (water uptake) and water use efficiency of wheat based on grain yield and above-ground dry matter of wheat plants at harvest

Water stress	Transpired water (L/plant)			Water use efficiency (g grains/L)			Water use efficiency (g dry matter/L)		
	Low	Medium	High	Low	Medium	High	Low	Medium	High
No stress	11.79	11.19	10.48	1.49	1.45	1.06	2.93	2.90	2.26
Early booting	10.08	10.04	9.13	1.24	1.26	0.99	2.64	2.79	2.14
Anthesis	8.57	8.44	8.02	0.57	0.42	0.37	1.89	1.75	1.60
Grain-filling	10.06	9.65	8.83	0.96	0.98	0.95	2.84	2.81	2.64
$LSD_{0.05}$:									
Subsoil salt × water regime		0.23			0.06			0.13	

Table 6 Effect of subsoil salinity and water stress on yield components of wheat

Water stress	Subsoil salt treatment			Mean
	Low	Medium	High	
Fertile tillers/plant				
No stress	11.65	10.35	8.00	10.00
Early booting	11.85	10.00	7.65	9.85
Anthesis	12.15	9.15	7.50	9.60
Grain-filling	11.80	10.15	8.50	10.15
Mean	11.85	9.90	7.90	
$LSD_{0.05}$:Subsoil salt, 0.75; Water stress, NS; Interaction, NS				
Kernel number/ear				
No stress	39.0	39.7	37.3	38.7
Early booting	31.0	33.3	33.0	32.4
Anthesis	18.0	17.0	17.3	17.4
Grain-filling	37.0	37.7	36.7	37.1
Mean	31.3	32.0	31.0	
$LSD_{0.05}$: Subsoil salt, NS; Water stress, 2.1; Interaction, NS				
1000- Kernel weight (g)				
No stress	38.7	39.0	37.6	39.0
Early booting	36.7	37.7	36.0	36.4
Anthesis	22.0	22.7	22.7	22.2
Grain-filling	23.0	24.3	20.0	22.9
Mean	30.0	30.9	29.0	
$LSD_{0.05}$: Subsoil salt, 1.0; Water stress, 1.3; Interaction, NS				

duced both kernel number and kernel weight, the effect of water stress imposed during grain filling was expressed only on kernel weight. There was no interaction between salt level and water regime for any of the yield components.

Increased subsoil salinity, as well as imposing water stress at different stages, significantly ($P<0.05$) reduced grain yield and harvest index of wheat (Table 7). Water stress following anthesis had the greatest affect on grain yield and harvest index. The effects of subsoil salinity on yields were not significantly greater in water-stressed plants than in well-watered plants. For example, in well-watered plants, increasing subsoil salinity from low to high reduced yields by 36%, whereas increased subsoil salinity reduced yield by 24.3, 39.1 and 13.4% when water stress was imposed during early booting, anthesis and grain-filling.

As with grain yield, water stress following anthesis had the greatest effect on harvest index, which was reflected most at medium and high subsoil salt. At low subsoil salt, imposing water stress at anthesis stage reduced the harvest index from 0.51 to 0.30, which was further depressed to 0.23 in plants at high subsoil salt.

Discussion

Results of significant reduction in root biomass, rooting depth, water uptake, water use efficiency and grain yield of wheat at high subsoil salinity even in no water stress plants and other water regimes suggest that high subsoil salinity like surface soil salinity has marked depressing effect on wheat irrespective of water regimes. There appeared to be no evidence to support the hypothesis that subsoil salinity would amplify the effects of drying the surface soil, when watering was withheld for periods of 20 days at any of the three different times. Although roots grew into salinised sub soil, they left residual water there that could have been used during grain filling (had the plants not been otherwise stressed), as evidenced by the low kernel mass of

Table 7 Effect of subsoil salinity and water stress on grain yield and harvest index of wheat

Water stress	Grain yield (g/plant)			Mean	Harvest index			Mean
	Low	Medium	High		Low	Medium	High	
No stress	17.60	16.20	11.15	15.00	0.51	0.50	0.47	0.49
Early booting	12.50	12.60	9.00	11.35	0.47	0.45	0.46	0.46
Anthesis	4.85	3.55	2.95	3.80	0.30	0.24	0.23	0.26
Grain-filling	9.70	9.50	8.40	9.20	0.34	0.35	0.36	0.35
Mean	11.15	10.45	7.90		0.41	0.39	0.38	
$LSD_{0.05}$: Subsoil salt	0.35					0.01		
Water regime	0.40					0.01		
Subsoil salt×water regime	0.65					0.02		

plants deprived of watering after mid grain filling. Thus any inability of wheat plants to utilise this subsoil water led to no greater yield reductions than would have occurred through the direct effects on the plants of salinity alone. The concentrations of water soluble carbohydrate in wheat stems were also highest in water stressed treatment with high subsoil salinity, showing that not even this labile store of assimilate was used to fill grains.

Roots in all treatments penetrated beyond 20 cm depth, and into subsoils that have medium and even high salinity. Even well-watered plants suffered a 36.6% loss in grain yield where the subsoil had high salinity. The soil properties in this treatment (salinity EC_e: 11.5 dS/m, ESP: 20, Cl: 1950 mg/kg) were typical of sites known to exhibit subsoil constraints to growth in North West of NSW Australia (Grewal et al. 2004). From this, it appeared that crops planted into these soils will suffer major yield penalties compared to crops in unconstrained soils, regardless of whether rainfall is such that crops would normally respond to the availability of subsoil water.

The increased level of salinity in the subsoil presumably had cumulative effects of reduced osmotic potential of soil solution (OP of about −90 kPa at low subsoil salt to −415 kPa at high subsoil salt), very high ECe (11.5 dS/m), ESP (20) and Cl (1950 mg/kg), which led to severe detrimental effects on root growth and consequently affected water uptake, and finally the grain yield and water use efficiency. Like the results of current study, Nuttall et al (2003) reported marked effect of high ECe and ESP on grain yield of wheat under field conditions in Victoria, Australia. High ESP in subsoil causes a build up of Na in plant tissues, which interferes with the uptake of nutrients and disturbs the plant growth (Naidu and Rengasamy 1993). The high level of Cl (1950 mg/kg) in subsoil in current appeared to cause Cl toxicity to roots and restricted the root elongation. Dang et al. (2008) reported soil Cl toxicity in subsoil as one of the major factors to affect the plant growth on many soils in northern Australia, and reported 854 mg Cl/kg as the critical level of Cl in subsoil to affect wheat yield. They reported soil Cl level as a more reliable indicator for the ability of roots to extract water from subsoil than ECe. Dang et al (2006) also demonstrated the presence of high Cl in many soils inhibited subsoil water extraction by wheat through the build up of toxic Cl ions in plant tissue. The Cl level of 1950 mg/kg in subsoil of current study was much greater than the critical level 854 mg Cl/kg reported by Dang et al. (2008) and there was also elevated concentration of Cl in wheat plants in current study.

Germination, emergence and initial growth of plants were unaffected by the subsoil salinity in current study, although these are commonly affected by surface soil salinity (Francois et al. 1986; Al-Karaki 2001). However, once roots penetrated beyond the surface soil that had low salinity into the salinised subsoil, reductions in growth occurred and visible symptoms were observed similar to surface salinity. It is clear that salinity can have serious effects on plant growth, even when confined to subsoils and plants have access to non-saline surface soils. These effects included reduced leaf size, leaf number, tiller number, necrotic spots on leaf tips and necrosis of leaf margins of older leaves, similar to the general responses to salinity reported by others (Munns et al. 1988; Mass and Grieve, 1990; Francois et al. 1986; Wang et al. 2001).

Results of this study indicate that the effects of high subsoil salinity and water stress imposed during early boot stage to grain filling stage to reduce grain yield were independent. Under high subsoil salinity, the reduction in grain yield was mainly due to reductions in number of ears per plant (effective tillers). However, the reduction in grain yield under water stress imposed at different stages commencing early boot stage was due to markedly lower number of grains per ear and reduced grain mass. Katerji et al. (2009) also reported that salinity and drought affect the grain yield of durum wheat differently. Under saline condition, they reported reduction in grain yield was due to reduction in number of grains per ear. However, the reduction in grain yield under drought condition was due to lower number of ears per plant. There was very high ECe (11.5 mS/m), Cl and ESP values in subsoil in current study as compared to EC and Cl values of saline water being used by Katerji et al. (2009). Probably this led to major variation in number of ears production in these two studies. The major reason for no effect of water regimes on number of ears per plant in current study in comparison to effects of drought in Katerji et al. (2009) study was due to fact that in current study water stress was commenced at early boot stage, anthesis and grain-filling stages, as tiller production is largely completed by these stages, so there was little or no effect of water regimes on tiller production. The main effects of water regimes in current study were only on number of grains per ear and grain mass, not on number of ears per plant (effective tillers).

Plants in the high subsoil salt environment adapted in a variety of ways. They concentrated roots (64% of total root dry weight) in the top 0–30 cm depth, a low-salt environment; increased water soluble carbohydrates in stems; and accelerated phenological development, to continue their growth and development for completion of their life cycle. But these plants were unable to compensate the yield losses in comparison to low subsoil salt.

Relative leaf water content decreased markedly with an increase in subsoil salinity in plants that were already water stressed, which has also been reported by other researchers (Sairam and Srivastava 2002). In the current study, there was little effect of increased salinity in the subsoil on relative leaf water content of no water stress plants, suggesting that the reduced plant growth of salt-stressed plants was primarily also attributable to something other than reduced cell turgidity caused by reduced osmotic potential.

There was a marked elevation in concentrations of Na and Cl and reductions in Ca/Na and K/Na ratios of flag leaf due to increase in subsoil salinity. Marked increases in the concentrations of Na and Cl in leaves of wheat at high subsoil salinity indicates that roots absorbed salts indiscriminately without exclusion of Na and/or Cl, which was transported to leaves. Necrotic leaf tips and margins observed at high subsoil salinity in current study were probably due to the accumulation of Na and Cl ions in leaf tissue. High Na concentration have been reported to be a limiting factor for plant growth in most crop plants (Francois et al. 1986; Munns 2002; Muranaka et al. 2002), although Na^+ is required in some plants, particularly halophytes (Glenn et al. 1999). Excessive Na has negative effects on electron transport and photosynthesis, and also through stomatal closure (Muranaka et al. 2002) which reduces assimilates supply. The grain yield of wheat has been reported to decrease progressively with an increase in Na concentration in leaves (Chippa and Lal 1995). The apparently excessive accumulation of Na^+ in plants at high subsoil salt in the present study markedly depressed the ionic balance (Ca/Na and K/Na ratios) of leaves. Calcium is known to play an important role in processes that preserve the structural and functional integrity of plant membranes, regulate ion transport and control activities of cell wall enzymes (Rengel 1992). Supplemental Ca supply has been reported to reduce Na accumulation in plants (Subbarao et al. 1990).

Increasing subsoil salinity also significantly reduced the K/Na ratio of flag leaf. Kara and Keser (2001) also observed reduction in K/Na ratio of maize plants by increasing the NaCl salinity. Tarakcioglu and Inal (2002) reported a marked decrease in K/Na ratio of lettuce plants by increased concentration of NaCl and/or Na in a solution culture experiment. Reduction of K uptake in plants by Na is a competitive process (Grattan and Grieve 1999). This competition could be at uptake level or transport level or both. In maize, salinity decreased growth due to K deficiency induced by excessive Na which caused reduction in uptake and translocation of K to shoots (Botella et al. 1997). A high level of K in young expanding tissue is associated with salt tolerance in many plant species (Storey et al. 1993; Khatun and Flowers 1995). Excessive Na^+ may also damage the plasma membrane and can increase the K^+ efflux from intercellular storage.

It is normally common though not universal that WUE based on above-ground dry matter basis increase a little with drought or salt stress due to stomata closer. However, in current study both high subsoil salt and water stress reduced WUE significantly based both on grain yield basis and above-ground dry matter basis, except there was no effect of salt on water use efficiency based on grain yield basis when watering ceased in mid grain filling. It appeared that the level high subsoil salt and water stress imposed at reproductive stage was so great that the wheat plants were unable to cope with the level of stress. Impact of high subsoil salt was, of course, greater on WUE based on grain yield compared with WUE based on above-ground dry matter basis. The impact of water stress at anthesis stage was greater on WUE compared with water stress at mid-grain-filling stage. Water stress at anthesis stage resulted in the rapid firing and loss of leaves that fell off the plants and were not included in the measurements of above-ground dry matter. Thus the loss in leaves contributed to decline in assimilate supply to grains as well as reduced aboveground biomass resulting in marked reduction in water use efficiency.

The results of this study have important implications for growing wheat on soils with a problem of subsoil with high ECe, ESP and Cl. Yields will be reduced on these soils regardless of seasonal rainfall conditions and any demand for subsoil water, unless more salt tolerant cultivars with improved water use efficiency are developed. Alternative profitable species with better salinity tolerance ability also need to be explored in these problematic soils.

Conclusions

The study demonstrates a significant impact of increasing subsoil NaCl salinity on wheat water uptake, grain yield and water use efficiency, regardless of water regimes. These effects appear to be due primarily to reduced root growth probably owing to osmotic potential effects of high EC and high concentration of Cl in rhizosphere and with their consequent effects on water uptake and over all plant growth. Water stress at anthesis stage had the most depressing effect on grain yield and water use efficiency of wheat followed by water stress at grain filling stage and early booting stage.

Acknowledgement The author acknowledges the help of Dr Shane Norrish, Professor Peter Cornish and technical staff (Mr Burhan Amiji, Mr Mark Emanuel and Ms Linda Allanson) for their help during this study. The work was supported by the Grains Research and Development Corporation of Australia (SIP08 project).

References

Al-Karaki GN (2001) Germination, sodium and potassium concentration of barley seeds as influenced by salinity. J Plant Nutr 24:511–522

Ashraf M, Athar HR, Harris PJC, Kwon TR (2008) Some prospective strategies for improving crop salt tolerance. Adv Agron 97:45–110

Barrs HD, Weatherley PE (1962) A re-examination of the relative turgidity technique for estimating water deficit in leaves. Aust J Biol Sci 15:413–428

Botella MA, Martinez V, Pardines J, Cerda A (1997) Salinity induce potassium deficiency in maize plants. J Plant Physiol 150:200–205

Chhipa BR, Lal P (1995) Na/K ratios as the basis of salt tolerance in wheat. Aust J Agric Res 46:533–539

Dang YP, Dalal RC, Mayer DG, McDonald M, Routley R, Schwenke GD, Buck SR, Daniells IG, Singh DK, Manning W, Ferguson N (2008) High subsoil chloride concentrations reduce soil water extraction and crop yield on Vertosols in north-eastern Australia. Aust J Agric Res 59:321–330

Dang YP, Routley R, McDonald M, Dalal RC, Singh DK, Orange D, Mann M (2006) Subsoil constraints in Vertosols: crop water use, nutrient concentration and grain yield of bread wheat, durum wheat, barley, chickpea and canola. Aust J Agric Res 57:983–998

Francois LE, Maas EV, Donovan TJ, Youngs VL (1986) Effect of salinity on grain yield and quality, vegetative growth and germination of semi-dwarf and durum wheat. Agron J 78:1053–1058

Glenn E, Brown JJ, Blumwald E (1999) Salt-tolerate mechanisms and crop potential of halophytes. Crit Rev Plant Sci 18:227–255

Grattan SR, Grieve CM (1999) Salinity-mineral nutrient relations in horticultural crops. Hort Sci 78:127–157

Grieve CM, Francois LE, Mass EV (1994) Salinity affects the timing of phasic development in spring wheat. Crop Sci 34:1544–1549

Grewal HS, Norrish S, Cornish P (2004) Subsoil salts affects root function, shoot growth and ionic balance of wheat plants. New directions for a diverse planet: In Proceedings of the 4th International Crop Science Congress. 26 Sep–1 Oct 2004. Brisbane, Australia.

Kara SM, Keser S (2001) Effect of salinity on plant growth and mineral constituents of maize (Zea mays). Indian J Agric Sci 71:371–374

Khatun S, Flowers TJ (1995) Effect of salinity on seed set in rice. Plant Cell Environ 18:61–87

Katerji N, Mastrorilli M, van Horn JW, Lahmer FZ, Hamdy A, Oweis T (2009) Durum wheat and barley productivity in saline-drought environments. Eur J Agron 31:1–9

Mass EV, Grieve C (1990) Spike and leaf development in salt-stressed wheat. Crop Sci 30:1309–1313

Mass EV, Lesch SM, Francois LE, Grieve CM (1994) Tiller development in salt stressed wheat. Crop Sci 34:1594–1603

Munns R (2002) Comparative physiology of salt and water stress. Plant Cell Environ 25:239–250

Munns R (2005) Genes and salt tolerance: bringing them together. New Phytol 167:645–663

Munns R, Gardner A, Tonnet ML, Rawson HM (1988) Growth and development in NaCl-treated plants. II. Do Na^+ or Cl^- concentration in dividing or expanding tissues determines growth in barley? Aust J Plant Physiol 15:529–540

Muranaka S, Shimizu K, Kato M (2002) Ionic and osmotic effects of salinity on single-leaf photosynthesis in two wheat cultivars with different drought tolerance. Photosynthetica 40:201–207

Naidu R, Rengasamy P (1993) Ion interactions and constraints to plant nutrition in Australia. Aust J Soil Res 31:801–819

Nuttall JG, Armstrong RD, Connor DJ (2003) Evaluating physiochemical constraints of Calcarosols on wheat yield in the Victorian southern Mallee. Aust J Agric Res 54:487–497

Rengasamy P (2002) Transient salinity and subsoil constraints to dryland farming in Australian sodic soils: an overview. Aust J Exp Agric 42:351–361

Rengasamy P (2006) World salinization with emphasis on Australia. J Exp Bot 57:1017–1023

Rengasamy P, Chittleborough D, Helyar K (2003) Root-zone constraints and plant-based solutions for dryland salinity. Plant Soil 257:249–260

Rengel Z (1992) The role of calcium in salt toxicity. Plant Cell Environ 15:625–632

Sairam RK, Srivastava GC (2002) Changes in antioxidant activity in sub-cellular fractions of tolerant and susceptible wheat genotypes in response to long term salt stress. Plant Sci 162:897–904

Storey R, Gorham K, Pitman MC, Hanson MG, Gage D (1993) Response of Melanthera biflora to salinity and water stress. J Exp Bot 44:1551–1561

Subbarao GV, Johnson C, Jana MK, Rao JVDK (1990) Effects of sodium/calcium ratio in modifying salinity response of pigeonpea (Cajanus cajan). J Plant Physiol 136:439–443

Tarakcioglu C, Inal A (2002) Changes induced by salinity, demarcating specific ion ratio (Na/Cl) and osmolality in ion and proline accumulation, nitrate reductase activity, and growth performance of lettuce. J Plant Nutr 25:27–41

Wang D, Shannon MC, Grieve CM (2001) Salinity reduces radiation absorption and use efficiency in soybean. Field Crops Res 69:267–277

Wong MTF, Asseng S (2007) Yield and environmental benefits of ameliorating subsoil constraints under variable rainfall in a Mediterranean environment. Plant Soil 297:29–42

Yemm EW, Willis AJ (1954) The estimation of carbohydrates in plant extracts by anthrone. Biochem J 57:508–514

Zarcinas BA, Carwright B, Spouncer LR (1987) Nitric acid digestion and multi-element analysis of plant material by inductively coupled plasma spectrometry. Commun Soil Sci Plant Anal 18:131–146