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## Root growth, soil water variation, and grain yield response of winter wheat to supplemental irrigation

Jianguo Man, Yu Shi, Zhenwen Yu and Yongli Zhang

Key Laboratory of Crop Ecophysiology and Farming System, Ministry of Agriculture, College of Agronomy, Shandong Agricultural University, Taian, P.R. China

### ABSTRACT

Water shortage threatens agricultural sustainability in the Huang-Huai-Hai Plain of China. Thus, we investigated the effect of supplemental irrigation (SI) on the root growth, soil water variation, and grain yield of winter wheat in this region by measuring the moisture content in different soil layers. Prior to SI, the soil water content (SWC) at given soil depths was monitored to calculate amount of irrigation water that can rehydrate the soil to target SWC. The SWC before SI was monitored to depths of 20, 40, and 60 cm in treatments of W20, W40, and W60, respectively. Rainfed treatment with no irrigation as the control (W0). The mean root weight density (RWD), triphenyl tetrazolium chloride reduction activity (TTC reduction activity), soluble protein (SP) concentrations as well as catalase (CAT), and superoxide dismutase (SOD) activities in W40 and W60 treatments were significantly higher than those in W20. The RWD in 60–100 cm soil layers and the root activity, SP concentrations, CAT and SOD activities in 40–60 cm soil layers in W40 treatment were significantly higher than those in W20 and W60. W40 treatment is characterized by higher SWC in the upper soil layers but lower SWC in the 60–100-cm soil layers during grain filling. The soil water consumption (SWU) in the 60–100 cm soil layers from anthesis after SI to maturity was the highest in W40. The grain yield, water use efficiency (WUE), and irrigation water productivity were the highest in W40, with corresponding mean values of 9169 kg ha<sup>-1</sup>, 20.8 kg ha<sup>-1</sup> mm<sup>-1</sup>, and 35.5 kg ha<sup>-1</sup> mm<sup>-1</sup>. The RWD, root activities, SP concentrations, CAT and SOD activities, and SWU were strongly positively correlated with grain yield and WUE. Therefore, the optimum soil layer for SI of winter wheat after jointing is 0–40 cm.

### ARTICLE HISTORY

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### KEYWORDS

Grain yield; root activity; root senescence; root weight density; soil water content; supplemental irrigation

Winter wheat (*Triticum aestivum* L.) production in the Huang-Huai-Hai Plain of China accounts for more than 60% of the entire wheat production in the country (National Bureau of Statistics of China, 2013); however, the production is threatened by severe water deficits. In this region, approximately 150–180 mm precipitation falls during the winter wheat growing season and more than 70% of irrigation water is required to maintain the high production of winter wheat (Liu et al., 2011; Zhang et al., 1999). In this region, traditionally, wheat crop is irrigated with up to 310 mm water and the water use efficiency (WUE) is approximately 13.2 kg ha<sup>-1</sup> mm<sup>-1</sup> (Sun et al., 2011; Zhang et al., 2011). Moreover, greater exploitation of groundwater use has caused the water table to recede at the rate of approximately 1 m per year for the last 20 years (Chen et al., 2009; Sun et al., 2006). To cope with these serious problems, supplemental irrigation (SI) techniques with limited water application at critical crop growth stages that substantially improve yield and WUE, should be urgently

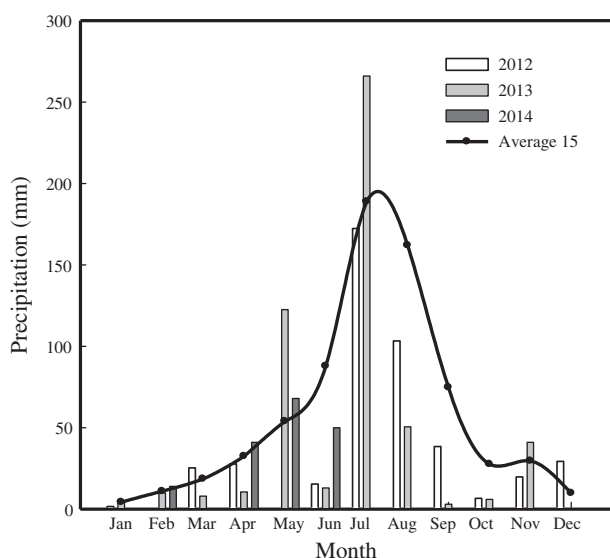
developed and adopted (Abourached et al., 2008; Garcia Garcia et al., 2009; Li et al., 2010; Oweis & Hachum, 2006; Wang et al., 2014).

The root system, an important organ that significantly influences crop growth and yield formation, functions in the absorption of soil moisture and nutrients (Kong et al., 2010; Monti & Zatta, 2009; Zhang et al., 2009). The soil water content (SWC) strongly affects the distribution of roots (Lv et al., 2010; Wang et al., 2004). Drought stress or waterlogging restricts root growth and reduces root density (Hayashi et al., 2013; Ma et al., 1999; Xue et al., 2003). Moderate SWC can increase root density, particularly in the deep soil layers, favors soil water absorption at depth and increases grain yield and WUE (Li et al., 2010; Qi et al., 2012; Zhang et al., 2009). Large variations in root growth are related to variations in SWC (Wang et al., 2006; Xue et al., 2003, 2010). Xue et al. (2003) showed that the winter wheat root water uptake, grain yield, and WUE are significantly higher in irrigated crops than in rainfed crops during

**CONTACT** Yu Shi  15065803101@163.com

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**Figure 1.** The monthly precipitation during 2012–2014, the average value during the previous 15 years (Average 15).

grain filling because of the higher root density in the irrigated crops. Zhang et al. (2004) found the distribution of water uptake from the soil layers and the distribution of root length density to be equivalent; the scarcity of roots in the deep soil layers restricts soil water use and limits wheat production.

Irrigation regimes applied during the winter wheat growing season significantly affect the root density, depth, and activity (Chen et al., 2014; Kang et al., 2002; Li et al., 2010; Wang et al., 2014; Zhang et al., 2004). Wang et al. (2014) reported that irrigation significantly increases the root weight density (RWD) in the 0–100 cm soil layers, particularly facilitating root growth in the deep soil layers. Suitable irrigation amount promotes root penetration, increases root density and utilization of water in deep soil, and consolidates the connection between water saving and high yield (Ram et al., 2013; Xue et al., 2010). Modulation in the activities of antioxidant enzymes may be one of the important factors in tolerance to environmental stress (Huseynova, 2012). Li et al. (2006) recommended that moderate irrigation can

increase soluble protein (SP) concentrations and root catalase (CAT) and superoxide dismutase (SOD) activities to a significantly larger extent than rainfed, seriously water stressed, or excessive irrigation conditions. Shi et al. (1998) showed that light water stress [60% field capacity (FC)] produces no differences between SP concentrations and root CAT and SOD activities in comparison with control treatment (80% FC) and 60% FC can be as the lower limit index of soil moisture for normal growth and development of wheat. However, studies of irrigation practices that seek to match soil moisture, especially at different soil layers, and their effect on root distribution, root activity and senescence, water use at different soil layers, and yield production of winter wheat are relatively limited.

In this study, a new SI method that recharges SWC at critical developmental stages in three soil layers (0–20, 0–40, and 0–60 cm) and brought the SWC in each measured soil layer to 65% FC at jointing and 70% FC at anthesis. The objectives of this study are (1) to investigate root distribution, RA, and root senescence of winter wheat in response to SI; (2) to determine the response of soil water changes during grain filling, soil water use, and yield production of winter wheat to SI by measuring soil moisture; and (3) to clarify the relationships between RWD, root activities, root antioxidant enzyme activities, soil water consumption, grain yield, and WUE of winter wheat.

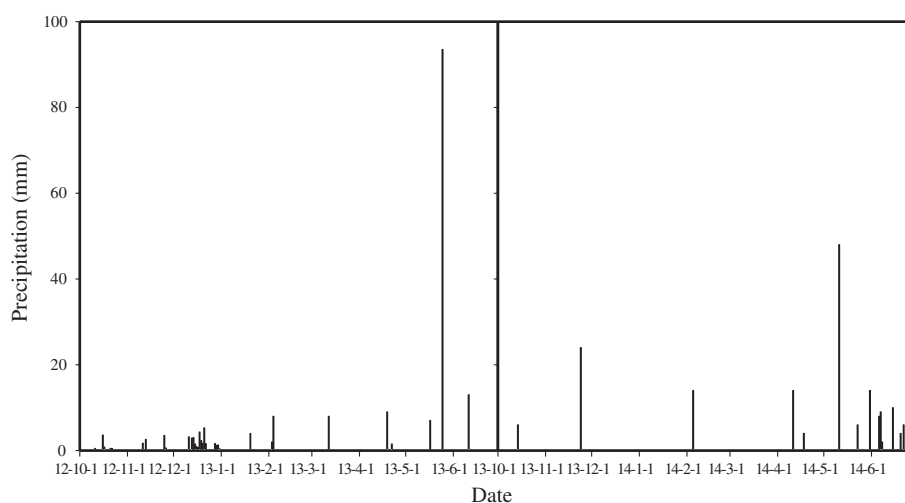
## Materials and methods

### Experimental site

Field experiments were conducted from October 2012 to June 2014 in the Shijiawangzi village, Yanzhou, Shandong Province, China (116°41'E, 35°42'N). The village is located in the centre of the Huang-Huai-Hai plain, and the environment is typical and representative of the plain. The area has a warm, temperate, semi-humid continental monsoon climate, an annual average temperature of 13.6 °C, annual accumulated

**Table 1.** Soil bulk density and soil water content of 20 cm layers in the top 0–200-cm soil profile of the experimental field.

Soil layers (cm)	Soil bulk density (g cm <sup>-3</sup> )		Field capacity (%)		Soil water content one day before sowing (%)	
	2012–2013	2013–2014	2012–2013	2013–2014	2012–2013	2013–2014
0–20	1.4	1.4	28.7	29.0	17.5	19.8
20–40	1.4	1.4	26.2	28.0	16.1	19.3
40–60	1.5	1.4	27.0	27.0	14.3	20.3
60–80	1.5	1.5	26.0	26.0	16.3	18.5
80–100	1.5	1.5	25.4	26.0	18.7	19.3
100–120	1.5	1.5	25.9	25.3	19.5	20.4
120–140	1.5	1.5	25.2	25.0	21.4	20.1
140–160	1.5	1.5	24.8	24.6	21.8	20.8
160–180	1.6	1.6	23.3	24.1	21.6	20.7
180–200	1.6	1.5	23.8	24.0	21.9	21.2



**Figure 2.** The daily precipitation during the 2012–2013 and 2013–2014 winter wheat growing seasons. Precipitations from July to December were not measured in 2014.

**Table 2.** The target relative soil water content ( $\theta_{tr}$ ) and actual relative soil water content ( $\theta_a$ ) in the supplemental irrigation treatments (W20, W40 and W60) after jointing and anthesis in 2012–2013 and 2013–2014 growing season, the amount of supplemental irrigation (CIR) is also indicated.

Treatment <sup>a</sup>	Thickness of the soil profile	SI at jointing			SI at anthesis			Total CIR (mm)
		$\theta_{tr}$ (%)	$\theta_a$ (%)	CIR (mm)	$\theta_{tr}$ (%)	$\theta_a$ (%)	CIR (mm)	
<i>2012–2013</i>								
W20	20	65.0	64.7	30.7	70.0	71.1	32.0	62.8
W40	40	65.0	63.5	49.6	70.0	68.2	51.2	100.8
W60	60	65.0	63.5	65.8	70.0	67.9	66.7	132.5
<i>2013–2014</i>								
W20	20	65.0	64.1	23.8	70.0	68.2	24.5	48.3
W40	40	65.0	63.4	44.0	70.0	70.3	45.5	89.6
W60	60	65.0	64.1	58.0	70.0	72.8	66.7	124.7

<sup>a</sup>The treatment of W20, W40, and W60 was supplemental irrigation by measuring 0–20-, 0–40-, and 0–60-cm soil layer moisture, respectively, and brought the soil moisture to 65% field capacity (FC) at jointing and 70% FC at anthesis.

sunshine of 2461 h, annual precipitation of 621.2 mm with 228.8 mm during the winter wheat growing season (the precipitation conditions are shown in Figure 1) and a groundwater depth of 25 m. The soil is cinnamon (Cooperative Research Group on Chinese Soil Taxonomy [CRGCST], 2001). The organic matter (15.9 g kg<sup>-1</sup>), total nitrogen (1.2 g kg<sup>-1</sup>), available phosphorus (30.9 mg kg<sup>-1</sup>), and potassium (114.5 mg kg<sup>-1</sup>) in the topsoil of the experimental plots were measured using the potassium dichromate colorimetric method, the Kjeldahl method, the sodium bicarbonate method, and the ammonium acetate method, respectively, details can be found in Lu (2000). The soil bulk density and SWC of each 20 cm soil layer of the top 0–200 cm are listed in Table 1. The precipitation during the 2012–2013 and 2013–2014 growing seasons was 225.0 and 170.0 mm, respectively. The precipitation distribution is shown in Figure 2.

### Experimental design

Four treatments were designed: a rainfed (W0) treatment with no irrigation as the control and three SI treatments: the SWC at given soil depths was monitored prior to SI to calculate amount of irrigation water that can rehydrate the soil to target SWC. The SWC before SI was monitored to depths of 20, 40, and 60 cm in treatments of W20, W40, and W60, respectively. All of the SI treatments brought the relative SWC in each measured soil layer to 65% FC at jointing (Z31, first node detectable) and 70% FC at anthesis (Z61, beginning of anthesis) (Zadoks et al., 1974). The required amounts of SI were calculated by the equation described by Cuenca (1989) and Jalilian et al. (2012):

$$CIR = 10y_{bd}D_h(\theta_t - \theta_n),$$

where  $CIR$  (mm) is the amount of SI;  $y_{bd}$  (g cm<sup>-3</sup>) is the soil bulk density;  $D_h$  (cm) is the thickness of the soil layer measured for SWC before SI (Table 2);  $\theta_t$  (%) is the target

SWC on a weight basis after SI; and  $\theta_n$  (%) is the SWC on a weight basis before SI.  $\theta_t$  was calculated as follows:

$$\theta_t = \theta_{\max} \theta_{tr} / 100,$$

where  $\theta_{\max}$  (%) is the FC and  $\theta_{tr}$  (%) is the target relative SWC (65% FC at jointing and 70% FC at anthesis in this study, respectively). A flow meter was used to measure the amount of water applied. CIR for each SI treatment (W20, W40 and W60) is shown in Table 2.

All treatments were replicated thrice and followed a randomized scheme. Each experimental plot was 4 × 4 m in size, with a 2.0-m wide zone without irrigation positioned between the two adjacent plots to prevent contamination.

### Crop management

All plots were supplied with 240 kg N ha<sup>-1</sup>, 150 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>, and 150 kg K<sub>2</sub>O ha<sup>-1</sup>. All P and K fertilizers and half of the N fertilizer were applied pre-sowing, and the remaining N fertilizer was top-dressed at the jointing stage. The high-yielding wheat (*Triticum aestivum* L.) cultivar Jimai22 was used in the experiments. Wheat seeds were sown at a density of 180 plants m<sup>-2</sup> on 10 October 2012 and 9 October 2013. Wheat seedling shoot growth ceased at the beginning of December and recommenced at the end of February. During this period, the average daily temperature was below 0 °C. Wheat plants were harvested on 12 June 2013 and 6 June 2014.

### Soil water measurement

Soil samples were collected using a soil corer (4.0 cm diameter) at 20-cm increments down to 200 cm in all experimental plots. SWC (gravimetric water content, %) was determined using the oven-drying method (Gardner, 1986).

$$SWC = (FW - DW) / DW \times 100,$$

where *FW* is the soil sample fresh weight (g); *DW* is the soil sample dry weight (g).

Measurements were performed before sowing (Z00), 1 day before irrigation, and 3 days after irrigation at both jointing (Z31) and anthesis (Z61) and at maturity (Z90). Three soil samples were collected at random locations from each plot.

### Root sampling and RWD

Roots were sampled from each treatment (three replicates) were taken at anthesis and 10, 20, and 30 days after anthesis (DAA). Following to the description of Xue et al. (2003) and Zhang et al. (2009), we carefully removed the aboveground parts prior to root sampling and collected

root samples at 20 cm increments down to 100 cm at anthesis and 20 cm increments down to 60 cm at 10 DAA, 20 DAA, and 30 DAA. Two cores per plot were collected: one within the crop row and one midway between rows. The resultant mixture of roots and soil was then placed in a polythene bag and washed with tap water. The soil and roots were carefully separated and refrigerated for further testing. The dry weight of the roots was determined by drying in an oven at 80 °C.

### Root biochemical assays

Roots were sampled from each experimental plot, as described in the method above, by 20-cm increments to 60 cm at 10 DAA, 20 DAA, and 30 DAA. According to Li et al. (2011), the root activity was determined using the TTC method (Lindström & Nyström, 1987), and represented by the triphenyl tetrazolium chloride (TTC) reduction activity. The root CAT activity was assayed by measuring the initial rate of H<sub>2</sub>O<sub>2</sub> disappearance (Kato & Shimizu, 1987); the SOD activity was assayed by measuring the inhibition of the photoreduction of nitro blue tetrazolium (NBT) following the method of Giannopolitis & Ries (1977); malondialdehyde (MDA) concentrations of flag leaves were assayed according to Quan et al. (2004); and SP concentrations of flag leaves were measured according to the coomassie brilliant blue G250 method described by Read & Northcote (1981). Details can be found in Wang et al. (2013).

### Crop water use

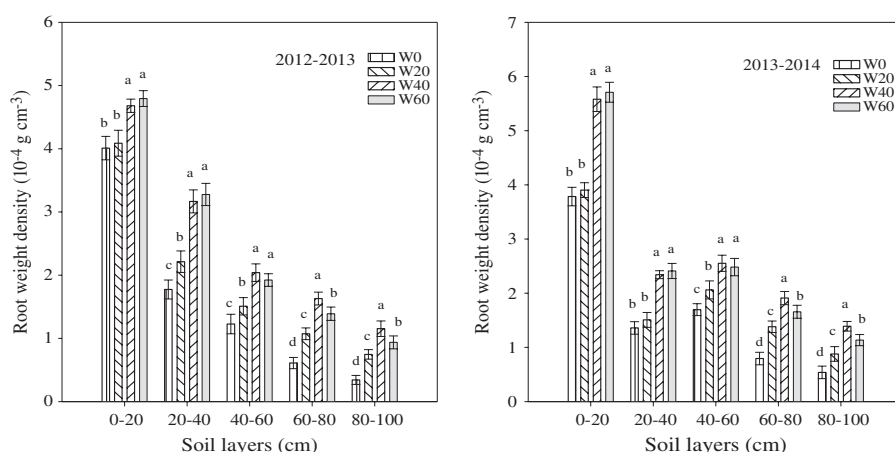
Total crop seasonal evapotranspiration (*ET<sub>c</sub>*) was calculated using the soil water balance equation (Chattaraj et al., 2013; Lv et al., 2011) for the growing season as follows:

$$ET_c = P + CIR + \Delta W - R - D,$$

where *ET<sub>c</sub>* (mm) is the total water consumption during the winter wheat growing season; *P* (mm) is the precipitation during the winter wheat growing season; *CIR* (mm) is the amount of SI;  $\Delta W$  (mm) is the soil water storage at sowing minus the soil water storage at harvesting in the 0–200 cm soil layers; *R* (mm) is the surface run-off; and *D* (mm) is the downward flux below the crop root zone. The soil water measurements indicated that the drainage at the site is negligible. Therefore, deep percolation was not accounted for in this study (Ali et al., 2007; Lv et al., 2011).

WUE of crop was determined as the ratio of grain yield to *ET<sub>c</sub>* (Wang et al., 2011); Irrigation water productivity (IWP) was calculated as follows:

$$IWP = \Delta GY / \Delta ET_c,$$



**Figure 3.** Responses of root weight density in the 0–100-cm soil layers to different treatments at anthesis in the 2012–2013 and 2013–2014 growing seasons, rainfed (W0), the soil water content before SI was monitored to depths of 20 cm (W20), 40 cm (W40) and 60 cm (W60), respectively, and bringing the soil moisture to 65% field capacity (FC) at jointing and 70% FC at anthesis. The same letter in the figure is not significant different at  $p = 0.05$  by LSD test. Vertical bars are standard errors.

**Table 3.** The mean value of triphenyl tetrazolium chloride (TTC) reduction activity in 0–60-cm soil layers during grain filling of root in 2012–2013 and 2013–2014 growing seasons under different treatments.

Treatment	TTC reduction activities ( $\text{mg g}^{-1} \text{h}^{-1}$ )		
	2012–2013	2013–2014	Mean
W0	21.5c	30.0c	25.8c
W20	35.0b	43.1b	39.0b
W40	43.0a	51.1a	47.0a
W60	42.7a	49.0a	45.8a

where  $\Delta GY$  is the difference of grain yield between irrigated treatment and rainfed treatment;  $\Delta ET_c$  is the difference of  $ET_c$  between irrigated treatment and rainfed treatment (Karrou & Oweis, 2012).

### Statistical analysis

Statistical analysis employed standard analysis of variance (ANOVA) using SPSS 13.0 software (SPSS Inc., Chicago, IL, USA.). Data for RWD, RA, root SP concentrations, CAT and SOD activities, MDA concentration, grain yield, and WUE were statistically analyzed at an  $\alpha = 0.05$  level of significance to determine whether significant differences exist among different treatments.

## Results

### Root distribution at anthesis

The vertical root distribution in the soil column at anthesis is presented in Figure 3. RWD decreased with depth in all treatments. The highest average RWD in the 0–100 cm soil layer ( $2.7 \times 10^{-4} \text{ g cm}^{-3}$ ) was associated with the W40 treatment. The RWD values for the W60, W20, and W0 treatments were  $2.6 \times 10^{-4}$ ,  $2.1 \times 10^{-4}$ , and  $1.7 \times 10^{-4} \text{ g cm}^{-3}$ , respectively, and the differences among treatments were significant.

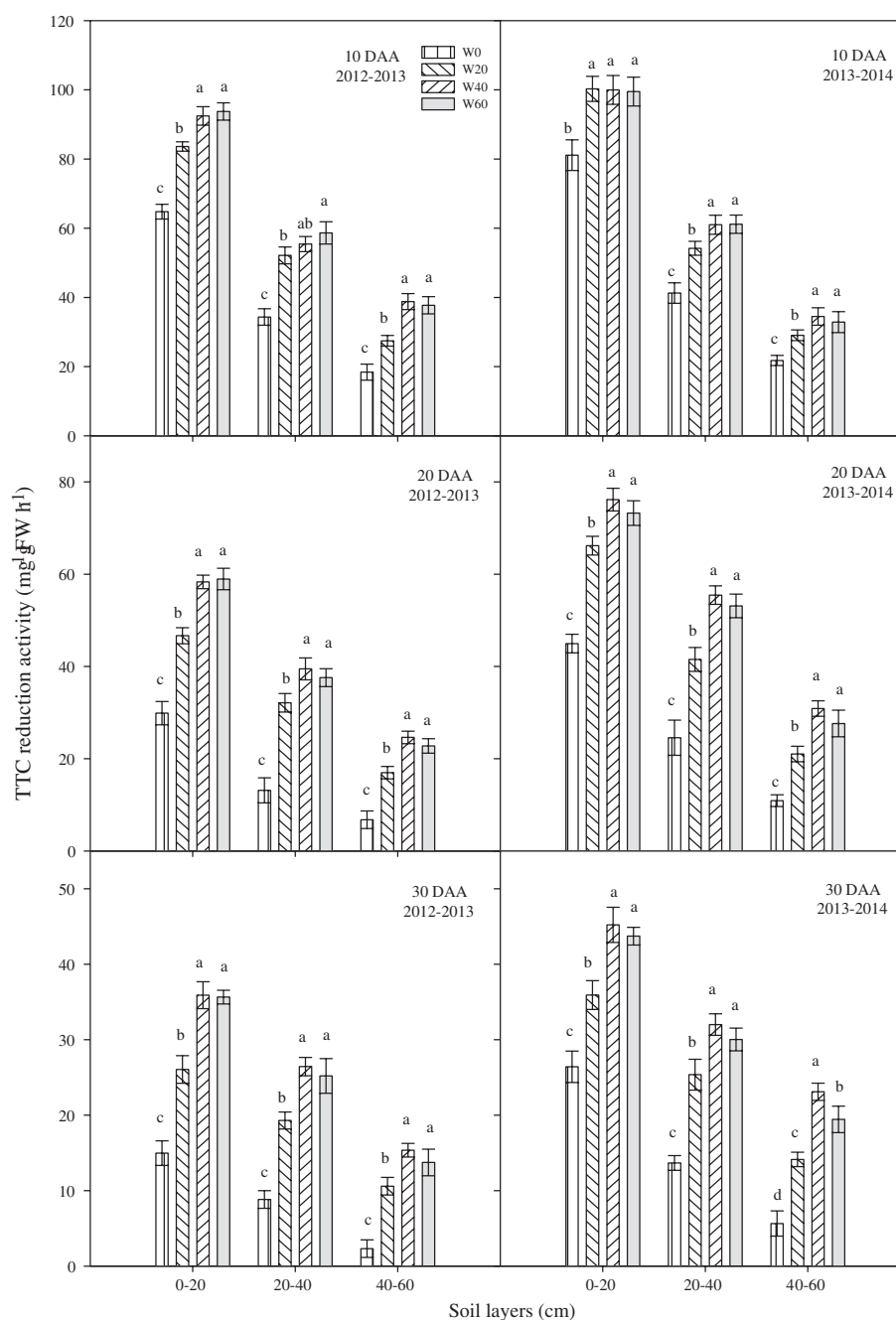
The 0–20-cm RWD between W40 and W60 did not differ and was significantly higher than that of W0 and W20. The RWD values in the 20–40-cm soil layer for W60 were higher than that for W40 and the difference was significant in the 2012–2013 growing season. In contrast, in the 40–100 cm soil layer, the RWD associated with W40 was higher than that in W60 and the difference was significant in the 60–100 cm soil layer. The lowest RWD was that of the 0–100 cm soil layer in the W0 treatment.

### Root activity after anthesis

The TTC reduction activity in 0–60 cm soil layers during grain filling in 2012–2013 and 2013–2014 growing seasons was shown in Table 3. The mean TTC reduction activity in W40 was the highest ( $47.0 \text{ mg g}^{-1} \text{h}^{-1}$ ), which did not differ from that of W60, but was significantly higher than those in W20 and W0 with corresponding mean values of 39.0 and  $25.8 \text{ mg g}^{-1} \text{h}^{-1}$ , respectively.

TTC reduction activity decreased with soil depth and grain filling (Figure 4). At 10 DAA, the TTC reduction activity in the 0–20, 20–40, and 40–60 cm soil layers did not differ between W40 and W60 and was higher than that for W20 and W0. At 20 DAA, the TTC reduction activity in the 0–20,





**Figure 4.** Changes in root TTC reduction activities in the 0–60-cm soil layers after anthesis of different treatments in the 2012–2013 and 2013–2014 growing seasons. The same letter in the figure is not significant different at  $p = 0.05$  by LSD test. Vertical bars are standard errors.

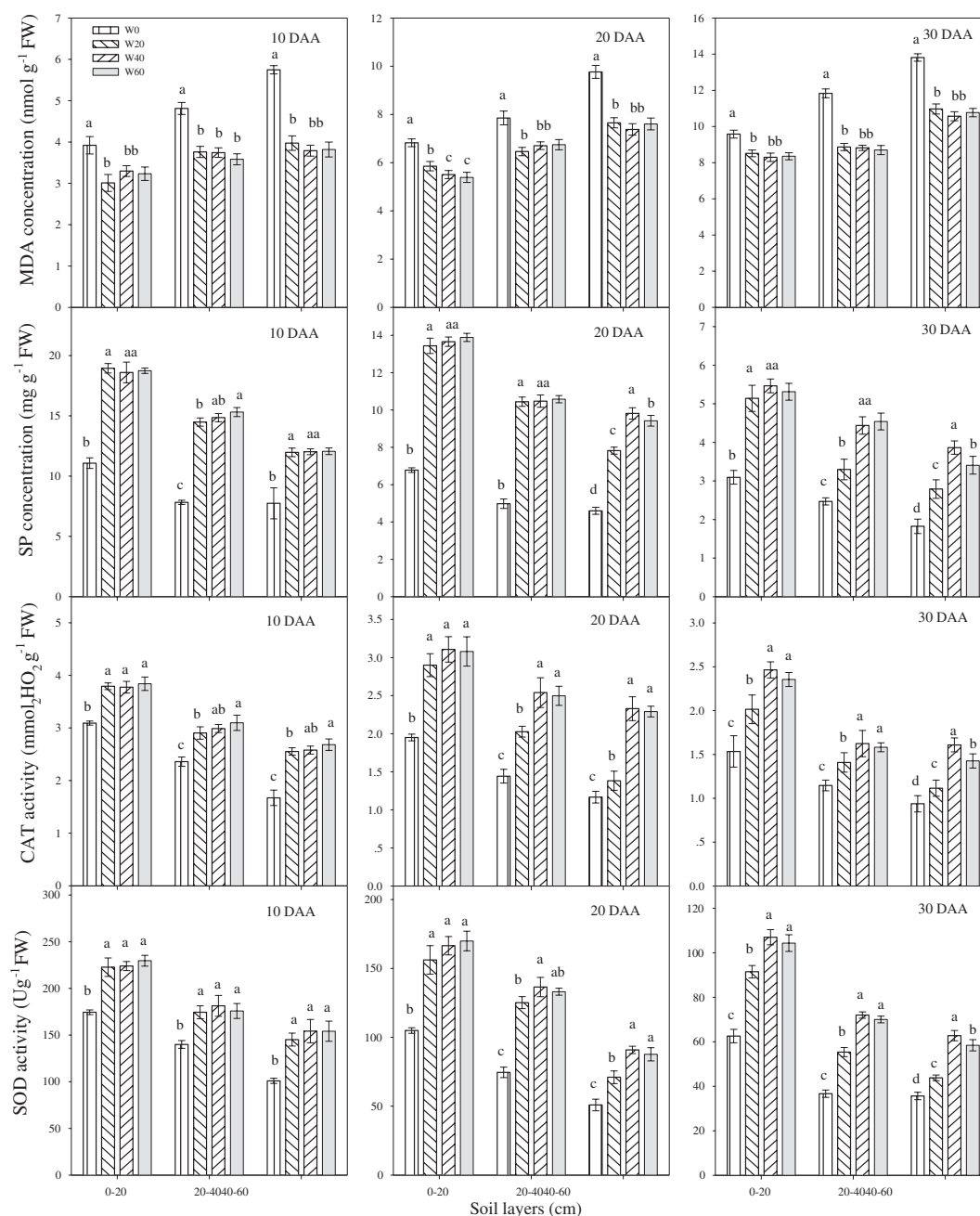
20–40, and 40–60 cm soil layers for W40 was higher than that for W20 and W0. However, the differences between W40 and W60 were not significant. At 30 DAA, the TTC reduction activity in the 0–20, 20–40, and 40–60 cm soil layers for W40 was higher than that for W60. The differences were significant in the 40–60 cm soil layer in the 2013–2014 growing season. The TTC reduction activity for W20 was significantly lower than that for W60. The W0 treatment was the lowest in TTC reduction activity in both growing seasons.

#### **Root senescence after anthesis**

The mean values of the root catalase (CAT) activities, the superoxide dismutase (SOD) activities, and the soluble protein (SP) concentrations in the 0–60 cm soil layer during grain filling are listed in Table 4. The W40 and W60 treatments resulted in significantly higher root CAT and SOD activities and SP concentrations than the W20 and W0 treatments. W0 treatment had the highest MDA concentration, whereas the MDA values for W20, W40, and W60 did not differ.

**Table 4.** The mean values of catalase (CAT) and superoxide dismutase (SOD) activities, soluble protein (SP) concentrations and malondialdehyde (MDA) concentrations of roots in 0–60 cm soil layers during grain filling under different treatments.

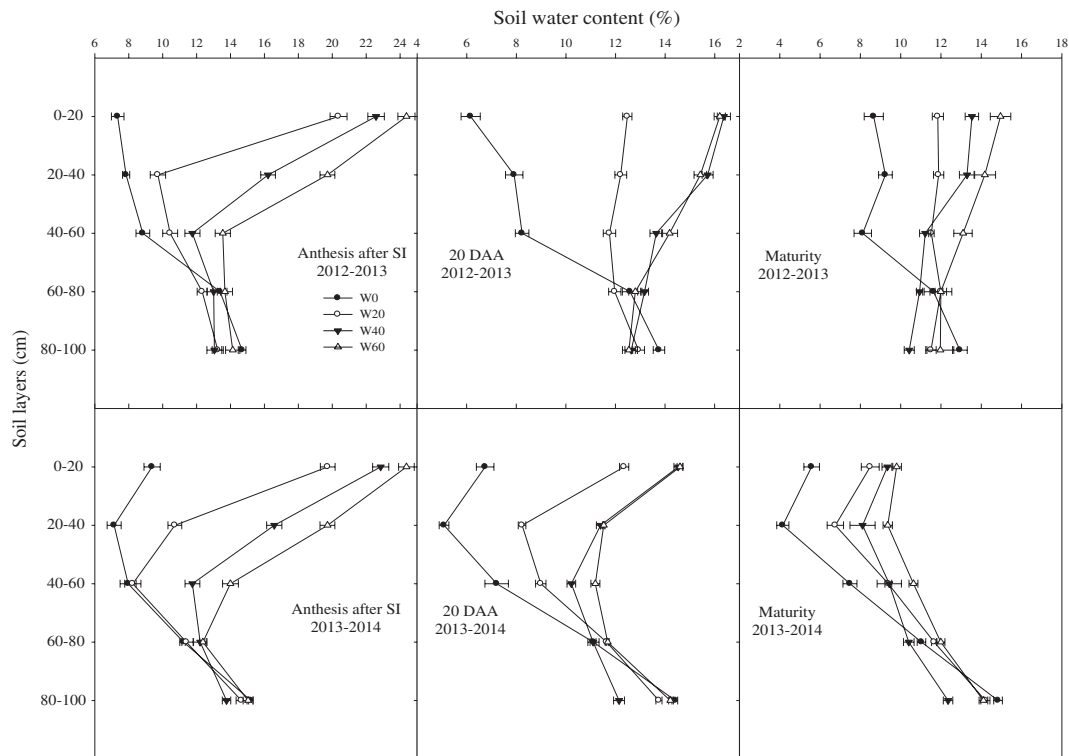
Treatment	CAT activities (mmol H <sub>2</sub> O <sub>2</sub> g <sup>-1</sup> FW)	SOD activities (Ug <sup>-1</sup> FW)	SP concentration (mg g <sup>-1</sup> FW)	MDA concentration (nmol g <sup>-1</sup> FW)
W0	1.70c	86.8c	5.60c	8.24a
W20	2.23b	120.6b	9.80b	6.56b
W40	2.56a	132.8a	10.4a	6.46b
W60	2.54a	131.5a	10.4a	6.47b

**Figure 5.** Changes in malondialdehyde (MDA) concentration, soluble protein (SP) concentration, and catalase (CAT) and superoxide dismutase (SOD) activities in winter wheat root at different soil layers after anthesis in different treatments in the 2013–2014 growing seasons. The same letter in the figure is not significant different at  $p = 0.05$  by LSD test. Vertical bars are standard errors.

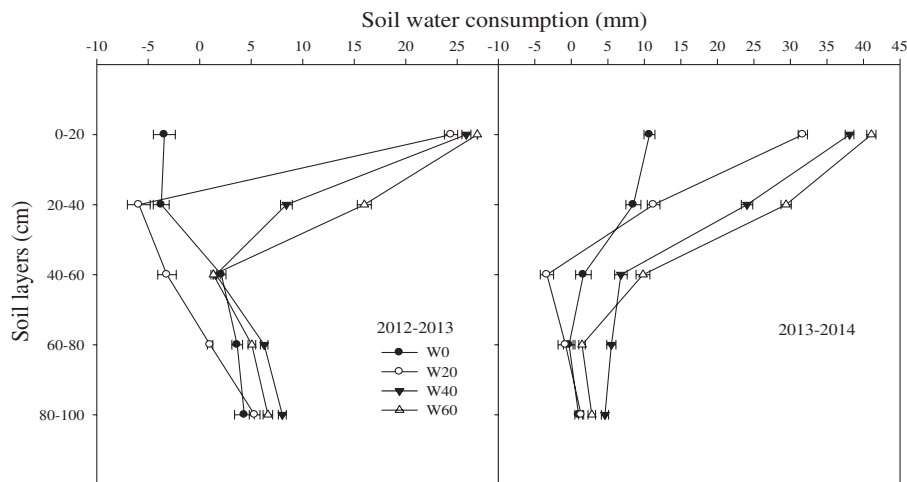
Root CAT and SOD activities and SP concentrations after anthesis decreased with soil depth (Figure 5), whereas the

MDA concentration increased. CAT and SOD root activities and SP concentrations in the 0–20-, 20–40-, and 40–60-cm





**Figure 6.** Soil water content (SWC) at anthesis after SI, 20 DAA and maturity in 0–100 cm soil layers for SI treatments in the 2012–2013 and 2013–2014 growing season. The horizontal bars are standard errors.



**Figure 7.** Soil water consumption (SWU) in 0–100-cm soil layers from anthesis after SI to maturity for SI treatments in the 2012–2013 and 2013–2014 growing seasons. The horizontal bars are standard errors.

soil layers did not differ among W20, W40, and W60 at 10 DAA. At 20 DAA, the CAT and SOD activities in the 20–40- and 40–60-cm soil layers and the SP concentrations in the 40–60-cm soil layer for W40 and W60 were significantly higher than those for W20. At 30 DAA, the CAT and SOD activities in the 0–20- and 20–40-cm soil layers and the SP concentrations in the 20–40-cm soil layer did not differ between W40 and W60. However, the CAT and SOD activities and SP concentrations in the 40–60-cm soil layer for W40 were significantly higher than

those for W60. The W20 treatment was the lowest in CAT and SOD activities in all soil layers. The W0 treatment had the highest MDA concentration and the lowest CAT and SOD activities and SP concentrations in each soil layer during grain filling.

#### **Soil water content and consumption after anthesis**

The SWC in the 0–100 cm soil layer was significantly affected by SI at jointing and anthesis (Figure 6). In the

**Table 5.** The crop evapotranspiration (ETc), grain yield, water use efficiency (WUE), and irrigation water productivity (IWP) in 2012–2013 and 2013–2014 growing seasons under different treatments.

Treatment	ETc <sup>a</sup> (mm)	Grain yield (kg ha <sup>-1</sup> )	WUE (kg ha <sup>-1</sup> mm <sup>-1</sup> )	IWP (kg ha <sup>-1</sup> mm <sup>-1</sup> )
<i>2012–2013</i>				
W0	352.1c	6123d	17.4d	
W20	398.4b	7179c	18.0c	22.8c
W40	439.4a	9077a	20.7a	33.8a
W60	442.6a	8701b	19.7b	28.5b
<i>2013–2014</i>				
W0	356.0c	6407d	18.0d	
W20	395.7b	7367c	18.6c	24.2b
W40	443.4a	9260a	20.9a	32.6a
W60	453.3a	8855b	19.5b	25.2b

<sup>a</sup>Within a column, values in the same growing season followed by different letters differ significantly ( $p < 0.05$ ).

**Table 6.** Correlation analysis between root weight density in different soil layers and soil water consumption (SWU) after anthesis after SI, grain yield and water use efficiency (WUE),  $n = 24$ .

	Root weight density at the depths				
	0–20 cm	20–40 cm	40–60 cm	60–80 cm	80–100 cm
SWU	0.818**	0.864**	0.768**	0.772**	0.783**
Yield	0.819**	0.836**	0.887**	0.853**	0.863**
WUE	0.654*	0.691**	0.803**	0.769**	0.781**

\*\* $p < 0.01$ ;

\* $p < 0.05$ .

2012–2013 growing season, at anthesis after SI, SWC in the 0–100 cm soil layers in W3 was the highest, and the values in the 0–80 cm soil layers in W40 were lower than that in W60, whereas the values in the 80–100-cm soil layer in W40 were higher than that in W60. At 20 DAA, SWC in the 0–80-cm soil layers did not differ between W40 and W60 and was significantly higher than that in W20. The SWC in the 80–100 cm soil layer did not differ in all treatments. At maturity, SWC in the 0–20- and 20–40-cm soil layers in W60 was the highest, followed by W40 and W20, but the value in W20 was lower than W40; the SWC in the 40–60 cm soil layer did not differ between W20 and W40; the SWC in the 80–100-cm soil layer in W20 and W60 was higher than that in W40.

In the 2013–2014 growing season, at anthesis after SI, SWC in the 0–20-, 20–40-, and 40–60-cm soil layers in W3 was the highest, followed by W40 and W20. The SWC in the 60–80 cm soil layer did not differ between treatments (Figure 6). At 20 DAA, the SWC in the 0–20-, 20–40- and 40–60-cm soil layers did not differ between W40 and W60 but was significantly higher than that in W20. The SWC in the 60–80- and 80–100-cm soil layers for W20 and W60 was significantly higher than W40. At maturity, the SWC in the 0–20-, 20–40-, and 40–60-cm soil layers in W60 was significantly higher than those in W40 and W20, and the SWC in the 60–80- and 80–100-cm soil layers in both W20 and W60 was higher than that in W40.

Figure 7 shows the soil water consumption (SWU) from anthesis after SI to maturity. The SWU in 0–60 cm

soil layers increased in the order of W60 > W40 > W20; however, the SWU in 40–60 cm soil layers for W40 and W60 did not differ in the 2012–2013 growing season. The SWU in the 60–100 cm soil layers increased in the order of W40 > W60 > W20 in both seasons.

### Grain yield and water use

The highest grain yield, WUE, and irrigation water productivity (IWP) with corresponding mean values of 9168 kg ha<sup>-1</sup>, 20.8 kg ha<sup>-1</sup> mm<sup>-1</sup>, and 35.5 kg ha<sup>-1</sup> mm<sup>-1</sup> were associated with the W40 treatment (Table 5) and were followed by those associated with W60 and W20. W0 was associated with the lowest grain yield and WUE. Compared with W40, the mean value of grain yield was lower by 20.7 and 4.3%, WUE was lower by 11.9 and 5.7% and IWP was lower by 20.3 and 18.7% in W20 and W60 in both growing seasons.

### Correlation analysis

The correlations between RWD and SWU, RWD and grain yield, and RWD and WUE in both seasons are analyzed and presented in Table 6. SWU, grain yield, and WUE showed significant positive correlation with RWD in all soil layers. The correlation between RWD and SWU in the 0–40 cm soil layer was stronger than in the 40–100 cm layer, whereas the RWD–grain yield and RWD–WUE correlations in the 0–40-cm soil layer were lower than in the 40–100-cm layer.

**Table 7.** Correlation analysis between root weight density (RWD), root TTC reduction activities (TTCRA), malondialdehyde (MDA) concentration, catalase (CAT), superoxide dismutase (SOD) activities, soluble protein (SP) concentration, soil water consumption (SWU) from anthesis after SI to maturity, grain yield and water use efficiency (WUE),  $n = 24$ .

	TTCRA	MDA	CAT	SOD	SP	SWU	Grain yield	WUE
RWD	0.855**	-0.615*	0.843**	0.887**	0.773**	0.891**	0.886**	0.794**
TTCRA		-0.771**	0.783**	0.971**	0.952**	0.897**	0.905**	0.817**
MDA			-0.645*	-0.743**	-0.821**	-0.782**	-0.769**	-0.667*
CAT				0.828**	0.779**	0.737**	0.782**	0.522*
SOD					0.939**	0.845**	0.824**	0.718**
SP						0.832**	0.777**	0.639*
SWU							0.956**	0.831**
Grain yield								0.911**

\*\* $p < 0.01$ ;\* $p < 0.05$ .

Hence, high RWD makes better use of the soil water, particularly in the 0–40-cm soil layer, and the high RWD in the 40–100 cm soil layer significantly improves the grain yield and WUE.

There was significant positive correlation among RWD, root TTC reduction activity, CAT and SOD activities, SP concentrations, SWU from anthesis after SI to maturity, grain yield and WUE; however, the MDA concentration was strongly negatively correlated with RWD, TTC reduction activity, CAT, SOD, SP, grain yield, and WUE (Table 7). SWU was strongly correlated with grain yield and WUE, whereas SWU, grain yield and WUE were more strongly correlated with RWD and TTC reduction activity.

## Discussion

Irrigation greatly affects the growth and development of crop root systems. Studies show that suitable amount of irrigation for wheat has greater root length density (Zhang et al., 2004) and RWD (Xue et al., 2003) than excessive or too little amount of irrigation for wheat and rainfed wheat. We found that irrigation significantly increases RWD in the 0–100-cm soil layer (Figure 3). Wang et al. (2014) and Xue et al. (2010) found that appropriate irrigation regimes (75–150 mm irrigation water) with lower water stress could increase RWD under field conditions and facilitate root growth in deep soil layers. Li et al. (2010) reported that under restricted irrigation regimes, irrigation of 120 mm only at jointing increased the root density in soil layers deeper than 30 cm, whereas irrigation of 40 mm at jointing, heading, and milking resulted in the highest root density in soil layers shallower than 30 cm. In the present study, the mean irrigation amount in W2 was 95.2 mm, which was significantly higher than that in W1 but lower than that in W3 (Table 2). However, RWD in W2 was the highest, particularly in the 60–80- and 80–100-cm soil layers (Figure 3). The RWD associated with the W1 treatment was the lowest in the 0–100-cm soil layer. The results suggest that the amount of irrigation determined by measuring the moisture of the 0–40-cm soil layer facilitate root growth

in the 60–100-cm soil layer because of water and nutrient absorption (Merrill et al., 2002; Qi et al., 2012).

Water stress and excessive water supply would limit root system and antioxidant enzyme activities (Li et al., 2006; Rasse & Smucker, 1998; Shi et al., 1998; Tang et al., 2014; Yang et al., 2010). Zuo et al. (2010) reported that after drought stress treatment, root activities decreased by 28.5% at 12 DAA and 36.3% at 24 DAA compared with normal irrigation treatment. Wang et al. (2012) found that appropriate soil water conditions (70–75% FC) could effectively promote root activity and increase the grain yield. It has been reported that SP concentrations and CAT and SOD activities decreased and MDA concentration increased with the water stress, thus accelerating plant senescence (Shi et al., 1998; Tang et al., 2014). The water content in the 0–40-cm soil layer was 63.4–63.5% FC at jointing and 68.2–70.3% FC at anthesis after SI in W40, which had the highest TTC reduction activity ( $45.8 \text{ mg g}^{-1} \text{ h}^{-1}$ ) (Figure 4). Root SP concentrations and CAT and SOD activities in W40 were the highest, particularly in the 40–60-cm soil layer (Figure 5). This suggests that SI, based on the 0–40-cm soil layer moisture, enhances the antioxidant enzyme activities and improves the root system activities, particularly in the 40–60-cm soil layer at the late growing stages of winter wheat.

Root growth in the soil layer is critical to the stored soil water uptake, particularly under water deficit conditions (Huang et al., 2012; Li et al., 2010; Shao et al., 2009). Xue et al. (2003) showed that the water uptake rate in 0–100-cm soil layers was significantly lower in rainfed than in irrigation treatments because of low root density. Li et al. (2010) reported that under restricted irrigation regimes, irrigation of 120 mm only at jointing results in the highest root length density, leading to the highest SWU in 0–160-cm soil layers. We found that the mean RWD in the 0–100-cm soil layer, particularly in the 60–100-cm layer, was the highest for W40 (Figure 3). Moreover, SWC was the lowest in the 60–100-cm soil layer at 20 DAA (Figure 6), indicating that the higher SWU of W2 in the 60–100-cm soil layer is due to the high RWD (Figure 7).

Wheat grain yield and WUE are closely related to the growth and development of the root system in the soil (Li et al., 2006; Shi et al., 1998; Wang et al., 2014). Increased RWD was found to be associated with improved water use efficiency and grain yield of wheat in Mediterranean environments (Liao et al., 2004). Zhang et al. (2009) reported that greater root biomass is significantly associated with greater aboveground biomass, which contributes to a higher grain yield. Li et al. (2010) observed that irrigation of 60 mm each at jointing and heading increased the root length density in the 60–100-cm soil layers, resulting in the highest 1000-grain weight, grain yield, and WUE. Wang et al. (2014) concluded that during the late growth stage, grain weight is closely positively correlated with RWD in the 0–100-cm soil layers. In the present study, grain yield and WUE were significantly positively correlated with RWD in all soil layers; a higher RWD, particularly in the 40–100-cm soil layers, can significantly improve grain yield and WUE (Table 3). The study also showed that grain yield and WUE had a significant positive relationship with root TTC reduction activity, CAT and SOD activities, and SP concentration (Table 4). Suitable irrigation at jointing and anthesis appears to increase RWD, root TTC reduction activity, and root antioxidant enzyme activity at the later stages of grain filling, which may be the reason why W40 achieved a high soil water absorption, grain yield, and WUE.

## Conclusions

SI at jointing and anthesis, determined by measuring the moisture of the 0–40 cm soil layer, improves RWD in the 0–100-cm soil layer and particularly in the 60–80- and 80–100-cm soil layers compared with other treatments, which are likely the reasons of the high SWU in these soil layers. It also increases the root TTC reduction activity, root SP and CAT and SOD activities at the late stages of grain filling. Maximal grain yield, WUE and IWP were obtained at suitable SWC (65% FC at jointing and 70% FC at anthesis) with the W2 treatment. Moreover, significant positive correlation among RWD, root TTC reduction activity, SP concentrations, CAT and SOD activities, SWU, grain yield and WUE was observed.

## Disclosure statement

No potential conflict of interest was reported by the authors.

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