



## Irrigation methods affect wheat flag leaf senescence and chlorophyll fluorescence in the North China Plain

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### Abstract

The water resource shortage in North China Plain is an increasing threat to the sustainability of wheat (*Triticum aestivum* L.) production. A two-year field experiment was conducted to examine the effects of two supplemental irrigation (SI) methods on wheat flag leaf senescence, chlorophyll fluorescence and grain yield. The following field treatments were conducted: no irrigation ( $W_0$ ); SI with 60 mm of water at jointing and anthesis stages (local quota SI,  $W_{ck}$ ); SI based on the relative soil water content (SWC) of 0–40 cm soil layers with 65% field capacity (FC) at jointing stage and 70% FC at anthesis stage ( $W_1$ ); SI based on SWC of same soil layers with 70% FC at the jointing and anthesis stage ( $W_2$ ); and SI based on the SWC of same soil layers with 75% FC at jointing stage and 70% FC at anthesis stage ( $W_3$ ). Results showed that  $W_0$  accelerated flag leaf senescence and had reduced grain yield. Among irrigation treatments,  $W_2$  (and  $W_3$  in 2013–2014) significantly increased flag leaf water potential from 7 to 28 days after anthesis (DAA) compared with  $W_{ck}$  and  $W_1$ . Superoxide dismutase activity, catalase activity of  $W_2$  increased by 15.41% and 14.96% compared with those in  $W_{ck}$ , resulting in the significantly decreased concentration of malondialdehyde and increased concentration of soluble protein at 14–28 DAA. The  $F_v/F_m$  at 21–28 DAA and the  $\Phi PSII$ , qP and NPQ at 14–28 DAA for  $W_2$  (and  $W_3$  in 2013–2014) were also significantly higher than those of  $W_{ck}$  and  $W_1$ . Eventually, grain yield, water use efficiency and irrigation benefit of  $W_2$  were 8704.54 kg ha<sup>-1</sup>, 20.86 kg ha<sup>-1</sup> mm<sup>-1</sup> and 31.44 kg ha<sup>-1</sup> mm<sup>-1</sup>, respectively, which were the highest among those of all the treatments. These values increased by 5.82%, 9.65% and 6.00%, respectively, relative to those of  $W_{ck}$ . In conclusion, the SI based on 0–40 cm soil layer and use of an appropriate relative SWC (both 70% FC at the jointing and anthesis stages) can reduce irrigation amount, delay leaf senescence and improve grain yield and water use efficiency.

**Keywords:** Supplemental irrigation based on soil moisture; Wheat; Superoxide dismutase activity; Actual photochemical efficiency of PSII; Grain yield.

### Introduction

The North China Plain produces approximately 53% of the wheat in China (Shan et al., 2011). In this region, the average precipitation is 550 mm per year and primarily concentrated in summer and thus can only satisfy 25%–40% of the water demand of wheat production (Fang et al., 2010). Thus, irrigation is essential to satisfy this demand in order to obtain high yield (Xiong et al., 2010). In North China Plain, only 519 m<sup>3</sup> of

water resource per capita, which is only 1/5 of the national average, is provided (Wu, 2006). This example of water resource shortage has become a serious problem for agricultural production. Therefore, the urgent development of water-saving irrigation technologies for this region is necessary for the improvement of rainfall and soil water storage use and maintenance of high yield and water use efficiency. At present, quota SI is commonly used in this region; for example, SI with 75 mm of water at the jointing and booting stages (Wang et al., 2014) or SI with 60 mm of water at the jointing and heading stages (Li et al., 2010). However, the amount of precipitation varies in different growing stages and years. Thus, irrigation based on a single quota presents several oversights. For high yield and water conservation, new methods must be developed to determine SI amount in which precipitation level, soil water storage and water requirements of wheat at different growth stages are considered.

The photosynthetic production of flag leaf generates 30%–50% of grain yield (Sylvester-Bradley et al., 1990). Therefore, delaying flag leaf senescence is important to ensure high grain yield (Zhang et al., 2006). During leaf senescence, reactive oxygen species (ROS) are produced and cause chlorophyll deterioration, protein degradation and lipid peroxidation, thereby affecting leaf photosynthesis, photochemical activities of PS II and grain filling (Lu and Zhang, 1998; Wu et al., 2012; Jia et al., 2013; Hafez and Gharib, 2016). Water stress disturbs the balance between ROS generation and clearance, which is maintained by the reactive oxygen scavenging system (consisting of superoxide dismutase [SOD], catalase [CAT] and other components), increases ROS accumulation, causes oxidative damage in cells and accelerates senescence (Miller et al., 2010; Singh et al., 2012). Under water stress, the decline of leaf water content and chlorophyll can be associated with the decrease in antioxidant enzyme activity (Pirnajmedin et al., 2015). Hasheminasab et al. (2012) showed that flag leaf SOD and CAT activities were elevated by 6.8 and 155.1  $\mu\text{g}^{-1}$  FW under water stress condition, whereas the malondialdehyde (MDA) content increased. Hameed et al. (2011) maintained the relative soil water content at 100% (well-watered), 75% (medium-watered) and 50% (low-watered) FC; their results showed that the CAT activity of the leaf under medium-watered condition was 200% of that under well-watered condition. Under water stress, the maximum photochemical efficiency of PS II ( $F_v/F_m$ ) and actual photochemical efficiency of PSII ( $\Phi\text{PSII}$ ) declined and photochemical quenching decreased by more than 60% compared with those under well-watered treatment (Zlatev, 2009; Wu et al., 2011). In addition, SI stages and quotas also affect the flag leaf senescence and chlorophyll fluorescence characteristics of wheat (Lin et al., 2009; Dong et al., 2014). Lin et al. (2009) showed that compared with a single irrigation application (60 mm at jointing stage), two applications (60 mm at both jointing and filling stage) significantly delayed flag leaf senescence at the late grain filling stage, whereas three applications (60 mm each at wintering, jointing and filling) had the same effect as the two applications.

Our previous studies showed that SI based on relative SWC of 0–40 cm soil layers can delay flag senescence and increase photosynthetic rate during grain filling, as well as improve grain yield (Guo et al., 2014; Man et al., 2015). However, a suitable relative SWC of SI based on 0–40 cm soil layers remains unknown. In the present experiment, different target levels of the relative SWC in 0–40 cm soil layers were set at the jointing and anthesis stages to guide SI, and the local quota SI treatment was used as control. This study aimed to determine the effect of SWC on flag leaf water potential and senescence, identify the changes in flag leaf chlorophyll fluorescence characteristics

during grain filling and evaluate the grain filling rate, grain yield and WUE of wheat to identify the optimal SI method. These objectives aimed to provide theoretical basis for water management for wheat in order to attain water-efficient and high-yielding cultivation.

## Materials and Methods

### Experimental site

The field experiment was conducted in 2012–2013 and 2013–2014 winter wheat growing seasons near the centre of the North China Plain in Shijiawangzi Village, Yanzhou, Shandong Province, China (116°41' E, 35°42' N). This region experiences a warm temperate and semi-humid continental monsoon climate and has an annual average temperature, accumulated sunshine and total precipitation of 13.6 °C, 2461 h and 621.2 mm, respectively. The groundwater depth in the area is 25 m. The soil is loam with a composition of 29.6% clay, 37.3% silt and 33.1% sand. Table 1 presents the precipitation level at different wheat growth stages during the field experiment. The soil nutrient of 0–20 cm soil layer, field capacity and soil bulk of 0–20 and 20–40 cm soil layers in 2012–2014 growing seasons are presented in Table 2.

Table 1. Precipitation at different wheat growth stages (mm).

Growing seasons	Sowing to jointing	Jointing to anthesis	Anthesis to maturity	Total
2012-2013	92	32.5	100.5	225
2013-2014	61	41	54	156

Table 2. Soil nutrient content, field capacity and soil bulk density of the experimental field.

Soil layers	Items	Growing seasons	
		2012-2013	2013-2014
0-20 cm soil layer	Soil organic matter (%)	1.47	1.43
	Total nitrogen (%)	0.12	0.13
	Available nitrogen (mg kg <sup>-1</sup> )	112.69	112.67
	Available phosphorous (mg kg <sup>-1</sup> )	35.53	38.50
	Available potassium (mg kg <sup>-1</sup> )	113.92	110.43
	Field capacity (%)	24.80	25.32
	Bulk density (g cm <sup>-3</sup> )	1.57	1.59
20-40 cm soil layer	Field capacity (%)	21.96	23.56
	Bulk density (g cm <sup>-3</sup> )	1.65	1.65

### Experimental design and irrigation management

The following four field treatments were performed in the 2012–2013 crop year: no irrigation ( $W_0$ ), irrigation at the jointing and anthesis stages with 60 mm of water each time (local quota SI practice,  $W_{ck}$ ), SI based on the relative SWC of 0–40 cm soil

layers with a target relative SWC of 65% FC at the jointing stage and 70% FC at the anthesis stage ( $W_1$ ) and 70% FC at the jointing and anthesis stages ( $W_2$ ). In the 2013–2014 crop year, a new treatment ( $W_3$ ) was added with a target relative SWC of 75% FC at the jointing stage and 70% FC at anthesis stage on the basis of the treatments in 2012–2013 (Table 3).

The amount of SI was calculated using the following equation (Guo et al., 2014):

$$I = 10 \cdot \gamma \cdot H \cdot (\beta_i - \beta_j), \text{ (Eq. 1)}$$

where  $I$  (mm) is the amount of SI,  $\gamma$  ( $\text{g cm}^{-3}$ ) is the soil bulk density,  $H$  (cm) is the depth of the soil layer measured for SWC prior to irrigation,  $\beta_i$  (%) is the target SWC on a weight basis following SI and  $\beta_j$  (%) is the SWC on a weight basis prior to irrigation.  $\beta_j$  was calculated as follows:

$$\beta_j = \beta_{\max} \cdot \beta_{tr}, \text{ (Eq. 2)}$$

where  $\beta_{\max}$  (%) is the FC and  $\beta_{tr}$  (%) is the target relative SWC. A flow metre was used to measure the quantity of applied water. Table 3 shows the total amount of irrigation for the different treatments.

Table 3. Target relative soil water content, relative soil water content after irrigation, relative error and irrigation amounts in the 0-40 cm soil layer of different treatments.

Growing seasons	Treatments	Jointing stage			Anthesis stage			Total irrigation amount (mm)
		TSRWC <sup>a</sup> (%)	RWCAI <sup>b</sup> (%)	I <sup>c</sup> (mm)	TSRWC (%)	RWCAI (%)	I (mm)	
2012-2013	$W_0$	—	—	0.00	—	—	0.00	0.00
	$W_{ck}$	—	70.93	60.00	—	77.36	60.00	120.00
	$W_1$	65	63.44	49.46	70	68.15	52.09	101.55
	$W_2$	70	67.74	56.98	70	69.14	50.06	107.04
2013-2014	$W_0$	—	—	0.00	—	—	0.00	0.00
	$W_{ck}$	—	67.52	60.00	—	95.42	60.00	120.00
	$W_1$	65	63.23	53.72	70	68.16	21.43	73.15
	$W_2$	70	68.27	61.63	70	68.76	14.96	76.59
	$W_3$	75	74.39	69.54	70	67.96	24.93	94.47

<sup>a</sup>TSRWC: Target soil relative water content. <sup>b</sup>RWCAI: Relative soil water content after irrigation. <sup>c</sup>I: supplemental irrigation amount.

All treatments were replicated three times in a randomised block design. Each experimental plot was 4 m × 4 m in size with a 2.0 m zone maintained between adjacent plots to minimise the effects of other treatments.

All plots were applied with 240 kg ha<sup>-1</sup> N, 150 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> and 150 kg ha<sup>-1</sup> K<sub>2</sub>O. All P and K fertilisers and 105 kg ha<sup>-1</sup> N fertilisers were surface-applied to the soil prior to tillage practices. At the jointing stage, 135 kg N ha<sup>-1</sup> was top-dressed to the soil at a depth of 4 cm by ditching between both rows of winter wheat. The high-yielding wheat

cultivar Jimai22 was used in the experiments. Wheat seeds were sown with type 2BJK-8 seeder (Yuncheng Gongli Co., Ltd) at a density of  $1.8 \times 10^6$  seeds  $\text{ha}^{-1}$  on October 9, 2012 and October 9, 2013. Wheat seedling shoots ceased growth at the beginning of December and started to grow again at the end of February of the subsequent year. During this period, the average daily temperature was below 0 °C. Wheat plants were harvested on June 14, 2013 and June 6, 2014.

#### *Measurement of SWC*

Soil samples were collected using a soil auger in 20 cm increments to a depth of 200 cm in all experimental plots. SWC (count as gravimetric water content, %) was determined using the oven-drying method (Jia et al., 2012). The measurements were performed before sowing, one day before irrigation and three days after irrigation at the jointing, anthesis and maturity stages. Three soil samples were obtained from random locations in each plot.

#### *Measurement of flag leaf water potential*

The water potential of pre-dawn flag leaves was measured using a portable PSYORO water potential system with a C-52 sample chamber (Li et al., 2008). Ten flag leaves with consistent growth were collected at anthesis and at 7, 14, 21 and 28 days after anthesis (DAA).

#### *Assay of antioxidant enzyme activities*

Twenty flag leaves were randomly collected at seven-day intervals from anthesis to maturity (that is, at 0, 7, 14, 21 and 28 DAA). Fresh samples were frozen in liquid nitrogen and then stored at  $-40$  °C until biochemical assays were performed.

Enzyme analyses were conducted by cutting 0.5 g of fresh flag leaf samples. The leaves were ground in a mortar with liquid nitrogen and then extracted with 5 mL of potassium phosphate buffer solution (pH 7.8) containing 0.2 mol of  $\text{L}^{-1}$   $\text{KH}_2\text{PO}_4$  and 0.2 mol of  $\text{L}^{-1}$   $\text{K}_2\text{HPO}_4$ . The homogenate was centrifuged at  $10,000 \times r$  (pm) for 20 min at 4 °C and the supernatant was dispensed into aliquots for enzyme analyses.

The SOD and CAT activities and concentrations of MDA and soluble protein in the flag leaves were measured in accordance with the methods described by Li et al. (2000).

#### *Measurement of flag leaf chlorophyll fluorescence parameters*

The chlorophyll fluorescence parameters were measured with a FMS-2 pulse modulated fluorometer (Hansatech, UK). The measurements for the 10 flag leaves were obtained in the morning (9:00 AM to 12:00 AM) under natural sunlight from anthesis to 28 DAA at seven-day intervals. The steady-state fluorescence ( $F_s$ ), maximum fluorescence ( $F_m$ ),  $F_v/F_m$  and  $\Phi\text{PSII}$  in light were measured in light-adapted flag leaves. After 30 min dark adaptation, the minimum fluorescence ( $F_o$ ) and maximum fluorescence ( $F_m$ ) were collected. The minimum fluorescence from light-adapted leaves ( $F_o'$ ),  $qP$  based on the puddle model and NPQ were calculated using the following equations (Marek et al., 2014):

$$Fo' = \frac{Fo}{Fv / Fm + Fo / Fm'}, \text{ (Eq. 3)}$$

$$qP = \frac{Fm' - Fs}{Fm' - Fo'}, \text{ (Eq. 4)}$$

and

$$NPQ = \frac{Fm - Fm'}{Fm'}, \text{ (Eq. 5)}$$

#### *Measurement of grain filling rate*

The emerging flowering spikes were all tagged on the same day. Twenty tagged spikes from each experimental plot were sampled at seven-day intervals from anthesis to maturity. Grain filling rate was estimated from the accumulated dry weight. At each sampling date, grains were separated from the glumes and then dried at 105 °C for 10 min and at 70 °C to constant weight. The total number of grains was counted and their dry weight was recorded (Guo et al., 2014).

#### *Measurement of evapotranspiration*

The total water consumption or crop evapotranspiration (*ET*) was calculated using the soil water balance equation for the growing season (Patanè and Cosentino, 2013):

$$ET = P + I + \Delta W - R - D, \text{ (Eq. 6)}$$

where *ET* (mm) is the total water consumption during the growing season, *P* (mm) is the level of precipitation, *I* (mm) is the amount of SI,  $\Delta W$  (mm) is the soil water storage at sowing minus the soil water storage at harvesting for the 0–200 cm soil profile, *R* (mm) is the surface runoff and *D* (mm) is the downward flux below the crop root zone. Soil water measurements indicated negligible drainage at the site. Therefore, deep percolation was not considered in the present study.

#### *Measurement of grain yield, WUE and irrigation benefit*

Grain yield was determined from 3 m<sup>2</sup> quadrat cuts from each experimental plot and reported on the basis of 12.5% moisture content.

WUE (kg ha<sup>-1</sup> mm<sup>-1</sup>) was defined as following (Sun et al., 2006):

$$WUE = Y / ET, \text{ (Eq. 7)}$$

where *Y* (kg ha<sup>-1</sup> mm<sup>-1</sup>) is the grain yield and *ET* (mm) is the total water consumption during growing season.

Irrigation benefit (IB, kg ha<sup>-1</sup> mm<sup>-1</sup>) was calculated as follows (Sun et al., 2006):

$$IB = \Delta Y / I, \text{ (Eq. 8)}$$

where  $\Delta Y$  (kg ha<sup>-1</sup> mm<sup>-1</sup>) is the post-irrigation increase in grain yield and  $I$  (mm) is the irrigation amount of SI.

### Statistical analyses

Statistical analyses included standard ANOVA and were performed using SPSS 13.0. The least significant difference method was used to determine differences among the treatments. The probability level for significance determination was 0.05.

## Results

### Changes in flag leaf water potential

Figure 1 shows the post-anthesis flag-leaf water potential. The flag-leaf water potential of the irrigation treatments was significantly higher than that of the W<sub>0</sub> treatment. No significant difference was observed among the flag-leaf water potentials of the irrigation treatments at 0 DAA. From 7 DAA to 28 DAA, W<sub>2</sub> (and W<sub>3</sub> in 2013–2014) exhibited the highest flag-leaf water potential, followed by W<sub>ck</sub> and W<sub>1</sub>. W<sub>0</sub> obtained the lowest flag-leaf water potential and the difference was significant.

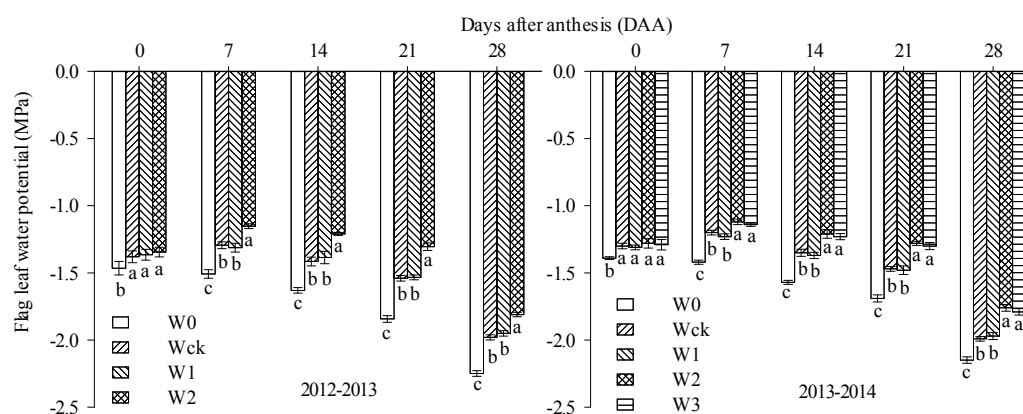


Figure 1. Flag leaf water potential after anthesis in 2012-2013 and 2013-2014.

No irrigation (W<sub>0</sub>), quota supplemental irrigation with 60 mm at both jointing and anthesis stages (W<sub>ck</sub>), supplemental irrigation based on soil relative water content of 0-40 cm soil layers, the target soil relative water content were 65% of field capacity (FC) at jointing and 70% FC at anthesis for W<sub>1</sub>, 70% and 70% FC for W<sub>2</sub> and 75% and 70% FC for W<sub>3</sub>. Error bars represent standard errors of the means. Different letters above error bars indicate significant difference among treatments by LSD test (P<0.05).

### SOD and CAT activities of flag leaves after anthesis

The SOD and CAT activities of the irrigation treatments were significantly higher compared with those under W<sub>0</sub> treatment in both growing seasons (Figure 2). During the

2012–2013 growing season, no significant difference was observed among the SOD and CAT activities of  $W_{ck}$ ,  $W_1$  and  $W_2$  from 0 DAA to 7 DAA. The SOD and CAT activities in  $W_2$  from 14 DAA to 28 DAA were significantly higher than those in  $W_{ck}$  and  $W_1$ . In 2013–2014, no significant difference was observed among irrigation treatments at 0 DAA. From 7 DAA to 28 DAA, SOD and CAT activities in  $W_2$  and  $W_3$  were significantly higher than those in  $W_1$  and  $W_{ck}$ .

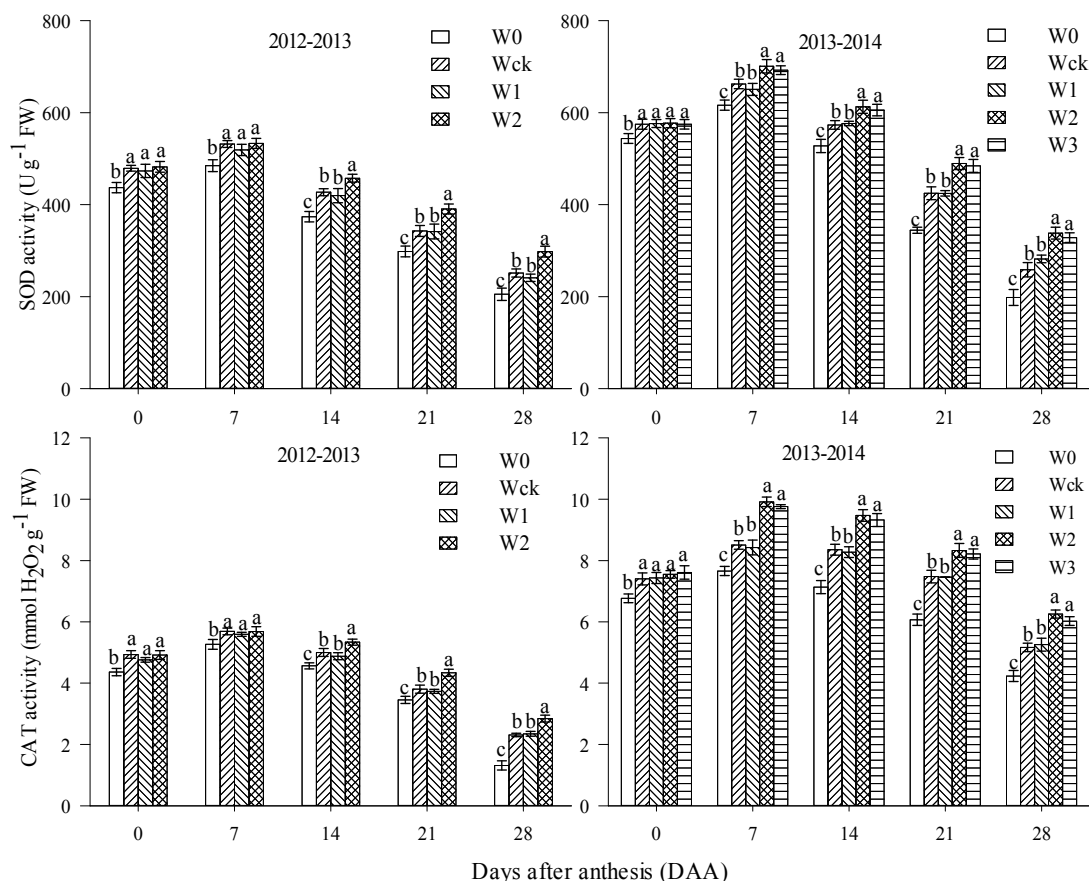


Figure 2. SOD and CAT activity of flag leaves after anthesis in 2012-2013 and 2013-2014.

Error bars represent standard errors of the means. Different letters above error bars indicate significant difference among treatments by LSD test ( $P < 0.05$ ).

#### *MDA concentration of flag leaves after anthesis*

Figure 3 shows no significant difference among the MDA concentrations of the treatments at 0 DAA.  $W_0$  exhibited the highest MDA concentration at 7 DAA and no significant difference was observed among  $W_{ck}$ ,  $W_1$  and  $W_2$  (and  $W_3$  in 2013–2014). From 14 DAA to 28 DAA, MDA concentration in  $W_2$  (and  $W_3$  in 2013–2014) was obviously lower than those in  $W_{ck}$  and  $W_1$  and the highest MDA concentration was observed in  $W_0$ .



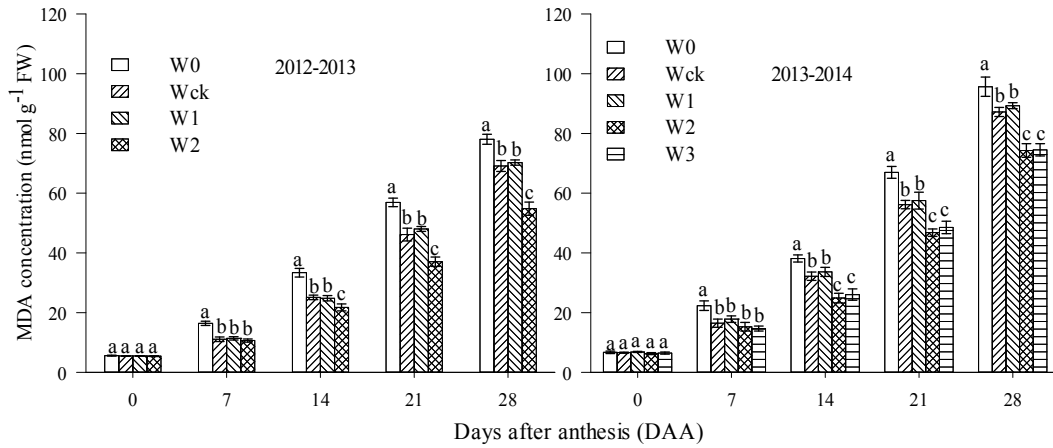


Figure 3. MDA concentration of flag leaves after anthesis in 2012-2013 and 2013-2014.

Error bars represent standard errors of the means. Different letters above error bars indicate significant difference among treatments by LSD test ( $P < 0.05$ ).

*Soluble protein concentration of flag leaves after anthesis*

In both growing seasons, the soluble protein concentration of flag leaves in all irrigation treatments was significantly higher than that in no irrigation treatment after anthesis (Figure 4). Among irrigation treatments, no significant difference was observed among the soluble protein concentrations of the irrigation treatments in 2012–2013 from 0 DAA to 7 DAA. From 14 DAA to 28 DAA, the soluble protein concentration in W<sub>2</sub> was significantly higher than those in W<sub>ck</sub> and W<sub>1</sub>. In 2013–2014, W<sub>2</sub> and W<sub>3</sub> obtained higher soluble protein concentrations from 7 DAA to 28 DAA compared with W<sub>ck</sub> and W<sub>1</sub> and the differences were significant.

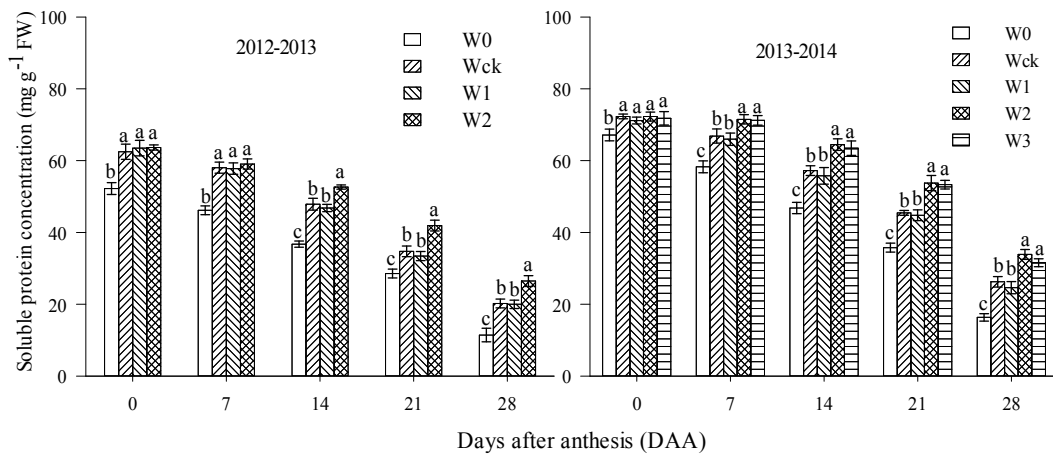


Figure 4. Soluble protein concentration of flag leaves after anthesis in 2012-2013 and 2013-2014.

Error bars represent standard errors of the means. Different letters above error bars indicate significant difference among treatments by LSD test ( $P < 0.05$ ).

### Flag-leaf chlorophyll fluorescence parameters

Figure 5 shows that no significant difference was observed among the  $F_v/F_m$  values of all the treatments from 0 DAA to 7 DAA. At 14 DAA, the  $F_v/F_m$  values of irrigation treatments were significantly higher than that in  $W_0$ . From 21 DAA to 28 DAA,  $F_v/F_m$  values in  $W_2$  and  $W_3$  in 2013–2014 were the highest and significantly higher than those in  $W_0$ ,  $W_{ck}$  and  $W_1$ .  $W_0$  obtained significantly lower  $F_v/F_m$  compared with  $W_{ck}$  and  $W_1$ .

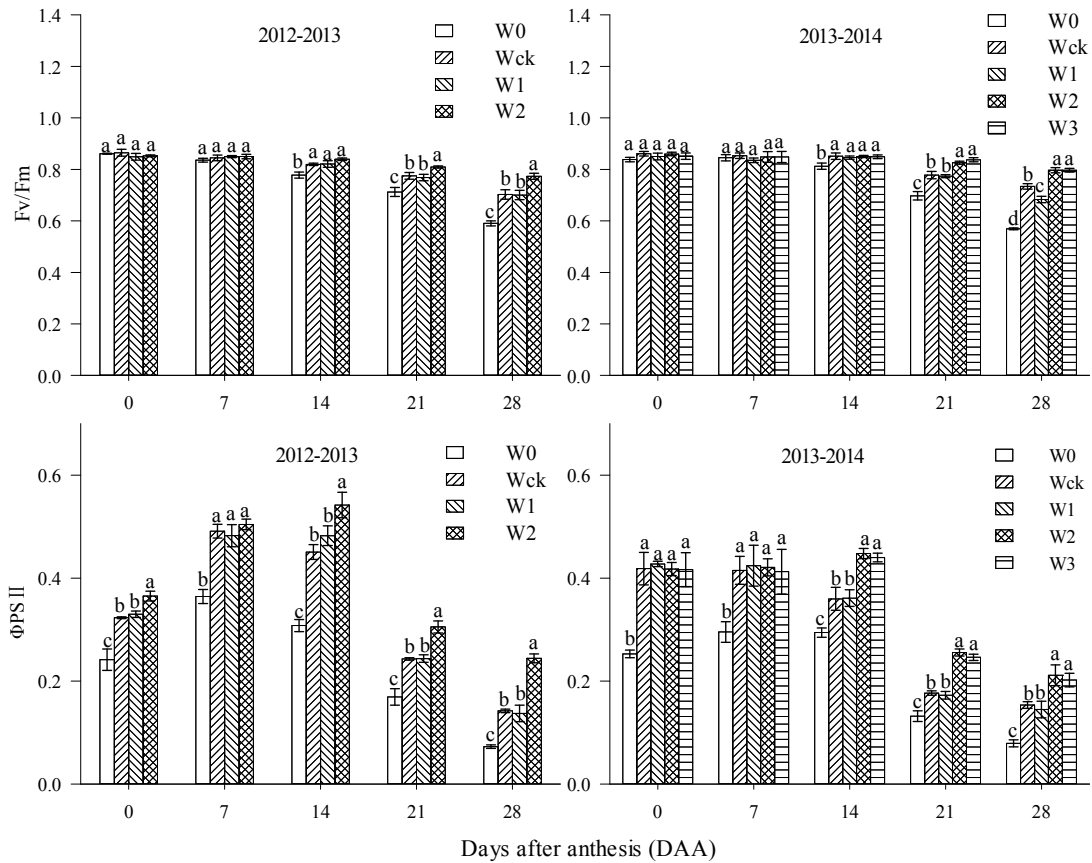


Figure 5.  $F_v/F_m$  and  $\Phi PS II$  of flag leaves after anthesis in 2012-2013 and 2013-2014.

Error bars represent standard errors of the means. Different letters above error bars indicate significant difference among treatments by LSD test ( $P < 0.05$ ).

In 2012–2013, the highest and the lowest  $\Phi PS II$  values were recorded in  $W_2$  and  $W_0$ , respectively, at 0 DAA. At 7 DAA, the  $\Phi PS II$  in  $W_0$  was significantly lower than those in irrigation treatments, but no significant difference was observed among  $W_{ck}$ ,  $W_1$  and  $W_2$ . Compared with  $W_{ck}$  and  $W_1$ , the  $\Phi PS II$  in  $W_2$  was significantly higher from 14 DAA to 28 DAA. In 2013–2014, the  $\Phi PS II$  value in  $W_0$  was significantly lower than those of irrigation treatments from 0 DAA to 7 DAA, without significant difference among irrigation treatments. From 14 DAA to 28 DAA, the  $\Phi PS II$  values in  $W_2$  and  $W_3$  was significantly higher than those in  $W_{ck}$  and  $W_1$ .

The  $qP$  and  $NPQ$  of flag leaf after anthesis are shown in Figure 6. In 2012–2013 period, the  $qP$  and  $NPQ$  values in  $W_0$  were significantly lower than those in irrigation treatments from 0 DAA to 7 DAA. From 14 DAA to 28 DAA, the  $qP$  and  $NPQ$  values

in  $W_2$  were significantly higher than those in  $W_{ck}$  and  $W_1$ . In 2013–2014, no significant difference was observed among the qP and NPQ values of irrigation treatments from 0 DAA to 7 DAA. From 14 DAA to 28 DAA, the qP and NPQ values in  $W_2$  and  $W_3$  were all significantly higher than those in  $W_{ck}$  and  $W_1$ .

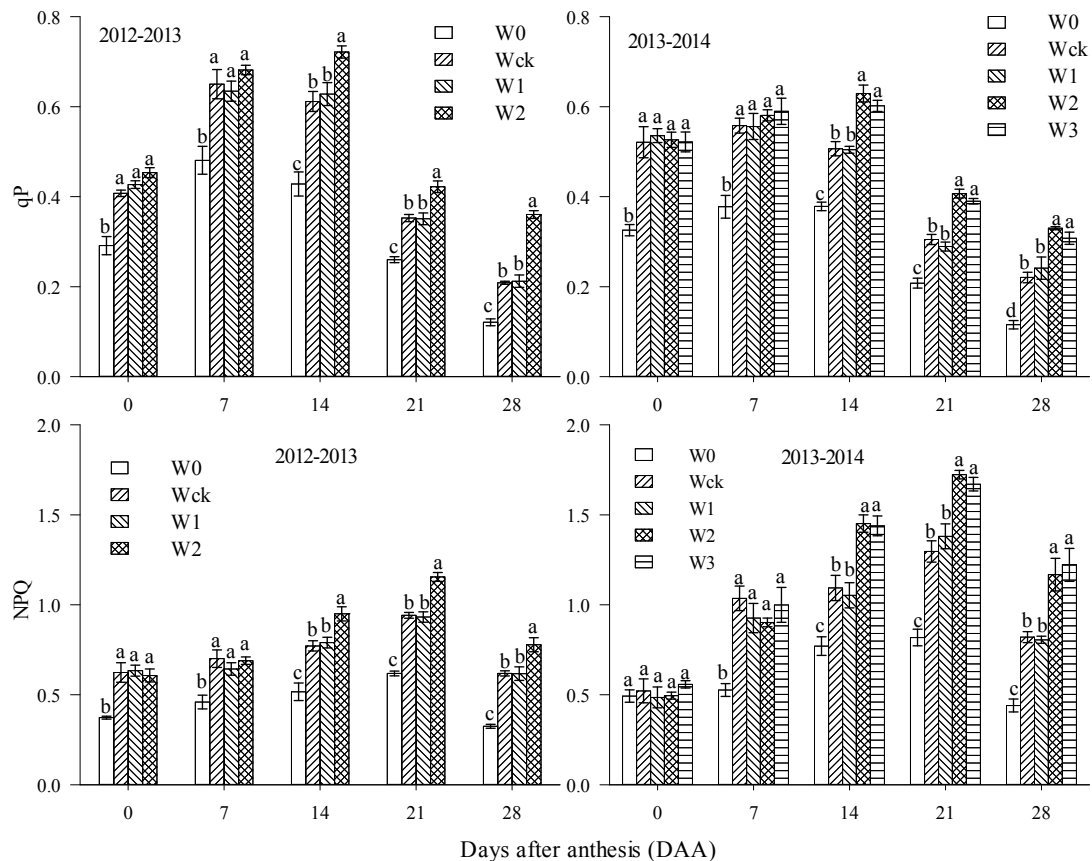


Figure 6. qP and NPQ of flag leaves after anthesis in 2012-2013 and 2013-2014.

Error bars represent standard errors of the means. Different letters above error bars indicate significant difference among treatments by LSD test ( $P < 0.05$ ).

### Grain filling rate

In 2012–2013, the grain filling rate of the no irrigation treatment was significantly higher than the irrigation treatments, no significant difference was observed among irrigation treatments at 0 DAA. At 7–28 DAA, the grain filling rate of  $W_0$  was significantly lower than that of irrigation treatments (Figure 7). Among irrigation treatments,  $W_2$  presented the highest grain filling rate, followed by  $W_{ck}$  and  $W_1$ , with significant difference. In 2013–2014, significant difference was observed between  $W_0$  and irrigation treatments at 0 DAA and  $W_0$  obtained the highest grain filling rate. At 7 DAA, the highest grain filling rate in  $W_0$  was significantly higher than that in  $W_{ck}$  and  $W_1$  but significantly lower than that in  $W_2$  and  $W_3$ . From 14 DAA to 28 DAA, the lowest grain filling rate was observed with  $W_0$ . Among irrigation treatments, grain filling rate in  $W_2$  and  $W_3$  were significantly higher than those in  $W_{ck}$  and  $W_1$ .

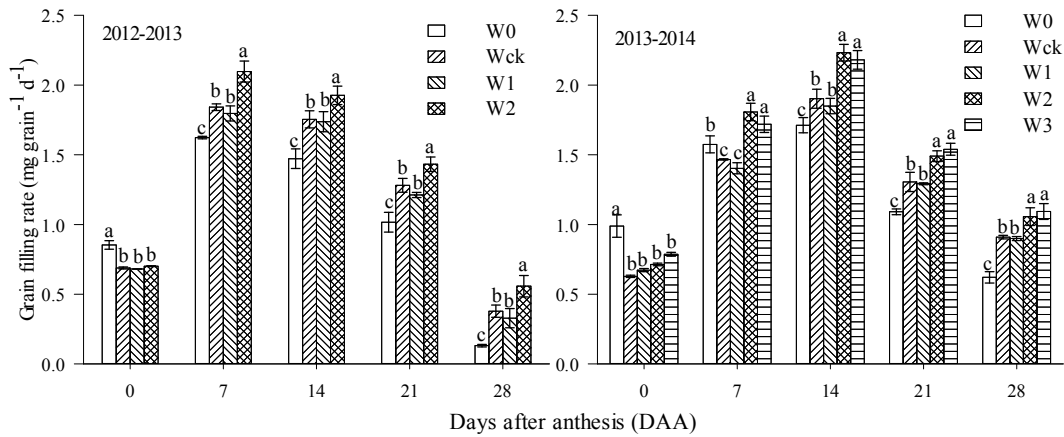


Figure 7. Grain filling rate of different treatments in 2012-2013 and 2013-2014.

Error bars represent standard errors of the means. Different letters above error bars indicate significant difference among treatments by LSD test ( $P < 0.05$ ).

### Grain yield, WUE and IB

Grain yield, ET and WUE in the  $W_0$  treatment were significantly lower than that in the irrigation treatments (Table 4). Among all the irrigation treatments,  $W_2$  presented the highest grain yield, followed by  $W_{ck}$  and  $W_1$  and the differences were significant. The SI amount of  $W_{ck}$  was significantly higher than those of  $W_1$  and  $W_2$ . Thus, the WUE and IB showed a significant decrease from  $W_2$  to  $W_1$  and to  $W_{ck}$ . In the 2013–2014, no significant difference in the grain yield was observed between  $W_2$  and  $W_3$ . However, the amount of SI and ET in  $W_3$  were significantly higher than that in  $W_2$ . Thus, the WUE and IB in  $W_3$  were evidently lower than that in  $W_2$ .

Table 4. Grain yield, total irrigation amount (TIA), evapotranspiration (ET), water use efficiency and irrigation benefit (IB) of different treatments.

Growing seasons	Treatments	Grain yield (kg ha <sup>-1</sup> ) <sup>a</sup>	TIA (mm)	ET (mm)	WUE (kg ha <sup>-1</sup> mm <sup>-1</sup> )	IB (kg ha <sup>-1</sup> mm <sup>-1</sup> )
2012-2013	$W_0$	5381.37 <sup>c</sup>	0.00	332.08 <sup>b</sup>	16.21 <sup>c</sup>	—
	$W_{ck}$	7941.55 <sup>b</sup>	120.00 <sup>a</sup>	412.63 <sup>a</sup>	19.25 <sup>b</sup>	21.33 <sup>c</sup>
	$W_1$	7948.50 <sup>b</sup>	101.55 <sup>b</sup>	406.11 <sup>a</sup>	19.57 <sup>b</sup>	25.28 <sup>b</sup>
	$W_2$	8397.37 <sup>a</sup>	107.04 <sup>b</sup>	410.16 <sup>a</sup>	20.47 <sup>a</sup>	28.18 <sup>a</sup>
2013-2014	$W_0$	6354.25 <sup>c</sup>	0.00	383.47 <sup>d</sup>	16.57 <sup>d</sup>	—
	$W_{ck}$	8509.50 <sup>b</sup>	120.00 <sup>a</sup>	452.56 <sup>a</sup>	18.80 <sup>c</sup>	17.96 <sup>d</sup>
	$W_1$	8454.34 <sup>b</sup>	75.15 <sup>c</sup>	422.73 <sup>c</sup>	20.00 <sup>b</sup>	27.95 <sup>b</sup>
	$W_2$	9011.70 <sup>a</sup>	76.59 <sup>c</sup>	424.14 <sup>c</sup>	21.25 <sup>a</sup>	34.70 <sup>a</sup>
	$W_3$	8893.27 <sup>a</sup>	94.47 <sup>b</sup>	436.97 <sup>b</sup>	20.35 <sup>b</sup>	26.88 <sup>c</sup>

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

## Discussion

Several studies in cotton and wheat showed that leaf-water potential decreased with the reduction of soil water potential, which is evidently related to irrigation (Grimes et al., 1987; Liang et al., 2002). Liang et al. (2002) reported that under alternating dry-wet conditions, the flag leaf water potential decreased from  $-0.4$  MPa to  $-1.3$  MPa when soil moisture decreased from 91% to 73%. Under field conditions, water stress at the booting stage significantly decreased flag-leaf water potential at the early stage of grain filling (Guóth et al., 2009). In the North China Plain, the irrigation amount decreased from 200 mm to 150 mm, flag-leaf water potential decreased by 16.7%. The reduction in soil water storage caused the decrease in flag-leaf water potential at grain filling stage (Gao et al., 2005). In the present study, the target SWC with 70%–75% at the jointing stage and 70% at anthesis were more appropriate and could facilitate wheat to maintain a high flag-leaf water potential and water content (Figure 2) and delay senescence. The relative SWC post-irrigation of quota SI treatment was excessive (77.36%–95.42%), possibly leading to the reduction of flag-leave water potential.

Water stress leads to oxidative stress, as reflected in the imbalance of electron transport rates and the increase in ROS levels (Beck et al., 2007; Štajner et al., 2011; Shi et al., 2015). ROS accumulation causes membrane lipid peroxidation, leading to MDA generation, resulting in secondary damage to enzymes and membranes (Beck et al., 2007; Hua et al., 2003). However, the enzymatic (such as SOD, CAT and POD) and nonenzymatic molecules (such as substances that facilitate osmotic adjustment, such as soluble sugars, proteins and free proline) can quench the ROS and protect the cell from abiotic stress (Farooq et al., 2009). In *Onobrychis viciifolia* Scop., water stress decreases dry matter yield and leaf relative water content while significantly increasing CAT, APX and SOD activity (Irani et al., 2015). Zhang and Kirkham (1994) reported that SOD and CAT activities remain constant or increase during the early phase of drought and subsequently decrease with further water stress. Fazeli et al. (2007) showed that under potted conditions, SWC is maintained at 100%, 75%, 50% and 25% of FC. The MDA concentration of leaves in 25% and 50% FC treatments was significantly higher than that in 75% and 100% FC treatments. Under rain-proofed pond conditions, the mid-watered treatment (60%–70% FC at anthesis) obtained the higher CAT activity in flag leaves compared with low-watered (40%–50% FC) and high-watered (80%–90% FC) treatments (Zhao et al., 2008). The results of this study under field conditions indicate that treatments of the target SWC with 70%–75% at the jointing stage and 70% at anthesis had the highest SOD and CAT activity (Figure 3) and soluble protein content (Figure 5), whereas the MDA (Figure 4) content decreased at the middle and late stages of grain filling. This phenomenon enables the flag leaf cell to maintain high osmotic substance content, enhance ROS scavenging, undergo osmotic adjustments and delay senescence.

Water condition regulates the expression of favourable gene-controlled maximal photochemical efficiency and potential activity of PSII (Bai et al., 2011). Water stress or waterlogging decreases photosynthetic rate, chlorophyll content,  $F_v/F_m$ ,  $\Phi$ PSII and qP and shortens the photosynthetic function period, thereby decreasing dry matter yield and grain yield (Fan et al., 2005; Tambussi et al., 2005; Tan et al., 2007; Ebrahimiyan et al., 2013; Majidi et al., 2015). Under water stress, the  $F_v/F_m$  and qP decreased by 5.5%–20.2% and 20.6%–76.1%, respectively, compared with the well-watered treatment (Zlatev et al., 2009).  $F_v/F_m$  and  $\Phi$ PSII decreased by 20% and 15%,

respectively, at 30 DAA when irrigation is halted during grain filling stage compared with those of the well-watered treatment (Tambussi et al., 2005). A similar finding was also reported by Wu et al. (2015). The highest qP and effective quantum yield of PSII of wheat flag leaf obtained in treatment involving 55% FC at anthesis compared with treatment containing 25% and 85% FC (Wu and Bao, 2011). In the present study, the  $F_v/F_m$  at 21 DAA to 28 DAA increased by 7.33% and the  $\Phi_{PSII}$ , qP and NPQ at 14 DAA to 28 DAA in  $W_2$  by 37.80%, 36.15% and 31.71%, respectively, compared with those in  $W_{ck}$  during the growing seasons (Figures 6 and 7), indicating that delaying leaf senescence decreases the down-regulation of photosynthetic electron transport (Lu and Zhang, 1998).

Yang and Zhang (2006) noted that soil drying during grain filling shortens the active grain filling period by 8 days, whereas the filling rate increases from  $1.40 \text{ mg d}^{-1}$  to  $1.94 \text{ mg d}^{-1}$ . Semcheddine and Hafsi (2014) conducted a rainfed ( $T_0$ ) and three irrigated treatments ( $T_1 = 50 \text{ mm}$  at the booting stage;  $T_2 = 50$  and  $15 \text{ mm}$  at the booting and heading stages, respectively;  $T_3 = 50$  and  $30 \text{ mm}$  at the booting and heading stages, respectively) under field conditions and found that the grain filling rates of  $T_3$  were 10.42%, 11.19% and 10.49% higher than those of  $T_0$ ,  $T_1$  and  $T_2$ , respectively. In the present study, relative SWC with 70%–75% at the jointing stage and 70% at the anthesis stage significantly improved the grain filling rate at the middle and late grain filling stages (Figure 8), extended the duration of the high grain filling rate and eventually increased grain yield.

Delaying leaf senescence can slow down the degradation of chloroplasts, decrease net photosynthetic rate and increase grain yield (Tian et al., 2012). Verma et al. (2004) reported that drought stress accelerates the senescence of wheat flag leaves and the green flag leaf area ratio at 35 DAA and grain yield decrease by 81.13% and 30.48%, respectively, compared with normal irrigation treatments. Supplemental irrigation based on the SWC of root zone, wheat grain yield achieved  $5.70 \text{ t ha}^{-1}$  under full SI, which increased by 0.52 and  $1.88 \text{ t ha}^{-1}$  compared with 2/3 SI and 1/3 SI. The highest WUE was observed at 2/3 SI, followed by 1/3 SI and full SI (Karrou and Oweis, 2012). However, Karam et al. (2009) reported that the highest wheat grain yield was obtained at 1/2 of full SI based on 0–90 cm SWC. In this study, the relative SWC with 70% FC at the jointing and anthesis stages had the highest grain yield, WUE and IB, which increased by 5.82%, 9.68% and 62.66% compared with those of  $W_{ck}$  during the growing season (Table 4). This outcome was associated with high grain filling rate, low ET and irrigation amount (Figure 7, Table 4).

## Conclusions

The SI regimes significantly affected water potential, senescence process and chlorophyll fluorescence of wheat flag leaf.  $W_2$  treatment obtained the highest grain yield, WUE and IB in both growing seasons because of the high grain filling rate in  $W_2$  during the late grain filling stage. Meanwhile, the high flag leaf water potential in  $W_2$  was observed during the late grain filling stage and it improved  $\Phi_{PSII}$  and qP. Moreover, such improvements were associated with the high SOD and CAT activities and low MDA concentration in flag leaves, which all reduced ROS damage in  $W_2$ .  $W_2$  treatment, with the target relative SWC set at 70% FC at the jointing and anthesis stages and based on 0–40 cm soil layers, was the optimum SI regime under this experimental condition.

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