Effect of different supplemental irrigation strategies on photosynthetic characteristics and water use efficiency of wheat

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ABSTRACT

Water resource scarcity has been increasingly becoming a threat to wheat (*Triticum aestivum* L.) production in North China Plain. Thus, a water-saving irrigation strategy should be urgently developed. We conducted a 2-yr field experiment to examine the effects of supplemental irrigation (SI) on the photosynthetic characteristics and water use efficiency (WUE) of wheat. This study employed two SI strategies: A quota SI using 60 mm water at jointing and anthesis stages (W1) and soil moisture testing SI (W2), which brings the target relative soil water content of 0-40 cm soil layer to 70% field capacity at jointing and anthesis stages. A non-irrigated treatment (W0) was used as control. Results showed that W2 significantly improved the water uptake in 80-160 cm soil layer compared with W1. Moreover, flag leaf photosynthetic rate, stomatal conductance, and transpiration 14-28 d after anthesis were highest in W2 successively followed by W1 and W0, and the difference was significant. Dry matter (DM) at maturity, DM accumulation post-anthesis and its contribution ratio to grain were significantly higher in W2 than in the other treatments. 13CO2 labeling results indicated that W2 promoted 13C-photosynthate accumulation in grain. In 2012-2013 growing season, the grain yield increased by 56.05% and 5.74% and WUE increased by 26.17% and 6.34% in W2 compared with those in W0 and W1, respectively. In 2013-2014 growing season, the grain yield increased by 41.82% and 5.90% and WUE increased by 23.1 and 21.0 mm lower than D40 in the two growing seasons, respectively, which were 15.3 and 22.0 mm lower than D60 in the two growing seasons, respectively. So D40 could be considered as a high yield and water-saving treatment.

Key words: Dry matter, photosynthesis rate, quota supplemental irrigation, *Triticum aestivum*.

INTRODUCTION

North China Plain is one of the major food-producing regions in China, producing approximately 50% of the country’s wheat supply (Fan et al., 2016). In this region, the annual precipitation is 550 mm, 20%-30% of which is received during the wheat growing season, and this precipitation level can meet only 25%-40% of the evaporanspiration (ET) in wheat (Fang et al., 2010). Supplemental irrigation (SI) is needed to ensure high wheat grain yield. However, water resource scarcity caused by excessive exploitation of groundwater has threatened crop production (Hu et al., 2010). The amount of irrigation needed in traditional SI practices reaches 310 mm, and the water use efficiency (WUE) of these practices is only 13-17 kg mm\(^{-1}\) (Sun et al., 2011; Zhang et al., 2011), which is lower than that in most wheat-producing areas worldwide (Zwart and Bastiaanssen, 2004). Therefore, effective water-saving irrigation techniques should be urgently developed.

Quota supplemental irrigation (SI) is a water-saving scheme that reduces irrigation frequency and amount per time. In this scheme, 60-75 mm irrigation water is used two to three times at key growing stages (jointing, booting, flowering, and milking stages) of wheat (Li et al., 2009; Zhao et al., 2013; Wang et al., 2014a). Quota SI with 60 mm water per time at jointing and heading stages is commonly employed in North China Plain (Li et al., 2009). However, there are some limitations in these studies, as they did not consider the effect of soil water conditions before irrigation on the irrigation amount, water consumption and wheat production. Our previous study by Guo et al. (2015) determined the irrigation amount by measuring the soil moisture at soil depths of 0-20 cm (D20), 0-40 cm (D40), and 0-60 cm (D60) to investigate the response of photosynthetic characteristics of flag leaves to the SI. The results showed that the total irrigation in the D40 treatment were 62.4 and 118.2 mm in 2011-2012 and 2012-2013 growing seasons, respectively, which were 15.3 and 54.0 mm higher than D20, but 23.1 and 21.0 mm lower than D60 in the two growing seasons, respectively. So D40 could be considered as a high yield and water-saving treatment.

Wheat grain yield depends on two mechanisms: Transfer of current assimilates directly into the grain post-anthesis and remobilization of stored assimilates in vegetative organs (Ercoli et al., 2008). The DM accumulates post-anthesis which is produced by photosynthesis of flag leaves and spikes accounts for more than 60% of grain yield (Fang et al., 2006), indicating a positive correlation between grain yield and DM accumulation post-anthesis (Ye et al., 2011; Meng et al., 2013). Water deficit post-anthesis reduces photosynthesis and DM accumulation,
resulting in reduced grain weight and grain yield (Plaut et al., 2004; Bahrani et al., 2011). Dong et al. (2013) used 60 mm water for each round of irrigation and found that DM accumulation post-anthesis and its contribution to the grain of the treatment group that was irrigated twice at the jointing and anthesis stages (W2) are higher by 16.66% and 16.51% than those irrigated only once at the jointing stage (W1), respectively. Correspondingly, the grain yield was 7324.4 kg ha\(^{-1}\), which is 349.7 kg ha\(^{-1}\) higher than that in W1, and the WUE of W2 was 19.22 kg ha\(^{-1}\) mm\(^{-1}\), which is 0.56 kg ha\(^{-1}\) mm\(^{-1}\) lower than that in W1.

In this experiment, soil moisture testing SI and quota SI treatments were used to investigate the effects of SI on the characteristics of flag leaf photosynthesis and DM production, as well as determine the most suitable water-saving and high-yielding irrigation strategy for wheat. The result is expected to serve as a theoretical basis for water management for wheat to facilitate water-efficient and high-yield cultivation.

**MATERIALS AND METHODS**

**Experimental site**

A field experiment was conducted during wheat growing seasons 2012-2013 and 2013-2014 in Shijiawangzi Village, Yanzhou (35°42’ N, 116°41’ E, 55 m a.s.l.), Shandong Province, which is located at the center of the North China Plain. This region presents a warm temperate semi-humid continental climate with an annual average temperature, accumulated sunshine, and precipitation of 13.6 °C, 2461 h, and 621.2 mm, respectively. The groundwater is 25 m deep. The precipitation during 2012-2013 and 2013-2014 wheat growing seasons was 225 and 156 mm, respectively. The precipitation events during 2012-2013 and 2013-2014 wheat growing seasons are shown in Figure 1.

The soil texture was loam consisting of 29.6% clay, 37.3% silt, and 33.1% sand. Table 1 shows soil nutrients at 0-20 cm soil layer, and field capacity (FC) and soil bulk at 0-40 cm soil layers.

![Figure 1. Precipitation events during 2012-2013 and 2013-2014 wheat growing seasons.](image)

| Items                        | Soil layer | 2012-2013 | 2013-2014 |
|------------------------------|------------|-----------|-----------|
| Soil organic matter, %       | 0-20 cm    | 1.47      | 1.43      |
| Total N, %                   |            | 0.12      | 0.13      |
| Available N, mg kg\(^{-1}\)  |            | 112.69    | 112.67    |
| Available P, mg kg\(^{-1}\)  |            | 35.53     | 38.50     |
| Available K, mg kg\(^{-1}\)  |            | 113.92    | 110.43    |
| Field capacity, %            | 20-40 cm   | 24.80     | 25.32     |
| Bulk density, g cm\(^{-3}\)  |            | 1.57      | 1.59      |
| Field capacity, %            |            | 21.96     | 23.56     |
| Bulk density, g cm\(^{-3}\)  |            | 1.65      | 1.65      |

**Table 1. Soil nutrient of 0-20 cm soil layer, and field capacity and soil bulk of 0-20 and 20-40 cm soil layers in 2012-2013 and 2013-2014 growing seasons.**
Experimental design
The SI treatments applied in 2012-2013 and 2013-2014 growing seasons are as follows: in quota SI treatment (W1), 60 mm water was applied each time; in soil moisture testing SI treatment (W2), water was applied based on the relative soil water content (SWC) of 0-40 cm soil layer, and the target relative SWC was 70% field capacity (FC). Field capacity is the amount of soil moisture after draining excess water, and the rate of downward movement has materially decreased (Twarakavi et al., 2009). An SI event occurred the next day after the relative SWC of 0-40 cm soil layer was tested at the jointing (Z31, first node is detectable) and anthesis (Z61, beginning of anthesis) stages (Zadoks et al., 1974). In SI treatments, water was sprayed evenly onto the experimental plots under pressure, and a flow meter was used to measure the amount of applied water. A non-irrigated treatment (W0) was set as control. Table 2 shows SI under different treatments.

The SI amount (I, mm) in W2 was calculated according to the following equation (Guo et al., 2015):
\[ I = 10 \times \frac{\gamma_{bd}}{D_h} \times \left( \theta_n - \theta_{tr} \right) \]
where \(\gamma_{bd}\) is the soil bulk density (g cm\(^{-3}\)), \(D_h\) is the thickness of the soil profile measured for relative SWC before irrigation (cm), \(\theta_n\) is the target SWC on a weight basis after SI (%), and \(\theta_{tr}\) is the actual SWC on a weight basis before irrigation (%). The value of \(\theta_{tr}\) was calculated as follows:
\[ \theta_{tr} = \theta_{max} \times \theta_n \]
where \(\theta_{max}\) is the FC (%) and \(\theta_n\) is the target relative SWC (%).

On the third day after SI, soil samples of 0-40 cm layer were collected to calculate the relative error between the actual and target relative SWC, each sample was replicated three times. The average relative errors in W2 were 2.23% and 2.12% (Table 2) in 2012-2013 and 2013-2014, respectively. These values indicated that the soil moisture testing SI had achieved the target relative SWC.

The experiment plots were in a randomized block design to remove the variability of soil fertility. Each experimental plot was 4 m × 4 m and replicated three times. A 2.0 m zone to remove the variability of soil fertility. Each experimental plot was 4 m × 4 m and replicated three times. A 2.0 m zone was set between adjacent plots to minimize the effects of water, and the rate of downward movement has materially decreased (Twarakavi et al., 2009). An SI event occurred the next day after the relative SWC of 0-40 cm soil layer was tested at the jointing (Z31, first node is detectable) and anthesis (Z61, beginning of anthesis) stages (Zadoks et al., 1974). In SI treatments, water was sprayed evenly onto the experimental plots under pressure, and a flow meter was used to measure the amount of applied water. A non-irrigated treatment (W0) was set as control. Table 2 shows SI under different treatments.

The experiment plots were in a randomized block design to remove the variability of soil fertility. Each experimental plot was 4 m × 4 m and replicated three times. A 2.0 m zone was set between adjacent plots to minimize the effects of other treatments. All plots were supplied with 240 kg N ha\(^{-1}\), 150 kg P\(_2\)O\(_5\) ha\(^{-1}\), and 150 kg K\(_2\)O ha\(^{-1}\). All P and K fertilizers and 105 kg N ha\(^{-1}\) of N fertilizers were surface-applied to the soil before performing tillage practices. At the jointing stage, 135 kg N ha\(^{-1}\) was applied as a topdressing to the soil at a depth of 4 cm by ditching between rows of wheat. The high-yield wheat ‘Jimai22’ was used in the experiments. Wheat seeds were sown using a 2BJK-8 seeder (Yuncheng County Gongli, Heze, China) at a density of 1.8 × 10\(^6\) seeds ha\(^{-1}\) on 9 October 2012 and 9 October 2013 and then harvested on 14 June 2013 and 6 June 2014, respectively.

Sample and data collection
Soil bulk density and SWC. The soil bulk density of 0-200 cm soil layers was determined using the method described by Wang et al. (2014b). Samples of 0-200 cm topsoil were collected in all experimental plots by using a soil auger at 20 cm increments, each sample was replicated three times. SWC on a weight basis was calculated using the oven drying method (Jia et al., 2012). Measurements were performed before sowing (Z00), 1 d before irrigation and 3 d after irrigation at both jointing (Z31) and anthesis (Z61) stages, and at maturity (Z90) stage.

Flag leaf photosynthesis parameters. Net photosynthesis rate (P\(_n\)), stomatal conductance (g\(_s\)), and transpiration rate (T\(_r\)) in flag leaf were observed from 09:00 to 11:00 h under natural light by using a portable photosynthesis system (CIRAS-2, PP-Systems, Hitchin, UK). Five measurements were obtained at 7-d intervals from 0 to 28 d after anthesis (DAA).

Dry matter production. Samples of the aerial plant parts were collected to monitor DM at anthesis (Z61) and maturity (Z90). Twenty adjacent plants in each plot were randomly cut at ground level and served as one sample. These plants were separated into stem + sheath, spike, and leaf at anthesis. At maturity, they were separated as stem + sheath, leaf, spike axis + glume, and grain. All samples were dried to a constant weight in a forced-draught oven at 70 °C, and their dry weights were recorded. The plant density was computed as the mean plant number within a 1 m\(^2\) plot.

The parameters related to DM accumulation and remobilization in wheat plants were calculated as follows (Zhang et al., 2008):
\[ \text{DM accumulation post-anthesis (DMA}_{anthesis} \text{, kg ha}^{-1} = \text{DM}_{anthesis} - \text{DM}_{maturity} \]

Table 2. Target relative soil water content, relative soil water content after irrigation, relative error and irrigation amounts in 0-40 cm soil layers of different treatments.

| Growing seasons | Treatments | Jointing stage | Anthesis stage |
|----------------|------------|----------------|---------------|
|                | TSRWC | RWCAI | RE | I | TSRWC | RWCAI | RE | I |
| 2012-2013      | W0 | - | - | - | 0.00 | - | - | - | 0.00 |
|                | W1 | 70 | 70 | 93 | 60.00 | - | 77.36 | - | 60.00 |
|                | W2 | 70 | 67.74 | 3.23 | 56.98 | 70 | 69.14 | 1.23 | 50.06 |
| 2013-2014      | W0 | - | - | - | 0.00 | - | - | - | 0.00 |
|                | W1 | 70 | 67.52 | 2.47 | 61.63 | 70 | 68.76 | 1.77 | 14.96 |

TSRWC: Target soil relative water content; RWCAI: relative soil water content after irrigation; RE: relative error; I: irrigation amount.
DM remobilization amount pre-anthesis (DMR, kg ha\(^{-1}\)) = DM plant aerial part at anthesis stage – (DM stem + sheath + leaf + spike axis + glume at maturity)

Contribution DMA\(_p\) to grain (GCDMA\(_p\), %) = (DMA\(_p\)/DM grains at maturity) × 100

Contribution DMR to grain (GCDMR, %) = (DMR/DM grains at maturity) × 100

Labelling of selected flag leaves with 13CO\(_2\). In 2013-2014 growing season, 10 flag leaves in each treatment were selected to be labelled with 13CO\(_2\) at 14 DAA. The flag leaf was overlapped by a 0.1-mm thick Mylar plastic bag, which could pass more than 95% of sunlight through. The bags were sealed with plasticine and then injected with 3.5 mL 13CO\(_2\). After 30 min of photosynthesis, the remaining leaf was overlapped by a 0.1-mm thick Mylar plastic bag, and the plastic bag was removed. The labelled samples were collected at ground level from 10 single tillers at maturity (Z90). Each tiller was separated into five parts as follows: flag leaves, other leaves, stem + sheath, spikes + glumes, and grain. All of the samples were oven-dried at 70 °C to a constant weight, and then their DM (g) values were recorded. The samples were subsequently milled into powder. Approximately 4 mg of samples were weighed and placed in tin capsules to determine their isotopic abundance by using a stable isotope ratio mass spectrometer (IsoPrime100, Isoprime, Cheadle, UK). Carbon isotope data were expressed in the typical delta notation (δ\(^{13}\)C), which is defined as follows (Yousif et al., 2012):

\[ \delta^{13}\text{C} = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} \right) \times 1000 \]

where \( R \) is the \(^{13}\text{C}/^{12}\text{C} \) isotope ratio. The international standard for \( C \) is Pee Dee Belemnite with a \(^{13}\text{C}/^{12}\text{C} (R_{\text{PDB}}) \) ratio of 0.01112372.

Atomic \(^{13}\text{C}\) was calculated as follows (Li et al., 2012):

\[ \text{Atomic} \ \delta^{13}\text{C} = \left( \delta^{13}\text{C} + 1000 \right) \times \frac{R_{\text{PDB}}}{\left( \delta^{13}\text{C} + 1000 \right) + 1000} \times 100 \]

The assimilated \(^{13}\text{C} \) in different organs was estimated as the difference between \(^{13}\text{C} \) contents of the labelled and non-labelled samples, as follows (Lu et al., 2002):

\[ ^{13}\text{C}_s = (\text{atomic} \ ^{13}\text{C}_l - \text{atomic} \ ^{13}\text{C}_n) \times TC_i \times DM_i \times 100 \]

where \(^{13}\text{C}_s \) is \(^{13}\text{C} \) assimilation in different organs (mg), \text{atomic} \ ^{13}\text{C}_l \) and \text{atomic} \ ^{13}\text{C}_n \) indicate the atomic \(^{13}\text{C} \) of labelled and non-labelled samples (%), respectively; \( TC_i \) is the total C content of sample (%), and \( DM_i \) is the dry weight of the sample (kg).

Net \(^{13}\text{C} \) assimilation of the entire plant is the sum of net \(^{13}\text{C}_s \) in flag leaves, other leaves, stem + sheath, spikes + glumes, and grain. The \(^{13}\text{C} \) distribution ratio of different organs was calculated as the ratio of \(^{13}\text{C}_s \) to net \(^{13}\text{C} \) assimilation of the entire plant (Lu et al., 2002).

ET and WUE. The total water consumption or crop ET during the growing season was calculated using the soil water balance equation (Patané and Cosentino, 2013):

\[ ET = P + I + \Delta W - R - D \]

where \( ET \) (mm) is the total water consumption during growing season, \( P \) (mm) is the precipitation, \( I \) (mm) is the SI amount, \( \Delta W \) (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. Run off and drainage are ignored in the North China Plain (including this experimental site, Lv et al., 2011).

WUE (kg ha\(^{-1}\) mm\(^{-1}\)) is defined as follows (Wang et al., 2013):

\[ WUE = Y \div ET \]

where \( Y \) (kg ha\(^{-1}\) mm\(^{-1}\)) is the grain yield and \( ET \) (mm) is the total water consumption during a growing season.

Statistical analyses
Data were statistically analyzed by using ANOVA and Fisher’s Least Significance Difference test (LSD) as the multiple pairwise comparison at \( \alpha = 0.05 \) to determine the significant effects of the treatments (SPSS 13.0 software).

RESULTS AND DISCUSSION
Relative SWC of 0-200 cm soil layers at jointing, anthesis, and maturity stages
Soil water condition at different growth stages affects photosynthetic characteristics, DM accumulation, and grain yield, which are directly affected by amount of irrigation (Liu et al., 2016). In our study, the average irrigation amounts in W1 and W2 in the two growing season were 60 and 59.31 mm at the jointing stage and 60 and 32.51 mm at the anthesis stage, respectively (Table 2). At jointing post-irrigation, the relative SWC of 0-40 cm soil layer was significantly higher in SI treatments (W1 and W2) than in W0 in both growing seasons (Figure 2). Nonsignificant difference was observed in the relative SWC of 60-200 cm soil layer among the treatments. At anthesis post-irrigation, W1 obtained the highest average relative SWC of 0-40 cm and 60-160 cm soil layer during the two growing season, followed by W2, W0 was the lowest, and the difference was significant. At maturity, in 2012-2013 growing season, the relative SWC in 80-180 cm soil layer of W1 was significantly higher than that of W2, W0 obtained the significant lowest relative SWC in 80-180 cm soil layer in comparison of W1 and W2. In 2013-2014 growing season, W1 produced the highest relative SWC in 80-160 cm soil layer at maturity, followed by W2 and W0, the difference was significant. This result showed that a decreasing irrigation amount increased water use in deep soil layer, consistent with the finding of Liu et al. (2011).

Net photosynthetic rate, stomatal conductance, and transpiration of flag leaf
Compared with the SI in W0, that at the jointing and anthesis stages in W1 and W2 increased the average flag leaf net photosynthetic rate, stomatal conductance, and transpiration in grain filling stage by 77.3%, 66.1%,...
and 51.9% in the two growing seasons, respectively (Figure 3). Similar results were reported by Xue et al. (2006), implying that non-irrigated treatment reduces the photosynthetic rate and stomatal conductance in flag leaf. Kang et al. (2002) and Wang et al. (2013) reported that reduced irrigation and the application of fertilizer maintain the high photosynthetic capacity of wheat and reduce ET, resulting in high grain yield and WUE. Compared with the wheat grown under full irrigation condition (75%-80% FC), that grown under mild drought stress condition (65%-70% of FC) during recovering to jointing stage maintains high canopy photosynthesis after anthesis (Liu et al., 2016). Our study found that setting the relative SWC of 0-40 cm soil layer to 70% both at the jointing and anthesis stages (W2) increased the net photosynthetic rate by 7.5%, 9.6%, and 21.4% at 14, 21, and 28 DAA compared with that in quota SI treatment (W1) during the 2012-2013 growing season. Gas exchange controlled by stomata is essential in maintenance of photosynthetic capacity (Oliver et al., 2009). Significant increase in stomatal conductance (11.7%, 6.3%, and 9.5% at 14, 21, and 28 DAA) and transpiration (10.8%, 7.6%, and 30.9% at 14, 21, and 28 DAA) was observed in W2 but not in W1, indicating that the photosynthetic reduction in W1 was due to stomatal conductance. A similar pattern was observed in W0, W1, and W2 in the 2013-2014 growing season.

**Dry matter accumulation in different organs at anthesis and maturity stages**

Dry matter accumulation and photosynthetic activity both influence wheat grain yield (Liang et al., 2010), which are associated with irrigation and SWC (Boutraa et al., 2010). Meng et al. (2013) found that DM at the regreening, stem elongation, anthesis, and maturity stages are all significantly correlated with grain yield, and the increase in grain yield from 7-9 t ha⁻¹ to > 9 t ha⁻¹ is mainly attributed to the increase in DM from stem elongation to anthesis stage. Compared with W1 and W2, W0 reduced the average total DM content in the two growing seasons by 32.15% in anthesis stage, and this phenomenon is mainly caused by DM reduction in stem + sheath (Table 3). Dry matter contents in stem + sheath, leaves, and spikes of W2 were greater by 1.71%, 3.05%, and 1.20% than those in W1 in 2012-2013 growing season and greater by 1.37%, 2.79%, and 2.17% than those in W1 in 2013-2014 growing season. Eventually, total DM in W2 increased by 217.66 and 253.75 kg ha⁻¹ compared with that in W1 in 2012-2013 and 2013-2014 growing seasons, respectively, although the difference was nonsignificant possibly due to the similar irrigation amount at jointing stages in W1 and W2.

At maturity, DM accumulation was highest in grain followed by those in stem + sheath, leaves, and spike axis + glume (Table 4). Moreover, W0 reduced DM in...
different organs compared with irrigation treatments, consistent with the results of Zhao et al. (2013). Kang et al. (2002) showed that setting SWC to 55% FC from the regrowth to stem elongation stages, to 70% FC from the booting stage to milk ripeness stage, and to 45%-55% FC from maturity to harvest obtained high biomass (DM at maturity), grain yield and WUE of wheat in Loess Plateau of China. In our study, DM content in stem + sheath, leaves, spikes axis + glume, and grain in W2 increased by 7.86%, 5.17%, 11.89%, and 5.74% in 2012-2013 growing season and by 6.64%, 5.61%, 6.26%, and 5.90% in 2013-2014 growing season, respectively, compared with that in W1. Eventually, total DM in

Table 3. Dry matter accumulation of different organs at anthesis.

| Growing seasons | Treatments | Stem + sheath | Leaves | Spikes | Total  |
|----------------|------------|---------------|--------|--------|--------|
|                |            | kg ha⁻¹        |        |        |        |
| 2012-2013      | W0         | 4799.60b       | 1627.60b | 1835.60b | 8262.80b |
|                | W1         | 7614.07a       | 2012.19a | 2194.48a | 11820.73a |
|                | W2         | 7744.00a       | 2073.60a | 2220.80a | 12038.40a |
| 2013-2014      | W0         | 5744.23b       | 1823.03b | 1860.20b | 9427.46b |
|                | W1         | 8661.07a       | 2856.22a | 2590.25a | 14061.38a |
|                | W2         | 8732.69a       | 2935.99a | 2646.45a | 14315.13a |

Within a column, values in the same growing season followed by different letters differ significantly (P < 0.05). Same as Tables 4, 6 and 7.

Table 4. Dry matter accumulation of different organs at maturity.

| Growing seasons | Treatments | Stem + sheath | Leaves | Spike axis + glume | Grains | Total  |
|----------------|------------|---------------|--------|-------------------|--------|--------|
|                |            | kg ha⁻¹        |        |                   |        |        |
| 2012-2013      | W0         | 2679.12c       | 1476.20c | 1239.55c          | 5381.37c | 10776.24c |
|                | W1         | 6175.56b       | 1676.33b | 1552.91b          | 7941.55b | 17346.34b |
|                | W2         | 6661.07a       | 1763.14a | 1737.67a          | 8397.37a | 18559.25a |
| 2013-2014      | W0         | 3442.99c       | 1806.14c | 1404.81c          | 6354.25c | 13008.19c |
|                | W1         | 7770.26b       | 2017.70b | 1768.76b          | 8509.50b | 20066.22b |
|                | W2         | 8286.21a       | 2130.96a | 1879.44a          | 9011.70a | 21308.31a |
W2 increased by 6.99% and 6.19% relative to that in W1 in 2012-2013 and 2013-2014 growing seasons, respectively. Moreover, $^{13}$C accumulation amount and ratio (Table 5) demonstrates that W2 increased the total $^{13}$C assimilation amount. $^{13}$C accumulation amount and ratio in grain at maturity were larger by 277.0% and 9.7% than those in W0 and larger by 63.8% and 3.7% than those in W1 during the same labeling time.

**Dry matter remobilization amount and ratio to grain at pre-anthesis stage**

The enhanced DM accumulation at maturity and high contribution ratio of DM accumulation post-anthesis (DMA$_p$) to grain resulted in high wheat grain yield in Huang-Huai-Hai Plain (Zhang et al., 2008). Appropriate irrigation increases DMA$_p$ and improves the DMA$_p$ contribution to grain (Ercoli et al., 2008; Liu and Ouyang, 2012). Ma et al. (2014) indicated that drought stress reduces grain yield by reducing DMA$_p$, which contributes approximately 87%, 82%, and 63% to grain yield under non-stress, moderate, and severe stress conditions, respectively. Table 6 shows the DM remobilization amount at pre-anthesis stage and accumulation amount at post-anthesis stage and their contribution ratio to grain. In both growing seasons, W2 reduced DM remobilization amount and its contribution ratio at pre-anthesis stage by 30.90% and 53.37%, respectively, compared with W0 and by 20.87% and 25.21% compared with W1. Moreover, DM accumulation amount at post-anthesis stage and its contribution ratio to grain in W2 were larger by 127.37% and 51.97% in W0 and by 17.24% and 10.80% than in W1. These results indicate that the increase in DM accumulation amount post-anthesis was the main factor for the increase in grain yield.

**Grain yield, WUE, and irrigation benefit**

Studies have shown that ET of wheat decreased with reduced irrigation amount, and non-irrigated treatment generally results in low ET and grain yield but high WUE compared with irrigation treatments (Guo et al., 2008; Dong et al., 2013). In our study, W0 reduced ET, grain yield, and WUE compared with SI treatments in both 2012-2013 and 2013-2014 growing seasons (Table 7), consistent with the findings of Yu et al. (2010). Moreover, Yu et al. (2010) found that a quadratic relationship exists between wheat grain yield, WUE, and water consumption, and increased grain yield and WUE were obtained when the ET ranged from 350 to 490 mm. Compared with well-watered condition, moderate water deficiency at grain filling stage resulted in high grain yield and WUE, corresponding to the increased DM mobilization and low ET (Zhang et al., 2008). Under mobile rain shelter condition, setting the relative SWC to 65%-70% FC at the grain filling stage resulted in the highest grain yield and WUE compared with that obtained under 75%-80% FC treatment (Liu et al., 2016). In our study, the ET of treatment which bring relative SWC to 70% at jointing and anthesis stage (W2) was 410.16 and 424.14 mm, which decreased by 2.47 and 28.42 mm than treatment which irrigate 60 mm water at jointing and anthesis stage (W1) due to the 12.96 and 43.41 mm reduction of SI in 2012-2013 and 2013-2014 growing season, respectively. Moreover, the grain yields in W2 were higher by 5.7% and 5.9% than in W1. Thus, the WUE and IB were significantly higher in W2 than in W1.

**Table 5. $^{13}$C accumulation amount and ratio in different organs at maturity in 2013-2014 growing season.**

| Treatments | W0 | W1 | W2 |
|------------|----|----|----|
| $^{13}$C accumulation, mg | 0.0008c | 0.0013b | 0.0021a |
| Other leaves | 0.0002c | 0.0003b | 0.0004a |
| Steam + sheath | 0.0007c | 0.0017b | 0.0019a |
| Spike axis + glumes | 0.0004b | 0.0006a | 0.0006a |
| Grain | 0.0070c | 0.0161b | 0.0264a |
| Total | 0.0091c | 0.0200b | 0.0314a |

**Table 6. Dry matter remobilization (DMR) amount pre-anthesis and accumulation amount post-anthesis (DMA$_p$) and their contribution ratio to grain in 2012-2013 and 2013-2014 growing seasons.**

| Growing seasons | Treatments | DMR | GCDMR | DMA$_p$ | GCDMA$_p$ |
|-----------------|------------|-----|-------|---------|-----------|
| 2012-2013 | W0 | 2867.93a | 53.29a | 2513.44c | 46.71c |
| W1 | 2415.93b | 30.42b | 5525.61b | 69.58b |
| W2 | 1876.52c | 22.35c | 6520.85a | 77.65a |
| 2013-2014 | W0 | 2773.52a | 43.65a | 3580.73c | 56.35c |
| W1 | 2504.66b | 29.43b | 6004.84b | 51.97b |
| W2 | 2018.52c | 22.40c | 6993.18a | 77.60a |

**Table 7. Grain yield, total irrigation amount, evapotranspiration (ET), water use efficiency (WUE) and irrigation benefit (IB) of different treatments.**

| Growing seasons | Treatments | Grain yield | Total irrigation amount | ET | WUE | IB |
|-----------------|------------|-------------|------------------------|----|-----|----|
| 2012-2013 | W0 | 5381.37c | 332.08b | 16.20c |
| W1 | 7941.55b | 383.47c | 21.33b |
| W2 | 8397.37a | 107.04b | 28.18a |
| 2013-2014 | W0 | 6354.25c | 383.47c | 16.75c |
| W1 | 8509.50b | 412.63a | 21.76b |
| W2 | 9011.70a | 28.18a | 34.70a |

**CONCLUSIONS**

In this study, soil moisture testing supplemental irrigation (SI) (W2) reduced the SI amount, thereby increasing water uptake in 80-160 cm soil layers and reducing evapotranspiration.
The flag leaf in W2 maintained high photosynthetic rate, stomatal conductance, and transpiration at the late grain filling stage. Therefore, dry matter at maturity, dry matter accumulation post-anthesis and its contribution ratio to grain were significantly higher in W2 than in other treatments. Consequently, W2 achieved the highest grain yield, water use efficiency, and irrigation benefit. In conclusion, soil moisture testing SI based on 0-40 cm layer with a relative soil water content of 70% field capacity at the jointing and anthesis stages was the most suitable irrigation strategy considering high yield and water conservation.

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