DOI: 10.1007/s11099-018-0833-5 PHOTOSYNTHETICA 56 (4): 1437-1446, 2018

Responses of photosynthesis, dry mass and carbon isotope discrimination in winter wheat to different irrigation depths

L.J. ZHENG*, J.J. MA*,+, X.H. SUN*, **, X.H. GUO*, J. JIANG*, R. REN*, and X.L. ZHANG*

*College of Water Resource Science and Engineering, Taiyuan University of Technology, Taiyuan 030024, China**
*Shanxi Conservancy Technical College, Yuncheng 044004, China***

Abstract

In order to test the effects of irrigation depth on winter wheat photosynthesis, four treatments were applied in a field experiment using PVC growth tubes (identical amounts of water were applied on the land surface, and at 60, 75, and 90% of the depth for the winter wheat root distribution, denoted as D0, D60, D75, and D90, respectively). Compared to the surface irrigation treatment D0, the leaf area index, chlorophyll content, net photosynthetic rate, transpiration rate, stomatal conductance, and intercellular CO_2 concentration increased with irrigation depths. The values of these indicators obtained by the underground irrigation treatment D75 were higher than those of D60 and D90, and thus D75 was found to be the optimum irrigation depth. Furthermore, a positive but not significant correlation (r = 0.62) between carbon isotope discrimination ($\Delta^{13}C$) and grain yield was found. This study improves our understanding of the mechanism of underground water distribution control with depth, and the efficiency of water-saving irrigation for winter wheat.

Additional key words: irrigation pattern; productivity; root distribution; soil water content; water-use efficiency.

Introduction

During the winter wheat growth period, rainfall is limited in arid and semiarid areas. To guarantee the crop productivity, the use of supplementary irrigation is widespread throughout northern China (Yang *et al.* 2015). However, surface irrigation results in a substantial water loss due to the evaporation from the soil surface and poor uniformity of the distribution of irrigation water in the soil profile (Marek *et al.* 2016). Therefore, it is necessary to develop water-conserving irrigation techniques to avoid the aforementioned disadvantages.

Underground irrigation, which changes the distribution of soil water at the root zone, substantially improves crop yield and irrigation water use efficiency (Khan *et al.* 2009). Knowledge of the irrigation depth is important for the design and management of underground irrigation system, thus delivering required amount of water to the plant. For winter wheat, the root distribution varied at different growth stages, but in practice, the irrigation depth was always maintained at a constant value (Zhang *et al*. 2011). It might cause the decrease of soil water availability in the deeper soil profile during the filling stage (Kirkegaard *et al*. 2007). Furthermore, using the water stable isotopes technology, Zhang *et al.* (2011), Sun *et al.* (2012), and Guo *et al.* (2016) reported that the main depth of root water uptake of irrigated winter wheat ranged from 20 to 180 cm during all growth stages, which mostly accounted for 40–90% of the depth of the winter wheat root distribution. Therefore, we hypothesized that determining the irrigation depth based on root distribution of winter wheat would be more reasonable than surface irrigation. Guo *et al.* (2016) found that deep underground irrigation, which irrigation depth applied at 90% of the depth of the winter wheat root distribution, could enhance yield and root growth compared to surface irrigation. However, observations of irrigation depth were limited to the root growth, which may not represent the whole plant characteristics. More research is needed to unravel its mechanism.

———

Received 14 April 2017, *accepted* 7 February 2018, *published as online-first* 21 June 2018.
+Corresponding author; e-mail: mjjsxty@163.com.
Abbreviations: Chl – chlorophyll; C_i – intercellular CO_2 concentration; D0 – water was irrigated on the land surface; D60, D75, and D90 – the same amount of water was irrigated from the surface to 60, 75, and 90% of the depth of the winter wheat root distribution; E – transpiration rate; g_s – stomatal conductance; GY –grain yield; LAI – leaf area index; P_N – net photosynthetic rate; RMD – root mass density; SWC – soil water content; WUE – water-use efficiency; $\Delta^{13}C$ – carbon isotope discrimination; ΔL – carbon isotope discrimination in leaf.
Acknowledgements: The research was supported by the National Natural Science Foundation of China (51579168) and the Program for Graduate Student Education and Innovation of Shanxi Province (2016BY065).

Extensive studies have been conducted to investigate the effects on photosynthesis in response to water stress (Liu *et al*. 2016, Luo *et al*. 2016). However, few published studies presented the physiological and biochemical responses of winter wheat to different irrigation depths based on root distribution. We hypothesized that there is optimum irrigation depth because the discovery of the optimum root–shoot ratio (Ma *et al*. 2009). Investigating the effects of different irrigation depths on photosynthesis and yield are important for proving this argument. The photosynthetic rate of leaves during the filling stage, which directly contributes to crop yield, is constrained by soil water availability in a deeper profile (Christmann *et al.* 2007, Ashraf and Harris 2013). In addition, carbon isotope discrimination ($\Delta^{13}C$) in leaf has been proposed as an indirect indicator of grain yield (GY) in wheat (Farquhar *et al*. 1989, Cao *et al*. 2009, Wahbi *et al*. 2011, Yasir *et al*. 2013). It was investigated whether a positive, negative, or no relationship between $\Delta^{13}C$ and GY existed (Monneveux *et al*. 2005, Elazab *et al*. 2015, Wang *et al*. 2016), and different coefficients were obtained to describe the same positive relationship. Thus, careful studies should be conducted before using $\Delta^{13}C$ as an indicator of GY under different conditions.

In this study, we measured the root distribution of winter wheat prior to each irrigation, then, four treatments with different irrigation depths were conducted in a pot experiment using PVC tubes (the same amount of water was applied on the land surface, and at 60, 75, and 90% of the depth of the winter wheat root distribution, denoted as D0, D60, D75 and D90, respectively). The objectives were to (*1*) investigate the effects of different irrigation depths on photosynthesis, dry mass, and $\Delta^{13}C$ during growth stages, and (*2*) find the optimum irrigation depth for improving the GY of winter wheat, as well as to study the relationship between $\Delta^{13}C$ and GY of winter wheat for different irrigation depths. The results of this study not only provide valuable information necessary for our further understanding of the control of water application at the plant–soil interface, but they also enhance the management capability for water-saving irrigation of winter wheat.

Materials and methods

Experimental site description: The experiment site was located in the southern region of Shanxi Province, China. The rainfall during the winter wheat growing season (October–May) was 177.8 mm in 2014–2015 and 212.6 mm in 2015–2016 (monthly rainfall data are shown in Fig. 1). The soil in the study region has been classified as loam and basic characteristics are shown in the table.

Soil depth [cm]	Bulk density [g cm^{-3}]	Field capacity [cm^3 cm^{-3}]	Soil texture composition [%] <0.002 mm	0.002–0.02 mm	0.02–2 mm	Organic matter [g kg^{-1}]
0-20	1.49	29.58	16.6	49.0	34.4	20.20
20-50	1.61	27.64	16.4	50.0	33.6	10.00
50-90	1.62	29.05	20.9	45.8	33.3	5.69
90-130	1.63	30.38	20.9	49.3	29.8	6.47
130-210	1.54	34.03	24.7	53.0	22.3	3.53
210-300	1.51	31.65	22.9	51.7	25.4	2.94

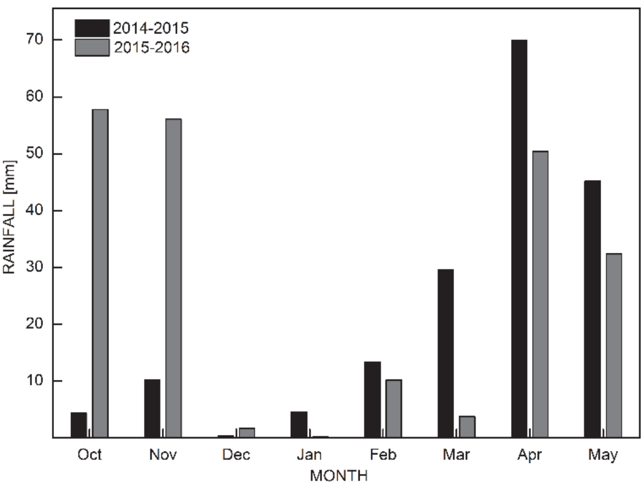

Fig. 1. The monthly rainfall during the winter wheat growth stage in 2014–2015 and 2015–2016.

The experiment was conducted using growth tubes during the winter wheat growing periods. The tubes consisted of PVC, with a depth of 3 m and an inner diameter of 18.6 cm. The tubes were sealed at the bottom *via* plastic film and were buried in soil. The top of each tube was leveled with the surrounding field of winter wheat. Tubes were filled with soil that was excavated from the study site. Soil samples were compacted every 5 cm with a rammer to ensure the required bulk density at the different soil layers. Before sowing, the field was fertilized with diammonium phosphate (75 kg km^{-2}). At the three-leaf stage, seedlings within each tube were thinned to three plants in 2014–2015 and twelve plants in 2015–2016.

Irrigation treatment: During the wintering stage in 2014, all winter wheat was irrigated on the soil surface with an amount of 67.5 mm, whereas no irrigation was provided in 2015 due to the larger rainfall during this stage. The experiment was conducted with four replicates for four irrigation depths during main growth stages (jointing, heading, and filling stages) of winter wheat, named D0 (irrigated on the land surface), D60, D75, and D90 (the same amount of irrigation from the surface to 60, 75, and 90% of the root distribution, respectively). According to the water requirement of winter wheat and the local standard practice (Guo *et al.* 2016), the amount of each irrigation was 67.5 mm and 75 mm in 2015 and 2016, respectively. The irrigation dates from the jointing to filling stages were 8 March, 11 April, 4 May, and 18 March, 18 April, 28 April, 9 May in 2016, respectively (Table 1S, *supplement available online*).

Prior to each irrigation, four growth tubes per each irrigation treatment were removed from the experiment site to sample the roots, and to determine the average maximum root depth and irrigation depth of different treatments. Then, water was added by infiltrating irrigation pipes at intervals of 20 or 30 cm (Fig. 1S, *supplement available online*). The number of irrigation pipes was chosen according to the results of a pilot experiment (Guo *et al.* 2016) and depths of different treatments. The irrigation amount for each infiltrating pipe was determined according to the following equation: $I = S \times D \times (\theta_t - \theta_n) \times \gamma$, where I [mL] represents the amount of each pipe, S [cm^2] represents the cross sectional area of growth tube (271.72 cm^2), D represents the thickness of soil profile (in this study it was 20 or 30 cm), γ [g cm^{-3}] represents the soil bulk density, θ_n represents the pre-irrigation soil water content in each measured soil profile, and θ_t represents the target relative soil water content, which was 85% of field capacity (Zhang *et al*. 2011), and the remaining water was applied at the surface to ensure the same total irrigation amount for different irrigation treatments.

Photosynthetic parameters: The net photosynthetic rate (P_N), transpiration rate (E), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i) of ten flag leaves per treatment were measured from 09:00 to 10:30 h at the early, mid, and final filling stages, using a *LI-COR 6400XT* portable photosynthesis system (*LI-COR, Inc*., Lincoln, Nebraska, USA) with natural light. After photosynthetic measurements, the Chl contents of the same leaves were analyzed using the method described by Wang *et al.* (2006). Then, the final values of P_N, E, g_s, C_i, and Chl were obtained *via* the average of all three measurements.

Leaf area index: Ten plants per tube were randomly chosen to calculate the leaf area (LA) at filling stage and the leaf area index (LAI) was calculated with the following equation: $LA = \sum(LL \times LW)/1.2$; $LAI = LA/S$, where LL represents the leaf length, LW represents the leaf width, and S represents the bottom area of the tube.

Carbon isotope discrimination ($\Delta^{13}C$) in leaf was measured in the flag leaf at the filling stage. Three identical healthy leaves for different irrigation depth treatments were sampled and dried at 70°C for 48 h to a constant mass, and grounded and sieved through an 80 mesh into a fine powder. The stable carbon isotope composition ($\delta^{13}C$) of different treatments was measured with an isotopic ratio mass spectrometer (*Delta V advantage*, *Thermo Fisher Scientific, Inc*., USA) interfaced with an element analyzer (*Flash EA1112 HT*, *Bremen, Germany*). Results were expressed as $\delta^{13}C$ [‰] = $[(R_{sample}/R_{reference}) - 1] \times 1{,}000$, $R = {}^{13}C/{}^{12}C$ in samples (R_{sample}) and in the standard PDB ($R_{reference}$, Pee Dee Belemnite) for carbon. The following formula was used to calculate the $\Delta^{13}C$ value (Farquhar *et al.* 1989):

$$\Delta^{13}C\ [‰] = \frac{\delta^{13}C_{air} - \delta^{13}C_{plant}}{1 - \frac{\delta^{13}C_{plant}}{1000}}$$

where $\delta^{13}C_{plant}$is the $\delta^{13}C$ of the sample and $\delta^{13}C_{air}$ is the $\delta^{13}C$ of the atmospheric CO_2. On the PDB scale, atmospheric CO_2 has a current deviation of approximately –8‰.

Dry mass: Every 10 cm of root samples were collected from the surface down to the 300 cm during the growth periods of winter wheat. Root mass density (RMD) refers to the unit volume of the soil root dry mass, which was determined *via* the following formula: RMD = M/V, where RMD [mg cm^{-3}] represents root mass density, M represents root dry mass, and V represents the volume of the soil sample. The proportion of each soil layer means the ratio of total root mass and root mass per soil layer. Plants in each tube were collected at maturity and separated into roots, leaves, stems, and spikes to estimate the dry mass of winter wheat. These samples were dried at 70°C until they reached a constant mass. Prior to harvest, the spike lengths, spike numbers, and number of kernel per spike were counted.

Soil water content and water-use efficiency: Weekly, both before and after irrigation, soil moisture content were measured using a time domain reflectometry with intelligent micro elements (*TRIME, IMKO*, Ettlingen, Germany). The measurements were made in 10-cm increments at depths of 0–160 cm and 0–300 cm in 2014–2015 and 2015–2016, respectively. The water consumption was calculated *via* the soil water balance equation: ET = 1 + P + K + ∆ S, where, ET [mm] represents the water consumption of winter wheat; I [mm] represents the irrigation amount; P [mm] represents the precipitation amount; ∆S [mm] represents the change of soil water storage, calculated as the difference of soil water content between the seeding and maturity stages for the 0–300 cm soil profile, and soil water content was determined using the oven-drying method; and K [mm] represents the amount supplied by underground water. In this study, the bottom of the PVC pipe blocked the underground water supply. Therefore, K was neglected when calculating ET. Water-use efficiency (WUE) [kg ha^{-2} mm^{-1}] was defined as: WUE = Y/ET, where, Y [kg ha^{-1}] represents the yield of winter wheat. Means were calculated for four replicates of the different treatments.

Statistical analysis: Analysis of variance (*ANOVA*) and correlation were calculated using *SPSS 17* software. One of the preconditions of *ANOVA* is the normality of para meters. The normality test was conducted by quantile – quantile (Q – Q) plots in *SPSS 17*. Logarithmic transformation was required if its distribution was not normal. Least significant difference (LSD) test was used to compare means and differences were considered significant at a level of $P<0.05$.

Results

Soil water content and root distribution at different soil profiles: the soil water content from jointing to maturity stages displayed significant differences between the various irrigation treatments (Fig. 2). During the growth stage in the 0–20 cm soil profiles, the SWC of D0 was maximal and the SWC of D75 was significantly higher than that of D90, but lower than that of D60 after the heading stage. However, in the 20–50 cm and 50–90 cm soil layers, the SWC of D0 were the lowest, and the variation range of SWC for D75 was the highest during the filling stage. In the 90–130 cm and below 130 cm soil profiles, the SWC of D0 decreased and tended to constant level during the growth stages, while the SWC from underground irrigation treatments still changed and sharply decreased after the filling stage. The SWCs of those layers for D60 and D75 were significantly lower than that of D0 in the 90–130 cm soil profiles at maturity.

The root mass density of winter wheat in four growth periods generally declined exponentially with the increasing soil depth and peaked at the heading stage (Fig. 3). Compared to the D0 treatment, underground irrigation treatments had a lower RMD and proportion of 0–20 cm, and the value of the D75 treatment was significantly higher than for the D90 treatment but lower than that from D60 during growth stages. However, the root mass density and the proportion below 50 cm were significantly higher under underground irrigation treatments. The total proportions of 0–20 cm soil layer were lower than those of 20–300 cm under D75 and D90 treatments during filling and maturity.

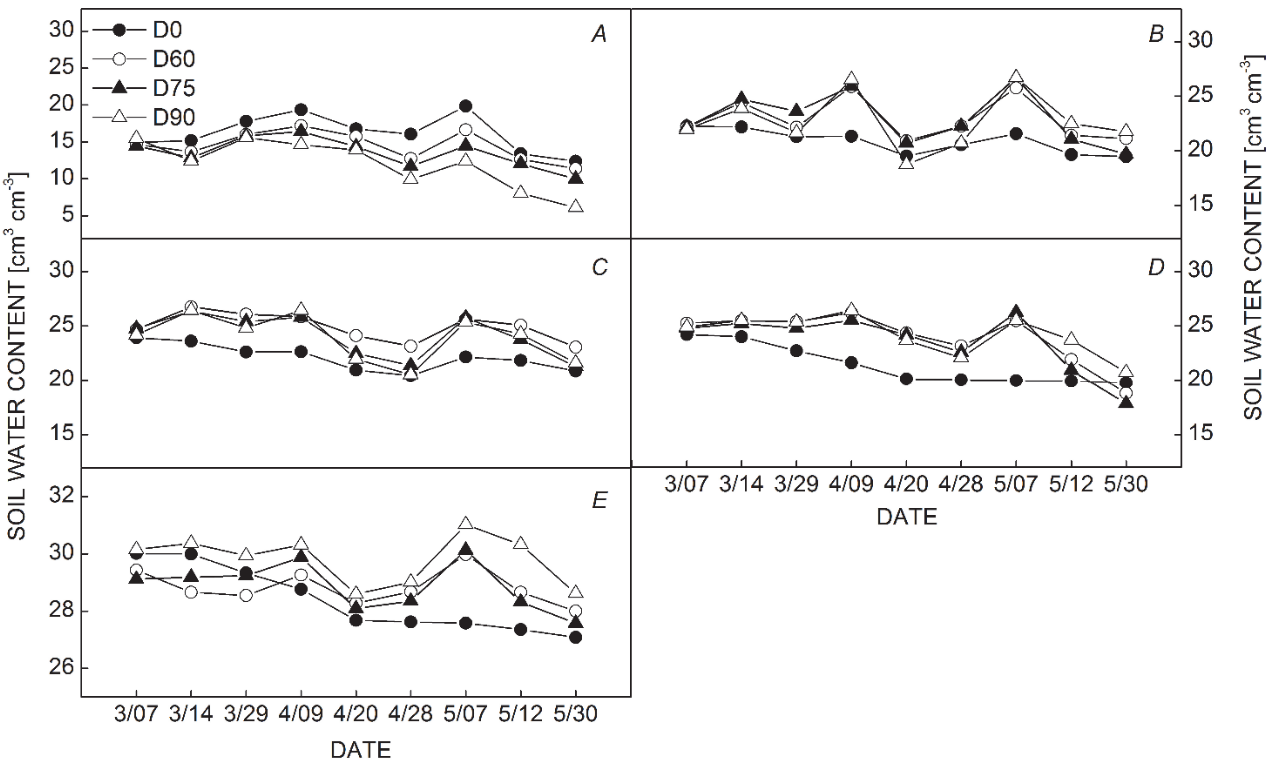

Fig. 2. Soil water content variation at 0–20 cm (*A*), 20–50 cm (*B*), 50–90 cm (*C*), 90–130 cm (*D*), and >130 cm (*E*) from filling to maturity stages in 2014-2015. D0 – surface irrigation; D60, D75, and D90 – the same amount of water was applied at 60, 75, and 90% of the depth of the root distribution.

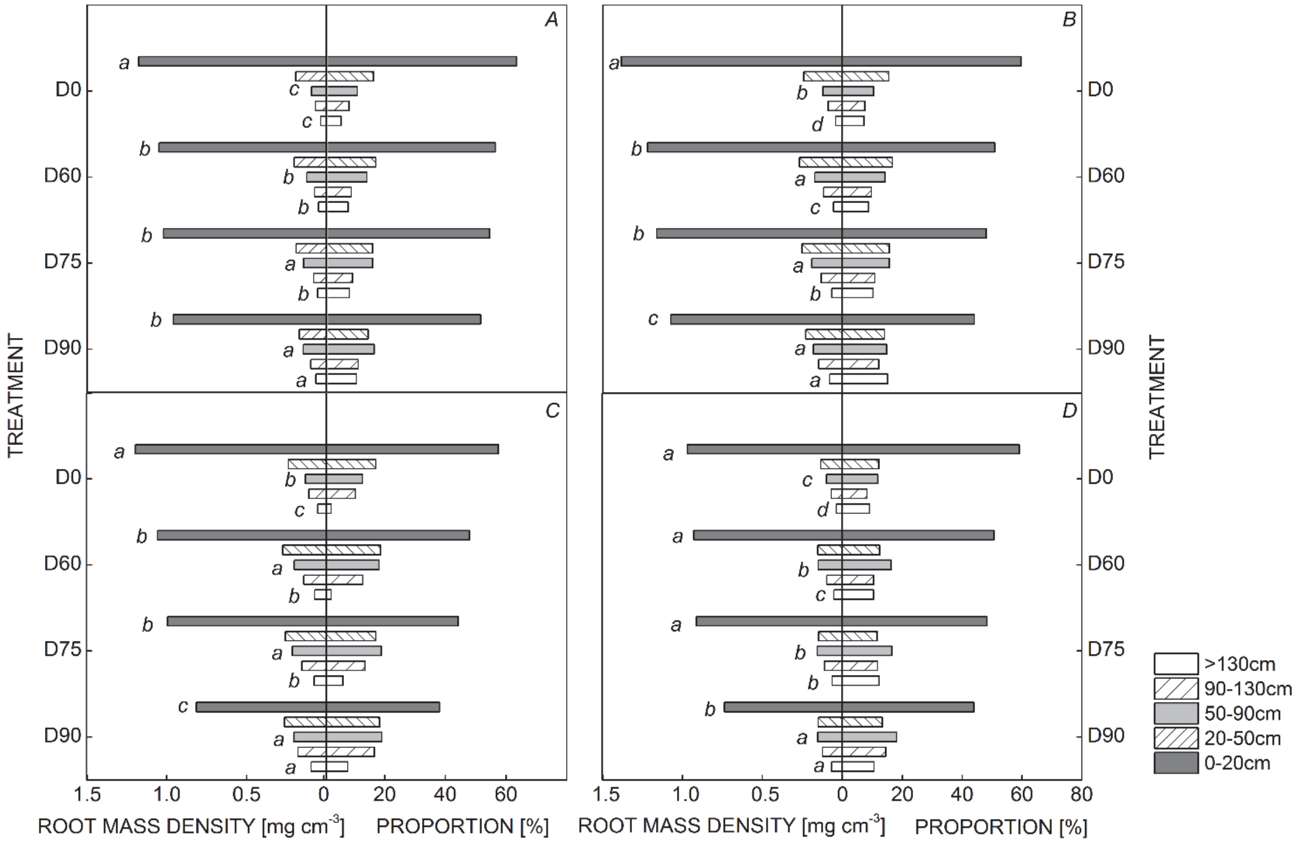

Fig. 3. Distribution of winter wheat root mass density and proportion under different irrigation treatments during the jointing (*A*), heading (*B*), filling (*C*), and maturity (*D*) stages in 2015. D0 – surface irrigation; D60, D75, and D90 – the same amount of water was applied at 60, 75, and 90% of the depth of the root distribution. The *different letters* in the figure are statistically different at $P<0.05$ by LSD test.

Leaf area index and photosynthetic parameters: As shown in Fig. 4*A*, the leaf area index (LAI) for the D60 treatment was significantly higher than that for D0 treatment, but lesser than that for D75 treatment at the filling stage. No significant LAI difference was observed between D60 and D90 treatments during both growth seasons. When comparing the leaf chlorophyll (Chl) content for different treatments (Fig. 4*B*), the value for the D75 treatment was significantly higher than those for D60 and D90 treatments in 2014–2015, while the values for D75 and D60 treatments were similar but significantly higher than those for D90 treatment in 2015–2016. The lowest Chl was obtained for the D0 treatment during both growing seasons.

The average net photosynthetic rate (P_N), transpiration rate (E), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i) values of winter wheat at the filling stage are presented in Fig. 4*C–F*. The maximum P_N value was observed under the D75 treatment, followed by D60 and D90 treatments, and the value for D0 treatment showed minima in 2014–2015 and 2015–2016 (Fig. 4*C*). Compared to the D0 treatment, g_s and E values increased for the underground irrigation treatments, and there was no significant difference between D60, D75, and D90 in 2014–2015, but at the 2015–2016 growing season, the D75 and D60 treatments resulted in significantly higher g_s and E values compared to other irrigation treatments (Fig. 4*D,E*). Furthermore, compared to the C_i value observed under the D0 treatment, the values for underground irrigation treatments increased but these differences were insignificant in 2015–2016.

Dry mass distribution at maturity: Fig. 5 shows the dry mass distribution in various plant organs at the maturity in 2015 and 2016. The total dry mass of winter wheat increased markedly under underground irrigation treatments compared to that for the D0 treatment for both growing seasons. The greatest mass of the spike was obtained for the D75 treatment in 2015 and for the D60 treatment in 2016. Compared to the lowest spike mass for D0, the values for D75 and D65 increased by 26.5 and 24.4%, respectively. At maturity, the highest dry mass of leaves was found for the D0 treatment, followed by D60 and D75 treatments without significant difference, and the value for D90 was the lowest during both growing seasons. However, dry mass in stems plus sheaths combined, and in roots did not significantly differ between D60 and D75 treatments in 2015; however, the value for D75 was significantly greater than those for D60 treatment in 2016. The lowest values were found for D0 treatment during both growing seasons.

GY values and components of winter wheat under different irrigation treatments are shown in Table 1. The highest GY value was obtained for D75, followed by D60 and D90, while the lowest was observed in D0 at maturity for both growing seasons. The number of kernels per spike and spike length of D60 and D75 were significantly higher than those of D90 and D0. Water-use efficiency (WUE) increased with increasing irrigation depth, and the highest value was observed for the D75 treatment in 2014–2015 and 2015–2016. However, there was no significant difference in the thousand kernel mass between different irrigation treatments.

Carbon isotope discrimination in leaf (ΔL) and relationships between ΔL and GY: Significant differences were found between the different irrigation treatments for carbon isotope discrimination in leaf (ΔL) at the filling stage, which ranged from 18.78 to 19.89‰ (Fig. 4*F*). The ΔL value increased for the deeper irrigation

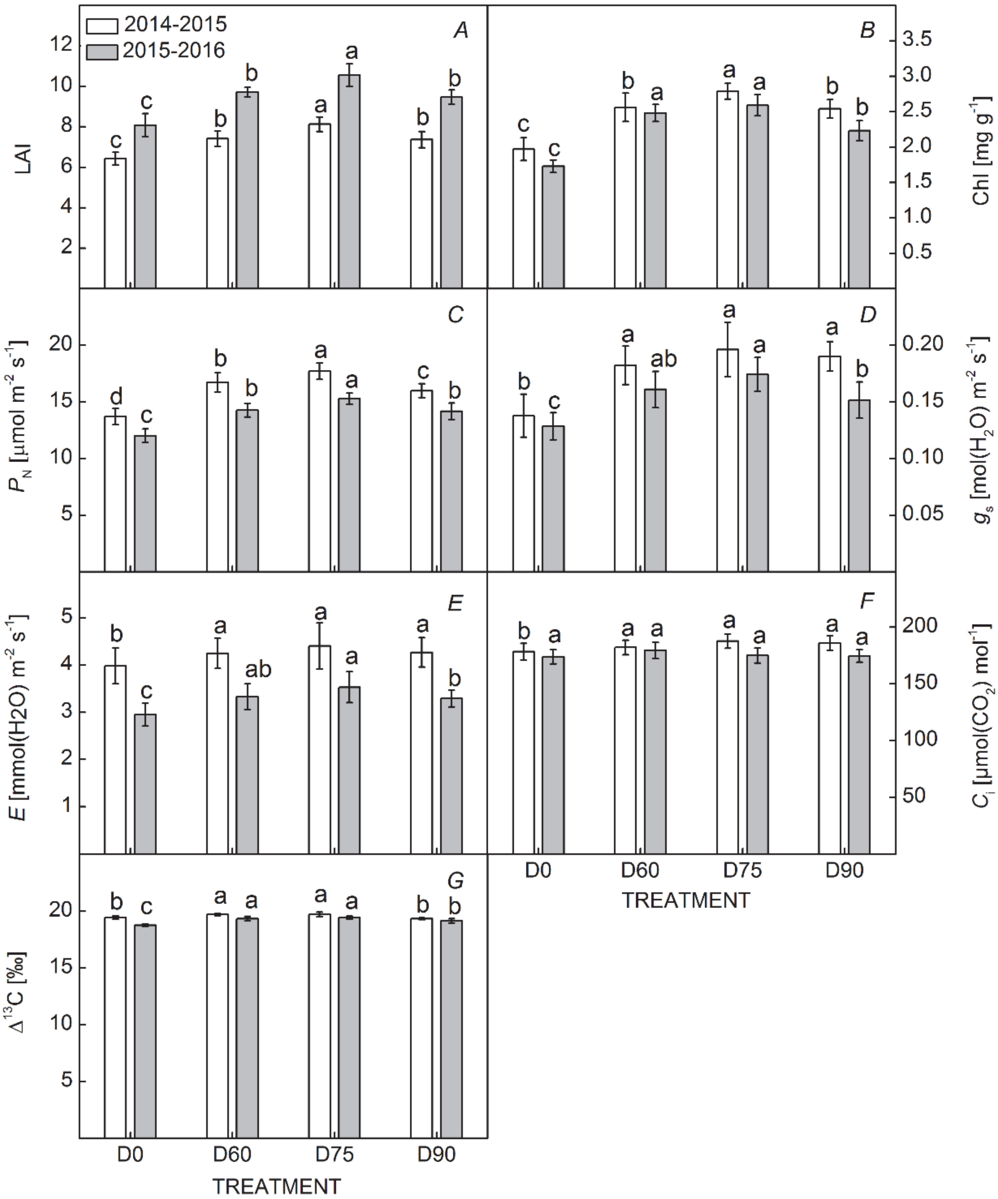

Fig. 4. Leaf area index (*A*), photosynthetic parameters (*B,C,D,E,F*), and carbon isotope discrimination (*G*) at the grain-filling stage in 2014–2015 and 2015–2016. Chl – chlorophyll; C_i – intercellular CO_2 concentration; D0 –surface irrigation; D60, D75, and D90 – the same amount of water was applied at 60, 75, and 90% of the depth of the root distribution. *E* – transpiration rate; g_s – stomatal conductance; LAI – leaf area index; P_N – net photosynthetic rate; WUE – water-use efficiency; $\Delta^{13}C$ – carbon isotope discrimination. The *different letters* in the figure are statistically different at $P<0.05$ by LSD test. *Vertical bars* represent the standard error.

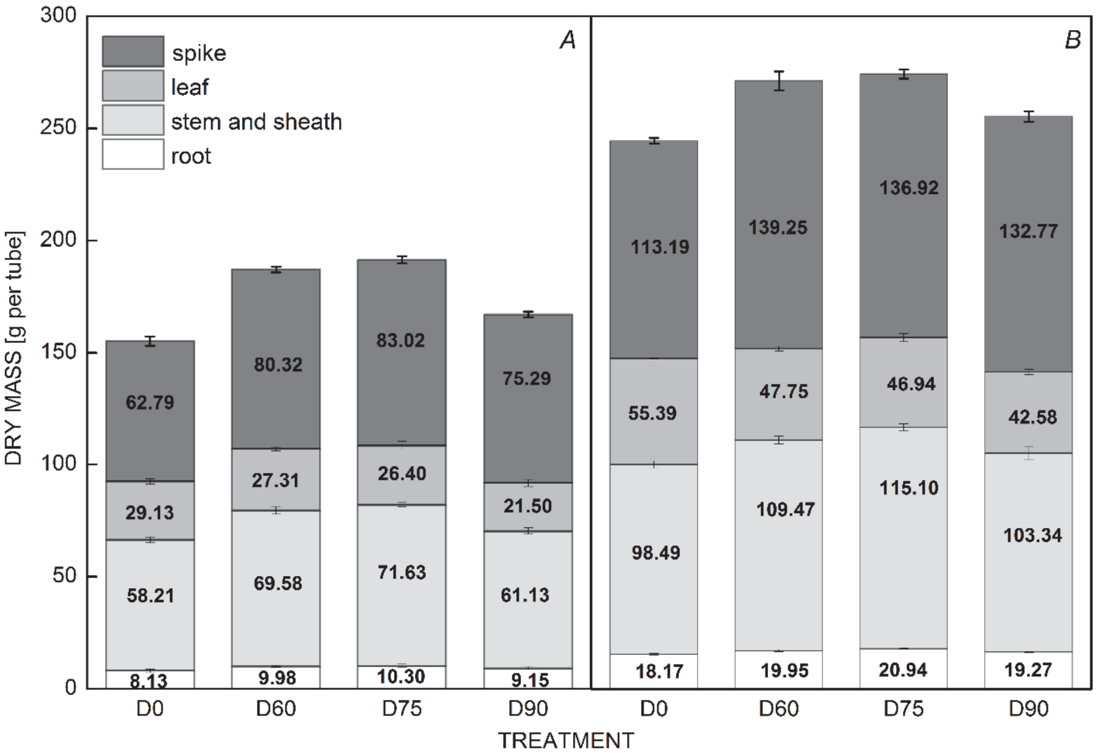

Fig. 5. Dry mass distribution in different organs of winter wheat at maturity for different treatments in 2014–2015 (*A*) and 2015–2016 (*B*). D0 – surface irrigation; D60, D75, and D90 – the same amount of water was applied at 60, 75, and 90% of the depth of the root distribution. *Vertical bars* represent the standard error.

Table 1. Grain yield and components of winter wheat under different irrigation treatments. D0 – surface irrigation; D60, D75, and D90 – the same amount of water was applied at 60, 75, and 90% of the depth of the root distribution; WUE – water-use efficiency. The *different letters* in the figure are statistically different at P<0.05 by LSD test.

Irrigation treatments	Spike length [cm]	Spike number per tube	Kernels per spike	Thousand kernel mass [g]	Grain yield [kg ha^{-2}]	WUE [kg ha^{-2} mm^{-1}]
2014–2015						
D0	7.0^{b}	29^{b}	40.08^{b}	42.66^{a}	6,259.98^{c}	11.27^{c}
D60	7.7^{a}	32^{a}	45.61^{a}	43.54^{a}	8,026.53^{ab}	15.38^{a}
D75	7.8^{a}	33^{a}	45.98^{a}	44.01^{a}	8,434.60^{a}	15.52^{a}
D90	7.3^{b}	35^{a}	41.00^{b}	40.75^{a}	7,566.15^{b}	14.23^{b}
2015–2016						
D0	7.3^{b}	46^{b}	39.68^{b}	47.82^{a}	6,540.04^{c}	11.98^{c}
D60	7.6^{a}	52^{a}	41.30^{a}	51.21^{a}	8,240.99^{a}	16.10^{a}
D75	7.7^{a}	51^{a}	42.17^{a}	52.70^{a}	8,492.76^{a}	15.96^{a}
D90	7.4^{b}	52^{a}	39.82^{b}	49.32^{a}	7,650.53^{b}	14.80^{b}

depth treatments compared to the surface irrigation treatment (D0). The highest ΔL value was found for D75, and the value of D60 was slightly lower than that of D75. There was no marked difference in ΔL between D90 and D0 in 2014–2015, however, the value of D90 treatment was significantly higher than that of D0 in 2015–2016.

Correlation analysis showed a positive relationship between ΔL and GY (r = 0.65), however, this correlation was not significant. GY was closely correlated to spike length (r = 0.92, P<0.01), spike number (r = 0.67, P<0.01), and number of kernels per spike (r = 0.94, P<0.05) for the different irrigation treatments. However, both thousand kernel mass and GY did not exhibit a strong positive correlation (r = 0.39). Thus, yield values were greatly determined by the growth in spike length and the number of kernels per spike.

Discussion

Soil water conditions in an irrigated root zone can affect the root structure and function of crops (Zegada-Lizarazu *et al.* 2005). The roots of winter wheat increase markedly at the jointing stage (Zuo *et al*. 2013). During the growing stage, underground irrigation treatment created a suitable soil water environment (the mean SWC of surface root zone (0–20 cm) and deep root zone (below 20 cm) maintained at 41–50% and 77–87% of field capacity), which favored root elongation and root water uptake in the deep soil profile (Guo *et al.* 2016). In this study, the variation of soil water content at different soil profiles indicated that plants under underground irrigation treatment could acquire more water from the deep soil (Fig. 2). Furthermore, the availability of soil water in deeper soil profile was significantly improved by the root mass of the deep soil layers (Xu *et al*. 2016), which was indispensable to improve crop yield (Martínez and Reca 2014). RMDs are widely used to measure the physiological functioning of crop roots (Wu *et al*. 2017). Compared to the D0 treatment, the RMD from the underground irrigation treatment decreased at the surface soil profile and increased in the deep soil depths; however, the maximum total root mass was obtained for the D75 at maturity. The larger root mass and more available soil water would be helpful for leaf growth and dry mass production (McDonald *et al.* 2006). Wang *et al*. (2003) suggested that with an increase in the vertical distribution of roots, the rate of decrease in Chl values in flag leaves slowed down. The highest LAI and Chl values in this study were obtained for the D75 treatment (Fig.4). Similar result was reported by Vieira *et al*. (2014). The higher LAI indicates higher photosynthetic capacity (Houborg *et al*. 2008, Liatukas *et al*. 2009) and the higher Chl values increased the amount of photosynthesis production during the filling stage (Plénet *et al*. 2000, Wu *et al*. 2008). In this study, the P_N value for D75 was higher than those in D60 and D90, and increased by 29.2 and 27.2%, respectively, compared to the D0 treatment. The g_s was directly related to the P_N (Zhu *et al*. 2016); however, no significant difference was found between D60, D75, and D90 treatments during both growing stages, while C_i value difference between D0 and D75 was insignificant in 2015–2016. These results indicate that nonstomatal effects inhibited the photosynthetic activity under underground irrigation treatment (Flexas *et al*. 2012, Liu *et al*. 2016).

In this study, the highest GY values and WUE were obtained for the D75 treatment, which increased by 34.7 and 37.8%, respectively, compared to surface irrigation (D0). Guo *et al*. (2016) also reported that higher yield and WUE were obtained by underground irrigation treatment. However, GY and WUE could not be improved *via* an increase in irrigation depth (Guo *et al*. 2014, Man *et al*. 2015). These values decreased by 10.3 and 8.3% for D90 compared to D75, respectively. Therefore, the irrigation depth might exhibit a certain threshold for these values, which decreased in the case of D90. This result might occur due to the following factors. (*1*) Underground irrigation is helpful for improving the root growth of winter wheat by increasing soil moisture at the deep root zone (Kirkegaard *et al*. 2007); however, roots require twice as much assimilates to produce one unit of dry mass compared to shoots (Passioura *et al*. 1983). Consequently, excessive root growth might consume a larger amount of soil water and more assimilates, which leads to smaller GY and WUE values (Ma *et al*. 2009). In this study, the RMD peaked at the heading stage, and the maximum total root mass and total water consumption amount were obtained for the D90 during the heading stage, which increased by 9.3 and 10.3%, respectively, compared to D0. However, at maturity, the total dry mass of shoot from D90 decreased by 7.2 and 14.5%, respectively, compared to D75. (*2*) Lower irrigation amounts applied to the top soil surface would reduce soil evaporation (Zhang *et al*. 2008) and increase water consumption in deep soil, but nutrients such as nitrogen and phosphorus are always distributed at the surface. Although hydraulic lift has been found to occur in winter wheat, the uneven spatial distribution of nutrients and water might affect the plant productivity (Shen *et al*. 2013). (*3*) Studies have highlighted that the depth of winter wheat root water uptake was 0–40 cm at the maturity stage (Zhang *et al*. 2011, Guo *et al*. 2016). However, the decreased RMD of surface soil profile from D90 was the highest one. Therefore, excessive underground irrigation depth would result in water deficiency in the main root uptake zone during the later growth stage.

The irrigation depth based on the 75% of root distribution was the optimum in this soil texture. In this study, hierarchical irrigation *via* infiltrating pipe was applied to achieve the wetted depth of the 75% of root distribution, with the purpose of avoiding the effect on the root growth of winter wheat in growth tubes. Then, in the field, underground irrigation system, such as optimized subsurface irrigation (Gunarathna *et al*. 2017) and alternative subsurface drip system (Martínez and Reca 2014), might be feasible to regulate the irrigation depth. However, the root distribution of winter wheat would be affected by soil texture and cultivars. The optimizing irrigation depth in different zone might not be the same; the study on this aspect needs to be strengthened in the future.

Many studies have demonstrated the relationship between $\Delta^{13}C$ and the GY value for various genotypes, crop tissues, sampling times, and environmental factors for winter wheat (Araus *et al*. 2003, Reynolds *et al*. 2007, Wahbi *et al*. 2011, Yasir *et al*. 2013, Wang *et al*. 2016), as well as under different soil water conditions such as irrigation amounts and methods (Elazab *et al*. 2015). However, little information exists about $\Delta^{13}C$ and GY for different irrigation depths based on root distribution. In this study, a positive (but not significant) correlation was found between ΔL and GY. A possible reason could be a correlation between ΔL and GY. Firstly, based on the physiological mechanism of $\Delta^{13}C$ and plant photosynthesis (Farquhar *et al.* 1989), g_s values were higher in response to higher irrigation depth compared to surface irrigation, and therefore would result in an increasing in $\Delta^{13}C$, while an increase in photosynthetic capacity would decrease the $\Delta^{13}C$ by decreasing C_i values, thus reducing the correlation between $\Delta^{13}C$ and GY. Secondly, a positive linear association between $\Delta^{13}C$ and soil water content has been pointed out (Wei *et al.* 2015). However, for GY values of winter wheat, the highest yield would not always be obtained with the highest soil water content (Zhang *et al.* 2008). Thirdly, high $\Delta^{13}C$ values in plant tissue might be due to the accumulation in early growth period (Yasir *et al.* 2013). Finally, some studies have highlighted that different irrigation treatments introduce different correlation between $\Delta^{13}C$ and GY (Monneveux *et al.* 2005). Future studies would benefit from investigating the different relationship for each irrigation depth.

Conclusion: Physiological characteristics of winter wheat can be important for increasing photosynthesis. These characteristics are affected by different irrigation depths based on the measurement of root distribution prior to irrigating at critical stages of water requirement. Compared to surface irrigation, the LAI, Chl content, photosynthetic parameters, GY, and WUE could be improved at underground irrigation depths. The present experimental shows that the optimum irrigation depth of D75 may be useful for improving GY while maintaining a relatively extensive root development and irrigation WUE. The positive, but not significant, relationship between $\Delta^{13}C$ and GY revealed that $\Delta^{13}C$ should be used cautiously as an indicator for GY of winter wheat at different irrigation depths.

References

Ashraf M., Harris P.J.C.: Photosynthesis under stressful environments: An overview. – Photosynthetica **51**: 163-190, 2013.

Araus J.L., Villegas D., Aparicio N. *et al.*: Environmental factors determining carbon isotope discrimination and yield in durum wheat under mediterranean conditions. – Crop Sci. **43**: 170-180, 2003.

Cao S.K., Feng Q., Si J.H. *et al.*: Relationships between foliar carbon isotope discrimination with potassium concentration and ash content of the riparian plants in the extreme arid region of china. – Photosynthetica **47**: 499-509, 2009.

Christmann A., Weiler E.W., Steudle E. *et al.*: A hydraulic signal in root-to-shoot signalling of water shortage. – Plant J. **52**: 167-174, 2007.

Elazab A., Bort J., Zhou B.W. *et al.*: The combined use of vegetation indices and stable isotopes to predict durum wheat grain yield under contrasting water conditions. – Agr.Water Manage. **158**: 196-208, 2015.

Farquhar G.D., Ehleringer J.R., Hubick K.T.: Carbon isotope discrimination and photosynthesis. – Annu. Rev. Plant Phys. **40**: 503-537, 1989.

Flexas J., Barbour M.M., Brendel O. *et al.*: Mesophyll diffusion conductance to CO_2: an unappreciated central player in photosynthesis. – Plant Sci. **193**: 70-84, 2012.

Gunarathna M., Sakai K., Nakandakari T. *et al.*: Optimized subsurface irrigation system (opsis): beyond traditional subsurface irrigation. – Water **9**: 599, 2017.

Guo F., Ma J.J., Zheng L.J. *et al.*: Estimating distribution of water uptake with depth of winter wheat by hydrogen and oxygen stable isotopes under different irrigation depths. – J. Integr. Agr. **15**: 891-906, 2016.

Guo Z.J., Yu Z.W., Wang D. *et al.*: Photosynthesis and winter wheat yield responses to supplemental irrigation based on measurement of water content in various soil layers. – Field Crop. Res. **166**: 102-111, 2014.

Houborg R., Boegh E.: Mapping leaf chlorophyll and leaf area index using inverse and forward canopy reflectance modeling and spot reflectance data. – Remote Sens. Environ. **112**: 186-202, 2008.

Khan M.J., Razzaq A., Khattak M.K. *et al.*: Effect of different pre-sowing water application depths on wheat yield under spate irrigation in Dera Ismael Khan District of Pakistan. – Agr.Water Manage. **96**: 1467-1474, 2009.

Kirkegaard J.A., Lilley J.M., Howe G.N. *et al.*: Impact of subsoil water use on wheat yield. – Aust. J. Agri. Res. **58**: 303-315, 2007.

Liatukas Ž., Ronis A., Ruzgas V.: The suitability of leaf area index for the selection of winter wheat (*Triticum aestivum* L.) breeding lines. – Zemdirbyste **96**: 3-15, 2009.

Liu E.K., Mei X.R., Yan C.R. *et al.*: Effects of water stress on photosynthetic characteristics, dry mass translocation and WUE in two winter wheat genotypes. – Agr.Water Manage. **167**: 75-85, 2016.

Luo H.H., Zhang Y.L., Zhang W.F.: Effects of water stress and rewatering on photosynthesis, root activity, and yield of cotton with drip irrigation under mulch. – Photosynthetica **54**: 65-73, 2016.

Ma S.C., Li F.M., Xu B.C. *et al.*: Effects of root pruning on the growth and water use efficiency of winter wheat. – Plant Growth Regul. **57**: 233-241, 2009.

Man J.G., Shi Y., Yu Z.W. *et al.*: Dry mass production, photosynthesis of flag leaves and water use in winter wheat are affected by supplemental irrigation in the huang-huai-hai plain of china. – PLoS ONE **10**: e137274, 2015.

Marek G., Gowda P., Marek T. *et al.*: Estimating preseason irrigation losses by characterizing evaporation of effective precipitation under bare soil conditions using large weighing lysimeters. – Agr.Water Manage. **169**: 115-128, 2016.

Martínez J., Reca J.: Water use efficiency of surface drip irrigation versus an alternative subsurface drip irrigation method. – J. Irrig. Drain. Eng. **140**: 1-8, 2014.

McDonald A.J., Riha S.J., Duxbury J.M. *et al.*: Wheat responses to novel rice cultural practices and soil moisture conditions in the rice – wheat rotation of nepal. – Field Crop. Res. **98**:116-126, 2006.

Monneveux P., Reynolds M.P., Trethowan R. *et al.*: Relationship between grain yield and carbon isotope discrimination in bread wheat under four water regimes. – Eur. J. Agron. **22**: 231-242, 2005.

Passioura J.B.: Roots and drought resistance. – Agr.Water Manage. **7**: 265-280, 1983.

Plénet D., Etchebest S., Mollier A. *et al.*: Growth analysis of maize field crops under phosphorus deficiency. – Plant Soil

223: 119-132, 2000.
Reynolds M., Dreccer F., Trethowan R.: Drought-adaptive traits derived from wheat wild relatives and landraces. – J. Exp. Bot. **58**: 177-186, 2007.
Shen Y.F., Li S.Q., Shao M.A.: Effects of spatial coupling of water and fertilizer applications on root growth characteristics and water use of winter wheat. – J. Plant Nutr. **36**: 515-528, 2013.
Sun N.X.: Study on Field Water Transformation Using Isotopes: a Case of Daxing District. Pp 57-70. China University of Geosciences, Beijing 2012.
Vieira G.H.S., Mantovani E.C., Sediyama G.C. *et al*.: Morpho-physiological indicators of water stress on sugarcane as a function of irrigation depths. – Biosci. J. **30**: 65-75, 2014.
Wahbi A., Shaaban A.S.A.: Relationship between carbon isotope discrimination (Δ), yield and water use efficiency of durum wheat in Northern Syria. – Agr. Water Manage. **98**: 1856-1866, 2011.
Wang F.H., Wang X.Q., Li S.J. *et al*.: [Effect of vertical distribution of root on senescence of flag leaf and partition of dry mass in high yielding winter wheat.] – J. Triticeae Crop **23**: 53-57, 2003.[In Chinese]
Wang X.K.: The Principle and Technology of Plant Physiology and Biochemistry Experiment. Pp. 130-134, 202, 280. Higher Education Press, Beijing 2006.
Wang Y.Z., Zhang X.Y., Zhang X.Y. *et al.*: Soil water regime affecting correlation of carbon isotope discrimination with yield and water-use efficiency of winter wheat. – Crop Sci. **56**: 760-772, 2016.
Wei J., Liu W.G., Cheng J.M. *et al*.: $\delta^{13}C$ values of plants as indicators of soil water content in modern ecosystems of the chinese loess plateau. – Ecol. Eng. **77**: 51-59, 2015.
Wu C.Y., Niu Z., Tang Q. *et al*.: Estimating chlorophyll content from hyperspectral vegetation indices: Modeling and validation. – Agr. Forest Meteorol. **148**: 1230-1241, 2008.
Wu Y., Wang H.Z., Yang X.W. *et al.*: Soil water effect on root activity, root weight density, and grain yield in winter wheat. – Crop Sci. **57**: 437-443, 2017.
Xu C.L., Tao H.B., Tian B.J. *et al*.: Limited-irrigaiton improves water use efficiency and soil reservior capacity through regulating root and canopy growth of winter wheat. – Field Crop Res. **196**: 268-275, 2016.
Yang B., Wen X.F., Sun X.M.: Irrigation depth far exceeds water uptake depth in an oasis cropland in the middle reaches of heihe river basin. – Sci. Rep. **5**: 1-12, 2015.
Yasir T.A., Min D., Chen X. *et al*.: The association of carbon isotope discrimination (Δ) with gas exchange parameters and yield traits in chinese bread wheat cultivars under two water regimes. – Agr. Water Manage. **119**: 111-120, 2013.
Zegada-Lizarazu W., Iijima M.: Deep root water uptake ability and water use efficiency of pearl millet in comparison to other millet species. – Plant Prod. Sci. **8**: 454-460, 2005.
Zhang X.Y., Chen S.Y., Sun H.Y. *et al*.: Dry mass, harvest index, grain yield and water use efficiency as affected by water supply in winter wheat. – Irrig. Sci. **27**: 1-10, 2008.
Zhang Y.C., Shen Y.J., Sun H.Y. *et al*.: Evapotranspiration and its partitioning in an irrigated winter wheat field: a combined isotopic and micrometeorologic approach. – J. Hydrol. **408**: 203-211, 2011.
Zhu M., Li F.H., Shi Z.S.: Morphological and photosynthetic response of waxy corn inbred line to waterlogging. – Photosynthetica **54**: 636-640, 2016.
Zuo Q., Zhang R.D., Shi J.C.: Characterization of the root lenth density distribution of wheat using a generalized function. – In:Timlin D., Ahuja L.R. (ed.): Enhancing Understanding and Quantification of Soil-Root Growth Interactions. Pp.93-117. American Society of Agronomy, Madison 2013.