DOI: 10.1007/s11099-016-0659-y PHOTOSYNTHETICA 55 (3): 454-466, 2017

Photosynthetic response of tetraploid and hexaploid wheat to water stress

Y.P. LI[*], Y.Y. LI[*], D.Y. LI[*], S.W. WANG[**], and S.Q. ZHANG[*,**,+]

State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau, Northwest A&F University, Yangling, Shaanxi 712100, China[*]
State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau, Institute of Soil and Water Conservation, Chinese Academy of Sciences and Ministry of Water Resources, Yangling, Shaanxi 712100, China[**]

Abstract

Photosynthetic characteristics of ear and flag leaves of wheat species, tetraploid *Triticum dicoccoides* Kom and hexaploid Bima1, were studied in plants grown under well-watered (WW) and water-stressed (WS) conditions. Compared to ears, flag leaves exhibited higher photosynthetic rate (P_N) at the filling stage, but more severe decrease under WS. P_N in the tetraploid wheat ear remained higher than that in the hexaploid wheat during the grain-filling stage. Water stress decreased P_N in both the organs; this decline was caused by a reduction in Rubisco activity, not by drought-induced stomatal limitation. Tetraploid wheat ears exhibited higher relative water content and water-use efficiency than that of hexaploid wheat, under WS. The change in phospho*enol*pyruvate carboxylase activity and carbon isotope composition indicated the absence of C_4 metabolism in the ears of both species under both conditions. The improved performance of the tetraploid wheat ears under WS was associated with better water relations.

Additional key words: carbon isotope; ear photosynthesis; enzyme activity; water relations.

Introduction

Wheat is one of the most important cereal crops worldwide. Drought stress is a major limiting factor in wheat production in arid and semi-arid areas (Araus *et al*. 2002). It is important to understand the mechanisms through which plants adapt to water-limited conditions and select wheat genotypes better suited to drought (Mingo *et al*. 2003, Araus 2008).

Photosynthesis plays a pivotal role in plants with leaves being key organs for photosynthesis in plants. However, it is now generally accepted that non-leaf organs may contribute to the grain yield (Blum 1985, Simmons 1987, Araus *et al*. 1993, Hetherington *et al*. 1998, Abbad *et al*. 2004, Tambussi *et al*. 2005). There are many reports of significant contributions of photosynthesis in green flowers or other photosynthetic parts to the total carbon and energy costs of reproduction (Bazzaz *et al.* 1979, Marcelis and Hofman-Eijer *et al.* 1995, Lytovchenko *et al.* 2011, Sawicki *et al.* 2016). Not only herbaceous or gramineous plants, also woody species contain chlorophyll (Chl) in several plant parts (Pfanz and Aschan 2001, Pfanz *et al.* 2002). Thus the photosynthesis of green tissues other than leaf mesophyll pay for their own carbon demands and contribute to the overall carbon gain of plants (Aschan and Pfanz 2003). In wheat, the ear has been suggested to be an important green organ for drought tolerance and to contribute a considerable portion of grain mass (Evans and Rawson 1970, Ram and Singh 1982, Wang *et al*. 2001, Reynolds *et al.* 2005). In particular, during drought, ear may be the main photosynthetic contributor to grain filling (Evans *et al*. 1972, Bort *et al*. 1994, Sánchez-Díaz *et al*. 2002). The contribution to assimilation made by ear photosynthesis ranges from 10 to 44%, depending on the environmental conditions and genotypes (Kriedemann *et al*. 1966). However, the mechanistic basis of the performance of photosynthesis in ears is not completely understood.

Received 18 January 2016, *accepted* 19 August 2016, *published as online-first* 19 September 2016.
[+]Corresponding author; phone: +86 29 87010897, e-mail: sqzhang@ms.iswc.ac.cn
Abbreviations: C_i – intercellular CO_2 concentration; C_o – ambient CO_2 concentration; Car – carotenoids; Chl – chloroplast; DAA – days after anthesis; E – transpiration rate; g_s – stomatal conductance; HS – heading stage; L_s – stomatal limitation; MDH – malate dehydrogenase; PEPC – phospho*enol*pyruvate carboxylase; P_N – net photosynthetic rate; RWC – relative water content; RuBP – ribulose-1,5- bisphosphate; WUE – water-use efficiency; WS – water-stressed; WW – well-watered.
Acknowledgement: This work was supported by the National Science and Technology Supporting Programs (2015BAD22B01), the 111 project of the Chinese Education Ministry (B12007), and Special Funds of Scientific Research Programs of State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau **(**A314021403-C5**).**

The importance of the ear as a source of assimilates appears to be related to its better photosynthetic performance under water stress conditions, being justified by the following points: (*1*) C_4 metabolism (either constitutive or drought-induced) occurs in the ear, (*2*) water-relation characteristics of ears are different (including xerophytic anatomy, osmotic adjustment, and a higher water-use efficiency), (*3*) refixation of the CO_2 respired by the developing grains occurs, (*4*) delayed senescence of ears compared to that of flag leaves (Tambussi *et al*. 2007).

Wheat species are classified according to the number of chromosomes found in their vegetative cells. Additionally, the A and B genomes of *Triticum dicoccum* Schuebl L. (4n = 28, AABB) have been identified as donors to the modern hexaploid bread wheat genome (6n = 42, AABBDD) (Gill *et al*. 1991, Kimber 1993). With an increase in ploidy and a shift from the wild to cultivated species, the sizes of grains and leaves and the duration of grain filling have increased, resulting in higher yields. However, the ability of wheat plants to compete and survive under natural conditions has decreased with the increase in ploidy (Evans *et al*. 1975). Liu *et al.* (2016) reported different wheat genotypes exhibit distinct photosynthetic traits, dry matter accumulation, and WUE under water stress conditions. Therefore, it is valuable to study a response of tetraploid and hexaploid wheat to water stress.

In recent years, the study of photosynthesis in wheat ears has focused on the contribution of ear photosynthesis to grain filling (Maydup *et al*. 2012) and the improved performance of ears in modern wheat varieties (Tambussi *et al*. 2005). However, few studies have examined the photosynthetic characteristics of ears in wheat species with different ploidy. A better understanding of the factors involved in drought-tolerance of ears, particularly in the maintenance of high photochemical efficiency, may help in designing strategies for improvement of the wheat yield under water stress conditions.

In this context, the present study was performed in order to examine the photosynthetic performance of flag leaves and ears in tetraploid and hexaploid wheat species and to analyze the mechanisms of the improved performance in tetraploid ears.

Materials and methods

Plant material and experimental settings: Two wheat species belonging to different ploidies were selected: tetraploid – *T. dicoccoides* Kom; hexaploid – Bima1.

Experiments were conducted in Yangling, Shaanxi Province, northwest China (34°16′56.24″N, 108°4′27.95″E; 460 m a. s. l.) over the winter–spring growing season (October to June in 2013 and 2014). Naturally dried soil with a net water content of 3.2% was collected from Shaanxi (local red loessial soil). Plastic pots (diameter 24 cm, height 24 cm) were filled with 7 kg of soil. Before sowing, an equivalent of 0.347 g(urea) kg^{-1}(soil) and 0.2 g(K_2HPO_3) kg^{-1}(soil) were incorporated into the soil in each pot. Twenty seeds, exposed to 25°C in an incubator for 2 d, were sown in each pot on 17 October, 2013. Seedlings were removed at three-leaf stage to keep ten plants per pot. Plants were exposed to well-watered (WW) or water-stressed (WS) conditions by ensuring the maintenance of soil moisture content of 75–85% or 35–45% of the field water capacity, respectively. Water control started at the booting stage employing the weighing method using irrigation as the water control standard every day.

Gas exchange: Gas-exchange parameters were measured in the morning (09:00–11:00 h) with five replicates used per treatment at the heading stage (HS) and at the early [5 d after anthesis (DAA)], middle (15 DAA), and late (25 DAA) grain-filling stages. Leaf gas exchange was measured using an open *LI-COR 6400* system (*LI-COR Inc*., Lincoln, NB, USA), with a mixed sequence across treatments in order to reduce the bias due to timing. Spike gas-exchange parameters were measured using a *LI-COR 6400* system equipped with a special man-made cylindrical measuring chamber under natural light (Jia *et al*. 2015). The chamber was irradiated with a PPFD of 1,000 μmol m^{-2} s^{-1} and the ear gas-exchange parameter measurements were conducted under a PPFD of natural light about 1,000 μmol m^{-2} s^{-1}. Plants were also irradiated with 1,000 μmol(photon) m^{-2} s^{-1} for at least 30 min prior to the measurement of photosynthesis. The spike surface area was calculated according to the formula of the fringe area (Teare and Peterson 1971).

Instantaneous water-use efficiency (WUE) was determined by the ratio of photosynthetic rate (P_N) and transpiration rate (E). Stomatal limitation (L_s) was calculated according to Berry *et al*. (1982): $L_s = 1 - C_i/C_o$, where C_i is the intercellular CO_2 concentration and C_o is the ambient CO_2 concentration.

Physiological measurements: In order to understand the mechanisms of ear photosynthetic performance, pigment and soluble protein contents, enzyme activity, carbon isotope contents, WUE, and relative water content (RWC) of the two wheat species were measured synchronously.

Sample collection: The flag leaf, awn, glume, lemma, and palea of wheat in the pot experiment were obtained at the HS and grain-filling stage (5, 15, and 25 DAA) from 09:00 to 11:00 h. Samples were quickly frozen in liquid nitrogen and stored at –80°C in a freezer. The Chl and soluble protein contents and enzyme activities were measured after all the samples were collected. The results of the ears were obtained by calculating the weighted averages of awn, glume, lemma, and palea contents.

Pigment and soluble protein content: Photosynthetic pigments were extracted from fresh samples in 80%

acetone. The absorbance of the extracts was measured at 470, 646, and 663 nm using a *Mini-1240* UV-Vis spectrophotometer (*Shimadzu,* Kyoto, Japan). Chl (*a*+*b*) and carotenoid (Car) contents were calculated using adjusted extinction coefficients (Lichtenthaler 1987). Pigment content was expressed as mg ml^{-1} g^{-1}(fresh mass, FM). The soluble protein content was measured according to the method of Bradford (1976).

Enzyme activity analysis: The activities of ribulose-1,5-bisphosphate (RuBP) carboxylase/oxygenase (Rubisco, EC 4.1.1.39), a key photosynthetic enzyme in C_3 plants, and phospho*enol*pyruvate carboxylase (PEPC, EC 4.1.1.31), the initial carboxylase in C_4 plants, were examined in order to evaluate the metabolic pathway in the flag leaves and ears.

For enzyme extraction, the procedure of Lilley and Walker (1974) was used with some modifications. Aliquots of the frozen samples were ground in a mortar with liquid nitrogen and extracted with 100 mM Tris-HCl (pH 7.8), 10 mM $MgCl_2$, 20 mM β-mercaptoethanol, 1.0 mM EDTA, and 2% (w/v) PVP. The extract was centrifuged at 13,000 × *g* for 10 min at 4°C. The supernatant was used for enzyme assays. Enzyme activity was determined spectrophotometrically (*UV-2550*, *Shimadzu*, Japan) at 340 nm and it was expressed in nmol(NAD) min^{-1} mg^{-1}(protein).

Rubisco activity measurements were carried out following the methods of Camp *et al*. (1982). The enzyme extract was added to the reaction mixture, which contained 50 mM Tricine-NaOH (pH 7.9), 15 mM $MgCl_2$, 10 mM $NaHCO_3$, 2 mM DTT, 10 mM KCl, 0.2 mM NADH, 1 mM EDTA, 4 U mL^{-1} 3-P-glycerate kinase (*Sigma*, USA), 2 U mL^{-1} creatine phosphokinase (*Sigma*, USA), 5 mM phosphocreatine (*Sigma*, USA), 5 mM ATP, and 4 U mL^{-1} glyceraldehyde-3P-dehydrogenase (*Sigma*, USA) in a final volume of 1 mL. Reactions were initiated by adding 0.5 mM RuBP.

Activity of PEPC was monitored spectrophotometrically as the reduction in NADH at 340 nm, according to the method of Blanke and Ebert (1992) with minor modifications. The enzyme extract was added to the reaction mixture, which contained 50 mM Tris-HCl (pH 7.8), 10 mM $MgCl_2$, 0.25 mM EDTA, 0.1 mM NADH, 5.0 mM $NaHCO_3$, 2.0 mM DTT, 4 U malic dehydrogenase (MDH) (*Sigma*, USA), and 2.0 mM phospho*enol*pyruvate (*Sigma*, USA), and the final volume was made to 1 mL. The reaction was initiated by adding the crude enzyme extract.

Carbon isotope: Carbon isotope was used to evaluate the integrated WUE and C_4 metabolism in the ear. Carbon isotope analyses of flag leaves and ear parts (awns, glumes, lemmas, and paleas) was performed on WW and WS plants in pot experiments at 15 DAA using an elemental analyzer (*Flash 1112 EA*; *ThermoFinnigan*, Bremen, Germany) coupled with an isotope ratio mass spectrometer (*Delta C IRMS*, *ThermoFinnigan*, Germany) operating in a continuous flow mode to determine the stable carbon ($^{13}C/^{12}C$) isotope ratios in the same samples. Approximately 1 mg of samples and reference materials were weighed in tin capsules, sealed, and then loaded into an automatic sampler (*ThermoFinnigan*, Germany) before the analysis. The $^{13}C/^{12}C$ ratio in the plant material was expressed in δ notation (Vohlidal 2008) as follows: $\delta^{13}C$ [‰] = $[(^{13}C/^{12}C)_{sample}/(^{13}C/^{12}C)_{standard} - 1] \times 1{,}000$, where "sample" refers to the plant material and "standard" refers to the international secondary standards of known $^{13}C/^{12}C$ ratios (IAEA CH_7 polyethylene foil, IAEA CH_6 sucrose, and USGS 40 L-glutamic acid) calibrated against Vienna Pee Dee Belemnite calcium carbonate (VPDB) with an analytical precision (SD) of 0.10‰. $\Delta^{13}C$ [‰] = $[(\delta^{13}C_{air} - \delta^{13}C_{sample})/(1 + \delta^{13}C_{sample})] \times 1{,}000$. The accepted value of $\delta^{13}C_{air}$ is −7.7‰.

Relative water content (RWC) was measured in flag leaves and ears. Measurements of plants subjected to WW and WS conditions in the pot experiments were taken at four stages (HS, 5, 15, and 25 DAA). RWC was determined using the FM measured immediately after the sample collection. Thereafter, the sample was saturated with distilled water for 12 h at a low temperature (4°C) in the dark to obtain the saturated mass (SM). Finally, the sample was dried at 80°C for 48 h to obtain the dry mass (DM). RWC was calculated according to Turner (1981) as follows: RWC [%] = (FM – DM)/(SM – DM) × 100.

Statistical analysis: Data populations obtained from the experiment had demonstrable normal distributions. A two-way analysis of variance (*ANOVA*) was used to assess the effects of treatments and species on all the dependent variables. The normal distribution was tested using *Levene*'s test. *ANOVA* was performed using *SPSS* statistical software (*Version 19.0* for *Windows*, *SPSS*, Chicago, IL, USA). A general linear model analysis of variance in the *SPSS* system was adopted. Differences between the means were evaluated using an independent-sample *t*-test. Data were averaged from three replicates. *Pearson*'s correlation analyses in the *SPSS* system were used to assess the correlations between the gas-exchange parameters.

Results

Photosynthetic traits throughout grain filling: Under both the watering conditions, hexaploid wheat showed higher flag leaf P_N than that of the tetraploid wheat (Fig. 1*A*), during the middle and late filling stages (Table 1). Tetraploid wheat had a higher ear P_N than the hexaploid wheat under both the watering conditions throughout the filling stages (Fig. 1*B*).

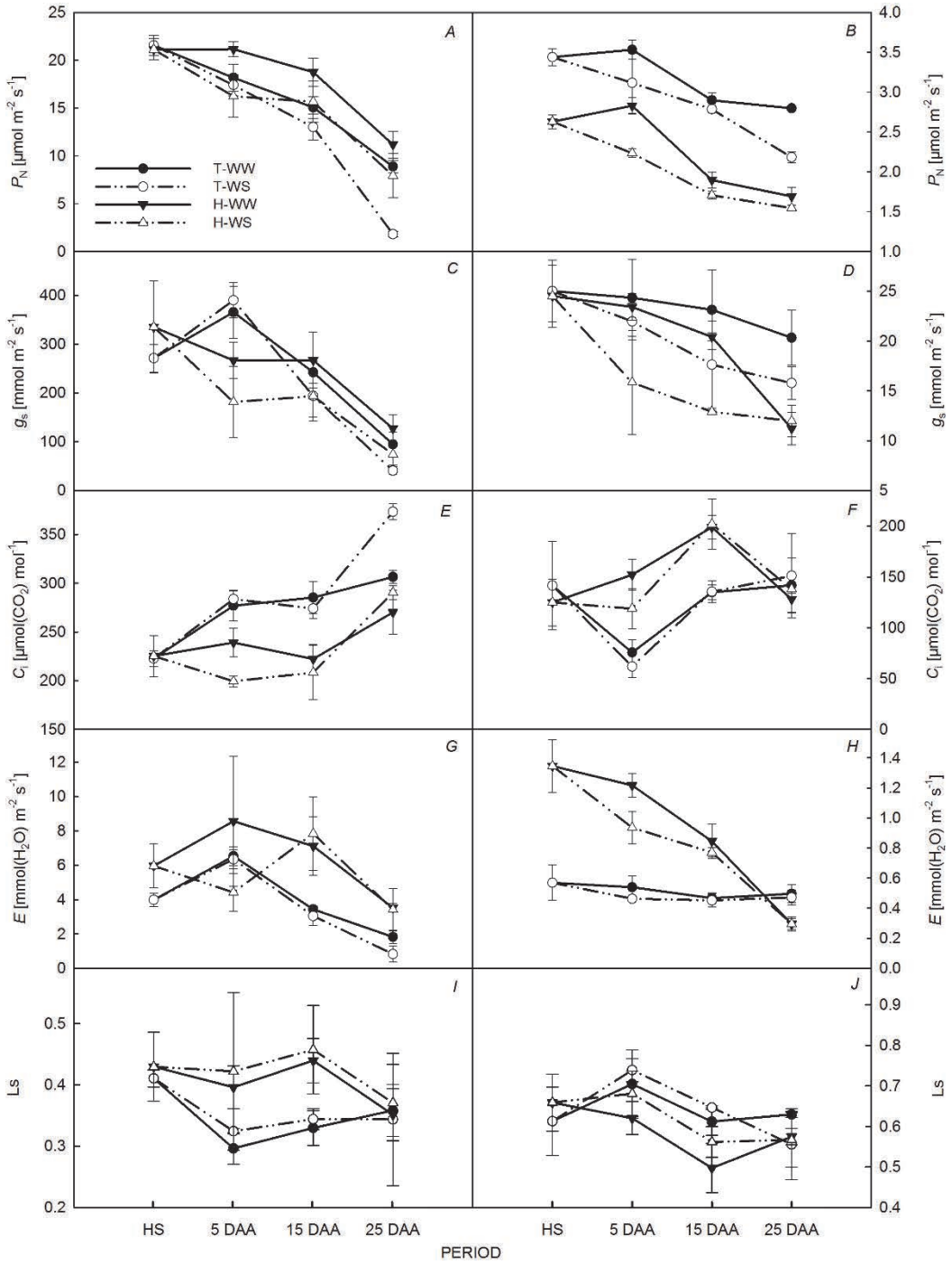

Fig. 1. Photosynthesis characteristics of flag leaves (*A*,*C*,*E*,*G*,*I*) and ears (*B*,*D*,*F*,*H*,*J*) measured at four stages in the pot experiments under well-watered (WW) and water-stressed (WS) conditions; P_N – photosynthetic rate; g_s – stomatal conductance; C_i – intercellular CO_2 concentration; E – transpiration rate; Ls – stomata limitation; T – *Triticum dicoccoides* Kom; H – Bima1; HS – deading stage; DAA – days after anthesis.

P_N of the two species showed similar trends during the filling stage in response to WS conditions. Nevertheless, P_N of the flag leaf decreased slightly from HS to 25 DAA. The differences in P_N between the four measurements were maximized in the plants subjected to WS conditions (Fig. 1*A*). In the ears, P_N increased to a maximum at 5 DAA and declined thereafter; this decrease was faster in hexaploid wheat compared to the tetraploid wheat under the both conditions (Fig. 1*B*). At 25 DAA, compared to the plants subjected to WW conditions at HS, the P_N of flag leaves decreased by 92 and 62% under WS conditions in tetraploid and hexaploid wheat, respectively (Fig. 1*A*), whereas the P_N of the ears only decreased by 37 and 42% in tetraploid and hexaploid wheat, respectively.

Table 1. Analysis of variance and differences between treatments and species of the measured physiological index. The *F* values followed by the letter ns are not significantly different *via Tukey*'s test. * – significant at $P \leq 0.05$; ** – significant at $P \leq 0.01$; *** – significant at $P \leq 0.001$. Ls – stomatal limitation; PEPC – phospho*enol*pyruvate carboxylase; P_N – net photosynthetic rate; RWC – relative water content; WUE – water-use efficiency.

Physiological index	Two-way *ANOVA*	Flag leaf			Ear		
		5 DAA	15 DAA	25 DAA	5 DAA	15 DAA	25 DAA
P_N	Treatment	11.32^{**}	7.92^{*}	40.45^{***}	17.06^{**}	12.22^{**}	94.85^{***}
	Species	1.17^{ns}	12.00^{**}	26.91^{***}	41.96^{***}	573.67^{***}	494.04^{***}
	Treatment×Species	6.03^{*}	0.31^{ns}	5.58^{*}	0.53^{ns}	0.78^{ns}	35.87^{***}
Ls	Treatments	0.47^{ns}	0.41^{ns}	0.003^{ns}	2.10^{ns}	4.65^{ns}	1.58^{ns}
	Species	6.34^{*}	19.69^{**}	0.07^{ns}	4.79^{ns}	19.00^{**}	0.46^{ns}
	Treatments×Species	0.001^{ns}	0.01^{ns}	0.18^{ns}	0.16^{ns}	0.41^{ns}	1.02^{ns}
Chlorophyll (*a*+*b*)	Treatments	41.63^{***}	36.38^{***}	900.49^{***}	15.55^{**}	13.09^{**}	16.08^{**}
	Species	465.51^{***}	119.71^{***}	46.50^{***}	4.27^{ns}	3.80^{ns}	11.60^{**}
	Treatments×Species	53.52^{***}	20.18^{**}	16.29^{**}	0.64^{ns}	0.01^{ns}	0.84^{ns}
Carotenoid	Treatments	6.68^{*}	73.66^{***}	248.80^{***}	10.19^{*}	2.93^{ns}	9.32^{*}
	Species	33.05^{***}	129.55^{***}	93.57^{***}	7.17^{*}	16.07^{**}	1.58^{ns}
	Treatments×Species	8.92^{*}	0.94^{ns}	16.61^{**}	0.52^{ns}	0.04^{ns}	0.15^{ns}
Rubisco activity	Treatments	43.42^{***}	108.84^{***}	317.93^{***}	94.75^{***}	9.97^{*}	68.46^{***}
	Species	0.90^{ns}	18.97^{**}	5.46^{*}	446.80^{***}	93.12^{***}	9.38^{*}
	Treatments×Species	4.06^{ns}	25.01^{**}	0.02^{ns}	0.39^{ns}	2.52^{ns}	0.08^{ns}
PEPC activity	Treatments	20.00^{**}	34.53^{***}	3.83^{ns}	$3{,}274^{***}$	848.36^{***}	15.42^{**}
	Species	7.08^{*}	30.07^{***}	5.43^{*}	$9{,}382^{***}$	$2{,}822^{***}$	38.09^{***}
	Treatments×Species	1.64^{ns}	2.48^{ns}	0.71^{ns}	$1{,}719^{***}$	218.66^{***}	5.69^{*}
Soluble protein	Treatments	216.21^{***}	65.48^{***}	54.35^{***}	0.47^{ns}	61.23^{***}	1.36^{ns}
	Species	133.86^{***}	8.10^{*}	60.72^{***}	20.42^{***}	89.82^{***}	482.75^{***}
	Treatments×Species	8.85^{*}	0.29^{ns}	12.12^{**}	0.56^{ns}	44.22^{***}	7.91^{*}
RWC	Treatments	23.64^{***}	109.11^{***}	174.00^{***}	1.61^{ns}	2.21^{ns}	23.68^{***}
	Species	0.07^{ns}	89.63^{***}	224.78^{***}	24.00^{***}	28.70^{***}	48.47^{***}
	Treatments×Species	0.08^{ns}	10.74^{*}	53.81^{***}	0.40^{ns}	2.24^{ns}	1.36^{ns}
WUE	Treatments	9.81^{*}	2.33^{ns}	2.68^{ns}	13.28^{**}	11.17^{**}	27.42^{***}
	Species	24.30^{**}	0.10^{ns}	4.74^{ns}	239.09^{***}	117.79^{***}	592.98^{***}
	Treatments×Species	0.01^{ns}	0.51^{ns}	0.06^{ns}	11.67^{**}	8.39^{*}	19.40^{**}

In flag leaves, g_s decreased by 85 and 78 % in tetraploid and hexaploid wheat (a decrease of 65 and 62% during the development), respectively, at 25 DAA under WS conditions compared to the plants subjected to WW during the heading stage (Fig. 1*C*). In contrast, the g_s of ears decreased by 30 and 51%, respectively (Fig. 1*D*). Water stress significantly increased the C_i of flag leaves in both the species at 25 DAA. Compared to the plants subjected to WW at the heading stage, the C_i of flag leaves increased by 68 and 29% under WS conditions in the tetraploid and hexaploid wheat, respectively, (an increase of 38 and 19% during the development; Fig. 1*E*), water stress had a minor effect on the C_i in the ears (Fig. 1*F*). Both the flag leaves and ears of hexaploid wheat exhibited higher *E* than tetraploid wheat under the two watering conditions (Fig. 1*G*,*H*). A more sharp decrease in *E* (by 78%) was observed in the hexaploid wheat compared to the 17% decrease in the tetraploid wheat under WS (Fig. 1*H*). The *E* of flag leaves of tetraploid and hexaploid wheat decreased by 79 and 43%, respectively (Fig. 1*G*). The flag leaves of hexaploid wheat presented higher L_s than that of tetraploid wheat (Fig. 1*I*); however, no obvious difference in L_s was observed in the ears of the two species (Fig. 1*J*). Water stress had a minor effect on L_s, both in the flag leaves and ears of the two species (Fig. 1*I*,*J*).

During the whole grain filling, P_N of flag leaves and ears were significantly and positively related to g_s in both the species and under both the watering conditions (Fig. 2*A–D*). *E* of the flag leaves was also significantly and positively related to g_s in both the species and under both the water conditions (Fig. 2*E*, *G*), but *E* of the ears was not significantly related to g_s, except in the case of hexaploid wheat ears under WW condition (Fig. 2*F*,*H*).

Pigment and soluble protein content: WS reduced Chl (*a*+b) and Car in both the species (Fig. 3*A–D*). A greater decline in Chl (*a*+*b*) occurred in the flag leaves of the tetraploid wheat (a significant difference from 5 to 25 DAA) than in those of the hexaploid wheat (a significant difference only found at 25 DAA; Fig. 3*A*, Table 1). In the ears, the hexaploid wheat showed greater decrease in the Chl (*a+b)* than that of the tetraploid wheat (Fig. 3*B*).

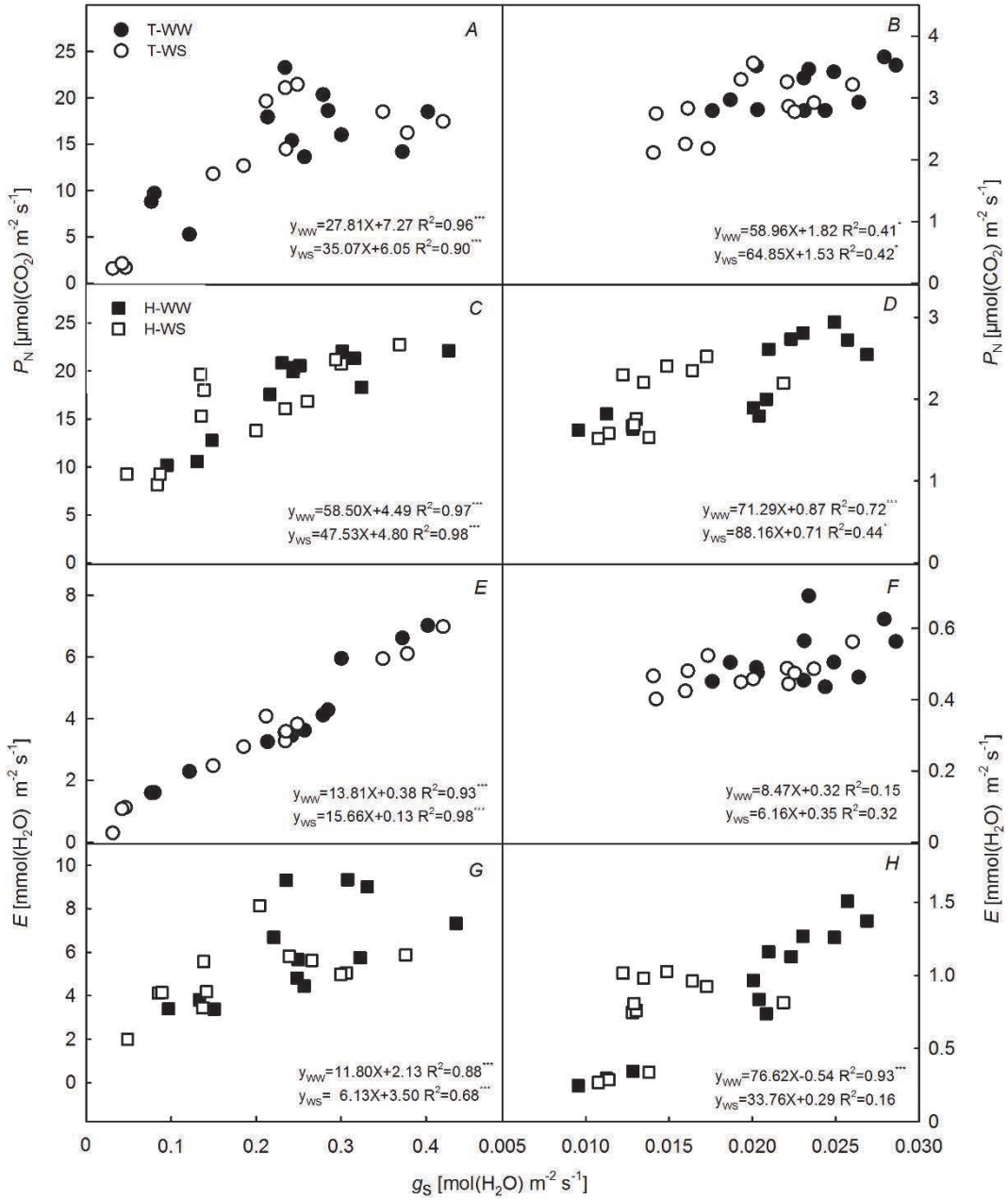

Fig. 2. Relationship between net photosynthetic rate (P_N) and stomatal conductance (g_s) (*A*,*B*,*C*,*D*), g_s and transpiration rates (*E*) (*E*,*F*,*G*,*H*) of flag leaf (*A*,*C*,*E*,*G*) and ear (*B*,*D*,*F*,*H*) during grain filling for tetraploid (□) and hexaploid (○) wheat species under well - watered (WW, *filled icon*) and water-stressed (WS, *open icon*) conditions. T – *Triticum dicoccoides* Kom, H – Bima1.

From HS to 25 DAA, the soluble protein content in the flag leaves under continuous WS conditions decreased more severely than those under WW conditions (Fig. 3*E*,*F*). Tetraploid wheat showed the higher soluble protein content than that of the hexaploid wheat at HS and 5 DAA stages, but it also showed a more pronounced decrease during the filling stage, especially under WS conditions. The soluble protein content declined by 61% under WS conditions from HS to 25 DAA (Fig. 3*E*). The decrease in the ears of the two species was lower than that in the flag leaves under WS conditions (Fig. 3*F*, Table. 1). The hexaploid wheat showed a decrease of 41% from HS under WW conditions to 25 DAA under WS conditions, whereas in the tetraploid wheat the decrease was 24% (Fig. 3*F*).

Changes in enzyme activities: Similar trends were observed for the changes in Rubisco activity in both the species. The activity in the flag leaves of tetraploid wheat was 48.37 nmol(NAD) min^{-1} mg^{-1}(protein) in the plants subjected to WW conditions at HS, while it decreased by 93% to 3.52 nmol(NAD) min^{-1} mg^{-1}(protein) in plants subjected to WS conditions at 25 DAA (Fig. 4*A*).

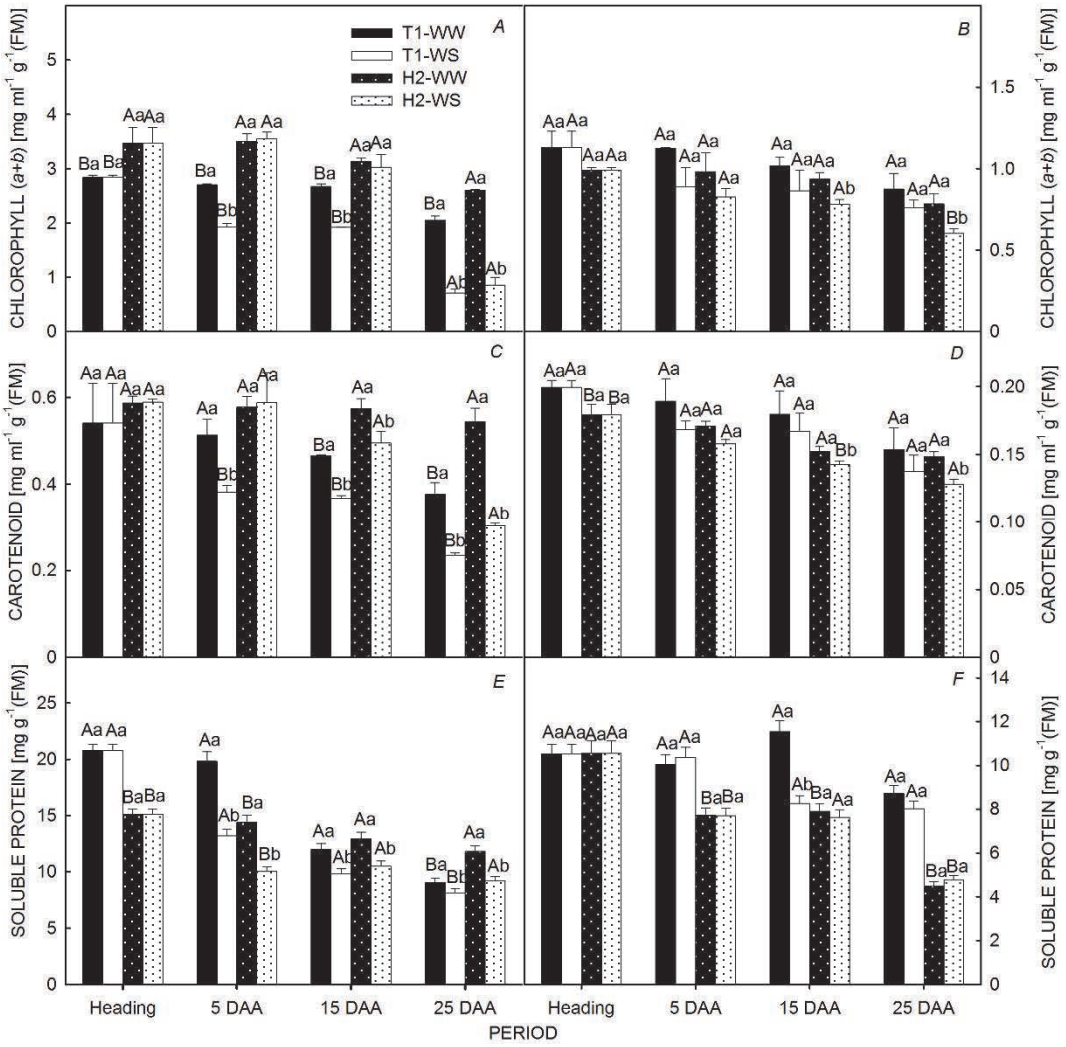

Fig. 3. Pigment contents (*A*,*B*,*C*,*D*) and soluble protein (*E*,*F*) contents of the flag leaf (*A*,*C*,*E*) and ear (*B*,*D*,*F*) for the two wheat species under well-watered (WW) and water-stressed (WS) conditions. The values are means ± SD of three replicates. The different *capital letters* above the bars denote significant differences for different species under the same water treatment (P=0.05); the different *lowercase letters* above the bars denote significant differences for different water treatments for the same species (P=0.05). T – *Triticum dicoccoides* Kom, H – Bima1.

In contrast, the Rubisco activity decreased more slowly in the flag leaves of hexaploid wheat, changing from 46.97 nmol(NAD) min^{-1} mg^{-1}(protein) in WW plants at HS to 5.90 nmol(NAD) min^{-1} mg^{-1}(protein) in the WS plants at 25 DAA, a decrease of 87% (Fig. 4*A*), which was moderate compared to the decrease by 46% in the WW plants at 25 DAA. However, in the ears, WS reduced the Rubisco activity from 72.20 nmol(NAD) min^{-1} mg^{-1}(protein) in the WW plants at HS to 7.39 nmol(NAD) min^{-1} mg^{-1}(protein) in the WS plants at 25 DAA, a decrease of 73%, in the tetraploid wheat. In contrast, a much greater decrease was observed in the ears of hexaploid wheat [a decrease of 90%, from 38.77 nmol(NAD) min^{-1} mg^{-1} (protein) in the WW plants at HS to 11.23 nmol(NAD) min^{-1} mg^{-1}(protein) in the WS plants at 25 DAA; Fig. 4*B*].

The PEPC activity decreased gradually from HS to 25 DAA, the decrease being significantly higher in the WS plants. The trends in the changes of the PEPC activity were similar during the measurement period for both the species; however, the reduction in PEPC activity was greater in the hexaploid wheat (70 and 75%) than that in the tetraploid wheat (47 and 68%) in the flag leaves and ears. The PEPC activity decreased more dramatically in the ears than that in the flag leaves (Fig. 4*C*,*D*).

RWC and its relationship to P_N: The changes in RWC of the plants subjected to WS conditions were more obvious than that in the plants under WW conditions, throughout the experiment, particularly for the hexaploid wheat. The RWC in the flag leaves under both the watering conditions exhibited a significant difference in both the species (Fig. 5*A*) with the RWC in the hexaploid wheat being higher than that in the tetraploid wheat, particularly at 15 and 25 DAA, in the plants subjected to WS conditions

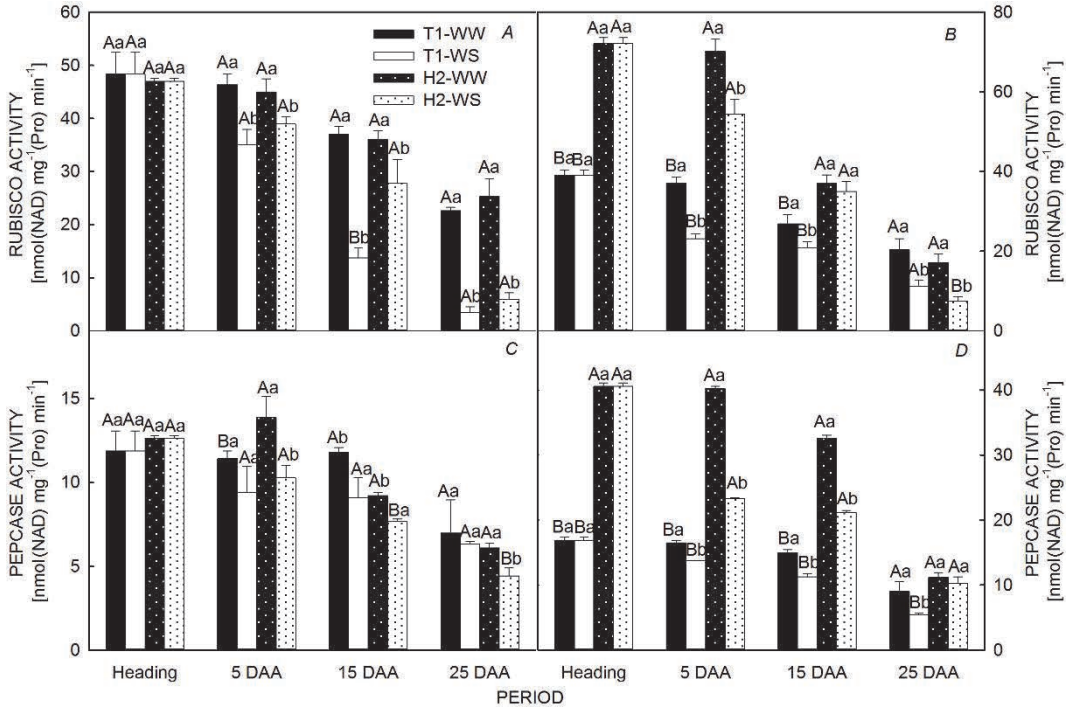

Fig. 4. Enzyme activity of the flag leaf (*A*,*C*) and ear (*B*,*D*) for the two wheat species under well-watered (WW) and water-stressed (WS) conditions. The values are means ± SD or three replicates. The different *capital letters* above the bars denote significant differences for different species under the same water treatment (P=0.05); the different *lowercase letters* above the bars denote significant differences for different water treatment for the same species (P=0.05). T – *Triticum dicoccoides* Kom, H – Bima1.

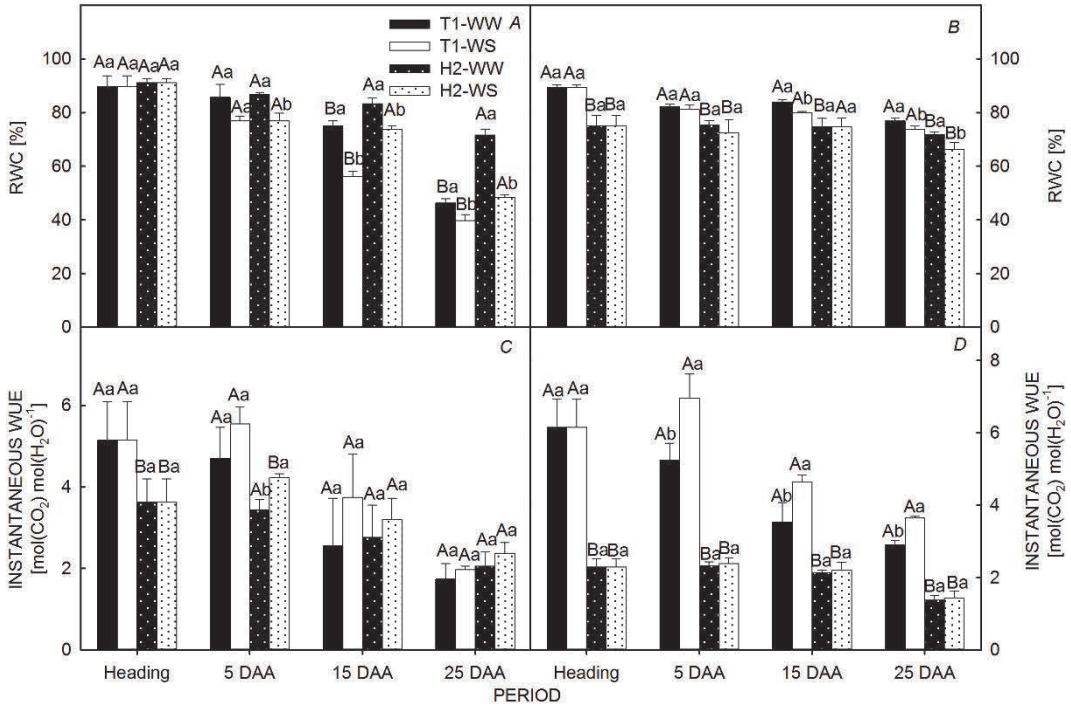

Fig. 5. Relative water content (RWC) and instantaneous water-use efficiency (WUE) of the flag leaf (*A*,*C*) and ear (*B*,*D*) for the two wheat species under well-watered (WW) and water-stressed (WS) treatments. The values are means ± SD of three replicates. The different *capital letters* above the bars denote significant differences for different species under the same water treatment (P=0.05); the different *lowercase letters* above the bars denote significant differences for different water treatments for the same species (P=0.05). T – *Triticum dicoccoides* Kom, H – Bima1.

Table 2. Stable carbon isotope discrimination of the two wheat species under well-watered (WW) and water-stressed (WS) treatments. Values are means ± SE. The different *capital letters* followed the mean values denote significant differences for different species under the same water treatment (P = 0.05); the different *lowercase letters* above the bars denote significant differences for different water treatment under the same species (P = 0.05).The F values followed by the letter ns are not significantly different *via Tukey*'s test. * – significant at $P \leq 0.05$; ** – significant at $P \leq 0.01$; *** – significant at $P \leq 0.001$.

Cultivars	Treatment	Organs ($\Delta^{13}C$) [‰]				
		Flag leaf	Awn	Glume	Lemma	Palea
Tetraploid	WW	23.48 ± 0.10^{Aa}	22.87 ± 0.20^{Aa}	22.13 ± 0.20^{Aa}	21.42 ± 0.22^{Aa}	20.29 ± 0.18^{Aa}
	WS	23.49 ± 0.20^{Aa}	21.59 ± 0.18^{Ab}	20.95 ± 0.17^{Ab}	20.34 ± 0.18^{Ab}	19.64 ± 0.20^{Ab}
Hexaploid	WW	21.03 ± 0.22^{Ba}	22.19 ± 0.17^{Ba}	22.47 ± 0.20^{Aa}	22.57 ± 0.26^{Ba}	22.47 ± 0.17^{Ba}
	WS	21.22 ± 0.18^{Ba}	20.60 ± 0.13^{Bb}	20.99 ± 0.20^{Ab}	21.18 ± 0.19^{Bb}	20.86 ± 0.29^{Bb}
Two-way *ANOVA*	Treatments	0.23^{ns}	208.00^{***}	153.58^{***}	99.53^{***}	82.65^{***}
	Species	497.15^{***}	70.43^{***}	1.58^{ns}	64.60^{***}	187.06^{***}
	Treatments× Species	1.83^{ns}	2.43^{ns}	3.22^{ns}	1.57^{ns}	14.91^{**}

Table 3. Yield of cultivars under well-watered (WW) and water-stressed (WS) treatments in the pot experiment. Values are means ± SE. The different *lowercase letters* above the bars denote significant differences for different water treatment under the same species (P=0.05).

Cultivar	Yield [g per pot]			1,000-grain mass [g]			Grain number per panicle		
	WW	WS	Decrease	WW	WS	Decrease	WW	WS	Decrease
Tetraploid	38.40 ± 4.50^{a}	31.85 ± 5.17^{a}	20.57%	40.91 ± 1.94^{a}	47.76 ± 2.44^{a}	-14.34%	42.00 ± 1.00^{a}	36.33 ± 3.06^{b}	13.50 %
Hexaploid	48.89 ± 3.81^{a}	35.33 ± 1.92^{b}	38.38%	45.37 ± 0.45^{a}	38.03 ± 1.05^{b}	19.30%	45.33 ± 4.93^{a}	43.33 ± 2.52^{a}	4.41%

(Fig. 5*A*). However, in the ears of both cultivars, no significant differences were observed in the RWCs between the plants subjected to WW and WS conditions, and no obvious decrease was observed at any of the experimental stages (Fig. 5*B*). The differences in RWCs between the two cultivars were found only at HS and 5 DAA (Fig. 5*B*). At 15 and 25 DAA, both the tetraploid and hexaploid wheat exhibited higher RWCs in the ears than that in the flag leaves.

Stable carbon isotope discrimination and WUE: Exposure to WS conditions increased the instantaneous WUE of the two species (Fig. 5*C*,*D*); however, significant differences between the two watering regimes were found only in the ears of the tetraploid wheat from 5 to 25 DAA and in the flag leaves of the hexaploid wheat at 5 DAA.

$\Delta^{13}C$ results for the different organs of the two cultivars are shown in Table 2. The $\Delta^{13}C$ value was higher under WW conditions than that under WS conditions for all organs (Table 2). However, differences in the $\Delta^{13}C$ values were not significant in the flag leaves. Compared to the flag leaves, all ear parts had lower $\Delta^{13}C$ values. Both under the WW and WS regimes, the $\Delta^{13}C$ values in the flag leaves and awns of the tetraploid wheat were much higher than those in the hexaploid wheat. In contrast, the higher $\Delta^{13}C$ values were observed for the other organs in the hexaploid wheat (Table. 2).

Yield and yield components: Compared to WW conditions, the grain yield of the two species both decreased under water stress; however, they displayed varying susceptibility to water stress. The grain yield, 1,000-grain mass, and the grain number per panicle of hexaploid wheat decreased by 38.4, 19.3, and 4.4%, respectively. For tetraploid wheat, the grain yield and the grain number per panicle decreased by 20.6 and 13.5%, respectively, while the grain mass increased by 14.3% (Table 3).

Discussion

Differences in photosynthetic performance between the flag leaves and ears: The importance of wheat ear photosynthesis to the grain yield, especially under water stress, has been under serious investigation (Maydup *et al.* 2010). The results of the present study revealed that the P_N in the flag leaves was significantly reduced by exposure to WS conditions; however, the influence of the drought treatment on the ear photosynthesis was not obvious, which is a finding consistent with those reported earlier (Tambussi *et al*. 2005). From the analysis of gas-exchange parameters (Figs. 1, 2), we concluded that the inhibition of P_N in the flag leaves and ears by water stress was not the result of stomatal closing, but occurred due to nonstomatal limitations. Moreover, compared to the hexaploid wheat,

the tetraploid wheat showed higher P_N and stronger tolerance to water stress in the ears, while the flag leaves were more sensitive to drought. The relatively higher Chl (*a*+*b*) and Car contents and lower *E* in the ears of the tetraploid wheat as compared to that of the hexaploid wheat suggested the relative stability of the tetraploid wheat ear photosynthetic apparatus. High instantaneous WUE might help in maintaining the ear photosynthesis during the grain filling period, especially in the plants under water stress.

As reported in previous studies, the reproductive structures, such as green flowers or developing fruits, are photosynthetically active and therefore can assimilate substantial amounts of carbon in many plants (Aschan and Pfanz 2003, Sawicki *et al*. 2016). In our study, the different response of the yield to water stress between the two wheat species might be related to the different response of P_N in the flag leaves and ears (Table 3). Therefore, we concluded that ear photosynthesis played an important role in wheat filling, particularly under drought stress. Further increases in the ear photosynthetic performance of modern wheat cultivars might be the most effective method for improving the yield potential in the future.

Decreased Rubisco activity resulted in a decline in photosynthesis under WS conditions: Photosynthesis is the most sensitive physiological process in response to water deficit (Wahid *et al*. 2007). Any reduction in photosynthesis affects both the growth and grain yield of wheat (Al-Khatib and Paulsen 1990). Our results revealed a substantial decrease in the Chl content and photosynthetic capacity of the flag leaves in wheat plants grown during the grain filling. Moreover, exposure to WS conditions aggravated the decrease in photosynthesis and eventually caused a significant decrease in the grain yield.

Rubisco acts as a crucial enzyme in CO_2 fixation. Upon exposure to high temperatures, Rubisco is deactivated either due to the acceleration of end-product formation or slower reactivation of Rubisco activase, resulting in a loss of the ability of Rubisco activase to maintain Rubisco activity and efficiency (Demirevska-Kepova *et al*. 2005, Salvucci and Crafts-Brandner 2004a,b). The decrease in P_N caused by reduced Rubisco content or activity is defined as a RuBP carboxylation limitation (Chen *et al*. 2005). In C_3 monocots, the activity of Rubisco is obviously reduced by water deficit in general (Monson *et al*. 1982, Kobza *et al*.1984, Grover *et al*. 1986). In the present study, the activity of Rubisco significantly decreased in both the cultivars under the WS conditions (Table 1). In the flag leaves, the Rubisco activity showed a sustainable and rapid reduction during water stress. In contrast, in the ears, although Rubisco activity decreased after the heat stress, minor changes in the activity occurred from HS to 25 DAA. These results suggested that the rapid decline in P_N due to water stress mainly resulted from the decline in Rubisco activity. The reduction in Rubisco activity resulted in a dramatic decrease in RuBP.

Previous studies have shown that the improved photosynthetic performance of the ears compared with that of the flag leaves under stress might be related to the degree of C_4 metabolism (Tambussi *et al*. 2007). Our results showed that the activity of PEPC, an essential enzyme in C_4 photosynthetic pathway, was not parallel with the drought tolerance. Compared to the hexaploid wheat, the tetraploid wheat presented relatively higher and lower PEPC activity in the flag leaves and ears, respectively (Fig. 4). These results suggest that higher P_N in the tetraploid wheat ears is not related to C_4 metabolism, and the higher PEPC activity in the wheat ears might play a role in other metabolic processes, such as in the assimilation of ammonium (Masumoto *et al*. 2010).

Improved water status stimulated photosynthetic performance in ears: The P_N in the ear was less affected by water stress than that in the flag leaves in our pot experiments. The better photosynthetic performance of the ears (compared to that of the flag leaves) under WS conditions, particularly in the tetraploid wheat, was associated with its capacity to maintain higher RWC (Fig. 5*A*,*B*). These results were consistent with the findings of a previous study (Tambussi *et al*. 2005) in which the capacity to maintain a high RWC was found to be the main cause of the higher drought resistance in wheat ears.

High WUE in the ears of C_3 cereals has been reported in some studies (Teare *et al*. 1972, Araus *et al*. 1993, Bort *et al*. 1994, Abbad *et al*. 2004). In our study, the WUE was higher in the ears than that in the flag leaves, which might also contribute to the better photosynthetic performance of the ears. The results revealed that the instantaneous WUE increased significantly in the ears of tetraploid wheat under WS conditions. Moreover, previous studies suggest that the WUE might have been underestimated in the gas-exchange studies because the respired CO_2 was not considered, and the *E* of ears was much higher than that of flag leaves; therefore, the total ear photosynthesis (and thus, instantaneous WUE) might have been dramatically underestimated. Taken together, the instantaneous WUE in the ears should be much higher than that in the flag leaves. In addition, the tetraploid wheat had a much higher instantaneous WUE at all the experiment stages, which could also partly explain the higher ear P_N.

The relationship between integrated WUE and ^{13}C isotopic discrimination ($\Delta^{13}C$) is well known. In C_3 species, $\Delta^{13}C$ is positively related to CO_2 concentrations in intercellular spaces and (given a constant vapor pressure deficit) negatively related to WUE (Farquhar and Richards 1984, Hubick and Farquhar 1989). In our study, the ear parts of tetraploid wheat showed lower $\Delta^{13}C$ values compared to those in the flag leaves, suggesting that the ears might have a higher WUE (Hubick and Farquhar 1989). Moreover, WS conditions significantly reduced the $\Delta^{13}C$ and tetraploid wheat had progressively lower $\Delta^{13}C$ values from the flag leaves to glumes and paleas (*i.e*., towards the organs closest to the respiring grains),

consistent with previous studies (Araus *et al.* 1993). However, the flag leaves of hexaploid wheat exhibited higher $\Delta^{13}C$ values than that of the ears, which might be associated with the lower P_N. Thus, both the higher instantaneous and integrated WUEs contributed to the improved performance of ear photosynthesis.

WS conditions decrease P_N (Lawlor 2002) and accelerate leaf senescence (Martinez *et al*. 2003). Compared to that in the flag leaves, the senescence rate of the ear parts was slowed down less under the WS conditions. Our results demonstrated that the key components of the photosynthetic machinery, *i.e*., Chl and soluble protein contents, decreased only slightly more in ears of the plants subjected to the WS conditions than in those of the WW plants, and the tetraploid wheat possessed higher Chl and soluble protein contents at 25 DAA. These traits have been proposed to account for the improved photosynthetic performance of the ear under WS conditions compared to that of the flag leaf under WS conditions. Therefore, delayed senescence under drought conditions might be associated with the improved performance of the tetraploid wheat ears.

Does the C_4 photosynthetic pathway exist in the ears? Previous studies have suggested a possible presence of C_4 metabolism in the ears of C_3 cereals (Nutbeam and Duffus 1976, Wirth *et al*. 1977, Singal *et al*. 1986, Ziegler-Jöns 1989b, Imaizumi *et al*. 1990). The evidence for C_4 metabolism includes C_4 metabolites detected, the higher C_4-enzyme activity, the C_4 gas-exchange properties, and oxygen insensitivity of the photosynthetic rate. In the current study, our results revealed that the ear parts had higher Rubisco (a key enzyme in the C_3 metabolism) and PEPC (a key enzyme in the C_4 metabolism) activities compared to that in the flag leaves, which was consistent with previous studies (Jia *et al*. 2015). However, the highest PEPC activity was observed in the hexaploid wheat ear parts, which had lower ear photosynthetic rates than that of the tetraploid wheat.

Although the ear parts showed lower carbon isotope compositions than the flag leaves, these values (ranging between approximately −26 and −30%) were within the normal range for C_3 species. Therefore, further studies are needed to examine C_4 metabolism performed in the ear parts and the contribution of C_4 metabolism to the improved photosynthetic performance.

Conclusion: In summary, the results of the present study illustrated that the WS conditions aggravated the process of wheat senescence by decreasing the contents of photosynthetic pigments and reducing the photosynthetic activity. WS conditions also led to changes in the functions of some active proteins, such as Rubisco. The observed decreases in the Rubisco activity inhibited the photosynthetic CO_2 assimilation capacity and were considered to be the main cause of the decline in P_N. The effects of water stress on the photosynthetic performance were species dependent. The flag leaves of the hexaploid wheat had greater water stability when compared to those of the tetraploid wheat; however, the ears of the latter one were more stable under the imposed WS conditions. Therefore, the improved performance of tetraploid wheat ears under water stress might be associated with its more efficient water utilization. C_4 photosynthesis occurring in the wheat ears needs to be characterized further.

References

Abbad H., Jaafari S.A., Bort J. *et al.*: Comparison of flag leaf and the ear photosynthesis with the biomass and grain yield of durum wheat under a range of water conditions and different genotypes. – Agronomie **24**: 19-28, 2004.

Al-Khatib K., Paulsen G.M.: Photosynthesis and productivity during high-temperature stress of wheat genotypes from major world regions. – Crop Sci. **30**: 1127-1132, 1990.

Araus J.L., Brown H.R., Febrero A. *et al.*: Ear photosynthesis, carbon isotope discrimination and the contribution of respiratory CO_2 to differences in grain mass in durum wheat. – Plant Cell Environ. **16**: 383-392, 1993.

Araus J.L., Slafer G.A., Reynolds M.P., Royo C.: Plant breeding and drought in C3 cereals: what should we breed for? – Ann. Bot.-London **89**: 925-940, 2002.

Araus J.L., Slafer G.A., Royo C., Serret M.D.: Breeding for yield potential and stress adaptation in cereals. – Crit. Rev. Plant Sci. **27**: 377-412, 2008.

Aschan G., Pfanz H.: Non-foliar photosynthesis – a strategy of additional carbon acquisition. – Flora **198**: 81-97, 2003.

Bazzaz F.A., Carlson R.W., Harper J.L.: Contribution to reproductive effort by photosynthesis of flowers and fruits. – Nature **279**: 554-555, 1979.

Berry J. A., Downton W.J.S.: Environmental regulation of photosynthesis. – In: Govindjee (ed.): Photosynthesis, Vol. II. Pp. 263-343. Academic Press, New York 1982.

Blanke M.M., Ebert G.: Phospho*enol*pyruvate carboxylase and carbon economy of apple seedlings. – J. Exp. Bot. **43**: 965-968, 1992.

Blum A.: Photosynthesis and transpiration in leaves and ears of wheat and barley varieties. – J. Exp. Bot. **36**: 432-440, 1985.

Bort J., Febrero A., Amaro T., Araus J.L.: Role of awns in ear water-use efficiency and grain weight in barley. – Agronomie **14**: 133-139, 1994.

Bradford M.M.: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. – Anal Biochem. **72**: 248-250, 1976.

Camp P.J., Huber S.C., Burke J.J., Moreland D.E.: Biochemical changes that occur during senescence of wheat leaves: I. Basis for the Reduction of Photosynthesis. – Plant Physiol. **70**: 1641-1646, 1982.

Chen G.Y., Yong Z.H., Liao Y. *et al.*: Photosynthetic acclimation in rice leaves to free-air CO_2 enrichment related to both ribulose-1,5-bisphosphate carboxylation limitation and ribulose-1,5-bisphosphate regeneration limitation. – Plant Cell Physiol. **46**: 1036-1045, 2005.

Demirevska-Kepova K., Holzer R., Simova-Stoilova L., Feller

U.: Heat stress effects on ribulose-1,5-bisphosphate carboxylase/oxygenase, Rubisco binding protein and Rubisco actives in wheat leaves. – Biol. Plant. **49**: 521-525, 2005.

Evans L.T., Bingham J., Jackson P., Sutherland J.: Effect of awns and drought on the supply of photosynthate and its distribution within wheat ears. – Ann. Appl. Biol. **70**: 67-76, 1972.

Evans L.T., Rawson H.M.: Photosynthesis and respiration by the flag leaf and components of the ear during grain development in wheat. – Aust. J. Biol. Sci. **23**: 245-254, 1970.

Evans L.T.,Wardlaw I. F., Fischer R. A.: Wheat. – In: Evans L.T. (ed.): Crop Physiology: Some Case of Histories. Pp. 101-150. Cambridge University Press, Cambridge 1975.

Farquhar G.D., Richards R.A.: Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. – Aust. J. Plant Physiol. **11**: 539-552, 1984.

Gill B.S., Friebe B., Endo T.R.: Standard karyotype and nomenclature system for the description of chromosome bands and structural aberations in wheat (*Triticum aestivum*). – Genome **34**: 830-839, 1991.

Grover A., Sabat S.C., Mohanty P.: Effect of temperature on photosynthetic activities of senescing detached wheat leaves. – Plant Cell Physiol. **27**: 117-126, 1986.

Hetherington S., Smillie R., Davies W.: Photosynthetic activities of vegetative and fruiting tissues of tomato. – J. Exp. Bot. **49**: 1173-1181, 1998.

Hubick K. T., Farquhar G. D.: Carbon isotope discrimination and the ratio of carbon gains to water lost in barley cultivars. – Plant Cell Environ. **12**: 795-804, 1989.

Imaizumi N., Usuda H., Nakamoto H., Ishihara K.: Changes in the rate of photosynthesis during grain filling and the enzymatic activities associated with the photosynthetic carbon metabolism in rice panicles. – Plant Cell Physiol. **31**: 835-843, 1990.

Jia S., Lv J., Jiang S. *et al.*: Response of wheat ear photosynthesis and photosynthate carbon distribution to water deficit. – Photosynthetica **53**: 95-109, 2015.

Kimber G.: Genomic relationships in *Triticum* and the availability of alien germplasm. – In: Damania A.B. (ed.): Biodiversity and Wheat Improvement. Pp. 9-16. Academic, Chichester 1993.

Kobza J., Uribe E.G., Williams G.J.: Temperature dependence of photosynthesis in *Agropyron smithii* Rydb. III. Responses of protoplasts and intact chloroplasts. – Plant Physiol. **75**: 378-381, 1984.

Kriedemann P.: The photosynthetic activity of the wheat ear. – Ann. Bot.-London **30**: 349-363, 1966.

Lawlor D.W.: Limitation to photosynthesis in water-stressed leaves: stomata vs. metabolism and the role of ATP. – Ann. Bot.-London **89**: 871-885, 2002.

Lichtenthaler H.K.: Chlorophyll fluorescence signatures of leaves during the autumnal chlorophyll breakdown. – J. Plant Physiol. **131**: 101-110, 1987.

Lilley R.M., Walker D.A.: An improved spectrophotometric assay for ribulosebisphosphate carboxylase. – BBA-Enzymology **358**: 226-229, 1974.

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

Lytovchenko A., Eickmeier I., Pons C. *et al.*: Tomato fruit photosynthesis is seemingly unimportant in primary metabolism and ripening but plays a considerable role in seed development. – Plant Physiol. **157**: 1650-1663, 2011.

Marcelis L.F.M., Hofman-Eijer L.R.B.: The contribution of fruit photosynthesis to the carbon requirement of cucumber fruits as affected by irradiance, temperature and ontogeny. – Physiol. Plantarum **93**: 476-483, 1995.

Martinez D. ., Luquez V. ., Bartoli C. ., Guiamét J. .: Persistence of photosynthetic components and photochemical efficiency in ears of water-stressed wheat (*Triticum aestivum*). – Physiol. Plantarum **119**: 519-525, 2003.

Masumoto C., Miyazawa S.I., Ohkawa H. *et al.*: Phospho*enol*-pyruvate carboxylase intrinsically located in the chloroplast of rice plays a crucial role in ammonium assimilation. – P. Natl. Acad.Sci. USA **107**: 5226-5231, 2010.

Maydup M.L., Antonietta M., Guiamet J.J. *et al.*: The contribution of ear photosynthesis to grain filling in bread wheat (*Triticum aestivum* L.). – Field Crop. Res. **119**: 48-58, 2010.

Maydup M. L., Antonietta M., Guiamet J. J., Tambussi E. A.: The contribution of green parts of the ear to grain filling in old and modern cultivars of bread wheat (*Triticum aestivum* L.): Evidence for genetic gains over the past century. – Field Crop. Res. **134**: 208-215, 2012.

Mingo D.M., Bacon M.A., Davies W.J.: Non-hydraulic regulation of fruit growth in tomato plants (*Lycopersicon esculentum* cv. Solairo) growing in drying soil. – J. Exp. Bot. **54**: 1205- 1212, 2003.

Monson R.K., Stidham M.A., Williams G.J. *et al.*: Temperature dependence of photosynthesis of *Agropyron smithii* Rydb. I. Factors affecting net CO_2 uptake in intact leaves and contribution from ribulose-1,5-bisphosphate carboxylase measured *in vivo* and *in vitro*. – Plant Physiol. **69**: 921-928, 1982.

Nutbeam A., Duffus C.: Evidence for C4 photosynthesis in barley pericarp tissue. – Biochem. Bioph. Res. Co. **70**:1198-1203, 1976.

Pfanz H., Aschan G., Langenfeld-Heyser R. *et al.*: Ecology and ecophysiology of tree stems: corticular and wood photosynthesis. – Naturwissenschaften **89**: 147-162, 2002.

Pfanz H., Aschan G.: The existence of bark and stem photosynthesis and its significance for the overall carbon gain. An ecopysiological and ecological approach. – Prog. Bot. **62**: 477-510, 2001.

Ram H., Singh R.: Chlorophyll content, photosynthetic rates and related enzyme activities in ear parts of two wheat cultivars differing in grain yield. – Plant Physiol Bioch. **9**: 94-102, 1982.

Reynolds M.P., Mujeeb-Kazi A., Sawkins M.: Prospects for utilising plant-adaptive mechanisms to improve wheat and other crops in drought & salinity-prone environments. – Ann. Appl. Biol. **146**: 239-259, 2005.

Salvucci M.E., Crafts-Brandner S.J.: Inhibition of photosynthesis by heat stress: the activation state of Rubisco as a limiting factor in photosynthesis. – Plant Physiol. **120**:179-186, 2004a.

Salvucci M.E., Crafts-Brandner S.J.: Mechanism for deactivation of Rubisco under moderate heat stress. – Plant Physiol. **122**: 513-519, 2004b.

Sánchez-Díaz M., García J.L., Antolín M.C., Araus J.L.: Effects of soil drought and atmospheric humidity on yield, gas exchange, and stable carbon composition of barley. – Photosynthetica **40**: 415-421, 2002.

Sawicki M., Courteaux B, Rabenoelina F. *et al.*: Leaf *vs*. inflorescence: differences in photosynthetic activity of grapevine. – Photosynthetica, doi 10.1007/s11099-016-0230-x.

Simmons S.R.: Growth, development and physiology. – In: Heyne E.G. (ed.): Wheat and Wheat Improvement. Pp. 77-113. Am. Soc. Agron. Inc., Madison 1987.

Singal H.B., Sheoran I.S., Singh R.: *In vitro* enzyme activities and products of $^{14}CO_2$ assimilation in flag leaf and ear parts of wheat (*Triticum aestivum* L.). – Photosynth. Res. **8**: 113-122, 1986.

Tambussi E.A., Bort J., Guiamet J.J. *et al.*: The photosynthetic role of ears in C3 cereals: metabolism, water use efficiency and contribution to grain yield. – Crit. Rev. Plant Sci. **26**: 1-16, 2007.

Tambussi E.A., Nogués S., Araus J.L.: Ear of durum wheat under water stress: water relations and photosynthetic metabolism. – Planta **221**: 446-458, 2005.

Teare I.D., Peterson C.J., Law A.G.: Size and frequency of leaf stomata in cultivars of triticum aestivum and other *Triticum* species. – Crop Sci. **11**: 496-498, 1971.

Teare I.D., Sij L.W., Waldren R.P., Goltz S.M.: Comparative data on the rate of photosynthesis, respiration and transpiration of different organs in awned and awnless isogenic lines of wheat. – Can. J. Plant Sci. **52**: 965-971,1972.

Turner N.C.: Techniques and experimental approaches for the measurement of plant water status. – Plant Soil **58**: 339-366, 1981.

Vohlidal J.: Explanatory glossary of terms used in expression of relative isotope ratios and gas ratios. – International union of pure and applied chemistry **30**, ISSN: 0193-6484, 2008.

Wahid A., Gelani S., Ashraf M., Foolad M. R.: Heat tolerance in plants: an overview. – Environ. Exp. Bot. **61**: 199-223, 2007.

Wang Z.M., Wei A.L., Zheng D.M.: Photosynthetic characteristics of non-leaf organs of winter wheat cultivars differing in ear type and their relationship with grain mass per ear. – Photosynthetica **39**: 239-244, 2001.

Wirth E., Kelly G.J., Fischbeck G., Latzko E.: Enzyme activities and products of CO_2 fixation in various photosynthetic organs of wheat and oat. – Z. Pflanzenphysiol. **82**:78-87, 1976.

Ziegler-Jöns A.: Gas-exchange of ears of cereals in response to carbon dioxide and light. II. Occurrence of a C3-C4 intermediate type of photosynthesis. – Planta **178**:164-175, 1989.