The Journal of Animal & Plant Sciences, 25(6): 2015, Page: 1633-1643
ISSN: 1018-7081

PHOTOSYNTHETIC PERFORMANCE IN PLOIDY LEVELS AND AMPHYPLOIDS OF WHEAT DURING DEVELOPMENTAL STAGES

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ABSTRACT

In order to determine the changing trends of some physiological characteristics, biological yield, and flag leaf area in wheat ploidy levels, while also comparing them with *Triticale* and *Tritipyrum* amphyploids, 23 genotypes were evaluated in a field-grown condition. Stomatal conductance (g_s), net photosynthesis rate (Pn), transpiration rate (Tr), substomatal CO_2 concentration (C_i), and water use efficiency (WUE) were measured through three developmental stages including tillering (Til), early grain filling (EGF) and late grain filling (LGF). Different traits in *Triticale* and *Tritipyrum* genotypes showed a significant difference in comparison with wheat ploidy genotypes. Besides, a significance difference was observed between the ploidy levels. At EGF stage, there was a considerable disparity among *Triticum* and *Triticale* amphiploids for C_i, Tr, and WUE. *Triticum* and *Tritipyrum* genotypes statistically demonstrated dissimilar, but meaningful trends in all of the assessed physiological traits except for Pn. Moreover, *Triticale* and *Tritipyrum* amphiploids represented to have various results in terms of g_s. Results showed that the amounts of g_s, Pn, and WUE have increased noticeably until EGF stage and they have declined by 50% at LGF stage. The highest mean values corresponding to C_i (Til, EGF, LGF), Pn (LGF), Tr (EGF), and g_s (EGF, LGF) traits were belonged to *Tritipyrum* species which likely relates the breeding capability of such newfound amphiploid genotypes. Correlation coefficients analysis revealed that Pn had positive association with g_s and Tr in the Til and LGF stages. Throughout the whole phases of the study, Tr and WUE have increased with increasing g_s and Pn. Reductions of g_s and Tr in EGF stage have caused WUE to increase. In 4x and 6x wheat genotypes, the biological yield was positively associated with Pn and Tr. In 4x wheat, the flag leaf area had a negative correlation with Pn and WUE, while in 6x wheat, along with the flag leaf area increasing, Pn and Tr have soared and C_i has decreased. This research was an attempt to assist wheat breeders in selecting well-suited breeding lines and physiological parameters, leading to producing high-yielding genotypes in *Triticum spp.*

Keywords: Developmental stages, photosynthesis performance, physiological responses, *Triticum* spp.

INTRODUCTION

All the world's existing wheat is related to *Triticum* genus and has about six thousand species categorized into three main groups: diploid, tetraploid and hexaploid wheat (Chantret *et al*., 2005; Jablonskytė-Raščė *et al*., 2013). Nowadays, the increasing use of improved cultivars and constant struggle with weeds in modern agriculture brought about a tangible reduction in genetic diversity of wheat. Wild grass species, which are considered as relatives of wheat, have almost desirable characteristics of transferability to bread or durum wheat (Li and Gill, 2006). Since the early 19th century, hybridization between species has been an important method for gene pool enrichment and transfers the desired traits of species to wheat. The most important goal of using wheat interspecific hybridization is to increase tolerance to environmental stress in the progeny (Sleper and Peohlman, 2006). *Triticale* (*Triticosecale* Wittmack) is obtained from the crossing of wheat and rye as female and male parents, respectively. Compared with the parents, it can produce a high-protein yield in marginal and infertile soils, due to its wider range of adaptability. Moreover, it is more compatible to the low temperature and dry conditions than other grains (Oettler, 2005). In addition, crosses between tetraploid wheat (with genome AABB and 2n=4x=28) as a female parent and salty beach (*Tinopyrum bessarabicum)* (with E^bE^b genome and 2n=2x=14) as a male parent led to the emergence of tolerant synthetic wheat to salinity which was known as *Tritipyrum* (Hassani *et al*., 2006).

Recently, scientists have paid more attention to genetic research or heritability of physiological traits and demonstrated that selection of varieties with high yield, based on these traits, can accelerate the breeding program

(Xiong *et al*., 2006; Chunyan *et al*., 2008; Hui *et al*., 2008; Maosong *et al*., 2008). Furthermore, photosynthetic traits measuring might have been one of the valuable methods to obtain qualitative and quantitative information about plant responses to stress conditions (Khajehpour, 2004).

The physiological traits of wheat are chiefly influenced by nuclear genome, and researchers believed that these traits could be improved by using genome of wheat ancestors (Del blanco *et al*., 2000; Chunyan *et al*., 2008). The increased expression of the 'D' genome of tetraploid wheat caused a plunge in the net photosynthesis rate and chlorophyll content (as the main cause of photosynthesis); however, this reduction depends on the source of 'D' genome. 'D' genome has also weakened the negative correlation between flag leaf area and gas exchange (CO_2 exchange rate– CER) (Del Belanco *et al*., 2000). Zhang *et al*. (2000) found that the 'A' genome chromosomes of wheat carry genes that control high net photosynthesis rate, water use efficiency and below transpiration rate. Studies indicated that, during the development process, by the increase in the ploidy levels of wheat (2x→4x→6x), photosynthetic rate and transpiration rate decreased occasionally (Austin *et al*., 1987; Huang *et al*, 2007). Variations in the number and size of leaf stomata, that caused subsequent changes in the exchange of $CO_{2,}$ are the most important factors to take into consideration in photosynthetic rate changes. On the other hand, reports by Planchon and Fesquet (1982) revealed that the photosynthetic rate per unit leaf area highly depends on leaf size and the presence of 'D' genome; besides, the diploid genotypes (with A or B genome) have higher photosynthetic rate, stomatal resistance and thus higher water use efficiency compared to the other ploidy levels.

The present research examined the effects of ploidy levels on some physiological traits and compared *Triticale* and *Tritipyrum* amphiplods with wheat for the studied traits. Moreover, the influence of developmental stages on the physiological traits and the correlation of these traits with each other have been investigated.

MATERIALS AND METHODS

Plant material and meteorological condition: Two diploid genotypes (*Triticum monococcum* subsp. *monococcum* with A^mA^m genome), nine tetraploid genotypes (*T. turgidum* with AABB genome), eight genotypes of hexaploid (*T. aestivum* with AABBDD genome), two genotypes of *Triticale* (*Triticosecal*e Wittmack with AABBRR genome), and two genotypes of *Tritipyrum* ($AABBE^bE^b$) were included in this study. All of the samples were obtained from the Agriculture and Natural Resources Research Centre of Shahrekord, Iran. Table 1 shows the list of genotypes, according to their country of origin, species and ploidy levels. The region has a cold semi-arid climate with hot summer days, mild summer nights, cool winter days and cold winter nights. Shahrekord has two main seasons such as wet (mid November–April/May) and dry (June–mid November) in a year. The mean annual temperature and rainfall in Shahrekord is 10.8°C and 334 mm, respectively. In the experimental site, rainfall normally started during the fall and winter and continued until mid-spring (May).

Site of study, experimental design and soil type: The experiments were carried out on the agricultural research farm (latitude 50˚ 49́ E, longitude 32˚ 21́ N, elevation 2050 m above mean sea level), Faculty of Agriculture of Shahrekord University during 2007-2008 (from 11th November 2007 through early July 2008) in randomized complete block design (RCBD) with three replications. The soil of the experimental site was of arid calcareous clay loam type, top layer naturally enriched with calcium carbonate (~30% in volume), low in nitrogen (0.06%), low in organic carbon (0.48%), alkaline in reaction, with pH (pH [H_2O] in 1:2.5 ratio) of 7.1 and EC =0.32 $dS.m^{-1}$.

Sampling procedures, growth conditions and measurements: Five plants in each experimental unit were randomly selected, tagged and were used for the record of physiological characteristics in the three developmental (tillering–Til, early grain filling–EGF, and late grain filling–LGF) stages. The assessments carried out on the uppermost plant leaf in tillering phase and flag leaf in the EGF and LGF stages. Achieved data associated with five samples per plot were then averaged and recorded for upcoming analysis. Aligned with the work of Khajehpour (2004), uniform seeds were immersed in distilled water at 25ºC for 24 h and planted in the experimental unit for seedling establishment on 11th November 2007. Each experimental plot consisted of 4 rows (2.5 m length) with 20 cm row spacing and plant to plant spacing of 3 cm. A month later, the uniform seedlings were selected for the experimental setup based on their uniformity in relation to stem height and the number of leaves and leaflets. Each experimental plot filled with 16 kg of yellow loam latosol which dried at room temperature and sieved to remove undesired elements. Acidity of substrate was adjusted to a pH of about 6.0, using macronutrients (nitrogen, N; phosphate, P; and potassium, K), by adding 30 g NPK (10: 10: 10, w/w/w) per plot. Throughout the experiment, the plants were grown under conditions with an average of diurnal photosynthetic photon flux (PPF) of 490 $\mu mol\ m^{-2}\ s^{-1}$, average of diurnal relative air humidity (RH) and air temperature (T_{ar}) of 80% and 28ºC, respectively. PPF was measured with a quantum sensor attached to a steady-state porometer (Li-1600; LiCor Bioscience, Lincoln, USA); then, RH and T_{ar} were registered with thermo hygrometer (m5203, Incoterm Ind., Porto Alegre, Brazil) placed in the experimental area. Irrigation was performed daily to maintain soil near field capacity by replacing evapotranspired water. Weeds were manually controlled weekly. Photosynthetic gas exchange was measured weekly during the first and second week in each developmental stages. Net photosynthesis rate or net assimilation rate (Pn in µmol CO_2 m^{-2} s^{-1}) was measured at fixed CO_2 concentration rate (ambient CO_2 – Ca at 400 µmol CO_2 m^{-2} s^{-1}), air temperature (32ºC) and relative humidity (60%). Photosynthetic light responses were measured according to Rouhi *et al*. (2007) using portable IRGA (Infra Red Gas Analyser) instrument (LI 6400, LI-COR, Lincoln, USA Int.). Beside the net photosynthesis rate, the gas exchange instrument also provided data on the stomatal conductance for water vapour (g_s in mol H_2O m^{-2} s^{-1}), intercellular CO_2 concentration (C_i in µmol CO_2 mol^{-1} air), and transpiration rate (Tr, µmol H_2O m^{-2} s^{-1}) at the tillering (Til), early grain filling (EGF) and late grain filling stages (LGF), corresponding to developmental stages 29, 73 and 83, respectively (Zadoks *et al*.,1974).

Table 1. Species, ploidy level, genotype name and origin of genetic materials used in this study.

Genome	Species	Ploidy level	Genotype	Origin/description
Diploid wheat (A^mA^m)	*T. moncoccum* ssp. *Monococcum*	14=2x=2n	5196	SPII[*]
	T. moncoccum ssp. *Monococcum*	14=2x=2n	3829	SPII
Tetraploid wheat (AABB)	*T. turgidum* ssp. *Durum*	28=4x=2n	Ajar	Shahrekord-Iran
	T. turgidum ssp. *Durum*	28=4x=2n	Dezful 548	Dezful-Iran
	T. turgidum ssp. *Durum*	28=4x=2n	Dezful 549	Dezful-Iran
	T. turgidum ssp. *Durum*	28=4x=2n	G9580B-FE1C	Canada
	T. turgidum ssp. *Durum*	28=4x=2n	AC Navigator	Canada
	T. turgidum ssp. *Durum*	28=4x=2n	Golden bal	Africa
	T. turgidum ssp. *Durum*	28=4x=2n	Dipper-6	CIMMYT [α]
	T. turgidum ssp. *Durum*	28=4x=2n	AJAia/…/gan	CIMMYT
	T. turgidum ssp. *Durum*	28=4x=2n	PI40098	CIMMYT
Hexaploid wheat (AABBDD)	*T. aestivum*	42=6x=2n	Arvand	Iran
	T. aestivum	42=6x=2n	Sardari	Iran
	T. aestivum	42=6x=2n	5242	SPII
	T. aestivum	42=6x=2n	5074	SPII
	T. aestivum	42=6x=2n	P89110G1D3	Canada
	T. aestivum	42=6x=2n	ES32	Canada
	T. aestivum	42=6x=2n	E0091&AC4CG	Canada
	T. aestivum	42=6x=2n	E0091&AC4AV	Canada
Triticale (AABBRR)	*Triticosecale* Wittmack	42=6x=2n	Ma_{45}	Kerman-Iran
	Triticosecale Wittmack	42=6x=2n	Canadian	Canada
Tritipyrum ($AABBE^bE^b$)	Longdom/*Thinopyrum bessarabicum*	42=6x=2n	La/b	Kerman-Iran [β]
	(Karim/*Thinopyrum bessarabicum*) × (Creso/*Thinopyrum bessarabicum*)	42=6x=2n	Cr/b×Kab	Kerman-Iran

Notes: [*]– Germplasm Collection Unit, Cereal Research Department, Seed and Plant Improvement Institute (SPII) Iran; [α] – International Maize And Wheat Improvement Center; [β] – Kindly Provided by Dr. Shahsavand Hasani, Bahonar University, Kerman, Iran.

Following Hui *et al*. (2008), Pn (µmol CO_2 m^{-2} s^{-1}) / Tr (mmol H_2O m^{-2} s^{-1}) ratio was regarded as an estimation criterion of the intrinsic water use efficiency (WUE, µmol CO_2 $mmol^{-1}$ H_2O) for all developmental phases of Til, EGF, and LGF. Measurements were performed on five plants in each plot on the flag leaf from the top, representing a fully expanded leaf. Only undamaged leaves were selected. Each week, measurements were conducted during two consecutive days from 10 am to 12 am. Plants were measured following a fixing order by species (*Triticum*, *Triticale* and *Tritipyrum*).

Leaf area (LA, mm^2) per plant was determined by means of a planimeter (AM 200, ADC Co., England). In addition, biological yield (g m^{-2}) was measured by harvesting one square meter from the longitudinal centre of each experimental unit at crop maturity. The samples were dried in an oven for 72 h and then were weighed.

Statistical analysis: All the statistical analyses were conducted using SAS program (SAS Institute, 2009). The analysis of variance with the PROC GLM procedure was applied to distinguish the effect of ploidy levels and amphyploid on the analysis traits. The mean separation was conducted through the t-test to eliminate the effect of ploidy levels and other factors at 1% significance level ($p < 0.01$). In addition, the PROC CORR procedure was used to determine the coefficient values of Pearson Correlation existing between all the traits. Photosynthetic parameters of each species were obtained after fitting the light response data obtained from each species to the equation describing the light response curve of photosynthesis according to Khajehpour (2004). Orthogonal comparisons were used to compare the spiciness' characteristics. In addition, the mean results of the developmental stages (Til, EGF and LGF) were compared by paired t-test for the removal of ploidy levels effect and other factors.

RESULTS

The results of the variance analysis showed that the genotype effect was significant ($p < 0.01$; Table 2) for stomatal conductance (g_s), net photosynthesis rate (Pn), transpiration rate (Tr), substomatal CO_2 concentration (C_i) and water use efficiency (WUE) throughout the three developmental phases, at tillering stage (Til), early grain filling stage (EGF) and late grain filling stage (LGF). The effect of the recorded phases in all of the corresponding traits was significant, with the exception of C_i. In addition, the interaction between the genotype and the stages was also found to be significant for all of the traits. Mean values of the physiological traits including g_s and orthogonal comparison of five *Triticea* species during the tillering, early-grain filling (EGF) and late-grain filling (LGF) stages are presented in Table 2.

In the tillering stage (Til), *T. turgidum* (4x) had significantly ($p < 0.01$) higher g_s than other species. At the early grain filling stage (EGF), *T. aestivum* (6x), *Triticale* and *Tritipyrum* showed higher g_s than tillering stage and the highest upsurge was related to *Tritipyrum* (0.05 mol m^{-2} s^{-1} to 0.13 mol m^{-2} s^{-1}) (Table 2). Moreover, in comparison with other species, *T. monococcum* (2x) had the lowest g_s. At this stage, the g_s decreased with the advancement of grain filling from EGF to LGF stage, and wheat ploidy levels showed lower g_s compared to *Triticale* and *Tritipryrum.*

Net photosynthesis rate in 2x (6.55 µmol m^{-2} s^{-1}) and 4x (7.05 µmol m^{-2} s^{-1}) wheat species at the tillering stage showed more Pn values compared to other species (Table 2). However, at the EGF stage, *Tritipyrum* (9.61 µmol m^{-2} s^{-1}), 2x (9.63 µmol m^{-2} s^{-1}) and 4x (9.38 µmol m^{-2} s^{-1}) wheat species had a higher photosynthesis rate (Pn) than *T. aestivum* (6x) and *Triticale*. The order of species for Pn at the LGF stage was *Triticale*> *Tritipyrum*> 4x> 6x> 2x, respectively.

In the tillering stage, 4x and 6x wheat species had the maximum (2.81 mmol $m^{-2}s^{-1}$) and minimum (2.14 mmol $m^{-2}s^{-1}$) values of transpiration rate (Tr) amongst all the species, respectively. At this stage, *Tritipyrum* (2.44 mmol $m^{-2}s^{-1}$) and 2x (2.47 mmol $m^{-2}s^{-1}$) species had higher Tr values in comparison with *Triticale* (2.34 mmol $m^{-2}s^{-1}$; Table 2). However, with increasing age from the tillering to early grain filling stage in *Triticale* and *Tritipyrum* amphiploids, the Tr has increased. Transpiration rate in 2x and 4x wheat species was higher than hexaploid wheat at early filling stage (EGF), but, however, in the LGF stage, 4x and 6x species had higher Tr in comparison with the 2x species.

The biological yield or total above-ground dry weight in diploid wheat (1648.1 g m^{-2}) species was superior to *Triticale* (1636 g m^{-2}; Table 2). The tetraploids produced as much dry matter as the hexaploids and did not show a significant difference by *Tritipyrum*. Furthermore, the highest and lowest mean values for flag leaf area was related to tetraploid (210.5 mm^2) and diploid (105.5 mm^2) wheat species, respectively, and they had significant differences in comparison with other species.

Mean values of five physiological traits (g_s, Pn, Tr, C_i, WUE) during the tillering, early- and late-grain filling phases for the targeted wheat genotype materials, and paired comparisons of these stages are shown in Table 3. Stomatal conductance (g_s) has increased from 0.065 mol m^{-2} s^{-1} at tillering stage to 0.075 mol m^{-2} s^{-1} at EGF stage; however, at the LGF phase, g_s has dropped about 50% (Table 3). In the tillering stage, the mean value of Pn was 6.29 µmol m^{-2} s^{-1}, while in the EGF stage the trend is upward, reaching a peak of 9.21 µmol m^{-2} s^{-1} and it bounces back into 3.48 µmol m^{-2} s^{-1} in the LGF phase. In addition, Tr at the EGF stage was more than tillering and LGF stages. The C_i in the tillering stage (150.74 µmol mol^{-1}) was more than EGF stage (136.54 µmol mol^{-1}). Although the C_i has been increased at LGF stage, this increase was not statistically significant (Table 3). Results on the effect of the different developmental phases on the WUE showed that, as compared to the tillering (2.58 µmol $mmol^{-1}$) stage, there was a significant increase in EGF (3.19 µmol $mmol^{-1}$) stage, which thereafter decreased up to 50% in LGF (1.31 µmol $mmol^{-1}$) stage.

Simple correlation coefficients (Table 4) showed that during the tillering and LGF stages, g_s and Pn had a significant positive relationship ($p < 0.01$). On the other hand, at these stages, high g_s was correlated closely with high Pn. Throughout the whole growth stages (i.e. Til, EGF, LGF), there existed a strong positive correlation between stomatal conductance and transpiration rate ($p < 0.01$). During the tillering and EGF stages, the g_s was positively correlated with C_i. During the early grain filling (EGF) stage, a strong and negative correlation between g_s and WUE was observed ($r = -0.62$; $p < 0.01$). At the tillering and LGF stages, by increasing the net photosynthetic rate, the transpiration rate has enhanced (Table 4). In other words, Pn maintained a positive relationship with Tr during the developmental stages of Til and LGF. Throughout the whole of growth stages, Pn and WUE were strongly positively correlated to each other ($p < 0.01$). Moreover, during the late grain filling stage, the Pn was negatively correlated with C_i ($r = -0.39$; $p < 0.01$). Associated with the increased trend of Tr there

Table2. Mean values of physiological traits and orthogonal mean comparison of five *Triticea* species during tillering (Til), early-grain filling (EGF) and late-grain filling (LGF) stages.

Species	**Mean values**																
	Stomatal conductance (g_s) mol m^{-2} s^{-1}			**Net photosynthetic rate (Pn) µmol $m^{-2}s^{-1}$**			**Transpiration rate (Tr) mmol $m^{-2}s^{-1}$**			**Substomatal CO_2 concentration (C_i) µmol mol^{-1}**			**Water use efficiency (WUE) µmol $mmol^{-1}$**			**Biological yield**	**Flag leaf area**
	Til	**EGF**	**LGF**	**Til**	**EGF**	**LGF**	**Til**	**EGF**	**LGF**	**Til**	**EGF**	**LGF**	**Til**	**EGF**	**LGF**	**(g m^{-2})**	**(mm^2)**
T. monococcum(2x)	0.05	0.05	0.024	6.55	9.63	2.34	2.47	2.82	2.00	155.11	143.58	148.74	2.64	3.47	1.17	1648.1	105.5
T. turgidum(4x)	0.09	0.08	0.034	7.06	9.38	3.43	2.81	2.93	2.55	150.45	125.80	145.50	2.57	3.22	1.34	1235.5	210.5
T. aestivum(6x)	0.05	0.07	0.034	5.53	8.91	3.18	2.14	2.73	2.49	146.24	132.78	145.84	2.61	3.32	1.28	1199.7	190.3
Triticale	0.06	0.08	0.051	5.98	8.86	5.40	2.34	3.51	3.45	154.76	160.74	149.43	2.56	2.90	1.56	1636.0	178.3
Tritipyrum	0.05	0.13	0.054	5.93	9.61	4.14	2.44	3.85	3.41	161.70	168.74	173.08	2.43	2.56	1.20	1353.7	188.2
	Orthogonal mean comparison of five species$^{\infty}$																
2x *vs* 4x	0.04**	0.03**	0.010**	0.51	0.25	1.09**	0.34**	0.11	0.55**	4.66	17.78**	3.24	0.02	0.25	0.17**	412.60**	105.0**
2x *vs* 6x	0.00	0.02**	0.010**	1.02*	0.72*	0.84**	0.33*	0.09	0.49**	8.87	10.8**	2.9	0.01	0.15	0.11*	448.40**	84.8**
2x *vs Triticale*	0.01	0.03**	0.027**	0.57	0.77	3.06**	0.13	0.69**	1.45**	0.35	17.16**	0.69	0.02	0.57**	0.39**	12.10	52.8**
2x *vs Tritipyrum*	0.00	0.08**	0.030**	0.62	0.02	1.80**	0.03	1.03**	1.41**	6.59	25.16**	24.34**	0.13	0.91**	0.03	294.40*	82.7**
4x *vs* 6x	0.04**	0.01*	0.000	1.53**	0.47*	0.25*	0.67**	0.20**	0.06	4.21	6.98**	0.34	0.01	0.10	0.06	35.80	20.2*
4x *vs Triticale*	0.03**	0.00	0.017**	1.08**	0.52	1.97**	0.47**	0.58**	0.90**	4.31	34.94**	3.93	0.00	0.32*	0.22**	400.50**	32.2*
4x *vs Tritipyrum*	0.04**	0.05**	0.020**	1.13**	0.23	0.71**	0.37**	0.92**	0.86**	11.25	42.94**	27.58**	0.10	0.66**	0.14*	118.20	22.3*
6x *vs Triticale*	0.01*	0.01	0.017**	0.45	0.05	2.22**	0.20	0.78**	0.96**	8.52	27.96**	3.59	0.01	0.42**	0.28**	436.30**	12.0
6x *vs Tritipyrum*	0.00	0.06**	0.020**	0.40	0.70*	0.96**	0.30*	1.12**	0.92**	15.46	35.96**	27.24**	0.15	0.76**	0.08	154.00	2.1
Triticale vs Tritipyrum	0.01	0.05**	0.003	0.05	0.75	1.26**	0.10	0.34*	0.04	6.94	8.00	23.65**	0.05	0.34	0.36**	282.30*	9.9

Notes: *, ** – Significant at 0.05 and 0.01 probability levels, respectively provided by *ANOVA*; ∞ – absolute value of species means differences are showed.

was a significant decrease of WUE (r = -0.70; $p < 0.01$) in the early grain filling stage, while the trend was positively correlated with C_i (r = 0.26; $p < 0.05$). This showed that at this stage, the C_i was significantly affected by transpiration rate.

Due to the importance of EGF stage and its role in biological yield, and moreover the flag leaf area as the most essential source for photosynthetic and dry matter production (Irani *et al*., 2010), the correlation between these two traits and some physiological properties of tetraploid (4x) and hexaploid (6x) wheat species were investigated during the EGF stage (Table 5). Since a large number of data were available, only those species having a key role in human consumption and cattle feeding (i.e. *T. turgidum* – 4x, *T. aestivum* – 6x) were selected for correlation study.

In both the 4x and 6x wheat species, with the increased amount of Pn and Tr, the biological yield has enhanced as well. In addition, as the g_s has increased in hexaploid wheat, the biological yield has increased as well. In tetraploid species, with more flag leaf area, net photosynthesis rate and water use efficiency were lower; however, in the hexaploid species with more flag leaf area, Pn and Tr have increased and C_i has declined.

Table 3. Mean values and comparison of tillering, early-grain filling and late-grain filling stages for stomatal conductance (g_s), net photosynthetic rate (Pn), and transpiration rate (Tr), substomatal CO_2 concentration (C_i) and water use efficiency (WUE) in all studied species.

Physiological stages	g_s mol m^{-2} s^{-1}	Pn µmol $m^{-2}s^{-1}$	Tr mmol $m^{-2}s^{-1}$	C_i µmol mol^{-1}	WUE µmol $mmol^{-1}$
Tillering (Til)	0.065	6.29	2.47	150.74	2.58
Early-grain filling (EGF)	0.075	9.21	2.98	136.54	3.19
Late-grain filling (LGF)	0.036	3.48	2.63	148.63	1.31
Til vs EGF	0.010	2.92^{**}	0.50^{**}	14.2^{*}	0.61^{**}
Til vs LGF	0.029^{**}	2.81^{**}	0.15	2.11	1.27^{**}
LGF vs EGF	0.039^{**}	5.73^{**}	0.35^{*}	12.09	1.88^{**}

*, ** –significant at 0.05 and 0.01 probability levels, respectively on the base of *t* test.

Table 4. Correlation coefficient (*r*) between physiological traits during developmental stages.

Physiological stages	Traits	g_s	Pn	Tr	C_i
Tillering (Til)	Pn	0.44^{**}			
	Tr	0.55^{**}	0.81^{**}		
	C_i	0.38^{**}	-0.13	0.17	
	WUE	-0.14	0.34^{**}	-0.11	-0.45^{**}
Early grain filling (EGF)	Pn	-0.05			
	Tr	0.70^{**}	0.08		
	C_i	0.35^{**}	-0.17	0.26^{*}	
	WUE	-0.62^{**}	0.41^{**}	-0.70^{**}	-0.37^{**}
Late grain filling (LGF)	Pn	0.67^{**}			
	Tr	0.83^{**}	0.78^{**}		
	C_i	0.19	-0.39^{**}	-0.12	
	WUE	0.07	0.65^{**}	0.11	-0.53^{**}

Notes: * – $p < 0.05$, ** – $p < 0.01$; explanation about Pn, Tr, C_i and WUE terms is given in Table 3.

Table 5. Correlation coefficient (*r*) between biological yield, flag leaf area and physiological traits during early-grain filling (EGF) phase for *Triticum turgidum* and *Triticum aestivum*.

Species	Traits	Stomatal conductance (g`$_s$)	Net photosynthetic rate (Pn)	Transpiration rate (Tr)	Substomatal CO_2 concentration (C_i)	Water use Efficiency (WUE)	Flag leaf area
Triticum turgidum (4x)	Biological yield	0.29_{ns}	0.46^{*}	0.38^{*}	0.27_{ns}	0.11_{ns}	0.08_{ns}
	Flag leaf area	-0.09_{ns}	-0.42^{*}	-0.01ns	0.04_{ns}	-0.40^{*}	1
Triticum aestivum (6x)	Biological yield	0.51^{*}	0.50^{*}	0.45^{*}	-0.08_{ns}	-0.04_{ns}	0.41^{*}
	Flag leaf area	0.31_{ns}	0.78^{**}	0.48^{*}	-0.50^{*}	0.06_{ns}	1

* – $p < 0.05$, ** – $p < 0.01$, ns – not significant.

DISCUSSION

Stomatal conductance (g_s): In the present study, wheat tetraploid species had the highest g_s in the tillering stage than other species; however, in EGF stage, g_s has increased in hexaploid wheat, *Tritipyrum* and *Triticale* (Table 2). In LGF stage, wheat ploidy had fewer amount of g_s in comparison with *Triticale* and *Tritipyrum*. The results indicated that some g_s attributed differences were influenced by the developmental stage, in addition to the effect of genome type. Zhang and Shan (2003) reported that g_s order were RR>DD>AA>BB in five diploid species. Different results were obtained in our study as the studied species were combination of these genomes. The high level of g_s in *Tritipyrum*, which is investigated for the first time in terms of physiological traits, especially in the EGF stage, might indicate that genes involved in the E^bE^b genome are effective on this trait. Significant changes that *Tritipyrum* showed in this trait probably indicate the effect of development period on this species. Superiority of g_s in tetraploid and hexaploid wheat over diploid species could be attributed to the increasing number of chromosomes within the cell nucleus. This affects cell number and volume, which may lead subsequently to an improved photoperiodic response and ultimately elevates the g_s.

Net photosynthetic rate (Pn): Comparing the wheat ploidy levels indicates that both 2x and 4x species in tillering stage have more Pn in comparison with hexaploid species and Pn has decreased from 2x to 6x in the EGF stage. However, unlike the two previous stages, 2x species had the lowest level of Pn in LGF stage. Several studies on the photosynthetic capacity of the various ploidy levels of wheat illustrated that the photosynthetic rate per unit leaf area basis declined with the increase in ploidy, *i.e*. from diploid to hexaploid species, whereas Pn per unit cell basis increased along with the increase in ploidy level (Srivalli and Khanna-Chopra, 2004; Huang *et al*., 2007; Maosong *et al*., 2008). Researchers stated that the reduction of photosynthesis from 2x to 6x is due to the enlarged mesophylle cells of leaf that reduce the surface area to volume ratio of the cells; hence, CO_2 exchange is carried out with greater resistance (Srivalli and Khanna-Chopra, 2004). Austin *et al.* (1987) observed that chlorophyll content may also reduce the amount of Pn in hexaploid species and, under the high light intensity, diploid wheats tend to have a higher ratio of chlorophyll a/b than hexaploid wheats. The higher chlorophyll a/b ratio indicates a higher concentration of photosystems per chlorophyll (*i.e*., a smaller photosynthetic unit size); therefore, 2x species has high net photosynthesis rate than other species. Del Blanco *et al.* (2000) claimed that differences in the diffusion pathway of CO_2 were the main reasons for the variation seen in Pn. Srivalli and Khanna-Chopra (2004) believed that Pn is considerably influenced by source-sink relationships, which are different in wheat across ploidies. Austin *et al.* (1982) found that the diploid species with 'A' genome have more net photosynthesis rate than *Aegilops tauschii* as the D-genome donor.

The results showed that, on each stage, the examined wheat species with maximum and minimum of Pn are different. For instance, similar to 6x, *Triticale* was low in tillering and EGF stages. However, in the LGF stage, Pn was higher in this amphyploids comparing to all species; it is apparent that in addition to the possible effect of genome, the effect of developmental stage is also striking in different species. Generally, g_s and Pn in wheat ploidy levels have declined abruptly in the LGF stage compared with the *Triticale* and *Tritipyrum* species; therefore, these amphiplods, especially *Triticale*, are more capable of maintaining photosynthetic rate stability. However, in wheat ploidy levels by approaching to later developmental stages along with g_s reduction, Pn also has decreased.

Transpiration rate (Tr): Hexaploid wheat had the lowest Tr in tillering and EGF stages compared with other species (Table 2). According to the results by Maosong *et al.* (2008), when ploidy levels in the wheat increase, the stomata become larger and its density decreases; therefore, the conductivity and photosynthetic rate in hexaploid wheat decreases. In the present study, Tr in tetraploid species in the grain filling period and moreover flag leaf size in this species was higher compared with the hexaploid species. Under the same environmental conditions and for a given stomatal resistance (or g_s), the transpiration per unit leaf area was higher for the broad leaf species. Thus, in the course of evolution in wheat, an increase in transpiration per unit leaf area has paralleled the increase in the leaf size. This can be clearly seen in the tetraploid (with AB genome) genotypes. However, the decrease in the g_s in the hexaploid (with ABD genome) cultivated types has reversed this trend and *Triticum aestivum* L. has a lower transpiration rate than AB genome tetraploids (Planchon and Fesquet, 1982).

Transpiration rate (Tr) in diploid and tetraploid species in the EGF stage was higher than hexaploid species; however, in the LGF stage, tetraploid and hexaploid species had a higher transpiration rate than the diploid ones. With the increasing age, especially in EGF and LGF stages, Tr has increased in *Triticale* and *Tritipyrum* amphiploids compared with wheat ploidy levels which probably caused an increase in g_s. One argument is that stomata in these amphiploids was more active compared with ploidy level; with increasing the plant age, even in the LGF stage, stomata action has not decreased greatly, which ultimately led to higher rate of Tr, g_s and Pn in the mentioned amphiploids during LGF stage.

Substomatal CO_2 concentration (C_i): In the three developmental stages, *Tritipyrum* had the highest rank for C_i (Table 2). There were not statistically significant differences between species in tillering stage, but *Triticale* and 2x wheat species showed greater C_i in comparison with 4x and 6x wheat. *Triticale* and *Tritipyrum* had similar levels of C_i in the EGF stage. In addition, on wheat ploidy levels, 2x and 4x showed the highest and the lowest C_i, respectively. In the LGF stage, *Tritipyrum* had the highest C_i and other species had no differences statistically. The results showed that almost the highest concentration of C_i in the three developmental stages belonged to the *Tritipyrum* species (Table 2). Seemingly, the E^bE^b genome effect has disabled *Tritipyrum* amphiploid from using C_i actively, to some extent. There was no significant difference between species in the tillering stage; however, in the EGF stage, C_i has increased in *Triticale* as well as *Tritipyrum* and these species had significant differences in wheat ploidy levels. Among ploidy levels, diploid and tetraploid had the highest and the lowest C_i, respectively. The highest C_i was observed in *Tritipyrum* in the LGF stage and other species did not differ statistically. In general, it can be said that hexaploid and tetraploid wheat species are likely more capable for the use of C_i, but the effect of growth stage, particularly for the *Triticale* amphiploid, should not be forgotten.

Water use efficiency (WUE): There was no significant difference between the species in tillering stage for WUE (Table 2). In EGF stage, wheat ploidy levels showed significantly ($p < 0.01$) higher WUE in comparison with *Tritipyrum* and *Triticale*. In this stage, the order of wheat species was 2x> 6x> 4x, respectively. Nonetheless, in LGF stage *Triticale* showed the maximum of WUE (1.56 μmol mmol^{-1}) and the least amount of it was related to 2x wheat species (1.17 μmol mmol^{-1}) and also 4x, 6x and *Tritipyrum* hovered around medium level. There was no significant difference between species for WUE in tillering stage (Table 2). Given that each species, in the time span, have shown different g_s, Pn and Tr, consequently between these species have not observed any significant difference for WUE. Since WUE leaf obtained by ratio of Pn/Tr, it is evident that a factor that affects the two traits will also influence WUE (Shao *et al.*, 2006; Hui *et al.*, 2008). In EGF stage, wheat ploidy levels had higher WUE compared with *Triticale* and *Tritipyrum*, and the high transpiration rate in *Tritipyrum* and *Triticale* causes WUE reduction in these species to a great extent. However, in LGF stage, WUE in *Triticale* and wheat diploid species increased and decreased respectively, and tetraploid, hexaploid wheat and *Tritipyrum* were in the moderate condition. It is apparent that RR and E^bE^b genomes in *Triticale* and *Tritipyrum* are effective for having higher g_s, Tr and lower WUE. Notwithstanding, in the LGF stage, the highest WUE in *Triticale* compared with other species probably illustrates that besides the genomic differences between these species, special attention should be paid to the environmental and developmental stages. Zhang *et al*. (2000) showed that chromosome 4R of addition lines of wheat-rye also contained genes conferring high WUE, while in the study of Cao *et al*. (2007), comparison of flag leaf WUE of different genomes was AA>BB>DD>RR. It can be stated that, there was no significant difference between ploidy levels in three stages of this study; however, some studies indicated that WUE of leaves and the power of water absorption have increased along with increasing ploidy levels (Zhang *et al*., 2000, Cao *et al*., 2007). Further, it may be the result of using different methods in WUE measurement.

Khazaei *et al.* (2009) indicated that 2x and 6x wheat have higher WUE than the 4x species which were poorly explained by differences in their capacity to extract water. The *Tritipyrum* genotypes, especially throughout grain development, almost had the highest mean value for Pn, g_s and C_i. These results probably indicate desirable genetic potential in replacing some chromosomes of *Tinopyrum bessarabicum* (E^bE^b genome) in *Tritipyrum* species with ‘D’ genome in wheat. Therefore, it provides a possibility, in terms of physiological traits, to achieve appropriate *Tritipyrum* genotypes. However, the low average of WUE in *Tritipyrum* can be attributed to high g_s and Tr. The concurrent increase of WUE and g_s is of outstanding importance because it enhances CO_2 attraction. It is clear that these traits should not be contradictory; therefore, this issue requires further study and analysis. It is apparent that *Tritipyrum* is a new amphyploid and still needs breeding measures for some of its traits (Hassani *et al*., 2006). Thus, low WUE and biological yield in *Tritipyrum* genotypes were not unexpected.

Flag leaf area: There was no significant difference between wheat ploidy, *Triticale* and *Tritipyrum* for flag leaf area. In *Triticale* and diploid species, compared with the rest of species, there were significant differences in terms of biological yield (Table 2). Maximum and minimum biological yield was observed in diploid and hexaploid species. The low tiller production in hexaploid (data not shown) compared with other species is likely one of the reasons for low biological yield in these genotypes. In a study on different ploidy levels, Khazaei *et al*. (2009) reported that the total dry matter in diploid genotypes was higher due to the high tillering (mostly sterile). Einkorn wheat (*Triticum monococcum* subsp. *monococcum*) was found previously to have a similar or even higher biomass compared with bread wheat, suggesting their prolonged growth period (Al Hakimi *et al*., 1996).

Higher leaf area, as the most essential source in photosynthetic product, probably will lead to an increase in the photosynthetic rate (Irani *et al*., 2010). The results of this study showed that diploid and tetraploid wheat species had the smallest and the largest flag leaf area, respectively (Table 2). Some studies (Maosong *et al*., 2008; Khazaei *et al*., 2009) indicated that flag leaf area had risen with the increase in wheat ploidy. Nonetheless, in the present study, most of the tetraploid wheat genotypes, compared with the hexaploid genotypes, had more length and leaf area. In their study, Araus *et al*. (1989) showed that flag leaf area in the tetraploid species were higher in comparison with hexaploid species and these species did not differ for Pn, g_s, C_i and WUE. On the other hand, Xiong *et al.* (2006) showed that ploidy levels of wheat were not significantly different in terms of flag leaf area.

The results of this study showed that g_s, Pn and WUE of genotypes increased in the EGF compared with tillering stage but reduced by almost half during the late grain filling (Table 3). Stomatal conductance and resistance depend on the frequency and the extent of stomata opening (characteristic that are various between leaves with different age, environmental conditions, and different genotypes). It is unanimously believed that the degree of stomatal opening also depends on the environmental factors such as leaf water potential, minerals, diseases, light, the concentration of CO_2, temperature, moisture and soil environment (Hay and Porter, 2006). Although at early growth stages, the young plants are exposed to low temperature and despite the fact that their g_s is moderate, the transpiration rate (Tr) is low. Gradually, with plant growth, temperature increases in the final stages of grain filling, and plant opens its stomata slightly which leads to g_s reduction. On the other hand, g_s reduction declines CO_2 intake and decreases the rate of photosynthesis. The temperature rise at the end of growth stage was effective in closing the stomata and reducing the transpiration rate.

Probably, one of the reasons behind the increase in photosynthetic rate and thus WUE, from tillering to the EGF stage, is due to the increase in g_s, which consequently activates the photosynthetic system to absorb C_i quickly and leads to more production. Comparing to tillering and LGF, C_i in EGF has decreased due to high photosynthetic rate; however, in the LGF stage, reduced g_s and other gas exchanges as well as aging of the plant and the photosynthetic organs along with reduced chlorophyll concentration and leaf nitrogen, decrease the photosynthetic rate eventually and C_i accumulates in the substomatal environment and its concentration increases. WUE has also changed due to a direct relationship between photosynthetic rate and this trait under different stages. In three stages of this study, C_i has decreased with increasing WUE (Table 4).

Correlations evaluation: Correlation coefficients between the traits indicated that with increasing g_s, Pn in tillering and LGF stages and also Tr and C_i have increased in the three stages (Table 4). The correlation between g_s and mentioned traits might be indicative of the fact that stomata can be considered as a main factor to limit the ability of photosynthesis and transpiration during crop growth. Wang *et al.* (2010) stated that g_s in wheat had a positive correlation with C_i, Tr and Pn, while it had a negative correlation with WUE. Typically, g_s is associated with photosynthesis and transpiration. This is related to the distribution route that is the same for CO_2 and water and it seems that with increasing in CO_2 for a fixed number of stomata, g_s and also WUE would increase. However, Del-Blanco *et al.* (2000) believed that CO_2 fixation can be increased without widening the stomatal opening, the stomatal conductance and transpiration; it denotes that photosynthesis will not necessarily increase with more water use. However, Chunyan *et al.* (2008) proposed that stomata were the main factor for net photosynthesis rate changing during the evolution of wheat.

Water use efficiency showed significant positive and negative correlation with Pn and $C_{i,}$ respectively in the three stages (Table 4), although correlation between WUE and the other physiological traits (g_s and Tr) was almost not clear in the stages. Hui *et al.* (2008) also showed that the absolute values of the correlation coefficients of corresponding traits to transpiration efficiency (TE) as the indicator of WUE should be ordered as $Pn>g_s>Tr>C_i$ in the early grain filling stage, $C_i>Tr>Pn>g_s$ in the middle grain filling stage, and $Pn>C_i>Tr>g_s$ in the late grain filling stage, respectively. In the present study, WUE has decreased with an increase in g_s and Tr, during the EGF stage, which represented a higher transpiration rate compared with g_s. Hence, the plants have lost more water, compared with CO_2 absorption.

In the three stages with Pn increasing and C_i reducing, WUE increased. In this regard, similar results were obtained by Baodi *et al.* (2008) and Wang *et al.* (2010). It seems that upsurge in CO_2 concentrations along with g_s and Tr increasing, are not appropriate to raise water use efficiency. Hui *et al.* (2008) believed that the indices of Tr and C_i had negative relationships with WUE in the middle-to-late grain filling stage; hence, it is important to select genotypes with low Tr and C_i during grain filling period. It should be noted that this relation also depends on the amount of air humidity. On the other hand, Zhang *et al.* (2010) illustrated that WUE with g_s and Pn had a positive correlation but only for Pn the correlation was significant.

In tetraploid and hexaploid species with increasing Pn and Tr, biological yield has increased in the EGF stage (Table 5). Furthermore, in the intervals, with the increase in g_s, biological yield in hexaploid species has increased. Net assimilation of CO_2 through the process of photosynthesis is the initial step for biomass production or biological yield (Del Blanco *et al*., 2000). It seems that selecting genotypes with high net photosynthesis rate in hexaploid and tetraploid wheat species can provide the possibility to achieve higher biological yield. Wang *et al.* (2010) believed that photosynthesis, as a key metabolic process for matter production in plants, is physiological basis of crop biomass yield. No significant relationship between biological yield and WUE was observed in the 4x and 6x wheat species. Khazaei *et al.* (2009) also showed that improvements in leaf water use efficiency in C_3 crop plants may not always translate into higher crop yield. In tetraploid wheat species, along with flag leaf area increasing, Pn and WUE have declined and it seems that smaller leaves have more Pn and WUE (Table 5). However, in the hexaploid wheat species, with flag leaf area rising, Pn and Tr have boomed and C_i has decreased. Nevertheless, Planchon and Fesquet (1982) believed that the Pn per unit leaf area depends markedly on the leaf size and on the presence or absence of the 'D' genome, and for the same leaf surface area the genotypes that do not bear the 'D' genome are more efficient than those which possess it. Zhang *et al*. (2010) reported no significant correlation between flag leaf area and water use efficiency.

In the 6x wheat species, probably a positive correlation between flag leaf area and biological yield demonstrated that the selection for high flag leaf area was beneficial to achieve genotypes with high Pn and biological yield. Planchon and Fesquet (1982) believed that an optimum flag leaf size might be determined accordingly, taking into account the different parameters involved in productivity such as photosynthesis, transpiration and translocation.

Conclusions: 1-Our results are influenced by genetic and environment along with the genome and developmental stages. In other words, a significant difference was observed between the ploidy levels, *Triticale* and *Tritipyrum.* As an example, in 4x and 6x wheat genotypes, a significant increase was observed in the amount of Pn, Tr and biological yield. Besides, the amounts of g_s, Pn, and WUE have increased noticeably from tillering to the EGF stage and they have all declined by 50% in the LGF stage. 2-This germplasm can play an important role in raising the biological yield and WUE through breeding in near future and could be useful to improve wheat resistance to stress conditions. 3-Till date, most of the researches conducted over physiological traits have only been concerned with the existed differences among varieties, chromosomal location of a specific gene, and molecular markers, but the genes directly relating to these traits remained unsolved or, in particular, the genetic relationship of physiological responses between these traits is still unclear. 4-The results of the study are expected to help wheat breeders to select appropriate breeding lines, parameters and threshold of characters which evolve high yielding genotypes in *Triticum* spp.

Acknowledgments: The authors would like to express the deepest gratitude to Shahrekord University for their financial assistance and the reviewers for their constructive comments on the improvement of the manuscript.

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