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RESEARCH ARTICLE

Evaluation of physiological markers for assessing drought tolerance and yield potential in bread wheat

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Abstract Carbon isotope discrimination (Δ) has been recognized as a valuable phenotyping tool in wheat breeding. However, technical expertise and analysis cost restrict its large-scale use. We examined the associations of ash content (AC), minerals content (Ca, K, Mg, Fe and Mn) and leaf chlorophyll content (*Chl*) with grain Δ and grain yield (GY) to assess their potential as substitute to grain Δ. We evaluated 49 wheat genotypes under two water deficit regimes (W_{120} and W_{200}) in a rain-out shelter. Leaf chlorophyll content (*Chl*) was strongly correlated with grain Δ and GY under moderate water deficit regime (W_{200}). Significant and negative correlations ($P < 0.01$) of AC and potassium concentration (K) with grain Δ, and between AC and GY was observed under both water regimes, while manganese concentration (Mn) was negatively correlated with grain Δ under W_{120} regime only and magnesium concentration (Mg) correlated negatively under the W_{200} regime only. Grain Δ was correlated ($P < 0.01$) positively with photosynthesis rate (*A*), stomatal conductance (g_s) and GY, while correlated negatively ($P < 0.01$) with intrinsic water use efficiency (iWUE) under both water regimes. Results confirm the role of grain Δ as an indirect selection criterion for drought tolerance under a wide range of drought conditions. Additionally, *Chl* is the most suitable trait to predict yield under moderate water deficit conditions. AC and K concentration in grain proved potentially useful and economical alternative criterion to grain Δ in the evaluation of differences in yield potential and drought tolerance in wheat under drought.

Keywords Physiological · Photosynthesis · Ash content · Carbon isotope discrimination · Leaf chlorophyll content · Mineral content

Introduction

Drought stress is a severe threat to the growth and productivity of bread wheat, which is one of the most cultivated and important food crops on the earth (Farooq et al. 2015). Therefore, most of the breeding programs focused on enhancing its yield capacity and tolerance to drought. However, the appraisal of suitable phenotyping traits remains a serious bottleneck in the successful breeding of cereal crops for drought tolerance.

Selection for morphological and physiological traits may be an effective methodology in conventional as well as in molecular breeding to uplift crop yield under different

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growing conditions (Razmjoo et al. 2008). However, insufficient knowledge about plant physiological processes that determine the yield capacity and adjustment to drought, together with the appropriate phenotypic evaluation tools, are the main factors restraining present breeding strategies (Richards et al. 2010).

Carbon isotope discrimination (Δ) is an effective physiological tool, which has been utilized in breeding approaches to develop wheat cultivars better acclimatized to drought conditions. It indicates a time-integrated measurement of the ratio of intercellular to ambient CO_2 (C_i/C_a) and water use efficiency (WUE), thus can be used as reciprocating method for screening cultivars capable of higher WUE (Slafer and Araus 2007). In C_3 plants, Δ is determined by C_i/C_a, and linking these values with each other owing to difference in the equilibrium between stomatal behavior and carboxylation capacity. Correlations among Δ and gas exchange parameters have been reported in wheat and other cereal crops under different water stress conditions (Monneveux et al. 2006; Yasir et al. 2013). Use of Δ in wheat breeding programs is advocated due to its high heritability, considerable genetic variation and insignificant genotype by environment interface. It is an integrated assessment over the whole life of the plant, thus can be measured at any point during plant growth. However, the high cost and the precise technical skills and facilities involved in Δ analysis have limited its extensive use (Tsialtas et al. 2002). Therefore, reliable surrogates for Δ are necessary to be developed as breeding tool for the screening of germplasm.

Leaf chlorophyll (*Chl*) content is an index of the photosynthetic potential of plants. Positive correlation between leaf chlorophyll content and photosynthetic capacity suggested its importance as an efficient trait for breeding crop plants with better yield (Teng et al. 2004). The association among *Chl* contents and grain yield has been found to be significant and positive in durum wheat (Guendouz et al. 2012), while significant and negative in bread wheat (Mohammadi et al. 2009). In addition, positive and negative correlations between Δ and leaf *Chl* contents have been reported in peanut (Rowland et al. 2012). Such kinds of relationships suggest the effectiveness of *Chl* contents as a useful, inexpensive and easily utilized tool to screen out the bulk of germplasm.

The minerals content, in vegetative tissues (Zhu et al. 2010) as well as in grains (Bogale and Tesfaye 2011) have been proposed as economical and easy method in forecasting yield and genotypic adaptation to drought in C_3 cereals. However, the studies on the relationships between Δ and individual mineral content and *Chl* content are scarce. Significant correlations between Δ and minerals content (P, K, Mg and Si) of plant organs of durum wheat, planted under water stressed environments, have been described by Merah et al. (2001). Zhu et al. (2010) also reported positive correlation of Δ with Mg and Ca and negative correlation with K in flag leaves of wheat under different water stress conditions. The mineral accumulation in vegetative tissues occurs during transpiration when passive movement of minerals takes place via xylem and in kernels via phloem (Nonogaki et al. 2007). Thus, mineral contents of vegetative parts give the indication of gas exchange taking place in transpiration process while mineral contents of kernel could give the status of photosynthesis activity and re-translocation process (Araus et al. 2001). However, this is important to evaluate whether the association between grain minerals and grain yield can be extended to different growing conditions, to determine the mechanism of such a relationship, and to test the feasibility of grain mineral as a complementary criterion to grain Δ while assessing genotypic differences in yield.

This study was executed to (1) estimate the effect of restricted irrigation during grain filling stage on Δ, grain yield, gas exchange parameters, leaf chlorophyll content, and minerals content (Ca, K, Mg, Fe and Mn), (2) to examine the relationships among Δ, grain yield, gas exchange parameters, leaf chlorophyll content, ash content and mineral concentrations, and (3) to determine the probable use of mineral contents and leaf chlorophyll contents substitute to Δ for the evaluation of yield and drought tolerance in a diverse group of bread wheat genotypes.

Materials and methods

Plant material

Forty-nine bread wheat (*Triticum aestivum* L.) genotypes being cultivated in winter and spring wheat regions of China were evaluated in this study. Out of which 46 were released cultivars, extensively cultivated in China and also used in different breeding projects, (Table 1). Genotypes also included a Chinese landrace and two Australian genotypes (Rebetzke et al. 2006). The detail about genotypes is given in Table 1.

Experimental conditions

This study was conducted at the Institute of Water Saving Agriculture in Arid Regions of China, Northwest A&F University, Yangling (latitude of 34°17.7′N, longitude of 108°4.05′E and altitude of 468 m), Shaanxi, China. To simulate controlled irrigation and to neglect the influence of erratic rainfall, the experiment was conducted in a partially controlled environment by growing the genotypes in a rainout shelter. The experimental soil was Loutu (Chinese

Table 1 List of genotypes with their planting regions and origins

No.	Genotype	Planting region	Origin[a]
1	Luohan 6	Huang-huai WWR	Henan
2	Xinmai 19	Huang-huai WWR	Henan
3	Zhou 17	Huang-huai WWR	Henan
4	Zhou 19	Huang-huai WWR	Henan
5	Luohan 21	Huang-huai WWR	Henan
6	Shijiazhuang 8	Huang-huai WWR	Hebei
7	Changwu 134	Huang-huai WWR	Shaanxi
8	Changwu 863	Huang-huai WWR	Shaanxi
9	Changwu 521-7	Huang-huai WWR	Shaanxi
10	Shaan 229	Huang-huai WWR	Shaanxi
11	Xiaoyan 6	Huang-huai WWR	Shaanxi
12	Shanmai 168	Huang-huai WWR	Shaanxi
13	Pubing 201	Huang-huai WWR	Shaanxi
14	Shaan 512	Huang-huai WWR	Shaanxi
15	Xiaoyan 22-3	Huang-huai WWR	Shaanxi
16	Pubing 143	Huang-huai WWR	Shaanxi
17	Xinong 389	Huang-huai WWR	Shaanxi
18	Liken 2	Huang-huai WWR	Shaanxi
19	Lantian 10	Huang-huai WWR	Shaanxi
20	Xinong 811	Huang-huai WWR	Shaanxi
21	Qinnong 712	Huang-huai WWR	Shaanxi
22	Jiufeng 22	Huang-huai WWR	Shaanxi
23	Ligao 6	Huang-huai WWR	Shaanxi
24	Changwu 58-61	Huang-huai WWR	Shaanxi
25	Yuanfeng 175	Huang-huai WWR	Shaanxi
26	Yuanfeng 139	Huang-huai WWR	Shaanxi
27	Fengchan 3	Huang-huai WWR	Shaanxi
28	Xinong 889	Huang-huai WWR	Shaanxi
29	Xinong 979	Huang-huai WWR	Shaanxi
30	Xinong 928	Huang-huai WWR	Shaanxi
31	Shaan 7859	Huang-huai WWR	Shaanxi
32	Hanyou 98	Huang-huai WWR	Shandong
33	Yunhan 22-33	Huang-huai WWR	Shanxi
34	Xifeng 20	Huang-huai WWR	Gansu
35	Zhonghan 110	Northern WWR	Beijing
36	Jing 411	Northern WWR	Beijing
37	Xiaoyan 81	Northern WWR	Beijing
38	Youmai 2	Northern WWR	Shandong
39	Jinmai 47	Northern WWR	Shanxi
40	Jinan 18	Northern WWR	Shandong
41	Jining 13	Northern WWR	Shandong
42	Jining 18	Northern WWR	Shandong
43	Jinmai 33	Northern WWR	Shanxi
44	Jing 2001	Northwest WWR	Gansu
45	Ningchun 45	Northwest SWR	Ningxia
46	Hongmangmai (landrace)	Northwest SWR	Ningxia
47	Mianyang 11	Southwestern WWR	Sichuan
48	Drysdale	Australia	New South Wales

Table 1 continued

No.	Genotype	Planting region	Origin[a]
49	Quarrion	Australia	New South Wales

WWR winter wheat region, *SWR* spring wheat region

[a] Chinese province/Australian state

soil Taxonomy) having mean bulk density of 1.4 g cm^{-3}, organic matter content of 0.84% and pH 7.7. The monthly average air temperature varied from − 1.7 to 13.8 °C during winter (germination to stem extension stages) and 16.3–25.1 °C during summer (booting to maturity stages), while monthly average relative humidity fluctuated from 55.7 to 86.5% over the crop growing period. All genotypes were planted on 20 October, 2011 in four-row plots (100-cm rows with 6.7-cm spacing between plants and 25-cm between rows), with a plant density of 60 m^{-2}. The treatments were set up under randomized complete block design with split plot arrangement having three replications. Restricted irrigation treatments (severe water deficit, W_{120} = 120 mm and moderate water deficit, W_{200} = 200 mm) were assigned in main plots while genotypes were assigned and randomized in sub plots under each main plot treatment. A light irrigation was applied in all treatments after sowing, to ensure homogeneous germination. Three restricted irrigations each at 40 mm were given to both the W_{120} and W_{200} water deficit treatments, by using a water-meter, at the tillering stage (BBCH 23), stem extension stage (BBCH 39) and booting stage (BBCH 49). An extra irrigation of 80 mm was applied to the W_{200} treatment at the fruit development stage (BBCH 73), while this irrigation was skipped in W_{120} in order to force moisture stress conditions at fruit development stages. The rest of the agronomic practices were kept uniform for all the treatments.

Leaf gas exchange parameters

Gas exchange parameters were measured, on fully expanded middle part of flag leaf between 09:00 and 13:00 time on a sunny and clear day at fruit development stages (BBCH 75 and 76), with a portable photosynthesis system (Li-Cor 6400; Li-Cor Inc., Lincoln, NE, USA). For measurements, five flag leaves per genotype per treatment were taken at random and their average value was used for final data. The Li-Cor 6400's LED light source was used to set PPFD at 1000 µmol m^{-2} s^{-1} while reference CO_2 was set at 400 ppm inside the chamber by keeping the flow rate constant at 500 µmol m^{-2} s^{-1}. Leaf temperature and chamber relative humidity were maintained at 28 °C and 40–50% respectively and reading was taken when the leaf was acclimated to the chamber environment. Photosynthesis rate (A), stomatal conductance to water vapour (g_s) and the ratio of intercellular CO_2 concentration to ambient CO_2 concentration (C_i/C_a) were calculated as described by Von Caemmerer and Farquhar (1981). The intrinsic water use efficiency (iWUE) was taken as A/g_s.

Carbon isotope discrimination

After threshing, 10 g grains were taken from each genotype per treatment and milled to a fine powder. The $^{13}C/^{12}C$ (R) were analyzed by using an Isotopic Ratio Mass Spectrometer (Delta V advantage, Thermo Fisher Scientific, Inc., USA) interfaced with an element analyzer (Flash EA1112 HT) by the Lab of Stable Isotopes, Chinese Academy of Forestry Sciences (Beijing). Results were stated as $\delta^{13}C$ (‰) = [(R sample/R reference) − 1] × 1000, R being $^{13}C/^{12}C$ ratio. A secondary standard (potato starch) calibrated against Pee Dee Belemnite (PDB) carbonate was used for comparison. The following formula was used to calculate Δ value (Farquhar et al. 1989):

$$\Delta(‰) = [(\delta a - \delta p)/(1 + \delta p)] \times 1000$$

where δp is $\delta^{13}C$ of sample and δa, the $\delta^{13}C$ of atmospheric CO_2. On the PDB scale, atmospheric CO_2 has a current deviation of approximately − 8‰ (Farquhar et al. 1989).

Ash and minerals content

Ash and minerals content were determined using the method 08-01 (American Association of Cereal Chemists, AACC, 1995). Grains from each genotype per treatment were milled to make fine powder and then dried by using oven at 72 °C for 48 h. Each sample was divided into two replicates and about 1.0 g of dry mass was taken to incinerate at 575 °C for 16 h. Ash contents (%) were calculated on a dry mass basis. Potassium (K), Magnesium (Mg), Calcium (Ca), Iron (Fe) and Manganese (Mn) contents were determined from the ash samples following Lin et al. (2003) by using flame atomic absorption spectrophotometer (Hitachi Z-2000, Japan).

Grain yield

At maturity (BBCH 99) spikes of ten plants from each genotype per treatment were separated and threshed, then, were dried to determine grain weight. The grain weight was converted into grain yield expressed as g m^{-2}.

Leaf chlorophyll content

The leaf chlorophyll content (*Chl*) was observed on fully expanded flag leaf of ten randomly selected plants from each genotype per treatment at fruit development stages (BBCH 75 and 76). Chlorophyll content was assessed by using optical measurements of leaf absorbance, at two different wavelengths of light i.e. red (650 nm) and near infra-red (940 nm), using portable chlorophyll meter (SPAD-502, Minolta Camera Co. Osaka, Japan) and the total chlorophyll content was expressed in relative SPAD units. This value is related to the chlorophyll content as the SPAD meter uses the two absorbance values to compute a dimensional numeric value (Padilla et al. 2018).

Statistical analysis

Analysis of variance (ANOVA) was done by using SAS GLM procedure (SAS version 8.01, SAS Institute, 2001, Cary, NC, USA) to estimate the overall significance of data. Treatments means were compared by using Duncan's multiple comparison test ($P < 0.05$). Pearson's correlation was used to find out the correlation between all pairs of measured parameters by using SPSS 16.0 (SPSS Inc. 2007).

Results

Effect of water regime and genotype on measured traits

Water regime, wheat genotypes and interaction between them had significant ($P < 0.01$) effect on grain yield (Table 2). Wheat genotypes sown under W_{200} had higher grain yield than W_{120} water deficit treatment, with a yield reduction of 36% in W_{120} treatment (Table 2). Among tested genotypes, grain yield ranged from 165.0 to 320.5 g m^{-2} and 299.2 to 489.4 g m^{-2}, in the W_{120} and W_{200} water treatments, respectively (Table 2). Grain Δ differed significantly ($P < 0.05$) both between water regimes and among genotypes (Table 2). The mean grain Δ values of 49 wheat genotypes were 18.37% and 18.99% under W_{120} and W_{200} water regimes, respectively. Gas exchange parameters (*A*, g_s, iWUE and C_i/C_a) were also significantly ($P < 0.05$) affected by water regimes, genotypes and the interaction of water regime and genotypes (Table 2). All the gas exchange parameters increased significantly from W_{120} to W_{200} treatment, but iWUE followed the opposite trend, by decreasing in the W_{200}

Table 2 Analysis of variance showing the mean squares of carbon isotope discrimination, grain yield, gas exchange parameters, leaf chlorophyll, ash content and minerals content of 49 bread wheat genotypes grown under two water regimes (W_{120} and W_{200})

Traits	Treatment (mean ± SD)		Source of variation			
	W_{120}	W_{200}	W	G	W × G	C.V
Δ (‰)	18.37 ± 0.29^b	18.99 ± 0.32^a	9.143**	0.132**	0.102**	4.20
GY(g m^{-2})	247.45 ± 86.8^b	385.54 ± 43.37^a	934,398.413**	3362.338**	2998.788**	5.74
A (μmol m^{-2} s^{-1})	14.88 ± 1.98^b	20.25 ± 2.34^a	1415.914**	12.256*	7.129NS	16.13
g_s (mol m^{-2} s^{-1})	0.31 ± 0.08^b	0.61 ± 0.14^a	4.187**	0.0349**	0.017NS	24.32
iWUE (μmol mol^{-1})	58.82 ± 12.24^a	37.71 ± 8.15^b	21,847.601**	287.030**	145.646NS	22.68
C_i/C_a	0.71 ± 0.05^b	0.80 ± 0.03^a	0.3712**	0.005**	0.002NS	5.97
Chl (SPAD value)	51.73 ± 2.69^b	58.69 ± 3.01^a	428.416**	26.885**	5.701**	7.74
AC (%)	1.60 ± 0.17^a	1.53 ± 0.17^b	0.276**	0.067**	0.049**	8.66
Ca (mg g^{-1})	5.86 ± 1.29^a	4.31 ± 1.22^b	117.774**	3.461**	2.863**	23.8
K (mg g^{-1})	43.04 ± 6.13^a	40.74 ± 5.14^b	258.661**	67.986**	59.926**	8.74
Mg (mg g^{-1})	13.16 ± 2.50^a	11.97 ± 1.65^b	68.651**	11.118**	6.910**	11.77
Fe (mg g^{-1})	0.60 ± 0.10^a	0.56 ± 0.06^b	0.067**	0.0135**	0.0123*	15.06
Mn (mg g^{-1})	0.55 ± 0.09^a	0.43 ± 0.08^b	0.716**	0.016**	0.013**	8.71

W_{120}, severe water deficit regime; W_{200}, moderate water deficit regime; *NS* not significant; *$P < 0.05$; **$P < 0.01$. Data are the mean ± SD of 98 values (corresponding to 49 genotypes and two replications per genotype with the exception of Δ, which is based on a single replication). Values for a given trait with different superscript letters are significantly different according to Duncan's multiple range test, $P < 0.05$. Mean square values from the analysis of variance of the same variables are shown for the water regime (W), genotype (G) and interaction (W × G) effects compared to the W_{120}, water stress treatment (Table 2). Furthermore, *Chl* content were also significantly ($P < 0.01$) affected by water regime, genotype and interaction (W × G) with an increase of 6.96 in the W_{200} over the W_{120} water treatment (Table 2). Grain ash content (AC) and individual mineral (Ca, K, Mg, Fe and Mn) content differed significantly ($P < 0.01$) between water regimes, genotypes and under their interaction effect. However, in contrast to grain yield, *Chl* and grain Δ, the grain ash contents increased by 5% from W_{200} to W_{120} water deficit treatment (Table 2).

Relationship between Δ, GY, ash content and mineral concentrations

Significant and positive correlations were observed between grain Δ and grain yield under each water regime (Table 3) and genotypes with higher Δ value corresponded to higher grain yield, while genotypes with lower Δ value corresponded to lower grain yield under both water conditions (Fig. 1e). In contrast, grain Δ had negative correlation with grain AC and K content under both water regimes, and had negative correlation with Mn content in the W_{120} and with Mg in the W_{200} water regimes. However, there was no correlation between grain Δ and either Ca or Fe content at either water regime (Table 3).

Ash content correlated negatively with grain yield (Fig. 2a) and positively with all individual mineral (K, Mg, Fe and Mn) content except Ca under both water regimes (Table 3). Mn was the only mineral that was correlated with grain yield under the W_{200} treatment, while the other minerals did not show significant correlation with grain yield under any of the water treatments. Ca positively correlated with Fe only in W_{200} while K was significantly correlated with Mg, Fe, Mn in W_{200} and with Mn in W_{120}. Mg content were significantly associated with Fe and Mn in both water conditions and Fe content were significantly associated with Mn under both water conditions (Table 3).

Relationship between Δ, grain yield, gas exchange parameters and *Chl*

Grain Δ correlated positively with *A*, g_s, C_i/C_a and *Chl*, while it correlated negatively with iWUE in both water regimes (Table 4). Furthermore, grain yield correlated positively with *A* and g_s under both water regimes and only with *Chl* in W_{200} treatment. *A* and g_s were strongly correlated with each other and C_i/C_a, while they correlated negatively with iWUE under both water regimes. iWUE correlated negatively with C_i/C_a in both water regimes and only *Chl* in W_{120}, while C_i/C_a correlated positively only with *Chl* in W_{120} regime (Table 4).

Discussion

Effect of water stress on grain Δ and grain yield

In this study, different water conditions induced significant variations in grain yield and grain Δ for 49 wheat genotypes, which were likely related to the differences in soil water availability during grain filling. A decrease of 36% in grain yield was recorded in in W_{120} regime as compared to W_{200} regime (Table 2). The mean grain Δ was decreased

Table 3 Correlations between carbon isotope discrimination (Δ), grain yield (GY), Ash content (AC) and minerals (Ca, K, Mg, Fe and Mn) content of 49 bread wheat genotypes under W_{120} (severe water deficit) and W_{200} (moderate water deficit) regimes in a rainout shelter

	Δ	GY	Ash	Ca	K	Mg	Fe
W_{120} (severe water deficit regime)							
GY	0.683**						
Ash	− 0.485**	− 0.298*					
Ca	− 0.230	− 0.145	0.118				
K	− 0.434**	− 0.233	0.761**	− 0.062			
Mg	− 0.241	− 0.207	0.451**	0.067	0.230		
Fe	− 0.198	− 0.025	0.478**	0.252	0.166	0.623**	
Mn	− 0.291*	− 0.187	0.544**	0.088	0.383**	0.331*	0.412**
W_{200} (moderate water deficit regime)							
GY	0.498**						
Ash	− 0.467**	− 0.313*					
Ca	− 0.020	0.054	0.120				
K	− 0.379**	− 0.279	0.858**	− 0.012			
Mg	− 0.303*	− 0.24	0.745**	0.096	0.767**		
Fe	− 0.076	− 0.089	0.287*	0.296*	0.336*	0.424**	
Mn	− 0.254	− 0.284*	0.506**	0.156	0.508**	0.653**	0.328*

*$P = 0.05$; **$P = 0.01$

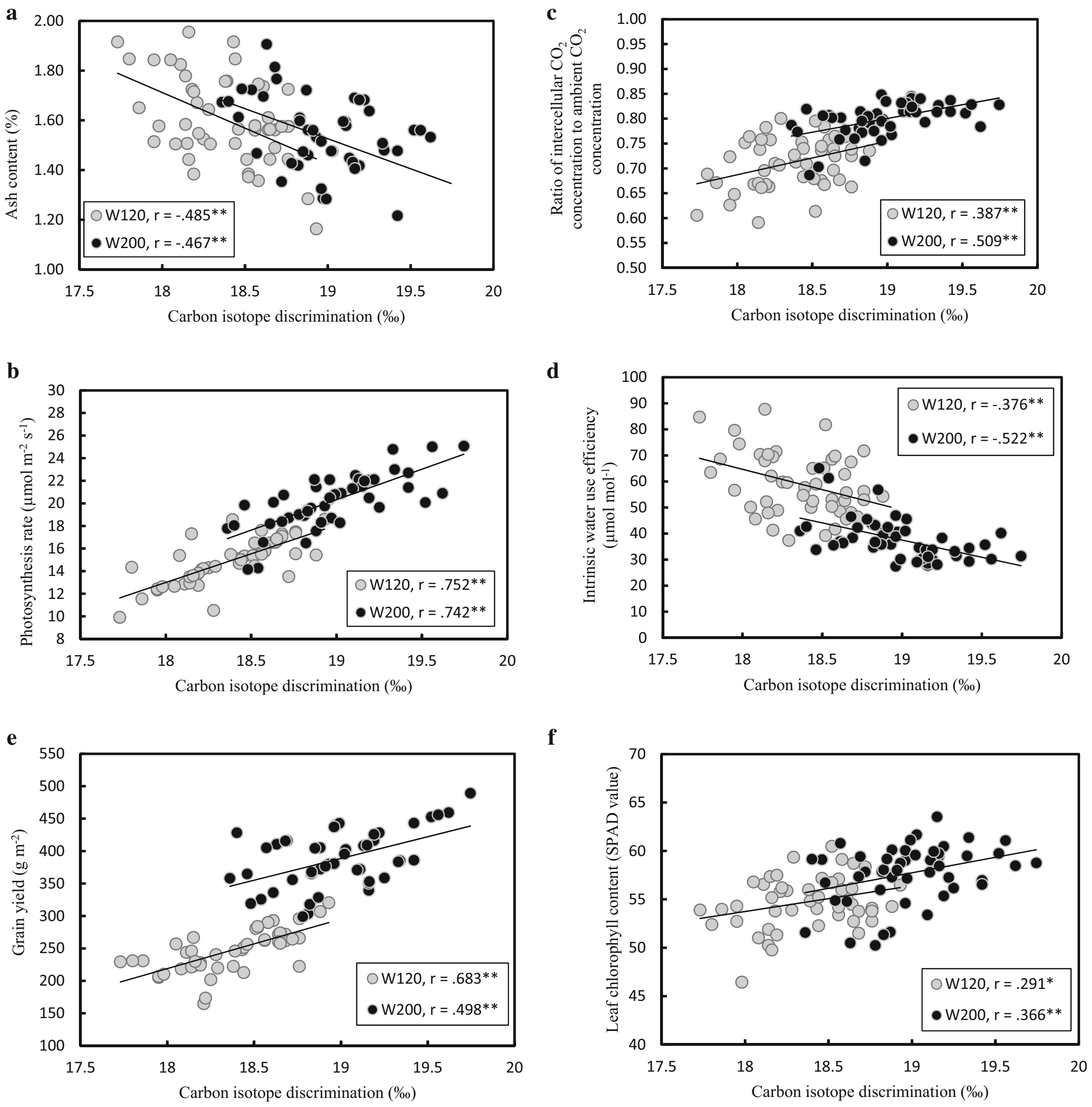

Fig. 1 Relationship between **a** carbon isotope discrimination and ash content, **b** carbon isotope discrimination and photosynthesis rate, **c** carbon isotope discrimination and ratio of intercellular CO_2 concentration to ambient CO_2 concentration, **d** carbon isotope discrimination and intrinsic water use efficiency, **e** carbon isotope discrimination and grain yield, **f** carbon isotope discrimination and leaf chlorophyll content, under two water deficit regimes (W_{120} and W_{200}). *$P < 0.05$; **$P < 0.01$

by 3.2% under W_{120} regime compared with W_{200} regime (Table 3). The lower grain yield and Δ value under W_{120} indicates the lower stomatal conductance in this treatment due to availability of reduced quantity of water in this treatment (Zhu et al. 2010; Yasir et al. 2013). The rise in evaporative demand owing to reduced soil moisture and humidity in the cropping area could probably be the reason of this low grain yield and grain Δ (Araus et al. 2003). The reduction in translocation of stored assimilates to the reproductive parts like wheat grains in W_{120} water regime might be the reason of reflecting low Δ values.

Effect of water stress on ash content and mineral concentrations

The higher grain ash and individual mineral (Ca, K, Mg, Fe and Mn) contents were noted in the W_{120} regime, which caused reduction in yield due to severe moisture stress in this treatment (Table 2). This increase can be explained by the mechanisms involved in mineral deposition in reproductive parts, which predominantly relies on the re-mobilization from vegetative parts and on the removal of minerals from these parts during senescence (Wardlaw 1990). Higher amount of photosynthates were produced in plants under W_{200} treatment due to the higher supply of

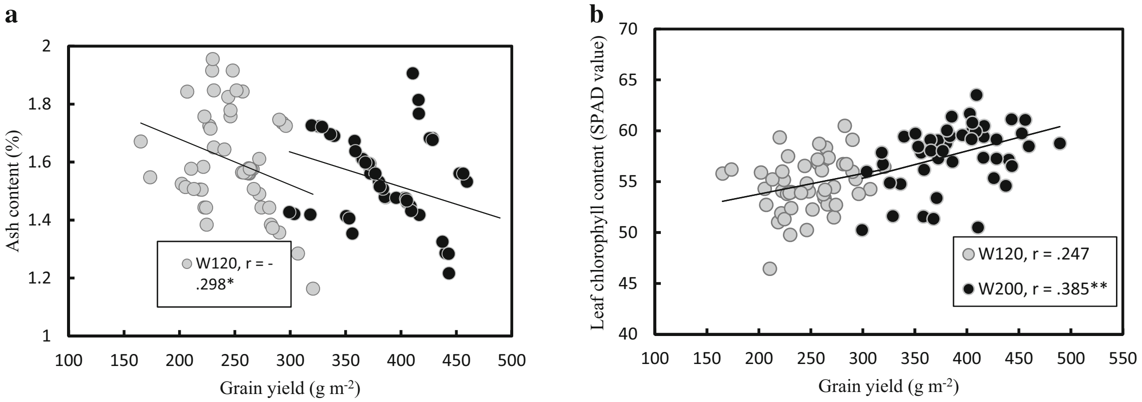

Fig. 2 Relationship between **a** grain yield and ash content, **b** grain yield and leaf chlorophyll content, under two water deficit regimes (W_{120} and W_{200}).). *$P < 0.05$; **$P < 0.01$

Table 4 Correlations between carbon isotope discrimination (Δ), grain yield (GY), gas exchange parameters (*A*, g_s, iWUE and C_i/C_a) and leaf chlorophyll content (*Chl*) of 49 bread wheat genotypes under W_{120} (severe water deficit) and W_{200} (moderate water deficit) regimes in a rainout shelter

	Δ	GY	*A*	g_s	iWUE	C_i/C_a
W_{120} (severe water deficit regime)						
GY	0.683**					
A	0.752**	0.535**				
g_s	0.596**	0.429**	0.701**			
iWUE	− 0.376**	− 0.298*	− 0.436**	− 0.804**		
C_i/C_a	0.387**	0.299*	0.437**	0.811**	− 0.999**	
Chl	0.291*	0.247	0.236	0.367**	− 0.368**	0.370**
W_{200} (moderate water deficit regime)						
GY	0.498**					
A	0.742**	0.440**				
g_s	0.678**	0.287*	0.866**			
iWUE	− 0.522**	− 0.249	− 0.671**	− 0.872**		
C_i/C_a	0.509**	0.243	0.646**	0.865**	− 0.994**	
Chl	0.366**	0.385**	0.240	0.316*	− 0.230	0.257

*$P = 0.05$; **$P = 0.01$

water, which resulted in increased proportion of carbohydrates in the starchy endosperm and decreased proportion of minerals in the grains. In moisture stress environments, the translocation of photosynthates is not affected as much as the process of photosynthesis (Loss and Siddique 1994). Therefore, remobilization of minerals from non-reproductive parts is therefore faster in drought stressed plants, leading to a relatively larger decrease of leaf mineral contents and a correspondingly larger increase of grain mineral contents compared to well-watered plants (Merah et al. 1999), as was observed in this study.

Effect of water stress on leaf chlorophyll content and gas exchange parameters

The reduction in *Chl* of leaves of the genotypes grown under W_{120} regime (Table 2) might be due to early leaf senescence which caused a reduction in chlorophyll concentration and an increase in chlorophyll degradation (Izanloo et al. 2008). Although, early leaf senescence and leaf abscission support plant existence under drought prone environments (Munné-Bosch and Alegre 2004), this situation can be responsible to yield reduction loss major crops (Rivero et al. 2007). The same was happened in our study in reduction of yield in W_{120} treatment due to early senescence.

In drought prone environments, the stomatal conductance controls the net flux of CO_2 into the leaf (Lawlor 2001). The decrease of the CO_2 assimilation rate is not only a function of stomata but the non-stomatic (metabolic) limitation can occur in conditions of long-lasting drought stress and cause reduction in leaf relative water content and water potential (Zivcak et al. 2013). To follow this fact, stomatal conductance caused a higher photosynthesis rate (20.25 $\mu mol\ m^{-2}\ s^{-1}$) under W_{200} and lower (14.88 $\mu mol\ m^{-2}\ s^{-1}$) under W_{120} (Table 2). There existed a significant difference in the mean values *A* and g_s under the two water stress treatments as the genotypes under W_{120} received 40% less water as compared to the genotypes under W_{200}. On average, *A* and g_s were reduced in W_{120} by 26% and 48%, respectively, as compared to W_{200} treatment (Table 2). The reduction of photosynthesis can be associated also with high temperature and, there exists the genotypic variance in photosynthesis and temperature response, especially between highly productive modern genotypes and landraces (Brestic et al. 2018).

Intrinsic water use efficiency (iWUE) was increased by 56% under W_{120} as compared to W_{200}. By contrast, lower values of *A* and g_s were observed in this treatment. Indeed, the iWUE is more closely related to g_s than photosynthesis rate (*A*), and therefore, reductions of g_s are accompanied by a greater increase in iWUE than reductions in *A* (Van den Boogaard et al. 1997). As for as the intensity of drought and its outcomes are concerned, the Stomatal conductance is rather a fine predictor than photosynthesis rate. In order to protect plants severe moisture stress, the stomata close, which confines the circulation of CO_2 into mesophyll parenchyma cells. This situation results in a greater decrease in water movement out of leaves than in photosynthesis rates, thus raising the WUE (Pou et al. 2008), as was observed in our study.

Relationship between Δ and gas exchange parameters

Δ and *A* were strongly correlated with g_s under the two water regimes (Figs. 1b, 3a, respectively), signifying that there was a stomatal constraint in both water treatments and the variation in *A* and C_i/C_a might be the result of variation in g_s existing in genotypes and are ultimately responsible for the variation in Δ (Zhu et al. 2010; Yasir et al. 2013). The highly significant and positive correlation between *A* and g_s (Fig. 3a) elaborates that the higher stomatal conductance under W_{200} than under W_{120} might enhance the entry of CO_2 into the chloroplast, which resulted in higher photosynthesis rate. Irrespective of the increased or decreased stomatal conductance, these gas exchange parameters have strong correlations among each others under both water stress conditions. It can be observed from the Fig. 3a that all genotypes have lower stomatal conductance and photosynthesis rate under W_{120} and more under W_{200}, but the magnitude of correlation is strong. The similar results have been reported in quinoa (Gonzalez et al. 2011), and rice (Centritto et al. 2009). In C_3 species, Δ is positively associated with C_i/C_a but negatively associated with WUE at the leaf level (Farquhar and Richards 1984). Consistent with these assumptions, in this study, Δ was correlated positively with C_i/C_a (r = 0.387, $P < 0.01$ and r = 0.509, $P < 0.01$ under W_{120} and W_{200}, respectively) but negatively with iWUE (Fig. 1c, d, respectively), under the two water treatments. Usually, photosynthetic capacity and the stomatal conductance are responsible to control C_i/C_a. The greater photosynthetic capacity or lesser stomatal conductance or their combination can be attributed to lower C_i/C_a and eventually lower Δ (Condon et al. 2002).

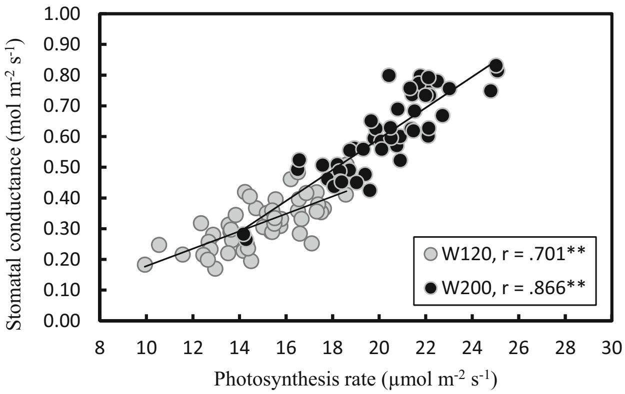

Fig. 3 Relationship between photosynthesis rate and stomatal conductance, under two water deficit regimes (W_{120} and W_{200}).). *$P < 0.05$; **$P < 0.01$

Relationship between Δ and grain yield

The magnitude of the relationship between Δ and grain yield was stronger in the severe water stress treatment (r = 0.683, $P < 0.01$) than under moderate water stress (r = 0.498, $P < 0.01$). Strong association between grain Δ and g_s obtained in our experiment under both water stress conditions (Table 4), can be explained by the theoretical understanding that both grain Δ and grain yield depend strongly on stomatal conductance. These results recommend that the grain Δ values can be utilized as selection criterions for higher yielding genotypes in water deficit environments.

Relationship of leaf chlorophyll content with grain yield and grain Δ

The positive correlations was observed between grain yield and chlorophyll content (Fig. 2b) under W_{120} (despite non-significant) and W_{200} treatments (r = 0.385, $P < 0.01$). This might be due to the positive relationship of leaf chlorophyll content with photosynthesis rate and stomatal conductance (Table 4). The chlorophyll content in leaves are found to be highly significant with photosynthetic capacity, nitrogen status of leaves and carboxylase activity of Rubisco in wheat and other crops (Seemann et al. 1987). Significant positive correlations between leaf chlorophyll content and Δ under both water regimes (Fig. 1f) might be the consequence of positive correlations between gas exchange parameters, *Chl* and grain yield. The nondestructive quantification of *Chl* and its relevancy with different essential nutrients has also been determined in tomato and maize (Kalaji et al. 2017). The results of current study have also confirmed the usefulness of leaf chlorophyll as an economic alternative for screening large numbers of germplasm for water use efficiency and, together with other traits, to predict yield under moderate water deficit conditions.

Relationship of ash content and minerals with grain Δ and grain yield

The relationships of ash content and individual mineral (Ca, K, Mg, Fe and Mn) content with grain Δ are variable in this study (Table 3). Negative correlations between grain yield and ash content (Fig. 2a) were found under both water deficit conditions. As the grain ash mainly contains minerals which are accumulated in grains through phloem transport, therefore mineral deposition in the grain chiefly depends upon translocation from the leaves. Due to this fact, the mineral proportion rather carbohydrates in the grain would be more under drought stress condition, which leads to negative correlation between ash content and grain yield. In plants, K is considered as a key osmotic solute and responsible for the movement of stomata. In moisture stress conditions, the stomata becomes more sensitive as the K concentration increases in the plant system. In a result, the stomatal conductance reduces and lowers the Δ value Likewise, the increase or decrease in K under different moisture conditions, the photosynthesis rate is also affected. In rice crop, the higher concentration of K gave rise in photosynthetic capacity (Fu et al. 1996). The higher concentration of K tandem with higher rates of photosynthesis could be responsible to produce low C_i/C_a and low Δ. This condition may magnifies the negative correlation between K concentration and Δ values. The same was experienced in our experiment under both water deficit treatments, while lower stomatal conductance led to lower grain Δ, the higher remobilization of minerals from vegetative parts led to an increase in grain mineral concentrations, and finally resulted in strong negative correlations between K and grain Δ. Negative correlation between Mn and grain Δ under W_{120} treatment was also found in this study, which has not been reported elsewhere. Moreover, we could not find any significant correlation (although it tended towards negative values) of Ca and Mg content with grain Δ in either water treatment.

Conclusions

The impact of drought was obvious in treatment W_{120}, where the values for grain yield, grain Δ, *Chl* and gas exchange parameters were significantly reduced and increased for AC and mineral contents. Grain Δ appeared as a prime predictor of iWUE and grain yield under both normal and severe water deficit conditions. Highly significant correlations of leaf chlorophyll content with grain Δ and grain yield under W_{200} treatment suggest that this trait could play a role to predict yield under moderate drought conditions. The consistent negative correlations of ash content with grain Δ and grain yield under both water deficit conditions suggest that the grain ash content could serve as useful and economical (Chinese RMB 2 per sample) alternatives to expensive grain Δ (Chinese RMB 90 per sample). The negative correlations of K and Mn contents with grain Δ under W_{120}, and of K and Mg contents with grain Δ under W_{200} treatment suggest that these mineral elements could also serve as indirect and suitable surrogates of Δ, particularly in drought prone conditions. The correlation among these physiological indicators under moderate to severe water deficit conditions validated their key role in estimation of grain yield, which could be helpful in designing future strategies for the prediction of yield under stressful environments.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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