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Research Article

Assessment of morphological and anatomical variability in *Triticum* species, *Aegilops* species, interspecific and intergeneric hybrids

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Abstract

Wheat species and wild relatives offer promising resources for wheat improvement and research in the current period of the genetic narrowing of modern wheat cultivars. The present study was performed to evaluate the morphological and anatomical traits of 20 diverse genotypes including *Triticum* and *Aegilops* species with intergeneric and interspecific wheat hybrids, which were compared with modern bread and durum wheat cultivars locally adapted to rainfed and irrigated conditions. The study showed that stomata density and size ranged from 55.3 to 108.6 stomata/mm^2 and 401.4 to 1296 µm^2, respectively, in the selected genotypes. Moving tetraploid to hexaploid genotypes, increased chromosome numbers yielded lower densities of large stomata in wheat species and hybrids. In this regard, the stomatal patterns of two hexaploid wheat hybrids and a wheat species including 'Agrotriticum', 'Aegilotriticum', and *T. compactum*, were of low density and large size stomata compared to *T. durum* cv. 'Kunduru 1149' with high density and small size stomata. Interestingly, the wild progenitor of the bread wheat D genome, *Ae. tauschii*, had a high density of the smallest stomata among the studied genotypes. The study further indicated that morphological parameters decreased under rainfed conditions compared to those under irrigated conditions, with levels varying among the genotypes. The rainfed flag leaf area and 1000-grain weight varied from 0.9 to 23.7 cm^2 and from 7.3 to 61.9 g, respectively under rainfed conditions, while they ranged from 1.2 to 35.7 cm^2 and 11.5 to 69.9 g under irrigated conditions. The flag leaf area had a significant and strong association with 1000-grain weight under rainfed (r^2= 0.79) and irrigated (r^2 = 0.77) conditions. *T. turanicum* and *T. polonicum* were characterized by the significantly highest 1000-grain weight in both rainfed and irrigated conditions. This study suggests that these wheat species with high 1000-grain weight might have promising alleles to be transferred into durum wheat to increase grain yield.

Keywords: genotypic variability; morphology; stomatal patterns; wheat species; wheat wild relatives

Introduction

Wheat is a staple source of nutrients for about 40% of the population worldwide (Giraldo *et al.*, 2019). In the wheat evolution process, wild tetraploid wheat, *Triticum dicoccoides*, was produced from hybridization between diploid wild wheat *Triticum urartu* (genome AA) and most likely *Aegilops speltoides* (genome BB) (Huang *et al.*, 2002; Peng *et al.*, 2011). Cultivated emmer wheat (*T. dicoccum*) was formed by plant selection of wild emmer and then evolved into the free-threshing ears of *T. turgidum*, *T. polonicum*, *T. turanicum*, and *T. carthlicum* by natural mutation (Peng *et al.*, 2011). Modern hexaploid bread wheat evolved through

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polyploidization between *T. turgidum* ssp. *durum* (genome AABB) and *Aegilops tauschii* (genome DD) 10.000 years ago (Feldman *et al.*, 1995).

Ancient wheat species, landraces, wheat wild relatives, and wheat hybrids offer biotic and abiotic stress tolerance, high biochemical and micronutrient contents, and quality in the improvement of new cultivars (Mathre *et al.*, 1985; Cakmak *et al.*, 2010; Arzani and Ashraf, 2017; Li *et al.*, 2018; Ullah *et al.*, 2018; Kishii, 2019). Wild wheat relatives and different wheat species have also been used as donors of drought tolerance in water deficit conditions (Peng *et al.*, 2013; Ikanović *et al.*, 2014; Suneja *et al.*, 2019). Particularly in rainfed farming, drought can lead to severe losses (Tigkas and Tsakiris, 2015). Water deficit may affect agriculture through limiting plant productivity by inducing stomatal closure and thereby reducing photosynthesis and growth (Németh *et al.*, 2002; Bibi *et al.*, 2012). Photosynthesis occurring in tissues through gas exchange has been described to be closely linked with stomata density and size (Chandra and Das, 2000; Buckley, 2005; Zwieniecki *et al.*, 2016). These characteristics of stomata on the upper and lower sides of the leaf considerably alter the gas exchange rate between inner and outer layers (Kardiman and Ræbild, 2018). Low density and large stomata could guarantee a proper photosynthetic rate and low stomatal conductance, implying that they would beneficially contribute to plants under conditions of increased CO_2 and decreased water availability in the future (Yin *et al.*, 2020). Also, in rice, stomata size has been proved to be related to grain yield, suggesting utilization possibilities for the improvement of yield in breeding programs (Limochi and Eskandari, 2013). Understanding how stomatal behaviour plays a considerable role in growth of different wheat species, hybrids, and wild wheat relatives is therefore important.

Considering the use of genetic resources, the present study aimed to determine morphological and anatomical traits and their relationships in many *Triticum* and *Aegilops* species together with their hybrids.

Materials and Methods

Experimental conditions

A field study was conducted in a randomized complete block design with three replications under rainfed and irrigated conditions in 2019 at Sarayönü Vocational School, Selçuk University, Konya, Turkey. A panel of 20 genotypes comprising different wheat species, *Aegilops* species, landraces, modern wheat cultivars, intergeneric and interspecific wheat hybrids was selected for the study (Table 1). *T. aestivum* cultivars ('Karahan 99' and 'Konya 2002') and *T. durum* cultivars ('Kunduru 1149' and 'Meram 2002') were used as control cultivars. Each one of locally adaptive modern cultivars represented an adverse genetic background with adaptations to rainfed and irrigated conditions, respectively.

The soil taken from the field (0-40 cm) and used in the experiment was clay-loam with low organic matter (1.60%) and high levels of $CaCO_3$ (31.9%) and Ca (6008 mg/kg). EC was 0.61 mmhos/cm. Soil pH was 7.76 and no salinity problem (0.02%) was observed. It was low in P_2O_5 (44.1 kg/ha) and Mn (6.17 mg/kg). K_2O (1128 kg/ha), Zn (0.74 mg/kg), and Cu (2.4 mg/kg) were found to be adequate. The soil was high in Mg (591.7 mg/kg) with a moderate level of Fe (3.9 mg/kg).

According to the meteorological data for the growing season regarding the long-term (1928-2018) and average annual rainfall (2019), the average rainfall values in the months of March, April, May, June, and July were very close to 136.9 mm in the long term and 92.8 mm in 2019. The relative humidity values were 53.8% and 48.2%, respectively, and the average temperature values were 15.2 °C and 17.2 °C, respectively.

Field experiment and measurements

The sowings were made in 5-cm rows with 20-cm spaces between rows. At sowing, DAP fertilizer (18% N, 46% P_2O_5) was top-dressed to plots at 130 kg ha^{-1}. In the tillering stage, ammonium nitrate (33% N) was applied by broadcasting. In total, 75 kg ha^{-1} nitrogen was used under rainfed conditions and 120 kg ha^{-1} was used under irrigated conditions. Weeds were controlled with a chemical herbicide. The plots under irrigated conditions were irrigated two times in the stem elongation and booting stages. Flag leaves were excised from plants (Figure 3). Six flag leaf areas (FLA) per replication were measured using ImageJ software (https://imagej.nih.gov/ij*)*. Harvesting was performed at the stage of full grain maturity (GS 92). Morphological parameters such as plant height per main stem, number of fertile tillers per plant, spike length per main spike, and number of spikelet's per main spike were determined for 10 plants per replication. 1000-grain weight (TGW) was determined by weighing dehulled seeds (4 × 100) per replication.

Table 1. Traits of genotypes involving *Triticum* species, *Aegilops* species, and wheat hybrids used in the study

Genotypes	Ploidy level	Inventory	Name	Origin
Aegilops species				
Ae. tauschii	2×	Clae 2	2016	Baluchistan, Pakistan
Ae. peregrina var. *brachyathera*	4×	PI 603247	TA 1885	Central, Israel
Ae. geniculate	4×	Clae 65	Sando Selection 253	Unknown
Ae. ventricosa	4×	PI 127000	M10	Unknown
Ae. neglecta	4×	PI 170199	2646	Turkey, Edirne
Triticum species and hybrids				
Elytritilops ssp.	-	PI 605347	Sando Selection 538	USA
Agrotriticum ssp.	8×	PI 550713	Calif. 6097	California, USA
Aegilotriticum ssp.	6×	PI 613322	CASS97B00063S	Mexico
T. aestivum ssp. *compactum*	6×	PI 114638	Gluclub	Victoria, Australia
T. ispahanicum var. ispahanorufum	4×	PI 330548	184	UK, England,
T. turgidum ssp. *turgidum*	4×	PI 134960	Pseudo-mirabile	Portugal
T. turgidum ssp. *carthlicum*	4×	PI 70738	Persian wheat	Iraq
T. turgidum ssp. *turanicum*	4×	PI 67343	Australian Pouland	Victoria, Australia
T. turgidum ssp. *polonicum*	4×	PI 56262	Milagre	Lisboa, Portugal
T. turgidum ssp. *dicoccoides*	4×	PI 346783	Nakhichevan	Pest, Hungary
T. durum cv. Meram 2002	4×	TR	Durum wheat	Konya, Turkey
T. durum cv. Kunduru 1149	4×	TR	Durum wheat	Eskişehir, Turkey
T. durum cv. Karakılçık	4×	TR	Landrace	Turkey
T. aestivum cv. Konya 2002	6×	TR	Common wheat	Konya, Turkey
T. aestivum cv. Karahan 99	6×	TR	Common wheat	Konya, Turkey

Elytritilops ssp. is a hybridization among *Elymus* × *Triticum* × *Aegilops;* Aegilotriticum ssp. from a hybridization between *Triticum* × *Aegilops*; '*Agrotriticum* ssp. from a hybridization between *Agropyron* × *Triticum*

Stomatal anatomy analysis

Microscopic slides pasted with a layer of super glue were pressed on the adaxial side of a fully expanded flag leaf selected from plants grown under irrigated condition in the pre-anthesis growth stage. Approximately 60 seconds later, the slides were taken off from the leaves and the epidermal tissues were peeled off from the leaf and attached on the slides. In total, three replicates were performed in each treatment. Stomata were imaged using Nikon Eclipse E400 light microscope equipped with DS-5M digital camera head and DS-L1 camera control unit (Nikon, Japan). The microscopic area was 0.8 mm^2 using a microscope resolution of 1280 × 960 pixel (96 dpi). Image analysis was performed using ImageJ software (*https://imagej.nih.gov/ij/*). Twenty-four stomata widths and lengths per replicate were measured for the stomatal size (area) ([stoma width × stomata length × π]/4). Stomatal density was calculated as number of stomata in six microscopic fields per replicate (Cortan *et al.,* 2017) (Figure 1).

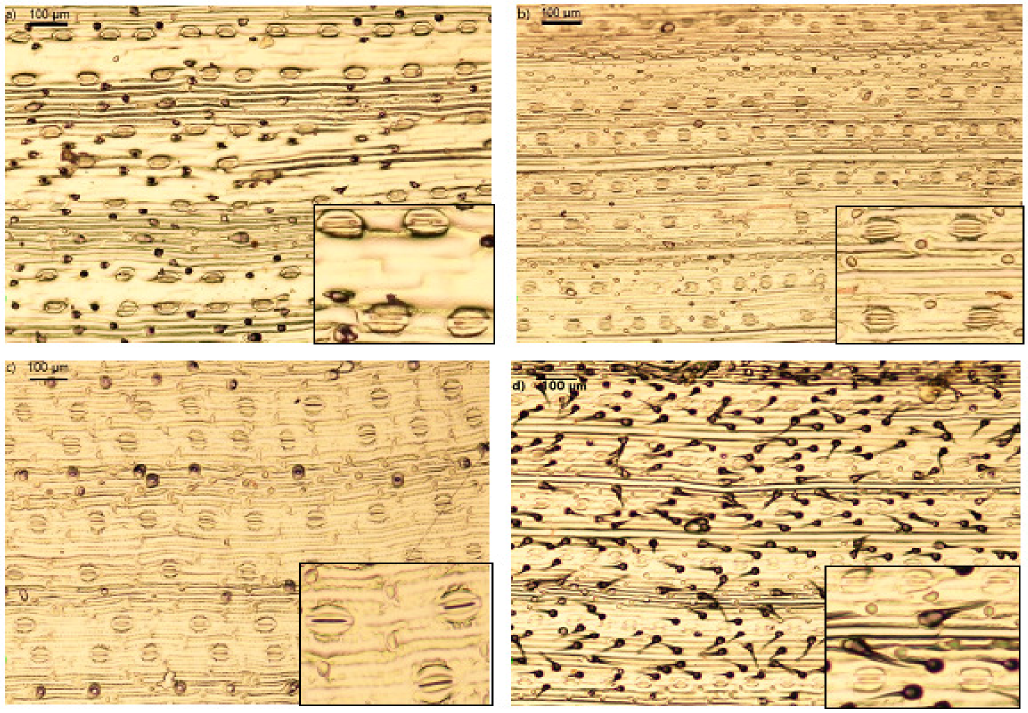

Figure 1. Stomatal diversity in wheat species and hybrids

Scale bar is 100 µm. The images are captured from the adaxial epidermal layers after imprinting of flag leaf tissues. Figure 1a is a representative of the fewest stomata in 'Agrotriticum' among the studied genotypes, figure 1b is a representative of the highest density of the smallest stomata in *T. durum* cv. 'Kunduru 1149'. Figure 1c indicates the largest size of stomata in 'Aegilotriticum' among the genotypes. Figure 1d shows stomata and typical more trichomes on epidermis of leaf in *T. carthlicum*. Black lined boxes are the zoomed part of the leaves showing stomatal features.

Statistical analysis

The morphological and anatomical results were evaluated by analysis of variance (general linear model procedure) and one-way Tukey's pairwise comparison test using Minitab Version 16 (Minitab Inc., State College, PA, USA). Regression analyses were performed in Microsoft Excel (Excel version in Microsoft Office 2016 for Windows) for significantly correlated traits.

Results

In this study, significant variations were observed within and between genotypes in terms of values for morphological and anatomical traits of *Triticum* and *Aegilops* species and their hybrids ($P < 0.0001$).

Stomatal anatomy

Stomata exhibited a diverse range of length, width, size, and density across different wheat and *Aegilops* species with hybrids. Stomata length ranged from 28 to 51.6 µm, stomata width from 18.3 to 32 µm, stomata size from 401.4 to 1296 μm^2, and stomata density from 55.3 to 108.6 stomata/mm^2 (Figure 2).

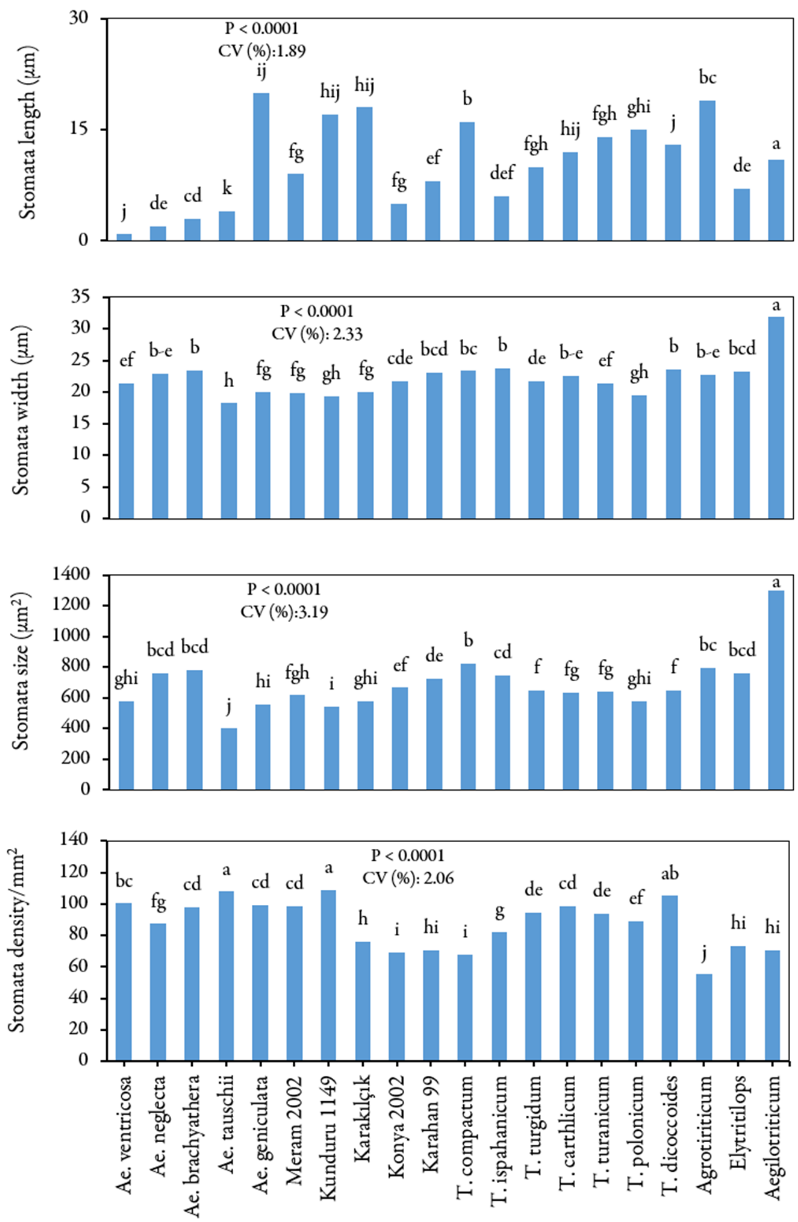

Figure 2. Stomatal length, width, size, and density of the studied genotypes

In the study, the hexaploid and tetraploid genotypes were discriminated precisely in terms of stomata density. The maximum stomatal length and width were observed in the 'Aegilotriticum' in wheat species and hybrids. Conversely, *T. dicoccoides* and *T. durum* cv. 'Kunduru 1149' had the lowest stomatal length and width, respectively. In general, the hexaploid wheat genotypes and hybrids possessed a higher stomata size compared to tetraploid wheat genotypes. In *Aegilops* species, *Ae. tauschii*, a unique genitor of bread wheat, indicated the lowest stomatal length and width (Figure 2). However, the highest values were obtained from *Ae. brachyathera*.

Table 2. Morphological traits of wheat species, *Aegilops* species, and wheat hybrids under rainfed conditions

	Plant height (cm)	Spike length (cm)	Spikelet number/spike	Tiller number/plant	Flag leaf area (cm^2)	1000-grain weight (g)
Ae. ventricosa	28.1^{g}	9.3^{b-e}	8.6^{g}	25.0^{bc}	2.4^{j}	11.1^{gh}
Ae. neglecta	26.0^{g}	4.1^{ij}	5.3^{g-h}	32.7^{ab}	1.1^{j}	11.7^{gh}
Ae. brachyathera	23.8^{g}	5.1^{g-j}	6.1^{g-h}	21.3^{b-e}	0.9^{j}	12.4^{gh}
Ae. tauschii	24.9^{g}	6.7^{fgh}	7.9^{g-h}	21.2^{b-e}	1.6^{j}	7.3^{h}
Ae. geniculata	22.6^{g}	3.5^{j}	3.9^{h}	42.3^{a}	1.2^{j}	14.8^{g}
Meram 2002	67.7^{def}	10.1^{abc}	19.5^{abc}	12.2^{c-f}	10.9^{fgh}	43.3^{c}
Kunduru 1149	82.2^{abc}	6.6^{gh}	16.0^{b-f}	7.0^{ef}	10.6^{fgh}	43.8^{c}
Karakılçık	64.0^{def}	6.2^{ghi}	13.9^{ef}	5.2^{f}	18.8^{bc}	43.4^{c}
Konya 2002	65.0^{def}	11.1^{abc}	21.2^{a}	6.2^{f}	13.0^{ef}	42^{c}
Karahan 99	59.4^{ef}	10.2^{abc}	15.5^{c-f}	9.7^{def}	15.3^{de}	34.2^{d}
T. compactum	59.1^{ef}	4.5^{hij}	15.8^{b-f}	9.9^{def}	15.2^{de}	32.5^{d}
T. ispahanicum	75.6^{bcd}	9.5^{bcd}	18.0^{a-d}	22.2^{bcd}	8.0^{hi}	40.4^{c}
T. turgidum	91.8^{a}	11.4^{ab}	19.9^{ab}	6.3^{f}	17.4^{cd}	42.6^{c}
T. carthlicum	72.4^{b-e}	9.0^{c-f}	18.2^{a-d}	9.6^{def}	8.5^{ghi}	31.7^{de}
T. turanicum	84.8^{ab}	11.1^{abc}	15.3^{def}	7.2^{ef}	21.9^{ab}	61.9^{a}
T. polonicum	75.6^{bcd}	7.3^{d-g}	17.3^{a-f}	6.3^{f}	23.7^{a}	52.6^{b}
T. dicoccoides	77.2^{bcd}	7.0^{efg}	15.1^{def}	11.5^{c-f}	6.4^{i}	26^{f}
Agrotiriticum	70.5^{cde}	12.1^{a}	13.6^{f}	11.3^{c-f}	8.0^{hi}	24.5^{f}
Elytritilops	76.5^{bcd}	11.9^{a}	14.8^{def}	10.1^{def}	13.5^{ef}	29.1^{f}
Aegilotriticum	54.5^{f}	10.1^{abc}	14.8^{def}	8.7^{def}	11.5^{fg}	30.4^{de}
Mean	60.1	8.3	14.0	14.3	10.5	31.8
P <	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
CV (%)	7.7	9.0	9.5	33.2	10.6	6.2

Morphological parameters

Significant variations in each parameter were observed among genotypes in both growth conditions. Plant height under rainfed and irrigated conditions ranged from 22.6 to 91.8 cm and 26.3 to 95.8 cm, respectively (Tables 2 and 3) and was generally greater in plants in the irrigated group than the rainfed group. *Aegilops* species had shorter plant height than wheat species and hybrids. The spike length ranged from 3.5 to 12.1 cm under rainfed condition and from 3.6 to 12.6 cm under irrigated condition (Tables 2 and 3). The average values in both conditions were relatively close for spike length and spikelet number, as well.

Significant variations in tiller number were also observed among genotypes in both growth conditions. The tiller number varied from 5.2 and 42.3 under rainfed condition and 7.4 and 33.7 under irrigated condition (Tables 2 and 3). *Aegilops* species and *T. ispahanicum* showed higher tillering capacity in both conditions than other wheat species and hybrids. The FLA ranged from 0.9 to 23.7 cm^2 under rainfed conditions and 1.2 to 35.7 cm^2 under irrigated conditions (Tables 2 and 3). There was a significant and strong relationship between FLA and TGW in rainfed ($r^2 = 0.79$) and irrigated ($r^2 = 0.77$) conditions (Figure 4).

The FLA under rainfed conditions was lower than that under irrigated conditions. Similarly, TGW values were reduced under rainfed conditions compared to irrigated conditions. The TGW varied widely among genotypes, ranging from 7.3 to 61.9 g under rainfed conditions and from 11.5 to 69.9 g under irrigated conditions (Tables 2 and 3). It was further observed that *Aegilops* species had lower TGWs than *Triticum* species and hybrids.

Table 3. Morphological traits of wheat species, *Aegilops* species, and wheat hybrids under irrigated conditions

Genotype	Plant height (cm)	Spike length (cm)	Spikelet number/spike	Tiller number/plant	Flag leaf area (cm^2)	1000-grain weight (g)
Ae. ventricosa	30.0^{g}	9.8^{c-f}	8.0^{de}	27.8^{bc}	3.3^{g}	11.5^{j}
Ae. neglecta	29.8^{g}	4.2^{ij}	5.2^{de}	26.6^{bcd}	1.2^{g}	15.7^{j}
Ae. brachyathera	27.7^{g}	5.7^{h-i}	4.7^{de}	29.3^{bc}	1.7^{g}	19.8^{ij}
Ae. tauschii	30.2^{g}	5.4^{hij}	5.4^{de}	25.5^{cde}	2.0^{g}	15.9^{j}
Ae. geniculata	26.3^{g}	3.6^{j}	3.5^{e}	33.7^{ab}	1.9^{g}	17.7^{ij}
Meram 2002	68.5^{def}	9.2^{def}	18.3^{ab}	11.3^{gh}	21.4^{bcd}	46.7^{cd}
Kunduru 1149	86.4^{abc}	6.7^{gh}	15.7^{ab}	11.8^{fgh}	19.0^{c-f}	46.3^{cd}
Karakılçık	66.6^{ef}	5.9^{hi}	9.6^{cd}	9.3^{h}	21.0^{cde}	47.2^{c}
'Konya 2002'	65.0^{f}	11.7^{ab}	20.3^{a}	8.5^{h}	22.1^{bc}	42.5^{cde}
'Karahan 99'	74.1^{c-f}	10.7^{bcd}	17.2^{ab}	17.5^{fg}	19.3^{c-f}	42.4^{cde}
T. compactum	63.5^{f}	4.5^{ij}	15.9^{ab}	19.2^{def}	19.5^{c-f}	33.8^{fg}
T. ispahanicum	95.8^{a}	10.2^{b-e}	17.9^{ab}	37.4^{a}	13.4^{ef}	43.2^{cde}
T. turgidum	93.1^{ab}	11.0^{abc}	20.3^{a}	7.5^{h}	20.9^{cde}	47.9^{c}
T. carthlicum	82.1^{a-d}	10.4^{b-e}	18.9^{ab}	17.5^{fg}	15.7^{def}	31.2^{fgh}
T. turanicum	88.1^{ab}	11.1^{abc}	16.5^{ab}	11.0^{gh}	27.6^{b}	69.9^{a}
T. polonicum	81.1^{bcd}	8.6^{ef}	16.6^{ab}	7.4^{h}	35.7^{a}	59.1^{b}
T. dicoccoides	89.9^{ab}	8.2^{fg}	15.1^{abc}	18.0^{efg}	18.1^{c-f}	24.7^{hi}
Agrotiriticum	79.4^{b-e}	12.6^{a}	14.2^{bc}	9.3^{h}	19.8^{cde}	28.8^{gh}
Elytritilops	85.1^{abc}	11.8^{ab}	14.3^{bc}	13.3^{fgh}	15.0^{ef}	36.2^{efg}
Aegilotriticum	63.9^{f}	9.7^{c-f}	15.2^{abc}	13.1^{fgh}	16.6^{c-f}	38.8^{def}
Mean	66.3	8.5	13.6	17.8	15.8	36.0
P <	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
CV (%)	6.8	6.9	13.8	13.8	12.8	7.5

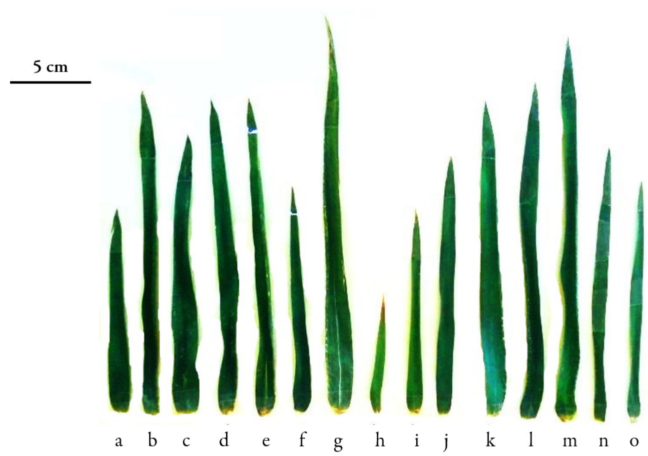

Figure 3. An image indicates the genotypic variations in FLA in rainfed condition
Letters are the representatives of *T. aestivum* cv. 'Karahan 99' (a), *T. durum* cv. 'Kunduru 1149' (b), *T. aestivum* cv. 'Konya 2002' (c), *T. durum* cv. 'Meram 2002' (d), 'Karakılçık' (e), 'Elytritilops' (f), *T. polonicum* (g), *T. dicoccoides* (h), 'Aegilotriticum' (i), *T. carthlicum* (j), *T. turgidum* (k), *T. compactum* (l), *T. turanicum* (m), 'Agrotriticum' (n), and *T. ispahanicum* (o). The fully expanded flag leaves were excised from plants. Scale bar is 5 cm.

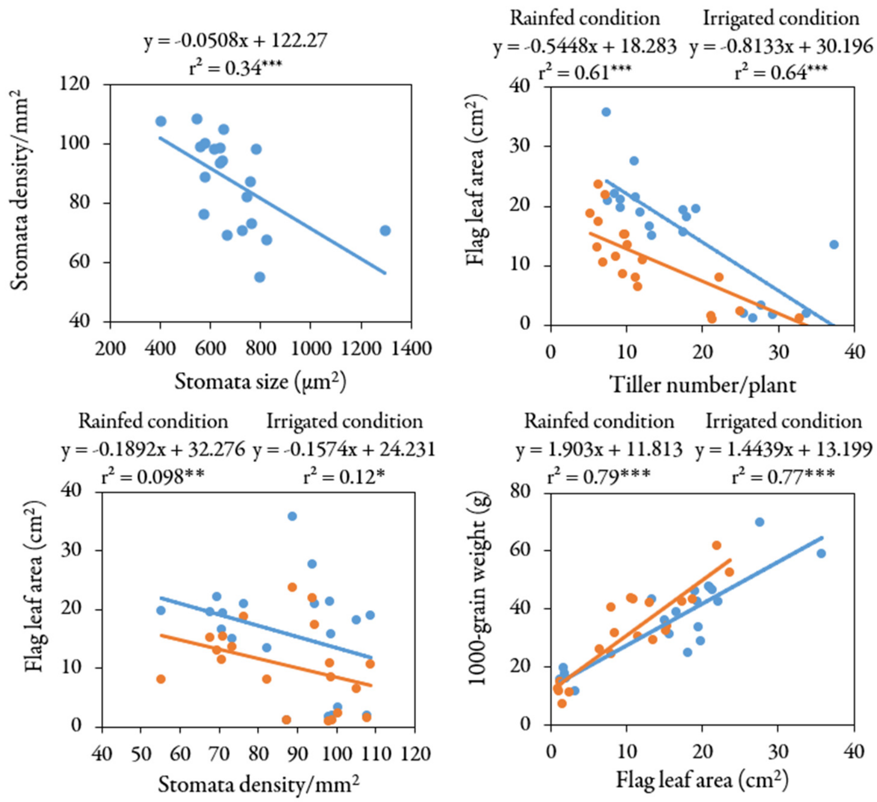

Figure 4. Relationship between/within stomatal and morphological traits of the studied genotypes
"*" indicates $P < 0.05$, "**" for $P < 0.01$, and "***" $P < 0.0001$

Discussion

Stomata size and density vary depending on genetic factors and environmental conditions; the size ranges from 10 and 80 µm in length with densities between 5 and 1000 mm^{-2} (Hetherington and Woodward, 2003). The present study showed that stomata density in two bread wheat cultivars ranged from 69.5 to 70.8 mm^{-2} (Figure 2). Similarly, the findings resulting from the current study were in agreement with those of Shahinnia *et al.* (2016) who found that adaxial stomata density in two bread wheat cultivars varied from 61 to 71 mm^{-2}. Mohammady *et al.* (2007) observed that stomatal traits varied from 44.8 to 51.9 µm of stomata length, 24.5 to 31.1 µm of stomata width. They also revealed that stomata density differed from 56.2 to 81.5 mm^{-2} in tetraploid and from 45.6 to 64.5 mm^{-2} in hexaploid wheat accessions.

The density of stomata on the leaves is a highly heritable characteristic (Hofmann and Dobrenz, 1983; Schoppach *et al.*, 2016). Hexaploid genotypes proved to have lower stomata density compared to tetraploid wheat genotypes. In this study, *T. durum* cv. 'Kunduru 1149' had a high density of small stomata compared to 'Agrotriticum', 'Aegilotriticum', and *T. compactum* with low densities of large stomata (Figure 1). These results were similar to the findings reported by Doheny-Adams *et al.* (2012), who concluded that smaller stomata were usually found in higher densities. Researchers have recently demonstrated pivotal results regarding appropriate stomata traits of plants. Reduced stomatal density has been proved to improve drought tolerance and water-use efficiency in bread wheat, rice, and wood plants (Caine *et al.*, 2019; Dunn *et al.*, 2019; Yin *et al.*, 2020).

In the present study, the hexaploid genotypes with low stomatal density may contribute to the improvement of new drought-tolerant cultivars in environments with reduced water availability. Regarding photosynthesis and yield-related stomata traits, leaves with a low density of large stomata have shown lower photosynthetic rates (Drake *et al.*, 2013). It was also suggested that high-yielding rice cultivars possessed a higher stomatal density and slightly shorter stomatal length (Ohsumi *et al.*, 2007). In the present study, tetraploid wheat species had high stomata density, short length, and small size. There are differences in the previous studies about ideal stomata traits in plants, suggesting a high density of small stomata for improved yield and photosynthesis but a low density of large stomata for drought tolerance and water-use efficiency. Hence, further work is needed to elucidate associations between stomatal traits and other physio-morphological, biochemical, and genetic traits under different environmental conditions.

Water deficit has been shown to lead to severe reductions in most morphological traits. It was described, for example, that precipitation was the key indicator for wheat yield evaluation (Tigkas and Tsakiris, 2015). Grain yield can be enhanced by 60% to 100% with 200 to 300 mm of irrigation (Zhang *et al.*, 1999). Meanwhile, in the current study, significant reductions under rainfed conditions compared to the irrigated condition were observed in morphological parameters, such as 9.4% for plant height, 19.7% for tillering, and 33.5% for FLA. The FLA is one of the key determinants underlying wheat grain yield, particularly under drought (Biswal and Kohli, 2013). Ideal FLA is therefore significant for sustaining optimal yield in water deficit conditions (Quarrie *et al.*, 1999). As wheat cultivars that have adapted to irrigated conditions, the FLAs of *T. aestivum* cv. 'Konya 2002' and *T. durum* cv. 'Meram 2002' were smaller under rainfed conditions and larger under irrigated conditions, whereas *T. aestivum* cv. 'Karahan 99' and *T. durum* cv. 'Kunduru 1149' adapted to rainfed condition possessed smaller leaf areas under irrigated conditions. Surprisingly, *T. polonicum*, *T. turanicum*, *T. durum* cv. 'Karakılçık', and *T. turgidum* maintained larger leaf areas in both conditions compared to other genotypes.

T. ispahanicum, *T. carthlicum*, and *T. dicoccoides* had lower FLA values in both growth conditions. Also, 'Aegilotriticum', *T. carthlicum*, 'Elytritilops', and *T. ispahanicum* were the most impacted genotypes by water deficit of 48.5% to 50% As expected, *Aegilops* species had inherently lower leaf areas than wheat species and hybrids. Among the *Aegilops* species, a progenitor of bread wheat, *Ae. tauschii*, was the least affected species.

This study showed that *T. ispahanicum* and *T. dicoccoides* were the most tillering species in both growth conditions among the studied genotypes. Longnecker *et al.* (1993) presented similar findings, indicating that tillering was dependent on genotypic and climatic factors. Another previous study reported that the tillering potential of bread wheat was greater than that of durum wheat (Pinthus, 1969). In the present study, there were no clear discriminations in terms of tillering between durum and bread wheat genotypes or tetraploid and hexaploid wheat genotypes. Indeed, *T. polonicum*, *T. turgidum*, *T. aestivum* cv. 'Konya 2002', and *T. durum* cv. 'Karakılçık' had less tillering capacity than other genotypes in both growth conditions.

Regarding plant height, *T. ispahanicum*, *T. turgidum*, *T. dicoccoides*, 'Elytritilops', and *T. durum* cv. 'Kunduru 1149' showed higher values in both growth conditions than other genotypes, while *T. compactum*, 'Aegilotriticum', *T. durum* cv. 'Karakılçık' (landrace), and modern wheat cultivars had shorter plant heights. Evaluating the spike length and spikelet number, genotypes under rainfed conditions had trends like those seen under irrigated conditions. Genotypes with high values maintained notably greater values in both conditions. The *Aegilops* species '*Ae. ventricosa*' had higher values regarding plant height, spike length, and spikelet number, while '*Ae. geniculata*' had lower values.

TGW was shown to be one of the significant indicators determining grain yield (Wu *et al.*, 2018). Previous studies indicated that TGW was significantly and strongly correlated with grain yield (Huang *et al.*, 2020; Öztürk *et al.*, 2020). A close look at the data in Tables 1 and 2 reveals that *T. turanicum* and *T. polonicum* had distinguishably higher TGW than the modern wheat cultivars and other studied genotypes in both rainfed and irrigated conditions. Consistent with the findings of the current study, Grausbruger *et al.* (2005) indicated that *T. turanicum*, known as Khorasan wheat, often had TGW values of greater than even 60 g. *T. turanicum* was suggested to be a natural hybrid between *T. polonicum* (Polish wheat) and durum wheat (Kuckuck, 1959). The caryopsis structure of '*T. turanicum*' has similarities with *T. polonicum*, *T. ispahanicum*

(Ispahan emmer), and *T. carthlicum* (Persian wheat) (Kosina, 1995). Also, *T. turanicum* was proposed to have wide genetic diversity with multiple agronomic adaptive traits (Lannucci and Codianni, 2019). Regarding *T. polonicum*, TGW can reach up to 80 g (Wang *et al*., 2002). In the present study, the TGW of *T. polonicum* in rainfed and irrigated conditions averaged 52.6 g and 59.1 g, respectively. The TGW of *T. turgidum* was close to that of modern durum wheat cultivars and higher than modern bread wheat cultivars in both growth conditions. *T. ispahanicum* possessed TGW values between those of modern bread and durum wheat cultivars in both rainfed and irrigated conditions. Other wheat species and hybrids had lower TGWs than modern wheat cultivars. Morphological parameters may be altered in response to water availability in the soil. It has been reported that wheat needs 450-650 mm of rainfall annually for optimal development (Zargar and Zargar, 2018). However, this desired rainfall may not occur in a timely manner in the wheat growth stages. Seasonal rainfall irregularity might further exacerbate the adverse effects on wheat growth under rainfed conditions. In the present study, the average TGW was reduced by 11.7% under rainfed conditions compared to the irrigated condition. *T. carthlicum* and *T. dicoccoides* maintained their TGWs under rainfed conditions.

In contrast, *T. aestivum* cv. 'Karahan 99', 'Elytritilops', and 'Aegilotriticum' were the most affected genotypes in terms of TGW under rainfed conditions compared to the irrigated condition among the wheat species and hybrids (Tables 2 and 3). In *Aegilops* species, the TGW was influenced most under rainfed conditions, excluding '*Ae. ventricosa*'.

Conclusions

Wheat species, wild relatives, and hybrids have recently attracted researchers' interest based on the genetic diversity in the gene pool. The present study has characterized the variability in stomatal and morphological traits of the studied genotypes. The variations among wheat species, wild relatives, and hybrids were about 2-fold for stomata density and 3.2-fold for stomata size. It was shown that 'Aegilotriticum', 'Agrotriticum', *T. compactum*, and *Ae. tauschii* particularly offered differential stomatal characteristics. '*Ae. tauschii*' had a high density of small stomata, while, conversely, *T. compactum*, 'Aegilotriticum', and 'Agrotriticum', a hexaploid wheat species and two man-made hybrids, possessed low densities of large stomata. Considering the value of genetic variability for the improvement of wheat in rainfed and irrigated conditions, the best-performing genotypes of *T. turanicum* and *T. polonicum* had considerably high TGW compared to adapted bread wheat cultivars, durum wheat cultivars, and other species. These genotypes with superior characteristics might be evaluated for hybridization studies to transfer valuable genes into wheat.

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Conflict of Interests

The author declares that there are no conflicts of interest related to this article. The author approved the final manuscript.

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