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Assessment of the suitability of *Triticum turgidum* accessions for incorporation into a durum wheat breeding program

Reza Mohammadi · **Ahmed Amri**

Abstract Expanding the genetic basis of modern wheat cultivars may improve yield stability and increase grain yields under climatic changes, to feed the growing human population. The main objective of this study was to evaluate the potential for incorporation into a durum wheat breeding program of 64 accessions of tetraploid wheats from six sub-species of *Triticum turgidum*, tested under rainfed (terminal drought stress) and irrigated (non-terminal drought stress) conditions during two cropping seasons. The accessions were assessed for twelve above-ground agro-physiological traits, drought tolerance and stability performance. The combined analysis of variance indicated that variability due to genotype, environment and genotype by environment (GE) interaction was highly significant ($P < 0.01$) for all traits tested into these experiments. Genotype, environment and GE interaction accounted for 34.2%, 27.8% and 25.3% of the total variation in grain yield, showing that this trait was mainly affected by genotypic variation. Based on the results, the genetic potential for high and stable grain yield and drought tolerance was found mainly in *T. turgidum* subsp. *turgidum* and *T. turgidum* subsp. *durum*, which could be explored in the durum breeding program. The cluster analysis revealed that the grouping patterns of tetraploid wheat accessions are not due to their geographical origin and their respective subspecies classification. Among the accessions, G56 (subsp. *turgidum*), G20, G63, G64, G6 and G4 (all belonging to subsp. *durum*) were the best in terms of providing high and stable grain yield in combination with breeder-preferred traits (i. e. earliness, higher kernel weight, and plant stature characteristics) adequate for drought tolerance. In conclusion, the identified accessions are valuable genetic resources that should be used directly for cultivar recommendation or to be crossed with native or commercial cultivars for improve yield productivity in durum wheat under the new circumstances of climatic change.

Keywords Tetraploid wheat · Agro-physiological traits · Phenotypic diversity · Drought tolerance

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Introduction

Genus *Triticum* encompasses species at three ploidy levels, diploid (2n = 14), tetraploid (2n = 4x = 28) and hexaploid (2n = 6x = 42), some of which are of considerable economic importance (Igrejas et al. 2020).

Durum wheat (*T. turgidum* L. subsp. *durum* Desf; BBAA, 2n=4x=28) and common wheat (*T. aestivum*; BBAADD, 2n=6x=42) are cultivated on large areas and make a major contribution to human food and animal feed (Tadesse et al. 2019; FAO 2021). Durum wheat is mainly used for pasta and other local end-use products such as semolina and couscous. It is an allotetraploid species that resulted from hybridization between *T. urartu*, a diploid species as the donor of genome A, and *Ae. speltoides* or a closely related species, which contributed genome B (Lilienfeld and Kihara 1934; Dvorak and Zhang 1990). This cross resulted in the wild emmer wheat (*T. turgidum* L. subsp *dicoccoides* (Korn. ex Asch. and Graebn.) Thell.), which was domesticated about 12,000 years ago by ancient farmers and gave rise to the primitive emmer wheat (*T. turgidum* subsp. *dicoccon*) with hulled grains and brittle ears (MacKey, 2005; Gupta et al. 2008). A selection from subsp. *dicoccon* evolved the naked type subspecies tetraploid wheat such as durum (*T. turgidum* L. subsp. *durum* (Desf.) Husn.), rivet wheat (*T. turgidum* L. subsp. *turgidum* (Desf.) Husn.) and the Polish wheat (*T. turgidum* subsp. *polonicum* (L.) Thell.), resulting in different landraces, many of which have been lost because of modern breeding programs with the consequent reduction in genetic variability (Autrique et al., 1996; Soriano et al., 2016; Kabbaj et al. 2017; Salsman et al. 2021).

Fortunately, genebanks conserve many accessions of these wild, primitive and cultivated subspecies, including a high proportion of local varieties that show a great diversity. The subspecies provide valuable traits for durum wheat improvement including adaptation to low input agriculture and tolerance to environmental stresses associated with climate change (Xynias et al. 2020). Extensive breeding and selection efforts over years have narrowed the genetic base in durum wheat, calling for mobilizing adaptive genes from landraces, primitive wheats and wild species conserved ex situ in gene banks to develop varieties adapted to climate change and having needed quality attributes (Sharma et al. 2021).

Durum wheat is mainly grown under Mediterranean type environment, where its productivity is severely affected by low and fluctuating rainfall. Assessing yield stability and adaptability of gene bank accessions has been investigated through different statistical models including joint regression

models (Finlay and Wilkinson 1963; Eberhart and Russell 1966), additive main effects and multiplicative interaction (AMMI) model (Gauch 1992), and genotype main effect and genotype by environment (GE) interaction (GGE) biplot model (Yan et al. 2000). The AMMI and GGE biplot models are among the most efficient methods for exploring genotypic diversity for yield stability, adaptability and for selecting accessions with specific adaptation to different agro-climatic regions. These methods are used extensively for exploring genetic diversity in global diversity panels of different crop species (Mallikarjuna et al. 2015; Das et al. 2019; Enyew et al. 2021; Aberkane et al. 2021; Singamsetti et al. 2021).

Climate change is projected to increase the incidence and severity of drought, and therefore future food security will seriously depend on the development of new drought tolerant wheat cultivars (Kumar et al. 2021). Recent studies have shown that interspecific crosses using wild *Triticum*, primitive wheats and *Aegilops speltoides* have allowed to develop durum wheat elite germplasm with enhanced tolerance to heat and drought and resistance to major diseases and insects calling for strengthening further pre-breeding efforts (Zaim et al. 2017; Aberkane et al. 2020).

In this paper, we investigated the phenotypic diversity and productivity of a diverse tetraploid wheat panel composed by new cultivars, landraces and primitive subspecies, all of them with putative drought and mild winter cold tolerance. The objectives were (a) to compare agro-physiological characteristics and drought tolerance of six *T. turgidum* subspecies, and (b) to evaluate the productivity and stability performance of accessions across environments. The ultimate goal is to select candidate accessions to be incorporated into durum wheat breeding program for the development of drought tolerant varieties adapted to the rainfed conditions of Iran.

Materials and methods

Plant materials

In this study, 62 tetraploid wheat accessions (Table S1) maintained at the gene bank of Dryland Agricultural Research Institute (DARI), Sararood station, Kermanshah, Iran along with two commercial

durum wheat cultivars were evaluated. These accessions were selected as a subset from 641 durum wheat accessions received from ICARDA gene bank in 2010 based on their level of cold tolerance and their agronomic performance. The accessions belonged to six different subspecies of *T. turgidum* including *carthalicum*, *dicoccon*, *durum*, *polonicum*, *turanicum* and *turgidum*. Among the 64 accessions, 27 accessions originated from Turkey (TUR), 19 from Russia (RUS), six from Azerbaijan (AZE), three from China (CHN), two from Armenia (ARM), two from Kazakhstan (KAZ), two from Iran (IRN), one from Afghanistan (AFG), one from Pakistan (PAK) and one from Tajikistan (TJK).

Experimental layout

In 2018–19, the accessions first were grown under rainfed condition to multiply the seeds. Then, the accessions were evaluated for the following two cropping seasons (2019–20, 2020–21), where in 2019–20 the experiment was carried out under rainfed condition and in 2020–21 the experiments were conducted under rainfed (terminal drought stress) and supplemental irrigation (no-terminal drought stress) conditions, resulting in three environments. The accessions in each experiment were carried out in a randomized complete blocks design with two replicates. Each accession was planted in a two-row plot with 2.5 m length and 0.25 m row spacing. Under the irrigated condition, two supplemental irrigations of 30 mm each were applied from heading to the mid-grain filling period to mitigate terminal drought stress. The experiments were conducted at Sararood dryland agricultural research station, DARI, (34° 19′N, 47°17′E; 1351 m a.s.l), the main station for breeding crops targeting regions with moderate cold climates in the west of Iran. The soil texture was silty-clay-loam at the research site. Weeds were controlled by herbicide complemented by hand-weeding when needed. Fertilizers were used at rates of 50 kg N ha^{-1} and 50 kg P$_2$O$_5$ ha^{-1} at the time of planting.

Data collection

Agronomic, physiological and phenological traits were recorded for each accession in each plot. Heading date (DHE) was recorded when about 50% of spikes fully emerged in each plot. Days to maturity (DMA) were recorded when about 50% of peduncles turned yellow in each plot. Phenotypic data for plant height (PLH), peduncle length (PL), flag-leaf length (FL), and spike length (SL) at maturity were collected from three randomly selected plants in each plot. PLH was measured as the height of the plant from the base to the tip of a spike (excluding awns). PL was measured as the distance from the top node to the base of the spike; FL was measured as the distance from the base to the tip of flag leaf, and SL was measured as the distance from the base to the tip of the spike (excluding awns). The number of grains per spike (NGPS) was determined as the average number of grains in five randomly selected matured spikes for each accession.

For measuring physiological traits such as chlorophyll content (SPAD index), normalized differences vegetation index (NDVI) and canopy temperature (CT), the data were recorded using standard protocols (Pask et al. 2012). The flag leaf chlorophyll content was determined during anthesis for three random flag leaves for each accession per replication using a SPAD 502 portable leaf chlorophyll meter (Minolta Co. Ltd., Tokyo, Japan). NDVI was recorded during anthesis for each plot using the GreenSeeker hand-held active sensor (Trimble GreenSeeker, USA) with 0.5 m horizontally above the canopy on a sunny day (Pask et al. 2012). CT was taken on a clear and sunny day with an infrared thermometer (Kimo KIRAY 100, UK) for all plots between 12:00 to 14:00 h and from a distance of about 0.5 m in the front of the canopy, (Pask et al. 2012).

At maturity, the plots were harvested with an experimental combine, and then plot yield is converted into kg ha^{-1}. After harvesting, a sample of grains was used to determine 1000-grain weight (TKW) for each accession.

Five drought tolerance and susceptibility indices, including stress tolerance index (STI; Fernandez 1992), geometric mean productivity (GMP; Fernandez 1992), mean productivity (Rosielle and Hamblin 1981), tolerance index (TOL; Hossain et al. 1990), and stress susceptibility index (SSI; Fischer and Maurer 1978), were calculated for each entry based on the grain yield under drought and irrigated environments, according to the following formulas, to differentiate the drought-tolerant and susceptible genotypes.

1. $STI = \dfrac{(Ys)(Yp)}{\left(\overline{Yp}\right)^2}$ (Fernandez 1992)
2. $GMP = \sqrt{(Ys)(Yp)}$ (Fernandez 1992)
3. $MP = \dfrac{(Ys+Yp)}{2}$ (Rosielle and Hamblin 1981)
4. $TOL = Yp_{\frac{Ys}{Yp}}Ys$ (Hossain et al. 1990)
5. $SSI = \dfrac{\left[1-\left(\frac{Ys}{Yp}\right)\right]}{1-SI}$; $SI = [1-\left(\overline{Ys}\right)/\left(\overline{Yp}\right)]$ (Fischer and Maurer 1978)

where Ys and Yp stand for the grain yield of each accession under drought and irrigated conditions, respectively; $\overline{Ys}$ and $\overline{Yp}$, represent mean yields of accessions under drought and irrigated conditions, respectively; and SI is stress intensity.

Data analysis

The data collected on measured traits from the three environments were subjected to combined ANOVA using a mixed linear model. The germplasm effect was treated as fixed and the environment, GE interaction, replications, and blocks were treated as random factors. For genetic and phenotypic correlation analyses among measured traits, best linear unbiased predictors (BLUPs) using the R software (R Core Team, 2016) with the package of META-R (Alvarado et al. 2015) was performed; and then the generated BLUPs data were subjected to all subsequent analyses.

A hierarchical cluster analysis was applied using Ward's (1963) method and Euclidean distance to classify the tetraploid accessions based on their subspecies taxon and their geographical origins. GGE biplot model, as described by Yan et al. (2000), was performed using the R software (R Core Team, 2016) with the package of GEA-R (Pacheco et al. 2016) to visualize the GE interaction (Yan 2002). A genotype by trait (GT) biplot analysis, as described by Yan and Rajcan (2002), was applied for trait profile of tetraploid wheat accessions and investigation of traits relationships.

Results

Forecasting data, combined ANOVA and phenotypic variations

The cropping seasons varied in the amount of annual rainfall and in its monthly distribution (Fig. 1),
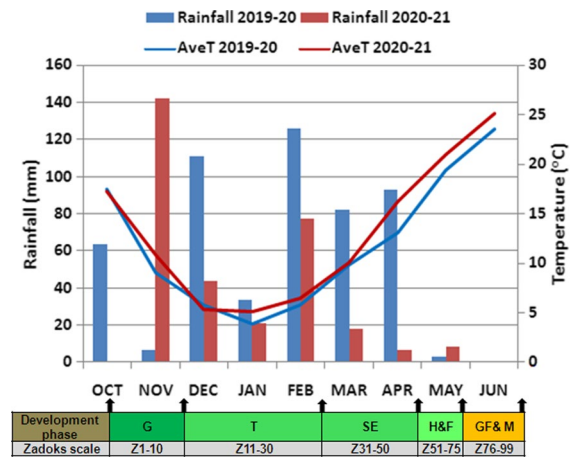

Fig. 1 Monthly rainfall patterns and average temperature during crop growth in two cropping seasons at Sararood experiment station, Iran. Growth stage of wheat experiments in accordance with the Zadoks (1974) scale to show phenological stage of wheat in regards to climatic condition. G: germination; T: tillering; SE: stem elongation; H&F: heading and flowering; GF&M: grain filling and maturity

providing different growing conditions. The annual rainfalls recorded in 2019–20 and 2020–21 were 518.8 and 317 mm, respectively. In the second cropping season, the experiments were subject to severe terminal drought due to the lack of rainfall combined with high temperatures during the grain-filling period (Fig. 1). Thus, based on drought stress experienced in 2019–20 (environment RF20) and 2020–21 (environment RF21), we refer to RF20 and RF21 respectively as moderate and severe drought-stressed environments, and the environment with supplemental irrigation (IR21) as near to normal conditions. Drought stress in 2019–20 started from late April and in 2020–21 from mid-March, which coincided with stem elongation and tillering stages, respectively (Fig. 1). No remarkable changes in monthly temperature were observed during the two cropping seasons.

The analysis of variance for investigated traits indicated significant effects of genotype, environment, and GE interaction. The variance due to the genotype and environment effects were highly significant ($P < 0.01$) for all measured traits (except for environment on spike length), while GE interaction was highly significant ($P < 0.01$) for grain yield and 1000-kernel weight, and significant for the number of grains per spike ($P < 0.05$) (Table 1). In the case of grain yield, the genotype accounted for the highest

Table 1 Combined analysis of variance for grain yield and agro-physiological traits of 64 tetraploid wheat accessions grown in three environments

		Source of variation					CV%
		Environment (E)	Rep/E	Genotype (G)	GE interaction	Error	
Traits	df	2	3	63	126	189	
YLD	MS	34,589,299**	68,904	1,347,502**	498,726**	166,471	17.2
	%TSS	27.8	0.1	34.2	25.3	12.7	
TKW	MS	954.7**	6.2	115.6**	14.5**	7.7	8.3
	%TSS	15.3	0.2	58.3	14.7	11.6	
NGPS	MS	3151.8**	18.3	154.8**	65.3**	34.6	14.35
	%TSS	20.4	0.2	31.6	26.7	21.2	
DMA	MS	1116.8**	15.0	29.5**	3.8 ns	4.7	1.3
	%TSS	40.6	0.8	33.7	8.7	16.2	
PLH	MS	56,889.6**	396.3	701.6**	76.9 ns	93.2	9.69
	%TSS	61.0	0.6	23.7	5.2	9.4	
PL	MS	3276.9**	76.1	119.7**	22.8 ns	26.3	12.5
	%TSS	29.6	1.0	34.0	13.0	22.4	
SL	MS	24.3 ns	14.9	299.5**	80.6 ns	161.6	11.99
	%TSS	4.2	2.6	51.6	13.9	27.8	
FL	MS	552.5**	14.3	9.0**	4.9 ns	5.3	11.56
	%TSS	33.0	1.3	17.0	18.6	30.1	
CT	MS	664.7*	67.4	4.6 ns	3.1 ns	2.5	5.5
	%TSS	49.7	7.6	10.9	14.5	17.4	
NDVI	MS	0.613**	0.018	0.010**	0.004 ns	0.003	9.2
	%TSS	41.1	1.8	21.3	15.5	20.3	
SPAD	MS	1261.2**	43.6	37.2**	8.6 ns	9.2	6.1
	%TSS	32.3	1.7	30.0	13.8	22.3	
DHE	MS	1409.1**	3.8	88.0**	7.3 ns	8.5	2.2
	%TSS	25.9	0.1	50.9	8.5	14.7	

df degree of freedom, *MS* mean squares, *%TSS* percentage relative to total sum of squares, *DHE* days to heading, *DMA* days to maturity, *NGPS* number of grains per spike, *PLH* plant height, *FL* flag-leaf length, *PL* peduncle length, *SL* spike length, *SPAD* SPAD-reading, *NDVI* normalized difference vegetation index, *CT* canopy temperature, *TKW* thousand kernel weight, *YLD* grain yield

** and ns indicate significant at 1% level of probability and non-significant, respectively

contribution to the total variation (34.2%), followed by environment (27.8%) and GE interaction (25.3%). For TKW, the genotype effect expressed the highest contribution (58.3%) to total variation, followed by environment (15.3%) and GE interaction (14.7%). Overall, the contribution of genotype to observed variation was the largest for grain yield, TKW, NGPS, SL, PL and DHE, while the contribution of the environment to total variation was the highest for PLH, CT, DMA, NDVI, FL, and SPAD.

Descriptive statistics including, mean, range values, and coefficient of variability (CV%), and broad-sense heritability (H^2b) for each measured trait in each environment are presented in Table 2. Under rainfed condition in 2019–20, *T. turgidum* subsp. *polonicum* and *T. turgidum* subsp. *dicoccon* were the earliest in heading (DHE = 134 days) and *T. turgidum* subsp. *turanicum* was the latest one (DHE = 140 days). Moreover, accessions with early

heading (DHE = 127 days) were found in subsp. *durum*. The highest values for SPAD reading were expressed in *T. turgidum* subsp. *turanicum* and *T. turgidum* subsp. *turgidum*, while the lowest value was observed for *T. turgidum* subsp. *durum*. The highest NDVI was recorded for subsp. *dicoccon* and the lowest was observed for subsp. *turanicum*. The highest mean number of grains per spike was found in the accessions of subsp. *turanicum* (51 grains/spike) while the lowest value was observed in subsp. *durum* and *dicoccon*. The maximum values for TKW (40.2 g) were found in subsp. *polonicum* followed by subsp. *durum* (38.5 g), and the accession with the highest mean yield (4392 kg/ha) was found in subsp. *turgidum*.

Under severe drought conditions of 2020–21, the accessions with the earliest heading date (DHE = 119 days) were found in subsp. *durum* and *turgidum* which also exhibited the maximum values

Table 2 Agro-physiological description of different subspecies of tetraploid wheat (*Triticum turgidum* L.) accessions and commercial cultivars under drought and irrigated environments during two cropping seasons

Sub-species	Statistics	DHE	SPAD	NDVI	CT	FL	SL	PL	PLH	DMA	NGPS	TKW	YLD
Moderate drought condition 2019–20 (RF20)													
Carthlicum (n=11)	Mean±SE	137±0.7	46.5±0.5	0.7±0.01	30.5±0.1	22.2±0.2	8.5±0.2	45.8±1.0	124.4±2.2	172±0.4	48±1.6	29.7±0.6	2237±123.7
	Range	132–141	43.2–492	0.63–0.76	30.1–31.4	21.2–23.9	8.0–9.9	40.8–50.6	111.7–134.7	170–175	40–58	26.8–33.6	1732–3045
	CV (%)	1.6	3.9	5.41	1.1	3.3	6.8	7.4	6	0.8	11	6.9	18.3
Dicoccon (n=3)	Mean±SE	134±0.9	46.5±0.6	0.73±0.57	30.7±0.1	22.2±0.3	8.0±0.1	48.7±2.3	130.6±2.8	170±0	45±1.2	29.5±1.7	2867±115.4
	Range	132–135	46.3–46.8	0.73–0.74	30.5–31.0	21.7–22.6	7.7–8.7	44.3–51.8	126.3–135.8	170–170	43–47	26.2–31.5	2637–2991
	CV (%)	1.1	0.6	0.57	0.8	2.1	7.1	8.1	3.7	0.2	4.6	9.8	7.0
Durum (n=39)	Mean±SE	135±0.5	46.3±0.3	0.69±0.01	30.5±0.1	22.4±0.1	7.9±0.1	47.3±0.7	124.3±1.5	171±0.3	45±0.9	32.2±0.6	2616±97
	Min	127–141	42.6–50.6	0.57–0.76	30–31.4	21.8–24.1	7–8.9	34.1–53.8	96.1–139.6	167–173	33–55	26.4–40.2	1165–38.7
	CV (%)	2.3	3.8	5.39	1.2	2.3	5.4	8.6	7.4	0.7	12.3	10.8	23
Polonicum (n=4)	Mean±SE	134±2	46.9±1.2	0.7±0.01	30.5±0.1	22.8±0.5	8.6±0.3	48.6±1.9	130±4	170±0.7	47±4.1	33.4±2.3	2544±168.9
	Range	130–137	44.4–49.2	0.68–0.71	30.2–30.8	21.3–23.4	7.8–9.4	45.6–54.2	121.6–140.6	169–172	36–54	28.3–38.5	2087–2903
	CV (%)	2.0	5.0	2.14	0.9	4.3	7.8	7.9	6.1	0.8	17.5	14	13.3
Turgidum (n=4)	Mean±SE	136±2.3	47.4±1.1	0.7±0.03	30.8±0.5	22.3±0.3	7.9±0.2	43.3±2.5	117.2±7	171±1.5	51±3.8	29.7±1.4	3047±607.5
	Range	131–141	45.9–50.2	0.62–0.74	30.1–32.2	21.3–22.8	7.7–8.4	39.2–50.4	98.5–131.3	168–175	40–57	27.4–33.9	1458–4392
	CV (%)	3.4	4.5	7.95	3.1	3	3.8	11.5	11.9	1.7	15.1	9.7	39.9
Turanicum (n=1)	Mean±SE	140	47.5	0.68	30.7	23.7	8.7	44.7	128	173	47	32.3	1821
Saji (Check)	Mean	127	51.3	0.62	31.3	21.5	7.4	44.8	105	167	56	33.5	3931
Zahab (Check)	Mean	128	50.6	0.66	30.8	21.8	7.6	43.6	105.7	167	56	36.3	3807
Overall experiment	Mean	135	46.6	0.7	30.6	22.4	8.1	46.8	124	170.7	46.7	31.7	2611.7
	H²b	0.76	0.64	0.53	0.42	0.35	0.53	0.7	0.67	0.75	0.85	0.84	0.89
	LSD	5.5	4.2	0.1	1.5	2.4	1.4	7.8	19.9	2.7	7.0	4.3	661.6
Severe drought condition 2020–21 (RF21)													
Carthlicum (n=11)	Mean±SE	131±0.5	48.2±0.2	0.59±0.01	28.7±0.1	19.1±0.1	7.7±0.2	37.4±0.7	89.7±1.3	166±0.4	38±0.3	30.6±0.9	1670±69.9
	Range	128–133	47.2–49.1	0.58–0.61	28.2–29.2	18.9–19.6	7.1–8.7	33.2–42.2	85.7–98.4	165–169	36–39	27–36.5	1349–2012
	CV (%)	1.1	1.2	1.72	1.1	1	6.9	6.3	4.7	0.8	2.2	10	13.9
Dicoccon (n=3)	Mean±SE	131±1.3	48.9±0.4	0.57±0.01	28.8±0.3	19.2±0	7.8±0.2	35.3±1.1	88.4±3.8	165±0.4	37±0.3	28.7±1.4	1825±178.6
	Range	128–133	48.4–49.6	0.55–0.6	28.4–29.2	19.1–19.3	7.5–8.1	33.7–37.5	83.3–95.8	164–165	37–38	26.8–31.5	1484–2087
	CV (%)	1.7	1.4	3.95	1.5	0.4	3.4	5.5	7.4	0.5	1.4	8.5	16.9
Durum (n=39)	Mean±SE	129±0.5	48.7±0.2	0.58±0.01	28.7±0.1	19.1±0	7.5±0.1	37.4±0.5	87.7±1	165±0.2	37±0.1	32.8±0.5	1797±47.9
	Range	119–135	46.3–50.6	0.54–0.62	27.9–29.9	18.8–19.6	6.3–8.7	31.4–42.5	65.7–104	161–169	35–40	25.5–38.7	1222–2494
	CV (%)	2.5	2.4	3.2	1.7	0.9	7.5	7.5	6.9	0.9	2.5	9.8	16.6

Table 2 (continued)

Sub-species	Statistics	DHE	SPAD	NDVI	CT	FL	SL	PL	PLH	DMA	NGPS	TKW	YLD
Polonicum (n=4)	Mean±SE	129±1.2	48.4±0.4	0.57±0.01	29.3±0.2	19.1±0.1	8.1±0.4	39±1.2	89.4±3.6	165±0.6	38±0.4	34.8±4.6	1637±141.4
	Range	127–131	47.5–49.2	0.56–0.6	28.9–29.7	19–19.2	7.1–9.1	36.3–42.1	79.4–95.8	164–166	37–39	27–48	1281–1941
	CV (%)	1.9	1.5	2.55	1.1	0.6	10.2	6.1	8	0.7	2.3	26.2	17.3
Turgidum (n=4)	Mean±SE	128±3.4	49.2±0.7	0.58±0.01	29.1±0.1	19.1±0.2	7.3±0.2	34.8±2	79.4±5.4	165±1.8	37±0.6	31.5±1.7	1834±377.9
	Range	119–135	47.3–50.9	0.55–0.61	29–29.5	18.7–19.5	6.8–7.8	30.7–38.5	65–88.9	160–168	36–39	26.7–34.1	1240–2880
	CV (%)	5.2	3	4.71	0.8	1.9	6.3	11.4	13.7	2.1	3.2	10.7	41.2
Turanicum (n=1)	Mean	133	48.2	0.61	28.5	19	7.5	40.9	102.3	166	37	33.5	1329
Saji (Check)	Mean	121	51.5	0.56	28.7	19	6.4	34.5	75.9	159	38	35.5	2241
Zahab (Check)	Mean	122	50.1	0.55	29.3	19.2	6.8	35.6	76.3	161	38	34.5	2346
Overall experiment	Mean	129.2	48.7	0.6	28.8	19.1	7.5	37.2	87.6	164.9	37.5	32.3	1777.1
	H^2b	0.83	0.38	0.52	0.31	0.11	0.61	0.67	0.75	0.71	0.14	0.76	0.73
	LSD	4.4	4.1	0.1	1.9	1.5	1.3	5.9	11.6	3.5	6.4	6.1	581.3
Irrigated condition 2020–21 (IR21)													
Carthlicum (n=11)	Mean±SE	130±0.5	52.3±0.2	0.58±0.003	26±0.1	18.7±0.1	7.7±0.2	39.6±0.2	91.1±1.2	168±0.3	41±1.5	34.4±0.9	2617±89.8
	Range	127–133	51.4–53.6	0.56–0.59	25.5–26.6	18.4–19.2	6.8–9.3	38.8–40.5	86.2–99.4	166–170	35–48	30.2–40.5	2248–3291
	CV (%)	1.3	1.4	1.67	1.4	1.5	8.4	1.3	4.3	0.6	12.2	8.5	11.4
Dicoccon (n=3)	Mean±SE	129±0.5	52.1±0.4	0.57±0.01	26.1±0.3	18.6±0.2	7.3±0.3	39.3±0.5	92.3±2.5	167±0.3	40±2.6	35.3±2.2	2596±222.4
	Range	128–130	51.4–52.8	0.56–0.58	25.5–26.7	18.3–18.9	6.7–7.7	38.3–39.9	88.3–96.9	166–167	36–45	30.9–38.1	2253–3013
	CV (%)	0.7	1.3	2.16	2.3	1.6	7.5	2.2	4.7	0.3	11.2	10.8	14.8
Durum (n=39)	Mean±SE	129±0.3	52.8±0.2	0.57±0.01	26±0.1	18.5±0.1	7.5±0.1	39±0.2	87.1±1	167±0.2	38±0.9	37.1±0.7	2751±70.33
	Range	125–134	51–55.8	0.54–0.6	25.2–26.8	17.8–19.4	6.4–8.8	36.3–40.9	64.2–98.5	165–169	23–51	29.2–44.8	1846–3612
	CV (%)	1.6	2.3	2.19	1.5	2	7.8	2.6	6.9	0.6	15.7	12	15.97
Polonicum (n=4)	Mean±SE	130±0.9	53.1±0.6	0.57±0	25.8±0.3	18.5±0.2	8.1±0.5	39.1±0.4	88.8±2	167±0.4	43±4	39.1±5.9	2648±150.7
	Range	128–132	51.7–54.1	0.57–0.58	25.3–26.5	17.9–19	7.5–9.7	38.1–40	83–92.5	166–168	31–48	31–56.2	2409–3088
	CV (%)	1.3	2.1	1.21	2	2.6	13.5	2	4.6	0.5	18.5	29.9	11.4
Turgidum (n=4)	Mean±SE	130±1.9	53.4±1	0.56±0.01	26.7±0.3	18.3±0.2	7.5±0.3	38.1±0.7	81.9±5.7	167±0.6	38±5.4	34.9±1.9	2745±316.1
	Range	125–134	51.8–56.3	0.54–0.59	26.1–27.6	17.9–18.6	6.8–8.3	36.2–39.6	65–89.6	165–167	22–46	30.4–39	2071–3572
	CV (%)	2.9	3.8	3.89	2.3	1.7	8.9	3.8	14	0.7	28.9	10.8	23
Turanicum (n=1)	Mean	131	53.7	0.59	25.8	18.5	7.1	39.4	92.9	169	33	39.4	2352
Saji (Check)	Mean	125	56.7	0.57	26.4	18.2	6.4	40.4	69.9	165	36	37.7	3584
Zahab (Check)	Mean	125	53.9	0.55	26.5	18.2	6.6	41.7	73.7	164	42	43.9	3443

Table 2 (continued)

Sub-species	Statistics	DHE	SPAD	NDVI	CT	FL	SL	PL	PLH	DMA	NGPS	TKW	YLD
Overall experiment	Mean	129.3	52.8	0.57	26.1	18.5	7.6	39.2	87.4	166.9	38.8	36.7	2731.2
	H^2b	0.64	0.49	0.31	0.38	0.20	0.65	0.21	0.67	0.51	0.86	0.92	0.68
	LSD	4.89	3.79	0.06	1.66	2.06	1.42	5.67	14.05	3.18	6.98	4.41	846.3

DHE days to heading, *DMA* days to maturity, *NDVI* normalized difference vegetation index, *CT* canopy temperature, *NGPS* number of grains per spike, *PLH* plant height, *FL* flag-leaf length, *PL* peduncle length, *SL* spike length, *SPAD* SPAD-reading, *TKW* thousand kernel weight, *YLD* grain yield, *TKW* thousand kernel weight, *SE* standard error, H^2b broad-sense heritability, *LSD* least significant difference ($P < 0.05$)

of SPAD reading and NDVI. Accessions with maximum TKW belonged to subsp. *polonicum* (48.0 g) and subsp. *durum* (38.7 g). The accessions belonging to subsp. *turgidum* performed well under severe drought conditions with an average grain yield of 1834 kg/ha (ranging from 1240 to 2880 kg/ha), followed by accessions belonging to subsp. *dicoccon* with an average grain yield of 1825 kg/ha (ranging from 1484 to 2087 kg/ha); and accessions belong to subsp. *durum* with an average grain yield of 1797 kg/ha (ranging from 1222 to 2494 kg/ha). The accessions from subsp. *turgidum* and subsp. *durum* expressed better performance than the two check cultivars.

Under irrigated conditions in 2020–21, the best yielding accessions belonged to subsp. *durum* (3612 kg/ha) and subsp. *turgidum* (3572 kg/ha), out-yielded the check cultivars. The maximum values for TKW were found among the subsp. *polonicum* (56.2 g) and subsp. *durum* (44.8 g). The highest number of grains/spike (average 43 grains; and ranged from 31 to 48) was shown by subsp. *polonicum* followed by subsp. *durum* (average 41 grains; varied between 35 and 48). The highest spike length was observed for subsp. *polonicum* (8.1 cm; ranged from 7.5 to 9.7 cm) and the shortest spike length was recorded for the check cultivars belonging to subsp. *durum*. Highest NDVI was expressed for subsp. *turanicum* which was also the latest in heading among the six *turgidum* subspecies.

Mean yield and stability performance of tetraploid wheat accessions

Evaluation of GE interaction and stability performance of 64 tetraploid accessions through GGE biplot analysis is presented in Fig. 2. The GGE biplot accounted for 88.46% of the total variation in grain yield. The which-won-where pattern of the GGE biplot indicated remarkable GE interaction between tetraploid accessions and environments and allowed the visualization of the best-performing accessions. In the polygon view of the GGE biplot (Fig. 2), the vertex accessions for grain yield were accessions G56, G37, G54, G60, G52, and G10, with G56 and G10 taking the vertices for the highest grain yield. Hence, these two accessions showed the highest response to environmental conditions for grain yield, where the accession G56 belonged to subsp. *turgidum* (originated from Pakistan) expressed the highest response

Fig. 2 Patterns of (i) "which-wins-where" and (ii) "mean *versus* stability" views of the GGE biplot for grain yield of the 64 tetraploid wheat accessions across three environments. The vertex accessions on convex hull (polygon) are the best in each mega-environment for the corresponding trait. The green arrowhead line that passes through the origin shows higher mean performance of an accession and the green dotted lines extending from the green arrowhead line show the stability of the accessions. The shorter the dotted line the higher the stability of the accessions. (Color figure online)

Fig. 3 Patterns of (i) "which-wins-where" and (ii) "mean *versus* stability" views of the GGE biplot for grain yield of the the six tetraploid subspecies across three environments. The green arrowhead line that passes through the origin shows higher mean performance of an accession and the green dotted lines extending from the green arrowhead line show the stability of the accessions. The shorter the dotted line the higher the stability of the accession. (Color figure online)

to drought environments (RF20 and RF21); and in contrast, G10 belonged to subsp. *durum* (originated from Turkey) was the best performer under irrigated environment (IR21).

In the polygon view of the GGE biplot (Fig. 2), lines from the origin of the biplot divide the biplot into different sections, which are resulting in different environment groups (Yan and Tinker 2006). Accordingly, two groups were identified for grain yield. Environments representing drought conditions (RF20 and RF21) jointly formed one group, whereas the irrigated environment (IR21) made a separate group for grain yield. The G56 was the best performing entry in drought environments. The other accessions which are far from the origin of the biplot also expressed good adaptation to these stressed environments. These accessions were: G20 belonging to subsp. *durum* with the origin of Turkey, G49, G4, and G6 (all belonging to subsp. *durum*) with the origins of Russia, Turkey and Russia, respectively, and G63 and G64 (check cultivars). Similarly, the genotype G10 followed by G15, G17, G11 and G26 were the best yielders in the section where environment IR21 was

positioned. The other genotypes which exhibited also relatively good adaptation to IR21 were: G17, G15 (Russia), and G11 (Turkey) all belonging to subsp. *durum*.

The genotypes were ranked for integrating mean yield and stability performance across environments using the average environment coordinate (AEC; Fig. 2). The AEC represented by a single arrowhead line that passes through the biplot origin shows a higher mean performance of a genotype. Genotypes G56, G20, G63, G64, G4, G6, G49, G12, G2, G17, and G34 expressed the highest mean yields across environments. In contrast, G54, G60, G58, G21, and G33 had the lowest yields across environments. Accessions far from the origin but positioned on the AEC or close to it are considered as best performing and stable accessions. Accordingly, G56, G63, G20, G64, G6, and G4 were the most stable genotypes with high mean yield, whereas G17, G37, and G10 with high mean yield had long distances from the AEC and then expressed the highest instability (Fig. 2).

For the assessment of mean yield and stability of tetraploid wheat accessions in the level of subspecies,

Fig. 4 Patterns of (i) "which-for-what" and (ii) "traits relationships" views of the GT-biplot for 12 traits of 64 tetraploid wheat accessions under rainfed condition in 2019–20 (**A**), under rainfed condition in 2020–21 (**B**) and under irrigated condition in 2020–21 (**C**). Vertex accessions on convex hull (polygon) are the best in each section for the corresponding trait. The dotted blue lines shows traits vectors and the angle between trait vectors shows correlation between traits. Acute angle shows positive correlation, obtuse angle indicates negative correlation and right angle represent for no correlation. (Color figure online)

a GGE biplot was constructed (Fig. 3). In the polygon view of the GGE biplot (Fig. 3), the vertex subspecies for grain yield were subsp. *turgidum*, *durum*, *carthlicum*, *turanicum* and *dicoccon*, showing the highest response to environmental conditions. The subsp. *turgidum* and subsp. *durum* took the vertices for the highest mean yields. The subsp. *turgidum* expressed the highest positive response to environments RF20 and RF21, and in contrast subsp. *durum* was the best performer for the environment IR21.

The *T. turgidum* subsp. *turgidum* followed by subsp. *durum* was the most stable with high mean yield, whereas subsp. *dicoccon* with a high mean yield expressed a longer distance from AEC and then showed the highest instability (Fig. 3). The subsp. *polonicum* with average mean yield showed the highest stability, while subsp. *turanicum* showed the lowest performance with average stability.

Trait relationships and trait profile of tetraploid wheat accessions

To study the traits profile of tetraploid wheat accessions and the relationships among investigated traits, a graphical genotype by trait (GT) biplot analysis was applied based on the genotype-by-trait matrix data for each environment (Fig. 4). The GT biplots explained 45.68–49.16% of the total variation. In the case of environment RF20 (Fig. 4a), the accessions and traits fell into six sections. The grain yield is separated into a single section, with G33 being the winner. The next section consisted of TKW, SPAD, NGPS, and FL, with G12 being the winner, while the third section comprised the accession G61 with the highest values of heading date and days to maturity. The fourth section comprised NDVI and PLH with G50 having the best combination for these traits. G52 expressed the highest value for peduncle length, while G54 showed
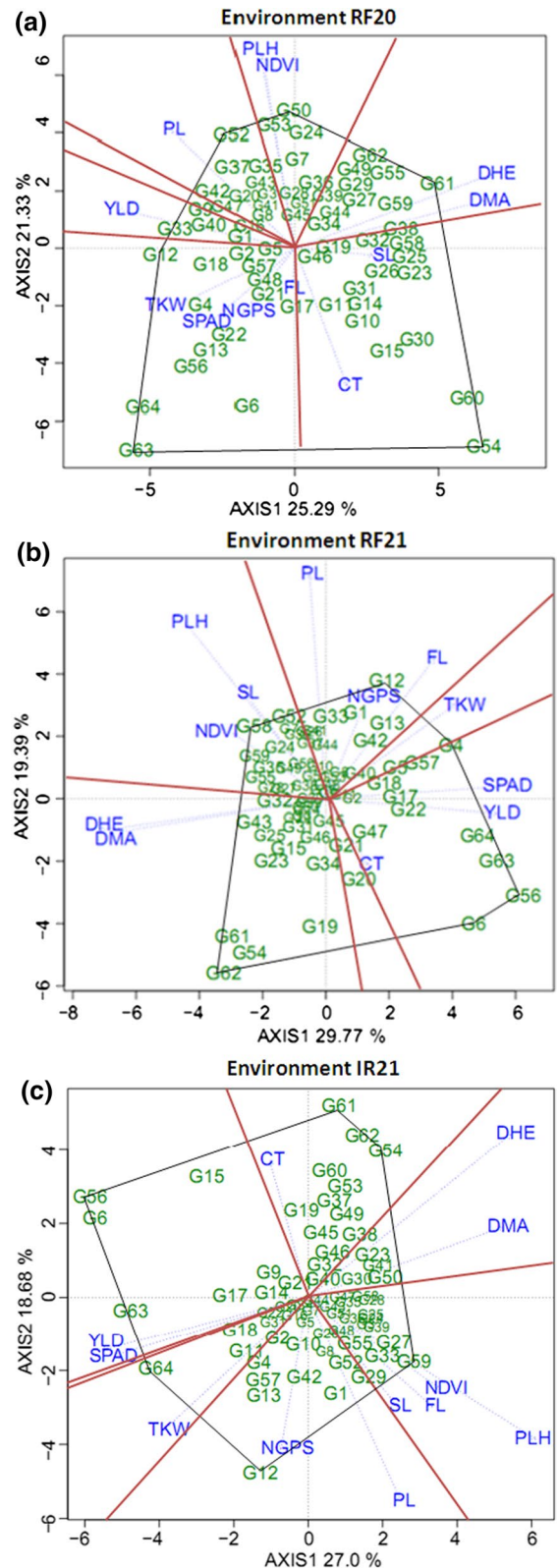
the highest combination level for traits of canopy temperature and spike length. Figure 4a also shows graphical relationships among studied traits. Most prominent positive correlations were found between NDVI and PLH, between DHE and DMA, between TKW, SPAD, and NGPS, and between grain yield and PL, as can be observed through the acute angles between their respective vectors. Accessions G33, G12, G40, G9, G42 as well as G63, G64, G56, G13, G22, G6, and G18 positively interacted with grain yield, TKW, SPAD, and grains/spike; while these accessions showed negative interaction with traits of days to heading and maturity, spike length and canopy temperature.

Under severe drought conditions in 2020–21 (Fig. 4b), the GT biplot explained 49.16% of the total variation. The traits fall into five sections and genotypes into six sections. Genotype G56 belonging to subsp. *turgidum* followed by G6, G63 and G64 had the best combination levels for grain yield, SPAD-reading, and canopy temperature. G58 had a high combination of plant height, spike length, and NDVI. G12 was characterized by the highest values of flag-leaf length, peduncle length, and the grains/spike; and G4 expressed the highest 1000-kernel weight. Genotype G12 followed by G54 and G61 exhibited the highest values of days to heading and maturity. Grain yield was positively associated with SPAD-reading, and G56, G63, and G64 positively interacted with these traits (Fig. 4b). Strong positive correlations were also observed between 1000-kernel weight, flag-leaf length and grains/spike, and G4, G57, G12, G13, G42, and G5 positively interacted with these traits. Plant height, peduncle length, spike length, and NDVI were positively associated in genotypes ranking, and G58 and G52 positively interacted with these traits. Days to heading and maturity were strongly correlated and showed negative or no correlation with other traits; where the G62, G54, G61, G43, G23, G25 and G15 positively interacted with these traits; and negatively interacted with grain yield and yield-related traits. Days to heading and maturity, plant height, peduncle length, grain yield and SPAD with highest long vectors showed the highest discriminating ability, while canopy temperature and NDVI exhibited the least discriminating ability.

In the case of environment IR21 (Fig. 4c), the GT biplot accounted for 45.68% of the total variation, and the traits and genotypes fell into six sectors. The genotype G56 followed by G6 and G63 expressed the highest combination level for grain yield and SPAD. Genotype G59 showed the highest values for plant height, NDVI, flag-leaf length and spike length. Genotype G12 expressed the highest combination of the grains/spike and peduncle length; and G61 expressed the highest value for canopy temperature. Genotype G64 expressed the highest 1000-kernel weight, while days to heading and maturity fell in the same section without any vertex genotype in their respective sector. Graphical analysis of trait relationships under irrigated conditions (Fig. 4c) indicated strong positive correlations between grain yield with SPAD and 1000-kernel weight; where G56, G6, G63, and G64 interacted positively with these traits. These traits were negatively associated with phenological traits (DHE and DMA), suggesting that selection for higher grain yield and TKW will enhance early maturity. Plant height, NDVI, flag-leaf length, spike length and peduncle length were positively correlated, and G59, G33, G29, G27, and G52 positively interacted with this group of traits. The grains/spike showed positive correlation with 1000-kernel weight and G12, G13, G57 and G64 expressed the highest values for these traits.

Genetic and phenotypic correlations of the 12 studied traits in the 64 tetraploid wheat accessions across environments are presented in Table 3. Significant positive genetic and phenotypic correlations were observed between TKW and SPAD with grain yield, indicating that accessions with higher TKW and SPAD-reading also produced higher grain yield. The significant negative correlation between days to heading, days to maturity, spike length and plant height with grain yield suggested that accessions with early heading and maturity and optimum plant stature tend to have higher productivity. Flag-leaf length showed a significant positive genetic and phenotypic correlation with TKW, suggesting that accessions with higher flag-leaf length tend to have higher kernel weights. NDVI was positively correlated ($P<0.01$) with days to heading and maturity and plant height, suggesting that the accessions with higher plant stature and late heading and maturity have higher values of vegetation index. The genetic and phenotypic correlation analyses between traits were in agreement with those reported by graphical analysis of trait relationships through GT biplot analysis.

Table 3 Genetic (diagonal lower) and phenotypic (diagonal upper) correlations of 12 studied traits in the diversity panel of 64 tetraploid wheat accessions across environments

Traits	DHE	SPAD	NDVI	CT	FL	SL	PL	PLH	DMA	NGPS	TKW	YLD
DHE		−0.52**	0.35**	−0.12	−0.21	0.11	−0.25*	0.33**	0.92**	−0.30*	−0.50**	−0.63**
SPAD	−0.66**		−0.18	0.01	0.21	−0.19	−0.02	−0.37**	−0.41**	0.43**	0.48**	0.53**
NDVI	0.46**	−0.27*		−0.61**	0.01	0.06	0.25*	0.56**	0.27*	−0.08	−0.24*	0.12
CT	−0.25*	0.05	−1.00**		−0.04	0.07	−0.23	−0.42**	−0.11	−0.21	0.01	−0.14
FL	−0.43**	0.34**	0.01	−0.23		0.50**	0.40**	0.20	−0.02	0.21	0.48**	0.04
SL	0.07	−0.23	0.14	0.09	0.72**		0.23	0.29*	0.21	−0.03	0.17	−0.36**
PL	−0.26*	0.01	0.29*	−0.30*	0.64**	0.34**		0.75**	−0.17	0.29*	0.18	−0.07
PLH	0.41*	−0.46**	0.60**	−0.64**	0.30*	0.36**	0.77**		0.35**	0.08	−0.11	−0.41**
DMA	0.96**	−0.53**	0.36**	−0.29*	−0.05	0.24*	−0.17	0.40**		−0.17	−0.37**	−0.66**
NGPS	−0.43**	0.66**	−0.25*	−0.33**	0.35**	−0.07	0.40**	0.10	−0.31*		0.12	0.21
TKW	−0.56**	0.54**	−0.32**	−0.07	0.81**	0.21	0.22	−0.13	−0.42**	0.16		0.26*
YLD	−0.74**	0.78**	−0.06	0.01	0.27*	−0.39**	−0.16	−0.64**	−0.81**	0.31*	0.30*	

DHE days to heading, *DMA* days to maturity, *NGPS* number of grains per spike, *PLH* plant height, *FL* flag-leaf length, *PL* peduncle length, *SL* spike length, *SPAD* SPAD-reading, *NDVI* normalized difference vegetation index, *CT* canopy temperature, *TKW* thousand kernel weight, *YLD* grain yield

*, ** significant at 5%, 1%, respectively

Quantifying drought tolerance/susceptibility in tetraploid wheat accessions

For evaluation of drought tolerance of the 64 tetraploid wheat accessions, five selection indices of STI, GMP, MP, TOL, and SSI were calculated (Table S2). The highest value of STI was obtained for G56 (belonging to subsp. *turgidum*) followed by G64, G63, G17, and G6 (belonging to subsp. *durum*), and the lowest was identified for G54 (subsp. *durum*) followed by G60 (subsp. *turgidum*), G58 (subsp. *turanicum*), G33 (subsp. *polonicum*) and G59 (subsp. *carthlicum*) (Table S2). Similar rankings of genotypes were obtained using GMP and MP.

The low value of the TOL index shows a low difference between the response of genotypes to drought and irrigated conditions; and then, no positive response to a favorable condition, which leads to finding stable genotypes with more tolerance to environmental changes. In contrast, a high value of TOL shows a positive response of genotypes to favorable conditions, leading to identifying genotypes specifically adapted to irrigated environments. Based on this, genotypes G15 (subsp. *durum*) followed by G2 (subsp. *durum*), G26 (subsp. *carthlicum*), G11 (subsp. *durum*) and G10 (subsp. *durum*) with the highest TOL values showed good response to the irrigated condition. In contrast, the genotypes G53, followed by G49, G37, G36, and G4 (all belonging to subsp. *durum*) exhibited the lowest values for TOL, which could be considered as resistant genotypes to environmental stresses.

In the case of SSI, genotypes with higher values are more susceptible to drought stress and those with lower values are more resistant. The highest value for SSI was obtained for G15 (subsp. *durum*) followed by G26 (subsp. *carthlicum*), G14 (subsp. *durum*), G11 (subsp. *durum*), and G2 (subsp. *durum*) should be characterized as most susceptible to drought stress. In contrast, the lowest value for SSI was found for G53, G49, G37, G4 (all belonging to subsp. *durum*), and G37 (subsp. *dicoccon*), indicating these genotypes are resistant to drought stress.

To better understand the relationships among drought selection indices and characterize the tetraploid wheat accessions, a biplot based on principal component analysis (PCA) was constructed based on the first two components (PC1 and PC2) that justified approximately 99.59% of the total variation (Fig. 5). The PC1 had high positive coefficients for STI, GMP, MP, Ys, and Yp. Thus, the first component could be named as a component of yield potential and tolerance to drought stress. STI, GMP, and MP were positively associated with Ys and Yp. This shows that the indices STI, GMP, and MP are suitable indices to identify
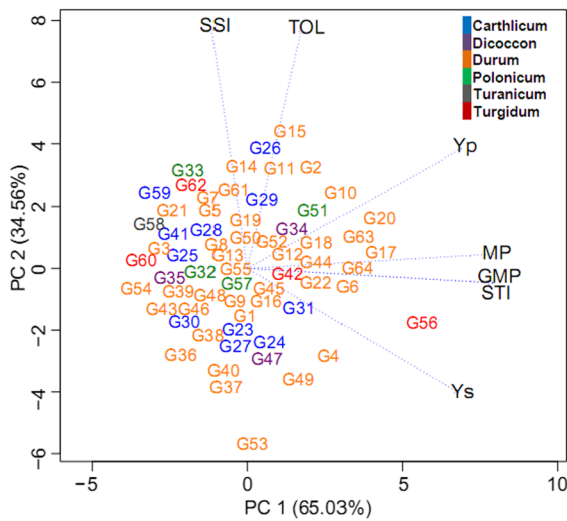
Fig. 5 PCA-based biplot for drought tolerance indices and mean yields under drought (Ys) and irrigated (Yp) conditions based on the first two principal components axes (PC1 and PC2) for 64 tetraploid wheat accessions. STI: stress tolerance index; GMP: geometric mean productivity; MP: mean productivity; TOL: tolerance index; SSI: stress susceptibility index

high-yielding and drought-tolerant genotypes under drought stress and non-stress conditions. Considering the high and positive values of PC1, genotypes with high values for these indices should be considered as drought-tolerant genotypes with high-yielding performance in both conditions. In this regard, accessions G56 (subsp. *turgidum*), G17, G20, G64, G63, and G6 (subsp. *durum*) positively interacted with these indices and should be considered as superior genotypes under both drought and irrigated conditions. The second component exhibited high positive coefficients for the indices SSI and TOL, thus could be named as a component of low yield and susceptibility to drought stress. Accessions G15 (subsp. *durum*), G26 (subsp. *carthlicum*), G14 (subsp. *durum*), G11 (subsp. *durum*), G33 (subsp. *polonicum*), G62 (subsp. *turgidum*), G61 (subsp. *durum*), G2 (subsp. *durum*), and G29 (subsp. *carthlicum*) with low PC1 and high PC2 showed a high level of susceptibility to drought stress and performed poorly under both conditions (Fig. 5).

Hierarchical clustering patterns of geographical origins

Cluster analysis based on the 12 measured traits using Ward's method was applied to analyze the relationships between tetraploid wheat accessions. The results of hierarchical cluster analysis for the geographical origins and their corresponding sub-species, allowed to identify eight accession groups that may be explored as potential genetic materials in durum wheat breeding programs. Group VII was the largest consisting of 21 accessions, followed by group VIII which contained 12 accessions. Groups II, III, V, VI, I, and IV, respectively, comprised 10, 10, 4, 3, 2, and 2 accessions. The clusters exhibited good correspondence with the stability and the performance of accessions obtained from the GGE biplot analysis. For example, accessions no. G56, G6, G63 and G64 in group V were among the high yielding accessions with high stability based on GGE ranking biplots. Furthermore, the two check varieties were grouped in cluster V. However, the results of cluster analysis do not support the relationship of the formed groups based on their geographical origins as well as on their respective sub-species. Based on the results, accessions from different regions were classified together and accessions with the same origins were classified into different groups. For example, among 27 accessions that were originally collected from Turkey, 12 accessions were classified in group VII, five in group VIII, four in group III, four in group II, and two accessions in group VI (Fig. 6). Such trends were also observed for the sub-species, as their corresponding accessions were distributed across different groups. For example in group V, three accessions belonging to subsp. *durum* were grouped with one accession belonging to spp. *turgidum*. The group IV consisted of two accessions, both originated from Azerbaijan, one belonged to subsp. *durum* and the other belonged to subsp. *turgidum*. Similar trends were also observed for other subspecies.

Discussion

This study was a part of pre-breeding efforts for durum wheat improvement under rainfed condition of Iran; and we are aimed at identifying genotypes from among several tetraploid wheat subspecies
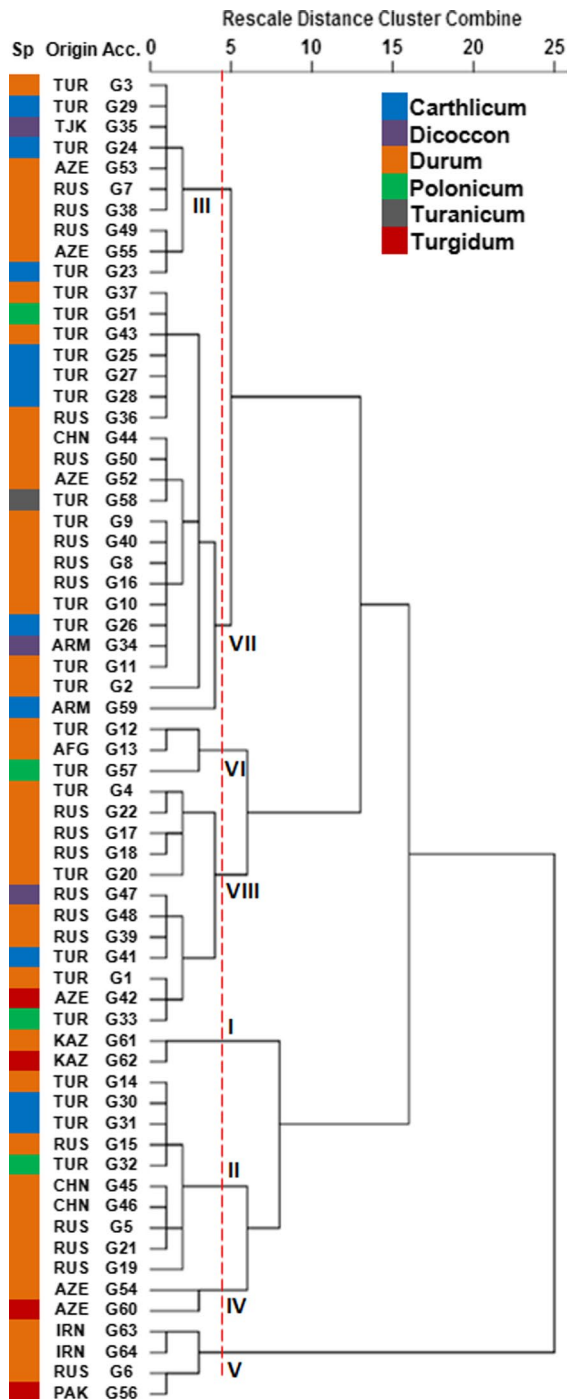
Fig. 6 Hierarchical cluster analysis generated using Ward's method based on the phenotypic traits measured for 64 tetraploid wheat accessions in relation to their geographical origins and respective subspecies. The dashed line represents the cutoff line for clusters (eight groups) according to discriminant analysis. Sp: Subspecies; Acc: Accession

that are candidates for contributing useful genes to the durum wheat breeding program. However, due to the limited genetic variability available in cultivated germplasm, pre-breeding is gaining importance in most crop improvement programs including wheat (Valkoun, 2001; Moore et al. 2015; Zaim et al. 2017). Furthermore, the increasing crop productivity under climate change requires the investigation, and subsequent exploitation of the genetic diversity preserved beyond the narrow range of commonly cultivated varieties (Moore et al. 2015). Under such situations, pre-breeding offers a unique tool to enhance the use of genetic variability present both in cultivated and wild type germplasm for better adaptation to the new circumstances of climatic change. Due to climate change, the Mediterranean area is expected to face more severe drought and an increase in average temperature in the near future, resulting in increased uncertainty in the performance of rainfed wheat (Mohammadi 2018). Under this situation, improving wheat productivity in drought-prone environments is a major challenge. Phenotyping available landrace collections and the integration of cost-effective technologies are crucial for achieving progress through plant improvement (Reynolds and Tuberosa 2008; Kyratzis et al. 2017; Mohammadi 2018).

It has been documented that the genetic diversity due to modern breeding has been increasingly narrowed in wheat and other crops (Autrique et al. 1996; Reif et al. 2005; Soriano et al. 2016; Salsman et al. 2021). This makes a serious challenge in breeding for higher crop performance and adaptation to biotic and abiotic stresses. Iran is one of the main centers for wheat domestication, and based on genetic and archaeological evidence, tetraploid wild emmer was domesticated from Fertile Crescent about 10,000 years ago (Salamini et al. 2002; MacKey, 2005; Oliveira et al. 2012).

Genetic variability plays an important role in plant breeding programs (Shukla et al. 2006). In durum wheat, species in the primary genepool, mainly landraces and primitive wheat belonging to subspecies of *Triticum turgidum* could contribute substantially to its improvement. The results of this study revealed a remarkable variation among the tetraploid wheat accessions for the measured traits. The considerable genotypic variation indicated in this study and in previous reports in durum landraces (Mohammadi et al.

2014; Royo et al. 2014; Kyratzis et al. 2017), suggest the existence of a great opportunity for selection and the use of landraces in durum wheat breeding programs. The highest variance due to the genotype effect indicates high genetic diversity in the tetraploid wheat accessions, which helps to select the best accessions for the target traits, and in such cases it is important to minimize the impact of the main environmental effects.

Previous studies have recommended GGE biplot as a useful tool for visualizing genotype stability and performance, genetic diversity between genotypes, and relationships with environments (Mohammadi et al. 2010; Das et al. 2019; Enyew et al. 2021). Using the GGE biplot method, the most responsive accessions to environmental conditions can be identified (Yan et al. 2000). In this study, accessions G56, G20, G63, G64, G4, G6, G49, G12, G2, G17, and G34 were the best performing for grain yield. The genotypes G56 belonged to subsp. *turgidum* followed by G20 G64, G64 G49, G4, and G6 belonging to subsp. *durum* showed the highest response to drought environments, and in contrast, genotypes G10, G15, G17, G11, and G26 (all belonging to subsp. *durum*) were the best performers under irrigated conditions. Differences in the relative performance of genotypes in different environments are also considered as a strong indicator of the existence of GE interaction (Gauch et al. 1997; Yan et al. 2000). High yielding genotypes under specific environments have been previously reported through GGE biplot analysis in different crop species such as in durum (Aberkane et al. 2021; Mohammadi et al. 2021) and bread wheat (Yan et al. 2000; Thungo et al. 2019). Using the GGE biplot, the top-ranking and stable accessions can be selected through the AEC axis (Yan 2002). In this study, G56 (subsp. *turgidum*), G20, G63, G64, G6 and G4 (subsp. *durum*) were found to be the most stable accessions with high mean yield, whereas G17, G37 and G10 (subsp. *durum*) with the highest mean yield but were instable. Previous reports on crop performance also showed that the high-yielding genotypes are not necessarily the most stable (Mohammadi et al. 2010; Yan et al. 2000; Enyew et al. 2021). The best accessions should express a larger projection on AEC which represents the highest mean yield and no distance from AEC refers to high stability (Yan and Tinker 2006). By this approach, many authors selected the best genotypes in durum wheat (Mohammadi et al. 2021)

and other cereal crops including bread wheat (Thungo et al. 2019), sorghum (Enyew et al. 2021) and Maize (Mallikarjuna et al. 2015).

Grain yield expressed significant positive phenotypic and genetic correlations with 1000-kernel weight and SPAD reading. This is in accordance with other reports in wheat (Mohammadi et al. 2018; Del Pozo et al. 2016). So, the positive correlation between yield and TKW indicates the possibility of simultaneous improvement of both traits through effective selection. Grain yield also exhibited significant negative phenotypic and genetic correlations with days to heading and maturity. This leads to the conclusion that selection for higher grain yield and TKW will be simultaneously improved by earliness. Negative correlations between grain yield with plant height, peduncle length, flag-leaf length, and phenological traits (heading and maturity) under drought conditions were confirming the results of other studies in durum wheat (Mohammadi et al. 2018; del Pozo et al. 2016). The negative correlations between grain yield and NDVI in 2020–21 were in agreement with Kyratzis et al. (2017) in durum wheat; while their positive correlation in 2019–20 was in agreement with other studies (Kyratzis et al. 2015; Mohammadi et al. 2021) in durum wheat.

The results verified that the clustering patterns of durum accessions are not mainly due to their geographical origin. This is in agreement with other reports in wheat that the accessions collected from the same geographical area are clustered in different groups (Mengistu et al. 2015; Yang et al. 2020). The clustering of accessions belonging to different regions in the same groups is probably due to the gene flow among the regions through market channels as well as the gradual exchange of grains among farmers. It could also be due to the fact that the used accessions were pre-selected for their cold tolerance and some agronomic traits.

The two national modern cultivars Saji and Zahab (belonging to subsp. *durum*), included in this study, were grouped in the same cluster (cluster V). These varieties are early in maturing and moderate in height with good yield potential. Accessions grouped with these check cultivars also should be considered as best accessions that combine high yielding with earliness and higher grain weight. Clustering of the best accessions identified by both GGE biplot and cluster analysis methods in

the same group shows that these accessions were selected for the same traits, particularly grain yield, which led to their greater similarity but showed more distance from other accessions. Multivariate analysis methods have been recommended to measure variability in the germplasm collection and to evaluate the relative contribution of different traits to the total variation in plant collections (Dodig et al. 2012; Rana et al. 2021; Das et al. 2019). These analyses allow the classification of germplasm into groups with similar traits. In this study, the results of biplot analysis for tetraploid wheat sub-species and geographical areas showed great diversity in the studied traits. The accessions with high mean yield tend to be early in maturity and to have a high SPAD index. These results show that grain yield is positively correlated with SPAD and negatively correlated with days to maturity. Similar results also are reported in wheat (Del Pozo et al. 2016; Roy et al. 2021), and rice (Saikumar et al. 2016). Based on the results, G56 (belonging to subsp. *turgidum*) and G20 (belonging to subsp. *durum*) were ideal accessions in terms of providing high mean yields and stability performance across environments. Furthermore, these accessions which expressed higher values of TKW and SPAD reading, were earlier in heading and maturity, and had optimum plant stature.

Considering the result of this study, it was observed that terminal drought stress decreased grain yield by 37% compared to irrigated conditions. Selection of accessions based on a combination of indices can be useful for improving drought tolerance with desirable yield under drought and irrigated conditions. Based on the results, genotypes G56 (subsp. *turgidum*), G17, G20, G64, G63, and G6 (subsp. *durum*) with higher yield performance under both stress and non-stress conditions were the most drought tolerant genotypes; and thus, should be recommended as sources of drought tolerance in durum wheat breeding.

In our investigation, G56 (belonging to subsp. *turgidum*) showed a wide adaptation to the environments that experience terminal drought conditions. This accession was one of the most stable accessions and performed better than the checks (Fig. 2). Therefore, this accession could be regarded as a potential genotype to be recommended for drought-prone environments. In addition, G20 (belonging

to subsp. *durum*) may qualify as another widely adapted and stable genotype, as its performance was greater than the checks. This accession also showed a positive response to the irrigated environment, as it can capture more benefits under favorable conditions. The development and release of high-yielding genotypes with high adaptation to different locations may allow the expansion of the areas used to grow durum wheat in Iran.

Conclusion

The results obtained in this study clearly showed that the tetraploid wheat sub-species are potential genetic resources with high diversity in grain yield and measured traits, which should be considered for the development of new high-yielding durum wheat cultivars. Among these, accessions with higher TKW, SPAD index and early in heading and maturity with optimum plant stature leads to higher grain yield under drought conditions in both cropping seasons. Additionally, genotypes with delayed leaf senescence (a higher SPAD index) would lead to higher grain yield and higher TKW. Therefore, SPAD reading, early heading and maturity dates that are significantly correlated with both TKW and grain yield are among the most useful traits for rapid evaluation of genetic material or for forward selection. The cluster analysis of accessions revealed that the clustering patterns of tetraploid wheat accessions are not mainly due to their geographical origin and respective subspecies. Among the accessions, the G56 (subsp. *turgidum*), G20, G63, G64, G6 and G4 (all belonging to subsp. *durum*) were the best in terms of providing high and stable grain yield in combination with breeder-preferred traits such as higher grain weight, optimum plant stature and drought tolerance. However, this does not make other sub-species less novel. This just highlights the closeness of turgidum to durum wheat compared to others in the evaluated traits. Selection of accessions based on a combination of drought selection indices can be useful for improving drought tolerance with desirable yield under drought and irrigated conditions. In summary, this study resulted in identifying several outstanding accessions that out-yielded the check varieties based on agronomic performance and drought tolerance which should be regarded for further evaluation in durum wheat breeding program.

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