



RESEARCH PAPER

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Analyzing winter wheat's drought stress tolerance via *in-vitro* and *in-vivo* screening

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Abstract

Occurrences of osmotic stress have profound impacts on global wheat production. Drought may be a global issue, in any wheat-producing region that can cause severe osmotic stress. In this study, a comparative study of drought tolerance screening techniques *in vitro* and *in vivo* was conducted using fifteen winter wheat cultivars. Under *in-vitro* screening, for simulating drought conditions, -0.45 MPa and -0.9 MPa osmotic potential were used. Polyethylene glycol 6000 was used to induce osmotic stress and seedling traits such as germination (%), shoot length, total root length, total root number, fresh and dry weights of shoots and roots, also proline content were studied. In the case of *in vivo* experiments, yield-contributing and biochemical traits were measured. The parameters included plant height, plant number, total tiller numbers, spike length, number of grains per spike, 1000-grain weight, grain yield per plant (g), and protein percentage, which were studied under irrigated and water-stress conditions. Drought stress significantly reduced seedling and yield-contributed traits. In the case of biochemical parameters (protein and proline), an increase was observed. The results can provide insight into the root trait development of wheat under -0.45 MPa and guide root architecture optimization and quality improvement in wheat. An analysis of correlations found significant correlations between most of the studied traits. According to the results, the cultivars Navid, Sabalan, Azar2, and Zare were identified as drought-tolerant while the cultivars Mihan, D92, and G31 were observed as drought-sensitive.

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Introduction

In the context of global warming, drought is expected to have the greatest impact on crop productivity (Andjelkovic, 2018). It is estimated that a 1°C increase in temperature can cause a 10–20% decrease in crop yield globally (Rose *et al.*, 2016). This effect can be even worse by the end of this century it is expected to be 2–4°C even more temperature rises which affect crop production (Rogelj *et al.*, 2016). Winter wheat cultivation is primarily rainfed, subject to variable rainfall patterns and often low rainfall, leading to drought (Hassini *et al.*, 2018). Drought plays a major role in limiting agricultural production in semi-arid and arid regions (Ahmad *et al.*, 2018), and negatively impacts wheat crops in terms of physiological, and biochemical, characteristics and also metabolic changes occur in all plant tissues, ultimately reducing yield performance (Cochard *et al.*, 2002). Worldwide, it is one of the most common causes of crop loss, reducing average agricultural yield by more than 50% (Wang *et al.*, 2003). For better adaptation of crop plants to reduce climatic variability and ensure food security, it is important to use and breed drought-resistant and highly efficient genotypes, change sowing times, sow and cultivate new crops, use more effective fertilizers, and improve agronomic water use efficiency (Koç, 2020). Abiotic stress, particularly water deficit, affects seed germination and seedling establishment in the majority of crop species (Bardees and Aldesuquy, 2017). Drought is documented to delay seed germination and suppress its rate. The influence of various seedling traits, grain yield, and yield components, also biochemical traits on drought tolerance, has been studied by various researchers (Cedola *et al.*, 1994; Larbi and Mekliche, 2004). A variety of artificial methods can be used to induce drought stress, including restricting water delivery, treating with abscisic acid (ABA), and injecting polyethylene glycol (PEG). Using PEG, a non-ionic water-soluble polymer, to induce drought stress in plants is extensively applied since it is not anticipated to enter plant cells (Djibril *et al.*, 2005; Vandana *et al.*, 2022). The study aimed to evaluate the responses of fifteen bread wheat cultivars to drought stress. Study objectives were as follows: studied the

characteristics in the seedling stage under in-vitro experiment, and studied yield contribute traits under in-vivo experiment.

Materials and methods

Experiment site and plant material source

In-vitro and in-vivo screenings were conducted at the “Arman Naghsh-Sabz Aras Co.” Aras free zone greenhouse site in IRI. Location is between 45°17' to 46°31' of the eastern longitude and 38°39' to 39°2' of the northern latitude. Annual rainfall is about 225 to 400 milliliters per year and the average temperature is about 15 degrees Celsius. The experimental material consisted of fifteen wheat cultivars (Table 1) collected from the East-Azerbaijan Agricultural and Natural Resources Research and Education Centre and also the Department of the Scientific Center of Agronomy and Plant Protection of RA.

In vitro screening

The study was evaluated on filter paper in Petri dishes in a growth chamber, for various seedling traits: germination percentage, total root length, total root number shoot length, fresh/dry weight of shoot and root, and free proline content. Factorial experiments were carried out in a completely randomized design with three replications. Drought stress was induced by Polyethylene glycol-6000 (Michael and Kaufmann 1973; Hoagland and Snyder 1933). Ten seeds of each cultivar were surface sterilized with 0.1% of HgCl₂ for 1 min, then washed thrice with distilled water to avoid fungal contamination, and then placed on Petri dishes and moistened with PEG-6000. Growth conditions included 14 hours of daylight and 10 hours of darkness, with temperatures of 25°C during the day and 20°C at night for two weeks (Faisal *et al.*, 2017). For drought conditions, -0.45 MPa and -0.9 MPa osmotic potential were used and the untreated seeds were used as a control. According to the International Seed Testing Association (Bousslama and Schapaugh, 1984), germination was measured after 48 hours. The growth of seedlings was evaluated by measuring the lengths of the shoots and roots and the fresh and dry weight (mg). Free proline content was measured by (Bates *et al.*, 1973) method.

Table 1. The pedigree of fifteen wheat cultivars used in the study

Cultivar	Pedigri	Year of release	Status
Sardari	Sardari	1930	Cultivar
Navid	7C/2-66/112-63(79 Kirkpinar)	2000	Cultivar
Alvand	077FC/5726-72-1	1996	Cultivar
Mihan	BKt/90Zhong87	2010	Cultivar
Azar2	Bb/Inia/Kvz/my 71/Maya S /Sefid	1999	Cultivar
Sabalan	2824-23-1/21AnF/809	1981	Landrace
Zare	Lira/3/Ymh/Tob/Mcd/4/Mo73/F35,70//130L1,11	2011	Cultivar
Pishgam	BKt/90-Zhong87/Barekat	2008	Cultivar
D92	—	2010	Cultivar
G31	—	2010	Cultivar
Sateni22	M574/51-M408	1998	Cultivar
Akhtamar	Bezostaya 1/Ferrigneum 127	1994	Cultivar
Armianka60	Lutescens 48 (K-482477)/Alborubrum 88	1987	Cultivar
Voskehask	Bezostaya 1/ Ae.taushai	1994	Landrace
Nairi68	Free pollination of Lutescens93	2000	Cultivar

In-vivo screening

The study was evaluated over two years in a randomized complete block design with three replications. The seeds were planted in plastic pots (inner dimensions 10.5 × 10.5 × 21 cm) with a 4:1 soil-to-sand ratio. Each pot contained two kilograms of dry soil mixed with sand. There were two drought treatments in the experiment: irrigated (70% of field capacity) water-stressed (35% of field capacity) and fifteen wheat cultivars (Table 1). According to standard procedures and protocols, yield-contributing traits such as plant height, total tillers number, spikelet per spike, grain number per spike, grain weight, thousand-grain weight, gain yield, and by (Bradford 1976) method protein percentage were measured.

Statistical analysis

The data of all parameters was statistically analyzed through analysis of variance techniques to check the significant differences among wheat cultivars at 0.01 probability level. Also, Pearson correlation analysis was done to explore the relationship among the traits.

Results and discussion

In vitro screening

The variations among the cultivars, stress levels, as well as cultivars × stress levels were found highly significant differences (Table 2). All parameters were adversely affected by drought stress. The data mean, data range, SD, and CV% in controlled (E1), -0.45 MPa (E2), and -0.9 MPa (E3) conditions are shown in

(Table 3). Seed germination in early developmental stages is cited as a basic prerequisite for successfully establishing wheat crops in drought conditions (Saha *et al.*, 2018). Germination was significantly affected by osmotic potential, cultivars, and their interactions. The average germination percentage of the control was 90.10%. Under -0.45MPa and -0.9 MPa, with 29.35% and 46.49% reduction, it was 63.66% and 48.21% respectively (Fig. 1). A similar decrease in PEG conditions was reported by (Jajarmi 2009; Rauf *et al.*, 2007; Sharma *et al.*, 2022). The GP% range was 80-100 in E1 and 46.6-83.3 in E2 (Table 3). Cultivars Navid, Voskehask, Sabalan, and Zare performed better and showed maximum GP% at higher osmotic potentials. The minimum reduction after the mentioned cultivars was observed in cultivar Azar2, whereas the maximum reduction was in the D92 cultivar. Chachar *et al.* (2016) found that increasing osmotic stress up to -0.9 Mpa caused a decrease in germination percentage in tolerant cultivars due to their developing biochemical and physiological functions. Shoot traits under control and different osmotic potentials are shown in (Fig. 2). Under the control conditions, the average shoot length was 13.16 cm, and it was decreased by 23.38%, and 46.19% at -0.45 MPa and -0.9 MPa respectively. Under -0.45 MPa, the range of data was 7.92-13.15 cm, while at -0.9 MPa it ranged from 4.61cm to 9.95 cm. The minimum mean reduction was detected in Navid and Voskehask. These results are confirmed by (Prakash *et al.*, 2015; Almaghrabi, 2012; Ahmad *et al.*, 2017; Sharma *et al.*, 2022) findings.

Table 2. The mean square of ANOVA analysis of germination and seedling traits of wheat cultivars under three levels of osmotic stress

Source of variance	df	SL	SFW	SDW	RL	RFW	RDW	RN	Prol
C	14	**	**	**	**	**	**	**	**
S	2	**	**	**	**	**	**	**	**
C*T	28	**	**	**	**	**	**	**	**

C: Cultivar, S: stress, ** = 1% level of significance; SL: Seedling length, SFW: shoot fresh weight, SDW: shoot dry weight, RL: Root length, RFW: Root fresh weight RDW: Root dry weight RN: root number, prol: proline.

Table 3. Mean data, range, SD, and CV% of parameters in controlled (E1), -0.45 MPa (E2), and -0.9 MPa (E3) conditions for fifteen wheat cultivars

Trait	Environment	Mean	Min	Max	SD	CV%
SL	E1	13.16	11.82	15.08	0.95	7.25%
	E2	10.08	7.92	13.15	1.72	17.04%
	E3	7.08	4.61	9.95	1.76	24.90%
SFW	E1	74.59	66.92	85.62	5.47	7.34%
	E2	58.44	46.05	76.05	9.85	16.86%
	E3	41.18	25.32	57.94	10.41	25.29%
SDW	E1	11.54	10.34	13.27	0.86	7.44%
	E2	8.97	7.03	11.74	1.55	17.23%
	E3	6.62	4.22	9.41	1.78	26.87%
RL	E1	10.35	8.03	12.32	1.14	11.00%
	E2	10.62	6.42	14.39	2.82	26.54%
	E3	5.82	2.42	10.33	2.87	49.31%
RFW	E1	41.62	32.46	49.77	4.93	11.84%
	E2	43.35	26.19	58.71	11.5	26.54%
	E3	23.95	9.15	42.15	12.14	50.67%
RDW	E1	6.8	5.25	8.06	0.77	11.36%
	E2	6.95	4.2	9.41	1.84	26.54%
	E3	3.9	1.4	7.08	2.01	51.63%
NOR	E1	7.59	6.7	8.4	0.57	7.54%
	E2	6.62	4.1	9.5	1.84	27.74%
	E3	4.39	2.42	6.2	1.3	29.70%
GP%	E1	90.11	80	100	6.66	7.40%
	E2	63.66	46.6	83.3	12.78	20.07%
	E3	48.21	30	66.6	10.27	21.30%
Proline	E1	1.55	1.25	1.75	0.17	10.78%
	E2	1.65	1.27	1.94	0.23	13.70%
	E3	1.7	1.33	2.03	0.24	14.19%

In the case of water stress, longer coleoptiles contribute to seedling emergence and establishment (Ahmad *et al.*, 2017; Chachar *et al.*, 2014). The reduction in the shoot and root lengths might be due to some disturbance posed by the osmotic stress conditions in cell division and elongation (Bayoumi *et al.*, 2008). Shoot fresh weight, under controlled conditions, the average value was 74.59 mg, which decreased by 21.64% and 44.75% at -0.45 MPa and -0.9 MPa, respectively. At controlled conditions, it ranged from 66.92 mg to 85.62 mg; under the highest osmotic potential, it ranged from 25.32mg to 57.94 mg. As drought stress increased, a decreasing trend was observed. The same result was reported by

Bayoumi *et al.* (2008) and Izabela *et al.* (2013).) Increasing osmotic potential reduced seedling fresh weight of both drought-tolerant and sensitive durum wheat cultivars (Sayar *et al.*, 2010). With increased PEG concentration in the growth media, shoot fresh weight decreased due to fewer leaves and smaller sizes (Ahmad *et al.*, 2017). The average shoot dry weight under control was 11.54 mg, which decreased to 8.97 mg and 6.42 mg at -0.45 and -0.9 MPa PEG. Shoot dry weight was less affected by the stress than shoot length and shoot fresh weight, which is confirmed by the finding of Fernandes *et al.* (2020). The reduction percentage was 46.19%, 44.75%, and 44.36 in shoot length, shoot fresh weight, and shoot

dry weight respectively at -0.9 MPa, that was shown that the length of the seedlings was more sensitive to drought. As a result of osmotic stress conditions, the shoot and root lengths may have been reduced (Bayoumi *et al.*, 2008; Fraser *et al.*, 1990).

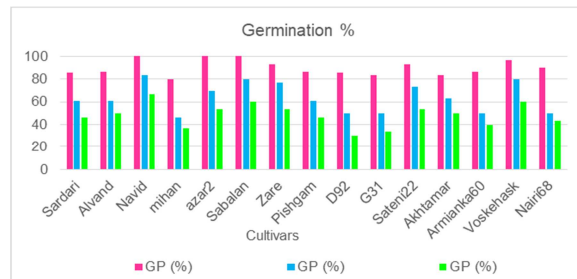


Fig. 1. Germination percentage under control and different osmotic potentials in fifteen wheat cultivars

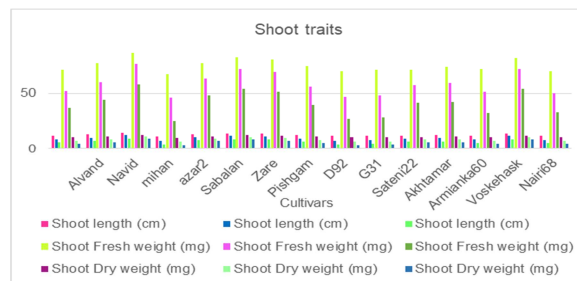


Fig. 2. Shoot traits under control and different osmotic potentials in fifteen wheat cultivars

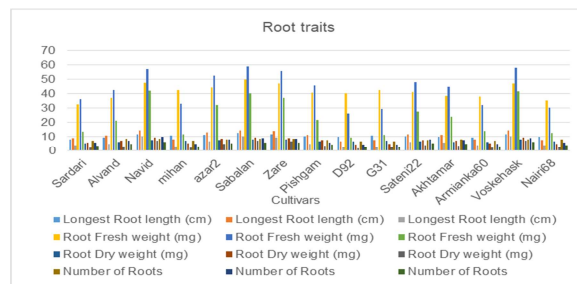


Fig. 3. Root traits under control and different osmotic potentials in fifteen wheat cultivars

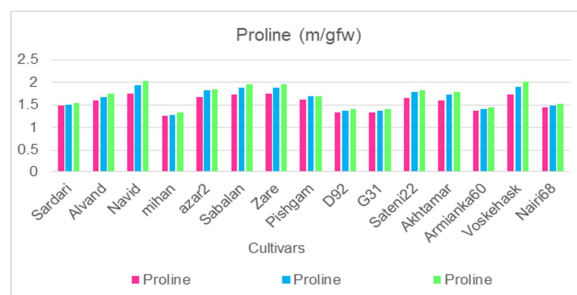


Fig. 4. Proline content under control and different osmotic potentials in fifteen wheat cultivars

Root traits under control and different osmotic potentials are shown in (Fig. 3). The root traits of wheat are among the most sensitive plant characteristics to drought (Kumar *et al.*, 2010; Ji *et al.*, 2014; Robin *et al.*, 2021). Different cultivars behaved differently as PEG concentrations increased. Under the control conditions, the average root length was 10.42 cm and ranged from 8.3 to 12.32 cm. It was increased by 2.16% at -0.45 osmotic potential, with a ranging of 6.42 and 14.39 cm, and then decreased by 44.08% at -0.9 potential with a ranging of 2.42cm and 10.33cm. The increase occurred in all cultivars, except Nairi68, Armianka60, Mihan, G31, and D92. Increasing percentages were different among cultivars. The most increasing value was observed in Voskehask (21.17%) followed by Navid (20.70%), Sablan (16.80%), Zare (16.51%), Azar2 (15.21%) and Sateni22 (15.25%). In cultivars Alvand, Pishgam, and Sardari, the incidence percentage ranged from 12.54% to 10.08%. This result was in line with the findings of (Jajarmi, 2009; Ghosh *et al.*, 2020), who reported that a reduction of turgor pressure and water uptake is caused by osmotic stress, eventually resulting in a reduction of root length. There can be a decrease in root length due to reduced relative turgidity and protoplasm dehydration, which reduce cell expansion and delay the division of cells (Mujtaba *et al.*, 2016). The average root number at controlled conditions was 7.59, which decreased to 6.62 and 4.39 at osmotic potential. An increase in the total number of roots at -0.45 MPa PEG, compared to the control was agreed with the findings of (Khanna-Chopra, 2012; Robin *et al.*, 2015). Increasing in length and number of roots in the cultivars Navid and Voskehask indicated their tolerance strategies under osmotic stress. Robin *et al.* (2021) reported that in the presence of higher osmotic stress, root formation activity was reduced, which was likely due to reduced substrate availability at the youngest positions of the phytomer. The influence of root traits on yield and other agronomic traits, especially under stress conditions, has been widely reported in all major crops (de Dorlodot *et al.*, 2007). The ranging of the root fresh weight under controlled conditions was 32.46 mg and 49.77mg with the average value of 41.62 mg, while it showed an increase under -0.45MPa osmotic potential and had the range of 26.19 mg and 58.71mg also the average value of 43.35 mg, which showed 4.15% increase.

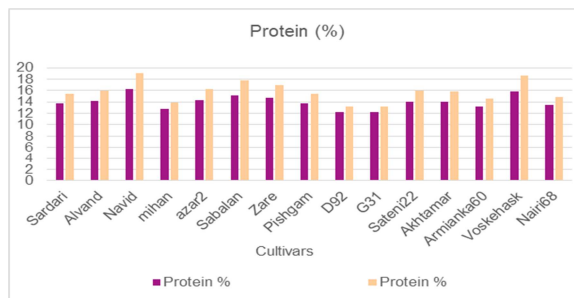


Fig. 5. Protein content under irrigated and water stress environments in fifteen wheat cultivars

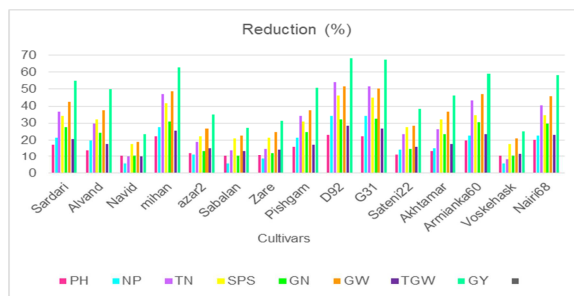


Fig. 6. Reduction of yield-associated traits under water stress conditions compared to irrigated environment

PH: plant height, TN: total tillers number, SPS: spikelet per spike, GN: grains number, GW: grains weight, TGW: thousand-grain weight, GY: gain yield, Pr: protein.

At -0.9 MPa osmotic potential, the average value decreased by 42.45 % in comparison to the control and was 23.95 mg. Drought-tolerant wheat cultivars have a higher fresh weight of seedlings under drought conditions (Ahmad *et al.*, 2013; Soltani *et al.*, 2006; Sharma *et al.*, 2022). When wheat seedlings were exposed to varying levels of osmotic stress, their dry biomass varied. The average values of root dry weight under control, -0.45 MPa, and -0.9 MPa were 6.80 mg, 6.95 mg, and 3.90 mg respectively. Between controlled and -0.45 Mpa conditions a 2.19% increase and between controlled and -0.9 MPa, it was observed a 43.85% decrease. Root fresh and dry weight increased under an osmotic potential of -0.45MPa, along with the main root length increasing. There was a decreasing trend in fresh and dry weight, as drought stress increased, which was confirmed by the previous studies (Kamran *et al.*, 2009; Marcińska *et al.*, 2013; Sharma *et al.*, 2022).

Proline content under control and different osmotic potentials are shown in (Fig. 4). Under control conditions, the proline had a mean value of 1.55 m/gfw with ranging of 1.25 m/gfw to 1.75 m/gfw, while at -0.45 MPa and -0.9 MPa osmotic potential, it increased to 1.64 m/gfw and 1.70 m/gfw, with increasing of 5.91% and 3.23% respectively. Under water deficit stress, proline accumulates widely in plants (Zhan *et al.*, 2011). The results are confirmed by the findings of (Sharma *et al.*, 2022). Navid and Voskehask have accumulated the most proline under higher osmotic potentials. Proline-rich cultivars performed better under stress (Kadam *et al.*, 2017). When exposed to environmental stresses, proline adjusts osmotic pressure, stabilizes subcellular structures, destroys free radicals, and buffers redox potential (Ashraf and Foolad, 2007).

In vivo screening

The yield of wheat grains is a complex trait that is affected by numerous factors, including number of tillers, weight of 1000 grains, plant height, etc (Bhattarai *et al.*, 2017). According to the ANOVA, variations among the cultivars, environment with different irrigation conditions, as well as interaction between C × E were found highly significant differences (Table 4). The mean, range, SD, and CV% of parameters in irrigated (E1) and water-stress (E2) conditions are shown in (Table 5). As shown in (Fig. 5), yield-associated traits are reduced under stressed conditions as compared to irrigated conditions. Different cultivars may differ in plant height due to their genetic makeup and environmental factors, including water scarcity, leading to decreased cell enlargement and more leaf senescence (Jaleel *et al.*, 2008). Under control and stressed conditions, the average plant height was 81.98 cm and 71.63 cm respectively. The minimum reduction percentage was 7.10% in the Sabalan cultivar, and under the E1 environment, the average value for Ph was 65.04 cm to 92.38 cm, whereas, with 12.69% reduction under stressed conditions, it ranged from 60.01 cm to 85.38 cm (Fig. 6). Drought stress reduced Plant height by 9.76% to 28.63% (Sharma *et al.*, 2022). A decline in cell size and more leaf senescence under drought stress could account for the reduction in plant height (Wang *et al.*, 2017; Manivannan *et al.*, 2007).

Table 4. The mean square of ANOVA analysis of yield-associated traits under water-stress conditions compared to irrigated conditions

Source of variance	df	PH	NP	Tillers	SPS	GN	GW	TGW	GY	Pr
C	14	**	**	**	**	**	**	**	**	**
I	1	**	**	**	**	**	**	**	**	**
C*I	14	**	**	**	**	**	**	**	**	**

C: Cultivar, I: irrigation, ** = 1% level of significance; PH: plant height, TN: total tillers number, SPS: spikelet per spike, GN: grains number per spike, GW: grains weight, TGW: thousand-grain weight, GY: gain yield, Pr: protein.

Table 5. Mean, range, SD, and CV% of parameters in irrigated (E1) and water-stress (E2) environments for fifteen wheat cultivars

Trait	Environment	Mean	Min	Max	SD	CV%
PH	E1	81.98	65.04	92.38	6.96	8.50%
	E2	71.63	60.01	85.38	7.94	11.46%
NP	E1	5.48	4.83	5.83	0.41	7.43%
	E2	4.52	3.17	5.5	0.83	18.25%
NT	E1	14.43	13.61	15.24	0.47	3.28%
	E2	10.13	6.24	13.75	2.47	24.41%
SPS	E1	24.72	22.51	26.4	1.46	5.91%
	E2	17.27	12.13	21.77	3.22	18.68%
GN	E1	42.35	35.44	46.14	3.29	7.76%
	E2	33.3	24.01	41.5	5.91	17.73%
GW	E1	1.99	1.73	2.32	0.2	10.23%
	E2	1.29	0.84	1.88	0.36	28.04%
TGW	E1	46.98	42.66	50.45	2.85	6.06%
	E2	38.24	33.05	45.44	4.21	11.02%
GY	E1	10.96	8.36	13.53	1.84	16.79%
	E2	6.12	2.66	10.34	2.7	44.08%
Pr	E1	14.01	12.2	16.2	1.18	8.45%
	E2	15.82	13.2	19	1.78	11.22%

The protoplasm is dehydrated and loses its turgidity during a drought so cell elongation, expansion, and mitosis are affected also the plant sheds its leaves to prevent moisture loss ultimately diminishing the height of the plant (Nonami, 1998; Khatiwada *et al.*, 2020). The number of plants was in the range of 4.52-5.48 per pot under controlled and ranged from 3.17 to 5.50 under stressed conditions. The reduction was the same at G31 and in D92 (34.37%) which was the maximum amount. On the other hand, Sabalan, Voskehask, and Navid showed the same amount of minimum reduction (5.66%) which was the minimum reduction value. As a result of control conditions, the average total tillers number was 14.43 per pot, while it had a 30.19% reduction and was 10.13 under drought conditions. Voskehask and Navid observed a minimum reduction of 8.09% and 9.78% respectively. The number of tillers per plant has a direct contribution to grain yield in wheat (Naushad *et al.*, 2020), and thus, it is an important trait to

measure. Pollen sterility increases when plants are exposed to drought at anthesis, and fewer kernels and tillers are produced per ear. The resulting reduction in yield also contributes to drought as well (Barnabás *et al.*, 2008; Anjum *et al.*, 2017). During control conditions, all cultivars had spikelets per spike above 22, with an average of 24.72, while during stressed conditions; the average spikelet per spike was 17.27, with 30.64% reduction. During stressed conditions, the maximum reduction occurred in G31 (12.38), while the minimum reduction occurred in Voskehask (8.09%) followed by Navid (9.78%). The number of spikelets per spike is one of the chief factors determining the total yield of the plant and it is highly affected by drought (Nawaz *et al.*, 2012).

The mean value for grain number under controlled conditions was 42.35 grains, while under stress, it showed a 21.85% reduction and was 33.30. It ranged from 35.44 to 46.14 under irrigated conditions and

was from 24.01 to 41.5 under deficient conditions. The reduction amount in Voskehask, Navid, and Sabalan was about 10%. Several factors contribute to grain number reduction due to water stress, including disrupted meristem development, floret abortion, and pollen sterility (Dolferus *et al.*, 2019; Dong *et al.*, 2017). The mean value of grain weight was 1.99 and 1.29 in irrigated and water-stress conditions, respectively, with a 36.06 % mean reduction. Minimum reduction grain weight was in Navid (18.97%). It ranged from 1.73 g to 2.32g in E1 and ranged from 0.84g to 1.88 g in E2. The drought stress also significantly affects the grain filling, thus leading to reduced grain size and a smaller number of grains (Sharma *et al.*, 2022; Maralian *et al.*, 2010). So ultimately this reduces grain and biological yields (Bayoumi *et al.*, 2008; Ding *et al.*, 2018). 1000-grain weights (TGW) ranged from 42.66 g to 46.98 g in the controls, with an average of 46.98 g. In the case of stressed conditions, the mean value was 38.24 g, with an 18.6% reduction. Results were confirmed by the findings of (Turan 2018) also mentioned that according to the reports of Bhatt (1973), grain yield in cereals is influenced more directly by the efficiency of a spike and 1000 seeds than by the direct effect. Therefore, when selecting, these characteristics are taken into account. In the case of grain yield, under controlled conditions, the total mean value was 11.04 g per pot while it was reduced by 44.56%, which showed 6.12 g per pot yield under water deficiency. According to the mean grain yield of two years under controlled conditions, cultivars Navid and Voskehask, followed by Sabalan and Zare, showed high yield, which also showed impressive performance under water-stress conditions with minimum reductions of 23.55%, 25.26%, 27.02%, and 31.32%, respectively. The average yield loss in wheat due to drought stress was estimated by cultivars Azar2 and Sateni20, which were 35.02% and 38.58%, respectively, which is lower than tolerated cultivars but higher than Alvand, Sardari, Pishgam, Nairi68, and Armianka60 cultivars. The highest yield reduction was recorded in cultivars D92 (68.18 %), followed by G31 (67.5%) and Mihan (62.5%). Previous studies have also reported a reduction in grain yield due to drought stress (Pour-

Aboughadareh *et al.*, 2019; Ahsan *et al.*, 2022). The total reduction in grain yield in this study was 46.58%, which was in line with the findings of (Hussain *et al.*, 2019), who reported that crops lose between 30% and 90% of their yields under drought conditions. Wheat cultivars exposed to drought stress exhibited a marked increase in protein concentrations compared to controls. In controlled conditions mean data was 14.01% which had an increase of 12.94% and reached 15.82% in drought conditions. Drought increased the average protein content of all cultivars. The results aligned with the findings of (Aneela *et al.*, 2017; Kim *et al.*, 1990; Ashrafi and Shaban, 2014).

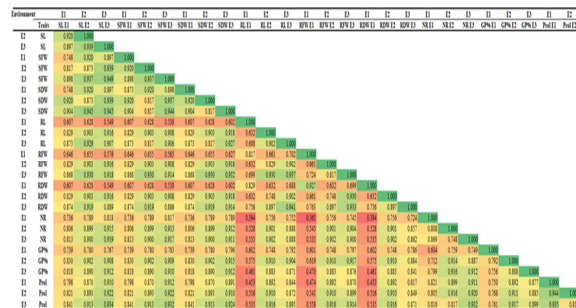


Fig. 7. Correlations of seedling traits in wheat cultivars under different osmotic potentials
SL: Shoot length, RL: Root length, SFW: Shoot fresh weight, SDW: Shoot dry weight, RFW: Root fresh weight, RDW: Root dry weight, NR: Number of roots, GP: Germination percentage, Prol: Proline content.

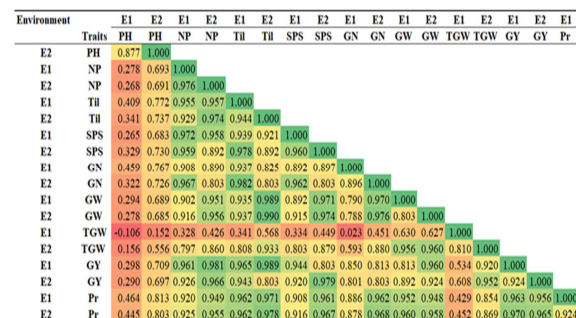


Fig. 8. Correlations of yield contributed traits in wheat cultivars in irrigated and water-stress environments
PH: plant height, TN: tillers number, SPS: spikelet per spike, GN: grains number, GW: grains weight, TGW: thousand-grain weight, GY: gain yield, Pr: protein.

Studies of correlation

Pearson's correlation was used to assess the associations between traits under control and drought stress conditions. Correlations of seedling traits in wheat cultivars under different osmotic potentials are shown in (Fig. 7). There was a significant correlation between seedling traits studied in this study. Therefore, any change to one of them will affect the others. Wheat drought tolerance is significantly influenced by these traits under PEG-induced drought stress conditions. A similar was found in the investigation of Ahmad *et al.*, (2013) where root length showed a strong correlation with shoot length and germination percentage as well as shoot length with germination percentage. Selecting a reliable seedling trait for osmotic stress and using it as a selection criterion will lead to improved seedling traits for drought conditions (Baloch *et al.*, 2012). Many studies have found positive correlations among wheat seedling traits under osmotic or water stress conditions (Bayoumi *et al.*, 2008; Dhanda *et al.*, 2004; Rauf *et al.*, 2007). Correlations of yield-contributed traits in wheat cultivars in irrigated and water-stress environments are shown in (Fig. 8). Agro-morphological and yield-related traits, such as plant height, tillers per plant, spikelet per spike, grains per spike, grain weight, and 1000-grain weight all showed positive and strong correlations with grain yield per plant. As reported by (Ojha, 2012; Thapa *et al.*, 2009; Bhattarai *et al.*, 2017) a significant correlation has been established between grain yield and the number of grains per spike, the average flag leaf duration, the average grain filling duration, and the plant height.

Conclusion

In this study under controlled and drought stress conditions, agro-morphological, physiological, and biochemical traits in wheat were investigated. Results showed significant variations in the mean values of various parameters, and there was a significant reduction in seedling traits and yield-related traits due to drought stress. The most reduction was observed in higher osmotic potential. Correlation analysis suggested that all the traits were closely

correlated with each other in different environments. Following in-vitro and in-vivo experiments, seedling traits, Yield and yield-related traits, and also biochemical traits were all found to be useful. All cultivars were grouped into four groups according to the data, mean values, and reduction percentages. The first group included cultivars Voskehask, Navid, Sabalan, and Zare, the most tolerant cultivars that could perform better under drought conditions. The Second group cultivars Azar2, Sateni20, and Akhtamar were moderately tolerant under water stress conditions. In contrast, Alvand, Pishgam, Sardari, Nairi68, and Armianka60 cultivars were moderately sensitive. Furthermore, cultivars Mihan, G31, and D92 showed high drought sensitivity. Drought-resistant cultivars can be used in breeding as well as genetical programs to increase yield under rainfed and water stress conditions.

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