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Evaluation of triticale genotypes for terminal drought tolerance using physiological traits

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ABSTRACT

Drought is a major constraint to crop production worldwide. The effect of terminal drought stress on the physiological traits of 18 triticale (*X. triticosecale*) lines comprising nine doubled haploid (DH) and nine corresponding advanced lines (F_{7-8}) and two bread wheat cultivars (control) was investigated under field conditions during two growing seasons of 2007–09. Plants were grown under full irrigation until the mid-jointing stage when drought stress was applied to the plant. Chlorophyll content, carotenoids content, proline content, relative water content (RWC), flag leaf area, flag leaf angle and grain yield were assessed. Triticale lines performed superior than wheat cultivars for drought tolerance considering both grain yield and majority of physiological traits. Grain yield positively and significantly correlated with the ratio of total chlorophyll (a+b) to total carotenoid and RWC under both environmental conditions. DH line number 4 possessed the greatest values of RWC, chlorophyll a, chlorophyll b, chlorophyll a+b and ratio of total chlorophyll (a+b) to total carotenoid under drought stress conditions. These accompanied by having high proline content and low grain yield reduction due to drought stress was ranked this DH line as superior drought tolerant genotype. The results revealed an inverse and significant relationship between grain yield loss due to drought stress with proline content under drought stress ($r = -0.61^{**}$), provided an evidence supporting the role of proline in drought tolerance.

Keywords: Drought tolerance, Physiological traits, *X. triticosecale*

Drought is a world-wide problem seriously influencing global crop production and it will become progressively important due to the global climate change.

Drought tolerance is a typical quantitative trait and breeding programmes to increase yield under reduced water supply conditions are rather challenging task. A physiological approach can complement empirical breeding to enhance the rate of yield improvement. Now-a-days, effort is focused on improving crop genotypes for drought-prone areas. Understanding of the mechanisms behind drought tolerance, which can lead to the restitution of physiological function and to hardening of plants under drought stress is essential for the achievement of such as a goal.

Several physiological traits, which can contribute to continued growth under water stress, have been identified. For example osmotic adjustment is a key mechanism enabling plant under drought to retain water absorption and cell turgor pressure, thus contributing to sustained higher photosynthetic rate and expansion growth. Nevertheless, there are a number of contrasting reports on the role of osmotic adjustment in crop plants. A comparative analysis of many studies devoted to osmotic adjustment has suggested that osmotic adjustment cannot be considered equally beneficial in all crops under every drought stress conditions but that a general positive association between yield and osmotic adjustment can be found under severe water stress (Serraj and Sinclair 2002). Likewise, the role of proline accumulation and its metabolism vis-a-vis tolerance to drought stress is a controversial issue. Water content and water potential of plant tissue are considered as the physiologically relevant integrators of drought effects (Jones 2007). Although, water potential was used to quantify the water status of plant, leaf relative water content (RWC) was introduced as a better indicator. This is due to the balance between water supply to the leaf and transpiration rate. Water loss can lower leaf water potentials, leading to reduced turgor, stomatal conductance and photosynthesis and ultimately to reduced growth and lighter yield (Kumar and Sharma 2010).

Photosynthesis related parameters have also been considered as the screening criteria in wheat for drought

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tolerant (Blum 2005). Carotenoids are lipid-soluble antioxidants produced by most photosynthetic organisms and belong to two classes of the carotens and xanthophylls. In plants, cartenoids perform as light collectors and protectors against photosensitization in chloroplasts.

Triticale (*X. triticosecale* Wittmack) is one of the most successful man-made cereals, was synthetized to obtain a cereal that combines unique grain quality of wheat (*Triticum* ssp) parent with tolerance to abiotic and biotic stresses of rye (*Secale* spp) parent (Lelley 2006). Triticale seems to be an interesting alternative to other cereals, particularly bread wheat, in environments where growing conditions are unfavourable or in low-input systems.

The objectives of this study were: (1) to evaluate the terminal drought tolerance of triticale DH and their corresponding F_{7-8} lines as well as two bread wheat cultivars (Roshan and Kavir), using physiological traits, and (2) to determinate the associations of physiological traits with grain yield under drought stress conditions in triticale.

MATERIALS AND METHODS

Field experiments were carried out during two growing seasons of 2007-09. Plant materials were grown in two separate experiments under stress and non-stress irrigation regimes in each year at the research farm of Isfahan University of Technology located at Lavark, Iran (40 km south west of Isfahan, 32° 32´N, 51° 23´ E, 1630 m asl). The soil at this site is silty clay loam, typic Haplargids of the arid tropic with *p*H=7.3-7.8. Mean annual precipitations and mean annual temperature were 140 mm and 14.5°C, respectively. Each experiment was conducted using a randomized complete block design with three replications. Each plot consisted of four 4m long rows spaced 25cm apart. Plant were grown under full irrigation until mid-jointing stage (43 growth stage of Zadoks scale) when water stress was applied. The soil-moisture regimes were established by irrigating after 70 mm evaporation from class-A Pan corresponded to soil water potential of –0.5 MPa (non-stress) and irrigating after 130 mm evaporation from class-A Pan corresponded to soil water potential of –1.2 MPa (drought-stress).

Experiments were conducted using nine DH lines and nine corresponding F_{7-8} lines of triticale derived from Polony Q /TW179 cross (Arzani and Darvey 2002) and two local bread wheat cultivars (Roshan and Kavir). Roshan as a drought tolerant and Kavir as a salt tolerant cultivars were included as control. DH line number 3 was registered in Australia as the cultivar Eleanor (Anon 2001).

The contents of photosynthetic pigments (PSP) (chl a, chl b, chl a+b, carotenoids (xanthophylls and carotenes (x+c)), the weight ratio of chl a and chl b (chl a/b) and the weight ratio of chl a and b to total carotenoids (chl (a+b)/(x+c))), proline, relative water content (RWC), flag leaf area, flag leaf angle and grain yield were evaluated. To measure the PSP content, 100 mg of tissue from 10 leaves obtained randomly from each plot was used. The tissues were then placed into a mortar and added 10 mL of 80% acetone and grind the tissue with a pestle. The leaf homogenate was poured through vacuum. This action repeated until the leaf above the pour became white. Then acetone was added the filtrate until the volume reached to 30 ml. After that the pigment extracts were centrifuged for 5 min. at 500 × g. Ultimately, the absorbance of photons at 663.2 nm, 646.8 and 470 nm was measured by spectrophotometer (Beckman UD-530). The amount of chlorophyll a, chlorophyll b and carotenoids were calculated according to Lichtenthaler and Buschman (2001) equations.

c_a(mg/g fw) = [(12.25 $A_{663.2}$ – 2.79 $A_{646.8}$) × ml Acetone 80%] / mg leaf tissue

c_b(mg/g fw) = [(21.50 $A_{646.8}$ –5.10 $A_{663.2}$) × ml Acetone 80%] / mg leaf tissue

$c_{(x+c)}$ (mg/g fw) = [{(1000 A_{470} – 1.82 c_a – 85.02 c_b /198) × ml Acetone 80%}]/ mg leaf tissue

The free proline content of leaves was determined by the method of Bates *et al.* (1973). First 0.1 g of plant material was homogenized in 10 ml of 3% aqueous sulfosalicylic acid and the homogenate filtered through whatman # 2 filter paper. Then two ml of filtrate was reacted with 2 ml acid ninhydrin and 2 ml of glacial acetic acid in a test tube for 1 hr at 100ºc, and the reaction terminated in an ice bath. Next the reaction mixture was extracted with 4 ml toluene, mixed vigorously with a test tube stirrer for 15–20 sec. After that the chromophore containing toluene was aspirated from the aqueous phase, warmed to room temperature and the absorbance read at 520 nm using toluene for a blank. Finally, the proline concentration was determined from a standard curve (A calibration curve was obtained with L-proline as the standard) and calculated on a fresh weight basis as follows:

[(µg proline/ml × ml toluene)/115.5 µg/µmole]/[(g sample)/5]=µmoles proline/g of fresh weight material

To measure the RWC, flag leaves were cut into 2 cm pieces and weighed (fresh weight = FW). The leaf pieces were then placed in distilled water for 4 hr and reweighed to obtain turgor weight (TW). The leaf pieces were oven dried, weighed and used as dried weight (DW). RWC was calculated using the formula proposed by Ritchie *et al.* (1990):

$$\%RWC = \frac{FW-DW}{TW-DW} \times 100$$

Leaf angle was measured the angle between flag leaf and stem using 20 leaves/plot. Length and width of flag leaf were measured using 20 samples from each plot and leaf area was then calculated.

Separate analysis of variance (ANOVA) and combined ANOVA were carried out using data from both drought stress and normal conditions and two growing seasons. Analyses of variances were carried out using PROC GLM of SAS (SAS Institute 1997). Contrast of triticale lines versus two wheat cultivars and F_{7-8} triticale lines versus DH triticale lines were conducted using orthogonal (independent)

comparisons. Mean comparisons were conducted using Fishers-least significant differences (LSD). Linear regression and correlation analyses were conducted to determine phenotypic relationship between the traits at two different field experimental conditions.

RESULTS AND DISCUSSION

In the present study, no significant differences were observed between two growing seasons (years) for the studied traits (Table 1), and hence data averaged on two years were used for statistical analyses. Combined ANOVA indicated the significant influence of drought and genotype on PSP contents (Table 1).

Mean of chl a, chl b, chl a+b and carotenoids content decreased due to drought stress (Table 2). Mean of chl a+b ranged from 0.66 mg/gfw for DH line number 9 to 1.11 mg/gfw for F_{7-8} line number 6 under non-stressed experiment and from 0.36 mg/gfw for DH line number 3 (Eleanor cultivar) to 0.86 mg/gfw for DH line number 4 under drought stressed experiment. Carotenoids content ranged from 0.21 mg/gfw (DH line number 5) to 0.36 mg/gfw (F_{7-8} line number 6) and 0.153 mg/gfw (DH line number 1) to 0.241 mg/gfw (F_{7-8} line number 7) for non-stressed and drought stressed environments, respectively (Table 2). Means of triticale lines and wheat cultivars did not differ significantly for carotenoids content under nonstress but wheat cultivars had significantly ($P < 0.01$) higher value of carotenoid content than triticale lines under the stressful conditions (Table 2). Carotenoids had positive and significant correlation with chl a ($r=0.46^{*}$), chl b ($r=0.62^{**}$) and chl a+b ($r=0.58^{**}$) under the non-stress conditions.

Mean of chl (a+b)/(x+c) ratio ranged from 2.67 for F_{7-8} line number 3 to 4.43 for DH line number 5 in the non-stressed experiment and from 1.91 for Eleanor to 5.09 for DH line number 4 in the drought stress experiment (Table 3). Mean of ratio chl (a+b)/(x+c) of genotypes were 3.45 and 2.90 in non-stress and drought stress environments, respectively. The weight ratio of chl (a+b)/(x+c) is an indicator of the greenness of plant (Lichtenthaler and Buschman 2001). There was not significant difference between mean of F_{7-8} and DH lines in control treatment but DH lines had higher ratio of chl (a+b)/(x+c) under drought stress conditions. Ratio of chl (a+b)/(x+c) of triticale lines and wheat cultivars did not differ significantly under non-stress treatment, but triticale lines were significantly ($P < 0.01$) superior under drought stress conditions for this trait (Table 3). Lower values for the ratio of chl (a+b)/(x+c) are an indicator of senescence, stress, and damage to the plant and the photosynthetic apparatus, which is expressed by a faster breakdown of chls than carotenoids. Chl (a+b)/(x+c) had positive and significant correlation with chl a+b ($r=0.50^{*}$), chl a ($r=0.54^{*}$) under non-stress conditions and this trait had positive and significant correlation with chl a+b ($r=0.80^{**}$), chl a ($r=0.80^{**}$) and chl b ($r=0.61^{**}$) under stress conditions.

The effects of drought and genotype were significant for proline content (Table 1). Drought stress caused an increase in proline content of the genotypes (Table 3). This result is consistent with that of Mallick *et al*. (2011) reporting increase of proline content under moisture stress.

Proline accumulation might respond to stresses such as temperature, drought and starvation (Sairam *et al.* 2002). High level of proline enables a plant to maintain low water potential and thus buffering the immediate effect of water storages within the organism. There was a negative correlation between reduction of grain yield and proline content under drought conditions ($r=-0.61^{**}$). Under both environmental conditions, F_{7-8} line number 4 possessed the greatest proline content (Table 3 and Fig 1) and this genotype had least reduction of grain yield (Table 4).

Drought significantly influenced RWC and caused

Table 1 Combined analysis of variance of the tested traits of triticale and wheat genotypes grown under drought and normal field conditions in two growing seasons

Source of variation	df	Mean square									
		chl a	chl b	chl a+ b	x + c	a+b/ x+c	Proline	RWC	Leaf area	Leaf angle	Grain yield
Year (Y)	1	0.0004	0.00003	0.0007	0.0002	0.187	61	1.33	6.58	37.92	0.26
Environment(E)	1	3.2^{**}	0.54^{**}	6.37^{**}	0.24^{**}	17.55^{**}	139868^{**}	13948^{**}	1509^{**}	2848^{**}	300.17^{**}
Y×E	1	0.0019^{**}	0.0006	0.0003	0.00005	0.0005	12.53	10.68^{*}	0.28	129.56^{*}	4.03^{**}
Replication (Y×E)	8	0.0003	0.0001	0.0006	0.0005	0.096	30	2.77	14.19^{**}	6.75	0.42
Genotype (G)	19	0.0558^{**}	0.0287^{**}	0.1307^{**}	0.0055^{**}	2.78^{**}	5601^{**}	115.14^{**}	107.32^{**}	167^{**}	4.96^{**}
Y×G	19	0.0033^{**}	0.0010^{**}	0.0046^{**}	0.0014^{**}	0.59^{**}	38	8.97^{**}	5.23	19.8	0.73^{**}
G×E	19	0.0149^{**}	0.0091^{**}	0.0374^{**}	0.0061^{**}	1.54^{**}	1540^{**}	63.19^{**}	17.27^{**}	116^{**}	1.64^{**}
G×E×Y	19	0.0019^{**}	0.0004	0.0024^{**}	0.00076^{*}	0.23^{**}	47^{*}	8.71^{**}	2.35	16	0.26
Residual	152	0.0003	0.0003	0.0006	0.0004	0.085	25.68	2.29	4.68	20.47	0.32
CV%		3.43	6.87	3.44	8.53	9.11	8.21	2.28	9.19	10.67	10.78

* $P < 0.05$, ** $P < 0.01$

Table 2 Means of the content of photosynthetic pigments of triticale and wheat genotypes grown under drought and normal field conditions and changes ratio in means of these traits affected by drought stress (drought/normal)

Traits	chl a			chl b			chl a+b			x + c		
Genotype	Non-stress(N)	Stress(S)	S/N	Non-stress(N)	Stress(S)	S/N	Non-stress(N)	Stress(S)	S/N	Non-stress(N)	Stress(S)	S/N
$F_{7\&8}$ lines												
1	0.734a	0.487a	0.67	0.307ef	0.201ef	0.64	1.041b	0.688b	0.66	0.251efg	0.179gh	0.72
2	0.537g	0.263j	0.48	0.260i-m	0.162ij	0.61	0.797h	0.425i	0.52	0.246efg	0.154i	0.6
3	0.495h	0.280i	0.56	0.252klm	0.164ij	0.64	0.747i	0.444i	0.59	0.281cd	0.194efg	0.68
4	0.572ef	0.308h	0.54	0.293fg	0.184fgh	0.62	0.865fg	0.493h	0.57	0.250efg	0.182gh	0.72
5	0.673bc	0.395de	0.58	0.336cd	0.210de	0.62	1.009c	0.605e	0.59	0.254d-g	0.222abc	0.88
6	0.725a	0.420c	0.58	0.382b	0.179ghi	0.47	1.107a	0.599e	0.54	0.363a	0.219bcd	0.61
7	0.542g	0.353g	0.65	0.306ef	0.284b	0.9	0.849g	0.637d	0.75	0.271cde	0.241a	0.89
8	0.497h	0.315h	0.64	0.271h-k	0.135k	0.48	0.768i	0.450i	0.58	0.267def	0.187fgh	0.7
9	0.674bc	0.368fg	0.55	0.284gh	0.178ghi	0.64	0.959d	0.546g	0.57	0.228gh	0.181gh	0.78
DH lines												
1	0.718a	0.369f	0.51	0.317de	0.119kl	0.38	1.035bc	0.487h	0.47	0.296bc	0.153i	0.5
2	0.599d	0.401d	0.67	0.268h-l	0.165hij	0.59	0.867fg	0.566fg	0.66	0.251efg	0.157i	0.64
3	0.552fg	0.245k	0.44	0.250lm	0.112l	0.44	0.801h	0.357j	0.45	0.240fg	0.188e-h	0.79
4	0.662c	0.501a	0.76	0.421a	0.362a	0.86	1.083a	0.863a	0.8	0.319b	0.170hi	0.53
5	0.577e	0.420c	0.72	0.352c	0.229cd	0.66	0.929e	0.649cd	0.7	0.210h	0.214cd	1
6	0.573e	0.436b	0.77	0.285gh	0.230c	0.82	0.859g	0.666bc	0.79	0.267def	0.202def	0.74
7	0.513h	0.389de	0.76	0.244m	0.156j	0.67	0.757i	0.545g	0.72	0.230gh	0.220bcd	0.96
8	0.684b	0.386e	0.56	0.255j-m	0.194efg	0.76	0.939de	0.580ef	0.62	0.260def	0.206cde	0.81
9	0.459i	0.318h	0.7	0.203n	0.185fgh	0.9	0.662j	0.503h	0.76	0.211h	0.206cde	1
Roshan	0.616d	0.307h	0.5	0.273hij	0.191efg	0.7	0.890f	0.498h	0.56	0.254d-g	0.221bcd	0.88
Kavir	0.545g	0.368fg	0.68	0.274ghi	0.297b	1.11	0.819h	0.665bcd	0.83	0.254d-g	0.234ab	0.92
LSD	0.021	0.016		0.019	0.02		0.028	0.028		0.028	0.02	

Means followed by the same letter within a column are not significantly different ($LSD_{0.05}$).

decrease in RWC (Table 3). Genotypes varied significantly for RWC under both environmental conditions (Table 3). RWC significantly correlated with grain yield under both environmental conditions. RWC had a positive and significant relationship with the ratio of chl (a+b)/(x+c) under drought stress conditions. There was significant difference between triticale lines and wheat cultivars for RWC with triticale lines being significantly ($P < 0.01$) superior. DH lines had higher level of RWC than F_{7-8} lines under drought stress conditions (Table 3). There was a negative and significant correlation (r=-0.46*) between RWC and carotenoids content under drought stress conditions. The results of present study are consistent with the recent work on cowpea (*Vigna unguiculata* L. Walp.), mungbean (*V. radiata* L.), snap bean (*Phaseolus vulgaris* L.) and wheat (*Triticum aestivum* L.), revealing that RWC is a good indicator of plant performance under drought stress conditions (Kumar and Sharma 2010).

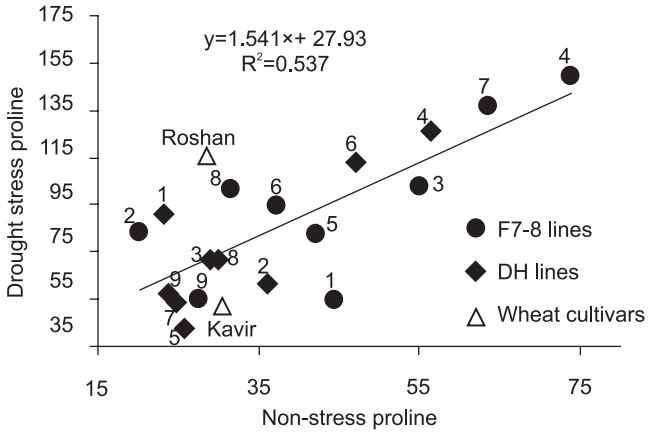

Fig. 1 Relationship between proline produced under drought stress and non-stress field conditions

Area of flag leaf was significantly reduced due to drought (Table 4). Leaf area had positive and significant relationship with RWC under non-stressed conditions, while this relationship was negative under water stress conditions. Blum (2005) suggested that small leaf area is benefical under drought stress because causes dehydration-avoidant.

Under drought stress environment, the genotypes varied significantly for flag leaf angle (Table 4). Analysis of combined variances showed that drought stress have significantly affected the flag leaf angle (Table 1). Flag leaf

Table 3 Means of the weight ratio of chl a+b to total carotenoids (chl (a+b)/(x+c)), proline and relative water content (RWC) of triticale and wheat genotypes grown under drought and normal field conditions and changes ratio in means of these traits affected by drought stress (drought/normal)

	chl a+b/x+c			Proline			RWC		
Genotype	Non-stress(N)	Stress(S)	S/N	Non-stress(N)	Stress(S)	S/N	Non-stress(N)	Stress(S)	S/N
$F_{7\&8}$ lines									
1	4.14ab	3.86b	0.93	44.44d	54.81j	1.23	76.9bcd	56.99f-i	0.74
2	3.24def	2.78d-g	0.86	20.26m	82.88g	4.1	70.64ij	56.49g-j	0.8
3	2.67h	2.29j	0.86	55.02c	102.27e	1.86	71.59hi	62.06c	0.87
4	3.47cde	2.71e-i	0.78	73.69a	149.61a	2.03	75.99cde	56.50g-j	0.74
5	3.97b	2.74e-h	0.69	42.15de	82.59g	1.96	74.37efg	52.91k	0.71
6	3.08fg	2.74e-h	0.89	37.21ef	94.56f	2.54	67.75k	57.52fgh	0.85
7	3.14efg	2.65f-i	0.84	63.46b	136.60b	2.15	74.07fg	58.57ef	0.79
8	2.87gh	2.41ij	0.84	31.50gh	102.13e	3.24	75.23def	55.75hij	0.74
9	4.22ab	3.01cde	0.71	27.73h-l	54.63j	1.97	77.43bc	59.66de	0.77
DH lines									
1	3.51cd	3.20c	0.91	23.27lm	90.67f	3.9	73.27fg	60.91cd	0.83
2	3.46cde	3.62b	1.05	36.12gh	62.38i	1.73	80.63a	67.19b	0.83
3	3.34c-f	1.91k	0.57	28.99h-k	71.27h	2.46	69.49jk	57.79fg	0.83
4	3.39c-f	5.09a	1.5	56.31c	126.29c	2.24	76.11b-e	72.27a	0.95
5	4.43a	3.05cd	0.69	25.82i-l	42.75k	1.66	74.04fg	57.37fgh	0.77
6	3.22def	3.31c	1.03	47.16d	113.86d	2.41	73.91fg	57.31fgh	0.78
7	3.32c-f	2.48g-j	0.75	25.14j-m	53.30j	2.12	71.02ij	58.70ef	0.82
8	3.62c	2.81def	0.78	29.96hij	71.63h	2.39	77.81b	57.56fgh	0.74
9	3.14efg	2.44hij	0.78	23.89klm	56.90ij	2.38	76.54bcd	55.46ij	0.72
Roshan	3.51cd	2.25j	0.64	28.61h-k	116.05d	4.06	71.94hi	55.05j	0.76
Kavir	3.23def	2.85def	0.88	30.60hi	51.78j	1.69	69.71j	57.50fgh	0.82
LSD	0.34	0.3		5.22	6.07		1.82	1.83	

Means followed by the same letter within a column are not significantly different ($LSD_{0.05}$)

angle was significantly increased due to drought (Table 4). Araus *et al.* (2002) stated that stress during plant development caused change in canopy feature and produce horizontal leaves.

Grain yield of all genotypes was significantly reduced by drought stress (Table 4). Under drought stress conditions, DH line number 4, F_{7-8} line number 1 and DH line number 2 produced higher grain yield and ranked as the superior genotypes. In these cases the breeders have selected plants characterized by high yield potential and high yield stability. This implies that traits maximizing productivity normally expressed in the absence of stress can still sustain a significant yield improvement under mild to moderate drought (Slafer *et al.* 2005).

The studied genotypes varied significantly for the tested physiological traits, with the exception of flag leaf angle under the non-stressful experiment. The orthogonal comparison showed that triticale lines and wheat cultivars differed significantly for yield and most of the physiological traits with triticale being significantly superior than wheat cultivars under both environmental conditions. There was, however, minor differences in the relative performance of DH and F_{7-8} triticale lines. F_{7-8} line number 1 and DH line number 2 were superior under both environmental conditions in view point of grain yield.

DH line number 4 possessed the highest values for RWC, chl a, chl b, chl a+b, Chl(a+b)/(x+c) under drought stress conditions. These accompanied by having high proline content and low grain yield reduction due to drought stress was ranked this DH line as superior drought-tolerant genotype. Grain yield produced under drought stress conditions positively and significantly correlated with chl a, chl a+b, RWC under non-stress conditions. Selection of genotypes with higher RWC value under non-stress condition leads to high grain yield under both environmental conditions. The results also show an inverse and significant relationship between grain yield loss due to drought-stress with proline content under drought stress ($r = -0.61^{**}$) and with carotenoids content ($r = -0.47^{*}$) under non-stress conditions, provided an evidence supporting the role of proline in drought tolerance. Moreover, the greatest proline content of F_{7-8} line number 4 accompanied with the least grain yield loss of this genotype provided further evidence supporting the role of proline in drought tolerance mechanisms in triticale.

Table 4 Means of leaf area, leaf angle and grain yield of triticale and wheat genotypes grown under drought and normal field conditions and changes ratio in means of these traits affected by drought stress (drought/normal).

Traits	Leaf area (cm^2)			Leaf angle (degree)			Grain yield (tonnes/ha)		
Genotype	Non-stress (N)	Stress (S)	S/N	Non-stress (N)	Stress (S)	S/N	Non-stress (N)	Stress (S)	S/N
$F_{7\&8}$ lines									
1	29.28abc	20.74ef	0.71	42.6	36.88g	0.86	8.42a	4.95ab	0.59
2	27.35bcd	19.61efg	0.72	36.69	41.37efg	1.13	6.36cde	3.57ghi	0.56
3	22.58ghi	19.54fgh	0.86	36.69	39.50fg	1.08	5.32h	3.70fgh	0.69
4	28.37bcd	22.30c-f	0.79	43.05	42.77d-g	0.99	5.43gh	4.45b-e	0.82
5	23.52fgh	23.78bcd	1.01	39.42	46.38c-f	1.18	7.14b	3.93e-h	0.55
6	27.42bcd	20.88def	0.76	37.83	43.03d-g	1.14	5.27h	3.84fgh	0.73
7	26.13c-f	22.60cde	0.86	37.83	43.21d-g	1.14	6.19c-f	4.08efg	0.66
8	25.94def	20.44ef	0.79	41.52	46.15c-f	1.11	5.96efg	4.08efg	0.68
9	30.01ab	21.33def	0.71	39.33	47.12cde	1.2	7.13b	4.18def	0.59
DH lines									
1	25.56d-g	20.55ef	0.8	38.11	42.83d-g	1.12	6.04efg	4.37cde	0.72
2	29.34abc	19.57fgh	0.66	37.17	42.07efg	1.13	8.09a	4.87abc	0.6
3	21.88hij	16.58hi	0.76	40.3	53.20bc	1.32	5.63fgh	3.43hi	0.61
4	19.26j	15.83i	0.82	39.92	58.58ab	1.47	6.72bc	5.16a	0.77
5	24.05e-h	20.58ef	0.86	35.12	41.52efg	1.18	6.08def	4.44b-e	0.73
6	26.90b-e	21.98c-f	0.82	35.72	41.39efg	1.16	6.39cde	3.97efg	0.62
7	27.57bcd	24.51abc	0.89	37.1	42.48d-g	1.14	6.67bcd	4.13def	0.62
8	30.13ab	25.71ab	0.85	40.13	46.90cde	1.17	7.13b	4.64a-d	0.65
9	32.54a	27.34a	0.84	44.02	48.47cde	1.1	6.79bc	3.78fgh	0.56
Roshan	20.16ij	16.92ghi	0.84	40.23	63.05a	1.57	5.22h	3.71fgh	0.71
Kavir	23.26f-i	20.13ef	0.86	35.67	49.33cd	1.38	5.29h	3.15i	0.6
LSD	3.29	2.99		6.43	7.21		0.62	0.52	

Means followed by the same letter within a column are not significantly different ($LSD_{0.05}$)

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