

# The genotypic and phenotypic responses and feature's associations of triticale genotypes to drought stress

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#### Abstract

In order to reflect the effect of water deficit on triticale and the probability of screening some suitable genotypes tolerant to the drought stress, a study on nine triticale genotypes under four irrigation regimes in two years was carried out. The irrigation regimes were containing control irrigation, and withholding irrigation at three stages: flowering, milky seed, and doughy seed. Different features including plant height, leaf angle, leaf weigh, total dry mater, spike length, spike weight, spike number, grain number, straw yield, harvest index, and grain yield were measured for all applied genotypes in this study. the results showed that lower number of irrigation and earlier withholding the water from the triticale plants can lead to a high decrease in productivity of triticale genotypes. Consequently, irrigation treatments and water availability are a significant factor for determining the type of breeding programs. Also, some genotypes showed a high potential for being considered for releasing cultivars. Under normal irrigation ET-83-20 and under severe water deficit ET-85-04 showed better performance than other genotypes. Estimation of genotypic features such as heritability and coefficient of variation showed that there is a high possibility and potential of producing cultivars with high productivity under either normal or stressed conditions. Additionally, high heritability and significant association with grain yield for some features such spike weight, spike number, and grain number suggest that they are suitable feature for indirect screening and selection criteria.

# Keywords: indirect selection, heritability, biplot, genotypic variation, water deficit.

#### 1. Introduction:

High demands for agricultural products as a result of growing population has led researchers to apply and review altered procedures and approaches so as to increase the production and adoptability of the crops (Vahdati and Lotfi, 2013). Many efforts have been done in order to combine varied capabilities of different plant species into one unique plant to increase the quantity and quality of the food product. Subsequently, over 100 years ago scientists had been able to made and release a new plant species by crossing between wheat (*Triticum sp.*) and rye (*Secale cereale*) plants (Chen et al., 2019). The intention was to increase the capability of wheat, as one of the most significant sources of food amongst cereals in the world, to resist the harsh environmental conditions such as drought stress. Different crossing between rye and wheat with altered polyploidy levels resulted in different types of plants with different properties inherited from different genome sets (Stepochkin, 2019). Triticale (*×Triticosecale Wittmack*) is a hexaploidy species (AABBRR) resulted from crossing between tetraploid wheat (T. durum Desf; 2n=28: AABB), known as durum wheat, and diploid rye (2n=14: RR). Final release of triticale inherited favorable features and properties from both of its progenitors such as higher resistance to drought stress and higher plant production in comparison to wheat plants (Mergoum et al., 2019).

Although triticale have shown several important improvements since its introduction as the first synthesized (man-made) crop, it still shows some shortages which require more attention of breeders (Daskalova and Spetsov, 2020; Riasat et al., 2019). This cereal crop, among all other cereal crops, is the

one that attracted lower attention by researchers and farmers while different studies indicated that it has a high potential to be used as a multiple purposes crop from direct human use to forage crop (McGoverin et al., 2011). Therefore, triticale can be referred to as a high genetically potential crop that its performance is still far from its highest capability. In addition, according to Guedes-Pinto et al. (2012), increase rate in cereals' consumption by the growing population has been around 3.5% per year, while the rate of grain yield increase is currently estimated to be around 2.4% per year. Accordingly, there is a high requirement to improve and extended methods capable of accelerating the efficiency of breeding programs leading to reach higher yield and stable cultivars.

Resistance to drought stress is a complex process involving different types of physiological and molecular networks and responses which has not been properly unveiled and addressed yet (Pirasteh-Anosheh et al., 2016; Vahdati et al., 2009). Drought stress, similar to the most of other environmental stresses, indirectly causes oxidative stress in plants as a result of disturbing the balance between production and detoxification of oxygen radicals (Behera et al., 2020; Cheniany et al., 2010; Saed-Moucheshi et al., 2014). In such condition, defense systems and stress resistance mechanisms in plants are activated to induce higher detoxification of oxygen radicals (Jariteh et al., 2011; Tabarzad et al., 2017).

Breeders have been performing different types of studies considering different methods on varied crops to improve their performance under drought stress condition (Aliakbari et al., 2013; Riasat et al., 2019; Riasat et al., 2020; Saed-Moucheshi et al., 2013; Saed-Moucheshi et al., 2017; Sallam et al., 2019; Tabarzad et al., 2017). In such conditions, generating synthesized variation within crop species and selecting genotypes with the highest yield and stability under altered environments, either natural or artificial, is one of the foremost aims of crop breeding studies.

In the current study, different synthesized genotypes of triticale were cultivated under different irrigation conditions to consider the probability of introducing new varieties tolerant to drought stress and changing the environmental conditions. In addition, the relationship of the morphological and yield related traits were evaluated by some advanced statistical methods in order to find possible traits suitable for indirect selection.

# 2. Materials and methods

# Field study and materials

In our study, nine newly produced triticale genotypes in two different years under different irrigation regimes were used. In each year four different irrigation regimes by withholding irrigation at flowering stage, seed milky stage, and seed doughy stage along with control condition with no stress were used. The experiment (growing season of 2017-2019) was performed in Zarghan location at the research field located in Zarghan Researches and Education Center for Agriculture and Natural Resources, Zarghan, Fars, Iran (52.7135° E, 29.7642° N, altitude 1600 m). It should be noted that the used plants parts in the present study complies with international, national, and institutional guidelines.

Nine advanced triticale genotypes (Table.1), originate from CIMMYT center, were sown early-November (10<sup>th</sup>) and mid-November (20<sup>th</sup>) in the first and second years, respectively. The irrigation schedule (Patel et al., 2019; Saed-Moucheshi et al., 2021c) was being set based on the period of depleting 40% of available water in the soil. Water soil capacity (SWC) was calculated according to following formula:

$$SWC(L) = FC(L) - WP(L)$$

SWC: soil water capacity, FC: field capacity, WP: wilting point.

In each year a split-plot design based on completely random block design with three replicates was used. The plots were comprised of four 2 m length rows with 20 cm space between the rows ( $1 \text{ m} \times 2 \text{ m}$  plot). The triticale seeds were sown on all four rows in each plot and had 5 cm distance from each other. The two central rows of each plot in both years were used for measuring grain yield at the harvest time.

Table 1. Mean comparison of interaction effect between irrigation and genotype in two years for measured feature in triticale plants

Irrigation withholds	Geneture	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2
	Genotype	Leaf weig	;ht	Plant he	eight	Spike leng	th
	Sanabad	0.08h-j	0.08i-k	128a	133.12a	95а-е	98.8a-c
	Pajh	0.09g-i	0.09g-j	122a	126.88a	82e-j	85.28c-h
	Javanilo	0.05l-l	0.05m-m	124a	128.96a	79.8g-l	82.99d-j
	ET-85-4	0.06k-l	0.06l-m	131a	136.24a	86.2c-i	89.65b-e
	ET-92-15	0.07j-k	0.07k-l	129a	134.16a	80.2f-l	83.41d-i
Flowering	ET-92-18	0.08h-j	0.08i-k	119a	123.76a	81.5e-k	84.76c-h
	ET-83-20	0.07j-k	0.07k-l	118a	122.72a	73.2h-m	76.13e-k
	ET-85-17	0.09g-i	0.09g-j	132a	133.12a	59.2n-n	61.57l-l
	ET-83-18	0.07j-k	0.07k-l	128a	131.84a	59.5m-n	61.88k-l
	Sanabad	0.09g-i	0.09h-j	128a	122.57a	100.1a-c	103.1a-b
	Pajh	0.1f-h	0.1f-i	119a	126.69a	89c-g	91.67b-d
	Javanilo	0.06k-l	0.06l-m	123a	135.96a	87c-h	89.61b-e
	ET-85-4	0.07j-k	0.07k-l	132a	131.84a	92b-g	94.76a-d
Milky	ET-92-15	0.08h-j	0.08i-k	128a	130.81a	89c-g	91.67b-d
	ET-92-18	0.09g-i	0.09h-j	127a	122.57a	90c-g	92.7b-d
	ET-83-20	0.08h-j	0.08i-k	119a	133.9a	81f-k	83.43d-i
	ET-85-17	0.09g-i	0.09h-j	130a	132.87a	68k-n	70.04i-l
	ET-83-18	0.08h-j	0.08i-k	129a	131.58a	67l-n	69.01j-l
	Sanabad	0.11c-f	0.11c-f	129a	122.4a	105a-b	107.1a-a
	Pajh	0.12b-e	0.12b-e	120a	125.46a	93a-g	94.86a-d
	Javanilo	0.08i-j	0.08j-k	123a	133.62a	90c-g	91.8b-d
	ET-85-4	0.09g-i	0.09h-j	131a	131.58a	95а-е	96.9a-d
Doughy	ET-92-15	0.1e-g	0.1e-h	129a	130.56a	93a-g	94.86a-d
	ET-92-18	0.12b-d	0.12b-d	128a	121.38a	94a-f	95.88a-d
	ET-83-20	0.11c-f	0.11c-f	119a	135.66a	86d-i	87.72c-g
	ET-85-17	0.13a-b	0.13a-b	129a	131.58a	72j-n	73.44g-l
	ET-83-18	0.11c-f	0.11c-f	128a	129.66a	70j-n	71.4h-l
	Sanabad	0.12b-d	0.12b-d	123a	124.6a	93a-g	107.38a-a
	Pajh	0.12b-c	0.12b-c	124a	125.61a	91c-g	94.21a-d
	Javanilo	0.08h-j	0.08i-k	130a	131.69a	96a-d	92.18b-d
	ET-85-4	0.1f-h	0.1f-i	129a	130.68a	93a-g	97.25a-d
Control	ET-92-15	0.11d-f	0.11d-g	128a	129.66a	94a-f	94.21a-d

ET-92-18	0.13a-b	0.13a-b	120a	121.56a	87c-h	95.22a-d
ET-83-20	0.12b-d	0.12b-d	133a	137.28a	106a-a	88.13c-f
ET-85-17	0.14a-a	0.14a-a	133a	134.73a	73i-n	73.95f-l
ET-83-18	0.12b-d	0.12b-d	129a	130.68a	72j-n	72.94h-l

### Measurements and statistical analysis

In this study different morphological and yield related traits containing leaf weight (LW), plant height (H), spike length (SpL), leaf angle (LA), spike weight (SpW), spike number (SpN), seed number (SN), 1000-seed weight (1000SW), total dry matter (DM), straw yield (), grain yield (), and harvest index (HI) were measured.

The obtained data related to field experiments were subjected to combined analysis of variance (combined ANOVA) and descriptive statistic's, such as mean and standard error. After that, the mean squares (MS) obtained from combined ANOVA for each of normal irrigation and drought stressed trials were used for estimating the residual (res) variance, variance of block (blk) within year (yr), genotype (gn) by year variance, genotypic variance, environmental variance, phenotypic variance, broad sense (general) heritability, genotypic coefficient of variation (GCV), and phenotypic coefficient of variation (PCV) based on the following formulas (Fan, 2018; Saed-Moucheshi, 2018). The analyses were performed in SAS-9.4 programming stored GitHub (M6) by using а code in repository (https://github.com/ArminSaed/GenoPheno SAS/issues/1). The following formulas were used to estimate different properties.

Parameter	Index	Formula
Residual variance	$\delta^2_{res}$	MS <sub>res</sub>
Block(year) variance	$\delta^2_{blk(yr)}$	(MS <sub>blk(yr)</sub> -MS <sub>res</sub> ) / (yr×gn)
Genotype by environment variance	$\delta^2_{gn\times yr}$	(MS <sub>gn×yr</sub> -MS <sub>res</sub> ) / blk
Genotypic variance	$\delta^2_{gn}$	(MS <sub>gn</sub> -MS <sub>res</sub> )/(blk ×yr)
Environmental variance	$\delta^2_{yr}$	$(MS_{blk(yr)}\text{-}MS_{gn\timesyr}+\delta^2{}_{res})/(blk\timesgn)$
Phenotypic variance	$\delta^2_{pn}$	$\delta^2_{gn} + [\delta^2_{yr} / (blk \times yr)] + [\delta^2_{gn \times yr} / yr]$
General heritability	h²	$\delta^2_{gn}/\delta^2_{pn}$
Genotypic coefficient of variation	GCV	$[(v\delta^2_{gn}) / Mean] \times 100$
Phenotypic coefficient of variation	PCV	$[(v\delta^2_{pn}) / Mean] \times 100$

Additionally, ANOVA and mean comparison were analyzed by proc GLM and proc MEANS in SAS statistical software version 9.6, respectively. The libraries "agricolae", "Nbclust", and "factoextra" in R statistical software (R 3.5) were applied for performing cluster analysis based on Ward method and Euclidian distance and biplot graph based on principal component analysis (PCA).

# 3. Results

The results of combined ANOVA for two years and four treatments along with nine genotypes showed that, except plant height and harvest index, all measured treatments were affected by year and irrigation

and there were significant differences among the genotypes. The interaction effect of the year by treatment, year by genotype, treatment by genotypes and the three-way interaction of the sources were mostly significant for the measured traits. Therefore, the mean comparisons were performed not for combined data but for the data obtained from different years and treatments. Mean comparison of leaf dry weight and spike length of triticale are presented in Table 1. The highest mean value for leaf dry weight in both years observed in ET-85-17 under control condition (normal irrigation). The minimum leaf weight in both years was recorded in Juanilo cultivar under withholding water at flowering stage. ET-83-20 in the first year and Sanabad in the second year showed the maximum values of spike length under control treatment, however, the minimum value of this traits was recorded for ET-85-17 under withholding irrigation at flowering stage for both years. The leaf angle of the leaf with the shoot was the smallest in genotype ET-85-4 and the highest in ET-92-15 regarding both consecutive years under irrigation treatment at flowering stage and control condition, respectively (Table 2). The highest mean values of spike weight and spike number was observed under irrigation treatment at flowering stage in Paj (with no significant difference from Juanilo) and ET-83-18, respectively. In the first year, ET-83-20 obtained the first place among triticale genotypes in both year for spike weight and number, respectively. In the second year, ET-85-4 showed the lowest spike weight while Juanilo showed the lowest spike number (Table 2).

A low variation was observed in the triticale genotypes under different irrigation treatments regarding 1000-seed weight. Similar results achieved for seed number per spike, however, the number of seed showed a significant decreasing pattern in response longer periods of irrigation withholding (Table 3). Total dry weight of the plants in genotype ET-85-17 was the minimum in both years, but Sanabad triticale cultivar showed maximum dry weight in both years (Table 3). The results of mean comparison of three-way effect of year by irrigation by genotype for straw yield, harvest index, and grain yield are presented in Table 4. The harvest index of the applied genotypes under all four treatment was similar and a low variation was observed for this trait. Longer periods of irrigation withholding treatments (at flowering and milky stages) showed almost less straw yield than the two other treatments in both years, but the differences among genotypes under each treatment were not significant.

In order to modeling the relationship between grain yield of triticale and the irrigation treatments, they transformed into the number irrigation the number irrigations. Figure 1 shows the relationship between irrigation number of triticale genotypes and grain yield. For both years, a linear relationship with a coefficient of determination (R-squared) greater than 0.89 was fitted to the data. This graph clearly shows that the greater number of irrigations leads to higher grain yield, however, the withholding irrigation at last stages i.e., doughy stage causes lower grain yield loss in comparison to other earlier stages. Also, the mean comparison between control condition and irrigation treatment at doughy stage showed low differences, and sometimes not significant differences, regarding used genotypes (Table 4). The highest mean value of grain yield in first and second years was achieved under control condition in ET-83-20 and Sanabad, respectively. ET-85-17 and ET-83-18 genotypes showed the lowest mean values of grain yield under irrigation treatment at flowering stage in both years. Moreover, the mean comparison between genotypes over al treatments showed that in both years ET-85-17 and ET-83-18 obtained the least grain yield while Sanabad and Paj, with a low difference, achieved the greatest triticale yield (Figure 1).



Figure 1. Triticale yield in response to irrigation interval (A) and the yield of triticale genotypes (B)

Genetic parameters were estimated by taking year, as environment, and genotype as random effects and calculating the expected values for each source of variation in ANOVA mixed model separately in each stress condition. Accordingly, environmental, genotypic, and phenotypic variance were extracted from data and transformed into standard indices i.e., heritability, phenotypic coefficient of variation (PCV), and genotypic coefficient of variation (GCV) in Table 5. The highest heritability was observed in spike length (98%) and it showed no significant difference from leaf weight and grain number (96%). Straw yield and harvest index showed the lowest percentage of heritability in (less than 20%). The heritability of grain yield was also high and significant (74%). Spike number and leaf weight showed the highest GCV (>14%) while the lowest PCV (>15%), on the other side. Plant height and leaf angle were the features with the same lowest percentage of PCV (<4%). The lowest GCV percentage was shared between plant height, leaf angle, and harvest index (Table 5).

The association between measured features in this study is portrayed by using correlation plot, biplot and three-way-plot based on principal component analysis (PCA) in Figure 2.



Figure 2. Correlation between the measured features (A), scree plot of principal component analysis (B), 2D-biplot (C) and 3D-biplot of measured features in triticale under different irrigation interval in two years.

Accordingly, the highest correlation of grain yield was belonged to dry mater. Spike number and straw yield also showed high positive correlation with grain yield of triticale. The correlation of harvest index with spy length, spike number, and 100 seed weight was positive while its correlation with other traits was negative. The biplot and 3D-plot showed almost similar results with the correlation plot, however, since the first three components (PC) are significant and they accounted for almost higher than 80 percent of variation among the data, the 3D-plot would be more suitable for determining the relationship among the features. The 3D-plot clearly shows that plant height and harvest index are placed in somewhere far from other features, and there is also a high space between these two features as well. Leaf weight and leaf angle along with spike weight and number and grain number showed to be the closest features to grain yield.

Irrigation withbolds	Constuna	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2	
ingation withious	Genotype	Leaf ang	gle	Spike wei	Spike weight		Spike number	
	Sanabad 35a-b		36.05a-c	1.94j-l	2.13i-j	390c-h	401.7c-h	
	Pajh	37a-b	38.11a-c	1.74 -	1.91j-j	380d-h	391.4d-h	
	Javanilo	34a-b	35.02b-c	1.74 -	1.91j-j	370e-h	381.1e-h	
	ET-85-4	33b-b	33.99с-с	1.99i-l	2.19h-j	350f-i	360.5f-i	
	ET-92-15	36a-b	37.08a-c	1.91j-l	2.1i-j	340g-j	350.2g-j	
Flowering	ET-92-18	37a-b	38.11a-c	1.99i-l	2.19h-j	330h-k	339.9h-k	
	ET-83-20	34a-b	35.02b-c	2.13g-k	2.34g-i	280j-l	288.4j-l	
	ET-85-17	34a-b	35.02b-c	1.89k-l	2.08i-j	260I-I	267.8I-I	
	ET-83-18	35a-b	36.05a-c	1.98j-l	2.18h-j	250I-I	257.5I-I	
	Sanabad	36a-b	37.08a-c	2.22e-k	2.39f-i	450c-c	463.5c-c	
	Pajh	39a-b	40.56a-b	1.96j-l	2.12i-j	395c-g	406.85c-g	
	Javanilo	35a-b	36.4a-c	2.01h-l	2.17h-j	390c-h	401.7c-h	
	ET-85-4	34a-b	35.36b-c	2.26c-k	2.45e-i	380d-h	391.4d-h	
Milky	ET-92-15	38a-b	39.52a-c	2.18e-k	2.35g-i	375e-h	386.25e-h	
	ET-92-18	37a-b	38.48a-c	2.22d-k	2.4e-i	330h-k	339.9h-k	
	ET-83-20	37a-b	38.48a-c	2.36b-i	2.55d-h	300i-l	309i-l	
	ET-85-17	36a-b	37.44a-c	2.17f-k	2.35g-i	290i-l	298.7i-l	
	ET-83-18	36a-b	37.44a-c	2.22e-k	2.39f-i	270k-l	278.1k-l	
	Sanabad	38a-b	39.71a-c	2.27c-j	2.43e-i	540a-b	561.6a-b	
	Pajh	37a-b	41.8a-a	2.26c-k	2.42e-i	520b-b	540.8b-b	
	Javanilo	36a-b	38.67a-c	2.26c-k	2.42e-i	410c-f	426.4c-f	
	ET-85-4	40a-a	37.62a-c	2.6a-c	2.78a-f	410c-f	426.4c-f	
Doughy	ET-92-15	40a-a	41.8a-a	2.4b-g	2.57c-h	400c-g	416c-g	
	ET-92-18	37a-b	41.8a-a	2.45a-g	2.62b-g	400c-g	416c-g	
	ET-83-20	37a-b	38.67a-c	2.6a-d	2.78a-f	390c-h	405.6c-h	
	ET-85-17	38a-b	38.67a-c	2.51a-f	2.69a-g	380d-h	395.2d-h	
	ET-83-18	38a-b	39.71a-c	2.55а-е	2.73a-g	350f-i	364f-i	
	Sanabad	40a-a	39.98a-c	2.37b-h	2.61b-g	580a-b	615.83a-a	
	Pajh	38a-b	42.08a-a	2.41b-g	2.65b-g	440c-d	600.3a-b	
	Javanilo	36a-b	39.98a-c	2.46a-g	2.71a-g	430с-е	455.4c-d	
	ET-85-4	40a-a	37.87а-с	2.6a-c	3.08a-a	420с-е	445.05c-e	
Control	ET-92-15	40a-a	42.08a-a	2.55а-е	2.86a-d	410c-f	434.7с-е	
	ET-92-18	38a-b	42.08a-a	2.7a-b	2.81a-e	410c-f	424.35c-f	
	ET-83-20	40a-a	39.98a-c	2.8a-a	2.97a-c	595a-a	424.35c-f	
	ET-85-17	38a-b	39.98a-c	2.67a-b	2.93a-d	400c-g	414c-g	
	ET-83-18	38a-b	39.98a-c	2.71a-b	2.98a-b	400c-g	414c-g	

Table 2. Mean comparison of interaction effect between irrigation and genotype in two years for measured feature in triticale plants

Irrigation	Conotuno	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2
withholds	Genotype	Seed nu	umber	1000-se	eed weight	Total dry matter	
	Sanabad	40g-g	40.4g-g	46a-d	46.92a-e	8920.8j-o	9812.88j-n
	Pajh	41f-g	41.41f-g	40c-d	40.8d-e	8496k-p	9345.6l-o
	Javanilo	41f-g	41.41f-g	40c-d	40.8d-e	7848m-q	8632.8m-p
	ET-85-4	42e-g	42.42e-g	45a-d	45.9а-е	8136l-q	8949.6I-p
	ET-92-15	43d-g	43.43d-g	42b-d	42.84с-е	8208k-q	9028.8I-p
Flowering	ET-92-18	44c-g	44.44c-g	43b-d	43.86b-e	7925.33m-q	8717.87m-p
	ET-83-20	45c-g	45.45c-g	45a-d	45.9а-е	7416o-q	8157.6n-p
	ET-85-17	46c-g	46.46c-g	39d-d	39.78е-е	6264q-q	6890.4р-р
	ET-83-18	47c-g	47.47c-g	40c-d	40.8d-e	6768p-q	7444.8о-р
	Sanabad	42e-g	42.63e-g	46a-d	47.38a-d	12075b-g	13041c-h
	Pajh	42e-g	42.63e-g	43b-d	44.29b-e	9799.65h-m	10583.62i-m
	Javanilo	43d-g	43.65d-g	43b-d	44.29b-e	9636.9i-n	10407.85i-n
	ET-85-4	43d-g	43.65d-g	49a-b	50.47a-b	10290g-k	11113.2h-l
Milky	ET-92-15	45c-g	45.68c-g	44a-d	45.32b-e	10269g-l	11090.52h-l
	ET-92-18	46c-g	46.69c-g	45a-d	46.35a-e	8820k-p	9525.6k-o
	ET-83-20	47c-g	47.71c-g	47a-c	48.41a-c	8767.5k-p	9468.9I-o
	ET-85-17	47c-g	47.71c-g	42b-d	43.26b-e	7875m-q	8505m-p
	ET-83-18	48b-f	48.72b-f	42b-d	43.26b-e	7560n-q	8164.8n-p
	Sanabad	45c-g	45.72c-g	48a-b	49.92a-c	13840b-c	14808.8b-c
	Pajh	48b-f	48.77b-f	42b-d	43.68b-e	13760b-c	14723.2b-c
	Javanilo	48b-f	48.77b-f	42b-d	43.68b-e	11033.07f-j	11805.38g-k
	ET-85-4	49a-e	49.78а-е	48a-b	49.92a-c	11900b-h	12733c-i
Doughy	ET-92-15	50a-d	50.8a-d	44a-d	45.76а-е	11150d-i	11930.5f-j
	ET-92-18	50a-d	50.8a-d	44a-d	45.76а-е	11403.33d-i	12201.57e-i
	ET-83-20	51a-c	51.82a-c	46a-d	47.84a-d	11700c-i	12519c-i
	ET-85-17	55a-b	55.88a-b	42b-d	43.68b-e	11100e-i	11877f-j
	ET-83-18	45c-g	56.9a-a	42b-d	43.68b-e	11500d-i	12305d-i
	Sanabad	48b-f	45.81c-g	46a-d	47.84a-d	16510a-a	18161a-a
	Pajh	48b-f	48.86b-f	44a-d	45.76а-е	12900b-f	18667a-a
	Javanilo	49a-e	48.86b-f	45a-d	46.8а-е	14040b-b	14190b-f
	ET-85-4	50a-d	49.88а-е	46a-d	53.04a-a	13200b-e	15444b-b
Control	ET-92-15	50a-d	50.9a-d	45a-d	47.84a-d	12300b-g	14520b-e
	ET-92-18	51a-c	50.9a-d	47a-c	46.8a-e	13300b-d	13530b-g
	ET-83-20	56a-a	51.92a-c	51a-a	48.88a-c	16970a-a	14630b-d
	ET-85-17	55a-b	55.99a-b	43b-d	44.72b-e	13100b-f	14410b-e
	ET-83-18	56a-a	57.01a-a	43b-d	44.72b-e	13300b-d	14630b-d

Table 3. Mean comparison of interaction effect between irrigation and genotype in two years for measured feature in triticale plants

		Year 1	Year 2	Year	Year	Year 1	Year 2
irrigation	Genotype			1	2		
withholds		Straw yield		Harves	st index	Grain yield	
	Sanabad		2252.13c	83.34	78.03	7340.53k-	7560.75m-
		-C	-d	а	а	m	р
	Рај	2081.04b	2738.19b	76.48	71.61	6414.96m-	6607.41p-
		-C	-d	а	а	р	S
	Juanilo	1601.85b	2199.27c	80.51	75.39	6246.15n-	6433.53q-
		-C	-d	а	а	р	S
	ET-85-4	1378.56c	1989.43d	76.88	78.48	6757.44l-o	6960.170-
		-C	-d	а	а		r
	ET-92-15	1920.72b	2552.9c-	80.49	71.99	6287.28n-	6475.9q-s
		-C	d	а	а	р	
Eloworing	ET-92-18	1547.63c	2148.83c	78.39	75.36	6377.71n-	6569.04q-
Flowering		-C	-d	а	а	р	S
	ET-83-20	1643.31b	2211.73c	77.35	73.4a	5772.69p-	5945.87s-s
		-C	-d	а		р	
	ET-85-17	1486.35c	1969.42d	71.33	72.42	4777.65q-	4920.98t-t
		-C	-d	а	а	q	
	ET-83-18	1965.51b	2498.24c	80.3a	66.8a	4802.49q-	4946.57t-t
		-C	-d			q	
	Sanabad	2402.16a	3077.98b	76.75	76.58	9672.84d-	9963.03e-
		-C	-d	а	а	f	g
	Рај	2274.58a	2832.8b-	79.29	73.2a	7525.07j-l	7750.82l-o
		-C	d	а			
	Juanilo	2048.2b-	2591.49b	81.3a	75.61	7588.7j-l	7816.36l-o
		С	-d		а		
	ET-85-4	1944.9b-	2517.74c	77.22	77.54	8345.1h-j	8595.46i-l
		С	-d	а	а		
Milley	ET-92-15	2339.25a	2922.88b	80.74	73.65	7929.75i-k	8167.64k-
IVIIIKY		-C	-d	а	а		n
	ET-92-18	1700.98b	2193.01c	78.49	77a	7119.02k-	7332.6n-q
		-C	-d	а		n	
	ET-83-20	1894.08b	2389.28c	77.76	74.85	6873.42I-o	7079.620-
		-C	-d	а	а		r
	ET-85-17	1759.54b	2206.07c	77.29	74.16	6115.460-	6298.93r-s
		-C	-d	а	а	р	

Table 4. Mean comparison of	f interaction	effect betw	een irrigatio	n and ge	enotype in	two	years for
measured feature in triticale	plants						

	ET-83-18	1756.3b-	2186.99c	80.12	73.71	5803.7р-р	5977.82s-s
		С	-d	а	а		
	Sanabad	2807.8a-	3335.31a	77.4a	77.87	11032.2b-	11473.49b
		С	-d		а	b	-b
	Рај	3164.48a	3703.86a	78.18	75.23	10595.52b	11019.34b
		-C	-d	а	а	-d	-d
	Juanilo	2678.91a	3117.06b	82.5a	75.99	8354.16h-j	8688.33i-l
		-C	-d		а		
	ET-85-4	2302.31a	2751.4b-	77.61	80.19	9597.69e-f	9981.6e-f
		-C	d	а	а		
Doughy	ET-92-15	2510а-с	2944.9b-	79.49	75.44	8640g-i	8985.6g-k
Doughy			d	а	а		
	ET-92-18	2583.33a	3028.77b	79.55	77.26	8820f-i	9172.8f-j
		-C	-d	а	а		
	ET-83-20	2584.53a	3038.91b	77.71	77.32	9115.47e-	9480.09e-i
		-C	-d	а	а	h	
	ET-85-17	2515.8a-	2949.43b	70.19	75.53	8584.2g-i	8927.57h-
		С	-d	а	а		k
	ET-83-18	3461.2a-	3944.65a	77.69	68.22	8038.8i-k	8360.35j-
		С	-d	а	а		m
	Sanabad	с 3818.65а	-d 5025.45a	a 75.86	a 73.1a	12590.64a	m 13135.55a
	Sanabad	с 3818.65а -b	-d 5025.45a -b	a 75.86 a	a 73.1a	12590.64a	m 13135.55a
	Sanabad Paj	c 3818.65a -b 3158.4a-	-d 5025.45a -b 5635.69a	a 75.86 a 76a	a 73.1a 71.38	12590.64a 9741.6d-f	m 13135.55a 13031.31a
	Sanabad Paj	c 3818.65a -b 3158.4a- c	-d 5025.45a -b 5635.69a	a 75.86 a 76a	a 73.1a 71.38 a	12590.64a 9741.6d-f	m 13135.55a 13031.31a
	Sanabad Paj Juanilo	c 3818.65a -b 3158.4a- c 3207.87a	-d 5025.45a -b 5635.69a 4107.44a	a 75.86 a 76a 77.45	a 73.1a 71.38 a 71.51	12590.64a 9741.6d-f 10832.13b	m 13135.55a 13031.31a 10082.56d
	Sanabad Paj Juanilo	c 3818.65a -b 3158.4a- c 3207.87a -c	-d 5025.45a -b 5635.69a 4107.44a -d	a 75.86 a 76a 77.45 a	a 73.1a 71.38 a 71.51 a	12590.64a 9741.6d-f 10832.13b -c	m 13135.55a 13031.31a 10082.56d -f
	Sanabad Paj Juanilo ET-85-4	c 3818.65a -b 3158.4a- c 3207.87a -c 3372a-c	-d 5025.45a -b 5635.69a 4107.44a -d 4232.75a	a 75.86 a 76a 77.45 a 74.87	a 73.1a 71.38 a 71.51 a 72.87	12590.64a 9741.6d-f 10832.13b -c 9828d-e	m 13135.55a 13031.31a 10082.56d -f 11211.26b
	Sanabad Paj Juanilo ET-85-4	c 3818.65a -b 3158.4a- c 3207.87a -c 3372a-c	-d 5025.45a -b 5635.69a 4107.44a -d 4232.75a -d	a 75.86 a 76a 77.45 a 74.87 a	a 73.1a 71.38 a 71.51 a 72.87 a	12590.64a 9741.6d-f 10832.13b -c 9828d-e	m 13135.55a 13031.31a 10082.56d -f 11211.26b -c
Control	Sanabad Paj Juanilo ET-85-4 ET-92-15	c 3818.65a -b 3158.4a- c 3207.87a -c 3372a-c 2890.5a-	-d 5025.45a -b 5635.69a 4107.44a -d 4232.75a -d 4348.02a	a 75.86 a 76a 77.45 a 74.87 a 76.86	a 73.1a 71.38 a 71.51 a 72.87 a 70.44	12590.64a 9741.6d-f 10832.13b -c 9828d-e 9409.5e-g	m 13135.55a 13031.31a 10082.56d -f 11211.26b -c 10171.98d
Control	Sanabad Paj Juanilo ET-85-4 ET-92-15	c 3818.65a -b 3158.4a- c 3207.87a -c 3372a-c 2890.5a- c	-d 5025.45a -b 5635.69a 4107.44a -d 4232.75a -d 4348.02a -d	a 75.86 a 76a 77.45 a 74.87 a 76.86 a	a 73.1a 71.38 a 71.51 a 72.87 a 70.44 a	12590.64a 9741.6d-f 10832.13b -c 9828d-e 9409.5e-g	m 13135.55a 13031.31a 10082.56d -f 11211.26b -c 10171.98d -e
Control	Sanabad Paj Juanilo ET-85-4 ET-92-15 ET-92-18	c 3818.65a -b 3158.4a- c 3207.87a -c 3372a-c 2890.5a- c 3348.07a	-d 5025.45a -b 5635.69a 4107.44a -d 4232.75a -d 4348.02a -d 3791.17a	a 75.86 a 76a 77.45 a 74.87 a 76.86 a 75.41	a 73.1a 71.38 a 71.51 a 72.87 a 70.44 a 72.31	12590.64a 9741.6d-f 10832.13b -c 9828d-e 9409.5e-g 9951.93c-	m 13135.55a 13031.31a 10082.56d -f 11211.26b -c 10171.98d -e 9738.83e-
Control	Sanabad Paj Juanilo ET-85-4 ET-92-15 ET-92-18	c 3818.65a -b 3158.4a- c 3207.87a -c 3372a-c 2890.5a- c 3348.07a -c	-d 5025.45a -b 5635.69a 4107.44a -d 4232.75a -d 4348.02a -d 3791.17a -d	a 75.86 a 76a 77.45 a 74.87 a 76.86 a 75.41 a	a 73.1a 71.38 a 71.51 a 72.87 a 70.44 a 72.31 a	12590.64a 9741.6d-f 10832.13b -c 9828d-e 9409.5e-g 9951.93c- e	m 13135.55a 13031.31a 10082.56d -f 11211.26b -c 10171.98d -e 9738.83e- h
Control	Sanabad Paj Juanilo ET-85-4 ET-92-15 ET-92-18 ET-83-20	c 3818.65a -b 3158.4a- c 3207.87a -c 3372a-c 2890.5a- c 3348.07a -c 4379.36a	-d 5025.45a -b 5635.69a 4107.44a -d 4232.75a -d 4348.02a -d 3791.17a -d 4329.75a	a 75.86 a 76a 77.45 a 74.87 a 76.86 a 75.41 a 83.82	a 73.1a 71.38 a 71.51 a 72.87 a 70.44 a 72.31 a 70.95	12590.64a 9741.6d-f 10832.13b -c 9828d-e 9409.5e-g 9951.93c- e 12691.35a	m 13135.55a 13031.31a 10082.56d -f 11211.26b -c 10171.98d -e 9738.83e- h 10300.25c
Control	Sanabad Paj Juanilo ET-85-4 ET-92-15 ET-92-18 ET-83-20	c 3818.65a -b 3158.4a- c 3207.87a -c 3372a-c 2890.5a- c 3348.07a -c 4379.36a	-d 5025.45a -b 5635.69a 4107.44a -d 4232.75a -d 4348.02a -d 3791.17a -d 3791.17a -d 4329.75a -d	a 75.86 a 76a 77.45 a 74.87 a 76.86 a 75.41 a 83.82 a	a 73.1a 71.38 a 71.51 a 72.87 a 70.44 a 72.31 a 70.95 a	12590.64a 9741.6d-f 10832.13b -c 9828d-e 9409.5e-g 9951.93c- e 12691.35a	m 13135.55a 13031.31a 10082.56d -f 11211.26b -c 10171.98d -e 9738.83e- h 10300.25c -e
Control	Sanabad Paj Juanilo ET-85-4 ET-92-15 ET-92-18 ET-83-20 ET-83-20	c 3818.65a -b 3158.4a- c 3207.87a -c 3372a-c 2890.5a- c 3348.07a -c 4379.36a 3506a-c	-d 5025.45a -b 5635.69a 4107.44a -d 4232.75a -d 4348.02a -d 3791.17a -d 4329.75a -d 4329.75a -d	a 75.86 a 76a 77.45 a 74.87 a 76.86 a 75.41 a 83.82 a 73.68	a 73.1a 71.38 a 71.51 a 72.87 a 70.44 a 72.31 a 70.95 a 69.32	12590.64a 9741.6d-f 10832.13b -c 9828d-e 9409.5e-g 9951.93c- e 12691.35a 9594e-f	m 13135.55a 13031.31a 10082.56d -f 11211.26b -c 10171.98d -e 9738.83e- h 10300.25c -e 9929.79e-
Control	Sanabad Paj Juanilo ET-85-4 ET-92-15 ET-92-18 ET-83-20 ET-83-20	c 3818.65a -b 3158.4a- c 3207.87a -c 3372a-c 2890.5a- c 3348.07a -c 4379.36a 3506a-c	-d 5025.45a -b 5635.69a 4107.44a -d 4232.75a -d 4348.02a -d 3791.17a -d 4329.75a -d 4329.75a -d 4329.75a	a 75.86 a 76a 77.45 a 74.87 a 76.86 a 75.41 a 83.82 a 73.68 a	a 73.1a 71.38 a 71.51 a 72.87 a 70.44 a 72.31 a 70.95 a 69.32 a	12590.64a 9741.6d-f 10832.13b -c 9828d-e 9409.5e-g 9951.93c- e 12691.35a 9594e-f	m 13135.55a 13031.31a 10082.56d -f 11211.26b -c 10171.98d -e 9738.83e- h 10300.25c -e 9929.79e- g
Control	Sanabad Paj Juanilo ET-85-4 ET-92-15 ET-92-18 ET-83-20 ET-83-17 ET-83-18	c 3818.65a -b 3158.4a- c 3207.87a -c 3372a-c 2890.5a- c 3348.07a -c 4379.36a 3506a-c 3551.2a-	-d 5025.45a -b 5635.69a 4107.44a -d 4232.75a -d 4348.02a -d 3791.17a -d 4329.75a -d 4329.75a -d 4329.75a -d	a 75.86 a 76a 77.45 a 74.87 a 76.86 a 75.41 a 83.82 a 73.68 a 73.68	a 73.1a 71.38 a 71.51 a 72.87 a 70.44 a 72.31 a 70.95 a 69.32 a 69.5a	12590.64a 9741.6d-f 10832.13b -c 9828d-e 9409.5e-g 9951.93c- e 12691.35a 9594e-f 9748.8d-f	m 13135.55a 13031.31a 10082.56d -f 11211.26b -c 10171.98d -e 9738.83e- h 10300.25c -e 9929.79e- g 10090.01d

•	•						
Feature	HV	EnV	PhV	H2	PCV	GCV	RS
LWt	0.000201	0.000007	0.000209	96.53	15.21	14.94	0.0287
Ht	20.22	1.05	21.28	95.05	3.6	3.51	9.03
SpL	128.63	1.36	129.99	98.96	13.23	13.17	23.24
LAng	2	0.09	2.08	95.8	3.82	3.74	2.85
SpW	0.02	0	0.02	91.99	5.54	5.32	0.25
SpN	3574.76	247.82	3822.58	93.52	15.61	15.1	119.11
GN	8.24	0.32	8.56	96.28	6.16	6.05	5.8
W1000	5.84	0.28	6.12	95.43	5.51	5.39	4.86
Yld	969061.8	344330.9	1313393	73.78	13.57	11.66	1741.89
DM	1270549	382579.4	1653128	76.86	11.43	10.02	2035.66
Hyld	70712.26	420563.6	491275.9	14.39	24.99	9.48	207.83
HI	5.35	17.93	23.28	22.99	6.35	3.05	2.28

Table 5. Genotypic and phenotypic estimated features based on the data from the both years of study.

#### 4. Discussion

Drought triggers a series of plant internal reactions which eventually affect the plant productivity. In this study the response of the applied genotypes to drought stress and change in environment was different. However, in most of the measured traits the triticale genotypes showed higher values than the triticale cultivars that were implemented in the experiment. This result indicates that there is high probability of introducing a new cultivar among these genotypes by continuing the breeding program on them. Also, the results indicated that the difference between control condition (full irrigation regime) and withholding the irrigation at doughy stage of seed was low, and in most of the cases not significant. On the other side, withholding the irrigation at earlier stages of plant development i.e., flowering and milky stage showed significantly lower values of measured yield-related traits than the both control and irrigation treatment at doughy stage. There are some other studies (Pakniyat et al., 2013; Riasat et al., 2020; Saed-Moucheshi et al., 2021b; Sallam et al., 2019) that shared similar results on cereal related to irrigation method and period with the current study. However, some studies (Bano et al., 2021; Gundaraniya et al., 2020; You et al., 2019) showed that even low stress related to the susceptible plant can lead to a high negative impact on its productivity. Therefore, these results are indicating that triticale is a tolerant plant under drought stress condition.

In this study, grain yield of triticale and all other yield-related and morphological features significantly responded to the effects of environment, irrigation treatments, and genotypes as the main sources of variations in the combined ANOVA. This result is indicating the differences among triticale genotypes in response to altered environments, which is alluding to the proficiency of the screening programs for introducing high potential genotypes. Thus, the estimation of genetic parameters would help the breeders to screen the genotype more precisely.

The heritability of almost all measured feature, excluding straw yield and harvest index, was significant in this study. Also, there were some high PCV, and GCV regarding features in this study showing the capability of screening programs among the used genotypes. One of the goals of the current study was to find proper methods to reach out to high potential genotype to tolerate drought stress condition. Accordingly, the assessments genotypes with higher capability and potential according to their genetic condition and their responses to

environmental changes using the measured traits is important. According to the results, screening the genotypes only based on their grain yield might no be very satisfying for the following generation in breeding programs as a result of its heritability. Thus, the features which have high variability and heritability as well as association with grain yield can be exploited as selection indicators to enhance selection efficiency of breeding programs. Grzesiak et al. (2003), Moucheshi et al. (2011), and Soloklui et al. (2019) identified traits such as leaf water potential, as proper indicators to select high-yield triticale genotypes under stress condition. Thus far, there are many studies concerning the effect of environmental stresses on triticale plants, however, few studies have focused on the impacts of other features on grain yield and their capability of being used as indirect criteria. Consequently, our study showed the efficiency of principal component analysis in screening and breeding programs and also showing a combination of relationships within features, genotypes, and cross relationships between features and genotypes.

Our results showed that the relationship of plant height and harvest index with grain yield were negative. In addition, these features showed low GCV and PCV indicating that they are not suitable to be used as selection criteria in triticale breeding programs. On the other side, leaf angle showed a high positive association with grain yield and it also had sufficient heritability making it a suitable candidate for selection criteria. However, it has been verified that the plants are using the lower angles of their leaves to limit the evaporation of the water in their leaves (Saed-Moucheshi et al., 2021a). Therefore, this feature might not be a good choice for being used for screening genotypes of triticale under drought stress condition. The features related to spike of triticale plant i.e., spike weight and spike number in line with seed number per spike showed significant and positive relationships with grain yield. These features also showed high heritability among all measured features. Subsequently, the traits related to spike of triticale plants are important and suitable feature for being used as selection criteria in triticale breeding programs under drought or normal conditions. Riasat et al. (2019) and Saed-Moucheshi et al. (2019) obtained similar results related to yield component traits and the indirect selection in triticale with the current study. In accordance with our results regarding the importance of using the other features than yield solely for screening, the relationship of biochemical features and grain yield of triticale was modeled in the study of Saed-Moucheshi (2018), without estimation of their heritability and other genotypic parameters, and it showed that showed that MDH, proline contents and SOD were the important features with significant impacts on triticale grin yield. Similarly, Riasat et al. (2019) nominated SOD activity as the most appropriate trait to obtain higher tolerant triticale genotypes. In addition, there are several studies on different crops that are in concordance with our results regarding the importance of indirect selection and the selection criteria (Ahmad et al., 2010; Lotfi et al., 2010; Riasat et al., 2020; Tabarzad et al., 2017; Vosough et al., 2019; Zahedi et al., 2016). Zahedi et al. (2016) showed that SOD along with CAT and proline content were the effective biochemical traits toward the grain yield of barley genotypes and they introduced these traits as the candidate features capable of being used as indirect criteria in barely breeding. However, in the study of Vosough et al. (2019), SOD was not able to be selected as an important feature toward grain yield of wheat landraces.

The overall results showed that lower number of irrigation and earlier withholding the water from the triticale plants can lead to a high decrease in productivity of triticale genotypes. Consequently, irrigation treatments and water availability is a significant factor for determining the type of breeding programs. Also, some genotypes showed a high potential for being considered for releasing cultivars. Under normal irrigation ET-83-20 and under severe water deficit ET-85-04 showed better performance than other genotypes. Estimation of genotypic features such as heritability and coefficient of variation showed that there is a high possibility and potential of producing cultivars with high productivity under either normal or stressed conditions. Additionally, high

heritability and significant association with grain yield for some features such spike weight, spike number, and grain number suggest that they are suitable feature for indirect screening and selection criteria.

#### Competing interests and consent to publish

The authors claim that there is no conflict or competing of interests regarding any detail of the current study. All authors have read the final manuscript and consent to its publication.

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