CHILEAN JOURNAL
of AGRICULTURAL RESEARCH

RESEARCH ARTICLE

Morphological and physiological traits of triticale as affected by drought stress

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Received: 12 September 2022; Accepted: 9 November 2022; doi: 10.4067/S0718-58392023000200203

ABSTRACT

Triticale (×*Triticosecale* Wittm.) is a major forage crop of arid and semiarid areas in China with yields much constrained by drought stress. Genetic improvement could benefit from a better understanding of drought effects on plant morphology and physiology. In this study, the morphophysiological traits of four triticale genotypes (line C31, varieties 'Gannong No.1', 'Gannong No.2', and 'Shida No.1') were determined on days 7, 14, 21, 35, 49, and 63 for treatments of the normal irrigation and drought stress, respectively. Rainfall of the drought stress treatments was controlled during the study using an anti-rain shed. Our findings suggest that drought stress had significant effects on all morphophysiological traits except for the relative number of leaves (RL) and catalase activities (RCAT). Among four genotypes, the averaged relative plant height (RH) of 'Gannong No.2' (0.90) was the highest, the averaged relative number of tillers (RT) of 'Gannong No.1' (0.77) was the highest, the averaged relative aboveground biomass yields (RAB) (0.53), relative leaf water content (RRWC) (0.96), and peroxidase activity (RPOD) (0.92) of C31 were the highest, respectively, and the averaged RCAT (1.06) of 'Shida No.1' was the highest. The interaction between genotype and drought stress days was significant for all morphophysiological traits except for the RT and RL. A comprehensive evaluation revealed that C31 was the most drought-tolerant genotype under persistent drought stress. These findings may be useful for the breeding and identification of drought-tolerant germplasms in the arid and semi-arid regions.

Key words: Comprehensive evaluation, drought tolerance, morphophysiological traits, relative values, ×*Triticosecale*.

INTRODUCTION

Drought is a global problem influencing crop production and it is becoming progressively more serious because of recent climate changes. Drought affects more than 99 million hectares across developing nations and more than 60 million hectares across developed parts of the world (Ahmad et al., 2015). Thus, drought continues to be an important challenge for agricultural researchers and plant breeders.

Triticale (×*Triticosecale* Wittm.) is a synthetic amphiploid cereal that was derived from a cross between wheat (*Triticum aestivum* L.) and rye (*Secale cereale* L.) Accordingly, it combines the advantages of wheat (high grain quality) and rye (tolerance to abiotic stresses, including drought, soil toxic elements, and low nutrient availability) (Lonbani and Arzani, 2011). Hence, triticale has been cultivated as an alternative crop in unfavorable environments worldwide.

Drought affects crop morphological, physiological, and biochemical processes, resulting in inhibited growth. Morphophysiological parameters are highly heritable, strongly correlated with stress tolerance, and can be easily assessed (Lonbani and Arzani, 2011). Morphological parameters, such as plant height, number of tillers and leaves, and biomass, are considered to be important for evaluating crop drought tolerance. Plant height as well as the number of leaves and productive tillers per plant are considerably influenced by environmental conditions, and are adversely affected by drought stress. Cultivating triticale under water-deficit conditions leads to decreased total biomass (Campuzano et al., 2012). Additionally, physiological parameters are also commonly used for evaluating crop stress tolerance. Water potential has been used to quantitatively analyze the water status of plants. The relative water content of leaves is a good parameter because it can reflect the extent of the effect of drought stress on plants as well as the balance between the water supply and transpiration rate (Lonbani and Arzani, 2011). The relative water content of leaves is influenced by drought, but it usually remains high in drought-tolerant cultivars exposed to drought stress (Zhang et al., 2008). Plants under water stress conditions accumulate soluble sugars as part of an adaptive mechanism, implying the soluble sugar content may indicate the severity of drought stress (Huang et al., 2011). In plants, reactive oxygen radicals are scavenged by antioxidative enzymes, including superoxide dismutase, peroxidase, and catalase, to minimize the damage caused by drought (Wang et al., 2018). Physiological parameters, such as the relative water content, soluble sugar content, and superoxide dismutase, peroxidase, and catalase activities in leaves, are very useful for evaluating the drought tolerance of triticale (Li et al., 2018).

The mechanism underlying the drought tolerance of plants is complex and certain parameters may not accurately reflect plant drought tolerance. Membership function analyses have been widely conducted to identify and comprehensively evaluate drought tolerance in alfalfa (*Medicago sativa* L.) (Wei et al., 2005), wheat (Chen et al., 2012), maize (*Zea mays* L.) (Tian et al., 2014), and other crops, but few studies using membership function analysis of morphophysiological parameters to comprehensively evaluate triticale drought tolerance have been reported. The purposes of this research were (1) to compare the morphophysiological parameters (plant height, number of tillers and leaves, dry weight of aboveground and underground parts, relative water content, soluble sugar content, superoxide dismutase activity, peroxidase activity, and catalase activity) of triticale genotypes responded to different drought stress days; and (2) to evaluate the drought tolerance of triticale genotypes under persistent drought conditions via principal component and membership function analyses. The results of our study may serve as a theoretical basis for exploiting drought-tolerant triticale genotypes suitable for cultivation in arid and semi-arid regions.

MATERIALS AND METHODS

Experimental site and materials

A field experiment was conducted at the Forage Experimental Station of Gansu Agricultural University in northwestern China (36°03′76″ N, 103°53′24″ E). This location has an average altitude of 1560 m a.s.l., average annual rainfall of 349 mm, an average annual temperature of 7.9 °C, and a frost-free period of 153 d. The soil texture used was loamy sandy soil, and the soil type was chestnut soil in the Chinese classification system and calcic-orthic Aridisol in the US soil taxonomic classification system (Zhang et al., 2017). The soil mixture had the following properties: 2.3 g kg^{-1} organic matter; 90.05 mg kg^{-1} alkali-hydrolyzed N; 172.8 mg kg^{-1} available K; 0.45 dS m^{-1} electrical conductivity; and pH 7.35. Field moisture capacity was determined by the Ring Knife method (Guo, 2021) before the experiment began.

Four triticale (×*Triticosecale* Wittm.) genotypes, line C31, varieties ‘Gannong No.1’, ‘Gannong No.2’, and ‘Shida No.1’ were used as the experimental materials, among which, line C31, ‘Gannong No.1’, and ‘Gannong No.2’ were bred by the College of Grassland Science, Gansu Agricultural University, China, using the traditional sexual hybridization techniques and a pedigree selection method. ‘Shida No.1’ was selected as the drought-resistant control based on previous studies (Li et al., 2018).

Experimental design and method

The experiments were conducted in a split-split-plot, in which, years (2017, 2018) was the main plots, drought stress days (0, 7, 14, 21, 35, 49, and 63 d) was the sub plots, and the four triticale genotypes (line C31 and varieties ‘Gannong No.1’, ‘Gannong No.2’, and ‘Shida No.1’) was the sub sub-plots. Three replicates were designed. Four triticale genotypes were sown on 20 March 2017 and 21 March 2018 with two seeds per hill at 4 cm depth with hills 20 cm apart. The plot size was 2 × 2 m. Land was prepared by deep ploughing, discing and harrowing. A basal fertilizer dose of 150 kg N ha^{-1} in the form of diammonium phosphate was applied just before sowing, and a top dressing of 150 kg urea-N ha^{-1} was applied at the seedling stage before irrigation. The irrigation was imposed by flood irrigation during both years. After irrigation, weeds were removed and seedlings were manually thinned to leave one healthy seedling per hill and 100 seedlings per subplot. After the first irrigation during the seedling stage (30 d after sowing), the drought stress treatment was initiated. During the experiment, the drought-stressed plants were protected from rainfall by an anti-rain shed, which was closed during rainy conditions. Phenological stages were evaluated according to the Zadoks phenological scale (Zadoks et al., 1974). The developmental stages of triticale plants at drought stress days 0, 7, 14, 21, 35, 49, and 63 d were seedling, seedling, tillering, tillering, booting, heading, and flowering stage, respectively (Table 1). The control plants were irrigated normally three times (i.e., at the booting, heading, and flowering stages) according to normal field management practices for triticale cultivation.

Table 1. Ratio of the soil moisture content to the field moisture capacity during drought stress.

Drought stress days	Ratio of the soil moisture content to the field moisture capacity (%)	Zadoks scale	Phenological stage
0	80.01	1	Seedling
7	58.82	1	Seedling
14	34.38	2	Tillering
21	32.03	2	Tillering
35	23.65	4	Booting
49	22.09	5	Heading
63	20.13	6	Flowering

Parameter determination

For each triticale genotype, the morphological and physiological parameters of the drought stressed and normal irrigated (control) plants were both determined at 0, 7, 14, 21, 35, 49, and 63 d after the drought stress initiated. At each determination, 10 plants were selected in each plot according to the sowing order, plant height (cm) from the ground to the tip of plants at the normal growth condition, number of tillers and leaves fully unfolded for each plant were determined. Dig up the whole plant using a shovel, each with 200 × 500 × 300 mm soil, wash the soil attached to the plants with a 1% sodium hydroxide solution, dry with filter paper, and separate the above- and under-ground at the joint of stem and root. Then the above- and underground parts were respectively cut into 10 cm long pieces and oven-dried separately at 103 ℃ for 0.5 h and 65 ~ 70 ℃ for 6 h. And the dry weight of the above- and under-ground biomass was determined when the sample was cooled at the normal temperature. The soil moisture content (%) was determined by weighing the fresh weight of the soil samples in field, taking them to the lab, drying them at 105 °C for 48 h in the oven (Yeoh et al., 2017), and weighing to get the value. After finishing the morphological determination, physiological parameters, including the relative leaf water and soluble sugar content, and activities of superoxide dismutase, peroxidase and catalase, were determined according to the following methodologies: Leaf relative water content (%) by weighing method (Ritchie et al., 1990; Lonbani and Arzani, 2011), soluble sugar content (mg g^{-1}) by anthrone colorimetry (Yemm and Willis, 1954), superoxide dismutase activity (U min^{-1} g^{-1} FW) by nitroblue tetrazolium method (Mishra and Fridovich, 1972; Nahakpam and Shah, 2011), peroxidase activity (U min^{-1} g^{-1} FW) by guaiacol method (Egley et al., 1983; Nahakpam and Shah, 2011), and catalase activity (U min^{-1} g^{-1} FW) by ultraviolet absorption method (Beers and Sizer, 1952; Nahakpam and Shah, 2011). In each plot, 10 plants were regarded as 10 replicates, and mean of the determined results was regarded as the result of this plot.

Calculation of relative values

When evaluating plant drought tolerance, the use of relative values (RV, i.e., relative to the control) can eliminate inherent differences among crop varieties and accurately reflect the drought tolerance of cultivars (Wang et al., 2012). The RV in this experiment was calculated using the following formula:

$$RV_{ij} = X_{ijDS}/X_{ijNI} \qquad (1)$$

In this formula, RV_{ij} is the relative value of parameter j for triticale genotype i, whereas X_{ijDS} and X_{ijNI} are the values of parameter j of triticale genotype i determined under the drought stress treatment (DS) and control (normal irrigation, NI), respectively, at the same number of drought stress days (Szira et al., 2008; Chen et al., 2012).

Statistical analyses

All RV data were analyzed using Excel 2010 and Prism 8.0.2 (GraphPad Software, San Diego, California, USA). Three-factor ANOVA analysis was performed on the parameters including the relative plant height (RH), number of tillers (RT) and leaves (RL), aboveground biomass (RAB), underground biomass (RUB), relative water content (RRWC), soluble sugar content (RSS), superoxide dismutase activity (RSOD), peroxidase activity (RPOD), and catalase activity (RCAT), using SPSS Statistics 19.0 (IBM, Armonk, New York, USA). If significant differences existed, Duncan's multiple range test was used to compare the differences. While doing the multiple comparison, differences of the single factors (drought stress days, triticale genotypes) were compared in SPSS software automatically, and differences of the interaction between the drought stress days and triticale genotypes was compared by regarding them as a single factor because there is no available package in the SPSS Statistics 19.0. The ANOVA results showed that nonsignificant difference existed between years, so the 2-yr data were averaged and analyzed.

Principal component analysis

Principal component analysis aims to simplify the data set by reducing the geometric dimensions of the mean values, while maintaining the largest contribution to the variance of the data set. All potentially relevant parameters could be simplified to several linearly irrelevant variables through dimension reduction (Sun et al., 2018). In this experiment, the principal component analysis was used to simplify the data set of RH, RT, RL, RAB, RUB, RRWC, RSS, RSOD, RPOD, and RCAT of four triticale genotypes (line C31, varieties 'Gannong No.1', 'Gannong No.2', and 'Shida No.1') under the drought stress days 63. Principal component analysis was conducted using SPSS Statistics 19.0 and CANOCO 5.0 (Microcomputer Power, Ithaca, New York, USA) (Ringnér, 2008). The results were automatically obtained after inputting the above data into the package of principal component analysis in SPSS software.

Comprehensive evaluation of drought tolerance

Triticale drought tolerance was evaluated on the basis of membership function values. The membership function of a fuzzy set is a generalization of the indicator function in classical sets and represents the degree of truth as an extension of valuation (Chen et al., 2012). For any set X, a membership function on X is any function from X to the real unit interval (0, 1). Related parameters were calculated as previously described (Szira et al., 2008; Chen et al., 2012).

The subordinate function values of various comprehensive parameters for different triticale genotypes were calculated as follows:

$$F(RV_j) = (RV_j - RV_{min})/(RV_{max} - RV_{min}),\ (j = 1, 2, 3, ...) \qquad (2)$$

The weights of various comprehensive parameters were calculated as follows:

$$W_j = P_j / \sum_{j=1}^{n} P_j,\ (j = 1, 2, 3, ...) \qquad (3)$$

The comprehensive drought tolerance of different triticale genotypes was determined using the following equation:

$$D = \sum_{j=1}^{n} \left[F(RV_j) \times W_j\right],\ (j = 1, 2, 3, ...) \qquad (4)$$

In these equations, RV_j indicates the j^{th} comprehensive trait; RV_{min} and RV_{max} represent the minimum and maximum values of the j^{th} comprehensive trait, respectively; W_j indicates the importance (weight) of the j^{th} comprehensive trait considering all comprehensive traits; P_j indicates the contribution rate of the j^{th} comprehensive trait of various triticale genotypes; and D indicates the comprehensive drought tolerance of various triticale genotypes under drought stress conditions on the basis of the comprehensive traits. The drought tolerance level was graded as $0.8 \leq D \leq 1$, $0.6 \leq D < 0.8$, and $0.4 \leq D < 0.6$, respectively, for highly, moderately and slightly drought tolerant. The grade $0.2 \leq D < 0.4$ indicated relatively drought sensitive and $0 < D < 0.2$ indicated drought sensitive (Zhao et al., 2019).

RESULTS

The ANOVA revealed highly significant differences in the RH, RRWC, RPOD, and RCAT data among triticale genotypes ($P < 0.01$) as well as significant differences in the RT and RAB data ($P < 0.05$). Regarding the effects of the number of days of drought stress, the mean squares were highly significant ($P < 0.01$) for RH, RAB, RUB, RRWC, RSS, and RSOD and significant ($P < 0.05$) for RT and RPOD. In terms of the interaction between the triticale genotypes and the number of drought stress days, there were highly significant differences in the RH, RAB, RUB, RRWC, RSS, RPOD, and RCAT data ($P < 0.01$) and significant differences in the RSOD data ($P < 0.05$) (Table 2). For the above parameters, multiple comparison needs to be done to compare differences among triticale genotypes, the number of drought stress days, and the interaction between triticale genotypes and the number of drought stress days, respectively.

Table 2. Results of ANOVA of morphophysiological parameters of drought tolerant triticale genotypes to drought stress. RH: Relative plant height; RT: relative number of tillers; RL: relative number of leaves; RAB: relative aboveground biomass; RUB: relative underground biomass; RRWC: relative -water content; RSS: relative soluble sugar content; RSOD: relative superoxide dismutase activity; RPOD: relative peroxidase activity; RCAT: relative catalase activity. *, **Significant difference at 5% and 1% probability level, respectively.

		F-value									
Variance	df	RH	RT	RL	RAB	RUB	RRWC	RSS	RSOD	RPOD	RCAT
Genotype (G)	3	2.47^{**}	1.80^{*}	0.24	2.18^{*}	0.34	10.28^{**}	0.21	0.89	4.83^{**}	2.46^{**}
Drought stress days (DS)	6	7.51^{**}	2.87^{*}	0.70	98.76^{**}	13.79^{**}	11.64^{**}	11.19^{**}	24.90^{**}	5.34^{*}	0.52
G × DS	18	5.84^{**}	1.56	1.13	90.94^{**}	2.96^{**}	42.01^{**}	11.36^{**}	1.86^{*}	3.85^{**}	8.99^{**}

Table 3. Differences in the relative morphophysiological parameters among triticale genotypes. RH: Relative plant height, RT: relative number of tillers, RAB: relative aboveground biomass, RRWC: relative water content, RPOD: relative peroxidase activity, RCAT: relative catalase activity. Values of a parameter (mean ± SE) labeled with different letters differ significantly (ANOVA followed by Duncan's multiple-range tests, $P < 0.05$).

Triticale genotypes	RH	RT	RAB	RRWC	RPOD	RCAT
Gannong No.1	0.73 ± 0.061b	0.77 ± 0.078a	0.42 ± 0.084ab	0.92 ± 0.009a	0.79 ± 0.045b	0.99 ± 0.026ab
Gannong No.2	0.90 ± 0.062a	0.71 ± 0.069ab	0.51 ± 0.068ab	0.93 ± 0.019a	0.75 ± 0.044b	0.87 ± 0.035b
C31	0.77 ± 0.043ab	0.72 ± 0.052ab	0.53 ± 0.071a	0.96 ± 0.012a	0.92 ± 0.028a	0.89 ± 0.042b
Shida No.1	0.72 ± 0.033b	0.54 ± 0.087b	0.33 ± 0.073b	0.84 ± 0.022b	0.74 ± 0.036b	1.06 ± 0.086a

Differences in morphophysiological parameters among the triticale genotypes

Among the four analyzed triticale genotypes, the average RH of 'Gannong No.2' under different drought stress days was significantly higher than that of 'Gannong No.1' and the 'Shida No.1' (Table 3); RH was lowest for the 'Shida No.1'. The average RT of 'Gannong No.1' was significantly higher than that of the 'Shida No.1'. Moreover, there were nonsignificant differences in the average RT between C31 and 'Gannong No.2'. The average RAB, RRWC, and RPOD of C31 were higher than the corresponding values of the other genotypes, including the 'Shida No.1', although some of the differences were nonsignificant. The average RCATs of 'Gannong No.2' and C31 were significantly lower than that of the 'Shida No.1', whereas the average RCAT of 'Gannong No.1' was only slightly lower than that of the 'Shida No.1'.

Differences of triticale genotypes among the number of drought stress days

As the number of drought stress days increased, the average RH and RT of the triticale genotypes decreased, with the exception of RH on drought stress days 14 and 35 as well as RT on drought stress day 35 (Figure 1A). Both RH and RT were lowest when the drought stress day 63. The average RAB and RUB of the four triticale genotypes also decreased as the drought stress days increased, with the exception of RAB on drought stress day 7 and RUB on drought stress days 14 and 35 (Figure 1B).

With increasing drought stress days, the average RRWC of the triticale genotypes decreased and was significantly lower on day 63 than on any other day, except for day 49. In response to the drought stress treatment, the average RSS of the triticale genotypes exhibited an inverted U-shaped trend. There were nonsignificant differences in the average RSS from day 7 to day 35. However, the average RSS subsequently decreased significantly, reaching its lowest level on day 63 (Figure 1C). The average RSOD of the triticale genotypes exhibited an M-shaped trend following the drought stress treatment, peaking on day 14. In contrast, the average RPOD was highest on day 7 and lowest on day 63 (Figure 1D).

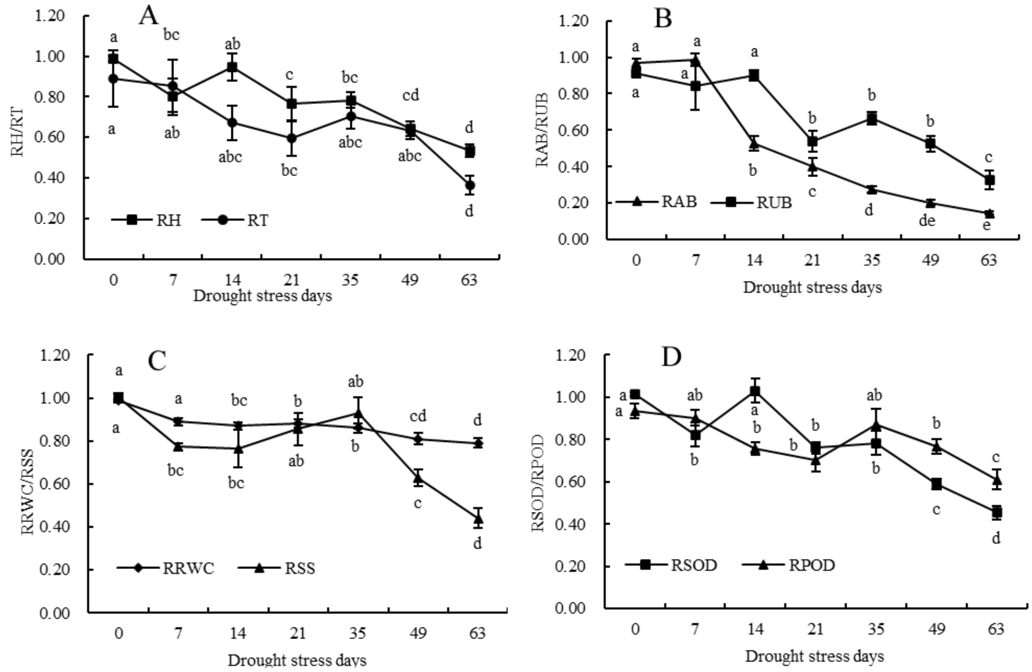

Figure 1. Difference of morpho-physiological parameters among the drought stress days. Relative plant height (RH) and number of tillers (RT) (A); relative aboveground biomass (RAB) and underground biomass (RUB) (B); relative leaf water content (RRWC) and soluble sugar content (RSS) (C); relative superoxide dismutase (RSOD) and peroxidase activity (RPOD) (D). Values are means ± SE. In columns with at least one common letter, there is nonsignificant difference in the level of 0.05 according to the Duncan test.

Differences of the interaction between triticale genotypes and number of drought stress days

RH: Under normal irrigation conditions (day 0), there were nonsignificant differences in RH (Figure 2A). Between day 7 and day 35, RH was higher for ‘Gannong No.2’ than for the other genotypes, including the ‘Shida No.1’, although some of the differences were nonsignificant. With increasing drought stress days, the differences in the RH among the four triticale genotypes decreased.

RAB: On day 0 (normal irrigation), RAB was significantly higher for ‘Gannong No.1’, ‘Gannong No.2’, and C31 than for the ‘Shida No.1’ (Figure 2B). On days 7 and 14, the RAB of ‘Gannong No.1’ was the highest, and significantly higher than the RAB of ‘Shida No.1’. When the drought stress was prolonged to 21 d, RAB was the highest for C31. On days 35 and 49, RAB was significantly higher for ‘Gannong No.2’ than for the ‘Shida No.1’. As the drought treatment period progressed, RAB decreased for all four triticale genotypes.

RUB: On day 7, RUB was significantly higher for C31 than for the ‘Shida No.1’ and the other genotypes (Figure 2C). Additionally, RUB decreased for all four triticale genotypes as the duration of the drought treatment period increased.

RRWC: On day 7, RRWC was significantly higher for C31 than for ‘Gannong No.1’, ‘Gannong No.2’, and the ‘Shida No.1’ (Figure 3A). On days 14 and 21, ‘Gannong No.2’ RRWC was highest, and significantly higher than the ‘Gannong No.1’ and ‘Shida No.1’ RRWCs. On days 35 and 63, the C31 RRWC was significantly higher than the ‘Shida No.1’ RRWC.

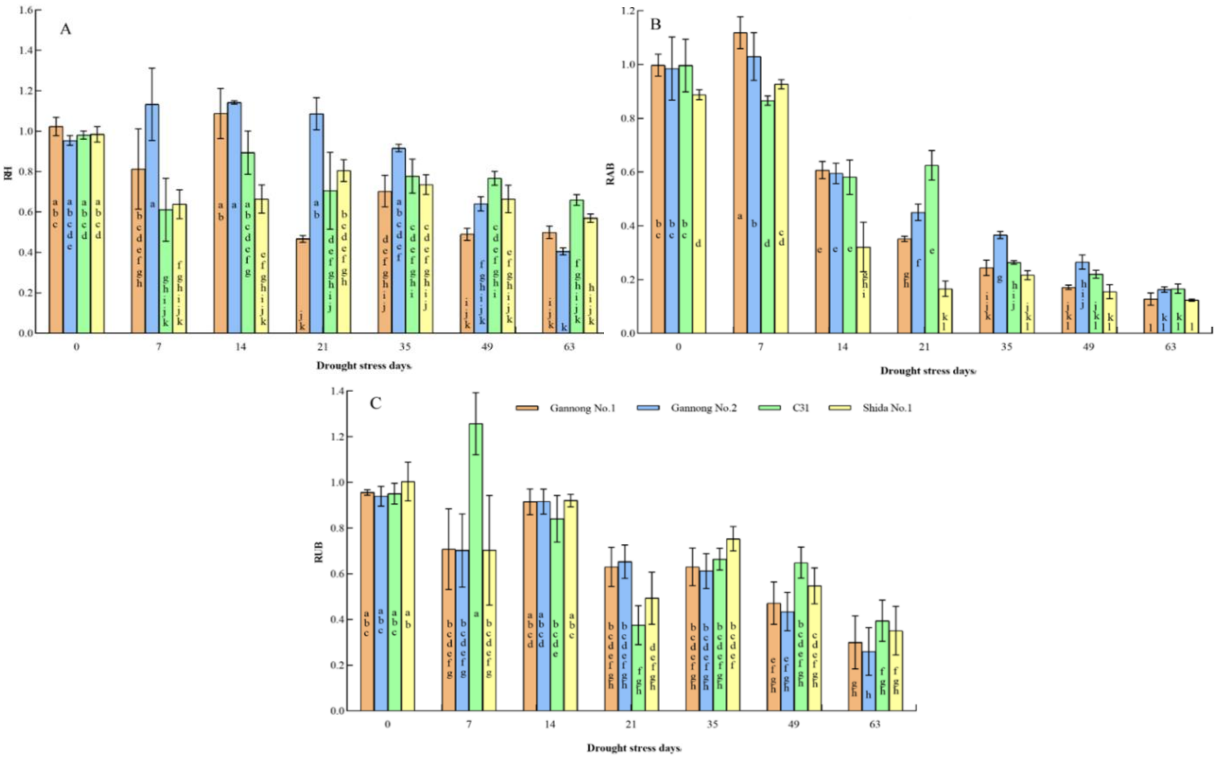

Figure 2. Differences of the relative plant height (RH) (A), relative aboveground biomass (RAB) (B), and relative underground biomass (RUB) (C) in triticale for the interaction of triticale genotypes and drought stress days. Values are means ± SE. In columns with at least one common letter, there is nonsignificant difference in the level of 0.05 according to the Duncan test.

RSS: When plants were normally irrigated (day 0), there were nonsignificant differences in RSS among the four triticale genotypes (Figure 3B). On day 7, the C31 RSS was highest, and significantly higher than the 'Gannong No.2' RSS. On day 14, RSS was significantly higher for 'Gannong No.2' than for the other genotypes. On day 21, RSS was significantly higher for 'Gannong No.1' than for the other genotypes and the 'Shida No.1'. On days 35 and 63, the 'Shida No.1 RSS was highest, and significantly higher than 'Gannong No.2' RSS.

RSOD: On days 7 and 14, RSOD was significantly higher for the 'Shida No.1' than for 'Gannong No.1' and 'Gannong No.2'. On day 21, RSOD was significantly higher for 'Gannong No.1' than for C31 (Figure 3C). On days 35 and 63, RSOD was significantly higher for C31 than for the other genotypes.

RPOD: On days 0 and 7, RPOD was significantly lower for 'Shida No.1' than for the other triticale genotypes (Figure 3D). On day 14, C31 RPOD was highest, and significantly higher than 'Gannong No.1' and 'Shida No.1' RPODs. When the drought stress was prolonged to 21 d, 'Gannong No.1' RPOD was the highest, and significantly higher than RPOD for the other genotypes and 'Shida No.1'. On days 35 and 63, C31 RPOD was significantly higher than RPOD of the other genotypes and 'Shida No.1', except on day 35.

RCAT: There were nonsignificant differences in RCAT among the four genotypes from day 0 to day 35. However, on days 49 and 63, 'Gannong No.2' and C31 RCATs were significantly lower than 'Shida No.1' RCAT. In contrast, 'Gannong No.1' RCAT was only slightly lower than 'Shida No.1' RCAT (Figure 3E).

Comprehensive evaluation of drought tolerance of triticale genotypes

Principal component analysis. Principal component analysis of 10 morphophysiological parameters of triticale genotypes under the drought stress days 63 indicated that the contribution rates of the first three comprehensive parameters (i.e., CI(1)-CI(3)) were 54.994%, 29.724%, and 15.282%, respectively (Table 4). The cumulative contribution rate was 100.00%. Thus, the original 10 parameters were transformed into three new independent comprehensive parameters. The first principal component mainly included RH, RUB, and RPOD; the second principal component mainly included RAB and RCAT; and the third principal component mainly included RT and RRWC. It can be seen from Figure 4 that the different triticale genotypes can respond and adapt to drought stress through the induction of different morphological and physiological responses.

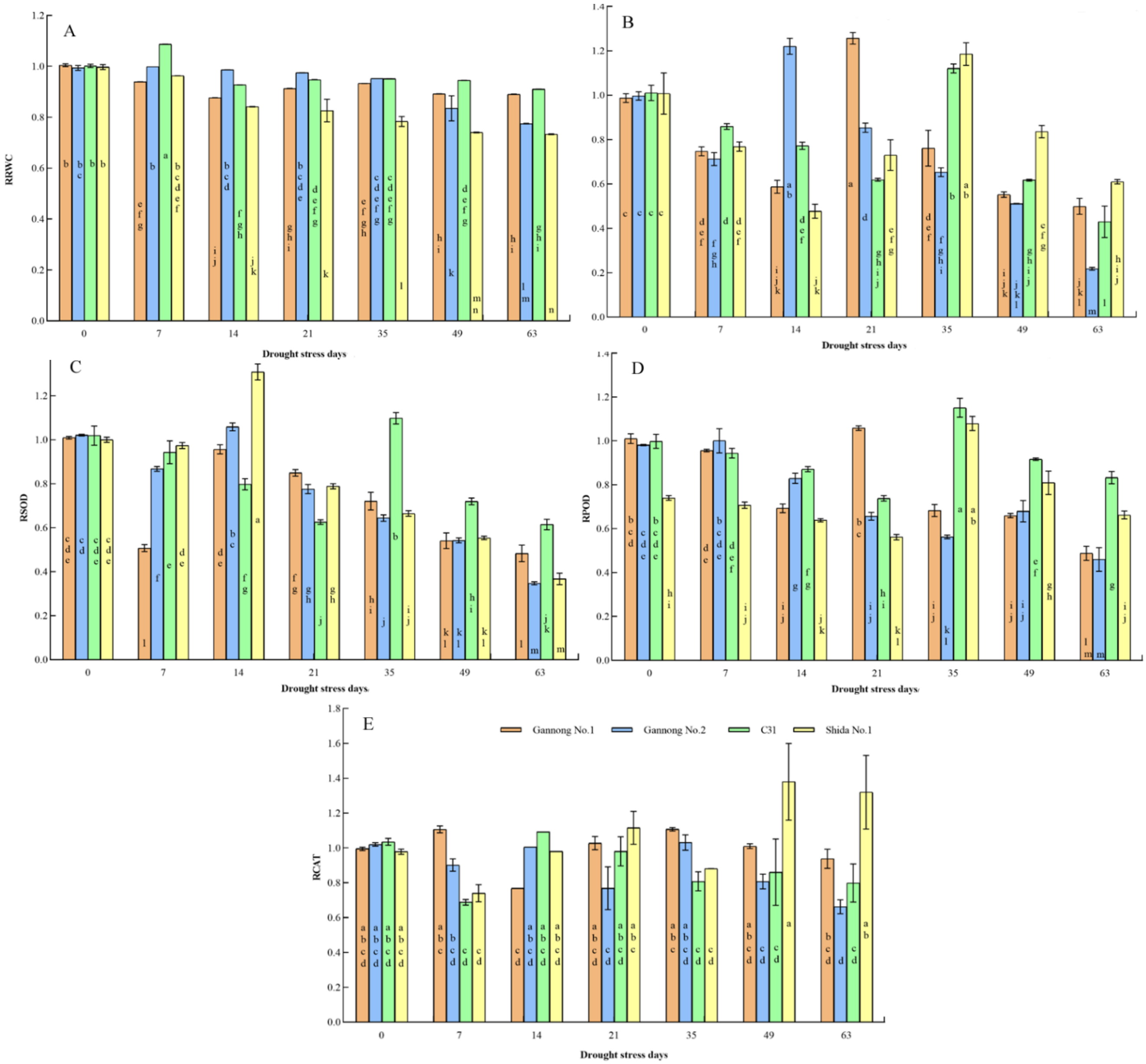

Figure 3. Differences of the relative leaf water content (RRWC) (A), relative soluble sugar content (RSS) (B) relative superoxide dismutase activity (RSOD) (C), relative peroxidase activity (RPOD) (D) and relative catalase activity (RCAT) (E) in triticale for the interaction of triticale genotypes and drought stress days. Values are means ± SE. In columns with at least one common letter, there is nonsignificant difference in the level of 0.05 according to the Duncan test.

Table 4. Coefficients of comprehensive parameters and contribution proportions of triticale genotypes. RH: Relative plant height, RT: relative number of tillers, RL: relative number of leaves, RAB: relative aboveground biomass, RUB: relative underground biomass, RRWC: relative water content, RSS: relative soluble sugar content, RSOD: relative superoxide dismutase activity, RPOD: relative peroxidase activity, RCAT: relative catalase activity.

Variable	Principle factors (63 d)		
	CI(1)	CI(2)	CI(3)
RH	0.990	-0.014	-0.139
RT	0.763	-0.057	0.643
RL	0.882	0.049	-0.470
RAB	-0.021	0.920	-0.392
RUB	0.971	-0.031	-0.237
RRWC	0.513	0.598	0.616
RSS	0.609	-0.772	0.184
RSOD	0.805	0.512	0.299
RPOD	0.910	0.130	-0.393
RCAT	0.324	-0.942	-0.088
Eigen values	5.499	2.972	1.528
Contribution, %	54.994	29.724	15.282
Cumulative contribution, %	54.994	84.718	100.000

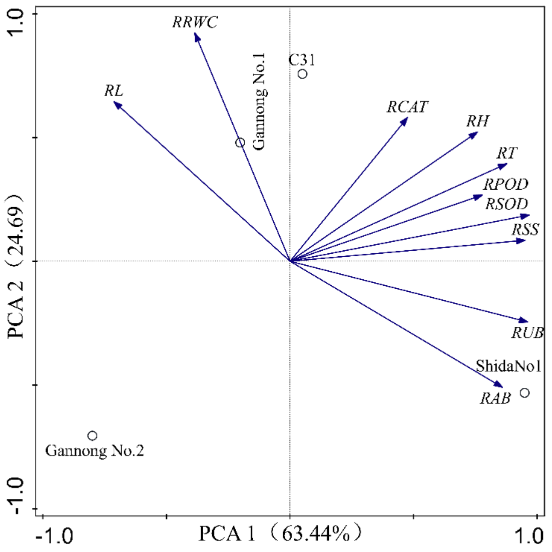

Figure 4. Principal component analysis on the relationship of triticale genotypes and parameters of morphological and physiological. RH: Relative plant height; RT: relative number of tillers; RL: relative number of leaves; RAB: relative aboveground biomass; RUB: relative underground biomass; RRWC: relative -water content; RSS: relative soluble sugar content; RSOD: relative superoxide dismutase activity; RPOD: relative peroxidase activity; RCAT: relative catalase activity.

Membership function analysis. The membership function values of various comprehensive parameters were calculated for each genotype using Equation 2. For the same comprehensive parameters (e.g., CI(1)), F(1) was the highest for C31 (1.000), implying that C31 was the most drought-tolerant genotype in terms of CI (1). In contrast, F(1) was the lowest for ‘Gannong No.2’ (0.000), suggesting that this variety was the least drought-tolerant genotype in terms of CI(1) (Table 5).

Table 5. Comprehensive parameter CI(x), weight parameter, membership function F(i), and D value for the comprehensive evaluation of the drought tolerance of four triticale genotypes under persistent drought conditions (63 d).

Triticale genotypes	CI(1)	CI(2)	CI(3)	F(1)	F(2)	F(3)	D values	Rank	Drought-tolerance classification
Gannong No.1	-0.787	0.605	1.125	0.030	0.772	1.000	0.399	3	Relatively drought sensitive
Gannong No.2	-0.850	-1.003	-0.722	0.000	0.000	0.112	0.017	4	Drought sensitive
C31	1.218	-0.680	0.511	1.000	0.155	0.724	0.707	1	Moderately drought tolerant
Shida No.1	0.419	1.079	-0.953	0.613	1.000	0.000	0.635	2	Moderately drought tolerant
Weight				0.550	0.297	0.153			

Weight determination. On the basis of the contribution rates of the various comprehensive parameters, the weights of the comprehensive parameters were calculated using Equation 3. On day 63, the weights of three comprehensive parameters (i.e., F(1)-F(3)) were 0.550, 0.297, and 0.153, respectively (Table 5).

The comprehensive drought tolerance of four triticale genotypes was calculated using Equation 4. In this study, the order of the drought tolerance of the four triticale genotypes on drought stress days 63 was C31 > ‘Shida No.1’ > ‘Gannong No.1’ > ‘Gannong No.2’, more specifically, C31 and ‘Shida No.1’ belongs to the moderately tolerant type, ‘Gannong No.1’ belongs to the relatively sensitive type, and ‘Gannong No.2’ belongs to the sensitive type (Table 5).

DISCUSSION

Drought tolerance of crops was a quantitative genetic trait controlled by multiple genes (Byzova et al., 2004; Wang et al., 2021) and must be assessed using several related parameters (Ozturk et al., 2019). Previous studies have shown that determined values play an important role in evaluating drought tolerance (Lonbani and Arzani, 2011; Saed-Moucheshi et al., 2019). For example, plant breeding programs mainly focus on the selection of high-yielding genotypes under conditions of yield potential (non-stress), followed by the selection of high-yielding genotypes under stress conditions (Blum, 2005). However, the relative value (drought stressed data divided by that of the normal irrigated) was always used to eliminate the inherent differences among different varieties to accurately reflect the drought tolerance of genotypes (Wang et al., 2012). So, in this study, relative values were used to evaluate the drought tolerance of different triticale genotypes. And on the basis of our previous studies (Li et al., 2018; Zhang, 2018), 10 morphological and physiological parameters were selected. The results of our study should be of vital importance for the selection of triticale genotypes for drought-prone areas.

Differences in morphophysiological parameters among triticale genotypes

Substantial biomass reductions have been observed in crops due to lowered water supply, even for a short period of time (Czyczyło-Mysza and Myśków, 2017). Rice (*Oryza sativa* L.) cultivar with good drought tolerance possessed relatively higher biomass accumulation under drought conditions (Ma et al., 2016). Productivities of drought-tolerant rye lines under drought stress conditions, as measured by biomass, were higher than those of drought-sensitive genotypes (Czyczylo-Mysza and Mysków, 2017). In this study, triticale line C31 obtained the highest RAB, and higher values in RH and RT while drought stressed for 63 d. These results were in accordance with which obtained from the actual determined results (unpublished data), which showed that triticale line C31 had the highest values in plant height, number of tillers per plant, and dry weight of the aboveground biomass while drought stressed for 63 d. So, this line can be used to breed new variety to serve for the agriculture and livestock industry.

The higher RWC level in the plants during drought stress indicates that the plants could limit the water loss and maintain a higher RWC, resulting in enhanced drought tolerance (Zhang et al., 2008). Khan et al. (2019)

observed a decline in RWC in chickpea (*Cicer arietinum* L.) due to drought stress and detected the highest RWC in the tolerant genotype. In our study, C31 had the highest average RRWC, which indicates that this genotype is able to sustain a higher RWC and maintain protoplast hydration for a longer span under drought stress conditions to ensure productivity (Khan et al., 2019). Antioxidant defense systems, which include antioxidant enzymes such as peroxidase, superoxide dismutase, and catalase, have been regarded as the most important defense system to protect the plants from oxidative stress by eliminating the accumulation of ROS (Wang et al., 2018). In the present investigation, 'Shida No.1' had the highest average RCAT, which indicates that 'Shida No.1' can efficiently alleviate the damaging effects of oxidative stress and adapt to drought stress (Wang et al., 2018).

Difference of morphophysiological parameters within the drought stress days

Drought stress inhibits plant growth by affecting the morphological, physiological, and biochemical processes. Previous studies have shown that the actual plant height of strawberry varieties decreased with the delay of drought stress time (Nezhadahmadi et al., 2015). In this study, the averaged relative plant height of the four examined triticale genotypes also decreased in response to increasing drought stress days (Figure 1A), which indicated that the drought stress had a major influence on plant height after eliminating the inherent differences among triticale genotypes. Regarding the number of tillers, the average RT of the four triticale genotypes was not significantly affected during the first 49 d of drought stress, but it decreased significantly on day 63 (Figure 1A). This result demonstrates that drought stress has adverse effects on the number of tillers, however, it also largely depends upon the duration of drought stress. Drought stress also adversely affects the biomass of plants (Campuzano et al., 2012). In the present study, increases in drought stress resulted in greater decreases in the biomass of the aboveground and underground plant parts (Figure 1B).

The relative water content has been used to evaluate the dehydration tolerance of plants. Earlier investigations demonstrated that the relative water content is lower for plants receiving limited irrigation than for sufficiently watered plants (Zhang et al., 2008). In this study, the average RRWC of four triticale genotypes decreased as the drought stress days increased (Figure 1C), likely because the cell membranes were damaged, leading to decreased water retention. Plants cope with various stresses via osmotic regulation and increases in the soluble sugar content. In the current study, the average RSS of four triticale genotypes increased from day 7 to day 35 of the drought treatment period, but it subsequently decreased (Figure 1C). Under drought conditions for 35 d, the triticale genotypes were likely actively accumulating soluble sugars and other substances in cells to decrease the osmotic potential and enhance water retention. This would help plants maintain normal metabolic activities and developmental processes, thereby increasing drought tolerance. However, the exposure to drought stress for 49 d disrupted plant metabolic activities, leading to decreased soluble sugar accumulation and accelerated degradation (Huang et al., 2011).

Superoxide dismutase, peroxidase, and catalase are key enzymes that function as scavengers of reactive oxygen species (Wang et al., 2018). Wang et al. (2012) proved that the abundance of antioxidative enzymes in *Brassica napus* plants increases in response to drought stress. In the current study, the changes in the average RSOD and RPOD in the examined triticale genotypes over the course of the drought treatment period were inconsistent with the reported results for alfalfa. This discrepancy between studies may reflect the diversity in how plant species respond to drought stress as well as the differences in the watering regimens in the two investigations.

Differences considering interaction between triticale genotypes and drought stress days

In terms of the interaction between triticale genotypes and number of drought stress days, we observed that drought stress days had variable effects on morphophysiological parameters of the four analyzed triticale genotypes. For example, on day 7, RH and RPOD were highest for 'Gannong No.2', whereas RAB and RCAT were highest for 'Gannong No.1'. Following an exposure to drought stress for 14 d, RH, RUB, RRWC, and RSS were highest for 'Gannong No.2', whereas RPOD and RCAT were highest for C31 and RAB was highest for 'Gannong No.1'. On day 63, RH, RAB, RUB, RRWC, RSOD, and RPOD were highest for C31, whereas RSS and RCAT were highest for the 'Shida No.1'.

The above-mentioned results demonstrate that plant drought tolerance is mediated by multiple factors. Moreover, the morphological and physiological changes induced by drought may vary depending on the onset of drought stress (e.g., rapid or gradual), drought stress day, and the plant developmental stage (e.g., tillering, stalk elongation, and maturity).

Comprehensive evaluation of drought tolerance of triticale genotypes

Principal component analyses can transform many individual parameters into relatively few comprehensive parameters, which are then used to calculate the D value according to membership functions to accurately reflect the drought tolerance of the tested varieties (Sun et al., 2018). In this study, a principal component analysis transformed 10 morphophysiological parameters of triticale genotypes under persistent drought conditions (63 d) into different independent comprehensive parameters (Table 4). More specifically, the original 10 parameters were transformed into three new independent comprehensive parameters. The calculated D values revealed that C31 was the most drought-tolerant genotype under persistent drought conditions.

The results of this study imply that different drought-tolerant triticale genotypes may initiate diverse defense mechanisms in response to varying levels of drought stress. The initiation of these distinct defense mechanisms might be associated with the differences in the physiological and molecular mechanisms underlying the adaptive responses of different varieties to drought stress. Therefore, the relationship between physiological parameters and the molecular mechanism regulating the varying characteristics of different drought-tolerant triticale genotypes will need to be clarified.

CONCLUSIONS

Crop drought tolerance is due to many factors and must be assessed using several related parameters. Our results showed that drought stress had significant effects on physiological and morphological traits of triticale. Among four genotypes, the averaged relative plant height of ‘Gannong No.2’ was the highest, the averaged relative number of tillers of ‘Gannong No.1’ was the highest, the averaged relative aboveground biomass yields, relative leaf water content, and peroxidase activity of genotype C31 were the highest, and the averaged relative catalase activity of ‘Shida No.1’ was the highest, respectively. A comprehensive evaluation revealed that C31 was the most drought-tolerant genotype under persistent drought conditions.

Author contributions

Conceptualization: D.Y., S.Y-Z. Methodology: D.Y., S.Y-Z. Software: D.Y. Validation: W.H-D., X.H-T. Formal analysis: D.Y., S.Y-Z. Investigation: S.Y-Z. Resources: S.Y-Z. Data curation: D.Y., S.Y-Z. Writing-Original Draft: D.Y., S.Y-Z. Writing-Review & Editing: W.H-D., D.Y. Visualization: W.H-D., X.H-T. Supervision: W.H-D., X.H-T. Project administration: W.H-D. Funding acquisition: W.H-D. All co-authors reviewed the final version and approved the manuscript before submission.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (32260339), Key R&D Program of Gansu province (20YF8NA129), and Breeding of Rye and Triticale Lines and Studies of their Seed Production Techniques (XZ202101ZD003N).

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