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Evaluation of drought resistance and index screening of foxtail millet cultivars

Xin Zhao*, Mingjing Huang, Xuefang Huang and Enke Liu
Shanixi Institute of Organic Dryland Farming, Shanxi Agricultural University, Taiyuan, Shanxi 030031, China
*Corresponding author. E-mail: interkiller@126.com

ABSTRACT

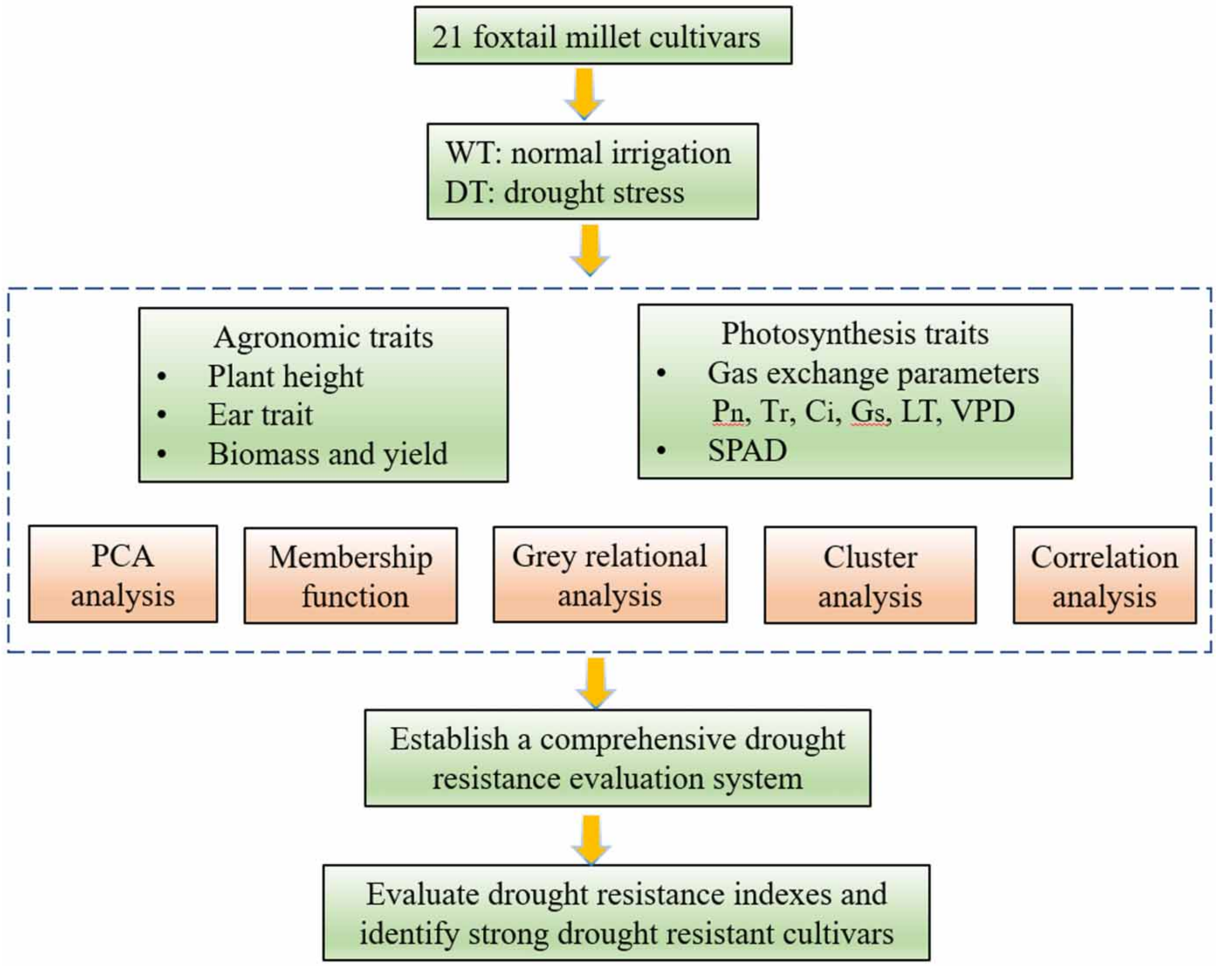
Screening for identification indicators and establishing a drought-resistance evaluation system can provide a basis for the selection and layout of drought-resistant foxtail millet cultivars. Twenty-one main foxtail millet cultivars in China were evaluated for drought resistance by measuring their main agronomic traits and photosynthetic indicators under the condition of drought stress and normal irrigation. Identifying the drought resistance is difficult when using a single index. The order of drought resistance of tested foxtail millet cultivars based on drought resistance comprehensive evaluation values (D value) and drought resistance index (DRI value) differed to an extent; therefore, a comprehensive evaluation method combining D and DRI values was appropriate and accurate. Gray correlation analysis showed that the grain weight per ear, yield, ear weight, and ear length could be used as indicators for drought-resistance evaluation of foxtail millet cultivars at the mature stage. According to the clustering analysis based on D and DRI values, the tested cultivars were divided into three grades of drought resistance and six foxtail millet cultivars with strong drought resistance were identified. The photosynthetic indicators showed that the net photosynthetic and transpiration rates and D and DRI values had correlation coefficients greater than 0.320.

Key words: agronomic indices, drought resistance index, drought stress, foxtail millet, gray correlation analysis, photosynthetic indices

HIGHLIGHTS

- Evaluation of drought resistance of foxtail millet based on D and DRI values was reliable.
- P_n and T_r could be used as reference indicators to evaluate the drought resistance.
- Drought seriously affected the most agronomic indices and several photosynthetic parameters.
- Using rainproof shed method with artificial water control can better predict the drought resistance of crop.

GRAPHICAL ABSTRACT



1. INTRODUCTION

Drought is one of the main restrictors of food production. With the intensification of global warming, drought problems have become more serious and posed a significant challenge to global food security (Davidson 2016). Arid and semiarid regions account for 52.5% of the total area of cultivated land in China. North China, as the main grain production area in the country, faces a serious water shortage problem. Foxtail millet (*Setaria italic* L.) is a crop that is mainly planted on dry and barren land in North China, because of its high water use efficiency, drought resistance, and barren tolerance (Sachdev 2021; Sun *et al.* 2021). However, not all foxtail millet varieties are highly drought tolerant. Therefore, to further explore drought-resistant varieties through the drought-resistance evaluation of germplasm resources is of great significance.

The main difficulty in the study of crop drought resistance is accurately identifying drought resistance and screening drought resistance indicators, which requires analysis of different indicators during different periods (Kamoshita *et al.* 2008). Researchers in recent years have proposed a variety of identification methods for drought resistance in different crops and studied these indicators in terms of morphology, physiology, and biochemistry (Albacete *et al.* 2014; Zhang *et al.* 2018). Identifying drought resistance at the germination, seedling, and mature stages is the main method for identifying drought resistance in foxtail millet germplasm resources (Cattivelli *et al.* 2008). By studying the drought resistance during different periods, researchers believed that the results of drought resistance identification at the mature stage of foxtail millet were relatively reliable (Zhang *et al.* 2010). In recent years, research on drought-resistant genes and proteins has accurately analyzed and identified the drought-resistance evaluation (Ghatak *et al.* 2016; Parvathi *et al.* 2019); however, it is difficult to apply on a large scale because of the high cost of detection. To avoid the one-sidedness and instability of a single index in the process of drought-resistance evaluation, researchers have used a combination of principal component, membership function, cluster, and stepwise regression analyses to carry out the evaluation of multiple indicators (Wang *et al.* 2017; Xiao *et al.* 2021, 2022).

However, the drought resistance of crops should ultimately be reflected in the yield. The previous evaluation method could not reflect the absolute yield of foxtail millet under drought conditions (Wang *et al.* 2017; Xiao *et al.* 2021, 2022); furthermore, previous evaluation methods usually lacked the research on physiological indicators. Therefore, further evaluating the yield of foxtail millet under dry land conditions in combination with the drought resistance index (DRI) method and analyzing the relationship between physiological traits and drought-resistant characteristics using a comprehensive evaluation method was necessary.

In addition, the present evaluation of foxtail millet drought resistance at the mature period is mostly conducted under natural conditions in the field (Zhang *et al.* 2012; Shah *et al.* 2020; Aberkane *et al.* 2021), which are greatly affected by different rainfall years; therefore, the research results may not be representative. Most studies on the drought resistance of foxtail millet focused on the germination and seedling stages (Dai *et al.* 2016), and the research at the mature stage was not systematic.

In this study, normal irrigation and drought stress treatments were set under artificial water control conditions by the rainproof shed. The agronomic and photosynthetic indices of 21 foxtail millet cultivars were utilized, and agronomic traits, yield, and photosynthetic traits were analyzed. The aims of the present study were to (1) investigate the effects of drought agronomic traits and photosynthesis traits, (2) to establish a comprehensive drought-resistance evaluation method, and (3) evaluate drought resistance indices and identify strong drought-resistant cultivars.

2. MATERIALS AND METHODS

2.1. Plant material

Twenty-one foxtail millet cultivars were tested, all of which were the main cultivars in China (Table 1).

2.2. Experimental designs

The experiment was conducted during the 2019 and 2020 seasons using the field simulation drought method with rainproof shed at Dongyang Experimental Station in China (112 °45′E, 37 °40′N). The area had a semi-humid continental monsoon climate with an annual average temperature of 9.7 °C, annual average precipitation of 445.5 mm, annual sunshine of 2,662.2 h, and annual frost-free period of 158.9 days. The soil in the field was classified as Calcisol with pH of 8.32.

The experiment used a random block design with three replicates at two irrigation levels. The treatments were as follows: (1) normal irrigation treatment (WT): soil water content of 65–75% (soil field capacity) during the growth period; and (2) drought stress treatment (DT): soil water content of 40–50% (soil field capacity) during the growth period. The soil water content was measured by neutron probe. Each plot was separated by concrete and had an area of 6.0 m^2 (3 m × 2 m).

Table 1 | Information of 21 foxtail millet cultivars

Code	Name	Origin	Code	Name	Origin
C1	Datong 34	Shanxi	C12	Qinhuang 2	Henan
C2	Changnong 47	Shanxi	C13	An-04	Henan
C3	Changsheng 07	Shanxi	C14	Jigu 41	Hebei
C4	Jingu 59	Shanxi	C15	Zhangza 13	Hebei
C5	Dungu	Shanxi	C16	Huangjinmiao	Hebei
C6	Changsheng 13	Shanxi	C17	Jinmiao K2	Inner Mongolia
C7	Jingu 21	Shanxi	C18	Gonggu 88	Jilin
C8	Honggu	Shanxi	C19	Jiugu 23	Jilin
C9	Zhonggu 2	Henan	C20	Jigu 22	Shandong
C10	Yugu 35	Henan	C21	Nenxuan 18	Heilongjiang
C11	Yugu 1	Henan			

2.3. Measuring items

At the mature stage, agronomic traits, including plant height (PH), grain weight per ear (GWE), ear weight (EW), ear length (EL), ear diameter (ED), kernel ratio (KR, EW/GWE), biomass (BM), and yield (Y) were recorded.

At the flowering stage, five representative plants were chosen from each plot to measure the photosynthetic parameters of the flag leaf at 9:00–11:00 pm under sunny conditions. Six gas exchange parameters, including transpiration rate (T_r), net photosynthesis rate (P_n), intercellular CO_2 concentration (C_i), stomatal conductance (G_s), leaf temperature (LT), and vapor pressure deficit (VPD), were measured by photosynthetic instrument (LI-6800, USA); relative chlorophyll content (SPAD) was measured by SPAD-502 Chlorophyll Meter.

2.4. Statistical analysis

The experimental data were analyzed using Excel 2013 software (Microsoft Corp., USA). Statistical analysis of the data was conducted using SPSS 18.0 software (SPSS Institute Inc., USA). The average values of all indices in 2019 and 2020 were used as basic data. A paired treatment *t*-test was used to determine the significance of the average difference between the measured values of each index. Drought resistance coefficient (DC) and DRI were calculated using Equations (1) and (2), respectively (Lan 1998; Liu *et al.* 2015).

X_i is the value of each indicator under DT treatment; CK_i is the value of each indicator under WT treatment; Y_a is the grain yield per square meter under DT treatment; Y_m is the grain yield per square meter under WT treatment; Y_M is the average grain yield per square meter of all cultivars under WT treatment; Y_A is the average grain yield per square meter of all cultivars under DT treatment.

$$\mathrm{DC} = \frac{X_i}{CK_i} \tag{1}$$

$$\mathrm{DRI} = \frac{(Y_a)^2}{Y_m} \times \frac{Y_M}{(Y_A)^2} \tag{2}$$

Correlation, frequency distribution, and principal component analyses were performed for the DC value of each indicator. According to Equations (3)–(5), the factor weight coefficient (ω_i), membership function value of each index of each genotype [$\mu(x_i)$] and drought resistance comprehensive evaluation value (D value) were calculated and analyzed (Xangsayasane *et al.* 2014; Bo *et al.* 2017).

P_i is the contribution rate of the *i*-th indicator, indicating the importance of the *i*-th indicator in all indicators; x_i is the value of the *i*-th indicator; $x_{i\max}$ is the maximum value of the *i*-th indicator; $x_{i\min}$ is the minimum value of the *i*-th indicator.

$$\omega_i = P_i \div \sum_{i=1}^{n} P_i \tag{3}$$

$$\mu(x_i) = \frac{x_i - x_{i\min}}{x_{i\max} - x_{i\min}} \tag{4}$$

$$D = \sum_{i=1}^{n} [\mu(x_i) \times \omega_i] \tag{5}$$

Based on the gray relational analysis (Wang *et al.* 2019), the correlation between the DC value of each index and the D and DRI values was analyzed. Finally, according to the D value and DRI value, the weighted pair group method average (WPGMA) was used for cluster analysis to classify the drought resistance level.

3. RESULTS

3.1. Representativeness and measured value of agronomic indices

Drought stress had a significant effect on the agronomic indices of foxtail millet cultivars (Table 2). The coefficient of variation (CV) of the agronomic indices among the different cultivars was between 4.4 and 25.4%, indicating that the tested foxtail millet cultivars were representative. The DT had significant effects for all cultivars, and the agronomic indices were sensitive to drought stress. In addition, the correlation coefficients of the measured values of the foxtail millet cultivars

Table 2 | Mean values of agronomic indices of foxtail millet cultivars

Item	PH (cm)		EL (cm)		ED (cm)		EW (g)		GWE (g)		KR		BM (t/ha)		Y (t/ha)	
	WT	DT	WT	DT	WT	DT	WT	DT	WT	DT	WT	DT	WT	DT	WT	DT
Average	142.7	110.8	23.71	16.79	2.64	1.88	18.8	14.8	15.2	11.4	0.812	0.771	17.52	13.65	6.83	5.04
Max	179.7	146.6	36.77	27.57	3.80	2.83	22.7	17.0	18.8	13.5	0.883	0.837	23.91	16.67	8.09	6.54
Min	93.2	62.6	17.30	10.39	1.94	1.26	12.6	11.4	10.4	8.8	0.731	0.679	12.30	10.86	4.88	4.02
CV (%)	16.1	17.7	18.2	22.7	15.8	25.4	12.0	10.4	12.0	11.8	4.4	6.1	16.9	12.4	12.8	15.0
t-value	23.494		30.313		29.636		44.085		59.548		10.262		27.059		50.006	
P-value	0.0001		0.0001		0.0001		0.0001		0.0001		0.003		0.0001		0.0001	
r	0.595		0.646		0.641		0.716		0.767		0.429		0.627		0.738	

Data are the mean across 2019 and 2020; WT, normal water treatment; DT, drought stress treatment; PH, plant height; EL, ear length; ED, ear diameter; EW, ear weight; GWE, grain weight per ear; KR, kernel ratio; BM, biomass; Y, yield; *r*, correlation coefficient.

under the WT and DT treatment ranged from 0.429 to 0.767, which further indicate that the sensitivity to drought stress differed for each index. Therefore, identifying the drought resistance of foxtail millet cultivars is difficult when using a single index.

3.2. DC analysis for agronomic indices

Compared with the normal irrigation treatment, the agronomic indices of different foxtail millet cultivars changed significantly under DT treatment (Table 3). The DC values of cultivars for different indicators were clearly different, with CV ranging from 5.587 to 17.279%. However, the DC values reflected different drought resistances among different cultivars, and the DC values of each index for the same cultivar also differed, indicating that the sensitivity of each index to drought stress is different.

In addition, the distribution times and frequencies of the DC values of the different indices in the same range varied greatly (Table 4). The distribution frequencies of PH, EL, ED, EW, GWE, KR, BM, and Y in the range of DC > 0.8 were 33.3, 14.3, 28.6, 52.4, 42.9, 100.0, 33.3, and 33.3%, respectively, indicating that EL and ED were more sensitive to drought stress. However, the superposition effect of the indices makes evaluating the drought resistance of cultivars difficult when using a single index.

Correlation analysis revealed various significant correlations were found among the indices (Table 5). Y was significantly and positively correlated with EW ($P < 0.01$), GWE ($P < 0.01$), BM ($P < 0.01$), PH ($P < 0.05$), EL ($P < 0.05$), and KR ($P < 0.05$).

3.3. Principal component analysis

Principal component analysis (PCA) was conducted on the DC values of the agronomic indices (Table 6). The first four principal components accounted for 87.73% of data variance. Therefore, PC1, PC2, PC3, and PC4 can accurately reflect differences in the drought resistance of foxtail millet cultivars. PC1 was highly correlated with GWE and Y, PC2 was highly correlated with ED, PC3 was highly correlated with BM and EW, and PC4 was highly correlated with the KR.

3.4. Comprehensive evaluation of drought resistance

Judging drought resistance using a single index is difficult because of the inconsistent order of the drought resistance coefficients for different indicators and cultivars. Therefore, evaluating the drought resistance of cultivars by analyzing the comprehensive evaluation value (D value) according to the weight coefficient of each index is necessary. The D value of the tested cultivars ranged from 0.177 to 0.886, with an average value of 0.562 and a CV of 37.3%, respectively. The drought resistance of the 21 tested foxtail millet cultivars was ranked according to the D value (Table 7). The cultivars with strong drought resistance were Zhangza 13, Yugu 1, Zhonggu 2, Honggu, and Jiugu 23, and those with weak drought resistance were An-04, Changnong 47, and Jigu 22.

However, the D and DC values did not reflect the high-yield character of cultivars under drought stress. Therefore, the DRI based on yield character should also be used to evaluate cultivars to compensate for the limitations of the D value method.

Table 3 | Drought resistance coefficients of agronomic indices in foxtail millet cultivar

Code	PH	EL	ED	EW	GWE	KR	BM	Y
C1	0.754	0.705	0.767	0.811	0.734	0.905	0.685	0.704
C2	0.704	0.561	0.634	0.651	0.596	0.916	0.686	0.610
C3	0.717	0.674	0.743	0.696	0.659	0.946	0.697	0.617
C4	0.751	0.721	0.715	0.643	0.604	0.939	0.927	0.866
C5	0.672	0.697	0.824	0.776	0.731	0.942	0.735	0.660
C6	0.821	0.676	0.715	0.806	0.697	0.865	0.723	0.702
C7	0.694	0.666	0.772	0.716	0.634	0.885	0.648	0.643
C8	0.758	0.750	0.874	0.902	0.850	0.943	0.774	0.823
C9	0.773	0.807	0.909	0.801	0.808	1.008	0.883	0.800
C10	0.876	0.658	0.842	0.792	0.746	0.942	0.798	0.712
C11	0.744	0.810	0.879	0.923	0.857	0.929	0.838	0.785
C12	0.786	0.790	0.658	0.879	0.861	0.980	0.888	0.751
C13	0.721	0.637	0.560	0.657	0.669	1.019	0.710	0.602
C14	0.788	0.667	0.517	0.712	0.702	0.986	0.904	0.721
C15	0.827	0.765	0.865	0.904	0.904	0.987	0.764	0.888
C16	0.923	0.828	0.629	0.798	0.755	0.946	0.795	0.719
C17	0.879	0.652	0.595	0.827	0.870	1.051	0.831	0.881
C18	0.790	0.658	0.561	0.919	0.858	0.934	0.716	0.897
C19	0.812	0.773	0.695	0.846	0.848	1.002	0.898	0.927
C20	0.669	0.643	0.541	0.746	0.617	0.827	0.673	0.570
C21	0.854	0.677	0.638	0.900	0.880	0.977	0.777	0.757
Average	0.777	0.705	0.711	0.796	0.756	0.949	0.779	0.744
CV (%)	8.995	9.842	17.279	11.437	13.529	5.587	11.040	14.405

PH, plant height; EL, ear length; ED, ear diameter; EW, ear weight; GWE, grain weight per ear; KR, kernel ratio; BM, biomass; Y, yield; C1～C21, code of foxtail millet cultivars, see Table 1.

Table 4 | Frequency distribution of drought resistance coefficients of agronomic indices in foxtail millet cultivars

	0 < DC < 0.4		0.4 < DC < 0.6		0.6 < DC < 0.8		0.8 < DC < 1.0		DC > 1.0	
Index	Times	Freq. (%)	Times	Freq. (%)	Times	Freq. (%)	Times	Freq. (%)	Times	Freq. (%)
PH	0	0	0	0	14	66.7	7	33.3	0	0
EL	0	0	1	4.8	17	81.0	3	14.3	0	0
ED	0	0	5	23.8	10	47.6	6	28.6	0	0
EW	0	0	0	0	10	47.6	11	52.4	0	0
GWE	0	0	1	4.8	11	52.4	9	42.9	0	0
KR	0	0	0	0	0	0	17	81.0	4	19.0
BM	0	0	0	0	14	66.7	7	33.3	0	0
Y	0	0	1	4.8	13	61.9	7	33.3	0	0

PH, plant height; EL, ear length; ED, ear diameter; EW, ear weight; GWE, grain weight per ear; KR, kernel ratio; BM, biomass; Y, yield.

The DRI values of the 21 tested cultivars ranged from 0.649 to 1.569, with an average value of 1.005 and a CV of 27.1%. The tested cultivars were ranked according to their DRI value; the cultivars with strong drought resistance were Gonggu 88, Jiugu 23, Zhonggu 2, Jinmiao K2, and Yugu 1, whereas those cultivars with weak drought resistance were Changnong 47, An-04,

Table 5 | Pearson correlation among drought resistance coefficients of agronomic indices in foxtail millet cultivars

Index	PH	EL	ED	EW	GWE	KR	BM	Y
PH	1							
EL	0.323	1						
ED	−0.062	0.487*	1					
EW	0.442*	0.521*	0.303	1				
GWE	0.553**	0.543*	0.254	0.915**	1			
KR	0.429	0.241	−0.028	0.149	0.533*	1		
BM	0.419	0.555**	0.073	0.174	0.38	0.575**	1	
Y	0.518*	0.479*	0.198	0.595**	0.717**	0.495*	0.618**	1

* and ** indicate significant correlation at $P < 0.05$ and $P < 0.01$, respectively; PH, plant height; EL, ear length; ED, ear diameter; EW, ear weight; GWE, grain weight per ear; KR, kernel ratio; BM, biomass; Y, yield.

Table 6 | Principal components analysis of agronomic indices in foxtail millet cultivar

	Factor loading			
Index	PC1	PC2	PC3	PC4
PH	0.335	−0.281	−0.291	−0.480
EL	0.360	0.289	0.368	−0.396
ED	0.159	0.630	0.394	0.271
EW	0.380	0.334	−0.468	0.005
GWE	0.449	0.102	−0.326	0.266
KR	0.305	−0.459	0.182	0.636
BM	0.338	−0.318	0.516	−0.246
Y	0.424	−0.084	−0.009	0.066
Characteristic root	4.033	1.410	1.008	0.567
Contribution rate (%)	50.42	17.62	12.60	7.09
Cumulative contribution (%)	50.42	68.04	80.64	87.73
Factor weight	0.575	0.201	0.144	0.081

PH, plant height; EL, ear length; ED, ear diameter; EW, ear weight; GWE, grain weight per ear; KR, kernel ratio; BM, biomass; Y, yield.

and Jigu 22. This result was different from that of drought resistance identification based on the D value, but the overall trend was similar.

3.5. Gray relational analysis

Gray relational analysis showed that the degree of correlation between the DC value of each index and the D value in turn was ranked from highest to lowest as Y, GWE, EW, EL, ED, BM, PH, and KR. The correlation degree between the DC value of each index and the DRI value was, ranked from highest to lowest, Y, GWE, EW, EL, BM, KR, PH, and ED (Table 8).

3.6. Cluster analysis

Twenty-one cultivars were clustered into three groups according to their D and DRI values (Figure 1). Group I was recognized as a strong drought-resistant cultivar and consisted of six cultivars, namely Zhonggu 2, Yugu 1, Zhangza 13, Jinmiao K2, Gonggu 88, and Jiugu 23, accounting for 28.6% of all cultivars. The D and DRI values of these six cultivars are in the top 10 among all tested cultivars. Group II consisted of nine medium drought-resistant cultivars, accounting for 42.9% of all cultivars. Group III consisted of six weakly drought-resistant cultivars, accounting for 28.6% of all cultivars.

The drought-resistance evaluation indicators of the tested cultivars were statistically analyzed according to the drought resistance level classification of the foxtail millet cultivars (Table 9). The membership function values of most indices, DRI

Table 7 | Evaluation results of membership functions and DRI in foxtail millet cultivars

	Membership function value							
Code	μ1	μ2	μ3	μ4	D value	Order	DRI value	Order
C1	0.450	0.787	0.431	0.713	0.537	13	0.807	15
C2	0.002	0.356	0.511	0.748	0.207	20	0.692	19
C3	0.225	0.603	0.631	0.802	0.406	16	0.784	16
C4	0.515	0.292	1.000	0.355	0.527	14	0.966	10
C5	0.403	0.824	0.657	1.000	0.573	10	0.943	12
C6	0.423	0.642	0.375	0.361	0.456	15	0.998	8
C7	0.163	0.770	0.576	0.757	0.393	17	0.769	17
C8	0.836	0.948	0.501	0.934	0.819	4	0.885	14
C9	0.875	0.787	0.885	0.851	0.858	3	1.379	3
C10	0.607	0.642	0.545	0.624	0.607	9	0.962	11
C11	0.891	1.000	0.615	0.752	0.863	2	1.311	5
C12	0.845	0.493	0.488	0.519	0.698	6	1.092	7
C13	0.169	0.175	0.506	0.835	0.273	19	0.676	20
C14	0.472	0.000	0.581	0.364	0.384	18	0.742	18
C15	1.000	0.856	0.419	0.968	0.886	1	1.184	6
C16	0.703	0.406	0.445	0.000	0.550	12	0.911	13
C17	0.869	0.133	0.252	0.798	0.627	7	1.321	4
C18	0.738	0.436	0.000	0.723	0.571	11	1.569	1
C19	0.980	0.422	0.558	0.625	0.779	5	1.469	2
C20	0.000	0.496	0.342	0.349	0.177	21	0.649	21
C21	0.772	0.446	0.147	0.686	0.610	8	0.986	9
Average	–	–	–	–	0.562	–	1.005	–
CV (%)	–	–	–	–	37.29	–	27.11	–

Table 8 | Correlation degree between DC value of all indices and D value together with DRI value in tested foxtail millet cultivars

Index	Correlation degree of DC with D	Rank	Correlation degree of DC with DRI	Rank
PH	0.469	7	0.426	7
EL	0.642	3	0.495	5
ED	0.532	5	0.352	8
EW	0.700	2	0.667	3
GWE	0.769	1	0.750	2
KR	0.413	8	0.440	6
BM	0.502	6	0.497	4
Y	0.631	4	0.835	1

PH, plant height; EL, ear length; ED, ear diameter; EW, ear weight; GWE, grain weight per ear; KR, kernel ratio; BM, biomass; Y, yield.

value, and D value decreased with an increase in the drought resistance level. The difference in both the D and DRI values at different drought resistance levels was large, which provides a basis for the classification of drought resistance levels among foxtail millet cultivar.

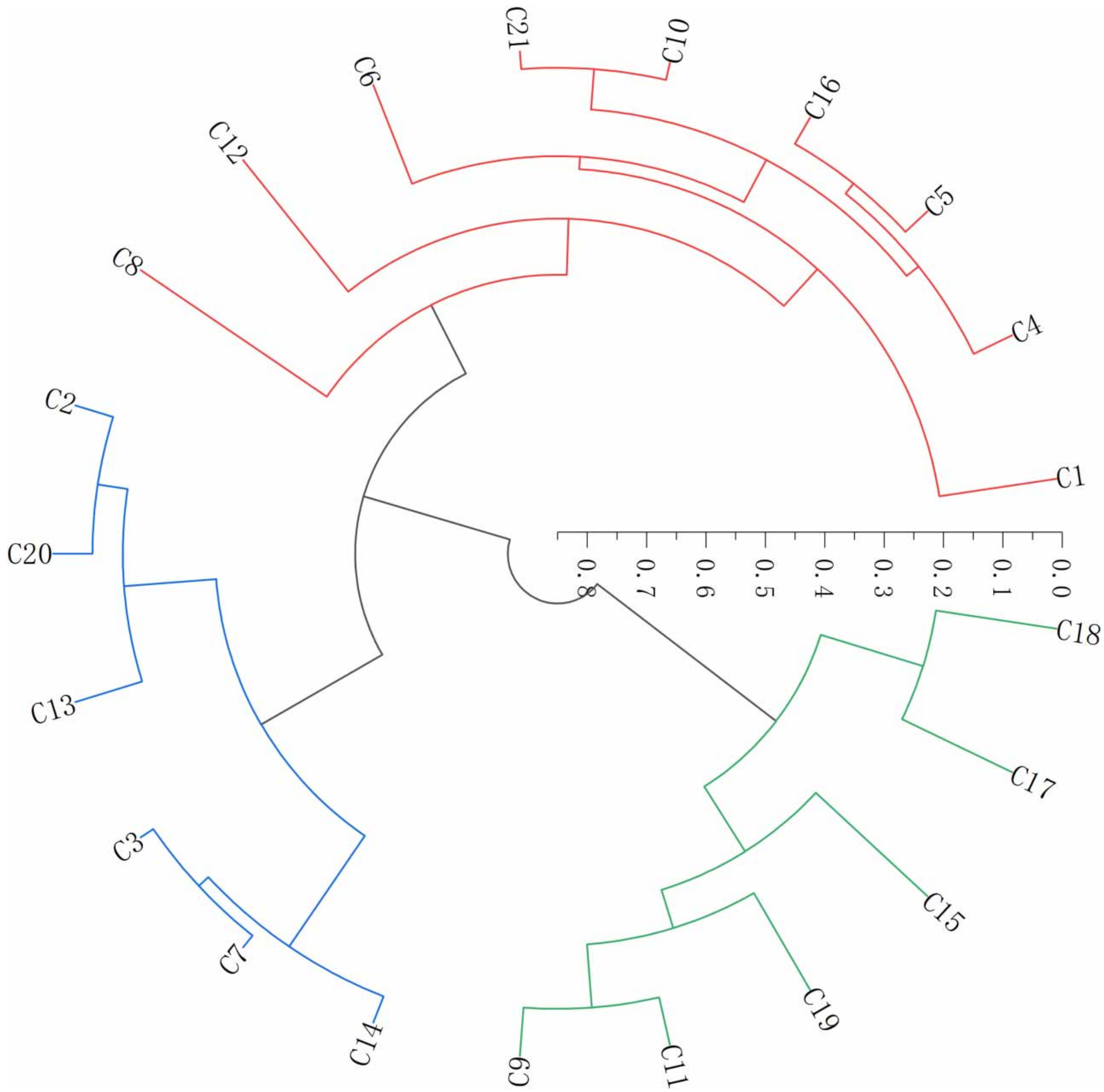


Figure 1 | Cluster diagram of each foxtail millet cultivars based on D value and DRI value.

Table 9 | Classification of drought-resistance evaluation indices in tested foxtail millet cultivar

	Membership function value		
Index	**1**	**2**	**3**
PH	0.532	0.514	0.183
EL	0.687	0.605	0.301
ED	0.596	0.568	0.282
EW	0.812	0.604	0.190
GWE	0.847	0.539	0.163
KR	0.705	0.493	0.458
BM	0.623	0.506	0.257
Y	0.822	0.487	0.161
D value	0.886	0.554	0.183
DRI value	0.854	0.363	0.084

PH, plant height; EL, ear length; ED, ear diameter; EW, ear weight; GWE, grain weight per ear; KR, kernel ratio; BM, biomass; Y, yield.

3.7. DC analysis of photosynthetic indices

A comparison of the average drought resistance coefficients of the photosynthetic indices for the tested cultivars (Table 10) showed that the drought resistance coefficients of T_r, P_n, C_i, and G_s were all lower than 0.9 under drought stress, indicating that they were greatly affected by drought stress. However, the T, VPD, and SPAD of most cultivars were approximately 1.000, indicating that drought stress had a relatively low impact on these three indices. In comparing the different drought resistance types (according to the cluster analysis), the drought resistance coefficients of C_i, T, VPD, and SPAD showed no significant differences. The P_n, T_r, and G_s of the different drought-resistant cultivars decreased with the weakening of drought resistance. The DC of the strong drought-resistant cultivars had a P_n that was significantly higher than those of the medium and weakly drought-resistant cultivars ($P < 0.05$), and the drought resistance coefficients of T_r and G_s of the strong and medium drought resistance cultivars were significantly higher than those of the weakly drought resistance cultivars ($P < 0.05$).

The correlation analysis between the DRI value and the photosynthetic indices showed a positive correlation with all indices except for VPD (Table 11). DRI was positively correlated with P_n ($P < 0.01$) and T_r ($P < 0.05$), while the correlations between G_s and SPAD and DRI values were not significantly but relatively high (0.231 and 0.212, respectively). Correlation analysis between the D value and photosynthetic indices showed a positive correlation for all indices. The D value was significantly positively correlated with P_n ($P < 0.05$), and the correlation coefficients of T_r and VPD with the D values were 0.322 and 0.229, respectively. The analysis of variance and correlation showed that P_n and T_r could be used as reference indices to evaluate the drought resistance of foxtail millet cultivars.

Table 10 | Drought resistance coefficients of photosynthesis indices in different types of foxtail millet cultivars

Type	T_r	P_n	C_i	G_s	LT	VPD	SPAD
SDC	0.879[a]	0.924[a]	0.787	0.861[a]	0.997	1.041	0.998
MDC	0.867[a]	0.879[ab]	0.781	0.851[a]	0.995	1.052	0.963
WDC	0.836[b]	0.834[b]	0.804	0.815[b]	0.988	1.011	0.975
Average	0.861	0.879	0.789	0.843	0.994	1.037	0.977
CV (%)	12.727	18.018	21.755	14.094	1.390	6.541	5.967

Different letters in each column indicate significant differences between different type of cultivars ($P < 0.05$).
SDC, strong drought-resistant cultivar; MDC, medium drought-resistant cultivar; WDC, weak drought-resistant cultivar; T_r, transpiration rate; P_n, net photosynthetic rate; C_i, Intercellular CO_2 concentration; G_s, stomatal conductance; LT, leaf temperature; VPD, vapor pressure deficit; SPAD, relative chlorophyll content.

Table 11 | Correlation coefficients among drought resistance coefficients of all photosynthetic indices with D value and DRI value

Index	T_r	P_n	C_i	G_s	T	VPD	SPAD	D value	DRI value
T_r	1								
P_n	0.444*	1							
C_i	0.723**	0.148	1						
G_s	0.878**	0.471*	0.770**	1					
LT	−0.008	−0.035	−0.323	−0.164	1				
VPD	−0.059	−0.197	−0.356	−0.440*	0.459*	1			
SPAD	0.054	0.166	0.208	0.051	0.08	−0.007	1		
D value	0.322	0.423*	0.077	0.164	0.129	0.229	0.178	1	
DRI value	0.438*	0.567**	0.104	0.231	0.116	−0.038	0.212	0.879**	1

* and ** indicate significant correlation at $P < 0.05$ and $P < 0.01$, respectively.
T_r, transpiration rate; P_n, net photosynthetic rate; C_i, Intercellular CO_2 concentration; G_s, stomatal conductance; LT, leaf temperature; VPD, vapor pressure deficit; SPAD, relative chlorophyll content.

4. DISCUSSION

4.1. Selection of drought-resistance evaluation methods

Crop drought resistance is a reflection of multiple drought resistance characteristics, which cannot be reflected in the results obtained using a single index for identification. The membership function method (D value) was used to evaluate drought resistance based on the comprehensive crop indices (Zou *et al.* 2020). This method emphasizes the absolute drought resistance of materials, which has significance in basic research on drought resistance breeding but does not reflect the high-yield character of cultivars under drought stress. The DRI method measures crop drought resistance based on the crop yield performance under drought stress and normal water condition (Blum 2005), in order to meet production needs and compensate for the limitations of the DC method.

Zhang *et al.* (2010) utilized the DRI method to identify the drought resistance of foxtail millet and analyzed the relationship between certain indices and DRI values. However, this study did not consider the importance of each index in the evaluation determined by the index variation coefficient. Some researchers believed that multiple agronomic traits combined with the D value can be used as an evaluation parameter to identify the drought tolerance of foxtail millet effectively and accurately (Lapuimakuni *et al.* 2018; Xiao *et al.* 2021, 2022), but it cannot precisely evaluate the yield under drought stress conditions.

In this study, the order of drought-resistant foxtail millet cultivars based on D and DRI values differed to a certain extent, and the DRI value can be used as a supplement to the D value to optimize the drought-resistance evaluation. The correlation analysis showed that the degree of correlation between the D value and yield was lower than that of the DRI value. Some cultivars have higher D values and lower DRI values, which indicates that the drought resistance based on several indices is good, but the yield under drought stress is not necessarily high. Combining the D and DRI values for system cluster analysis of drought resistance on the tested cultivars not only considers the importance of each index but also considers the production demand; therefore, the evaluation results are objective and reliable.

4.2. Selection of drought resistance indicators

Drought resistance in crops is a complex quantitative trait that involves multiple mechanisms and factors. Therefore, screening for suitable indicators is key for determining the drought resistance. Drought resistance in foxtail millet is a concentrated expression of plant morphology and yield after a series of adaptive changes in morphological structure, physiology, and biochemistry of cells under drought stress (Kamoshita *et al.* 2008; Song *et al.* 2017; Gupta *et al.* 2020). Generally, growth and yield-related indices are reliable indicators of drought resistance. According to the study on drought resistance of foxtail millet, yield character is most sensitive to drought stress (Xiao *et al.* 2021). Previous studies also revealed that the effect of drought stress on the physiological and biochemical indicators of wheat was greater than that on agronomic indicators; however, the physiological and biochemical indices were greatly affected by the test period (Chernyad'ev & Monakhova 2003; Subrahmanyam *et al.* 2006).

In this study, drought stress affected agronomic and photosynthetic indices to different degrees, with more significant effects on agronomic indices. The range of drought resistance coefficients of agronomic indices was 0.705–0.796 (except for KR), whereas those of photosynthetic indices were all above 0.800 (except for Ci). However, foxtail millet indices were affected by drought stress to different degrees, with certain correlations between each index. Therefore, evaluating the drought resistance of cultivars objectively and accurately using these indicators is difficult. Gray correlation analysis showed that the four traits with a higher degree of correlation with the D value were GWE, Y, EW, and EL. Unlike wheat and other crops (Subrahmanyam *et al.* 2006; Cattivelli *et al.* 2008), the PH and ED of foxtail millet showed a weak correlation with drought resistance and were not suitable to be used as the key indices for screening of drought-resistant cultivars. In addition, variance analysis and correlation analysis showed that the P_n and T_r could also be used as reference indicators to evaluate the drought resistance of foxtail millet. Therefore, in addition to using agronomic traits as screening indicators of drought resistance, further research must focus on analyzing physiological and biochemical indicators. However, these are unstable and difficult to control during the measurement period and are easily affected by factors such as growth environment and period (Reddy *et al.* 2004). Research on physiological, biochemical, and molecular mechanisms (metabolomics and transcriptomics) must be strengthened in the further drought-resistance evaluation (Mishra *et al.* 2012; Shah *et al.* 2020).

5. CONCLUSION

Drought stress had a significant effect on most agronomic indices and some photosynthetic indices of foxtail millet cultivars at a mature stage. The combination of D and DRI values was determined to be an appropriate drought resistance identification index. Foxtail millet cultivars with strong drought resistance at a mature stage were selected. The GWE, Y, EW, and EL of foxtail millet can be used as simple and intuitive drought-resistance evaluation indicators. P_n and T_r could also be used as reference indicators to further determine the drought resistance of foxtail millet.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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