CHILEANJAR
CHILEAN JOURNAL of AGRICULTURAL RESEARCH

RESEARCH

Principal component analysis and comprehensive evaluation on drought tolerance difference of canola cultivars at germination and emergence stages

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Received: 6 April 2021; Accepted: 15 July 2021; doi:10.4067/S0718-58392021000400557

ABSTRACT

Drought stress in autumn is an important factor restricting canola (*Brassica napus* L.) germination and emergence in the Yangtze River basin in China. Screening drought tolerance varieties in canola is a cost-effective and practical way. The objective of this study was to explore the methods of drought tolerance evaluation and to screen canola varieties with drought tolerance at germination and emergence stages. The experiments were conducted with 53 cultivars under drought treatment and non-stress control. In this research, 16 investigated indicators were synthesized into six independent indices which could represent 87.5% characters information related to drought resistance of canola varieties. With this analysis and evaluation, 53 varieties could be classified into three categories: five varieties as YY10, JLYT, LY9, ZS11, and YY50 owned drought-resistant; 16 varieties as ZY589, QZ7 and DAZ600 and so on, were moderately drought-resistant; and the other 32 varieties belonged to non-drought-resistant. The study proved that there were six indices as mean germination time, germination index, seedling emergence time, fresh and dry weight per plant and 1000-grains weight, which could be used as identifying indicators related to crop drought resistance at germination and seedling stage.

Key words: Canola, drought resistance, germination, principal component analysis, seedling.

INTRODUCTION

Southwest China is an important winter canola (*Brassica napus* L.) planting area located in the upper reaches of the Yangtze River (Yin and Wang, 2012). Although the annual precipitation is high during winter canola growing season in this region, the precipitation periods are uneven which have led to seasonal drought frequently (Aksenova et al., 1994; Bai et al., 2014). Seasonal rainfall shortage is a main factor limiting the plant growing and seedling establishment for autumn sowing canola, and canola yield is considerably influenced in drought autumn which commonly coincided with the canola sowing time in this region (Li et al., 2015; Channaoui et al., 2019). In addition, during canola seedling stage, seasonal drought would lead to seedling emerging unevenly, slow emergence of leaves, reduction of green leaf area, which seriously affects harvesting yield and seed quality (Xie et al., 2013; Bai et al., 2014; Li et al., 2015; Jian et al., 2016). Seed germination and successful seedling establishment are two important prerequisites for yield determination (Xie et al., 2013; Bai et al., 2014). Hence, varieties with high germination potential and seed vigor should be identified to ensure optimum seedling establishment for high seed yield (Maataoui et al., 2005; Nourgholipour et al., 2018). Therefore, it is the top priority for screening of canola genotypes with drought tolerance at germination stage and evaluation of selecting indices for canola production.

In recent years, different methods have been reported for crop drought tolerance identification, which are involved in the physiological, biochemical, morphological identification indicators (Li et al., 2010; Xie et al., 2013; Bai et al., 2014; Li et al., 2015; Jian et al., 2016; Channaoui et al., 2019). On the one hand, canola variety with drought resistance could be identified by some single indicator, but be vulnerable to environmental difference due to the combinative and comprehensive characters determining canola drought resistance (Xie et al., 2013; Jian et al., 2016). On the other hand, only using the method of membership function to evaluate multiple indicators would lead to the overlapping of information provided by each single indicator; because there has been different correlation among different indicators, it would be difficult to obtaining a concise and convenient rule for canola drought resistance identification (Li et al., 2010; Bai et al., 2014; Channaoui et al., 2019). Principal component analysis could integrate a linear combination of multiple indicators into fewer comprehensive indices. There is no correlation among these comprehensive indicators, which could present overall information from the original multiple indices related to canola drought resistance. It is gradually applied to evaluate some crops drought tolerance comprehensively (Liu et al., 2016; 2019a; Pavia et al., 2019).

Some researchers have already focused on screening a single indicator to identify canola varieties with drought tolerance. In our research, 53 canola varieties with different drought resistance in the Yangtze River Basin were analyzed through the correlation analysis, principal component analysis, membership function and cluster analysis. Much drought resistance indices were weighted and analyzed to obtain the comprehensive evaluation value of drought resistance for the experimental varieties. The purpose of this study aimed to provide an efficient method selecting drought-resistant genotypes in winter canola. At the same time, this study could also provide theoretical reference for drought resistance breeding and canola production in the Yangtze River Basin of China.

MATERIALS AND METHODS

Plant material

The experiment was carried out at Tongren University in China. In this experiment, 53 canola (*Brassica napus* L.) varieties were chosen, which have been widely planted in the planting areas of the Yangtze River basin. Seeds of these 53 varieties were collected, dried and stored in a refrigerator at 4 °C until this experiment. The studied canola varieties were provided by the College of Plant Science and Technology in Huazhong Agricultural University and were numbered from 1 to 53, and these varieties were as follows (in the bracket, every variety was represented with seed yields, phenological periods, sowing dates in the field and suitable planting regions):

(1) ZYY8 (widely planted in the upper reaches of the Yangtze river of China, which seed yield was about 2983 kg ha^{-1} with a growing period of 219 d after sowing (das) at any time from October 1 to 10), (2) YY57 (upper reaches of the Yangtze river, seed yield about 2916 kg ha^{-1} with 218 das at any time from October 1 to 15), (3) YY817 (upper reaches of the Yangtze river, seed yield about 2670 kg ha^{-1} with 222 das growing period at any time from October 15 to 30), (4) YY10 (upper reaches of the Yangtze River, about 2381 kg ha^{-1} with 214 das from October 1 to 10), (5) ZY589 (in the middle and lower reaches of the Yangtze river, about 2295 kg ha^{-1} with 217 das from October 1 to 10), (6) ZY28 (in the middle and upper reaches of the Yangtze river, about 2587 kg ha^{-1} with 212 das from October 1 to 15), (7) FY520 (in the middle and upper reaches of the river, about 2559 kg ha^{-1} with 218 das from October 1 to 10), (8) ZNY9 (in the middle and upper reaches, about 2742 kg ha^{-1} with 232 das from October 5 to 15), (9) YG2009 (in the middle and upper reaches, about 2470 kg ha^{-1} with 217 das from October 5 to 15), (10) YY50 (in the middle and upper reaches, about 2319 kg ha^{-1} with 219 das from October 1 to 10), (11) ZYZ19 (in the lower reaches, about 2930 kg ha^{-1} with 230 das from October 1 to 15), (12) ZS12 (in the middle and upper reaches, about 3135 kg ha^{-1} with 230 das from October 1 to 10), (13) ZS9 (in the middle and lower reaches, about 2385 kg ha^{-1} with 220 das from September 15 to 30), (14) ZYZ7 (in the middle and lower reaches, about 2520 kg ha^{-1} with 219 das from September 20 to October 5), (15) ZS11 (in the middle and lower reaches, about 2479 kg ha^{-1} with 234 das from September 25 to October 10), (16) CZY3 (in the middle reaches, about 2525 kg ha^{-1} with 217 das from September 20 to October 5), (17) FY792F1 (in the middle reaches, about 2325 kg ha^{-1} with 216 das from September 20 to October 20), (18) XZY814 (in the middle reaches, about 2608 kg ha^{-1} with 220 das from September 20 to October 10), (19) HXY12 (in the middle and lower reaches, about 2761 kg ha^{-1} with 219 das from October 5 to 15), (20) BYZ12 (in the upper reaches, about 2235 kg ha^{-1} with 190 das from October 10 to 25), (21) JXY11 (in the middle reaches, about 2427 kg ha^{-1} with 218 das from September 15 to October 5), (22) CZY2 (in the middle reaches, about 2422 kg ha^{-1} with 215 das from September 20 to October 15), (23) CYZ83 (in the middle and lower reaches, about 2578 kg ha^{-1} with 215 das from September 15 to October 15), (24) SXY9 (in the middle reaches, about 2362 kg ha^{-1} with 220 das from September 25 to October 30), (25) LY9 (in the middle reaches, about 2618 kg ha^{-1} with 218 das from October 15 to 25), (26) QY7 (in the

Yangtze river basin, about 3000 kg ha^{-1} with 225 das from September 25 to October 10), (27) RHY6 (in the lower reaches, about 3231 kg ha^{-1} with 226 das from October 5 to 15), (28) KLY1 (in the lower reaches, about 2562 kg ha^{-1} with 204 das from September 25 to October 10), (29) BY517 (in the upper reaches, about 2528 kg ha^{-1} with 224 das from October 1 to 10), (30) GYZ6 (in the middle reaches, about 2025 kg ha^{-1} with 202 das from October 5 to 15), (31) CJZY3 (in the upper reaches, about 2792 kg ha^{-1} with 214 das from September 25 to October 10), (32) QDJ (in the middle and upper reaches, about 2823 kg ha^{-1} with 208 das from October 1 to 10), (33) CSY (in the middle and upper reaches, about 2856 kg ha^{-1} with 208 days after sowing from October 1 to 10), (34) JLY5 (in the upper reaches, about 2715 kg ha^{-1} with 210 das from September 25 to October 10), (35) JLY7 (in the upper reaches, about 2737 kg ha^{-1} with 216 das from September 25 to October 10), (36) JLY10 (in the upper reaches, about 2802 kg ha^{-1} with 210 das from October 1 to 10), (37) YBS (in the upper reaches, about 2887 kg ha^{-1} with 210 das from October 5 to 15), (38) JLYT (in the upper reaches, about 2730 kg ha^{-1} with 210 das from October 5 to 15), (39) YB (in the upper reaches, about 2718 kg ha^{-1} with 210 das from October 5 to 15), (40) DAZ600 (in the Yangtze river basin, about 2875 kg ha^{-1} with 210 das from October 1 to 10), (41) XDZY9 (in the upper reaches, about 2794 kg ha^{-1} with 227 das from October 10 to 25), (42) DZY10 (in the Yangtze river basin, about 2455 kg ha^{-1} with 224 das from October 1 to 10), (43) JHH (in the upper reaches, about 2250 kg ha^{-1} with 190 das from October 20 to 30), (44) DZY5 (in the middle and upper reaches, about 2473 kg ha^{-1} with 223 das from September 10 to October 10), (45) XY50 (in the middle reaches, about 2340 kg ha^{-1} with 216 das from September 20 to October 10), (46) ZYZ108 (in the lower reaches, about 3094 kg ha^{-1} with 226 das from October 10 to 25), (47) ZY18 (in the lower reaches, about 2221 kg ha^{-1} with 219 das from October 15 to 30), (48) YY9 (in the upper reaches, about 2298 kg ha^{-1} with 220 das from October 1 to 10), (49) BDHZ (in the middle reaches, about 2100 kg ha^{-1} with 188 das from October 20 to 30), (50) FYJ (in the upper reaches, about 2725 kg ha^{-1} with 220 das from September 15 to October 15), (51) YB (in the Yangtze river basin, about 2745 kg ha^{-1} with 225 das from October 5 to 15), (52) QY20 (in the upper reaches, about 2260 kg ha^{-1} with 235 das from September 15 to October 10), (53) QZ7 (in the upper reaches, about 2485 kg ha^{-1} with 195 das from April 5 to May 10).

Experimental design and management

The experiment was a two-factor in a completely randomized design with five replicates for each treatment. The main-factor was the two water potential treatments, including no drought stress (control) which was the nutrient solution of Hoagland; and drought stress treatment simulated by adding 10% polyethylene glycol (PEG6000, 10% in w/v, MW) to the Hoagland nutrient solution (-0.15 MPa, Michel and Kaulmann, 1973). The concentration of 10% PEG6000 was based on the results of our previous research on the same canola cultivars, where seeds germination was affected at water potentials lower than the solution concentration of 10% PEG6000. Sub-factor was different canola varieties.

Germination trials were carried out in polyethylene boxes (12 cm length × 12 cm width × 6 cm height), which were sterilized with 1% NaClO solution for 15 min and rinsed before use. Three sheets of sterile filter paper were moistened with 10 mL designed solution and placed in the bottom of every germination box. For each variety, the control treatments were Hoagland nutrient solution and the drought stress treatments were 10% PEG in Hoagland solution. The experimental seeds of each variety were surface sterilized with 1% NaClO for 15 min, and then rinsed with distilled water. Thereafter, seeds were dried to remove excess water and allowed to dry at room temperature until their initial weight. After that, 50 seeds were sown on the moistened paper sheets in every germination box. And 10 boxes were sown for each variety, five of them suffered drought stress from the 10% PEG in Hoagland solution, the rest five had no stress with Hoagland solution. Hoagland nutrient solution contained: macro elements (mM), 2.0 K_2SO_4, 5.0 $Ca(NO_3)_2$, 0.50 $Ca(H_2PO_4)_2$, 2.0 $MgSO_4$, and micro elements (μM) 40 $FeSO_4$, 0.6 $CuSO_4$, 1.6 $ZnSO_4$, 10 $MnCl_2$, 100 H_3BO_3, 0.2 H_2MoO_4.

After sowing, germination boxes were placed in the growth chamber, where the temperature was maintained at 28 °C/22 °C based on a 12:12 h photoperiod (4000 lx). Each germination box was supplied the corresponding nutrient solution every day, and the pH of both solutions was maintained at 5.50 ± 0.05 by adding 1 mol L^{-1} HCl or 1 mol L^{-1} NaOH to the solutions during the germination and seedling stage.

Sampling, data processing and analysis

A seed was considered to be germinated when the 1-2 mm radicle appeared. Germination percentage was recorded each day until the 7th day when seedlings were harvested. Ten seedlings were measured for root and shoot length. Mean germination time (MGT), germination index (GI), seedling vigor index (SVI) and mean seedling emergence time

(MST) were calculated with the following Equations 1-4 (Zhang et al., 2013b):

$$MGT = \sum(Dn)/\sum n \quad (1)$$

$$GI = \sum(D/n) \quad (2)$$

where n is the number of seeds germinated on day D (D representing the number of days since sowing).

$$SVI = \text{Percentage germination} \times \text{Seedling length (Shoot length + Root length)} \quad (3)$$

$$MST = \sum(Dm)/\sum m \quad (4)$$

in the Equation 4, m is the number of seedling emerged on day D (D representing the number of days since sowing).

Drought tolerance coefficient (DTC) and membership function value of each comprehensive index of each genotype $U(X_j)$ were obtained by the following Equations 5-8 in the statistical analysis (Toscano et al., 2017):

$$DTC = \text{Drought stress character value/Control treatment character value} \quad (5)$$

$$U(X_j) = (X_j - X_{min})/(X_{max} - X_{min}) \qquad j = 1, 2, \ldots, n \quad (6)$$

in the Equation 6, X_j represents the j-th comprehensive index of each genotype, X_{min} is the minimum value of j-th comprehensive index of each genotype, and X_{max} is the maximum value of j-th comprehensive index of each genotype.

$$W_j = P_j/\sum_{j=1}^{n} Pj \qquad j = 1, 2, ..., n \quad (7)$$

in the Equation 7, W_j is the importance of the j-th comprehensive indicator in all comprehensive indicators; P_j is the contribution rate of the j-th comprehensive indicator of each genotype.

$$D = \sum_{j=1}^{n} [U(Xj) \times Wj] \qquad j = 1, 2, ..., n \quad (8)$$

in the Equation 8, D value is the comprehensive evaluation value of drought tolerance of each genotype under the condition of drought stress during the germination stage of canola.

In this research, R software version 3.6.2 (R Foundation for Statistical Computing, Vienna, Austria) was used for statistical analyses, principal component analysis, regression analysis and cluster analysis. The differences between the means were compared by Fisher's protected least significant difference test ($P < 0.05$).

RESULTS

Drought tolerance coefficient of various characters in canola

The drought tolerance coefficient of each single character was calculated by the results of each single character of the 53 canola varieties under drought stress and control treatments (Table 1). The average value of 14 single characteristic index under drought stress was lower than that of the control (except for the mean germination time (MGT), ratio of shoot and root dry weight (SRTD), ratio of shoot and root fresh weight (SRTF) of the 53 varieties during canola germination stage. Drought stress differently affected the indexes of germination-related characters of all varieties, MGT, germination index (GI), SRTF and root fresh weight per plant (RFW) changed greatly comparing the other 11 characters, and the changed degrees of the four characters were 14.7%-147.4%, 55.4%-8.9%, 49.5%-182.0% and 84.5%-43.1%, respectively. The variation range of each character was different among canola varieties, so it was one-sided to evaluate the canola drought tolerance only according to the drought tolerance coefficient of a single character. Moreover, the correlation analysis showed that there was a large or small correlation between the drought tolerance coefficients of each character (Table 2), which made the information overlapped. At the same time, the contribution for each character greatly differed under drought stress. Therefore, these characters could not accurately evaluate the drought tolerance difference of each variety.

Principal component analysis

According to the principal component analysis (Table 3), contribution rates of the first six comprehensive indexes (CI_1, CI_2, CI_3, CI_4, CI_5 and CI_6) were 32.3%, 17.5%, 12.3%, 10.7%, 8.2% and 6.5%, respectively, and their cumulative contribution rate was 87.5%. Consequently, the weak contributions from other index could be ignored compared with the first six comprehensive indexes. In this way, the original 16 single indicators were transformed into six new independent comprehensive indicators, representing 87.5% of the information of the original 16 single indicators. The first principal component was mainly determined by the six characters components, i.e., 1000-seeds weight (OSW), seedling dry weight

Table 1. Contrast coefficients of morphological and physiological traits of canola varieties.

Variety	MGT	GI	SVI	RL	SL	MST	RSTD	RSTF	RFW	SFW	RDW	SDW	FWPP	DWPP	OSW	SD
ZYY8	1.02	1.02	0.94	0.95	1.06	0.83	1.03	1.51	0.41	0.63	0.81	0.84	0.61	0.83	1.08	1.12
YY57	0.86	1.08	0.83	0.80	0.85	0.80	1.13	1.66	0.48	0.80	0.63	0.71	0.77	0.69	0.53	0.84
YY817	0.87	1.08	0.76	0.74	0.81	0.78	0.99	1.23	0.41	0.51	0.49	0.49	0.50	0.49	0.75	0.95
YY10	0.87	1.08	0.75	0.62	1.05	0.81	1.30	2.33	0.59	1.37	0.92	1.20	1.29	1.14	1.17	1.14
ZY589	1.01	1.03	0.84	0.83	0.99	0.95	1.11	1.68	0.57	0.95	0.83	0.92	0.92	0.91	0.91	1.03
ZY28	0.94	1.05	0.73	0.73	0.75	0.87	1.14	1.11	0.47	0.52	0.44	0.50	0.51	0.49	0.69	0.97
FY520	2.44	0.46	0.38	0.83	0.95	1.02	0.83	1.04	0.33	0.35	1.04	0.87	0.35	0.91	0.69	0.91
ZNY9	2.47	0.45	0.37	1.01	1.14	1.23	0.86	1.07	0.36	0.39	1.09	0.93	0.39	0.97	0.76	0.98
YG2009	1.05	1.00	0.93	0.95	1.05	0.87	0.84	1.42	0.45	0.64	0.79	0.66	0.62	0.69	0.69	0.94
YY50	0.93	1.05	0.87	0.82	1.02	0.85	1.29	1.66	0.55	0.91	0.88	1.13	0.88	1.08	0.99	1.05
ZYZ19	1.60	0.65	0.58	0.57	0.76	1.29	0.94	2.49	0.28	0.71	0.94	0.88	0.67	0.89	0.98	0.96
ZS12	1.01	1.03	0.78	0.77	0.91	0.87	1.01	1.16	0.48	0.56	0.63	0.63	0.55	0.63	0.82	0.99
ZS9	1.09	0.97	0.95	0.99	1.00	0.95	0.65	1.30	0.52	0.67	0.88	0.57	0.66	0.63	0.95	0.97
ZYZ781	0.96	1.03	1.12	1.19	0.93	0.88	0.83	1.03	0.71	0.73	0.88	0.73	0.72	0.76	0.82	0.93
ZS11	1.03	0.99	0.93	0.93	0.93	0.95	0.94	1.02	0.99	0.89	0.96	0.88	0.97	0.94	0.97	0.98
CZY3	1.76	0.64	0.73	0.85	0.99	1.18	1.33	1.64	0.34	0.57	0.38	0.50	0.55	0.47	0.51	0.84
FY792F1	0.91	1.06	0.72	0.64	0.89	0.87	1.06	1.09	0.62	0.67	0.69	0.73	0.67	0.72	0.89	0.96
XZY814	0.91	1.06	0.64	0.57	0.80	0.84	0.94	1.43	0.31	0.44	0.75	0.71	0.43	0.72	0.79	0.98
HXY12	1.01	0.98	0.88	0.88	0.90	0.96	0.74	1.54	0.43	0.67	0.92	0.67	0.64	0.72	0.67	0.93
BYZ12	0.91	1.07	0.60	0.54	0.74	0.81	0.89	1.82	0.28	0.50	0.88	0.78	0.48	0.80	0.79	0.93
JXY11	1.03	0.99	0.58	0.52	0.77	0.92	1.74	1.99	0.44	0.87	0.44	0.76	0.83	0.69	0.84	0.98
CZY2	1.06	1.01	0.74	0.61	1.23	0.87	1.39	1.48	0.57	0.84	0.69	0.96	0.82	0.90	0.92	0.92
CYZ83	1.21	0.85	0.74	0.78	0.81	1.15	1.10	1.13	0.57	0.65	0.63	0.68	0.64	0.67	0.69	0.93
SXY9	1.20	0.94	0.69	0.72	0.85	1.11	1.62	0.75	1.19	0.89	0.58	0.95	0.92	0.87	0.97	1.00
LY9	0.94	1.04	0.80	0.73	0.96	0.85	1.49	1.08	1.14	1.23	0.75	1.12	1.22	1.04	0.90	0.97
QY6	1.44	0.78	0.84	0.94	0.98	1.22	1.47	0.91	0.88	0.80	0.56	0.83	0.81	0.77	0.72	0.94
RHY6	1.40	0.77	0.69	0.70	0.91	1.15	1.33	0.91	0.78	0.71	0.50	0.66	0.71	0.63	0.77	0.99
KLY1	1.08	0.89	0.79	0.72	0.95	1.26	2.26	2.82	0.16	0.44	0.39	0.89	0.41	0.79	0.81	1.01
BY517	0.92	1.06	0.69	0.69	0.72	0.85	0.81	1.51	0.31	0.47	0.69	0.55	0.45	0.58	0.69	0.95
GYZ6	0.87	1.06	0.87	0.91	0.72	1.01	1.12	0.73	0.60	0.44	0.58	0.65	0.45	0.64	0.69	0.93
CJZY3	0.85	1.09	0.89	0.84	0.97	0.78	1.37	1.91	0.41	0.79	0.63	0.86	0.75	0.81	1.11	1.00
QDJ	0.92	1.05	0.95	0.93	1.00	0.93	1.22	0.50	1.29	0.65	0.50	0.61	0.71	0.59	0.84	1.03
CSY	0.96	1.01	1.09	1.10	1.02	1.05	0.56	1.47	0.34	0.50	1.38	0.77	0.48	0.90	0.95	1.02
JLY5	0.91	1.05	0.80	0.77	0.81	0.86	0.51	0.66	0.91	0.61	1.25	0.64	0.64	0.77	0.80	1.00
JLY7	0.94	1.04	0.88	0.87	0.89	0.82	1.32	0.84	0.83	0.69	0.63	0.83	0.71	0.78	0.84	1.00
JLY10	0.87	1.08	0.86	0.84	0.86	0.79	0.90	0.96	0.91	0.87	0.69	0.62	0.87	0.63	0.79	0.98
YBS	0.92	1.06	0.90	0.85	1.03	0.92	0.77	1.81	0.52	0.94	1.00	0.77	0.90	0.82	0.88	1.00
JLYT	0.96	1.01	0.84	0.82	0.82	1.03	0.57	0.71	1.43	1.02	1.52	0.87	1.06	1.00	0.98	1.06
YB	1.14	0.93	0.99	1.05	0.92	1.03	0.74	0.73	1.38	1.01	0.94	0.70	1.04	0.75	0.92	1.04
DAZ600	1.12	0.93	0.75	0.74	0.86	1.10	0.93	1.15	0.40	0.46	1.09	1.01	0.45	1.03	0.90	1.03
XDZY9	0.88	1.08	0.87	0.88	0.83	0.93	1.10	0.94	0.88	0.83	0.54	0.60	0.83	0.59	0.91	0.98
DZY10	1.34	0.79	0.51	0.79	0.91	1.22	1.01	1.26	0.41	0.52	0.98	0.99	0.51	0.99	0.81	0.96
JHH	0.87	1.08	0.66	0.58	0.84	0.78	0.89	1.11	0.60	0.67	0.43	0.38	0.66	0.39	0.72	0.90
DZY5	1.24	0.86	0.50	0.45	0.62	1.04	2.26	1.21	0.42	0.50	0.38	0.85	0.49	0.75	0.82	0.98
XY50	1.06	1.01	0.72	0.76	0.80	0.82	0.42	0.69	0.97	0.67	1.13	0.47	0.69	0.60	0.77	0.96
ZYZ108	1.35	0.80	0.85	0.87	0.98	1.16	1.43	1.61	0.44	0.71	0.56	0.80	0.68	0.75	0.91	0.92
ZY18	1.11	0.96	0.76	0.77	0.89	0.88	1.15	0.52	1.37	0.72	0.50	0.58	0.78	0.56	0.81	0.96
YY9	0.92	1.06	0.78	0.75	0.86	0.81	0.86	1.50	0.48	0.73	0.79	0.68	0.70	0.71	0.90	0.92
BDHZ	1.20	0.93	0.44	0.39	0.71	0.90	0.49	1.10	0.49	0.54	1.13	0.55	0.54	0.67	0.65	0.88
FYJ	0.99	1.02	0.84	0.85	0.87	0.84	0.90	1.38	0.44	0.61	0.75	0.67	0.59	0.69	0.82	0.97
YBA	0.95	0.83	0.82	0.82	1.07	1.14	1.20	2.13	0.26	0.55	0.63	0.75	0.52	0.72	1.00	1.01
QY20	1.06	1.01	0.70	0.73	0.68	1.00	1.25	2.25	0.59	1.34	0.63	0.78	1.27	0.75	0.84	1.02
QZ7	1.05	1.03	0.77	0.81	0.74	0.86	1.47	2.31	0.39	0.90	0.69	1.01	0.85	0.94	0.83	0.99

MGT: Mean germination time; GI: germination index; SVI: seedling vigor index; RL: root length; SL: shoot length; MST: mean seedling emergence time; RSTD: ratio of shoot and root dry weight; RSTF: ratio of shoot and root fresh weight; RFW: root fresh weight per plant; SFW: shoot fresh weight per plant; RDW: root dry weight per plant; SDW: shoot dry weight per plant; FWPP: fresh weight per plant; DWPP: dry weight per plant; OSW: 1000-seeds weight; SD: seed diameter.

Table 2. Correlation matrix of drought-tolerance coefficients of different characters.

Index	DWPP	FWPP	GI	MGT	MST	OSW	RDW	RFW	RL	RSTD	RSTF	SD	SDW	SFW
FWPP	0.439**													
GI	0.031	0.359**												
MGT	0.293*	-0.038	-0.661**											
MST	0.287*	-0.082	-0.397**	0.535**										
OSW	0.640**	0.509**	0.361**	-0.040	0.158									
RDW	0.610**	0.234	0.019	0.315*	0.074	0.340*								
RFW	0.147	0.656**	0.211	0.081	-0.061	0.245	0.285*							
RL	0.253	0.220	0.110	0.254	0.231	0.270*	0.364**	0.343**						
RSTD	0.246	0.294*	-0.002	0.258	0.243	0.220	-0.331*	0.136	-0.030					
RSTF	0.360**	0.230	0.043	0.189	0.135	0.302*	0.097	-0.338*	-0.027	0.494**				
SD	0.330*	0.156	0.398**	-0.141	0.371**	0.585**	0.063	-0.025	0.172	-0.050	-0.031			
SDW	0.957**	0.474**	0.015	0.293*	0.293*	0.628**	0.387**	0.129	0.192	0.482**	0.446**	0.283*		
SFW	0.462**	0.994**	0.346**	-0.017	-0.084	0.513**	0.237	0.583**	0.203	0.327*	0.326*	0.133	0.505**	
SL	0.432**	0.209	0.061	0.282*	0.326*	0.487**	0.225	0.087	0.565	0.103	0.149	0.428**	0.420**	0.212
SVI	0.147	0.422**	0.589**	-0.286*	-0.049	0.427**	0.198	0.393**	0.743**	0.062	0.086	0.247	0.126	0.407**

*, **Significant at 0.05 and 0.01 level.

DWPP: Dry weight per plant; FWPP: fresh weight per plant; GI: germination index; MGT: mean germination time; MST: mean seedling emergence time; OSW: 1000-seeds weight; RDW: root dry weight per plant; RFW: root fresh weight per plant; RL: root length; RSTD: ratio of shoot and root dry weight; RSTF: ratio of shoot and root fresh weight; SD: seed diameter; SDW: shoot dry weight per plant; SFW: shoot fresh weight per plant; SL: shoot length; SVI: seedling vigor index.

per plant (DWPP), shoot dry weight (SDW), shoot fresh weight (SFW), seedling fresh weight per plant (FWPP), and shoot length (SL), which could be summarized as biomass character factors. The second principal component was determined by MGT, GI, mean seedling emergence time (MST), seedling vigor index (SVI), SDW and root fresh weight per plant (RFW), which could be summarized as biomass factors and germination time factors. The third, fourth, fifth and sixth principal components were determined by root length (RL), ratio of shoot and root dry weight (RSTD), average RFW, seed diameter (SD) and ratio of shoot and root fresh weight (RSTF), which could be roughly categorized as biomass factors and quantitative factors.

Comprehensive evaluation and cluster analysis of drought tolerance coefficient

The membership function value was calculated by the Equation 6 for all representative indexes of each variety. Equation 7 was used to calculate the weight of six representative indexes. The comprehensive evaluation D-value of drought resistance could be obtained according to the U-value and weight of each representative index of each variety by Equation 8 (Table 4). The drought tolerance could be evaluated comparing the D-value of each variety. In this research, such four varieties with higher D-value had higher drought resistance as YY10 (0.790), LY9 (0.701), JLYT (0.684) ZS11 (0.668) and YY50 (0.658). Euclidean distance and maximum distance method were used to cluster D-value to evaluate varieties drought tolerance difference comprehensively (Figure 1), and in this way, the 53 varieties could be classified into three categories. The first-class varieties with the strongest drought resistance were YY10, JLYT, LY9, ZS11, and YY50; there were 16 varieties belonging to the second one with moderate drought resistance, such as QZ7, DAZ600, ZYZ19 and so on; and the third category was the rest 32 varieties with the weakest drought resistance, which were BY517, JHH, CZY3 and so on.

Table 3. Coefficients of comprehensive index (CI) and their contribution rate (CR).

Comprehensive index	DWPP	FWPP	GI	MGT	MST	OSW	RDW	RFW	RL	RSTD	RSTF	SD	SDW	SFW	SL	SVI	CR (%)
CI_1	-0.3493	-0.3332	-0.1468	-0.0772	-0.1081	-0.3543	-0.2139	-0.205	-0.2324	-0.1562	-0.165	-0.1965	-0.3468	-0.336	-0.2669	-0.2565	32.3
CI_2	-0.2148	0.1925	0.464	-0.4807	-0.3935	0.0364	-0.0818	0.2187	0.0303	-0.1672	-0.1955	0.0447	-0.2319	0.1662	-0.1138	0.3104	17.5
CI_3	0.0363	0.2523	0.0345	-0.0888	-0.2003	-0.0098	-0.2456	-0.0259	-0.439	0.4292	0.3554	-0.2391	0.1681	0.2878	-0.3157	-0.2316	12.3
CI_4	0.0457	-0.2319	0.2736	-0.3137	0.1075	0.2424	-0.255	-0.5064	-0.1654	0.0562	0.2281	0.4755	0.0827	-0.2047	0.1453	0.0241	10.7
CI_5	-0.3441	0.0163	-0.0281	0.1158	0.2565	-0.1284	-0.5316	0.111	0.3018	0.4788	0.0545	-0.0464	-0.1769	0.0096	0.2042	0.3023	8.2
CI_6	-0.0079	0.1526	-0.0711	0.0008	0.3184	0.1182	-0.2338	0.3438	-0.2981	0.0401	-0.5292	0.4453	0.0174	0.0805	-0.0887	-0.316	6.5

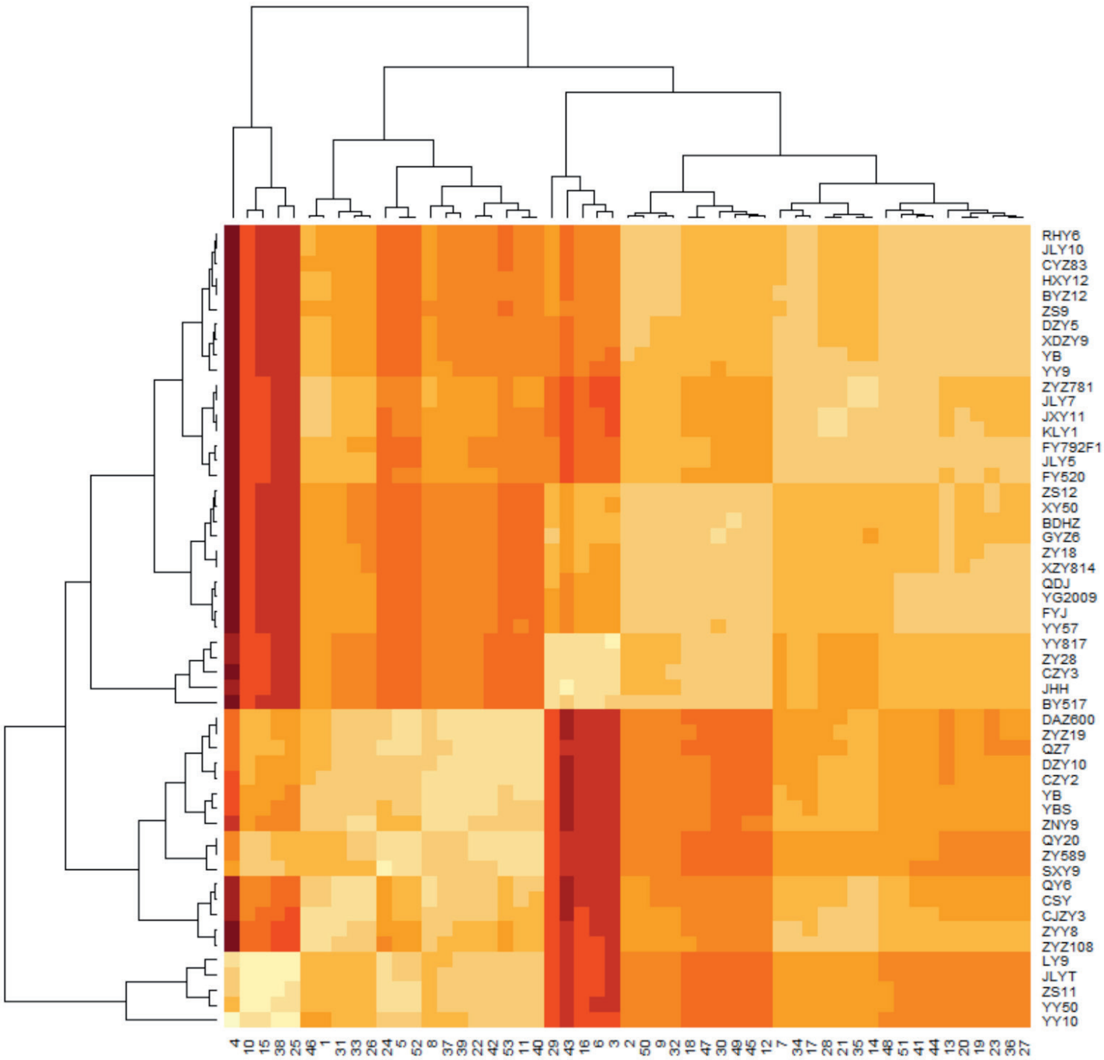
DWPP: Dry weight per plant; FWPP: fresh weight per plant; GI: germination index; MGT: mean germination time; MST: mean seedling emergence time; OSW: 1000-seeds weight; RDW: root dry weight per plant; RFW: root fresh weight per plant; RL: root length; RSTD: ratio of shoot and root dry weight; RSTF: ratio of shoot and root fresh weight; SD: seed diameter; SDW: shoot dry weight per plant; SFW: shoot fresh weight per plant; SL: shoot length; SVI: seedling vigor index.

Table 4. The value of comprehensive index (CI), index weight (IW), membership function (U(X)), evaluation (D) and prediction (P) of drought tolerance of each variety.

Varieties	CI_1	CI_2	CI_3	CI_4	CI_5	CI_6	$U(X_1)$	$U(X_2)$	$U(X_3)$	$U(X_4)$	$U(X_5)$	$U(X_6)$	P	D	Rank
ZYY8	45.592	34.910	46.317	28.850	30.702	35.630	0.591	0.274	0.896	0.104	0.097	0.865	0.142	0.485	20
YY57	48.259	36.547	48.183	31.933	26.951	35.513	0.401	0.449	0.991	0.006	0.039	0.028	-0.009	0.384	39
YY817	35.933	28.487	36.968	23.712	23.423	28.917	0.134	0.164	0.992	0.013	0.011	0.356	-0.087	0.251	52
YY10	69.759	49.833	64.864	46.906	29.333	43.021	1.000	1.000	0.987	0.008	0.069	1.000	0.292	0.790	1
ZY589	55.771	41.061	54.315	36.872	29.589	38.763	0.689	0.604	0.903	0.096	0.331	0.606	0.180	0.590	8
ZY28	35.817	28.262	36.717	23.823	22.958	28.491	0.130	0.176	0.939	0.055	0.178	0.272	-0.074	0.259	51
FY520	30.314	21.560	31.242	19.714	22.938	22.459	0.689	0.000	0.017	0.981	0.474	0.273	0.275	0.441	26
ZNY9	33.629	23.126	35.346	22.065	25.400	24.919	0.771	0.043	0.000	1.000	0.882	0.382	0.354	0.526	16
YG2009	44.767	34.296	46.184	28.799	29.562	35.547	0.401	0.290	0.859	0.123	0.183	0.267	0.036	0.379	41
YY50	55.894	41.463	53.719	36.141	31.065	38.468	0.919	0.563	0.946	0.050	0.136	0.729	0.252	0.658	5
ZYZ19	42.692	31.689	41.416	28.075	23.420	30.100	0.672	0.339	0.320	0.463	1.000	0.718	0.288	0.564	11
ZS12	39.326	30.544	39.796	25.744	25.368	30.508	0.320	0.219	0.907	0.094	0.182	0.460	0.017	0.352	46
ZS9	45.915	34.863	47.444	29.950	29.753	36.675	0.320	0.329	0.815	0.148	0.349	0.668	0.054	0.399	38
ZYZ781	51.262	38.911	52.851	32.968	34.314	41.263	0.493	0.399	0.916	0.068	0.202	0.470	0.081	0.453	22
ZS11	61.095	44.891	59.211	40.430	33.988	43.250	0.816	0.691	0.862	0.091	0.437	0.742	0.247	0.668	4
CZY3	38.100	28.727	40.675	25.052	24.560	30.679	0.113	0.210	0.306	0.558	0.797	0.000	0.017	0.270	50
FY792F1	42.863	32.816	41.480	28.582	24.448	30.617	0.441	0.342	0.958	0.036	0.186	0.580	0.064	0.431	28
XZY814	33.207	26.628	32.680	21.426	22.112	25.324	0.435	0.091	0.956	0.036	0.132	0.426	0.046	0.362	43
HXY12	44.615	34.140	45.337	28.905	28.304	34.741	0.447	0.314	0.839	0.099	0.352	0.230	0.064	0.408	34
BYZ12	34.997	27.631	33.967	22.792	21.949	25.831	0.551	0.141	0.964	0.036	0.072	0.416	0.083	0.409	33
JXY11	47.128	35.007	45.296	32.122	21.878	31.361	0.406	0.513	0.841	0.109	0.280	0.503	0.063	0.448	24
CZY2	50.662	37.800	48.335	33.306	26.873	33.879	0.683	0.499	0.872	0.125	0.179	0.615	0.168	0.552	13
CYZ83	41.981	31.777	42.034	27.727	25.344	31.440	0.378	0.309	0.624	0.218	0.721	0.263	0.089	0.403	37
SXY9	52.690	38.738	49.625	36.342	26.700	35.229	0.643	0.606	0.765	0.216	0.652	0.688	0.213	0.605	6
LY9	66.166	47.678	61.538	45.104	29.909	41.923	0.873	0.922	0.928	0.052	0.135	0.595	0.225	0.701	2
QY6	50.753	37.418	50.559	33.767	29.555	37.027	0.510	0.488	0.517	0.364	0.875	0.319	0.175	0.509	17
RHY6	43.610	32.550	42.951	29.533	24.331	31.318	0.320	0.386	0.511	0.339	0.724	0.386	0.091	0.405	36
KLY1	36.249	29.285	37.839	22.333	26.558	30.203	0.533	0.068	0.695	0.141	0.951	0.447	0.173	0.448	25
BY517	33.884	26.993	34.596	22.234	22.511	27.213	0.257	0.115	0.954	0.041	0.150	0.262	-0.031	0.291	49
GYZ6	36.555	29.278	37.713	23.427	27.068	30.572	0.332	0.115	0.961	0.013	0.449	0.270	0.022	0.345	48
CJZY3	49.908	37.795	49.645	32.320	29.209	36.865	0.562	0.430	1.000	0.000	0.000	0.904	0.112	0.501	19
QDJ	46.634	36.036	46.674	31.875	29.348	36.817	0.262	0.388	0.947	0.041	0.295	0.496	0.002	0.377	42
CSY	43.222	33.952	45.238	26.311	34.113	36.988	0.677	0.142	0.872	0.065	0.527	0.658	0.195	0.507	18
JLY5	42.472	32.737	41.369	28.650	26.882	32.231	0.504	0.305	0.945	0.036	0.169	0.438	0.076	0.433	27
JLY7	47.008	35.820	46.382	30.925	28.967	35.074	0.527	0.380	0.923	0.057	0.086	0.501	0.084	0.453	23
JLY10	51.423	38.434	50.563	35.123	27.482	37.325	0.326	0.555	0.990	0.010	0.028	0.420	-0.009	0.406	35
YBS	55.086	40.855	54.391	36.459	29.912	39.598	0.574	0.582	0.954	0.042	0.284	0.563	0.123	0.536	15
JLYT	59.920	43.797	56.133	41.607	30.700	40.997	0.821	0.755	0.878	0.065	0.488	0.711	0.249	0.684	3
YB	60.342	44.133	59.273	41.598	32.537	44.186	0.476	0.736	0.750	0.180	0.488	0.625	0.126	0.542	14
DAZ600	37.849	29.606	36.843	23.337	27.462	28.558	0.853	0.112	0.747	0.164	0.635	0.582	0.277	0.565	10
XDZY9	49.995	37.532	49.712	34.081	27.530	37.051	0.262	0.513	0.980	0.019	0.303	0.610	0.009	0.414	31
DZY10	37.997	27.874	37.444	24.733	24.936	26.949	0.798	0.174	0.534	0.302	0.858	0.451	0.284	0.556	12
JHH	39.356	30.321	39.059	27.356	20.567	28.919	0.000	0.336	0.982	0.013	0.007	0.314	-0.140	0.231	53
DZY5	33.446	26.094	31.855	21.940	19.829	22.930	0.481	0.157	0.643	0.237	0.518	0.466	0.126	0.412	32
XY50	42.614	32.223	41.900	29.787	24.685	31.967	0.285	0.367	0.873	0.128	0.087	0.383	-0.007	0.354	45
ZYZ108	46.397	34.866	47.003	29.862	28.589	35.016	0.487	0.354	0.554	0.308	0.757	0.598	0.169	0.482	21
ZY18	45.892	34.663	44.753	32.504	25.387	33.987	0.228	0.456	0.799	0.161	0.211	0.445	-0.008	0.360	44
YY9	45.214	34.317	44.763	29.962	25.996	33.316	0.424	0.376	0.957	0.039	0.074	0.589	0.049	0.422	29
BDHZ	33.186	25.524	31.163	23.016	18.014	22.626	0.378	0.201	0.755	0.216	0.237	0.211	0.040	0.350	47
FYJ	42.006	32.382	42.671	27.214	27.189	32.849	0.401	0.258	0.889	0.083	0.133	0.468	0.042	0.382	40
YBA	40.420	31.038	41.834	25.559	27.332	32.367	0.447	0.188	0.601	0.062	0.706	0.748	0.131	0.416	30
QY20	65.111	46.323	62.547	45.132	25.958	42.093	0.481	0.972	0.882	0.125	0.446	0.504	0.109	0.591	7
QZ7	52.718	39.106	51.628	34.637	28.256	37.111	0.741	0.532	0.916	0.125	0.156	0.477	0.177	0.574	9
IW							36.9	20.0	14.1	12.2	9.4	7.4			

P: Prediction value of drought tolerance; D: comprehensive evaluation value of drought tolerance; CI_1~CI_6: value of first (CI_1), second (CI_2), third (CI_3), fourth (CI_4), fifth (CI_5) and sixth (CI_6) comprehensive index, respectively; $U(X_1)$~$U(X_6)$: value of the membership function of first ($U(X_1)$), second ($U(X_2)$), third ($U(X_3)$), fourth ($U(X_4)$), fifth ($U(X_5)$) and sixth ($U(X_6)$) comprehensive index, respectively.

Figure 1. Dendrogram based on D value by cluster analysis.



Rescaled distance cluster combine.

Screening indices and drought tolerance coefficient in canola

The mathematical model evaluating drought resistance was established and predicted the drought resistance. The stepwise regression analysis of 16 characteristic indicators was carried out to screen the drought resistance indicators of different canola varieties during germination and emergence stage. The optimal regression equation was established, and took the comprehensive evaluation value of drought tolerance (D-value) as the dependent variable and the drought tolerance coefficient of each single indicator as the independent variable: $D = -0.601 + 0.225X_1 + 0.105X_2 + 0.207X_3 + 0.182X_5 + 0.110X_6 + 0.036X_{13}$ ($F = 23\,836.39^{**}$, $R^2 = 0.9999$), and in this formula, X_1, X_2, X_3, X_5, X_6 and X_{13} represented DWPP, FWPP, GI, MST, OSW and SDW, respectively. It would be inferred from the equation that among the 16 single indicators, the above six indicators significantly influenced drought tolerance of canola seedlings at germination period. Therefore, these six indexes could be determined selectively to evaluate difference in drought tolerance. The prediction value of drought tolerance (P) was significantly correlated with the comprehensive evaluation value of drought tolerance (D-value) for the 53 canola varieties ($r = 0.8399^{**}$), which effectively proved the equation accuracy in predicting varieties drought tolerance difference.

DISCUSSION

Selecting drought tolerance characters and identifying canola varieties with drought tolerance during germination and emergence stages

Crop drought tolerance identification was a procedure identifying, screening, evaluating and classifying different varieties with varied drought tolerance capacity (Li et al., 2010; Bai et al., 2014; Channaoui et al., 2019). The most important step was to select clear and effective identification indices of drought-resistant varieties in a much earlier growing period (Xie et al., 2013). Some research showed that there could be a single physiological indicator for drought resistance identification in canola, such as crop canopy temperature difference, detached leaves dehydration rate, leaves water potential and osmotic potential, pod osmotic adjustment ability, chlorophyll stability in leaves, germination rate under low osmotic condition and relative vigor index (Li et al., 2010; Xie et al., 2013; Bai et al., 2014; Li et al., 2015; Channaoui et al., 2019).

However, crop drought tolerance was a quantitative genetic trait controlled by multiple genes (Byzova et al., 2004; Jian et al., 2016). Different varieties owned different traits related to drought tolerance, which was affected by certain genes and growing environment (Byzova et al., 2004; Muller et al., 2005; Zhao et al., 2005; Li et al., 2010; Zhang et al., 2013a; Jian et al., 2016). It was difficult to evaluate drought tolerance comprehensively and accurately through a single index. So, the multi-index membership function method was advised to evaluate drought tolerance in some crops. Although it would reflect many drought-tolerance characters of crops relatively comprehensively, the membership function showed much certain limitation.

In recent years, the comprehensive evaluation had already been preliminarily applied to identify drought-resistant germplasm, such as wheat, corn, soybean and other crops (Khodarahmpour and Motamedi, 2011; Ali et al., 2018; Farid and Ridwan, 2018; Liu et al., 2019b; Pavia et al., 2019). The comprehensive evaluation was involved in the principal component analysis, membership function analysis, regression analysis and cluster analysis of drought tolerance indices correlation for different varieties. In this study, 16 indicators including morphological and agronomic characters were selected in germination and emergence stages when canola was most prone to drought damage. These 16 single indicators were synthesized into six independent comprehensive indexes to identify drought tolerance difference among the studied 53 varieties with principal component analysis. The obtained six indexes were screened by stepwise regression analysis, and regression equation was established. Lastly, these 53 canola varieties could be classified in the drought tolerance difference by cluster analysis.

Drought tolerance characteristics during canola germination and emergence stages

Winter canola greatly required water during the whole growing season, and its adaptability was poor to drought stress (Hamzei and Soltani, 2012; Shabani et al., 2014). Drought stress would affect root development, plant growth, and ultimately lead to a significant decline in yield and yield components (Marcinkeviciene et al., 2013; Shabani et al., 2014). The variety with strong drought tolerance would have a stronger adaptability to arid environment, which could significantly reduce water transpiration and improve the capacity of osmoregulation substance metabolism in the case of drought stress (Borisjuk et al., 2013; Kuai et al., 2016).

In this experiment, 1000-seeds weight, fresh and dry seedling weight, germination time and some root characters occupied the first, second and third principal component positions respectively. The results showed that DM accumulation, germination time and root characters were the important performance indexes related to drought tolerance among different canola varieties under some drought conditions (Xie et al., 2013; Channaoui et al., 2019). Under drought conditions, some excellent characters' performance in canola germination and emergence stages might be benefit for obtaining more nutrients, water, light and other resources in the growing environment; and the performance could contribute to the competition ability of the individual seedling (Borisjuk et al., 2013; Bai et al., 2014).

However, the effects of drought stress on seedlings were not only performed in the phenotypic parameters at the germination stage, but also in the specific physiological and biochemical processes (Li et al., 2010; Jian et al., 2016; Channaoui et al., 2019). In the next research, it will be necessary to further explore and improve the comprehensive evaluation method of canola drought tolerance with combining physiological characters which could be measured easily and conveniently.

CONCLUSIONS

Based on the principal component analysis of the drought tolerance coefficients of 16 characters of the 53 canola varieties, the original single characters related to drought tolerance were transformed into six independent comprehensive indexes, representing 87.5% of the total related data in this experiment. The comprehensive evaluation value (D-value) of each variety was calculated by means of the membership function and cluster analysis. The 53 varieties could be divided into three categories, five varieties with higher drought tolerance relatively, 16 varieties with medium drought tolerance and the rest 32 ones with relatively lower drought tolerance.

By stepwise regression analysis, six drought resistance indexes were selected, such as dry weight per plant, fresh weight per plant, germination index, mean seedling emergence time, 1000-seeds weight and shoot dry weight per plant, and the optimal regression equation was established, which made the prediction quicker and more convenient for canola varieties difference in drought tolerance. Therefore, the integrated method including the principal component analysis, cluster analysis and stepwise regression analysis was more reliable for the comprehensive evaluation of drought tolerance difference during canola germination and emergence period. It not only avoided the one-sidedness and instability of the single indicator evaluation, but also revealed the relationship between drought tolerance coefficients and characters related drought tolerance in canola germination and emergence stage.

ACKNOWLEDGEMENTS

The research and publication were supported by the National Natural Science Foundation of China (31660354), Program for Top Talents in Science and Technology of Education Department in Guizhou (KY [2016]108) and Scientific Research Foundation for Doctors of Tongren University (trxyDH1608).

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