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RESEARCH ARTICLE

Drought resistance indicators screening and drought resistance evaluation of *Salvia miltiorrhiza* Bunge germplasm resources at seedling stage

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ABSTRACT

Drought is a significant abiotic constraint in *Salvia miltiorrhiza* production, particularly during seedling cultivation. Therefore, researching drought resistance is crucial. This study aimed to investigate the effects of drought stress on the agronomic and physiological characteristics of *S. miltiorrhiza* at the seedling stage, evaluate drought resistance of various germplasms, and identify drought resistance markers. To simulate drought stress, five different concentrations of polyethylene glycol (PEG) (0%, 5%, 10%, 15%, and 20%) were applied to four *S. miltiorrhiza* germplasms at the seedling stage. Agronomic traits, root morphology, and physiological and biochemical indicators were measured. The data were then analyzed using the membership function method, correlation analysis, and stepwise regression analysis. The results revealed that as the PEG concentration increased, the aboveground agronomic traits and underground root morphology of the four *S. miltiorrhiza* germplasms showed a decline. The activities of catalase and peroxidase initially increased and then decreased, while the contents of soluble sugar (SS), soluble protein, and proline increased compared to the control. Based on the membership function values of 16 morphological and physiological indicators, the drought resistance order of the four *S. miltiorrhiza* germplasms was as follows: Yudanshen VD > Yudanshen VA > Yudanshen VB > Yudanshen VC. Additionally, an optimal regression equation ($D = 0.887X_1 - 0.003X_2 + 0.142X_3$) was developed through stepwise regression analysis, indicating that X_1, X_2, and X_3 represent the maximum root length, root surface area, and SS content, respectively. Consequently, this study presents a comprehensive and reliable method for evaluating drought resistance in *S. miltiorrhiza* germplasm resources.

Key words: Comprehensive drought resistance evaluation, drought resistance indicators screening, *Salvia miltiorrhiza*, seedlings.

INTRODUCTION

As the Earth warms, more and more ecosystems are being exposed to increasing periods of drought or arid conditions (Varshney et al., 2020). Drought is a major abiotic stress for terrestrial plants and can cause decreased growth, reduced fruiting/reproduction, or even death (Berg et al., 2016). It is responsible for more agricultural financial loss than all other environmental factors combined (Bahrami et al., 2014). Plants are being exposed to increasingly arid conditions, and the ensuing water stress could be responsible

for decreasing food security and dwindling availability of grown resources. Native and local communities face a particular challenge, the loss of traditional ethnobotanical medicines threatened by encroaching drought stress (Hosseini et al., 2018).

Salvia miltiorrhiza Bunge is a traditional Chinese herbal medicine, whose medicinal parts are roots and stems. It has been extensively used for the treatment of cardiovascular and cerebrovascular diseases such as coronary heart disease, chronic renal failure, atherosclerosis, myocardial infarction, and angina pectoris (Li et al., 2018; Shi et al., 2019). The demand for *S. miltiorrhiza* has steadily increased due to its significant medicinal value in recent years, and the planting area of *S. miltiorrhiza* is expanding with the increasing demand in China. It is mainly planted in arid and semi-arid mountain areas in China.

To date, commercial *S. miltiorrhiza* herb mainly relies on the cultivated resource; in most planting areas is mainly multiplied by seeds. Germination and early seedling growth are critical stages of plant development and are vulnerable to various stress conditions. Therefore, obtaining fast and orderly seedlings is crucial for high yields. The optimum temperature range for *S. miltiorrhiza* seed germination is between 20 and 25 °C (He et al., 2014). Seedlings are generally raised in June-July and transplanted in October-November during autumn. When planting *S. miltiorrhiza* in summer, the hot and dry climate with high surface temperature may lead to a low emergence rate of seedlings due to drought stress (Huang et al., 2018). Therefore, drought is one of the main problems *S. miltiorrhiza* cultivation is facing currently.

Previous studies have shown that appropriate concentrations of germination inducers and seed coating can increase the germination rate and drought resistance of *S. miltiorrhiza* seeds. In addition, cultivating germplasm with strong drought resistance during the germination period is also an effective method to improve the yield and quality of *S. miltiorrhiza* (Zuo et al., 2010; Huang et al., 2018; Zhang et al., 2018). A series of changes occur in plants under drought stress, such as plant height, root length, water content, osmotic regulation substance content and antioxidant enzyme activity. The sensitivity of plants to drought can be determined by the changes in morphological and physiological indices after drought stress. Drought tolerance evaluation of germplasm resources employing morphological combined with physiological indicators could increase the accuracy of screening and identification of drought-tolerant germplasm. Though the results of some research are not quite the same, scholars have reached a consensus on taking different aspects into consideration rather than simply using one index to reflect the drought resistance of plants. The comprehensive drought resistance measurement value (D-value) integrates the correlation and contribution rate of various indicators, which can eliminate the limitations of a single indicator and obtain more accurate resistance evaluation, and is widely used in drought resistance evaluation of crops.

Due to the complexity of soil composition and the difficulty of effectively distinguishing drought stress effects from other effects in the soil, the study of drought resistance is more complex. The approach of polyethylene glycol (PEG) (PEG-6000) osmotic stress simulated-drought can better address these issues. The PEG molecules are inert, highly hydrophilic, and non-ionic, they do not enter the cell wall space and PEG molecules with a molecular weight greater than 3000 are apparently not absorbed by plants (Tarkow et al., 1996), not having any toxic effects (Emmerich and Hardegree, 1990). It could regulate the osmotic pressure of the solution, thus have usually been used to induce water stress. Therefore, PEG is ideal for simulating infiltration and is a commonly used osmoregulator. The PEG osmotic method for simulating drought stress is simple, reproducible, and stable, and is suitable for rapid identification of drought tolerance in a large number of varieties (lines) during germination and seedlings. The genetic potential of drought resistance using PEG solution with an osmotic potential of -1.03 MPa was evaluated in 20 different sorghum seedlings (Bibi et al., 2012). Abouzaid et al. (2016) tested five concentrations of PEG in guava, among which the optimum drought concentration for screening was determined as 8%.

Existing work has already established that reduced water access and simulated drought stress can negatively impact the growth of *S. miltiorrhiza*, but has not provided research solutions that may help native stakeholders. Most plants' seedling growth stages are susceptible to water changes. Uniyal and Nautiyal (1998) proposed to evaluate plants stress resistance by the growth status of seedlings. Therefore, this study was based on 16 agronomic and physiological indicators and researched the drought resistant characteristics of *S. miltiorrhiza* at seedling stage. The purpose of our research herein was to establish a

new method of simulated drought stress using polyethylene glycol (PEG-6000) hypertonic solution. This experimental protocol allows for the large-scale identification of drought resistance in a variety of *S. miltiorrhiza* contemporaneously. In view of the foregoing, the objectives of this study were to evaluate *S. miltiorrhiza* seedling's agronomic and physiologic traits that are associated with drought tolerance and mathematical statistical analysis was applied to comprehensively evaluate different germplasm and screen the drought resistance indicators.

MATERIALS AND METHODS

Test materials and experimental design

The materials selected for the experiment were all obtained from wild germplasm resources screened by the group and collected by the College of Agronomy, Henan Agricultural University, Henan, China, and were recorded as YudanshenVA, YudanshenVB, YudanshenVC, and YudanshenVD (Table 1).

Table 1. Origin and basic traits of *Salvia miltiorrhiza* seedlings tested.

Germplasm	Origin	Basic traits
YudanshenVA	Song Country, Henan, China	Plants erect, tall and compact. Stem green, less villous, leaflets dark green, obovate, thick and shiny, surface shriveled, margin circular serrate. The number of branches per plant is 2-7. The root is vermilion. The dry weight of the root per plant is 135.00 g, salvianolic acid B content is 4.30%, and total tanshinone content is 0.55%
YudanshenVB	Song Country, Henan, China	Plants erect, tall and compact. Stem green, leaflets dark green, lanceolate and glossy, surface shriveled and flat. The number of branches per plant is 2-5. The root is brick-red. The dry weight of the root per plant is 113.00 g, salvianolic acid B content is 4.34%, and total tanshinone content is 0.41%
YudanshenVC	Mianchi Country, Henan, China	Plants loose. Stem mostly green, less pubescence. Leaflets green and lanceolate, occasionally margin purple, surface significantly shriveled. The number of branches per plant is 2-4. The root is brick-red. The dry weight of the root per plant is 94.00 g, salvianolic acid B content is 3.27%, and total tanshinone content is 0.37%
YudanshenVD	Mianchi Country, Henan, China	Plants erect, tall and compact. Stem purple, long villous, leaflets dark green, obovate, thick and shiny, surface slightly shriveled, margin crenate, dark purple, petioles purple. The number of branches per plant is 3-5. The number of lateral roots is large. The root is vermilion. The dry weight of the root per plant is 82.00 g, salvianolic acid B content is 4.88%, and total tanshinone content is 0.42%

Salvia miltiorrhiza Bunge seeds were sterilized with 1% NaClO solution for 15 min after rinsing with distilled water. After that, 50 seeds of each germplasm were sown on 10 cm × 10 cm × 10 cm (length × width × height) plastic pots containing 1 kg soil mixture of 65% nutrient soil and 35% vermiculite. The experiment was arranged according to a randomized design with four replicates of 96 pots. Polyethylene glycol (PEG) concentrations of 5% were used to cause the effects of water stress. We used distilled water in control pots to ensure controls were not additionally stressed. After sowing, all pots were well-watered. Irrigation was conducted by watering each pot with 20 mL tap water at 2 d intervals. After seedling emergence, 150 mL distilled water containing the prescribed PEG-6000 treatment was added every 7 d, three times in total over the course of the experiment. The experiment was carried out in the light incubator where the temperature was maintained at 25 °C day and night, and the daylight length was 12 h (4000 lx).

Determination of indices and methods

Investigation and sampling were carried out 21 d after PEG-6000 treatment. In this study, a total of 14 traits were investigated. Plant height, leaf length and leaf width were measured with a measuring tape. Stem diameter were measured using digital calipers (ABS Digimatic caliper 500-197-30, Mitutoyo Corp., Kawasaki, Japan). We counted the number of leaves on each sample by hand. Maximum root length, root surface area, apical number, total root projection area, and mean root diameter were measured using a digital scanner (Perfection V750, Epson, Suwa, Japan) and WinRHIZO root analysis software (Regent Instruments, Quebec, Canada).

Extraction medium for superoxide dismutase (SOD), guaiacol peroxidase (POD) and catalase (CAT) consisted of 0.05 M phosphate buffer (pH 7.0). The homogenate was prepared by crushing 0.5 g fresh tissue in 5 mL ice-cold extraction medium in Grinder (JXFSTPRP-24, Shanghai jingxin, Shanghai, China). The extract was centrifuged at 12000 rpm for 20 min at 4 °C (centrifuge 5430R, Eppendorf, Hamburg, Germany), then supernatant was used for estimation of SOD, POD and CAT. The activity of SOD was determined by the method of nitro blue tetrazolium (Li, 2006), POD by the method of guaiacol (Wang, 2006). The CAT activity was measured by the ultraviolet absorption method (Wang, 2006). Soluble protein (SP) content was measured by Coomassie brilliant blue G-250 staining (Li, 2006). Determination of soluble sugar (SS) by anthrone colorimetry (Li, 2006). Proline (Pro) was determined by the ninhydrin method (Wang, 2006).

Statistical analysis

Drought tolerance coefficient (DC) and membership function value of each comprehensive index of each germplasm μ (X_{ij}) were obtained by Equations 1-4 in the statistical analysis (Toscano et al., 2017):

$$DC = \frac{\text{Measured value under drought stress}}{\text{Measured value under control}} \quad (1)$$

The ability of drought resistance for different germplasm was determined by the drought tolerance coefficient (DC) of single indicators, that can eliminate differences among varieties.

$$\mu(X_{ij}) = \frac{X_j - X_{jmin}}{X_{jmax} - X_{jmin}} \qquad j = 1, 2, 3, \ldots, n. \quad (2)$$

In Equation 2, X_j represents the j^{th} comprehensive index of each germplasm, X_{jmin} is the minimum value of j^{th} comprehensive index of each germplasm, and X_{jmax} is the maximum value of j^{th} comprehensive index of each germplasm.

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

In Equation 3, W_j is the importance of the j^{th} comprehensive indicator in all comprehensive indicators;

$$D = \sum_{j=1}^{n} [\mu(X_j) \times W_j] \qquad j = 1, 2, 3, \ldots, n. \quad (4)$$

In Equation 4, D value is the comprehensive evaluation value of drought tolerance of each germplasm under the condition of drought stress during the seedling stage of *S. miltiorrhiza.*

The statistics of data including ANOVA, principal component analysis and the membership function analysis were conducted by using SPSS 24.0 and Excel 2019. The correlation analysis figures were constructed using Origin 2022 (OriginLab Corporation, Northampton, Massachusetts, USA). The differences between the means were compared by Fisher's protected least significant difference test ($P < 0.05$).

RESULTS

Changes in aboveground agronomic characteristics, underground morphological characters, physiological indexes in response to drought stress

Table 2 indicates that the plant height of *S. miltiorrhiza* was inhibited by PEG treatments, Yudanshen VA, Yudanshen VC, and Yudanshen VD reached their minimum height at 20% PEG treatment, whereas Yudanshen VB at 15% PEG treatment. The percentage reductions in plant height compared to the control were 79.68%, 67.91%, 42.35%, and 62.54%, respectively. The stem thickness of both Yudanshen VA and Yudanshen VB was observed to be inhibited to varying degrees under PEG treatment, with a progressive reduction in thickness as the PEG concentration increased. However, for Yudanshen VC and Yudanshen VD, their stem thickness initially increased and then decreased. Specifically, the maximum stem thickness was observed at 5% PEG for Yudanshen VC and 10% PEG for Yudanshen VD, with increases of 72.00% and 43.48%, respectively. The effect of PEG treatment on leaf length and width was consistent across Yudanshen VA, Yudanshen VB, Yudanshen VC, and Yudanshen VD, where both parameters decreased in proportion to the level of drought stress induced by the PEG treatment. At 20% PEG concentration, leaf length of Yudanshen VA, Yudanshen VB, Yudanshen VC and Yudanshen VD, was significantly reduced by 73.49%, 66.13%, 50.41%, and 19.35%, and leaf width was significantly reduced by 82.93%, 60.83%, 44.83%, and 22.50%, respectively, compared to the control. Leaf number also decreased with increasing PEG concentration in all germplasms.

Table 2. Effects of drought stress on morphological indicators of the shoot at seedling stage. Different letters following the average values of various indicators corresponding to different treatments under the same type indicate significant differences at the 0.05 probability level. CK: Control group without PEG treatment.

Germplasm	PEG treatment	Plant height	Stem thickness	Leaf length	Leaf width	Number of leaves
		cm	mm	cm	cm	
Yudanshen VA	CK	7.58 ± 0.75^{a}	1.16 ± 0.15^{a}	3.32 ± 0.22^{a}	2.46 ± 0.26^{a}	7.20 ± 1.09^{a}
	5%	5.64 ± 0.56^{a}	0.95 ± 0.23^{bc}	2.10 ± 0.34^{b}	1.56 ± 0.09^{b}	5.80 ± 0.45^{b}
	10%	4.22 ± 0.55^{b}	1.00 ± 0.14^{ab}	1.28 ± 0.22^{c}	1.16 ± 0.23^{bc}	5.20 ± 1.10^{b}
	15%	2.20 ± 0.20^{c}	0.84 ± 0.12^{bc}	0.96 ± 0.22^{c}	0.86 ± 0.21^{c}	5.20 ± 0.84^{bc}
	20%	1.54 ± 0.52^{c}	0.67 ± 0.04^{c}	0.88 ± 0.08^{c}	0.42 ± 0.08^{d}	3.60 ± 0.89^{c}
Yudanshen VB	CK	6.62 ± 0.45^{a}	1.43 ± 0.26^{a}	3.10 ± 0.28^{a}	2.40 ± 0.25^{a}	6.60 ± 0.55^{a}
	5%	5.42 ± 0.51^{ab}	1.14 ± 0.18^{ab}	2.16 ± 0.46^{b}	1.64 ± 0.33^{b}	6.00 ± 0.23^{ab}
	10%	3.58 ± 0.33^{c}	0.99 ± 0.05^{b}	1.24 ± 0.05^{c}	1.30 ± 0.11^{bc}	5.40 ± 0.89^{bc}
	15%	2.48 ± 0.27^{d}	0.76 ± 0.05^{c}	1.10 ± 0.21^{c}	1.04 ± 0.19^{c}	5.00 ± 0.71^{c}
	20%	3.62 ± 0.27^{c}	0.86 ± 0.04^{bc}	1.05 ± 0.39^{c}	0.94 ± 0.15^{c}	3.80 ± 0.45^{d}
Yudanshen VC	CK	8.60 ± 1.29^{a}	0.75 ± 0.17^{bc}	2.46 ± 0.30^{a}	1.74 ± 0.18^{a}	7.00 ± 1.00^{a}
	5%	6.70 ± 0.36^{b}	1.29 ± 0.12^{a}	1.84 ± 0.25^{b}	1.52 ± 0.19^{ab}	6.60 ± 0.89^{a}
	10%	5.42 ± 0.32^{bc}	0.83 ± 0.02^{bc}	1.48 ± 0.20^{bc}	1.36 ± 0.15^{b}	5.40 ± 0.89^{a}
	15%	3.90 ± 0.43^{cd}	0.66 ± 0.17^{c}	1.30 ± 0.34^{c}	1.06 ± 0.29^{c}	5.60 ± 0.75^{a}
	20%	2.76 ± 0.52^{d}	0.88 ± 0.05^{b}	1.22 ± 0.28^{c}	0.96 ± 0.23^{cd}	4.80 ± 0.84^{a}
Yudanshen VD	CK	6.14 ± 0.56^{a}	0.69 ± 0.19^{c}	1.86 ± 0.23^{a}	1.60 ± 0.27^{a}	5.60 ± 0.89^{a}
	5%	5.04 ± 0.29^{b}	0.84 ± 0.08^{ab}	1.84 ± 0.33^{ab}	1.36 ± 0.17^{b}	5.50 ± 0.36^{a}
	10%	4.26 ± 0.40^{bc}	0.99 ± 0.02^{a}	1.70 ± 0.40^{ab}	1.34 ± 0.27^{b}	5.80 ± 0.45^{a}
	15%	3.70 ± 0.48^{cd}	0.91 ± 0.08^{a}	1.64 ± 0.23^{bc}	1.30 ± 0.21^{b}	4.20 ± 0.45^{b}
	20%	3.54 ± 0.58^{d}	0.90 ± 0.07^{a}	1.50 ± 0.17^{ab}	1.24 ± 0.22^{b}	4.80 ± 0.30^{b}

Table 3 shows that the PEG-simulated drought stress altered the root morphology and inhibited the root growth in *S. miltiorrhiza*. With the increase of PEG the maximum root length, root surface area, and the apical number of Yudanshen VA and Yudanshen VB decreased at 5% and 10% PEG, increased at 15% PEG, and significantly decreased by 60.19%, 86.91%, 76.72%, 83.63%, 31.15%, 67.23% compared with the control at 20% PEG. The maximum root length decreased with the increasing PEG stress, with the greatest decrease of 7.55% at 15% PEG concentration for Yudanshen VD and 81.40% at 20% PEG concentration for Yudanshen VC. The PEG-simulated drought stress significantly inhibited total root projection area of Yudanshen VA, Yudanshen VB, and Yudanshen VC, the greatest decrease was predictably observed under the most severe induced drought conditions, with significant reductions of 73.37%, 76.39% and 58.75%, respectively. The largest reduction of the maximum root length, root surface area, apical number and root projection area was all Yudanshen VB, which decreased by 86.91%, 83.63%, 67.23% and 76.39% respectively compared with the control, indicating that the root morphology of Yudanshen VB was most sensitive to PEG stress. The PEG-induced drought stress significantly inhibited the average root diameter of Yudanshen VB, Yudanshen VC and Yudanshen VD, this inhibition decreased proportionally with increasing concentrations of PEG. Under the most severe simulated drought conditions (20% PEG), average root diameter decreased by 55.17%, 66.67%, and 35.48% in Yudanshen VB, Yudanshen VC, and Yudanshen VD, respectively, compared to the control.

Table 3. Effects of drought stress on root system indicators of *Salvia miltiorrhiza* seedlings. Different letters following the average values of various indicators corresponding to different treatments under the same type indicate significant differences at the 0.05 probability level. CK: Control group without PEG treatment.

Germplasm	PEG treatments	Maximum root length	Root surface area	Apical number	Total root projection area	Mean root diameter
		mm	mm^2		mm^2	mm
Yudanshen VA	CK	60.74 ± 1.40^{a}	5.37 ± 0.18^{a}	269.67 ± 2.82^{a}	1.99 ± 0.06^{a}	0.29 ± 0.01^{a}
	5%	42.30 ± 4.03^{b}	2.74 ± 0.24^{c}	250.00 ± 2.83^{ab}	0.87 ± 0.08^{c}	0.21 ± 0.01^{b}
	10%	47.24 ± 5.73^{b}	2.18 ± 0.79^{c}	122.00 ± 3.93^{d}	0.69 ± 0.15^{c}	0.21 ± 0.01^{b}
	15%	62.07 ± 3.56^{a}	4.29 ± 0.53^{b}	225.26 ± 5.76^{b}	1.37 ± 0.17^{b}	0.22 ± 0.02^{b}
	20%	24.18 ± 1.01^{c}	1.25 ± 0.07^{d}	185.67 ± 1.32^{c}	0.53 ± 0.02^{c}	0.32 ± 0.04^{b}
Yudanshen VB	CK	170.52 ± 7.96^{a}	9.41 ± 0.12^{a}	495.33 ± 20.13^{a}	4.87 ± 0.04^{a}	0.29 ± 0.01^{a}
	5%	131.04 ± 12.78^{b}	8.64 ± 1.75^{a}	253.67 ± 3.20^{bc}	2.75 ± 0.56^{b}	0.21 ± 0.01^{b}
	10%	34.87 ± 3.69^{c}	2.64 ± 0.26^{b}	190.00 ± 3.50^{c}	1.84 ± 0.08^{c}	0.24 ± 0.01^{ab}
	15%	162.79 ± 4.64^{a}	9.78 ± 1.86^{a}	209.00 ± 11.25^{b}	3.11 ± 1.23^{b}	0.19 ± 0.01^{bc}
	20%	22.32 ± 1.85^{c}	1.54 ± 0.44^{b}	162.33 ± 1.37^{c}	1.15 ± 0.14^{c}	0.23 ± 0.02^{b}
Yudanshen VC	CK	137.86 ± 5.12^{a}	5.97 ± 0.09^{a}	301.90 ± 1.17^{a}	2.36 ± 0.03^{a}	0.60 ± 0.01^{a}
	5%	58.61 ± 2.51^{b}	3.85 ± 0.17^{b}	204.00 ± 4.38^{bc}	1.23 ± 0.07^{b}	0.22 ± 0.02^{b}
	10%	41.74 ± 4.18^{bc}	2.73 ± 0.29^{c}	173.00 ± 8.33^{bc}	0.97 ± 0.09^{bc}	0.21 ± 0.01^{b}
	15%	59.47 ± 3.21^{b}	3.73 ± 0.47^{b}	152.50 ± 6.50^{bc}	1.19 ± 0.02^{b}	0.20 ± 0.01^{b}
	20%	25.64 ± 1.08^{c}	2.99 ± 0.36^{bc}	264.33 ± 5.05^{b}	0.95 ± 0.13^{bc}	0.20 ± 0.02^{b}
Yudanshen VD	CK	42.50 ± 1.46^{c}	3.62 ± 0.20^{b}	168.33 ± 4.88^{c}	1.15 ± 0.06^{ab}	0.31 ± 0.08^{a}
	5%	62.22 ± 6.89^{a}	4.18 ± 1.24^{a}	216.33 ± 5.47^{a}	1.33 ± 0.10^{a}	0.21 ± 0.01^{b}
	10%	50.18 ± 2.07^{b}	3.66 ± 0.74^{a}	146.33 ± 4.89^{c}	1.17 ± 0.24^{ab}	0.23 ± 0.01^{b}
	15%	39.29 ± 6.49^{c}	2.40 ± 0.36^{c}	170.33 ± 15.77^{b}	0.76 ± 0.02^{c}	0.19 ± 0.01^{b}
	20%	41.85 ± 4.35^{bc}	2.67 ± 0.20^{c}	336.00 ± 12.92^{a}	1.09 ± 0.04^{b}	0.20 ± 0.01^{b}

From Table 4, under PEG treatment the SOD activity of Yudanshen VA increased compared to the control, while that of Yudanshen VB decreased. POD activity of all germplasms initially increased before decreasing with the increase of PEG concentration. The POD activities of Yudanshen VA, Yudanshen VC, and Yudanshen VD reached the maximum at 5% PEG concentration, increasing by 438.71%, 7.34% and 186.89%, respectively. The Yudanshen VB's largest increasing amplitude on POD activities was 114.29% at 10% PEG concentration. At different PEG concentrations, the CAT activities of both Yudanshen VA and Yudanshen VB were higher than the control while Yudanshen VC and Yudanshen VD were lower than the control. The results may suggest that PEG stress induced upregulated CAT activities in the Yudanshen VA and Yudanshen VB to endure oxidative damage caused by drought-induced production of reactive oxygen species. The CAT activities of Yudanshen VB and Yudanshen VC under all PEG treatments were significantly different from the well-watered control plants. Soluble sugar and soluble protein content increased gradually with increasing drought stress, although both metrics ultimately decreased in the most severely drought-stressed plants receiving a 20% PEG treatment. The highest increase in soluble sugar (SS) and soluble protein (SP) content both belonged to Yudanshen VC which showed a 479.64% (15% PEG) and 282.43% (20% PEG) significant increase, respectively, in comparison with the control. Except for Yudanshen VD, the SP content of all germplasm were significantly higher than the control. The Pro content of the leaves of all four *S. miltiorrhiza* cultivation types was higher than normal under PEG stress, and all showed an increasing trend with the intensification of drought. It shows that *S. miltiorrhiza* can alleviate membrane lipid damage and enhance its water absorption capacity by increasing Pro content under drought stress.

Table 4. Effects of drought stress on physiological traits of *Salvia miltiorrhiza* at seedling stage. Different letters following the average values of various indicators corresponding to different treatments under the same type indicate significant differences at the 0.05 probability level. SOD: Superoxide dismutase activity; POD: peroxidase activity; CAT: catalase activity; SP: soluble protein content; SS: soluble sugar content; Pro: proline content.

Germplasm	PEG treatment	SOD	POD	CAT	SP	SS	Pro
		u g^{-1} min^{-1}			mg g^{-1}	μg mL^{-1}	μg g^{-1}
Yudanshen VA	CK	106.557 ± 4.279^{d}	137.503 ± 58.803^{b}	98.519 ± 6.974^{b}	15.982 ± 1.808^{b}	8.656 ± 0.226^{c}	4.397 ± 0.173^{e}
	5%	262.595 ± 12.067^{c}	740.741 ± 26.029^{a}	113.704 ± 6.820^{b}	42.899 ± 3.689^{a}	9.074 ± 0.453^{c}	5.046 ± 0.001^{de}
	10%	440.801 ± 5.540^{a}	505.655 ± 81.305^{a}	103.704 ± 20.859^{b}	36.871 ± 3.876^{a}	10.243 ± 0.986^{bc}	8.260 ± 0.017^{c}
	15%	363.388 ± 5.276^{b}	133.067 ± 20.132^{b}	128.889 ± 11.192^{b}	41.123 ± 0.873^{a}	12.396 ± 0.986^{b}	9.046 ± 0.089^{b}
	20%	354.281 ± 5.485^{b}	135.285 ± 15.525^{b}	142.963 ± 3.533^{a}	47.619 ± 5.279^{a}	14.732 ± 0.392^{a}	12.431 ± 0.089^{a}
Yudanshen VB	CK	344.262 ± 22.364^{a}	232.868 ± 10.096^{b}	88.889 ± 5.609^{c}	16.122 ± 16.122^{b}	3.870 ± 0.679^{d}	0.978 ± 0.034^{d}
	5%	289.617 ± 32.213^{ab}	319.361 ± 9.464^{ab}	115.556 ± 9.814^{b}	36.217 ± 7.730^{a}	4.549 ± 0.392^{d}	1.251 ± 0.001^{d}
	10%	285.064 ± 36.566^{ab}	499.002 ± 16.236^{a}	148.148 ± 10.760^{a}	41.077 ± 2.444^{a}	11.564 ± 0.599^{c}	1.713 ± 0.059^{c}
	15%	182.149 ± 60.734^{b}	137.503 ± 3.998^{c}	172.593 ± 17.324^{a}	42.245 ± 1.607^{a}	14.958 ± 0.599^{b}	12.379 ± 0.286^{b}
	20%	289.617 ± 22.750^{ab}	190.730 ± 8.737^{b}	143.704 ± 10.784^{a}	46.684 ± 5.197^{a}	20.389 ± 0.226^{a}	24.875 ± 0.140^{a}
Yudanshen VC	CK	217.668 ± 78.340^{ab}	392.548 ± 17.097^{a}	159.259 ± 7.689^{a}	11.963 ± 8.169^{b}	5.001 ± 0.816^{d}	6.636 ± 0.180^{d}
	5%	157.559 ± 43.668^{b}	421.379 ± 11.397^{a}	111.852 ± 5.740^{b}	32.945 ± 3.132^{a}	5.231 ± 0.226^{d}	7.456 ± 0.078^{c}
	10%	389.799 ± 44.654^{a}	223.996 ± 12.765^{b}	77.037 ± 1.949^{c}	40.609 ± 1.239^{a}	9.980 ± 1.708^{c}	9.405 ± 0.001^{b}
	15%	159.381 ± 66.503^{b}	197.383 ± 9.482^{b}	68.148 ± 2.634^{c}	35.329 ± 1.637^{a}	10.988 ± 0.391^{b}	12.294 ± 0.045^{b}
	20%	315.118 ± 68.567^{ab}	361.499 ± 3.356^{a}	114.444 ± 7.551^{b}	45.750 ± 2.391^{a}	16.090 ± 0.857^{a}	17.644 ± 0.017^{a}
Yudanshen VD	CK	266.849 ± 3.970^{b}	167.753 ± 15.788^{c}	129.740 ± 11.418^{b}	41.778 ± 3.126^{a}	6.002 ± 0.816^{d}	1.570 ± 0.062^{d}
	5%	230.419 ± 60.570^{b}	481.260 ± 18.886^{a}	105.185 ± 15.286^{b}	38.647 ± 8.431^{a}	8.001 ± 0.226^{d}	3.508 ± 0.030^{c}
	10%	347.905 ± 51.992^{a}	246.174 ± 12.734^{b}	104.074 ± 22.236^{b}	45.282 ± 1.439^{a}	9.980 ± 1.428^{c}	3.644 ± 0.017^{c}
	15%	244.080 ± 50.141^{b}	419.162 ± 10.393^{a}	177.778 ± 12.341^{a}	46.311 ± 1.331^{a}	18.918 ± 0.392^{a}	5.491 ± 0.045^{b}
	20%	367.942 ± 59.950^{a}	252.828 ± 5.323^{b}	79.259 ± 5.958^{c}	45.937 ± 4.533^{a}	16.090 ± 0.784^{b}	18.807 ± 0.017^{a}

Optimal PEG concentration for simulated drought stress at seedling stage and correlation analysis

Most of the 16 indices measured in this study reached significant differences from the control at 10% PEG concentration, therefore, 10% was the optimum PEG concentration for identifying drought resistance in *S. miltiorrhiza* seedlings. The drought tolerance coefficient of each single character was calculated by the results of each single character of *S. miltiorrhiza* under drought stress (10% PEG) and control treatments (Table 5). It can be seen from Table 5 that the variation coefficient of the relative values of the 16 indexes measured varies from 11.33% ~ 139.14%, which indicates that the selected materials of *S. miltiorrhiza* are rich and representative.

For the correlation coefficient matrix of every single indicator (Figure 1), there is a very significant correlation between plant height and stem diameter, leaf length was significantly correlated with plant height and stem thickness, number of leaves and root surface were significantly correlated, root surface area and apical number were significantly correlated, mean root diameter was significantly correlated with plant height, stem diameter, leaf length and apical number, SOD activity was significantly correlated with number of leaves, root surface area. There was a correlation between every two indices, resulting in overlapping of the information they provided. At the same time, the function of every single indicator under drought stress was also different, the evaluation of cotton drought resistance was not accurate by using every single indicator directly. The responses of every single indicator of drought stress were also different, there is an antagonistic and synergistic relationship between growth and physiological indicators. The evaluation of *S. miltiorrhiza* drought resistance was not accurate by using every single indicator directly. To make up for the deficiency of drought resistance evaluation of each indicators, it is necessary to further use membership function analysis, stepwise regression analysis, and correlation analysis for comprehensive evaluation.

Table 5. Drought resistance coefficients of four *Salvia miltiorrhiza* germplasm.

Indicators	Yudanshen VA	Yudanshen VB	Yudanshen VC	Yudanshen VD	Mean	Coefficient of variation (%)
Plant height	0.56	0.54	0.63	0.69	0.61	11.33
Stem thickness	0.86	0.69	1.11	1.43	1.02	31.47
Leaf length	0.39	0.40	0.60	0.91	0.58	42.33
Leaf width	0.47	0.54	0.78	0.84	0.66	27.39
Number of leaves	0.72	0.82	0.77	1.04	0.84	16.84
Maximum root length	0.78	0.20	0.30	1.18	0.62	73.79
Root surface area	0.41	0.28	0.46	1.01	0.54	59.70
Apical number	0.45	0.38	0.57	0.87	0.57	38.13
Total root projection area	0.35	0.38	0.41	1.02	0.54	59.43
Mean root diameter	0.72	0.83	0.35	0.74	0.66	32.14
SOD activity	4.14	0.83	1.79	1.30	2.02	72.95
POD activity	3.68	2.14	0.57	1.47	1.97	66.76
CAT activity	1.05	1.67	0.48	0.80	1.00	50.39
SP content	2.31	2.55	3.39	1.08	2.33	40.93
SS content	1.18	2.99	2.00	1.66	1.96	39.14
Pro content	1.96	1.76	1.42	2.32	1.87	139.14

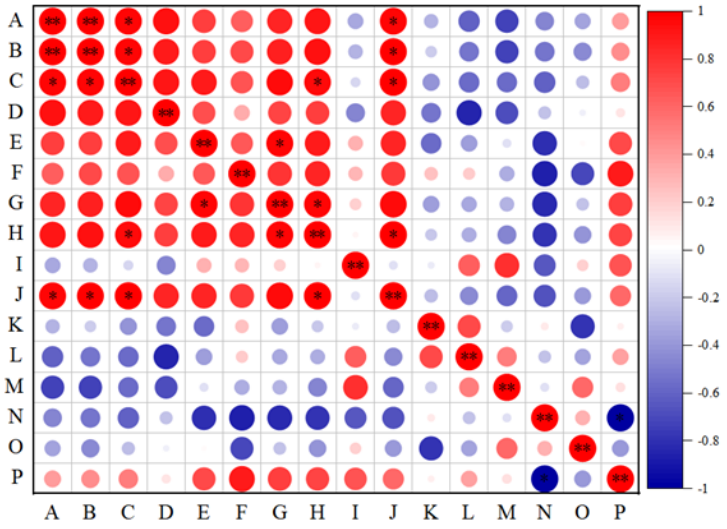

Figure 1. Correlation analysis of drought resistance coefficient of various indicators under drought stress. **Correlation is significant at the 0.01 level (2-tailed), *Correlation is significant at the 0.05 level (2-tailed); A: Plant height; B: stem thickness; C: leaf length; D: leaf width; E: number of leaves; F: maximum root length; G: root surface area; H: apical number; I: total root projection area; J: mean root diameter; K: superoxide dismutase activity; L: guaiacol peroxidase activity; M: catalase activity; N: soluble protein content; O: soluble sugar content; P: proline content.

Identification of drought resistance and screening of drought resistance indicators

The drought resistance coefficient of all indexes is further analyzed by principal component analysis (Table 6), contribution rates of the first two comprehensive indicators (PC1, PC2,) were 60.328%, and 21.725%, respectively, and their cumulative contribution rate was 82.035%. In this way, the original 16 single indices can be converted into two new independent comprehensive indices, and 82.035% of the information carried by original indices could be represented. As shown in Table 7, the first principal component has three characteristics that acceptably correlated with component: Plant height, stem thickness, leaf length, root surface area and apical number. So, the first principal component may be defined as comprehensive indicators of the aboveground morphology and root growth. The second principal component has two characteristics: SP content and SOD activity, it can be defined as comprehensive indicators of osmoregulation substances and antioxidant enzyme activity.

Table 6. Eigenvalues and contribution rate of principal components.

Principal components	Initial Eigenvalues		
	Eigenvalues	Contribution rate	Cumulative contribution rate
		%	%
1	9.414	58.835	58.835
2	3.889	24.306	83.141

Table 7. Eigenvector matrix by principal component analysis. SOD: Superoxide dismutase activity; POD: peroxidase activity; CAT: catalase activity; SP: soluble protein content; SS: soluble sugar content; Pro: proline content.

Indicators	Principal component	
	1	2
Plant height	0.311	-0.142
Stem thickness	0.315	-0.099
Leaf length	0.321	-0.076
Leaf width	0.271	-0.278
Number of leaves	0.288	0.081
Maximum root length	0.258	0.265
Root surface area	0.323	0.063
Apical number	0.325	-0.026
Total root projection area	0.315	0.088
Mean root diameter	-0.015	0.451
SOD activity	-0.090	0.186
POD activity	-0.139	0.430
CAT activity	-0.174	0.291
SP content	-0.232	-0.347
SS content	-0.120	-0.130
Pro content	0.208	0.390

The membership function value was calculated by using the comprehensive index value (PC1, PC2) of the drought tolerance of the four *S. miltiorrhiza* germplasm resources at seedling stage (Equation 2). According to the contribution rate of each comprehensive index, the weights of the two comprehensive indices were calculated successively, which were 58.835% and 24.305%, respectively (Equation 3). Then, the comprehensive membership function value (D value) is obtained by adding the product of membership function value and weight (Equation 4). The drought tolerance could be evaluated by comparing the D value of each germplasm, usually the greater the D value, the stronger the drought resistance. According to D value, drought resistance of *S. miltiorrhiza* germplasm was ranked, Yudanshen VD > Yudanshen VA > Yudanshen VB > Yudanshen VC (Table 8). The D value of Yudanshen VD is the largest, which is 0.922, indicating that this germplasm has the strongest drought resistance among the tested germplasm, and the D value of Yudanshen VC is the smallest, which is 0.192, indicating that its drought resistance is the weakest.

Table 8. Membership function analysis of each indicator.

Germplasm	PC1	PC2	FC1	FC2	D value	Rank
Yudanshen VA	0.668	1.990	0.180	1.000	0.501	2
Yudanshen VB	0.226	1.007	0.000	0.625	0.245	3
Yudanshen VC	1.001	-0.632	0.315	0.000	0.192	4
Yudanshen VD	2.683	1.469	1.000	0.801	0.922	1
Weight, %			62.496	37.501		

A stepwise regression analysis of 16 indexes was carried out to accurately predicted drought resistance *S. miltiorrhiza* germplasm. Stepwise regression allows for identification of potential candidates which may suggest or identify drought resistance. The regression used drought tolerance (D value) as the dependent variable and the drought tolerance coefficient of every single indicator as the independent variable: $D = 0.887X_1 - 0.003X_2 + 0.142X_3$ ($R^2 = 0.999$), and in this formula, X_1, X_2, X_3 represented maximum root length, root surface area, SS content, respectively. It can be inferred from the equation, that among the 16 single indexes, the above three indexes significantly influenced the drought tolerance of *S. miltiorrhiza* at seeding stage. So, these three indexes could be determined selectively to evaluate differences in drought tolerance.

DISCUSSION

The seedling stage is the most sensitive stage for plants to water stress. If drought occurs at this time, it will have adverse effects on subsequent growth, development, yield and quality, and even cause death, plants will respond to drought stress through morphological, physiological and metabolic changes and utilize various mechanisms to reduce the negative effects of stress (Ravelombola et al., 2020). The effect of stress on morphological traits is one of the most noticeable effects of drought stress. Li et al. (2022a) found through experiments that plant height, leaf number and other indicators are sensitive to water and can be used as representative indicators for drought resistance identification and analysis. In this study, the plant height, leaf length, leaf width, the number of leaves of the four *S. miltiorrhiza* germplasm resources were significantly inhibited under PEG stress. This change is conducive to maintaining the balance between water absorption organs and water evaporation organs, and reducing water loss in plants. Other scientists have similar reports such as Borges et al. (2016) that showed that water limitation significantly affected the development of aerial parts of parsley. Among the five morphological traits studied in this study, plant height and leaf length were most affected by drought stress, indicating that the impact of drought on phenotype mainly manifests as inhibiting plant height and reducing leaf area.

When water became the limiting factor for plant growth, the roots first sense water deficit, then a variety of responses in plants would occur to resist drought, including increased root-to-shoot ratio, enhanced fine root growth, deeper taproots, and accumulation of solute, so as to maintain their functional behaviors (Du et al., 2020). Pirnajmedin et al. (2015) found that there is a significant positive correlation between root dry weight, root volume, total root absorption area, and drought resistance of varieties. In this study, under drought stress the root growth of tested materials was inhibited, total root projection area, mean root diameter, maximum root length, root surface area and apical number decreased compared with the control. Krouma (2010) found similar results, they reported that root fresh and dry weight remarkably decreased with increase in drought stress in *Cicer arietinum*. However, Wang et al. (2020) found that root length, lateral root number, root surface area, root volume, and root-canopy ratio increased with the PEG-simulated drought stress. The different results may be due to the fact that mild drought stimulates plant root growth, which is inhibited when drought is severe and exceeds the self-regulatory capacity of the plant. Drought resistant germplasm Yudanshen VD adapts to water stress by increasing the maximum root length, root surface area, number of root tips, and total root projection area under mild drought stress (5%, 10% PEG), preferentially allocate biomass to roots to maintain root growth to beneficial toward increasing water absorption.

Production of reactive oxygen species under drought stress has deleterious effects on plant cells. The accumulation of superoxide radicals, hydrogen peroxide and hydroxyl radicals cause induction of lipid peroxidation, denaturation of protein, inactivation of enzymes, destruction of nucleic acids and damage of the cell membrane (Fathi and Tari, 2016). Plants under drought stress usually regulate a series of physiological and biochemical reactions to resist the damage caused by stress. This study shows that POD activity, CAT activity, SS content and SP content of *S. miltiorrhiza* increased under drought stress to reduce active oxygen damage and maintain cellular turgor pressure. Drought tolerant germplasm can maintain high POD and CAT activities in arid environments. A similar trend was observed by Zhang et al. (2018) in American ginseng. Similarly, high activities of antioxidant enzymes also improved drought tolerance of

cultivars of three evergreen shrubs (Xu et al., 2022), Lanzhou lily (Li et al., 2022b). *Salvia miltiorrhiza* can increase osmoregulation substances such as soluble sugar and soluble protein, resulting in the decrease of tissue water potential, so as to maintain its own cell turgor, to ensure the normal operation of its own physiological and metabolic activities.

Drought stress is an important environmental limiting factor that affects the physiological and biochemical reactions of medicinal plants and changes the process of secondary metabolism of plants. Deng et al. (2021) found that the content of soluble protein, soluble sugar, and proline showed an upward trend with increasing drought stress, while the content of malondialdehyde significantly increased. The activity of antioxidant enzymes (SOD, POD, and CAT) increased with increasing drought stress. In addition, mild drought was conducive to the accumulation of phenolic acids and tanshinone active components. Breeding excellent varieties of *S. miltiorrhiza* with strong drought resistance is also an effective way to improve yield and quality. Evaluating drought resistance of different germplasm reasonably, and screening for drought resistance identification indices, are helpful to tap drought-tolerant germplasm resources and cultivate drought tolerant varieties, which is of great significance to ensure the stability of yield and quality of *S. miltiorrhiza* in drought-prone areas.

Crop drought tolerance evaluation was a procedure identifying, screening, evaluating and classifying different varieties with varied drought tolerance capacities. Drought tolerance of plants is often a complex quantitative trait controlled by multiple genes, the evaluation of drought tolerance of plants using single character cannot reflect the drought resistance of varieties comprehensively and accurately (Bo et al., 2017). In recent years, the drought comprehensive evaluation had already been widely applied to wheat, corn, soybean and other crops (Bibi et al., 2012; Du et al., 2020).

At present, drought resistance of crops is mainly identified by soil drought method and hypertonic solution simulated drought method, and the latter is widely used because of its simple operation, time and effort saving, and relatively accurate control of stress levels. The selection of appropriate concentration of PEG is necessary to study the drought resistance of different crops and different developmental periods. In this study, different concentrations of PEG solution were used to analyze the drought resistance of *S. miltiorrhiza* seedlings. It was found that the drought resistance of the test materials in the 10% treatment group was significantly different, and 10% was taken as the optimal PEG concentration for the drought resistance identification of *S. miltiorrhiza* seedlings.

Principal component analysis can reduce multiple variables to several potential factors without little loss of information as much as possible. Bedane et al. (2015) found that the 11 traits can be explained by two comprehensive components (PC1 and PC2). They found that PC1 is mainly the three indicators of plant height, ear length and biomass, and PC2 is mainly the number of tillers per plant and grain yield. The membership function method can eliminate the one-sidedness caused by individual indicators and make the drought resistance of all tested materials comparable, and also integrate drought resistance coefficients (DC) of different traits (Nouri-Ganbalani et al., 2009), which can effectively reflect the comprehensive performance of plants under drought stress. At present, membership function analysis combined with principal component analysis or correlation analysis and stepwise regression analysis has been widely applied in the study of crop drought. In research on the drought resistance of irises, principal component analysis is combined with the membership function analysis to identify "Purple Flower" and "Bloodstone" had the highest drought resistance capability (Bo et al., 2017). Song et al. (2017) used membership function value of drought tolerance to screen wheat materials with strong drought resistance. Thus, measuring the 16 morphological and physiological indicators related to drought resistance of *S. miltiorrhiza* in this study, using principal component analysis, 16 single indices were converted into two comprehensive indices, combined with membership function analysis, the comprehensive evaluation value of drought resistance (D value) was applied to evaluate four *S. miltiorrhiza* germplasm comprehensively.

When evaluating the drought resistance of plants, the selection of suitable evaluation indexes is a key factor for accurate evaluation. At present, the evaluation of plant drought resistance is mainly carried out in terms of phenotypic indicators, physiological and biochemical indicators, and yield traits. In research on the drought resistance of irises, principal component analysis is combined with regression analysis to screen out the water

loss rate, POD activity, malondialdehyde content (Bo et al., 2017). In this study, PEG-6000 was used to simulate drought stress to identify the drought resistance of four *S. miltiorrhiza* germplasm resources at seedling stage, and 10 indexes such as plant height, stem diameter, leaf length, leaf width, maximum root length, SOD activity, and SS content were measured. Finally, the optimal regression equation of the result table obtained by establishing the optimal regression equation of drought resistance: $D = 0.887X_1 - 0.003X_2 + 0.142X_3$, three single indices namely, maximum root length, root surface area, SS content, were selected. Plant height, effective fruit branch number, single boll weight, transpiration rate and chlorophyll were selected as the key indicators to evaluate the drought tolerance of cotton (Sun et al., 2021). Ma (2018) suggested that root-to-crown ratio, root length, and root surface area were more representative in seedling drought stress, which was generally consistent with the results of this study.

CONCLUSIONS

The 10% PEG 6000 stress treatment of *Salvia miltiorrhiza* seedlings can be used for simple and rapid screening of drought resistance in *S. miltiorrhiza* germplasm. Under PEG-induced drought stress, different *S. miltiorrhiza* germplasms exhibited varying degrees of response to drought stress. At 5% PEG and 10% PEG, *S. miltiorrhiza* can reduce its own drought stress damage by regulating the activity of antioxidant enzymes and increasing the content of osmoregulation substances, thereby improving its drought resistance, indicating that *S. miltiorrhiza* has a certain degree of drought resistance.

Based on the principal component analysis of the drought tolerance coefficients of 16 morphological and physiological indicators of the *S. miltiorrhiza*, the original single characters related to drought tolerance were transformed into two independent comprehensive indicators, representing 83.141% of the total related data information in this experiment. The comprehensive evaluation value (D value) of each germplasm was calculated by means of the membership function. The Yudanshen VD germplasm has the strongest drought resistance among the tested germplasm. By stepwise regression analysis, three drought resistance indicators were selected, such as maximum root length, root surface area, and the optimal regression equation was established, which made the prediction quicker and more convenient for *S. miltiorrhiza* difference in drought tolerance.

Author contributions

Conceptualization: H.Z. Methodology: H.Z., L.Z. Software: L.Z., M.L., P.M., H.H. Validation: P.M., H.H. Formal analysis: H.Z., L.Z., M.L. Investigation: L.Z., M.L., P.M., H.H. Resources: H.Z. Data curation: H.Z., Z.W. Writing-original draft: H.Z., L.Z. Writing-review & editing: H.Z., Z.W., F.Y. Visualization: H.Z. Supervision: Z.W., F.Y. Project administration: H.Z. Funding acquisition: H.Z. All co-authors reviewed the final version and approved the manuscript before submission.

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