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Evaluation of genetic diversity and drought tolerance among thirty-three dichondra (*Dichondra repens*) genotypes

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Abstract

Dichondra (*Dichondra repens*) is an important ground cover plant and is also used as a herbal medicine in China. Objectives of this study were to evaluate phenotypic and genetic diversities among 33 genotypes by using 18 simple sequence repeat (SSR) markers and to further identify the drought tolerance of these germplasms based on five physiological parameters. Results showed that natural variations in phenotypes including plant height, leaf area, leaf thickness, and petiole length were observed among 33 genotypes under well-watered conditions. All 18 SSR primer pairs were found to be polymorphic and significant genetic variation was found in these genotypes. In addition, there were obvious differences in leaf relative water content (RWC), electrolyte leakage (EL), chlorophyll (Chl) content, photochemical efficiency (Fv/Fm), and performance index on absorption basis (PI_{ABS}) among 33 genotypes in response to a prolonged period of drought stress (46 d). Drought tolerance of 33 genotypes was then ranked by using subordinate function value analysis (SFVA) and the most drought-tolerant or -sensitive genotypes were identified as Dr5 or Dr29, respectively. Principal component analysis (PCA) further classified 33 genotypes into group I (drought-tolerant), group II (drought-sensitive), and group III (medium types). Current findings showed that 18 selected SSR primers could be potentially used to analyze genetic diversity and varietal identification in dichondra species. Drought-tolerant wild dichondra resources provide a rich genetic base for breeding of new cultivars.

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INTRODUCTION

Dichondra (*Dichondra repens*) is a perennial convolvulaceous plant that is wildly used as a ground cover for landscaping, ecological restoration, and weed control due to its ability to form a dense and low-growing sward[1,2]. Previous studies have demonstrated that dichondra was able to establish a denser greensward for weed suppression than other ground cover plants such as creeping red fescue (*Festuca rubra*) and white clover (*Trifolium repens*) in an apple orchard[3,4], but did not cause reduction in fruit yield[5]. In addition, dichondra is also a main constituent in many traditional herbal beverages in China and its extracts including *n*-butanol, vanillin, umbelliferone, and scopoletin exhibit antinociceptive effect, antibacterial activity, and anti-inflammation for treatment of icterohepatitis, dysentery, hydrops, or other diseases[6–8]. There are more than five species of the genus *Dichondra* in the world and most of them are distributed in the Americas. Up to now, only one wild species is found in China[8]. Requirement for new dichondra cultivars to be used for park and home landscaping is increasing in virtue due to their creeping growth habit and no need for mowing. However, the breeding of dichondra species is far behind other ground cover plants.

Global warming aggravates the frequency of extreme weather events such as high temperature and drought worldwide. Drought stress causes a lack of water availability in plants resulting in growth retardation and a decline in utility value[9]. Screening and evaluation of relative drought-tolerant genotypes play pivotal roles in breeding for stress-tolerant new cultivars. Multiple molecular markers including microsatellite or simple sequence repeat (SSR), restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), and random-amplified polymorphic DNA (RAPD) markers have been applied for selection and evaluation of diverse plant resources[10]. Among them, SSR markers exhibit outstanding characteristics of chromosome-specific location, co-dominant inheritance, and better interspecific transferability, and has become an important tool for molecular breeding[11]. Earlier studies by Varshney et al. and Powell et al. proved that SSRs were found to be more polymorphic than other molecular markers[12,13]. Kumar et al. reviewed the importance of SSR markers for molecular breeding of salt-tolerant *Brassica* genotypes[14]. Maqbool et al. evaluated drought tolerance of 40 chickpea (*Cicer arietinum*) genotypes based on the change in seed yield and genetic diversity *via* SSR markers, which provided basic information for breeding of drought-tolerant chickpea genotypes[15].

Understanding of genetic diversity and drought tolerance of different dichondra genotypes could help geneticists or breeders to interpret germplasm architecture or breed new cultivars. In addition, selection and utilization of drought-tolerant dichondra genotypes could be propitious to decrease in maintenance and management costs in the field. Objectives of this study were to evaluate morphological variation, genetic diversity *via* SSRs, and drought tolerance based on changes in five physiological parameters including leaf relative water content (RWC), electrolyte leakage (EL), chlorophyll (Chl), photosystem II photochemical efficiency (Fv/Fm), and

performance index on absorption basis (PI_{ABS}) of 33 dichondra genotypes (three cultivars and 30 wild genotypes collected from southwest China). These physiological parameters have been widely used for evaluating stress tolerance in various plant species, since they indicate water status, cell membrane stability, and photosynthetic capacity[16–19]. Current findings will provide potential materials for breeding program and further exploration of drought-resistant mechanism by using drought-tolerant and -sensitive dichondra genotypes.

RESULTS

Changes in morphological characteristics and genetic diversity among 33 dichondra genotypes

Figure 1 showed leaf sizes among 33 genotypes under normal conditions. There were significant variations in plant height, leaf area, leaf thickness, and petiole length among 33 genotypes (Fig. 2a–d). Dr9 exhibited the highest plant height and the greatest leaf area, whereas Dr12 showed the shortest plant height and Dr28 had smallest leaf area compared to other genotypes (Fig. 2a & b). The value of leaf thickness of all genotypes was more than 0.1 mm except Dr20 (Fig. 2c). The biggest value of leaf thickness was also observed in Dr9 (Fig. 2c). Dr26 and Dr28 had smaller petiole lengths than other genotypes under normal condition (Fig. 2d). Table 1 showed amplification results using 18 SSR primers. A total of 256 bands were amplified by these SSR primers and the total number of polymorphic bands reached 228 (Table 1). Primer C24 or IBM13 exhibited the highest or lowest polymorphism information content (PIC) than other primers, respectively (Table 1). Cluster analysis found that the average variation range of genetic similarity coefficient was from 0.56 to 0.89 among 33 genotypes (Fig. 3). New cultivars 'Xiaoshao' (Dr32) and 'Duliujiang' (Dr33) had closer genetic relationship, and commercial cultivar 'Silver Falls' (Dr30) showed closer genetic relationship with Dr31 (Fig. 3).

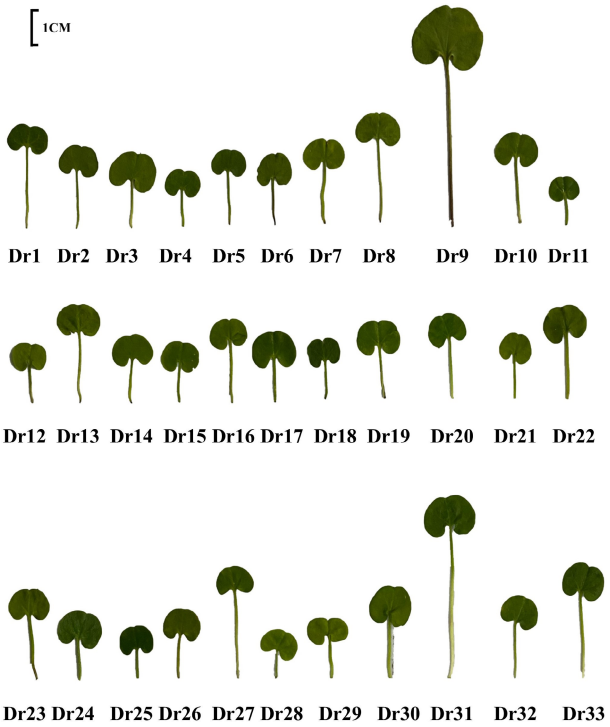

Fig. 1 Phenotypic differences in leaves of 33 *Dichondra repens* genotypes under normal conditions.

Changes in physiological parameters among 33 dichondra genotypes in response to drought stress

Dr29 completely died after 46 d of drought stress, so no physiological parameters were detected (Figs 4 & 5). Obvious variations in RWC and EL among 33 genotypes were observed, as demonstrated by drought stress index (DSI) of RWC and EL (Fig. 4a & b). Dr5, Dr18, and Dr33 showed higher DSI of RWC than other genotypes, and smallest DSI of RWC was detected in Dr8 and Dr27 (Fig. 4a). Dr9 or Dr33 had the biggest or smallest DSI of EL than other genotypes, respectively (Fig. 4b). Dr5, Dr9, Dr3, and Dr4 exhibited higher DSI of Chl as compared to other genotypes, whereas Dr28, Dr27, Dr20, and Dr30 had lower DSI of Chl than other genotypes (Fig. 5a). DSI of Fv/Fm of Dr1, Dr2, Dr3, Dr4, or Dr5 was greater than 1.0, but DSI of Fv/Fm of Dr8,

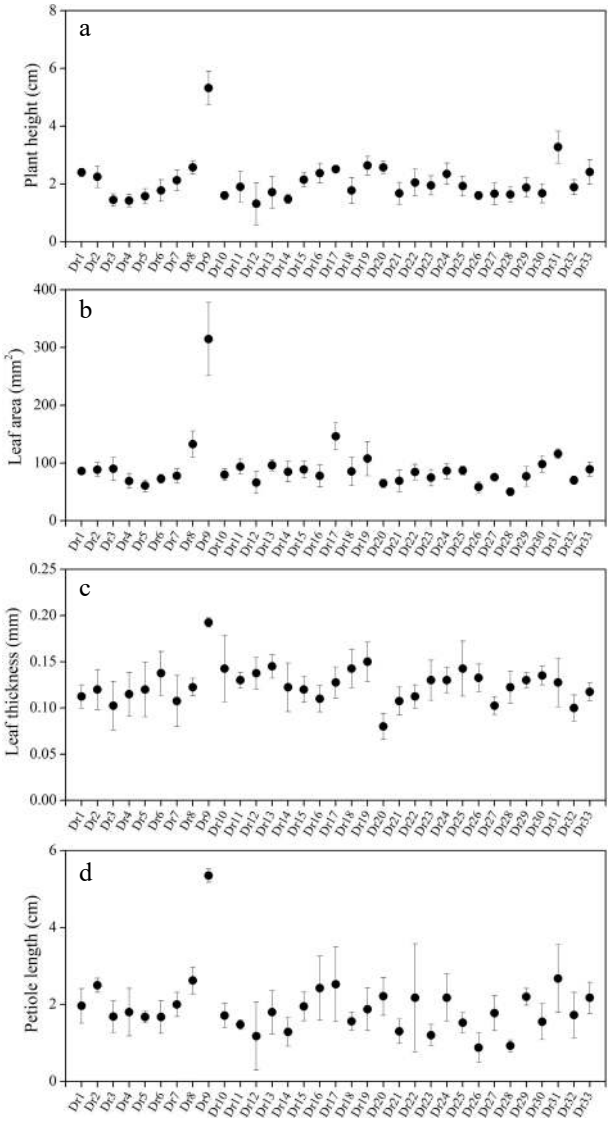

Fig. 2 Differences in (a) plant height, (b) leaf area, (c) leaf thickness, and (d) petiole length among 33 *Dichondra repens* genotypes under normal conditions. Vertical bars represent standard errors of the mean (n = 10).

Table 1. Amplification results among 33 *Dichondra repens* using different SSR primers.

Primer name	Total number of amplified bands	Number of polymorphic bands	PPB (%)	*PIC*
C24	18	18	100.00	0.338
C27	19	19	100.00	0.295
C30	17	9	52.94	0.163
C33	13	13	100.00	0.314
C51	11	10	90.91	0.354
C60	18	18	100.00	0.193
C66	13	13	100.00	0.342
C67	8	8	100.00	0.230
C71	23	19	82.61	0.230
Z25	21	13	62.90	0.163
Z37	3	3	100.00	0.266
Z57	19	16	84.21	0.266
Z69	9	8	88.89	0.215
Z113	16	16	100.00	0.292
Z135	11	11	100.00	0.279
SSR11	12	12	100.00	0.305
IBM13	4	4	100.00	0.111
IBM445	21	18	85.71	0.292
Total	256	228	—	—
Average	14.22	12.67	91.59	0.258

Dr14, or Dr27 was less than 0.5 (Fig. 5b). DSI of Fv/Fm of other genotypes ranged from 0.5 to 1.0 (Fig. 5b). The highest DSI of PI_{ABS} was detected in Dr5, and DSI of PI_{ABS} of Dr7, Dr8, Dr14, or Dr27 were close to 0.0 (Fig. 5c).

Comprehensive evaluation of drought tolerance among 33 dichondra genotypes

Drought tolerance among 33 genotypes was evaluated synthetically based on subordinate function value (SFV) (Table 2). Dr5 had the largest SFV of RWC, and the second or third largest SFV of RWC was found in Dr18 and Dr33, respectively. Dr33 (top), Dr31 (second), and Dr32 (third) showed bigger SFV of EL than other genotypes (Table 2). Maximum SFV of Chl was detected in Dr5. The top three genotypes with bigger SFV of Fv/Fm than other genotypes in the sequences were Dr3, Dr5, and Dr4. Dr5 had the maximum SFV of PI_{ABS} as compared to other genotypes, whereas Dr27 exhibited the minimum SFV of PI_{ABS} than other genotypes except Dr29. Comprehensive ranking showed Dr29, Dr28, and Dr27 exhibited lower drought tolerance than other genotypes. Out of the 33 genotypes, drought tolerance of Dr5, Dr33, or Dr3 ranked first, second, or third, respectively (Table 2). Heat map showed variations in five physiological parameters among 33 genotypes in response to drought stress (Fig. 6a). 33 genotypes could be divided into three groups based on principal component analysis (PCA) analysis (Fig. 6b). The first group included eight genotypes (Dr5, Dr33, Dr9, Dr1, Dr2, Dr32, Dr3, and Dr4) with better tolerance than other genotypes, and the second group consisted of four genotypes (Dr29, Dr27, Dr8, and Dr14) which had lower drought tolerance than other genotypes. The remaining 21 genotypes were aggregated to form the third group and their drought tolerance was

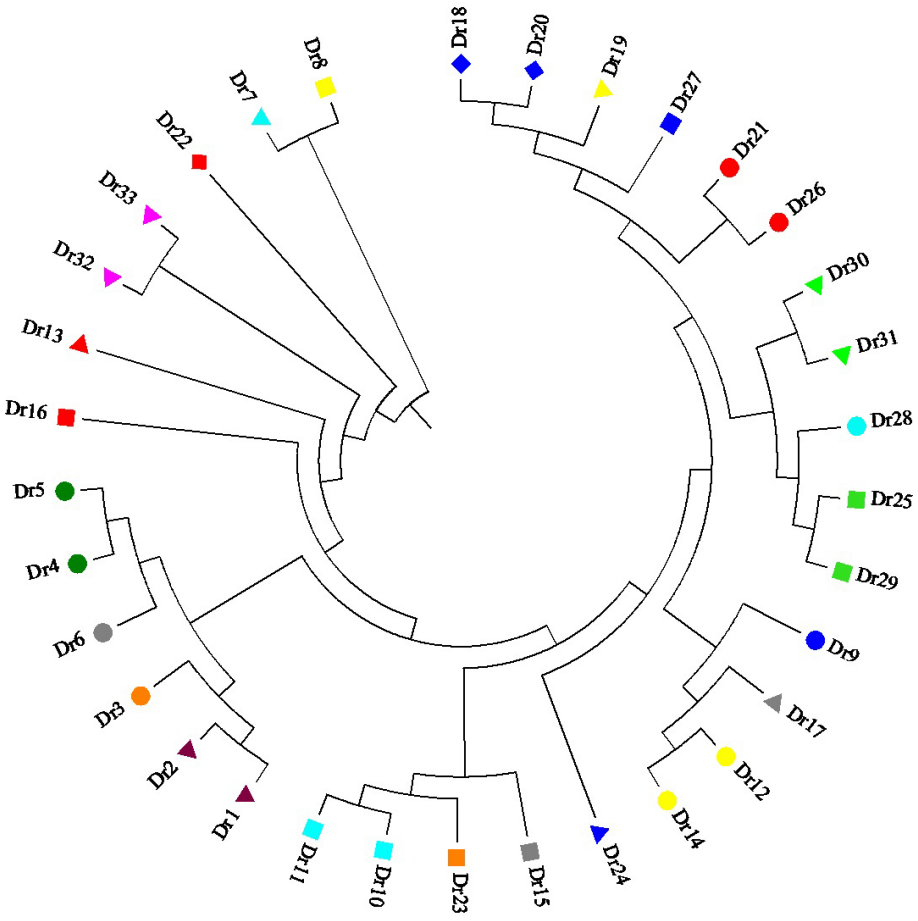

Fig. 3 Cluster analysis of 33 *Dichondra repens* genotypes based on SSR markers.

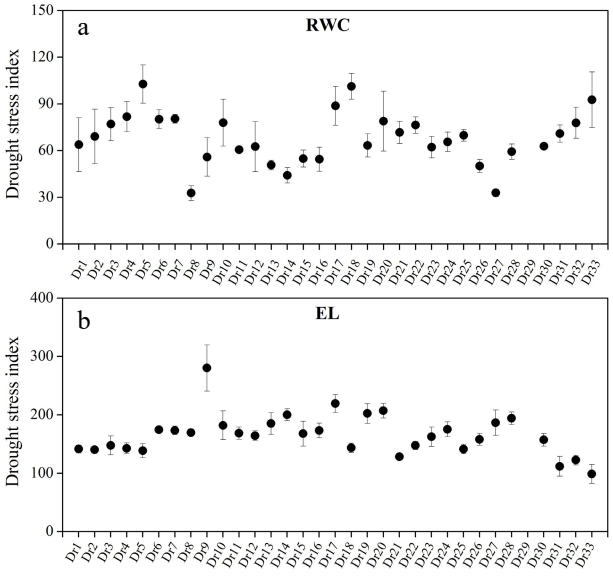

Fig. 4 Differences in drought stress index of (a) relative water content (RWC) and (b) electrolyte leakage (EL) among 33 *Dichondra repens* genotypes. Vertical bars represent standard errors of the mean (n = 4).

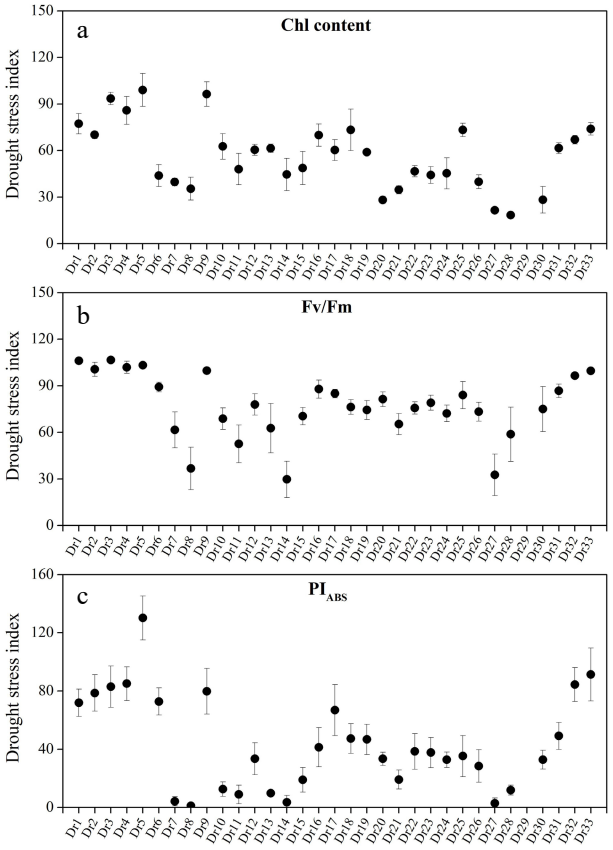

Fig. 5 Differences in drought stress index of (a) chlorophyll (Chl), (b) photosystem II photochemical efficiency (Fv/Fm), and (c) performance index on absorption basis (PI_{ABS}) among 33 *Dichondra repens* genotypes. Vertical bars represent standard errors of the mean (n = 4).

intermediate between the first group and second group (Fig. 6b).

Table 2. Membership function values of five physiological parameters and comprehensive evaluation of drought tolerance among 33 *Dichondra repens* genotypes.

Material No.	RWC	EL	Chl	Fv/Fm	PI_{ABS}	Average	Order
Dr5	0.836	0.641	0.932	0.879	0.642	0.786	1
Dr33	0.758	0.855	0.719	0.849	0.474	0.731	2
Dr3	0.652	0.618	0.898	0.904	0.455	0.706	3
Dr4	0.681	0.612	0.847	0.868	0.462	0.694	4
Dr32	0.642	0.706	0.637	0.823	0.461	0.654	5
Dr2	0.575	0.613	0.722	0.839	0.433	0.636	6
Dr1	0.536	0.572	0.798	0.842	0.391	0.628	7
Dr18	0.824	0.577	0.752	0.658	0.312	0.625	8
Dr31	0.595	0.766	0.696	0.742	0.295	0.619	9
Dr9	0.458	0.328	0.917	0.851	0.444	0.600	10
Dr6	0.686	0.434	0.570	0.764	0.412	0.573	11
Dr25	0.592	0.545	0.745	0.723	0.253	0.572	12
Dr17	0.735	0.314	0.708	0.727	0.361	0.569	13
Dr16	0.498	0.440	0.736	0.757	0.296	0.545	14
Dr22	0.656	0.569	0.510	0.652	0.252	0.528	15
Dr19	0.539	0.396	0.703	0.643	0.306	0.517	16
Dr10	0.667	0.452	0.703	0.592	0.173	0.517	17
Dr12	0.512	0.430	0.672	0.671	0.248	0.506	18
Dr23	0.523	0.453	0.545	0.679	0.274	0.495	19
Dr21	0.608	0.617	0.428	0.573	0.208	0.487	20
Dr24	0.558	0.422	0.455	0.634	0.272	0.468	21
Dr7	0.717	0.371	0.579	0.539	0.106	0.462	22
Dr15	0.485	0.444	0.541	0.605	0.207	0.456	23
Dr30	0.537	0.484	0.363	0.647	0.243	0.455	24
Dr26	0.425	0.455	0.523	0.635	0.230	0.453	25
Dr13	0.431	0.391	0.718	0.547	0.163	0.450	26
Dr11	0.512	0.483	0.652	0.460	0.142	0.450	27
Dr20	0.676	0.290	0.188	0.700	0.255	0.422	28
Dr8	0.362	0.412	0.567	0.329	0.144	0.363	29
Dr14	0.406	0.281	0.620	0.278	0.145	0.346	30
Dr28	0.505	0.271	0.272	0.514	0.156	0.344	31
Dr27	0.323	0.295	0.321	0.294	0.113	0.269	32
Dr29	0.000	0.000	0.000	0.000	0.000	0.000	33

DISCUSSION

Wild dichondra is widely distributed in southwest China, but the problem is that lack of enough research has slowed down breeding and utilization of these wild resources. In the past 30 years, SSRs have been widely used to evaluate genetic diversity in various plant species[20–22]. In our current study, significant genetic variation was detected among 33 dichondra genotypes through using 18 selected SSR markers that were developed from convolvulaceous sweet potato (*Dioscorea esculenta*). Excellent transferability of SSR markers cross related species has been demonstrated in many previous studies. For example, SSR markers from barley (*Hordeum vulgare*) exhibited good interspecific transferability in wheat (*Triticum aestivum*) and rye (*Secale cereale*)[23]. Mutual transferability of SSR between wheat and rye was also very high[24]. In addition, tall fescue (*Festuca arundinacea*) SSR markers could be applied for evaluation of genetic relationships in meadow fescue (*Festuca pratensis*), tetraploid fescue (*Festuca arundinacea*), and ryegrass (*Lolium perenne*)[25]. Our study found that all 18 primers were found to be polymorphic when they were applied to 33 dichondra genotypes, which indicated these primer pairs could be used for analysis of genetic diversity and cultivar identification in dichondra species. In addition, phenotypic variations in plant height, leaf area, leaf thickness, and petiole length were also

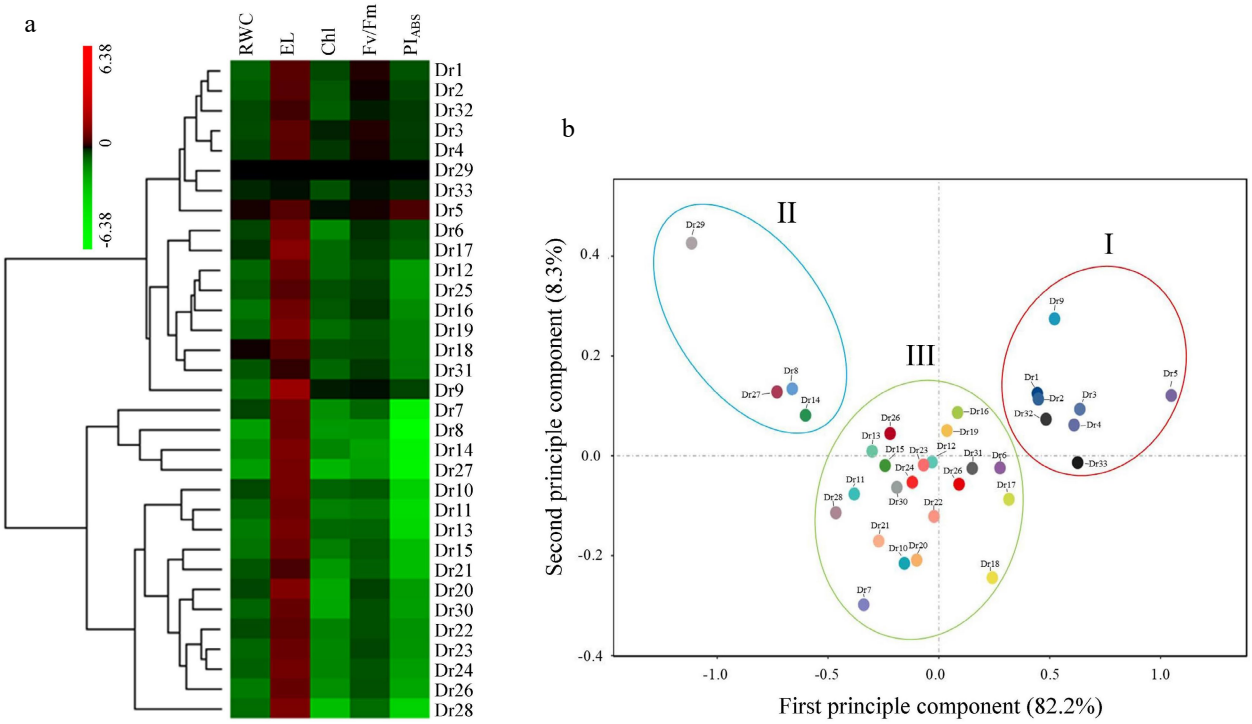

Fig. 6 Changes in (a) heat map and (b) principal component analysis (PCA) based on five different physiological parameters. RWC, relative water content; EL, electrolyte leakage; Chl, chlorophyll; Fv/Fm, photosystem II photochemical efficiency; PI_{ABS}, performance index on absorption basis.

observed among 33 dichondra genotypes under well-watered condition. Diverse morphological variability and genetic variation are beneficial to screen suitable accessions for stress adaptation, because variation in morphological characters often indicates genetic differences in one particular plant species, which provides abundant gene resources for screening new cultivars differing in drought tolerance.

Although many previous studies have been conducted to identify drought-tolerant plant genotypes in the field or under controlled conditions[26–29], selection and identification of dichondra genotypes with better drought tolerance have not been reported so far. Leaf RWC and EL are two important indicators of drought tolerance, as the RWC reflects leaf water status and the EL indicates cell membrane stability when plants suffer from drought stress[30]. Both of them have been applied to evaluate plant drought tolerance. Ahmed et al. found that drought tolerance of wheat was positively related to higher RWC and cell membrane stability which could be used to screen drought-tolerant genotypes at the seedling stage[31]. Drought-tolerant bermudagrass (*Cynodon dactylon*) also showed higher RWC and lower EL than drought-sensitive accessions in response to drought stress[18]. Dhanda et al. reported that cell membrane stability was the most important trait for evaluation of drought tolerance among thirty wheat cultivars[32]. Our current study demonstrated that Dr5, Dr18, and Dr33 could maintain higher leaf RWC and lower EL than other dichondra genotypes, whereas Dr8 exhibited the lowest RWC and the highest EL under drought stress. Those genotypes with higher leaf RWC and lower EL in response to drought stress could be recognized as potential breeding materials for developing drought-tolerant varieties.

Drought-tolerant plants could delay Chl degradation to maintain higher photosynthesis under water-deficit condition[33]. It has been found that the maintenance of higher Chl content is a common characteristic in drought-tolerant plant genotypes[18,31]. Apart from Chl content, Fv/Fm and PI_{ABS} also are critical parameters for evaluation of stress tolerance in plant species, as Fv/Fm represents photosystem II photochemical efficiency and PI_{ABS} indicates health status of photosynthetic organs[34,35]. It has been found that higher Chl content, Fv/Fm, or PI_{ABS} were the superior indicators with regard to better tolerance to heat stress in creeping bentgrass (*Agrostis stolonifera*) accessions[17], salt stress in white clover germplasm[16], and drought stress in sour cherry (*Prunus cerasus*) genotypes[36]. Dichondra genotypes exhibited significant variations in Chl content, Fv/Fm, and PI_{ABS} in response to a prolonged period of drought stress. Higher Chl content, Fv/Fm, and PI_{ABS} were found in Dr3, Dr4, Dr5, Dr9, and Dr33 which could be potential drought-tolerant genotypes.

Drought tolerance evaluated by one particular parameter is often one-sided. Subordinate function value analysis (SFVA) has been applied to comprehensively evaluate drought tolerance of diverse plant accessions based on different parameters[28,37,38]. The most promising drought-tolerant dichondra genotypes (Dr5, Dr33, Dr3, Dr4, and Dr32) were screened based on the SFVA in our current study. In addition, those 33 dichondra genotypes were classified into three distinct groups according to the analysis of PCA. Group I included 8 genotypes (Dr1, Dr2, Dr3, Dr4, Dr5, Dr9, Dr32, and Dr33) which were identified to be drought-tolerant candidates and group II contained four genotypes (Dr8, Dr14, Dr27, and Dr29) which were recognized as drought-sensitive accessions. The remaining 21 dichondra genotypes were classified into group III, which was intermediate between group I and III for drought tolerance. Similar results were found in the study of Badr et al. who reported that PCA analysis could clearly separate out

drought-tolerant maize (*Zea mays*) genotypes from 40 accessions[39]. Analytic results from SFVA were consistent with the findings based on the analysis of PCA. These selected drought-tolerant genotypes offer available materials for breeders to develop new dichondra cultivars.

CONCLUSIONS

A total of 18 SSR primer pairs were applied to evaluate genetic diversity of 33 dichondra genotypes and all primer pairs were found to be polymorphic. Natural variations in phenotypes including plant height, leaf area, leaf thickness, and petiole length were also observed among 33 genotypes under the well-watered condition. Drought tolerance of 33 genotypes was ranked by using SFVA, and the most tolerant genotype was Dr5 and most drought-sensitive genotype was Dr29. In addition, PCA analysis could classify 33 genotypes into group I (drought-tolerant), group II (drought-sensitive), and group III (medium types). Current findings showed that 18 selected SSR primer pairs could be used to potentially analyze genetic diversity and varietal identification in dichondra species. Selected drought-tolerant wild resources provide a rich genetic base for the breeding of new cultivars.

MATERIALS AND METHODS

Plant growth and treatments

Thirty wild dichondra genotypes and three commercial cultivars 'Silver falls', 'Xiaoshao', and 'Duliujiang' were collected from the Field Gene Bank at Sichuan Agricultural University (Table 3) and transplanted into polyvinyl chloride (PVC) tubes (33 cm in length, and 11 cm in diameter). All PVC tubes were filled with same mixtures of soil and sand (v:v, 1:1). Plants were cultivated in a greenhouse from July 14th to August 30th, 2020 (average temperature about 27/18 °C day/night and 800 μmol $m^{-2}·s^{-1}$ photosynthetically active radiation) and fertilized weekly with full Hoagland's solution[40]. For drought treatment, plants were then divided into two groups: one group was irrigated three times a week to avoid soil drought as well-watered control, and another group was subjected to drought stress by stop irrigating for 46 d. Leaves were collected for detecting physiological parameters and SSR markers. Each genotype was replicated four times (four tubes) under normal condition or drought stress.

Measurements of phenotypic and physiological parameters

A vernier caliper was used to measure leaf thickness and leaf area (S) which was calculated based on the formula $S = \pi \times [(\text{length} + \text{width}) / 4]^2$. Plant height and petiole length were measured by using a ruler, and 10 independent plants were selected randomly from each tube for the measurement of these phenotypic parameters. For leaf RWC, fresh leaves were cut from plants and weighted instantly to record fresh weight (FW). These leaves were then soaked in deionized water for 10 h and turgid weight (TW) was weighted. All leaves were put in an oven at 80 °C for 72 h to detect dry weight (DW). The RWC was calculated as RWC (%) = [(FW − DW) / (TW −DW)] × 100)[41]. To detect leaf EL, fresh leaves (0.15 g) were soaked in 40 mL of deionized water for 24 h at 25 °C and initial conductivity of solution ($C_{initial}$) was measured by using a conductivity meter (YSI Model 32, Yellow Spring, OH). Max conductivity of solution (C_{max}) was detected after leaves were autoclaved at 120 °C for 20 min. The EL was calculated as the ratio of $C_{initial}$ to C_{max}[42]. For Chl content, leaves were soaked in 15 mL of dimethyl sulfoxide for 48 h and absorbance was detected at 645 and 663 nm with a spectrophotometer (Spectronic Instruments, Rochester, NY, USA)[43]. For Fv/Fm and PI_{ABS}, leaves were kept in darkness for 15 min and a fluorescence meter (Pocket PEA Chl Fluorimeter, Hansatech Instruments Ltd, UK) was used to record Fv/Fm and PI_{ABS}[44].

Table 3. Test 33 *Dichondra repens* materials and their sources.

Material No.	Origin	Altitude (m)
Dr1	Zhongjiang, Sichuan	600
Dr2	Pingtang, Guizhou	848
Dr3	Dushan, Guizhou	1010
Dr4	Tianzhu, Guizhou	350
Dr5	Naxi, Sichuan	404
Dr6	Dayi, Sichuan	310
Dr7	Bishan, Chongqing	350
Dr8	Jining, Yunnan	1890
Dr9	Xifeng,Guizhou	990
Dr10	Xishui, Guizhou	1169
Dr11	Sinan, Guizhou	730
Dr12	Jiangkou, Guizhou	475
Dr13	Tongren, Guizhou	415
Dr14	Zhenyuan, Guizhou	382
Dr15	Danzai, Guizhou	894
Dr16	Sandu, Guizhou	500
Dr17	Sandu, Guizhou	780
Dr18	Dujun, Guizhou	842
Dr19	Shuicheng, Guizhou	1193
Dr20	Liuzhi, Guizhou	1035
Dr21	Anshun, Guizhou	1278
Dr22	Qinglong, Guizhou	1393
Dr23	Jin'an, Guizhou	1336
Dr24	Panzhou, Guizhou	1532
Dr25	Xingren, Guizhou	1336
Dr26	Anlong, Guizhou	1250
Dr27	Wangmo, Guizhou	653
Dr28	Ziyun, Guizhou	1160
Dr29	Huishui, Guizhou	980
Dr30 ('Silver Falls')	USA	-
Dr31	Xichou, Yunnan	1108
Dr32 ('Xiaoshao')	Yiliang, Yunnan	1970
Dr33 ('Duliujiang')	Sandu, Guizhou	600

DNA isolation, PCR amplification, and evaluation of polymorphism

Total DNA was extracted from approximately 0.1 g of fresh leaf tissues by using an assay kit purchased from Tiangen Biotech Co., LTD, Beijing, China. A Hoefer Dyna Quant 200 (Amersham Biosciences, Piscataway, NJ, USA) was used to detect DNA concentration which was adjusted to 10 ng · μL^{-1} of final concentration using purified water. PCR reaction was conducted by using 7.5 μL of 2× Mix (P2015, Dongsheng Biotech), 3 μL of DNA, 1.5 μL of 0.6 μmol·L^{-1} each primer, and 3 μL of purified water. A total of 18 primer sequences which were developed from sweet potato and their annealing temperature were recorded in Supplmental Table S1[45]. PCR products were electrophoresed in 6% polyacrylamide denaturing gels under 200 V for 30 min and then 400 V for 1.5 h. For SSR bands detection, gels were silver-stained and then captured using a camera. Gel images were analyzed by using the software Gel Analyzer 19.1 (www.gelanalyzer.com) to estimate base pair size

of bands. Polymorphism was determined based on absence or presence of SSR locus.

Statistical analysis

Variations in phenotypic and physiological parameters were analyzed by Statistix 8.1 (Tallahassee, FL, USA). PCA biplot analysis was performed by using SPSS 20 (IBM, Armonk, NY, USA). Drought tolerance was evaluated by using SFVA based on five physiological parameters (RWC, EL, Chl, Fv/fm, and PI_{ABS})[17]. DSI was calculated according to the formula DSI = (value of parameter under drought stress) / (value of parameter under normal condition) × 100. Cluster analysis of 33 *Dichondra micrantha* genotypes based on SSR markers was conducted by using NTSYSPC2.10e and MEGA 6 (Tokyo Metropolitan University, Hachioji, Tokyo, Japan)[46].

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Conflict of interest

The authors declare that they have no conflict of interest.

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