Genetic diversity and relatedness among ornamental purslane (*Portulaca* L.) accessions unraveled by SRAP markers

Sizhen Jia^{1,2} · Zhiming Yan^{1,2} · Yuanhua Wang^{1,2} · Yue Wei^{1,2} · Zhenqiang Xie^{1,2} · Fei Zhang³

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Abstract Ornamental purslanes (*Portulaca* L.) are a popular annual bedding and container plant for landscaping. Little information is available concerning the genetic characterization of ornamental purslane resources thus far. The purpose of this study was to investigate the genetic diversity and relationships present in a collection of ornamental purslanes from *Portulaca umbraticola* and *P. grandiflora* cultivated in China, using sequence-related amplified polymorphism (SRAP) markers. The genotyping showed that 16 SRAP primer combinations totally produced 261 informative fragments and averaged 16.31 per primer combination. The major allele frequency and Nei's gene diversity was calculated at 0.78 and 0.31 across the loci, indicative of a moderate low diversity. Both unweighted pair group method with arithmetic average (UPGMA) clustering and a Bayesian-based approach apparently assigned the whole accessions into two subgroups: *P. umbraticola* and *P. grandiflora*, well concordant with the botanical classification and flower type. The findings provide a brandnew understanding of genetic diversity and population structure present in ornamental purslane, and benefit a sound design of breeding programs in future.

Keywords Genetic diversity · Population structure · *Portulaca* L. · Sequence-related amplified polymorphism marker

Introduction

Portulaca L.—an annual succulent plant of the family Portulacaceae—is distributed worldwide, mainly in the Neotropical region and in tropical African. The genus also occurs in subtropical regions where a few species can be found in Australia, Europe and Asia (Coelho et al. 2010). The number of species in the genus is uncertain, with estimates ranging from a few morphologically variable species (Geesink 1969) to over 100 (Carolin 1993; Eggli and Ford-Werntz 2002). One of the reasons for such differences is that most of the species present high levels of phenotypic plasticity, which is usually associated with the wide range of distribution (Coelho et al. 2010).

Within *Portulaca* species, most were of no major commercial importance, except *P. oleracea*, *P. grandiflora*, and *P. umbraticola* (Ocampo and Columbus 2012). *P. oleracea*, often known as common purslane, is cultivated as a wild vegetable and medicinal plant (Chan et al. 2000; El Jack 2004), and its consumption is known to reduce the incidence of cancer and heart diseases (Thangavel and Subburam 1998; Lim and Quah 2006). Whereas, *P. grandiflora* and *P. umbraticola*, often called as moss-rose purslane or large-flowered purslane, is a small, but fast-growing annual ornamental widely grown in temperate regions for ornamental purpose. The flowers of large-flowered purslane are often single with five petals, variably red, orange, pink, white, and yellow. Recently, numerous cultivars have been selected for double flowers with additional petals and for attractive flower color. In previous

✉ Fei Zhang
zhangfei@njau.edu.cn

¹ Department of Agronomy and Horticulture, Jiangsu Vocational College of Agriculture and Forestry, Jurong 212400, Jiangsu Province, China

² Jiangsu Engineering and Technology Center for Modern Horticulture, Jurong 212400, Jiangsu Province, China

³ College of Horticulture, Nanjing Agricultural University, No. 1 Weigang, Nanjing 210095, Jiangsu Province, China

studies, portulaca plants showed an excellent tolerance towards moderate drought (Rahdari and Hoseini 2012), salinity (Yezici et al. 2007; Teixeira and Carvalho 2009), and a considerable accumulation capacity for heavy metals (Mihailovic et al. 2015). Additionally, portulaca plants have a high regeneration potential after pruning. Repeated pruning after flower withered usually keeps ornamental purslanes spouting new axillary buds or branches, and subsequently in blossom all summer long. Therefore, ornamental purslane is popularly grown as an annual bedding or as a container plant for landscaping. Currently, various ornamental purslane cultivars have been released for the floricultural market. Unmatched with its commercial importance, the genetic diversity and relationships within ornamental purslanes have remained largely unknown, and most of the existing references focused mainly on the phylogenetic relationship of the wild species (Ohsaki et al. 1999). In a recent important literature by Ocampo and Columbus (2012) exhibited the phylogenetics of 59 species in genus *Portulaca*, including only three ornamental purslane cultivars.

As we know, *P. umbraticola* and *P. grandiflora* are not originated in China, and almost all the commercial ornamental purslanes in China flower market are imported overseas, for example, from Brazil and Argentina. Owing to irregular communication, the cultivar names or pedigree of the commercial ornamental purslanes in China market are largely unknown, which bring a problem for future breeding activities. Thus, a complete characterization of ornamental purslane resources is an important link between the conservation and utilization of these resources. The sequence-related amplified polymorphism (SRAP) technique is an open reading frames-based marker system that targets functional genes (Li and Quiros 2001) and has been used for various purposes, say, cultivar or hybrid discrimination (Zhang et al. 2012; Yu et al. 2016), linkage map construction (Zhang et al. 2011), and genetic diversity evaluation (Bar et al. 2015; Li et al. 2016; Valdez-Ojeda et al. 2014) as well. The objectives of the present study were to unravel the genetic diversity and structure among ornamental purslane accessions from *P. grandiflora* and *P. umbraticola* collected in China using SRAP makers. The findings from this study add new understanding of genetic diversity of ornamental purslane and will be beneficial for its future improvement.

Materials and methods

Plant materials

A total of thirty-eight ornamental purslane accessions, including eight from *P. umbraticola* and thirty from *P. grandiflora* Hook., were collected in this study. These

accessions were chosen because they are a representative sample of the commonly cultivated members of large-flowered purslanes in China. Two morphological traits, flower type and leaf type, for each accession are summarized in Table 1 and Fig. 1.

DNA isolation

Fresh and young leaves from five plants of each accession were randomly collected and mixed for genomic DNA isolation. The total genomic DNA was extracted using a modified CTAB procedure (Khanuja et al. 1999). Integrity and quality of DNA were evaluated in comparison with known concentrations of Lambda DNA on 0.8% agarose gel.

SRAP genotyping and data scoring

A total of 36 SRAP primer combinations from 6 forward and 6 reverse primers were initially screened for polymorphism with each two accessions of *P. umbraticola* and *P. grandiflora* in this study (Table 2), and the primer combinations yielding informative patterns were then used to genotype 38 accessions. The primers were designed according to Li and Quiros (2001). All PCR amplifications were performed in 10 µl reaction volume containing 1 × PCR buffer, 3 mM Mg²⁺, 200 µM dNTP, 0.5 U *Taq* DNA polymerase (Takara, Dalian, China), 10 µM of each primer and 25 ng DNA template. The PCR was performed following the procedure recommended by Li and Quiros (2001): an initial denaturing step was performed at 94 °C for 5 min followed by 5 cycles at 94, 35 and 72 °C for 1 min, subsequently followed by 35 cycles at 94, 50 and 72 °C for 1 min with a final extension step at 72 °C for 7 min. PCR products were electrophoresed through 8% non-denaturing polyacrylamide gels run at 300 V for 2.5 h in 0.5 × TBE buffer, and visualized by silver staining. The resulting data were scored in the form of a 1/0 matrix.

Data analysis

The number of polymorphic markers, allele frequency, polymorphism information content (PIC) for each marker type were derived from the 1/0 matrices using PowerMarker 3.25 software (Liu and Muse 2005). Cluster analysis was performed using unweighted pair group method with arithmetic mean (UPGMA) method based on the Nei's distance index (Nei and Li 1979). This analysis was implemented with the SAHN module of NTSYS-pc 2.2 (Rohlf 2005). The robustness of the clusters was estimated via a 1000 replicate bootstrap analysis (Felsenstein 1985). The genetic structure of the accessions was characterized using a Bayesian-based STRUCTURE software

Table 1 List of ornamental purslanes under study

No.	Accession	Species	Flower type	Leaf type
1	PL11001	<i>P. ambraticola</i>	Single	Dent
2	PL11002	<i>P. ambraticola</i>	Single	Dent
3	PL11003	<i>P. ambraticola</i>	Single	Dent
4	PL11004	<i>P. ambraticola</i>	Single	Dent
5	PL11005	<i>P. ambraticola</i>	Single	Dent
6	PL11006	<i>P. ambraticola</i>	Single	Dent
7	PL11007	<i>P. ambraticola</i>	Single	Dent
8	PL11008	<i>P. ambraticola</i>	Single	Dent
9	PL11011	<i>P. grandiflora</i> Hook.	Single	Pine needle
10	PL11009	<i>P. grandiflora</i> Hook.	Single	Pine needle
11	PL11012	<i>P. grandiflora</i> Hook.	Double	Pine needle
12	PL11010	<i>P. grandiflora</i> Hook.	Double	Pine needle
13	PL11013	<i>P. grandiflora</i> Hook.	Double	Pine needle
14	PL11014	<i>P. grandiflora</i> Hook.	Double	Pine needle
15	PL11015	<i>P. grandiflora</i> Hook.	Double	Pine needle
16	PL11016	<i>P. grandiflora</i> Hook.	Double	Pine needle
17	PL11017	<i>P. grandiflora</i> Hook.	Double	Pine needle
18	PL11018	<i>P. grandiflora</i> Hook.	Double	Pine needle
19	PL11019	<i>P. grandiflora</i> Hook.	Double	Pine needle
20	PL11020	<i>P. grandiflora</i> Hook.	Double	Pine needle
21	PL11021	<i>P. grandiflora</i> Hook.	Double	Pine needle
22	PL11022	<i>P. grandiflora</i> Hook.	Double	Pine needle
23	PL11023	<i>P. grandiflora</i> Hook.	Double	Pine needle
24	PL11024	<i>P. grandiflora</i> Hook.	Double	Pine needle
25	PL11025	<i>P. grandiflora</i> Hook.	Double	Pine needle
26	PL11026	<i>P. grandiflora</i> Hook.	Double	Pine needle
27	PL11027	<i>P. grandiflora</i> Hook.	Double	Pine needle
28	PL11028	<i>P. grandiflora</i> Hook.	Double	Pine needle
29	PL11029	<i>P. grandiflora</i> Hook.	Double	Pine needle
30	PL11030	<i>P. grandiflora</i> Hook.	Double	Pine needle
31	PL11031	<i>P. grandiflora</i> Hook.	Double	Pine needle
32	PL11032	<i>P. grandiflora</i> Hook.	Double	Pine needle
33	PL11033	<i>P. grandiflora</i> Hook.	Double	Pine needle
34	PL11034	<i>P. grandiflora</i> Hook.	Double	Pine needle
35	PL11035	<i>P. grandiflora</i> Hook.	Double	Pine needle
36	PL11036	<i>P. grandiflora</i> Hook.	Double	Pine needle
37	PL11037	<i>P. grandiflora</i> Hook.	Double	Pine needle
38	PL11038	<i>P. grandiflora</i> Hook.	Double	Pine needle

package (Pritchard and Falus 2009). The number of assumed sub-populations, K , was assigned from 1 to 10. Five independent runs were carried out, each involving a burn-in period of 100,000 steps followed by 100,000 Monte Carlo Markov Chain iterations for each tested K value (Hubisz et al. 2009). The most likely value of K was deduced following an ad hoc measure ΔK as proposed by Evanno et al. (2005). Of the five independent runs, the one delivering highest likelihood value was used to assign the entries into the indicated sub-populations.

Those entries associated with membership probabilities (Q values) < 0.8 were considered as an admixed group.

Results

SRAP polymorphism

The 16 selected SRAP primer combinations that generated a clear and informative banding pattern were used to

Fig. 1 Representative accessions of the investigated ornamental purslanes from *P. umbraticola* (a–e) and *P. grandiflora* (f, g with a single flower; h, l with a double flower)

Table 2 The SRAP primer sequences used for genotyping in this study

Forward primer	Sequence (5' to 3')	Reverse primer	Sequence (5' to 3')
Me20	GAGTATCAACCCGGATT	Em1	GACTGCGTACGAATTAAT
Me21	GTACATAGAACCGGAGT	Em2	GACTGCGTACGAATTTGC
Me22	TACGACGAATCCGGACT	Em3	GACTGCGTACGAATTGAC
Me23	CACAGTCATGCCGGAAT	Em4	GACTGCGTACGAATTTGA
Me24	GACCAGTAAACCGGATG	Em5	GACTGCGTACGAATTAAC
Me25	CAGGACTAAACCGGATA	Em6	GACTGCGTACGAATTGCA

genotype the whole panel. The 16 SRAP primers produced 261 valid polymorphic fragments and averaged 16.31, varying from 3 (M25E5) to 33 (M22E1) polymorphic alleles generated per primer combination (Table 3). Estimates of major allele frequency and Nei's gene diversity index based on the SRAP data averaged at 0.78 and 0.31 across the loci, respectively. The PIC values ranged from 0.17 for M21E6 to 0.34 for M21E2, and averaged at 0.25 (Table 3).

Cluster analysis

Clustering based on the Nei's genetic distance matrix is depicted in Fig. 2. The cophenetic was calculated at 0.91, indicative of a good fit between the dendrogram and the original similarity matrix. In this dendrogram, the ornamental purslanes were grouped into two major clusters, corresponding well with their botanical classification. Cluster I included the 8 members from *P. umbraticola* with

Table 3 SRAP marker diversity measurements for the 38 ornamental purslane accessions included in this study

Marker	Polymorphic alleles	Major allele frequency	Gene diversity	PIC
M20E2	24	0.82	0.27	0.23
M20E3	28	0.78	0.32	0.26
M20E4	27	0.84	0.25	0.21
M21E2	23	0.65	0.44	0.34
M21E3	18	0.79	0.31	0.25
M21E4	5	0.73	0.37	0.30
M21E6	7	0.88	0.20	0.17
M22E1	33	0.79	0.30	0.25
M22E2	5	0.77	0.35	0.28
M22E4	15	0.79	0.30	0.25
M23E1	18	0.83	0.26	0.22
M23E2	5	0.76	0.36	0.29
M23E3	12	0.77	0.33	0.26
M23E4	16	0.80	0.29	0.24
M25E5	3	0.76	0.32	0.25
M25E6	22	0.77	0.32	0.26
Mean	16.31	0.78	0.31	0.25

a dent leaf, and cluster II contained the other 30 accessions from *P. grandiflora* with a pine-needled leaf. Moreover, cluster II could be further divided into two sub-groups. Subgroup-a contained the two *P. grandiflora* accessions with a single flower, while the 28 accessions in subgroup-b showed a double flower, indicating a correlation between sub-groups and flower type. Bootstrap analysis showed that 40.5% bootstrap values associated with the phylogenetic dendrogram lay at a moderate support value >50% (Fig. 2).

Bayesian-based population structure

The population structure of the 38 ornamental purslane accessions was evaluated based on the SRAP data with Bayesian-based STRUCTURE package. The ad hoc statistic ΔK based on the rate of change in the $L(K)$ between successive K values showed the highest value at $K = 2$. Therefore, the panel was divided into two main populations, designated as Pop 1 and Pop 2, respectively (Fig. 3a). Pop 1 contained eight accessions from *P. umbraticola*, and Pop 2 included 28 accessions from *P.*

Fig. 2 Dendrogram of 38 ornamental purslane accessions clustering based on the SRAP marker-based Nei's distance index. The bootstrap values larger than 50% are shown on corresponding clustering nodes. The accession codes on the right of the dendrogram refer to Table 1

Fig. 3 Genetic structure of the 38 ornamental purslane accessions, derived from STRUCTURE analysis, based on an admixture model with $K = 2$ (a), and $K = 3$ (b). Each entry is represented by a thin

vertical line. The identified sub-groups are indicated in different colors. The accession numbers refer to Table 1

grandiflora. With a membership probability threshold of 0.80, two accessions from *P. grandiflora* (PL11009 and PL11010) were assigned to the admixed group. However, PL11009 and PL11010 were assigned to a dependent subpopulation when $K = 3$ (Fig. 3b).

Discussion

A complete knowledge and understanding of the genetic diversity present in a panel of plant resources can aid in its effective management and genetic improvement. Ornamental large-flowered purslane is a popular annual landscaping plant, whereas the lack of genetic characterization hinders the proper breeding programs for the species. To fill this gap, the genetic diversity and population structure of a working set of ornamental purslane accessions, representative for the cultivated gene pool in China, were investigated using SRAP markers in the present study.

SRAP marker proves effective for genetic diversity analysis in plant species (Aneja et al. 2012). Recently, Zhang et al. (2012) reported 40 polymorphic fragments were averagely generated for each SRAP primer combination in ornamental bromeliads. Yu et al. (2016) found that roughly 10 polymorphic amplicons were produced per SRAP primer combination in *Hibiscus* F_1 progenies. Simsek et al. (2017) reported that all the 216 fragments amplified by 14 SRAP primer pairs were polymorphic in *Cyclamen* species. In this context, an average of 16.3 informative fragments per primer combination was yielded in ornamental purslanes (*Portulaca* L.), indicating the efficiency of SRAP-based genotyping. However, the estimates of mean major allele frequency (0.78) and Nei's

gene diversity (0.31) across the loci are suggestive of a moderate low genetic variation harbored in the ornamental purslanes. However, it is worthy to note that the genetic variation might be underestimated by the limited sample size of the investigated ornamental purslanes (38 accessions here).

Understanding the patterns of population structure is essential for efficient germplasm organization (Zhang et al. 2012). Zhang et al. (2012) detect definite population structures in ornamental bromeliads, well accordant with their genera relationships. In chrysanthemum, however, Klie et al. (2013) failed to identify any structure and concluded that the lack of any detectable population structure reflected history of repeated backcrossing and germplasm exchanges between breeders. In this study, however, the investigated ornamental purslanes from *P. umbraticola* and *P. grandiflora* were grouped well by species and horticultural traits (i.e., flower and leaf types). In *P. grandiflora* cluster, particularly, the two accessions (PL11009 and PL11010) with a single flower in subgroup-a were apparently discriminated from the other *P. grandiflora* accessions with a double flower in subgroup-b. The structuring results approve the efficiency of SRAP markers in characterizing ornamental purslanes. Moreover, some 40% of the bootstrap values related to the phylogenetic dendrogram were larger than 50% (Fig. 2), thus indicative of a moderate robustness of the clustering.

To give a more sensible populations structure present in the ornamental purslanes, a Bayesian-modeled STRUCTURE were implemented. The result revealed that, the *P. umbraticola* accessions were assigned to the Pop-1, and *P. grandiflora* accessions with a double flower to the Pop-2 at the membership probability 0.80. The two *P. grandiflora*

entries, PL11009 and PL11010, belonged to admixed structure with germplasm introgression from the two subpopulations (Fig. 3a) at $K = 2$ (Fig. 2a), but was assigned to a dependent subpopulation when $K = 3$ (Fig. 3b). Thus, the classification of PL11009 and PL11010 may be debatable. Aside from markers specific to *P. umbraticola* or *P. grandiflora* accessions, some unique markers were detected for the two entries PL11009 and PL11010 (data not shown). In morphology, PL11009 and PL11010 (Fig. 1f, g) share pine-needled leaf type with other *P. grandiflora* accessions, but same single flower type with *P. umbraticola* accessions. Therefore, we infer that germplasm of both *P. grandiflora* and *P. umbraticola* are principally involved in the formation of the entries PL11009 and PL11010.

In conclusion, SRAP marker was proved to be effective in characterizing the genetic diversity and relationships of ornamental purslanes. Results from this research revealed a relatively low genetic diversity and a definite population structure present in the current ornamental purslanes from *P. umbraticola* and *P. grandiflora*. As little information is reported regarding breeding related researches in *Portulaca* species (Venu-Babu et al. 1996; Wickramasinghe et al. 2009), the findings benefit a sound breeding program in ornamental purslanes. Specifically, it can be predicted that crossbreeding between ornamental purslanes of the two species will enable new cultivars of interesting traits.

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Compliance with ethical standards

Conflict of interest The authors declare they have no conflict of interest.

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