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Original Article

Genetic Diversity of Purslane Populations of Iran Based on Some Morphological and Biochemical Traits

Running title: Genetic Diversity of Purslane Populations of Iran

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Abstract

Portulaca oleracea L. is a medicinal plant belonging to Portulacaceae family, which exhibited a wide scope of pharmaceutical properties such as pain killer, antipyretic, anti-viral and anti-fungal. The aim of the present study was evaluation of morphological diversity 18 populations of *P. oleracea*. The seeds of 18 populations were cultivated based on a completely randomized design with three replicates in the research farm of Medicinal Plants Research Center, Shahed University, Tehran, Iran. The morphological traits, chlorophyll and protein contents were measured in the vegetative stage. The analysis of variance showed that there was a significant difference among populations of *P. oleracea* in terms of the most studied morphological and biochemical traits. The highest shoot dry weight (84.84±12.70 g per plant) belonged to the Zanjan population, while the lowest shoot dry weight (18.89±1.72 g per plant) belonged to the population of Lourdgan. The correlation between most of the studied traits was positively significant. The cluster analysis of the 18 *P. oleracea* populations based on morphological and biochemical traits produced three main clusters. Overall, the outcomes of the present study were indicated the presence of high genetic variability among the *P. oleracea* populations. Our findings suggested that collection of the plants from different regions can be used for hybridization to generate useful recombinants in the segregating generations and improve breeding varieties of *P. oleracea* and can be utilized for preservation and maintenance the germplasm of this medicinal plant.

Keywords: Biochemical, Genetic diversity, Morphological, *Portulaca oleracea*.

Introduction

Portulaca oleracea commonly known as Purslane is an herbaceous annual in the family of Portulacaceae. The plant is distributed all over the world including Europe, America, Canada, India, New Zealand, Australia, China and Japan [1]. It is particularly well adapted to the warm, moist conditions found in irrigated agricultural and ornamental sites. The plant requires a moist light rich well-drained soil in a sunny position. The plants will not produce good quality leaves when growing in dry conditions. The plants take about six to eight weeks to produce a crop from seed and can then be harvested on a cut and come again principle, providing edible leaves for most of the summer. The plant has a round, smooth, procumbent, succulent stem, with small, oblong, wedge-shaped, sub-sessile, alternate or sub-opposite dark-green leaves. The flowers are small, yellow, solitary or clustered, stalkless, placed above the last leaves on the branches, blooming in June and July, and opening only for a short time towards noon. Seeds are reddish brown to black, oval, and tiny [2,3].

The plant is rich in fatty acids, proteins, and vitamins (mainly vitamin A, C, E, and some vitamin B and carotenoids), as well as dietary

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minerals, such as magnesium, calcium, potassium, and iron with about 70% of its fatty acids being unsaturated and about 50% containing only omega-3 fatty acids. At the present there are two types of betalain alkaloid pigments, the reddish betacyanins and the yellow betaxanthins [4]. According to Iranian traditional medicine sources, purslane is an antinociceptive, anesthetic, antiseptic, anti-ascorbate, anti-inflammatory, anti-inflammatory, blood purifier, anti-fungal and reducing swelling and abscesses, insect bite and scorpion sting [5].

Evaluation of genetic variation of plants is one of the important requirements for plants in order to achieve high yield and desirable quality. In breeding programs, genetic diversity is the first step that has been created over the years and is one of the most important factors in the survival of creatures, including plants, for adaptation to environmental changes [6]. Genetic diversity can be estimated based on genealogy analyzes using various methods including morphological traits, protein markers, and molecular markers [7,8]. Initially, morphological and agronomic characteristics are often used for basic characterization [9]. Interspecific and intergeneric hybridisation has been the major source of genetic variation in breeding new cultivars of ornamental plants [10,11]. With respect to genetic diversity, several proteins and molecular polymorphic have been reported in the purslane (*Portulaca oleracea* L.) accessions [12-14].

P. oleracea is a very variable species, cytologically, *P. oleracea* is characterized by a sequence of polyploids, from a base number of x=9. The variability expressed by the existence of diploid (2n=18), tetraploid (2n=36) and hexaploid (2n=54) populations. Based on seed size and seed-coat cell morphology, up to ten subspecies has been differentiated. The cultivated forms (2n=54), which usually have taller plants and larger seeds, can best be distinguished botanically at cultivar level [15, 16].

In *P. oleracea*, genetic diversity of 10 purslane accessions has been evaluated using AFLP markers [17]. Alam *et al*. [18] evaluated 45 Malaysian accessions of *P. oleracea* using EST-SSR marker and reported 71.87% variation within population and 28.13% among populations. Also, Alam *et al*. [18] in a study on genetic diversity of 45 *P. oleracea* accessions using ISSR marker reported 89% variation within population and 11% among populations. Each marker explores a part of the differences and similarities within the genome. Because of the commercial and pharmaceutical importance of this plant and the growing trend of its use and cultivation, improving the plant with high yield and secondary metabolites cultivars using wild plants or selected ecotypes as well as conservation of natural germplasm resources in the country seems necessary. The objective of this study was to evaluate morphological and biochemical diversity among 18 populations of *P. oleracea* collected from different states of Iran to estimate genetic relationships among the population which can be utilized for breeding program and phytochemical study.

Material and Methods

Plant Materials

Seeds of 18 populations of *P. oleracea* seeds were collected from different regions of Iran (Table 1).

Growth Conditions

The seeds were surface sterilized with 10% sodium hypochlorite (NaClO) solution for 10 minutes and thoroughly rinsed with distilled water [19]. The seeds of 18 populations were cultivated based on completely randomized design with three replicates in the Research farm of Medicinal Plants Research Center, Shahed University, Tehran, Iran. Each population was cultivated in 1 × 1.2 m plots with three rows and 25 cm spacing and a density of 20-30 plants per plot. The plants were irrigated every day until flowering stage. Before flowering stage ten plants were randomly selected from each experimental unit and the data on morphological and biochemical traits such as average shoot length (cm), stem diameter (mm), number of branches, stem color, shoot fresh weight (g per plant), shoot dry weight (g per plant) and chlorophyll (µg/g FW) and protein contents (µg/g FW) were measured. The dry shoot weights after drying at the 68 ^{o}C for 48 hours were measured.

Determination of Chlorophyll and Protein Content

The chlorophyll content of leaves was measured using Chlorophyll‑Meter‑XT‑SPAD-502 equipment (Konica Minolta, Inc., Tokyo, Japan) [20]. To determine the protein content, one gram fresh leaf from the 18 different *P. oleracea* population was grounded in liquid nitrogen using pre-cooled mortar and pestle to obtain a fine powder and then homogenized with 2 mL of the

HEPES/KOH buffers according to the method of Talei *et al*., [21]. Finally, the total protein concentration was determined by the Bradford method [22] at 595 nm, using a spectrophotometer (Lambda 25, UV/VIS).

Statistical Analysis

The analysis of variance and Duncan's multiple range tests were performed using SPSS25 software at significance level of P≤0.01, and the cluster analysis and PCA analysis were performed using JMP software version 13. The formula used to estimate the broad-sense heritability is [23]:

$$h^2 = \frac{\delta_G^2}{\delta_P^2} \quad and \quad \delta_P^2 = \delta_G^2 + \delta_e^2$$

Where δ_G^2 is the genetic variance, δ_P^2 is the phenotypic variance and δ_e^2 is the environmental variance.

Table 1 Geographical origins of the of *Portulaca oleracea* L. population from different part of Iran.

code	Region originated	Latitude	Longitude	Altitude (m)
1	Varamin	35º 12' 36.513"	51º 40' 26.832"	842
2	Malard	35 º 41' 29.949"	50º 50' 2.720"	1159
3	Karaj	35º 49' 11.727"	50º 56' 11.296"	1282
4	Qom	34º 37' 35.903"	50º 55' 37.719"	911
5	Abhar	36º 10' 29.042"	49º 15' 20.246"	1574
6	Zanjan	36º 38' 41.781"	48º 30' 54.880"	1642
7	Qazvin	36º 17' 52.241"	50º 2' 41.569"	1338
8	Bandar Turkman	36º 49' 45.880"	54º 3' 19.337"	-26
9	Talesh	37º 52' 26.749"	48º 54' 24.150"	-6
10	Hamedan	34º 49' 52.081"	48º 30' 56.491"	1756
11	Razan	35º 23' 18.355"	49º 1' 29.424"	1839
12	Izeh	29º 28' 0.801"	51º 16' 18.382"	102
13	Babol	36º 29' 39.799"	52º 42' 25.769"	8
14	Tonekabon	36º 46' 15.806"	50º 50' 49.824"	48
15	Lourdgan	30º 39' 18.529"	51º 36' 53.288"	1843
16	Boroujerd	33º 52' 32.840"	48º 45' 32.557"	1533
17	Kashan	33º 57' 12.852"	51º 22' 9.576"	1015
18	Folad Shahr	32º 27' 55.425"	51º 24' 13.244"	1708

Table 2 Variance analysis of 18 purslane populations in terms of some morphological and biochemical traits.

S.O.V	df	Plant height	Stem diameter	Branches number	Fresh weight	Dry weight	Chlorophyll content	Protein content
Rep	2	727.04 **	5.53 *	4.22 **	2301.30 ^{ns}	620.78 ^{ns}	195.339 **	0.022 ^{ns}
Genotype	17	62.06 **	3.01 **	0.41 ^{ns}	31588.23 **	822.50 **	40.705 ^{ns}	0.016 *
Error	34	36.82	2.06	0.26	7484.56	116.58	35.024	0.007
CV (%)		9.62	15.99	15.93	25.83	23.62	12.80	18.93

*, **: Significant at 5% and 1% probability level, and ns; non-significant.

Result and Discussion

The results of analysis of variance showed that there is a significant difference between the purslane populations based on most of the studied traits (P≤0.01), while there was no significant difference between the population based on number of branches and chlorophyll content (Table 2).

The highest (70.57±3.12 cm) and lowest plant height (54.85±2.35 cm) belonged to Abhar population from the Zanjan state and Bandar Turkman population from the Golestan state, respectively. The highest stem diameter (10.91±0.32 mm) belonged to the Boroujerd population from the Lorestan state, while the lowest stem diameter (7.55±0.30 mm) belonged to the population of Bandar Turkman.

The results indicated that the highest (84.84±12.70 g per plant) and the lowest shoot dry weight (18.89±1.72 g per plant) belonged to the Zanjan and Lourdgan populations, respectively. The results also showed that the highest protein content (0.583±0.04 µg.g^{-1} FW) belonged to the Bandar Turkman population, while the lowest protein content (0.306±0.04 µg.g^{-1} FW) belonged to the Zanjan population. There were no significant differences among different population in terms of chlorophyll contents, nevertheless chlorophyll content variation due to different population was between 40.98 to 55.80 µg.g^{-1} FW (Table 3). Therefore, according to the results, it can be said that there are many differences in measured traits among the populations, which can be used for breeding programs and selection of the desired genotypes.

Mohebodini, *et al*., [13] studied twenty purslane plant populations for morphological and agronomic traits. The reason for the high variability among the purslane in terms of measured traits could be the pursuit of free purslane pollination and the possibility of pollen transfer between the species [24]. The correlation between the most morphological traits was very significant and positive, while the correlation between protein content and plant height, and fresh and dry shoot weight was significantly negative. The highest correlation was found between shoot fresh weight and shoot dry weight (r = 0.94), and the lowest correlation between number of branches and protein (r = 0.25) (Table 4).

Table 3 Mean comparison of different purslane populations based on some studied traits.

Genotype	Plant height (cm)	Stem diameter (mm)	Fresh weight (g)	Dry weight (g)	Protein content (µg/g FW)
Varamin	64.50±2.15 a-d	10.44±0.30 ab	424.37±30.21 ab	71.46±3.76 ab	0.39±0.07 bc
Malard	64.00±4.57 a-d	8.51±0.31 a-c	187.33±53.18 de	48.46±7.86 c-e	0.44±0.08 a-c
Karaj	67.13±4.97 a-d	9.52±0.28 a-c	479.60±21.66 a	45.45±3.88 c-e	0.40±0.07 bc
Qom	64.03±5.73 a-c	8.66±0.18 a-c	246.33±61.74 c-e	35.34±7.82 d-f	0.39±0.09 bc
Abhar	70.57±3.12 a	8.89±0.45 a-c	255.93±19.75 c-e	46.98±3.95 c-e	0.40±0.08 bc
Zanjan	67.41±3.61 a-c	7.85±0.31 bc	357.30±51.92 a-c	84.84±12.70 a	0.31±0.04 c
Qazvin	64.67±0.99 a-d	9.07±0.37 a-c	187.50±7.47 de	47.53±3.07 c-e	0.49±0.01 ab
Bandar Turkman	54.85±2.35 d	7.55±1.09 c	233.03±50.17 c-e	55.08±7.81 b-d	0.58±0.04 a
Talesh	59.48±1.49 a-d	8.94±0.67 a-c	190.47±30.15 c-e	60.54±6.11 bc	0.52±0.03 ab
Hamedan	68.20±4.77 ab	8.75±0.33 a-c	229.60±2.66c-e	29.46±0.59 e-f	0.47±0.05 ab
Razan	58.07±6.21 b-d	9.81±0.72 a-c	194.27±29.13 c-e	23.83±5.51 f	0.53±0.06 ab
Izeh	59.10±1.81 a-d	7.77±0.44 bc	173.13±28.12 de	24.86±4.32 f	0.48±0.05 ab
Babol	68.57±5.98 ab	7.86±0.27 bc	234.73±24.61 c-e	54.68±2.69 b-d	0.36±0.02 bc
Tonekabon	55.87±3.85 cd	10.70±1.84 a	155.10±30.41 de	44.96±4.47 c-e	0.41±0.02 bc
Lourdgan	58.80±2.08 a-d	8.19±0.65 a-c	135.87±15.09 e	18.89±1.72 f	0.37±0.02 bc
Boroujerd	61.63±3.84 a-d	10.91±0.78 a	441.83±68.98 ab	47.26±8.34 c-e	0.47±0.03 ab
Kashan	64.90±1.24 a-d	9.27±0.86 a-c	302.57±71.80 b-e	45.05±8.91 c-e	0.52±0.02 ab
Folad Shahr	63.73±5.00 a-d	8.70±0.80 a-c	319.43±66.58 b-d	38.07±7.36 d-f	0.47±0.03 ab

Mean values ± SE are from three independent replicates and values superscripted by different letters are significantly different by Tukey's multiple range tests ($P \leq 0.01$).

Table 4 Correlations among some morphological and biochemical traits in purslane.

Traits	Plant height	Stem diameter	Branches number	Shoot fresh weight	Shoot dry weight	Chlorophyll
Stem diameter	-0.34	-	-	-	-	-
Branches No	-0.36	0.64 **	-	-	-	-
Shoot fresh weight	0.70 **	0.66 **	0.73 **	-	-	-
Shoot dry weight	0.58 **	0.38	0.47	0.94 **	-	-
Chlorophyll	0.51 *	0.31	-0.51*	0.43	-0.42	-
Protein	-0.63 **	0.38	-0.25	-0.52*	-0.51 *	-0.47

**, *= Significant at 1 and 5% probability levels.

Mohebodini, *et al*., [13] in a study of 20 different genotypes of purslane, reported that there was a significant positive correlation between plant height, tiller number, leaf length and width, internode length and essential oil, but negative correlation with leaf width. Alam, *et al*., [12] in a study of 45 different populations collected from different states of Malaysia showed that there was a high level of genetic diversity in the germplasm.

Cluster Analysis

Cluster analysis was performed to classify the genotypes into homogenous groups based on the similarity/dissimilarity percentage between them. The unweighted pair group method with arithmetic mean (UPGMA) cluster analysis of the 18 populations of *P. oleracea* based on morphological and biochemical traits produced four clusters. Cluster 1 contained four populations (Varamin, Karaj, Zanjan and Babol), cluster 2 contained three populations (Abhar, Kashan and Boroujerd), cluster 3 contained nine populations (Malard, Qazvin, Hamedan, Qom, Folad Shahr, Izeh, Lourdgan, Bandar Turkman and Talesh) and cluster 4 including two populations (Razan and Tonekabon) (Figure 2). The distance between leader and joiner accessions in cluster analysis based on all measured traits was 5.29. The highest distance (5.29) was obtained among Malard and Varamin accessions, while the lowest distance (0.86) was obtained among Malard and Qazvin accessions. Clustering pattern confirmed that the genetic diversity is independent of a geographical area as accessions collected from different regions were accumulated in same clusters and those from the same region were also distributed into different clusters randomly. The findings of research suggest that plants belonging to different clusters can be used in the hybridization program to produce recombinant genotyping, genetic diversity, and reproductive breeding programs for *P. oleracea* population.

Alam *et al*., [25] studied the morphological physiological and mineral traits of 45 Malaysian *P. oleraaea* populations and finally divided their cluster analysis into five general groups. The pattern of population variation in this study partly follows the geographical pattern, and the external masses are clustered in the central and western provinces. In another study, the diversity of 9 Iranian purslane populations with 2 foreign populations from Germany and Japan was investigated for morphological and phytochemical characteristics. Cluster decomposition results using WARD algorithm grouped them into four different groups. Qazvin population had more genetic diversity than other populations.

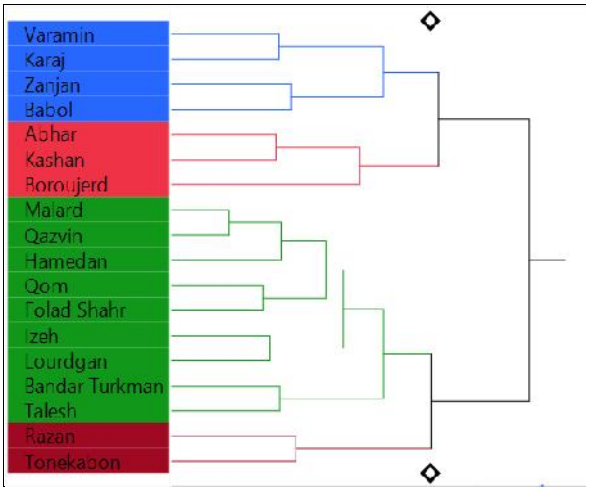

Fig. 2 A dendrogram generated by UPGMA clustering method of 18 populations of *Portulaca oleracea* L. based on all measured traits.

Principal Component Analysis (PCA)

Principal Component Analysis (PCA) is one of the multivariate analyses which reduces the more

number of correlated variables to limited number uncorrelated individual variables known as Principal Components (PC), thereby, simplifies the multivariate data set. Therefore, for a better understanding of the effectiveness of the studied traits, principal components analysis (PCA) was performed using seven characteristics on the biplot (Figure 3). The PCA revealed two principal components (PC) with Eigen value ranging from 0.21 to 2.36, which made up 55.5% of the total data variance (Figure 3). The PCA also indicated that Babol and Zanjan populations had more plant height and shoot dry weight, while Razan and Tonekabon populations had more protein content. In addition, the PCA results confirmed the results of the clustering. The PCA revealed that the most important character in PC1 was shoot fresh weight and plant height, while chlorophyll content was the least effective, while the most important character in PC2 was stem diameter and chlorophyll content, while number of branches was the least effective (Table 5). Nevertheless, since PC1 and PC2 represented 33.8% and 21.7% of the total variance respectively, so, the importance of the mentioned traits in PC1 and PC2 should be further looked into in future studies.

Table 5 Component matrix of morphological and biochemical traits in 18 populations of *Portulaca oleracea* L.

Variables	PCA1	PCA2	PCA3
Shoot fresh weight	0.861	0.295	-
Plant height	0.674	-0.33	-
Shoot dry weight	0.616	-0.282	-
Number of branches	0.598	0.053	-
Protein	-0.567	0.419	-
Stem diameter	0.248	0.768	-
Chlorophyll	0.229	0.688	-
Eigen value	2.358	1.508	0.944
Variance%	33.79	21.74	13.49
Cumulative Variance%	33.79	55.53	69.09

Broad-sense Heritability

Broad-sense heritability calculated for all characters revealed that among morphological and biochemical characters, the broad-sense heritability (h^2) of shoot dry weight was the highest (66.87) and chlorophyll content was the lowest (5.15) (Table 4). Bhatti *et al.* [26] have reported the higher heritability of shoot and root length in *Gossypium hirsutum* L.

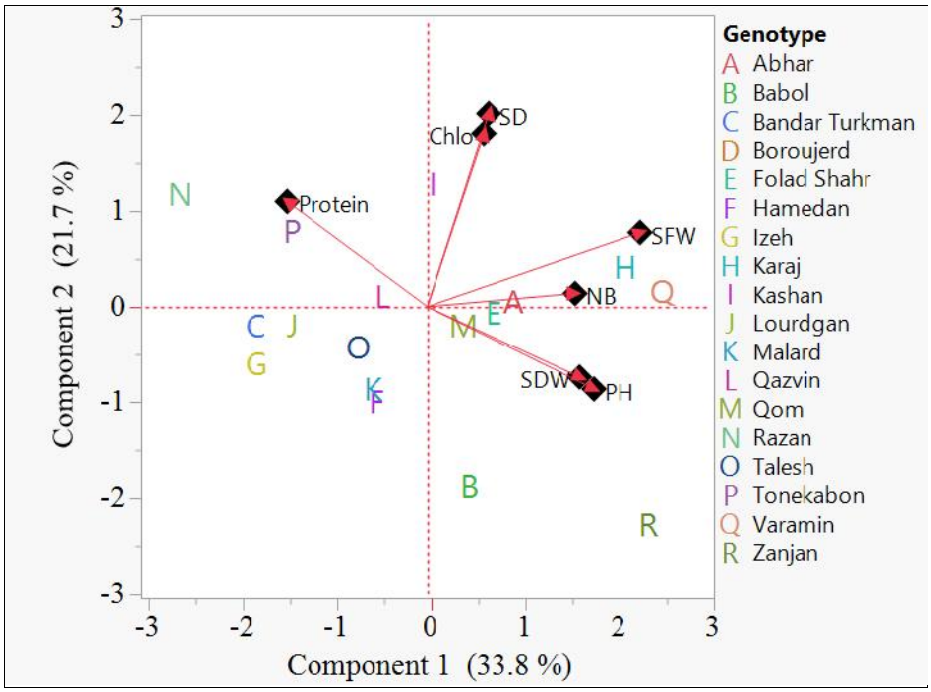

Fig. 3 The principal components analysis (PCA) of studied traits in eighteen population of *Portulaca oleracea* L. The PCA revealed two principal components (PC). Plant height (PH), stem diameter (SD), number of branches (NB), shoot fresh weight (SFW), shoot dry weight (SDW), chlorophyll (Chlo).

Table 4 Components of variance and broad-sense heritability of morphological and biochemical characters in eighteen population of *Portulaca oleracea* L.

S.O.V	Plant height	Stem diameter	Branches number	Fresh weight	Dry weight	Chlorophyll content	Protein content	E(MS)
Genotype	62.06 **	3.01**	0.41ns	31588.23 **	822.50 **	40.71 ns	0.016 *	$\pm^2_e + r\pm^2_g$
Error ($\sigma_{\frac{}{}}$	36.82	2.06	0.26	7484.56	116.58	35.02	0.007	$\pm^2_e$
σ	8.41	0.32	0.05	8034.56	235.31	1.90	0.003	
σ	45.23	2.38	0.31	15519.12	351.89	36.92	0.01	
h	18.59	13.45	16.13	51.77	66.87	5.15	30.00	

σ^2_G: Genetic variance, σ^2_P: phenotypic variance and h^2_B: broad-sense heritability.

The present results confirm high heritability of shoot and root dry weight and total dry weight among agronomic traits as well as plant height and number of leave. Therefore, the underlying genetic mechanisms can be considered as direct criterion for assessing genetic diversity.

Conclusion

In general, the results of the study revealed that there was a high genetic diversity between the *P. oleracea* populations provides important baseline data and a better understanding of conservation, management, and collection strategies for germplasm of this species. The findings of research indicated that the geographically different populations are placed in identical clusters. Also, in general, there is no relation between geographical position and population relatives. Therefore, the findings of research suggest that plants belonging to different clusters can be used in the hybridization program to produce recombinant genotyping, genetic diversity, and reproductive breeding programs for *P. oleracea* populations.

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