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Protein patterns and their association with photosynthetic pigment content, agronomic behavior, and origin of purslane accessions (*Portulaca oleracea* L.)

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Abstract

In this study, the proteomic, morphometric, and photosynthetic pigment data of purslane (*Portulaca oleracea*) accessions were combined together to show their impact on genetic variation in order to establish a relationship between protein patterns and phenotypic behavior of the plant. Seeds of 18 collected purslane accessions were cultivated based on a completely randomized design with three replicates. Before the flowering stage, the data on morphology, photosynthetic pigment content, and seed proteins were obtained. The results showed a significant difference among purslane accessions in terms of the most studied agronomic characteristics and the content of photosynthetic pigments and proteins. The cluster analysis of the 18 purslane accessions based on agronomic data, and photosynthetic pigment content, and protein pattern data produced three main clusters. Moreover, the seed protein analysis revealed that the two polymorphic protein bands of size 40 kDa (protein "a") and 30 kDa (protein "b") effectively diversified the agronomic, photosynthetic pigment, and phylogenetic relationships among the purslane accessions. Interestingly, protein "a" was produced in plants growing in low altitude areas and played a suppressive role for TDW, while protein "b" was produced in plants growing in high altitude areas and functioned as an activator agent for this trait. Overall, the outcomes of the present study indicated the presence of high genetic variability (77.6%) among the purslane accessions. These findings suggest that these proteins should be sequenced for further proteomic analyses and can be used for hybridization to generate useful recombinants in segregating generations and improve breeding varieties of *P. oleracea*.

Key words: chlorophyll, genetic diversity, morphometric, *Portulaca oleracea*, protein pattern

Abbreviations

Chlo	–	chlorophyll
FW	–	fresh weight
HEPES	–	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
TDW	–	total dry weight
SDS-PAGE	–	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
UPGMA	–	unweighted pair group method with arithmetic average

Introduction

Portulaca oleracea, commonly known as purslane, is an herbaceous herb from the Portulacaceae family. Purslane occurs worldwide, including Europe, America, Canada, India, New Zealand, Australia, China, and Japan (Movahedian et al., 2007). 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 area. 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, placed above the last leaves on the branches, and bloom open only for a short time toward the noon. Seeds are reddish brown to black, oval, and tiny (Okafor et al., 2014).

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The plant is rich in fatty acids, proteins, and vitamins (mainly vitamins A, C, E, and B and carotenoids) as well as dietary minerals such as magnesium, calcium, potassium, and iron. Approximately 70% of its fatty acids are unsaturated, and approximately 50% of these fatty acids comprise only omega-3 fatty acids (Masoodi et al., 2011). According to folk medicine sources, purslane has antinociceptive, anesthetic, antiseptic, antiascorbate, anti-inflammatory, anti-fungal, and blood purifying properties; reduces swelling and abscesses; and heals insect bite and scorpion sting (Zhou et al., 2015).

Genetic diversity can be estimated using various methods including morphological traits, protein markers, and molecular markers (Aharizad et al., 2012; Talei et al., 2014). Initially, morphological and agronomic characteristics have often been used for basic genetic characterization (Okpul et al., 2005). However, morphological and agronomical traits have several limitations such as low polymorphism and heritability and may be controlled by epistasis and pleiotropic gene effects (Danaeipour et al., 2019), while biochemical and molecular markers reflect the genotype more directly, independent of environmental impact (Ernst et al., 2017). Biochemical markers and specifically seed protein markers are valuable tools to identify cultivar, register new varieties, and characterize plant species to study genetic diversity (Kutka Hlozakova et al., 2016; Sharma and Krishna, 2017). Most of the proteins are unique and suitable to be used as markers in genetic diversity analyses at inter- and intra-population levels (Asante et al., 2009). Morphological data could also be used as reliable tools in phylogenetic analysis when they are correlated with biochemical data (Huang et al., 2013). With regard to genetic diversity, several polymorphic proteins have been reported in the genus *Mentha* (Hassan et al., 2003), *Ocimum* species (Mustafa et al., 2006), wheat (El-Bakatoushi, 2010), and Brassicaceae (Khurshid and Rabbani, 2012) and purslane (*P. oleracea* L.) accessions (Alam et al., 2015). The reason for the high variability among purslane in terms of measured traits could be the pursuit of free purslane pollination and the possibility of pollen transfer between the species (Wickramasinghe et al., 2010). Hence, the present research aimed to evaluate seed protein profiles and attempted to utilize them along with morphometric data and photosynthetic pigment data, not only to illustrate genetic diversity but also to discover a relationship among protein patterns, photosynthetic pigments, and morphometric behavior in the herb's accessions. In other words, we assessed whether there is a possibility to detect any specific protein band or bands in relation to a particular photosynthetic pigment and morphometric behavior.

Materials and methods

Genetic material and growth conditions

Seeds of 18 accessions of *P. oleracea* were collected from different geographical origins of Iran (Table 1). The seeds were surface sterilized with 10% sodium hypochlorite (NaClO) solution for 10 min and thoroughly rinsed with distilled water (Talei et al., 2011). The seeds were cultivated based on a randomized complete block design with three replicates in the research farm of Medicinal Plants Research Center, Shahed University, Tehran, Iran. Each accession was cultivated in 1 × 1.2 m plots with three rows and 25 cm spacing, at the density of 20–30 plants per plot. The plants were irrigated every 2 days until the flowering stage. Before the flowering stage, 10 plants were randomly selected from each experimental unit, and the data on morphological and photosynthetic pigment traits such as average shoot length (cm), stem diameter (mm), number of branches, shoot fresh weight (FW) (g per plant), shoot dry weight (g per plant), and chlorophyll a (μg/g FW) and chlorophyll b content (μg/g FW) were obtained. The dry shoot weights were measured after drying at 68°C for 72 h.

Determination of chlorophyll content

To determine the content of chlorophyll a, chlorophyll b, and total chlorophyll, approximately 0.5 g fresh leaf tissue was extracted with 5 ml of 100% acetone with three replicates. The extracted sample was filtered through a Whatman No. 2 filter paper. The filtrate sample was then centrifuged at 3000 *g* for 5 min. Next, 50 μl pigment extract was added to 950 μl of 80% aqueous acetone, and the respective absorptions were measured using a Shimadzu UV-1201 model spectrophotometer at 645 and 663 nm (Arnon, 1967).

Determination of protein content

To determine the content of seed proteins, 1 g of collected seeds were grounded in liquid nitrogen using a pre-cooled mortar and pestle to obtain a fine powder, and the powder was then homogenized with 2 ml

Table1. Geographical origins of the of *P. oleracea* accessions from different parts of Iran

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

of HEPES/KOH extraction buffer as described by Talei et al. (2013). Finally, the Bradford method (1976) was used to determine the total protein concentration using a spectrophotometer at 595 nm (Lambda 25, UV/VIS). The experiments were performed in triplicate.

Protein separation by SDS-PAGE

The protein samples were subjected to SDS-PAGE using Laemmli method (1970). Twenty microliters of the protein samples was mixed with 4 µl loading buffer (0.125 M Tris base, pH 6.8; 20% (w/v) glycerol; 2% (w/v) SDS; 2% (v/v) 2-mercaptoethanol; and 0.01% (w/v) bromophenol blue) and loaded into the wells of the 12% separating gel. Electrophoresis was performed at 100 V over 90 min by using a Mini Protein electrophoresis system (Bio-Rad, USA).

The gels were stained with 0.25% Coomassie Brilliant Blue R-250 (Sigma) in 40% (v/v) methanol and 7% (v/v) acetic acid for 1 h and de-stained with 40% (v/v) methanol and 7% (v/v) acetic acid until the background was clear. Gel scanning and visualization were performed using a densitometer (GS-800, Bio-Rad). The analysis was conducted using UVIDoc Analyzer software.

Statistical analysis

SPSS v.25 software was used for all statistical analyses, including the raw data normality test and the main data analysis as well as for analysis of variance and Duncan's multiple range test ($P \leq 0.01$). JMP software version 13 (SAS Institute Inc., 2009b) was used for cluster analysis.

Results

Analyses of agronomic characteristics and photosynthetic pigments of the purslane accessions

The variance analysis of the investigated characteristics revealed significant differences among the purslane accessions for most of the studied traits ($P \leq 0.01$), while no significant differences in stem diameter, number of branches, and root dry weight were observed among the accessions (Tables 2 and 3). These results indicated that the accessions selected for this study

Table 2. Variance analysis of 18 purslane accessions in terms of agronomic traits

S.O.V	df	Mean square						
		shoot length	stem diameter	branches number	shoot fresh weight	shoot dry weight	root length	root fresh weight
Rep	2	200.0**	6.3 ns	2.6**	2864.3 ns	176.6 ns	46.9**	207.9**
Genotype	17	70.8*	3.0 ns	0.4 ns	31588.2*	621.8**	8.5*	16.6 ns
Error	34	32.5	2.1	0.3	7484.6	216.9	4.0	4.5
CV [%]		9.0	16.0	15.9	22.8	22.2	9.47	8.9

*, ** – significant at 5% and 1% probability level; ns – non-significant

Table 3. Variance analysis of 18 purslane accessions in terms of some agronomic traits, protein and photosynthetic pigments contents

S.O.V	df	Mean square					
		root dry weight	total dry weight	protein	chlorophyll a	chlorophyll b	total chlorophyll
Rep	2	10.3**	155.9 ns	0.67**	4.2 ns	181.4**	240.1**
Genotype	17	0.5 ns	618.3**	0.02**	17.0**	64.8**	78.7**
Error	34	0.5	218.0	0.01	4.9	13.7	26.1
CV [%]		18.7	23.8	2.3	4.4	17.9	7.1

were significantly different from each other based on photosynthetic pigments and agronomic traits.

The highest (71.4 ± 5 cm) and lowest (49.1 ± 1.7 cm) shoot length were shown by Abhar accession from the Zanjan state and Bandar Turman accession from the Golestan state, respectively. The highest stem diameter (10.9 ± 0.8 mm) was exhibited by Boroujerd accession from the Lorestan state, while the lowest stem diameter (7.5 ± 1.1 mm) was shown by Bandar Turkman accession. Talesh and Lourdgan accessions showed the highest (411.93 ± 30.1 g per plant) and lowest shoot FW (142.6 ± 15.1 g per plant), respectively. The highest shoot dry weight (74.4 ± 5.4 g per plant) was shown by Zanjan accession, while the lowest shoot dry weight (21.7 ± 3.1 g per plant) was observed for Foulad shahr accession. No significant differences in root fresh and dry weights were observed among the different accessions; nevertheless, root fresh and dry weights varied from 20.5 to 29.9 and from 3.2 to 4.9 g per plant, respectively, among the different accessions. Table 3 shows the comparison of mean values obtained using Duncan's multiple range test for the studied traits at $P \leq 0.01$.

The results showed significant differences in root length among the accessions, with the variation ranging from 17.7 to 24.3 cm. The highest (88.8 ± 6.1 g per plant) and lowest total dry weight (TDW) (22.9 ± 4.1 g per plant) were observed for Zanjan and Lourdgan accessions, respectively. The highest (3.3 ± 0.12 mg/g FW) and lowest (3.0 ± 4.1 mg/g FW) protein content was shown by Bandar Turkman and Hamadan accessions, respectively. The highest (53.2 ± 0.9 μg/g FW) and lowest (43.3 ± 3.8 μg/g FW) chlorophyll a content was observed for Zanjan and Bandar Turkman accessions, respectively, while the highest (28.5 ± 1.1 μg/g FW) and lowest (12.4 ± 4.2 μg/g FW) chlorophyll b content was observed for Qazvin and Zanjan accessions, respectively. The total chlorophyll content varied from 64.6 to 81.1 μg/g FW among different accessions (Table 4). On the basis of these results, it could be stated that the differences in the measured traits among the accessions can be used for breeding programs and selection of the desired genotypes.

The variance analysis based on 13 states showed significant differences among the accessions in shoot length, shoot fresh and dry weights, TDW, protein content, and chlorophyll content in each state. The highest (68.1 ± 4.5 cm) and lowest (49.1 ± 1.8 cm) shoot length was observed for the Gilan state and Golestan state,

Table 4. Mean comparison of different purslane accessions based on some studied traits

Genotype	Shoot length [cm]	Shoot fresh weight [g]	Shoot dry weight [g]	Root length [cm]	Total dry weight [g]	Protein [mg/g fw]	Chlorophyll a [µg/g fw]	Chlorophyll b [µg/g fw]	Total chlorophyll [µg/g fw]
Varamin	66.3 ± 1.6 $^{a–d}$	330.4 ± 30.2 $^{a–d}$	62.9 ± 8.4 ^{ab}	21.0 ± 0.6 $^{a–e}$	75.1 ± 8.4 ^{ab}	3.2 ± 0.10 ^{ab}	48.2 ± 2.8 ^{b}	16.4 ± 1.2 ^{ef}	64.6 ± 3.9 ^{e}
Malard	62.7 ± 2.2 $^{a–c}$	335.5 ± 53.2 $^{a–d}$	60.4 ± 12.7 $^{a–c}$	20.0 ± 0.6 $^{c–e}$	52.7 ± 13.1 $^{c–e}$	3.3 ± 0.09 ^{ab}	51.9 ± 0.7 ^{ab}	21.0 ± 2.0 $^{b–d}$	73.0 ± 2.7 $^{a–e}$
Karaj	65.5 ± 3.5 $^{a–c}$	313.8 ± 21.7 $^{a–d}$	48.6 ± 3.7 $^{a–e}$	22.0 ± 0.0 $^{a–c}$	49.6 ± 3.8 $^{c–e}$	3.1 ± 0.11 $^{a–d}$	52.2 ± 0.3 ^{ab}	25.6 ± 0.9 ^{ab}	77.8 ± 1.3 $^{a–c}$
Qom	65.9 ± 2.9 $^{a–c}$	271.3 ± 61.7 $^{a–d}$	53.3 ± 9.1 $^{a–d}$	24.0 ± 1.5 ^{ab}	38.8 ± 9.5 $^{d–g}$	3.2 ± 0.13 ^{ab}	52.4 ± 0.1 ^{ab}	27.7 ± 1.7 ^{ab}	80.0 ± 1.7 ^{ab}
Abhar	71.4 ± 5.0 ^{a}	203.3 ± 19.8 $^{a–d}$	49.2 ± 5.3 $^{a–e}$	21.3 ± 1.3 $^{a–e}$	49.9 ± 6.3 $^{c–e}$	3.2 ± 0.09 ^{ab}	52.8 ± 0.6 ^{a}	17.8 ± 3.0 $^{c–e}$	70.6 ± 2.4 $^{b–e}$
Zanjan	63.2 ± 5.6 $^{a–c}$	240.2 ± 51.9 $^{a–d}$	74.4 ± 5.4 ^{a}	20.3 ± 2.0 ^{a}	88.8 ± 6.1 ^{a}	3.1 ± 0.09 $^{b–d}$	53.2 ± 0.9 ^{a}	12.4 ± 4.2 ^{f}	65.6 ± 3.4 ^{de}
Qazvin	63.5 ± 2.1 $^{a–c}$	205.9 ± 7.5 $^{a–d}$	42.3 ± 7.4 $^{b–e}$	21.0 ± 0.6 $^{a–e}$	51.8 ± 7.4 $^{c–e}$	3.2 ± 0.08 ^{ab}	52.7 ± 0.1 ^{a}	28.5 ± 1.1 ^{a}	81.1 ± 1.0 ^{a}
Bandar Turkman	49.9 ± 1.7 ^{d}	370.8 ± 80.2 $^{a–c}$	60.1 ± 4.6 $^{a–c}$	17.7 ± 0.3 ^{e}	59.9 ± 4.8 $^{b–d}$	3.3 ± 0.12 ^{a}	43.3 ± 3.8 ^{c}	24.4 ± 6.6 $^{a–c}$	67.7 ± 9.4 ^{de}
Talesh	68.1 ± 3.8 ^{ab}	411.9 ± 30.1 ^{a}	35.9 ± 17.3 $^{b–e}$	21.7 ± 1.5 $^{a–d}$	64.7 ± 16.6 ^{bc}	3.3 ± 0.14 ^{a}	52.8 ± 0.4 ^{a}	15.6 ± 0.1 ^{ef}	68.4 ± 0.5 $^{c–e}$
Hamedan	61.4 ± 7.5 $^{a–c}$	193.7 ± 2.7 $^{a–d}$	28.6 ± 4.8 ^{de}	18.0 ± 1.1 ^{de}	33.4 ± 4.9 $^{e–g}$	3.0 ± 0.10 ^{e}	49.8 ± 0.1 ^{ab}	16.5 ± 0.7 ^{ef}	66.3 ± 0.7 ^{de}
Razan	65.9 ± 1.8 $^{a–c}$	181.2 ± 29.1 $^{b–d}$	32.8 ± 7.0 $^{c–e}$	22.7 ± 2.7 $^{a–c}$	27.5 ± 7.5 ^{g}	3.2 ± 0.12 ^{de}	52.6 ± 0.3 ^{a}	25.4 ± 4.3 ^{ab}	78.0 ± 4.0 $^{a–c}$
Izeh	62.9 ± 1.2 $^{a–c}$	287.0 ± 28.1 $^{a–d}$	51.9 ± 5.1 $^{a–d}$	22.0 ± 3.1 $^{a–c}$	28.5 ± 5.1 ^{fg}	3.3 ± 0.12 ^{ab}	49.0 ± 1.5 ^{ab}	17.5 ± 0.5 $^{c–e}$	66.5 ± 2.0 ^{de}
Babol	63.1 ± 4.5 $^{a–c}$	400.2 ± 24.6 ^{ab}	57.5 ± 9.7 $^{a–c}$	20.7 ± 0.5 $^{a–e}$	58.7 ± 9.5 $^{b–d}$	3.2 ± 0.15 ^{ab}	50.8 ± 0.6 ^{ab}	22.3 ± 0.3 $^{a–d}$	73.0 ± 1.0 $^{a–e}$
Tonekabon	65.8 ± 2.1 $^{a–c}$	301.7 ± 30.4 $^{a–d}$	41.9 ± 12.6 $^{b–e}$	20.3 ± 0.9 $^{b–e}$	48.9 ± 12.3 $^{c–f}$	3.2 ± 0.15 ^{ab}	50.2 ± 0.9 ^{ab}	22.2 ± 4.0 $^{a–d}$	72.3 ± 4.9 $^{a–e}$
Lourdgan	56.6 ± 2.6 ^{cd}	142.6 ± 15.1 ^{d}	24.5 ± 3.7 ^{de}	24.3 ± 1.5 ^{a}	22.9 ± 4.1 ^{g}	3.2 ± 0.07 $^{a–c}$	51.8 ± 0.6 ^{ab}	17.8 ± 1.1 $^{c–e}$	69.6 ± 0.6 $^{c–e}$
Boroujerd	57.9 ± 2.4 $^{b–d}$	182.7 ± 15.1 $^{b–d}$	36.7 ± 3.4 $^{b–e}$	20.9 ± 0.7 $^{a–e}$	51.4 ± 2.9 $^{c–e}$	3.2 ± 0.11 $^{a–c}$	51.4 ± 0.1 ^{ab}	23.7 ± 0.1 $^{a–c}$	75.1 ± 0.2 $^{a–d}$
Kashan	63.3 ± 2.0 $^{a–c}$	217.2 ± 71.8 $^{b–e}$	40.0 ± 11.8 $^{b–e}$	20.3 ± 1.0 b–e	48.9 ± 11.5 $^{c–f}$	3.1 ± 0.13 ^{ab}	49.7 ± 0.9 ^{ab}	22.4 ± 4.5 $^{a–d}$	72.2 ± 5.4 $^{a–e}$
Folad Shahr	62.9 ± 5.6 $^{a–c}$	158.9 ± 66.6 ^{cd}	21.7 ± 3.1 ^{e}	21.4 ± 2.0 $^{a–e}$	42.0 ± 2.5 $^{d–g}$	3.2 ± 0.18 $^{c–e}$	51.7 ± 0.8 ^{ab}	15.8 ± 1.2 ^{ef}	67.5 ± 0.4 ^{de}

Different letters indicate a significant difference between the values of pairs of treatment within columns (mean values ± standard error of mean) at $P \leq 0.01$

Table 5. Component matrix of measured agronomic characteristics in 18 populations of *Portulaca oleracea*

Variables	PCA1	PCA2	PCA3	PCA4	PCA5
Total chlorophyll	0.787	0.132	0.125	0.566	−0.040
Protein	−0.710	−0.144	0.235	0.384	0.016
Chlorophyll b	0.694	0.132	−0.085	0.597	0.097
Root fresh weight	0.619	0.180	0.089	−0.212	0.573
Chlorophyll a	0.484	0.050	0.476	0.148	−0.295
Total dry weight	−0.389	0.653	0.122	−0.063	0.068
Shoot dry weight	−0.470	0.619	−0.067	0.258	0.058
Shoot fresh weight	−0.396	0.554	0.131	0.346	0.209
Root dry weight	0.498	0.545	−0.128	−0.473	0.186
Stem diameter	−0.177	−0.055	0.697	−0.103	−0.044
Shoot length	0.206	0.451	0.534	−0.300	−0.427
Root length	0.370	−0.339	0.411	−0.032	0.205
Branches number	−0.342	−0.238	0.482	0.005	0.551
Eigen value	3.327	1.882	1.538	1.405	1.044
Variance [%]	25.593	14.478	11.828	10.809	8.027
Cumulative variance [%]	25.593	40.072	51.900	62.709	70.736

respectively. The highest (69.3 ± 15.1 g per plant) and lowest (22.9 ± 21.7 g per plant) TDW was exhibited by Zanjan, and Chaharmahal and Bakhtiari states, respectively. The protein content in different states varied from 3.0 to 3.3 g/ml FW.

The principal component analysis (PCA) revealed five principal components (PCs) with eigenvalue ranging from 0.02 to 3.33, which made up 70.74% of the total data variance (Table 5). According to the PCA, 40.07% of total variance was explained by the first two components. Total chlorophyll content had the highest positive correlation (> 0.787) with PC1, while stem diameter showed the least correlation (> −0.177). The most important characteristic in PC2 was TDW (> 0.653), while chlorophyll a showed the least correlation (> 0.050) (Table 5). The UPGMA cluster analysis of the 18 accessions of *P. oleracea* based on the measured agronomic characteristics using the minimum variances (ward method) generated three clusters at the Euclidean distance of 6.82. The first cluster (green cluster) included seven accessions, the second cluster (red cluster) contained eight accessions, and the third cluster (blue cluster) contained three accessions (Fig. 1). The first cluster was associated with the highest mean of TDW (61.2 g per plant) and protein content (3.24 g/ml FW), while the second cluster had the lowest mean of TDW (33.1 g per plant) and protein content (3.05 g/ml FW).

Seed protein patterns and protein-based diversity

Seed storage proteins of the 18 collected *P. oleracea* accessions from different geographical origins were extracted and separated on SDS-PAGE. The results showed the presence of 15 different types of protein bands ranging from 15 to 100 kDa. The results indicated that most of the bands were similar in all accessions. However, two protein bands (13.3%) with low molecular weight (almost 30 kDa and 40 kDa) were found to be polymorphic. These two polymorphic proteins included a 40 kDa protein (designated as band “a”), which was absent only in Karaj, Razan, and Kashan accessions, and a 30 kDa protein band (designated as band “b”), which was present in Karaj, Talesh, Bandar Turkman, Razan, Tonekabon, and Kashan accessions (Fig. 2). These protein bands can also serve as potential useful markers for hybridization and breeding programs in future studies.

The UPGMA cluster analysis of the 18 accessions of *Portulaca oleracea* based on the protein patterns using the minimum variances (ward method) generated three clusters at the Euclidean distance of 4.88 (Fig. 3). The first cluster with green color contained 12 accessions,

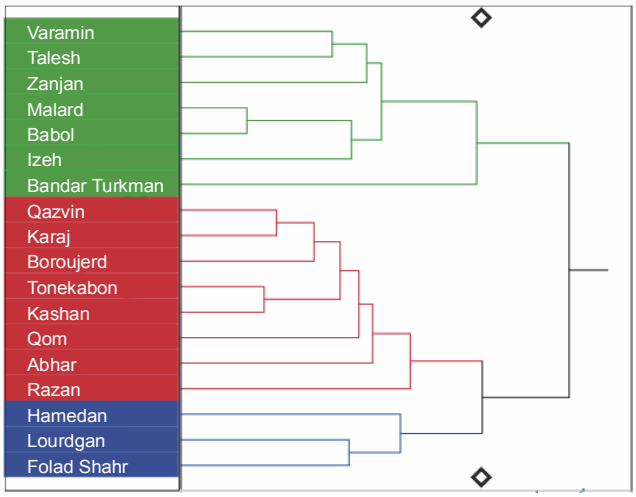

Fig. 1. Dendrogram generated by UPGMA clustering method based on the studied agronomic behavior and photosynthetic pigments in the 18 accessions of *Portulaca oleracea*

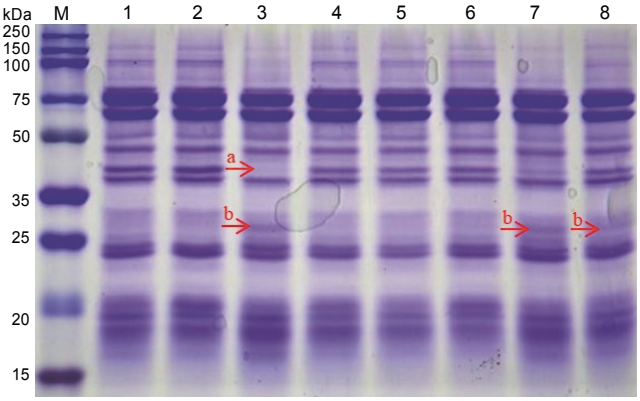

Fig. 2. The seed protein patterns of *P. oleracea* accessions on 12% polyacrylamide gel; the lane M represents the protein ladder (Thermo Fisher Scientific, 15–250 kDa), 1) Varamin, 2) Malard, 3) Karaj, 4) Qom, 5) Abhar, 6) Zanjan, 7) Bandar Turkman and 8) Talesh; protein samples were loaded with equal amount of 15 µg of proteins

indicating close similarity among most accessions; the second cluster (the red cluster) contained 3 accessions; and the third cluster (the blue one) also comprised 3 accessions. Karaj, Razan, and Kashan accessions, which were lacking protein "a", were located separately in the third cluster. The cluster analysis indicated that the accessions possessing protein "b" are much more related together than those lacking this protein and were located in the second cluster (Fig. 3).

Altitude and proteomic association of the accessions

Grouping the accessions based on the presence or absence of the polymorphic proteins and comparing the

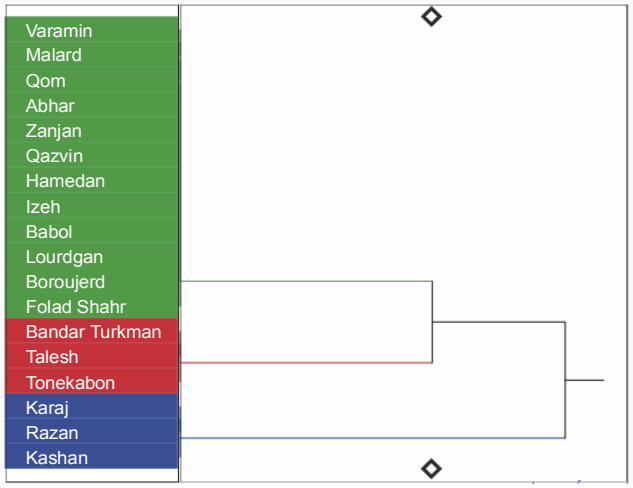

Fig. 3. Dendrogram generated by UPGMA clustering method based on seed storage protein patterns showing the phylogenetic relationships of the 18 accessions of *P. oleracea*

groups in terms of the altitude data using independent sample t-test showed significant differences between the groups (Table 5). The results revealed that the presence or absence of these proteins might be related to the altitude of geographical origins. The mean of altitude in accessions containing protein "a" was lower than that for accessions lacking this protein, while the mean of altitude in accessions containing protein "b" was higher than that for accessions lacking this protein (Table 6). In other words, protein "a" was produced by accessions growing in low altitude areas, while protein "b" was produced by accessions growing in high altitude areas.

Relationships between accessions based on agronomic traits, photosynthetic pigments, and protein data

The cluster analysis based on agronomic traits, photosynthetic pigments, and protein data provided evidence to prove the existence of differences among the accessions. Grouping the accessions based on the presence or absence of the polymorphic proteins and comparing the groups in terms of TDW, chlorophyll b content, and total chlorophyll content using independent samples t-test indicated significant differences between the groups (Table 7). The mean of TDW in accessions containing protein "a" was higher than that for accessions lacking this protein, while the mean of TDW in accessions containing protein "b" was lower than that for accessions lacking this protein. The mean of chlorophyll b and total chlorophyll content in accessions containing protein "a" was lower than that for accessions lacking this protein,

Table 6. The comparing the two groups of *Portulaca oleracea* accessions based on presence and absence of protein "a" and protein "b" in terms of altitude of geographical origins using *t*-test

Variable	Groups	Protein "a"			Protein "b"		
		N	mean ± SEM	sig	N	mean ± SEM	sig
Altitude	presence	45	962.1 ± 108.2	0.017	21	1285.6 ± 136.1	0.031
	absence	9	1378.7 ± 121.4		33	869.9 ± 121.3	

Table 7. The comparing the two groups accessions of *Portulaca oleracea* based on presence and absence of protein "a" in terms of agronomic traits using *t*-test

Protein	Groups	N	Total dry weight		Chlorophyll b		Total chlorophyll	
			mean ± SEM	sig	mean ± SEM	sig	mean ± SEM	sig
Protein "a"	presence	45	51.2 ± 2.9	0.034	20.0 ± 0.9	0.039	70.8 ± 1.0	0.043
	absence	9	42.0 ± 3.8		24.5 ± 1.9		76.0 ± 2.2	
Protein "b"	presence	21	43.2 ± 3.4	0.041	22.9 ± 1.5	0.037	73.4 ± 1.9	0.049
	absence	33	53.8 ± 3.4		19.4 ± 0.9		70.5 ± 1.0	

while the mean of chlorophyll b and total chlorophyll content in accessions containing protein "b" was higher than that for accessions lacking this protein (Table 6). In other words, the presence of protein "a" caused an increase in dry weight and the presence of protein "b" caused a decrease in dry weight, while the presence of protein "a" caused a decrease in the chlorophyll content and the presence of protein "b" caused an increase in the chlorophyll content.

Discussion

The study of biochemical and biomass differences among plant species of different geographical origins is important to obtain essential baseline data and to enable a better understanding of conservation, management, and collection strategies for germplasm of these species (Lou et al., 2015). In the current study, agronomic traits and protein patterns revealed significant differences among the studied purslane accessions; these results agreed well with the findings of Alam et al. (2014), who showed a high genetic diversity among purslane populations, which could be due to free pollination and the possibility of pollen transfer between the species (Wickramasinghe et al., 2010).

Marker techniques, ecotypes, and their origins are important parameters that affect the observed genetic polymorphisms in plants (Binova et al., 2020). Protein pattern analysis and clustering of accessions based on protein patterns are useful in plant breeding programs. The gel analysis detected two polymorphic protein bands sized approximately 40 kDa (band "a"), which was absent in Karaj, Razan, and Kashan accessions, and 30 kDa (band "b"), which was present in Karaj, Qazvin, Bandar Turkman, Razan, Lourdgan, Kashan, and Folad Shahr accessions. Three clusters were observed in protein-based data analysis. Karaj, Razan, and Kashan accessions, which lacked protein "a", were located separately in the third cluster, and the accessions possessing protein "b" were much more related together than those lacking this protein and were located in the second cluster.

In Iran, purslane accessions are distributed in various geographical regions with different climates, which results in adaptation to ecological factors and genetic variation. Climate plays an important role in local adaptation of the plant species and genetic diversity of the plant populations (Mosca et al., 2012). In the present study, grouping the accessions based on the presence or absence of these protein bands and comparing the groups according to the altitude of geographical origins, TDW, and chlorophyll b and total chlorophyll content using independent samples *t*-test showed significant differences between the groups (Tables 5 and 6). The relationship between protein patterns and geographical origins indicated the impact of ecological conditions on adaptation and differentiation of purslane accessions;

this result agreed well with the findings of Zhao et al. (2006) on *Stipa grandis* populations and of Huang et al. (2016) on *Caragana microphylla,* who reported influence of some climatic factors on genetic diversity in these plants. Our results showed the explicit impact of seed proteins in improving (protein b) or decreasing (protein a) agronomic traits and photosynthetic pigments. For instance, the absence of protein "a" and presence of protein "b" in Karaj, Razan, and Kashan accessions resulted in lower TDW and lower chlorophyll content than that in other accessions (Table 4). Thus, it could be concluded that the agronomic variation observed in these accessions might be due to the specific protein profiling. There is little information on the relationship between protein patterns produced by seed storage proteins in electrophoresis, agronomic characteristics, and geographical origins. Talei et al. (2014) studied *Andrographis paniculata* accessions and reported that the polymorphic proteins effectively differentiated the morphological characteristics and phylogenetic relationships among the accessions and these proteins acted as activator agents for agronomic characteristics such as TDW. Andrews et al. (2006) in a study on tobacco (*Nicotiana tabacum*) without emphasizing on a specific protein claimed that generally, shoot protein concentration influences shoot and root growth. A linear regression model incorporating leaf soluble protein concentration and plant dry weight has also been reported. Victor et al. (2010) in a study on *Vitis riparia* Michx. grapevines reported that day length could act as an abiotic factor for alteration of protein patterns and can lead to a different expression pattern of agronomic characteristics in plants. Gherekhloo et al. (2020) studied *Phalaris minor* populations and reported a high genetic similarity among the populations in different regions. Bahraminrjad and Mohammadinejad (2013) reported that different cultivation conditions influenced the agro-morphological traits of cumin ecotypes.

There is little information on how the detected protein patterns could be directly attributed to the agronomic characteristics, although recent studies have tended to make a more accurate link between specific proteins and agronomic diversity (Hanada et al., 2009). Our results agreed well with the findings of Akhila and Beevy (2011) who used protein patterns as markers to describe the morphological variation between the *Sesamum* species as it was noted that a speci c protein was the unique feature of the cultivated species, whereas the two other protein bands were characteristics of the wild species. Interestingly, sometimes, a speci c protein seems to simultaneously play the role of a repressor and an activator in plants' architecture (He et al. 2010). Notably, in our present study, these roles were performed by separate proteins, where in the suppressive role was performed by protein "a" for TDW in some accessions, whilst protein "b" played the activator role for this trait in different accessions. The results also indicated that the simultaneous presence of the activator and suppressor proteins in an accession could lead to an unstable increase in different morphological traits. The results of the present study suggest that the protein variation among the accessions of *P. oleracea* can be used as an effective strategy in plant breeding programs. In addition, the differences observed among the accessions are quite important for the development of the *P. oleracea* bank and may be used in hybridization and breeding programs. Nevertheless, there are few questions that need to be addressed: which mechanism could lead to an increase or decrease in TDW? Can the altitude of geographical origins be effective in the synthesis of these proteins? What is the biochemical combination of these proteins? Are these proteins encoded by a specific gene or by a couple of genes? And finally, are these proteins probably associated with the photosynthetic pigment content of the plant?

Conclusions

Although seed proteins, agronomic traits, and photosynthetic pigments were used for providing data to study the diversity and inter-relationships of *P. oleracea* accessions, our main purpose was to find the association between protein patterns and the altitude of geographical origins, agronomic traits, and photosynthetic pigment content. The two polymorphic protein bands of 40 kDa (protein "a") and 30 kDa (protein "b") effectively diversified the agronomic behavior, photosynthetic pigments, and phylogenetic relationships among the purslane accessions. Interestingly, protein "a" was produced in accessions growing in low altitude areas and played a suppressive role for TDW, while protein "b" was produced in accessions growing in high altitude areas and served as an activator agent for this trait. Overall, the outcomes of the present study indicated that the polymorphic pro-

teins effectively diversified the agronomic behavior and photosynthetic pigments among the purslane accessions. These findings suggest that these proteins should be sequenced and perfectly established for further proteomic analyses. Finally, these proteins can be used for hybridization to generate useful recombinants in segregating generations and in improving breeding varieties of *P. oleracea*.

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