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Research Paper

Evaluation of some Iranian watercress (*Nasturtium officinale* L.) populations using agro-morphological traits

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Abstract: Watercress (Nasturtium officinale L.) is a perennial member of the Brassicaceae family and naturally grows in abundance near springs and open-running waterways in Europe, America and Asia. The plant seeds are rich sources of glocunasturtiin which its hydrolysis product, 2-phenethyl isothiocyanate (PEITC), is believed to be cancer preventing. The present study was aimed to evaluate twenty-four wild growing populations of N. officinale collected from six provinces of Iran using agro-morphological traits. According to the results, plants of Noshahr population had the minimum number of silique per plant (8.71) and the lowest length of flowering stems (4.86 cm). The highest plant fresh weights were recorded in plants collected from Sarab (50.73 g) and Mehraban (40.94 g). The highest anthocyanin content was visually observed in plants of Uromieh 1 and Uromieh 2 populations. Three kinds of leaf shape were identified in studied populations. Correlation studies revealed significant relationships between some measured characteristics such as number of leaves with the leaf width and leaf length and leaf width with the plant height (correlation coefficients 0.825, 0.720, 0.880, respectively). Cluster analysis divided populations in question into four main groups including 13, 8, 2 and 1 members. Members of the first group had the lowest plant fresh weights and visually lacked anthocyanin. The similarity point of plants in the second group was based on the traits of leaf length, leaf width and the existence of anthocyanin. Plants of the third group were similar from the perspective of plant fresh weight and the plant height, and finally there was clear difference in the fourth group (including Noshahr population) from the perspective of silique and the leaf thickness. Considering morphological diversity observed in the present study, Iranian watercress populations have the potential to be used in the breeding programs of this plant.

Keywords: Cluster analysis; Genetic diversity; Glocunasturtiin; Morphological traits; Watercress

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Introduction

Watercress (Nasturtium officinale L.) is a perennial herb from the Brassicaceae family which found in abundance near springs and open-running waterways in Europe, America and Asia, and is produced commercially beside the natural water current and as the hydroponic in the greenhouse (Biddington et al., 1983). This plant has high nutritional value so that the iron level is more than spinach, its vitamin C is more than the orange and its calcium level is more than the milk (Palanisvamy et al., 2003). One hundred gram fresh leaf of watercress is including 43 mg vitamin C, 4700 unit vitamin A and 34 mg Alpha-tocopherol (Hadas et al., 2006).

Watercress is a rich source of 2-phenethyl glucosinolate (gluconasturtiin) (Gerard et al., 2006). Glocunasturtin is a kind of glocusinolate and glocusinolates are group of organic compounds, which include solphur and nitrogen resulting from breaking glucose and one amino acid, they are found as the secondary metabolite in Brassicaceae family (Mithen, 2001). The hydrolysis product of gluconasturtiin, 2-phenethyl isothiocyanate (PEITC), is believed to be cancer preventing (Hecht et al., 1999 and 1995).

Three species of 'watercress' are recorded in Europe; Rorippa nasturtium-aquaticum (L.) Hayek (common or green watercress, 2n = 32), R. × sterilis (Airy-Shaw) (brown watercress, 2n = 48) and R. microphylla (Boenn.) Hyland (wild watercress, 2n =64) (Howard and Manton, 1946). Together R. africana (Braun Blanquet) from North Morocco, the closely allied R. valdes-bermejoi (Castroveijo) from Spain, and R. gambellii and R. floridanum (Al-Shehbaz and Rollins) from North America, they have been proposed to constitute a genus Nasturtium distinct from Rorippa (Al-Shebaz and Price, 1998). It is proposed that these species are more closely related to Cardamine than Rorippa, supporting this contention with evidence from sequencing of the gene rbcL (Les, 1994).

According to researches and visiting different regions of the Iran, it was determined that the watercress exists as a native plant and the people of that region used it as fresh leafy vegetable, the other consumption of the watercress is its pharmaceutical consumption which was used to treat the kidney pain, the body booster, digestive system disinfectant. Since this plant has not been cultivated in Iran, Iranian people use it as a wild plant. The recent droughts have been resulted in decrease of water resources. According to this fact that this plant grows beside the water, there is a serious threat to survive it and the indiscriminate use of nature has led to this valuable plant is in danger of extinction. So, the attempt to maintenance habitats and especially their genetic resources by identification, permanent protection, propagation and its cultivation and development of its production is necessary to maintenance and survive this plant. One efficient strategy to solve loss of plant diversity consist of exploiting wild germplasm genomes of species, which preserve a good part of their adaptive factor, diseases tolerance, and their genetic richness (Mahjoub et al., 2009).

Understanding genetic diversity and the origin may prove helpful in designing an effective way for managing of plants species (Choudhary et al., 2001; Zhange et al,. 2008). Morphological traits are useful and practical markers in breeding programs that reveal different level of polymorphism (Pirkhezri et al., 2010) Also, perception of these traits can be used in breeding programs for developing new commercial cultivars (Balmer and Blanke, 2005; Hrotko et al., 2008). Actually, selection as a breeding program is practiced based on genetic diversity. With the increasing diversity of populations the selection range becomes more extended (Basafa and Taherian, 2009). Traditionally, diversity is assessed by measuring variation in phenotypic traits, which are of direct interest to users (Farshadfar and Farshadfar, 2008).

Grevesen and Kack (1997) evaluated twelve spinach cultivars based on morphological traits in a field experiment in 1991 and 1992 in both spring and autumn crops. Morphological characteristics such as colour, leaf size, and smoothness of the leaves are important for industrial use in vegetable leafy. The content of nitrate and oxalic acid and the colour of a spinach product can be influenced by the choice of cultivar and the amount of stalk allowed in the harvested material. Vahabi Sedehi et al. (2008) evaluated morphological traits of the grass pea landraces in Iran and the results of this research verified possibility of use morphological traits for assessment genetic diversity, management of genetic resources

and determination of repetitive populations in grass pea. Pahlavani (2005) evaluated ten breeding lines of safflower (Carthamus tinctorius L.) from Iran in respect technological and morphological traits for their utilization aspects in both food industry and agricultural applications. Classification of these lines on the basis of morphological traits such as plant height, number of heeds per plant and number of days to flowering showed that Iranian germplasm could be economically beneficial.

This research was carried out to introduce the best watercress populations from the perspective of agriculture traits, to preserve valuable germplasms, and to identify watercress distribution centers in Iran.

Martials and Methods

The collection of watercress wild samples was begin on the spring 2011 from 24 points of Iran. The regions include six provinces from different regions of Iran including Ilam, West Azerbaijan, East Azerbaijan, Mazandaran, Alborz and Central (Figure 1). The geographical features of habitats including the latitude and longitude and the height from the sea level are given in Table 1. Ten plants were selected randomly from every region. The studied characteristics included 17 quantitative and qualitative characteristic (Tables 3). The measurement of qualitative characteristics was done based on the scoring. The Pearson correlation coefficients were used for the quantitative traits and the Spearman correlation coefficients were used for the qualitative traits. The negative, positive and very significant correlations among different traits were observed based on the results obtained from the correlation calculation among different traits. Digital caliper was used to measure the quantitative characteristics, the leaf thickness, width and height of silique and the ruler was used to measure leaf length, the leaf width and the plant height and a balance with an accuracy 0.1 gram was used to measure the plant fresh weight. The data analysis resulting from the experiment including the factors analysis, diplot, triplot, correlation analysis and cluster analysis was done using the software SPSS 19.

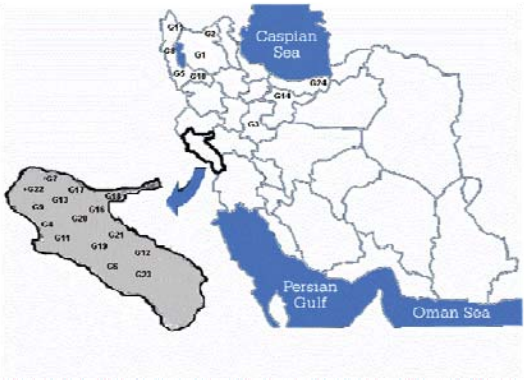

Figure 1. Selection regions of watercress populations from Iran

Table 1. Geographical distribution of watercress in Iran

Population	Symbol	Altitude (m)	Longitude		Latitude		Province	Climate
			Degree (°)	Minute	Degree (°)	Minute		
Mehraban	G1	1700	47	58	37	48	East Azerbaijan	Cold mountainous
Sarab	G2	1680	47	41	37	56	East Azerbaijan	Cold mountainous
Jasb	G3	1525	49	47	34	37	Central	Temperate mountainous
Karazan	G4	1450	46	18	33	10	Ilam	Cold and Semi Arid
Shahin degh	G5	1406	47	31	36	21	West Azerbaijan	Temperate mountainous
Meimeh	G6	1360	47	11	32	11	Ilam	Cold and Semi Arid
Aliabad olia	G7	1350	46	33	33	47	Ilam	Cold and Semi Arid
URoumieh 1	G8	1332	45	2	37	23	West Azerbaijan	Temperate mountainous
Job sorkhe olia	G9	1320	46	30	33	36	Ilam	Cold and Semi Arid
Miandoab	G10	1314	46	20	36	24	West Azerbaijan	Temperate mountainous
Mishkhas	G11	1310	46	29	33	26	Ilam	Cold and Semi Arid
Kolm	G12	1300	46	20	33	19	Ilam	Cold and Semi Arid
Job shirali	G13	1300	46	87	33	41	Ilam	Cold and Semi Arid
Fashand	G14	1215	50	6	35	11	Alborz	Cold and humid
URoumieh 2	G15	1200	45	19	37	52	West Azerbaijan	Temperate mountainous
Sartang	G16	1160	46	29	33	59	Ilam	Cold and Semi Arid
Sang sefid	G17	1140	46	43	33	31	Ilam	Cold and Semi Arid
Shabab	G18	1100	46	22	34	6	Ilam	Cold and Semi Arid
Badre	G19	1100	46	1	33	16	Ilam	Cold and Semi Arid
Cheshmehrashid	G20	1050	46	40	33	11	Ilam	Cold and Semi Arid
Pakal	G21	1050	46	46	33	21	Ilam	Cold and Semi Arid
Lomar	G22	950	46	15	33	13	Ilam	Cold and Semi Arid
Gorab	G23	890	47	34	33	33	Ilam	Cold and Semi Arid
Noshahr	G24	-3	51	12	36	9	Mazandaran	Warm temperate and humid

Results and Discussion

The samples of Mehraban and Sarab were collected from the height near 2000 m from the sea level and Noshahr samples were collected from the height -3 m from the sea level. The greatest difference in view of latitude was between populations of Meimeh (32° & 11 min) and Sarab (37° & 56 min). The flowering season of watercress was began from early March depending on the region climate and continued till June and its silique fruits ripe from June till October. This plant exists as evergreen beside the water stream and as vegetative phase. Its propagation is often asexual, so that every part of plant in contact with the water produces adventitious root and develop a complete plant. Vegetative traits were evaluated before flowering and reproductive traits were evaluated at the peak flowering, except for fruit-related traits that were evaluated in the

final stages of growth. The Noshahr and Lomar populations were the earliest flowering and Sarab and Mehraban populations were the latest flowering. All populations had the compound leaves and glabrous organs, hollow stem, four white petals, four sepals and hermaphrodite and the stamens were higher proportional to the stigma. The color of immature fruit was green and the color of riped fruit was brown. There was no anthocyanin in the leaf of populations, but there was anthocyanin in the stem and petiols of some populations including Meimeh, Uroumieh 1, Uromieh 2, Shahindegh, Noshahr, Miandoab and Fashand special in the terminal parts of stem and near the terminal buds. The correlation coefficients were calculated among the traits and are provided in the Table 4. There was significant correlation between leaf length and leaf width (0.929**), leaf number and plant height (0.880**) and between the number of peripheral stems and leaf thickness (0.490*) and there was negative correlation between the leaf shape and the leaf number (-0.541*). The factors analysis was done using the software SPSS 19 which its result was shown in Table 5. Four major and independent factors accounted for 79.433% total variance. In the first factor, the traits of leaf length, leaf width, flower number in the inflorescense, silique length, leaf number, plant height, number of peripheral and fresh weight in second factor with the higher coefficients accounted for 43.559% total variance. The traits of leaf thickness, length of flowering stems, number of silique, silique width and leaf shape accounted for 15.413% in the third factor, the traits of flower number, the number of inflorescense, the existence or lack of anthocyanin accounted for 11.524% total variance and finally the traits of leaflet number per leaf in the forth factor accounted for 8.936% total variance. According to the results of analysis, there was the greatest difference among the populations for leaf length, leaf width, leaf thickness, flower number, inflorescense number, number of flower in the inflorescence which produced the greatest variance among the populations. The factor analysis reduced 17 traits to four main factors, which the first and second factors had the largest variance.

Table 3. Average, minimum, maximum and the variance of measured quantitative & qualitative traits in Iranian watercress.

Measured traits	Abbreviation	Unit	Average	Maximum	Minimum	Coefficient of variation
Leaf lenght	LL	cm	9.27	18.4	4.73	31.98
Leaf width	LW	cm	4.71	13	2.04	45.88
Leaf thickness	LT	mm	0.43	0.88	0.24	24.59
Number of leaflet in leaf	NLL	-	7	9	5	14.57
Fresh weight	FW	gr	19.81	50.73	9.66	52.04
lateral branch number	NLB	-	4.6	10.33	1.55	42.43
Plant height	PH	cm	41.78	122.6	13.99	45.58
Leaf number	NL	cm	22.19	79.93	14.13	58.74
flower number	NFB	-	84.77	155.49	13.35	50.3
Inflorescense number	NI	-	4.82	8.8	1.97	35.08
flower number in inflorescence	NFI	-	17.75	35.06	5.97	40
Flower stem lenght	FSL	cm	7.36	14.6	4.44	28.61
Silique number	NS	mm	26.35	59.11	7.93	44.92
Silique lenght	SL	mm	15.47	35.3	6.51	33.72
Silique width	SW	-	2.58	16.93	1.28	122.4

Qualitative traits and their states and codes

measured traits	Abbreviation	1	2	3
Leaf form	LF	Almost circular, with small teeth	Circular without teeth	Serrated oval
Anthocyanin	A	No anthocyanin	anthocyanin	

Table 4. Correlation coefficient between traits of Iranian watercress population (**&* significant correlation respectively at level 1% & 5%, respectively)

	LL	LW	LT	NFB	NI	NFI	FSL	NS	SL	SW	NL	PH	NSSH	FW	NLL	LF	A
LL	1																
LW	0.929**	1															
LT	0.499*	0.624**	1														
NFB	0.429*	0.375	0.149	1													
NI	0.630**	0.635**	0.564**	0.784**	1												
NFI	0.627**	0.705**	0.422*	0.791**	0.679**	1											
FSL	0.383	0.508*	0.686**	0.201	0.568**	0.377	1										
NS	-0.065	-0.061	0.322	0.323	0.517**	0.012	0.390	1									
SL	0.464*	0.577*	0.761**	0.206	0.482*	0.510*	0.694**	0.089	1								
SW	0.025	0.074	0.202	0.013	0.072	0.141	0.212	0.241	0.322	1							
NL	0.720**	0.825**	0.694**	0.182	0.531**	0.575**	0.717**	-0.070	0.749**	0.121	1						
PH	0.746**	0.880**	0.625**	0.268	0.526**	0.644**	0.728**	0.025	0.699**	0.263	0.880**	1					
NSSH	0.771**	0.777**	0.490*	0.426*	0.561**	0.684**	0.469*	0.067	0.434*	0.153	0.699**	0.726**	1				
FW	0.715**	0.588**	0.069	0.503*	0.433*	0.534**	0.130	-0.136	0.231	-0.056	0.474*	0.493*	0.717**	1			
NLL	0.065	0.121	0.115	-0.085	-0.182	0.015	-0.175	-0.054	0.084	-0.016	-0.167	0.025	-0.062	0.025	1		
LF	0.278	0.163	-0.503*	0.266	0.137	0.170	-0.135	-0.163	-0.140	-0.217	0.541**	-0.043	0.257	0.412*	-0.374	1	
A	-0.099	-0.378	-0.099	-0.338	-0.099	-0.391	0.232	0.139	0.298	0.351	0.258	-0.324	-0.212	-0.020	-0.183	0.290	1

Cluster analysis was done based on the four major factors by the metod of average linkage between groups and using the software SPSS 19 and the populations were divided into four major categories including three groups and one independent population (Figure 2). The populations of Badreh, Aliabad Olia, Cheshmeh Rashid, Shirali stream, Sorkhe Olia stream, Sarab, Mishkhas, Pakal, Karezan, Kolm, Shahin Degh, and Miandoab were placed in group 1, which in leaf length, leaf number, leaf shape and leaf width were different with others. The second group included Uromieh 1, Fashand, Meimeh, Uromieh 2, Sang Sefid, Lomar, Sartang and Jasb, which in number of inflorescence, silique width, existence and lack anthocyanin were different with others. Two populations of Sarab and Mehraban were placed in the third group which had the greatest number of peripheral stem, the fresh weight and height among the populations, finally Noshahr population was placed alone in one group which was different with others in leaf thickness, flower number and silique number.

Table 5. Quantities of the variance and the cumulative percentage of variance for the four main factors

Total Variance Explained

	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
Component	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	7.405	43.559	43.559	7.405	43.559	43.559	6.490	38.175	38.175
2	2.620	15.413	58.973	2.620	15.413	58.973	2.875	16.913	55.087
3	1.959	11.524	70.497	1.959	11.524	70.497	2.535	14.915	70.002
4	1.519	8.936	79.433	1.519	8.936	79.433	1.603	9.431	79.433

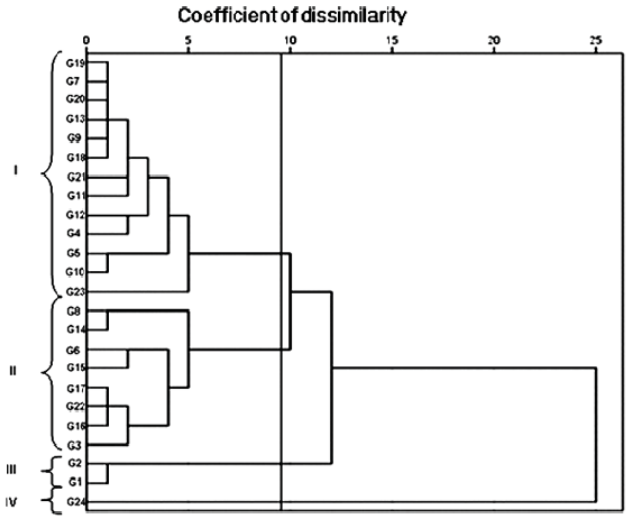

Figure 2. Cluster analysis diagram of 24 Iranian watercress populations

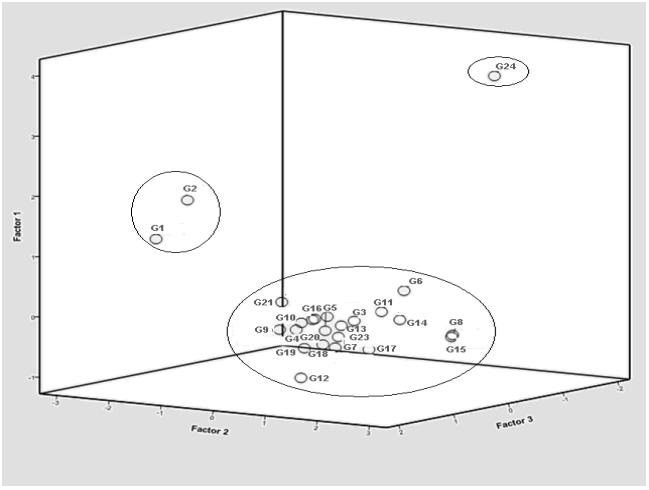

Figure 3. Three-dimensional analysis of studied watercress populations

Figure 4. Shabab (A) and Meimeh (B) populations habitats, Meimeh population, early April 2011 (the plants are at peak flowering) (C), watercress Leaves (population of Meimeh) (D&F), Stem with anthocyanin and without anthocyanin (E).

Diplot analysis was done based on two first factors having high amounts and accounted for 58.973% total variance. The results obtained from diplot analysis confirmed the results obtained from the cluster analysis and Noshahr population which was placed in one group in the cluster analysis was separated from other populations. Sarab and Mehraban accessins were separated according to the results obtained from the cluster analysis. Other populations were placed near each other.

In triplot analysis, the first three major factors which accounted for 70.497% total variance were used (Figure 3). According to the results obtained from triplot analysis, Sarab and Mehraban populations were separated from other populations and Noshahr population was placed alone in the plot which was different from some traits. The triplot analysis in comparison to diplot analysis clearly indicated the populations distance relative to each other and this subject indicates the clear role of flower number, inflorescense number and existence or lack of anthocyanin in grouping the populations.

A B C D

Figure 5. Flower (A), seed and silique (B), seeds (C) and flowering stem (D) of waterccress (population of Noshahr).

Conclusion

This study revealed considerable phenotypically (and presumably genetic) diversity among watercress populations growing naturally in Iran. Cluster analysis grouped the populations according to their taxonomic classification and probable potential use. The variability observed was good from a genetically diverse germplasm. Therefore, from the breeding standpoints, this germplasm showed good candidates according to our objectives for environmental adaptability. According to the results, Iranian watercress populations had high diversity in studied traits and so important to protect them and to use in the breeding programs. Sarab and Mehraban populations having the greatest fresh weight and plant height, Meimeh, Fashand and Uromieh 1 having the greatest anthocyanin amount, Karazan population having the greatest flower and fruit and the populations of Shahin Degh and Sarab have the ability to grow in the temperature -15°C. These populations could be in the breeding programs for high yield, pharmaceutical traits, seed product tolerance to freezing respectively.

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