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Full Length Research Paper

Genetic diversity of Iranian *Bunium persicum* germplasm by morphological markers and essential oil components

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Medicinal and aromatic plants have a traditional history of use, with strong roles in cultural heritage, and in the appreciation of food and its links to health. Black caraway (*Bunium persicum* Boiss. Fedtsch) belonging to the family of Apiaceae, is an economically important medicinal and aromatic plant found naturally growing in dry temperate regions of Iran and some other areas. The present investigation assessed the genetic variability of its ecotypes from Iran by employing morphological markers and essential oil components. The samples were collected from fifteen locations of Iran and were planted for evaluating morphological characters and also, the essential oil were identified by gas chromatography (GC) and gas chromatography-mass spectrometry (GC/MS) methods. Dendrogram of clustering with UPGMA algorithm and Euclidean distance separated 15 ecotypes to 5 main groups which in each group, ecotypes were same in geographical and habitat situation and biplot of PC1 and PC2 approved it. Principal components analysis (PCA) reduced the original 16 variables to 2 principal components that accounted for 95.83% of the total variance. The results of factor analysis showed that the first four factors mostly reflected most of the trait variations. Heritability of the morphological traits was high and the ecotypes exhibited the high genetic variations from the viewpoint of coefficient of variations. Based on our assessments, we showed high genetic variations for Iranian *B. persicum* germplasms.

Key words: *Bunium persicum*, genetic diversity, morphological markers, essential oil components, medicinal plant.

INTRODUCTION

Medicinal plants are natural products used for ancient medicine. This practice has existed since prehistoric times. In other words herbs and spices have a traditional history of use, with strong roles in cultural heritage, and in the appreciation of food and its links to health (Azimzadeh et al., 2008; Tapsell et al., 2006). These plants synthesize substances (such as secondary metabolites) that are useful to the maintenance of health in humans and other animals (Azimzadeh et al., 2008; Tapsell et al., 2006; Lai and Roy, 2004). There are many hundreds of aromatic and medicinal plants that can be grown in temperate and semi temperate climates like Iran and there are probably great deals with properties as yet undiscovered (Omidbaigi, 2005).

Scientific name of “Black caraway” or “Kala zira” is *Bunium persicum* Boiss. Fedtsch hereafter, in this paper we use the name “*B. persicum*”. *B. persicum* is a perennial aromatic and medicinal plant belonging to the family of Apiaceae. It is a temperate plant, economically important, naturally occurring in the dry temperature and elevated regions where the winter is severe and the

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ground is under snow in winter, because a long chilling period is essential for germination of seeds. These regions include mainly Iran and some other areas such as Afghanistan, Pakistan, Tajikistan and north India (Hanelt et al., 2001; Panwar, 2000; Panwar et al., 1993).

The seeds and oils of *B. persicum* are used in perfumery industries, indigenous and industrial medicine; also, they are used as spices (Panwar et al., 1993; Gincarlo et al., 2006). *B. persicum* is mainly known as an important medicinally plant and used in medicinal formulations. Seeds are stimulants and carminative and are used in treating diarrhea, dyspepsia, fever, flatulence, stomachic, hemorrhoids, hiccoughs, for antihistaminic and more numerous uses in medicine industries (Hanelt et al., 2001; Peter, 2004; Panda, 2004; Boskabadi and Moghaddas, 2004). This plant has several therapeutic effects on digestive and urinary tract disorders and is well known as an anti-convulsion, anthelmintic, anti-asthma and antidyspnea in Iranian folk medicine. *B. persicum* oil is capable of suppressing the initial stage of an inflammatory process (Boskabadi and Moghaddas, 2004; Salehi et al., 2008). Also, this plant is used for culinary purposes and for flavoring foods and beverages (Pourmortazavi et al., 2005).

The plant habit is dwarf or tall, spreading or compact, the height ranging from 30 to 80 cm. The plant is branched herb, roots are tuberous, leaves 2 to 3 needle-like, finely dissected, white flowers, borne on compound umbels, fruit oblong, 3 to 4 mm long dark brown, ridges prominent, stylopodium flattened, styles reflexed. The crop is naturally cross-pollinated. The propagation is both vegetative (through bulbs) or through seeds. The chromosome number of the plant is 2n=14 and is not subjected to any vigorous crop improvement work and there are no approved varieties or improved cultivars (Panwar et al., 1993; Panwar, 2000; Peter, 2004). In the case of the assessment of *B. persicum* genetic diversity using morphological markers and essential oil components there is no significant reports till now. Despite knowing high economical value and high quality genetic sources of *B. persicum*, there are lots of unknown and unstudied cases about this amazing species.

In 1992, 100 plants from a domesticated population of *B. persicum* were evaluated for 7 yield components. Plants exhibited a wide range of variation for primary and secondary umbels per plant, tuber weight and seed yield per plant. Yield per plant was significantly and positively correlated with primary and secondary umbels per plant, umbellate, secondary umbel and tuber weight per plant (Kapila et al., 1997). Devi (2004) assessed twenty collections of *B. persicum* for fourteen characters. Most characters were statistically significant between the collections, which is an indication of existing high amount of variability between the collections. With respect to essential oil percent, best results were shown by collections made from Shong and Kupa areas of Kilmaur district followed by Bharmour area of Chamba district.

Maximum range was found for number of seeds per plant (553.93 to 1363.38). High genetic gains of the order of 62.97, 53.49, 48.57 and 40.19% were recorded with regard to four characters including number of seeds per plant, number of umbels per plant, number of secondary branches per plant and number of primary branches per plant, respectively. At the phenotypic level, out of total 91 character combinations, 27 combinations showed significant positive associations. The environmental correlation coefficient for 16 character combinations was significant. Path coefficient analysis indicated that overriding characters contributing directly towards seed yield per hectare were number of umbels per plant, number of secondary braches per plant, number of primary branches per plant and number of seeds per umbel (Devi, 2004). Majeed and Sharma (2006) assessed the genetic variability of *B. persicum* by employing morphological and Random Amplification of Polymorphic DNA (RAPD) markers. Among 40 random decamer primers, 37 primers obtained reproducible patterns and 36 primers produced 168 polymorphic bands. Dendrogram based on RAPD analysis classified accessions into two clusters. Cluster I consisted of Gurez, Sangla and Khrew samples from Himachal Pradesh state whereas, Cluster II included Kalpa and Harwan samples from Jammu and Kashmir State. High coefficients of variation were observed for characters like plant height and number of seeds per plant, which indicate that a fair amount of variability exists for these traits.

Genetic diversity of 23 ecotypes of *B. persicum* from Iran, one ecotype from Pakistan and 3 accessions of Cumin (*Cuminum cyminum* L.) were studied using RAPD markers. 15 random primers were selected for study of the ecotypes that 220 polymorphic bands were obtained. Cluster analysis using Jaccard similarity coefficient and UPGMA method was grouped the ecotypes into 9 different clusters. Eight ecotypes from the Kerman province along with one ecotype from the Semnan province and 3 populations from the Khorasan province were classified in the same group. Six other ecotypes from the Kerman province and Saghafi ecotype of the Isfahan province and Alamoot from Ghazvin province were represented as individual branches in the cluster. Maximum similarity among the ecotypes of *B. persicum* detected between Sirach and Jiroft-Darin and minimum between Bam and Sistan (Pezhmanmehr et al., 2007).

Amplified fragment length polymorphism (AFLP) markers have been used to characterize the genetic diversity among 20 ecotypes of *B. persicum* from Iran. Seventeen AFLP primer combinations led to amplify 303 scorable bands and of these, 228 (75%) were polymorphic across the 20 ecotypes. Dendrogram based on Dice method and UPGMA algorithm separated the 20 ecotypes into four main groups. Ecotype collected from Koohbanan in Kerman province was represented as an individual group while 9 other ecotypes from the Kerman

province together with all other ecotypes from the Semnan, Khorasan, Ghazvin and Esfahan provinces were placed in the same group. Ecotypes of Bam and Kooshk from the Kerman province were represented in the same group in close relationship with another group, which included ecotypes of Bidooeie and Khebr from the same province (Jahansooz et al., 2007). Regarding chemical studies there are only some reports about percentage of components not comparison studies.

In one of these studies, *B. persicum* oil was extracted by steam distillation of crushed seeds. The oil content was about 5 to 14% in fresh seeds and 3 - 6% in dried seeds. The straw contains *B. persicum* herb oil to the extent of 1.25%. The commercial products are seed, seed oil and solvent extracted oleoresin (Panwar et al., 1993). The hydro-distillation of *B. persicum* seeds has been reported, previously. According to these reports, *B. persicum* seeds contain essential oil rich in monoterpene aldehydes, the main components are cuminaldehyde, ρ-mentha-1, 3-dien-7-al and ρ-mentha-1,4-dien-7-al, terpene hydrocarbons are γ-terpinene, ρ-cymene and β-pinene. The latter compounds are thought to reduce the quality of the spice (Salehi et al., 2008; Thappa et al., 1991; Baser et al., 1997; Foroumadi et al., 2002).

Pourmortazavi et al. (2005) were obtained volatile constituents of *B. persicum* seeds by supercritical carbon dioxide extraction and analyzed by Gas Chromatography-Mass Spectrometry (GC-MS). A total of 16 compounds accounting for 99.36% of the *B. persicum* supercritical fluid extraction extracts were identified. γ-terpinene (37.98%), cuminaldehyde (11.48%) and α-methyl-benzenemethanol (25.55%) were the major compounds in *B. persicum* (Pourmortazavi et al., 2005). Sardari et al. (1998) examined extracts of 40 Iranian and Canadian plants for their antifungal activity against several species of *Aspergillus*, *Candida* and *Cryptococcus* and out of the 40 plant extracts, 26 (65%) showed activity against at least one fungal strain. The extracts with the most wide spectrum of activity were related to *Diplotaenia damavandica*, *Heracleum persicum*, *Sanguisorba minor* and *Zataria multiflora*. However, cytotoxicity test results in cells, showed *D. damavandica* and *B. persicum* had the lowest cytotoxicity among these plants (Sardari et al., 1998). Takayuki et al, (2007) studied on antifungal effects (against *Fusarium oxysporum*) of volatile compounds from 52 plants including *B. persicum* and some other spices and herbs and the result showed that the *B. persicum* has strongest effect, followed by cumin and cardamom. GC/MS analysis of *B. persicum* identified seven volatile compounds, γ-terpinene, limonene, p-cymene, β-pinene, α-pinene, cuminaldehyde, and myrcene. Among these, cuminaldehyde and p-cymene showed the strongest antifungal activities against *F. oxysporum*, suggesting roles in the antifungal activity of *B. persicum.*Eventually, they mentioned that the cuminaldehyde is the main antifungal compound in *B. persicum* (Takayuki et al., 2007). Shahsavari et al. (2008) studied antioxidant activity and chemical characterization of essential oil of *B. persicum.* Essential oil study were done by GC/MS technique and the major components out of 29 components were caryophyllene (27.81%), γ-terpinene (15.19%), cuminylacetate (14.67%), cuminaldehyde (5.96%), p-cymene (5.25%), pinocarvyl acetate (4.36%), limonene (3.88%), α-methyl-benzene methanol (3.88%), croweacin (2.89%) and β-pinene (2.23%) (Shahsavari et al., 2008).

Azizi et al. (2009) examined essential oil content and constituents of *B. persicum* from Iran during field cultivation (domestication). Fruit oils from wild type (WT), first (CY1) and second year (CY2) cultivars (fourth and fifth year plants) analyzed by GC and GC/MS. The essential oil content of the WT (9.1% v/w) was higher than that of the CY1 (6.2% v/w) and CY2 (5.1% v/w). No significant differences found with respect to the oil constituents. The main constituents were γ-terpinene (WT: 44.2, CY1: 40.8 and CY2: 36.8%), associated with cuminaldehyde (WT: 16.9, CY1: 14.1, and CY2: 11.8%), and g-terpinen-7-al (WT: 10.5, CY1: 10.6, and CY2: 18.7%). In total 35 components could be identified covering 95.4% (WT), 94.9% (CY1) and 96.3% (CY2) of the oil content (Azizi et al., 2009). We assessed the genetic diversity of Iranian germplasm of *B. persicum* as the world's first research in this field that considers multi ecotype from different regions of Iran and also considers morphological, physiological and chemical traits (7 main essential oils of the plant) for genetic diversity analysis, synchronously.

MATERIALS AND METHODS

Identification of essential oil components

Plant material

The plant materials were fifteen ecotypes of wild *B. persicum* which collected from natural habitats of the plant in Iran. The ecotypes seed at maturity were collected for chemical analysis of the essential oil. The plants were identified by comparison with the voucher specimens that were in the herbarium of the Research Institute of Forests and Rangelands (Tehran, Iran). The ecotypes name or collection locations, province, climate conditions, longitude, latitude and elevation of fifteen analyzed samples are shown in Table 1.

Isolation of the essential oil

The seeds of each sample were ground separately in a blender to produce a powder and subjected to hydro-distillation for two hours, using a clevenger-type apparatus (Shanghai Heqi Glassware Co., China). The oils were dried over anhydrous sodium sulfate and their percentage contents were calculated on basis of the fruits dry weight (Sefidkon and Rahimi, 2002; Sefidkon and Jamzad, 2005).

Gas chromatography analysis

GC analysis was performed using the Shimadzu GC-9A gas chromatograph (Shimadzu Co., Japan) equipped with a DB-5 fused silica column (30 × 0.25 mm with 0.25 µm film thickness). Oven

Table 1. Specification of 15 Iranian *B. persicum* ecotypes*.

Ecotype code and collection location	Province	Climate condition	Longitude		Latitude		Elevation (M)
			Deg.	Min.	Deg.	Min.	
1- Masih	Yazd	Temperate-Semi arid	31	54	2200	54	17
2- Mehriz	Yazd	Semi temperate – Semi arid	31	35	2400	54	26
3- Hormozgan 390	Hormozgan	Temperate- Humid	27	13	1220	56	22
4- Natanz	Isfahan	Semi temperate – Semi arid	33	32	2100	51	54
5- Saghafi	Isfahan	Semi temperate – Semi arid	33	47	1600	55	05
6- Hosein Abad	Kerman	Semi temperate – Semi arid	30	15	2200	56	58
7- Joopar	Kerman	Semi temperate – Semi arid	29	28	3200	55	41
8- Hormozgan 351	Hormozgan	Temperate- Humid	27	13	1220	56	22
9- Alamoot	Ghazvin	Semi temperate – Semi humid	36	15	2300	50	03
10- Ray sample	Isfahan	Semi temperate – Semi arid	33	47	1600	55	05
11- Sirch	Kerman	Semi temperate – Semi arid	30	48	2650	56	34
12-Mashhad university.	Khorasan	Semi temperate – Semi humid	36	16	2600	59	38
13- Semnan	Semnan	Semi temperate – Semi arid	35	35	1700	53	33
14- Kalat Naderi	Khorasan	Semi temperate – Semi humid	36	16	2600	59	38
15- Khoro Biabanak	Isfahan	Semi temperate – Semi arid	33	47	2000	55	05

*In this table, Deg, Min and M respectively mean: degree, minute and meter.

temperature was held at 50°C for 5 min and then programmed to 250°C at a rate of 3°C/min. Injector and detector (FID) temperature were 290°C; helium was used as carrier gas with a linear velocity of 32 cm/s (Sefidkon and Rahimi, 2002; Sefidkon and Jamzad, 2005).

Gas chromatograph / spectrometry analysis

GC-MS analyses were carried out on the Varian 3400 GC-MS system (Varian, Inc. USA) equipped with a DB-5 fused silica column (30 m × 0.25 mm with 0.25 µm film thicknesses). Oven temperature was 50 to 220°C at a rate of 3°C/min, transfer line temperature 240°C., injector temperature 230°C., carrier gas was helium with a linear velocity of 31.5 cm/s. Sample was injected with split ratio of 1:60, flow rate was 1.1 ml/min, ionization energy 70 electron volt, scan time 1 s, mass range 40 - 350 Amu (Sefidkon and Rahimi, 2002; Sefidkon and Jamzad, 2005).

Identification of the components

The components of the essential oils were identified by the comparison of their mass spectra with those of a spectral computer library or with authentic compounds and confirmed by the comparison of their retention indices, either with those of authentic compounds (Sefidkon and Rahimi, 2002; Sefidkon and Jamzad, 2005).

Morphological markers

Plant material: The tuberous roots of samples which were collected from fifteen natural habitats locations in Iran were used for this research section.

Plantation through tuberous roots: Random tubers of the ecotypes were selected and planted in the field. Land preparation is necessary before the plantation. Land was ploughed to the depth of 30 to 35 cm to bring it to fine tilth. This helps in aeration of soil, better development of tuberous roots and also keeps the field free from weeds. The tuberous roots are planted at a distance of 50 cm between rows. The optimized manuring, weeding and regular irrigation periods are also essential for this crop. Finally, the seed was manually harvested.

Morphological traits and sampling: In this research some of the more important traits which may be useful for the next breeding research of *B. persicum* were assessed. Data from 3 plants of each genotype (3 sample of each ecotype) were recorded on the following 7 phenotypic traits: number of primary umbels, number of secondary umbels, number of stems, plant height (cm), first stem height from field surface (cm), diameter of stem (mm), thousand grain weight (g) and earliness (quantitative scores from 1 to 3 for showing degree of earliness, 1 for most earliness, 2 for mid-earliness and 3 for non-earliness).

Statistical analysis

ANOVA for morphological traits*:* ANOVA based on randomized complete block design (RCB) with 3 replications was performed using SAS software, version 9.0 (SAS Institute Inc., Cary, North Carolina, USA) for determining differences of the ecotypes. The means were compared using Duncan's multiple range tests at $P \leq 0.05$.

Cluster analysis, Principal Components Analysis (PCA) and Factor Analysis (FA): Cluster analysis was performed using Euclidean distance and UPGMA (Un-weighted Pair Group Method with Arithmetic Mean) algorithm to show the structure of the diversity. Cophenetic correlation coefficient was calculated to evaluate capability of the cluster analysis. PCA using correlation matrix was carried out to further analyze the structure of the genetic diversity and first and second principal components (PC1 and PC2) were bipolted for the ecotypes. Factor analysis using principal component method was also performed by SAS software, version 9.0 (SAS Institute Inc., Cary, North Carolina, USA). Cluster analysis and PCA were performed with the software of NTSYS-pc computer program (Rohlf, 2001). It is mentionable that data were transformed to Z scores with NTSYS-pc computer program and then cluster analysis was performed. Heritability by using expected values of

Table 2. Chemical characters of 15 *B. persicum* ecotypes.

Ecotype	Oil yield (%)	Cuminaldehyde (%)	α-Pinene (%)	γ- Terpinene (%)	Limonene (%)	ρ-Cymene (%)	α-Terpinene-7-al (%)	γ-Terpinene-7-al (%)
1-Masih	2.2	14.45	1.65	28.85	11.1	8.2	2.1	7.6
2-Mehriz	2.72	21.65	1.54	34.8	12.14	8.57	2.4	8.6
3-Hormozgan 390	2.76	20.83	2.1	25	4.77	24.98	1.18	19.47
4-Natanz	2.9	17.06	2.27	32.44	15.13	12.18	2.3	7.89
5-Saghafi	4.9	33.91	2.52	25.59	6.27	10.72	3.04	5.95
6-Hosein Abad	2.7	8.9	1.29	47.7	1.09	12.34	0.9	15.63
7-Joupar	2.54	19.52	1.13	44.4	1.08	10.67	2.24	13.26
8-Hormozgan351	3.1	25.42	1.55	22.79	8.41	9.91	0.65	24.83
9-Alamoot	4.1	7.96	1.79	44.23	9.35	7.96	0.57	17.55
10-Ray sample	2.24	7.93	1.26	46.77	0.95	12.1	0.88	15.33
11-Sirch	3.4	13.73	2.12	35.28	5.72	7.69	4.2	21.49
12-Mashhad University	3.9	16.65	3.65	43.34	10.56	7.7	1.9	9.89
13-Semnan	1.4	26.83	1.9	31.77	5.49	17.2	1.74	6.03
14-Kalat Naderi	3.2	17.34	1.94	44.15	6.33	10.71	2.63	7.59
15-Khoro Biabanak	3.4	14.45	1.723	38.39	7.27	7.71	1.1	18.48
Coefficient of variation	28.17	40.91	32.78	23.27	59.50	40.79	54.30	46.13

mean squares and coefficient of variation of each trait were calculated. Also, correlation coefficients were calculated for all character combinations. All correlation coefficients were Pearson's correlation coefficients except the earliness which are Spearman's correlation coefficients because of ranking score of this characteristic. These later parameters which explain were done by SAS software, version 9.0.

RESULTS

In chemical analysis seven main essential oils and oil yield percentage (totally 8 traits) were evaluated on *B. persicum* samples (Table 2). Also, eight morphological traits were evaluated in 3 replications which the means of each trait for each ecotype are shown in Table 3. In the Table 4, average of compositions percentage of the oil components of fifteen Iranian *B. persicum* ecotypes was shown. In this Table the retention index (RI) of each component shown for more information. The components have been ranked based on their percentage from high to low. Also in the Figure 1, the oil yield percentage of fifteen Iranian *B. persicum* ecotypes showed in the schematically graph to better illustration and comparison of the ecotypes in this trait. Analysis of variance results on seven quantitative morphological traits showed that the ecotypes were significant for number of primary umbels, plant height, diameter of stem, first stem height from field surface and thousand grain weights at one percent probability level. Also, the ecotypes for number of secondary umbels were significant at five percent probability level. But, number of stems was not significant. Duncan's multiple range tests of the ecotypes mean are shown in Table 4.

Hosein Abad ecotype had the highest number of primary umbels. The range of means for the number of primary umbels, were between 27.33 (Hosein abad) and 13.33 (Ray sample). For the number of secondary umbels Alamoot ecotype had the highest means and the lowest was Joupar. Because, the number of stems was non-significant in ANOVA, all of the ecotypes had not significant difference. But, plant height in Kalat naderi was the highest and Khoro Biabanak had the lowest height. Also, Duncan's multiple range tests showed that Saghafi had the highest mean in first stem height from field surface and the range of means was between 3.16 and 1.30. For diameter of stem the highest mean belonged to Mashhad University ecotype and the lowest mean to Hormozgan 351.

Finally, highest mean for earliness was in Natanz and the lowest in Hormozgan 390 (Table 4). Coefficients of variations for each trait have shown on the Tables 2 and 4. The highest Coefficients of variations was for Limonene (59.5) and the lowest was for plant height (15.05). The correlation matrix between traits showed in Table 5. Out of total 120 character combinations, 15 combinations showed significant associations. The number of primary umbels was found positively and significantly correlated with only the number of secondary umbels (+0.61) at five percent probability level. The number of secondary umbels and the plant height had significant positive correlation (+0. 51) at five percent probability level. The number of stems was found significantly correlated with the cuminaldehyde percentage (+0.52), the γ-terpinene percentage (-0.56) and γ-terpinene-7-al percentage (-0.58) at five percent probability level. The diameter of stem was found positively and significantly correlated with the plant height (+0.59) and the first stem height surface (+0.32) at five percent probability level and also, was found negatively

Table 3. Average of compositions percentage of the oil components of Iranian *B. persicum* ecotypes.

Components (%)	Retention Index (RI)	Composition average for all 15 ecotypes (%)
γ- terpinene	1061	36.37
Cuminaldehyde	1253	17.78
γ-terpinene-7-al	1319	13.31
ρ-cymene	1025	11.24
Limonene	1030	7.04
α-pinene	938	1.9
α-terpinene-7-al	1252	1.86

Table 4. Mean comparison of morphological traits in 15 *B. persicum* ecotypes.

Ecotype	Number of primary umbels	Number of secondary umbels	Number of stems	Plant height (cm)	First stem height from field surface(cm)	Diameter of stem (mm)	Thousand grain weight (g)	Earliness
1- Masih	14.33^{d}	9.33^{e}	9.66^{a}	52.00^{bc}	1.53^{ef}	3.76^{b-f}	4.10^{gh}	1
2- Mehriz	25.33^{ab}	11.00^{b-e}	10.33^{a}	49.00^{b-d}	1.40^{f}	3.76^{b-f}	4.10^{gh}	2
3- Hormozgan 390	15.66^{cd}	11.66^{a-e}	7.33^{a}	44.66^{cd}	2.33^{bc}	3.30^{d-f}	3.63^{h}	2
4- Natanz	22.66^{a-c}	14.33^{a-d}	11.00^{a}	54.33^{bc}	2.53^{b}	4.70^{ab}	7.13^{a}	2
5- Saghafi	22.66^{a-c}	15.00^{a-d}	9.33^{a}	60.66^{ab}	3.16a	4.13^{a-e}	6.70^{ab}	2
6- Hosein Abad	27.33^{a}	16.00^{ab}	7.00^{a}	54.00^{bc}	1.66^{d-f}	4.53^{a-c}	4.80^{e-g}	1
7- Joupar	16.00^{cd}	7.33^{e}	6.66^{a}	44.33^{cd}	1.53^{ef}	3.26^{ef}	5.50^{c-e}	3
8- Hormozgan 351	21.00^{a-d}	13.33^{a-d}	8.33^{a}	45.00^{cd}	1.30^{f}	1.90^{g}	4.56^{fg}	3
9- Alamoot	21.00^{a-d}	17.00^{a}	7.00^{a}	50.33^{b-d}	1.53^{ef}	3.76^{b-f}	6.10^{bc}	3
10-Ray sample	13.33^{d}	10.66^{b-e}	6.33^{a}	55.33^{bc}	1.93^{c-e}	3.90^{b-f}	4.36^{g}	2
11- Sirch	19.00^{b-d}	10.00^{c-e}	6.66^{a}	44.00^{cd}	1.60^{d-f}	3.10^{f}	5.63^{cd}	2
12-Mashhad University.	24.66^{ab}	14.00^{a-d}	7.66^{a}	51.33^{bc}	1.50^{f}	5.06^{a}	5.10^{d-f}	1
13- Semnan	20.66^{a-d}	10.66^{b-e}	10.33^{a}	43.33^{cd}	1.36^{f}	3.60^{c-f}	4.26^{gh}	3
14-Kalat Naderi	21.00^{a-d}	15.33^{a-c}	6.33^{a}	68.33^{a}	2.00^{cd}	4.30^{a-d}	4.40^{fg}	1
15-Khoro Biabanak	15.33^{cd}	12.00^{a-e}	5.66^{a}	39.00^{d}	1.93^{c-e}	3.30^{d-f}	5.40^{c-e}	3
Coefficient of variation	22.02	21.99	21.61	15.05	28.29	20.38	20.08	38.65

In each column, means followed by the same letters are not significantly different (Duncan's multiple range tests, P ≤ 0.05).

and significantly correlated with the earliness (-0.67) and the percentage of γ-terpinene-7-al (-0.67). The earliness had significant negative correlation with the plant height (-0.64) and the diameter of stem (-0.67) at one percentprobability level. The oil yield was found positively and significantly correlated with the thousand grain weight (+0.60) and the α-pinene percentage (+0.52) at five percent probability level. The cuminaldehyde percentage showed significant positive correlation (+0.52) at five percent probability level with the number of stems and also, significant negative correlation with the γ-terpinene percentage (-0.71) at one percent probability level. The limonene percentage and the α-pinene percentage had significant positive correlation (0.45) at five percent probability level. The both of α-terpinene-7-al percentage and ρ-cymene percentage had not significant correlation with any trait. The γ-terpinene-7-al percentage was found negatively and significantly correlated with the diameter of stem (-0.67) at one percent probability level.

Our analysis demonstrates that heritability estimates are varies by more than 23% up and down between traits. The highest heritability percentage was for the first stem height from field surface (98.7%) and the lowest was for the number of stems (75.4%). Also, the heritability percentage for the number of primary umbels was 90.7 and 88.7% for the number of secondary umbels, 92.9% for the plant height, 95.1% for the diameter of stem and finally 98.4% for the thousand grain weight.

The UPGMA algorithm grouped 15 Iranian ecotypes of *B. persicum* in to 5 main groups (Figure 2). The first group includes two ecotypes from Yazd province (Masih Yazd and Mehriz Yazd) and one ecotype from Semnan province that have the same climate and geographical situation. The second group includes Isfahan, Ghazvin and Kerman province ecotypes (Joopar, Alamoot,

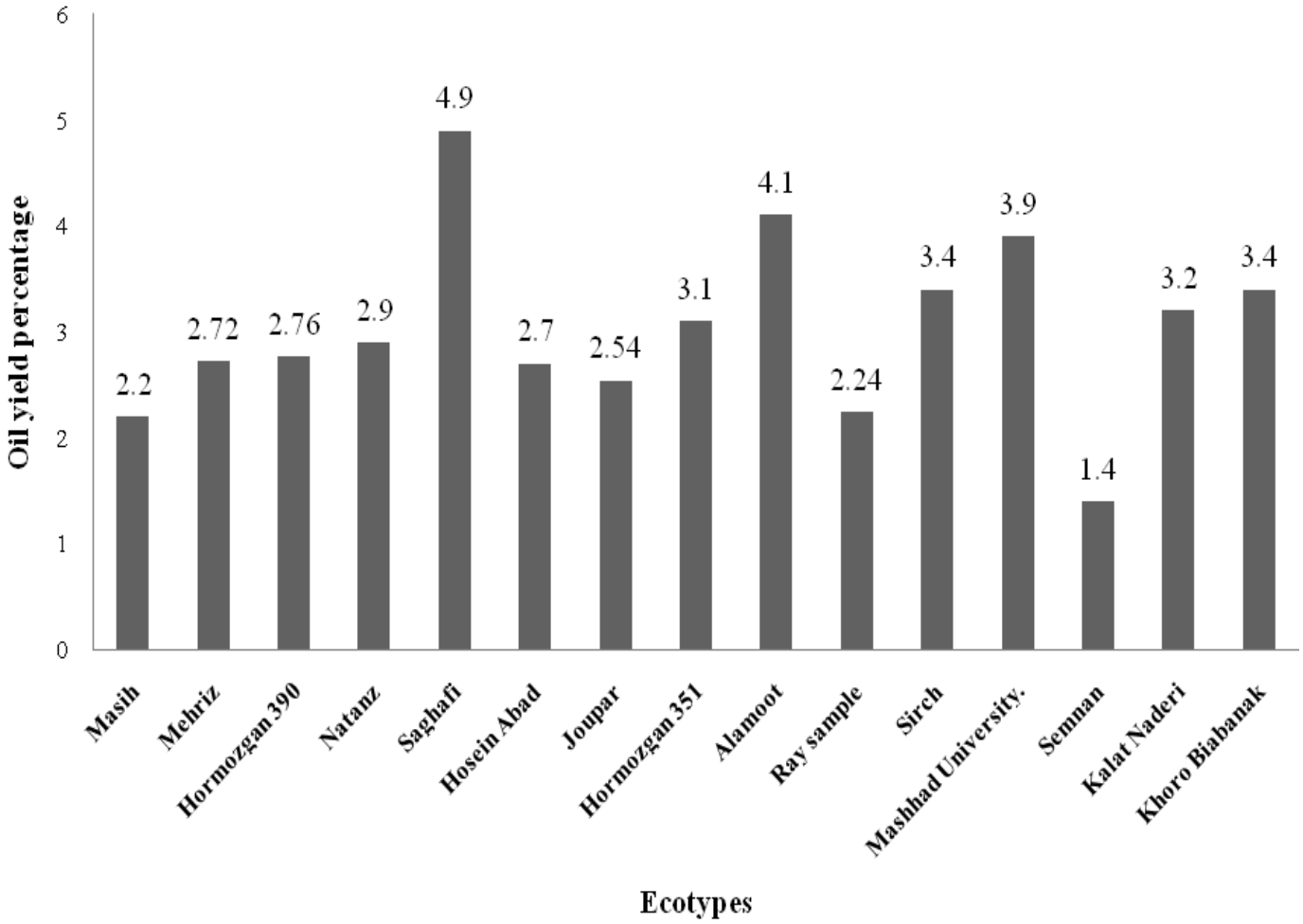

Figure 1. The oil yield percentage of 15 ecotypes of Iranian *B. persicum.*

Ashaee 350, Khoro Biabanak and Sirch) that have the same climate and geographical specification of habitat. The third group includes Hormozgan province samples (Hormozgan, 390 and Hormozgan, 351) which are approximately the same. The next group had Kalat naderi, Mashhad University ecotypes which are from Khorasan province and one ecotype from Isfahan province (Hosein Abad). Finally, the best ecotypes which were created the final group. This group includes Saghafi and Natanz ecotypes from Isfahan province. In the cluster analysis, Masih and Mehriz ecotypes had the lowest distance and Natanz and Mashhad University ecotypes had the highest distance. Cophenetic correlation coefficient for the dendrogram was 0.70 that showed the goodness of fit to the dissimilarity and cophenetic matrices and goodness of our algorithm (UPGMA). PCA revealed that the first two principal components explained 92.77 and 3.06% of the total variation, respectively. Factor analysis was performed using all of the traits for the mean of 15 ecotypes (Table 6). Based on the screen plot and pre-analysis, four factors were selected. For the first factor, the diameter of stem (0.75), plant height (0.68), α-pinene (0.66), number of secondary umbels (0.59), number of primary umbels (0.62), α-terpinene-7-al (0.40) and γ-terpinene-7-al (-0.63) were the traits with the highest factor loading. This factor was named “agronomical traits” factor. The second factor had the highest factor loading on cuminaldehyde (0.82), γ-terpinene (-0.90) and also number of stems (0.70). This factor was named “main essential oils” factor. The third factor was named as “yield” factor and had the highest factor loading on the thousand grain weight (0.60), oil yield (0.72) and earliness (0.53). Finally, the fourth factor was named as “plant height and some essential oils” factor and reflected mostly the first stem height from field surface (0.71), limonene (-0.55) and ρ-cymene (0.64).

DISCUSSION

Average of compositions percentage for the oil components of fifteen Iranian *B. persicum* ecotypes revealed the genetic diversity of these ecotypes from

Table 5. Correlation coefficients between the traits of 15 *B. persicum* ecotypes.

Traits	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. No. of primary umbels	1															
2. No. of secondary umbels	0.61*	1														
3. Number of stems	0.35	-0.03	1													
4. Plant height	0.30	0.51*	0.06	1												
5. First stem height surface	-0.04	0.31	0.11	0.45	1											
6. Diameter of stem	0.43	0.41	0.15	0.59*	0.32*	1										
7.Thousand grain weight	0.26	0.35	0.11	0.13	0.48	0.26	1									
8. Earliness	-0.27	-0.18	-0.05	-0.64**	-0.28	-0.67**	0.22	1								
9. Oil yield	0.31	0.58	-0.20	0.28	0.46	0.16	0.60*	-0.02	1							
10. Cuminaldehyde	0.16	-0.09	0.52*	-0.01	0.33	-0.23	0.05	0.23	0.14	1						
11. α-Pinene	0.32	0.33	0.18	0.16	0.28	0.47	0.28	-0.24	0.52*	0.25	1					
12. γ-Terpinene	0.12	0.14	-0.56*	0.23	-0.27	0.47	0.05	-0.15	-0.03	-0.71**	-0.16	1				
13. Limonene	0.29	0.22	0.62	0.04	0.01	0.19	0.26	-0.13	0.20	0.13	0.45*	-0.37	1			
14. ρ-Cymene	-0.23	-0.08	0.10	-0.11	0.28	-0.07	-0.37	-0.28	-0.38	0.25	-0.04	-0.32	-0.31	1		
15. α-Terpinene-7-al	0.11	-0.27	0.20	0.20	0.24	0.15	0.28	0.18	0.19	0.31	0.31	-0.15	0.15	-0.23	1	
16. γ-Terpinene-7-al	-0.26	-0.02	-0.58*	-0.52*	-0.27	-0.67**	-0.10	0.01	0.10	-0.31	-0.27	-0.03	-0.29	0.03	-0.36	1

*, ** : Significant at 0.05 and 0.01 probability level, respectively.

ecotypes of other regions and also the role of environment in producing the secondary metabolites such as essential oils. In the Table, the γ- terpinene percentage (36.37%) was the highest percentage among these seven main components, and the α-terpinene-7-al percentage (1.86%) was the lowest. This result was in agreement with Pourmortazavi et al. (2005), Takayuki et al. (2007) and Azizi et al. (2009) studies that reported the γ-terpinene was the highest percentage in all of the components. However, in the Shahsavari et al. (2008) research the caryophyllene (27.81%) was the highest. The significance of the six out of seven traits from and also, the high range of means variability among ecotypes for each trait, revealed the high genetic diversity of Iranian *B. persicum* ecotypes.

In the correlation results, the oil yield was significantly and positively correlated with the thousand grains weight and α-pinene. It showed that the yield components (oil yield and thousand grains weight) and oil goodness for industry usage were associated with each other. Our high heritability values of all of the traits demonstrated that the genetic variance of the traits of *B. persicum* had high values in relation to environment variance. Heritability values in this study were higher than those of Devi (2004). This finding may be due to different studied ecotypes or environmental conditions between the studies.

In cluster analysis all five groups contained ecotypes that had the same situation in their geographical and habitat specifications. Two ecotypes that had good agronomical and essential oil components were in one group. The high genetic variation of ecotypes can be seen in the dendrogram of clustering. Same results were reported by Majeed and Sharma (2006) so that dendrogram based on RAPD analysis revealed that the samples belonging to the same locations were grouped in the same cluster (Majeed and Sharma, 2006). However, in the other molecular studies that assessed by Jahansooz et al. (2007) and Pezhmanmehr et al. (2007) the ecotypes from similar locations were not classified into the same groups by cluster analysis (Pezhmanmehr et al., 2007; Jahansooz et al., 2007).

PCA reduced the original 16 variables to 2 principal components that accounted for 95.83% of the total variance. The Biplot of PC1 and PC2 for the ecotypes showed that these two components grouped 15 Iranian *B. persicum* ecotypes into 5 main groups almost same as the cluster analysis result (Figure 3). There is no

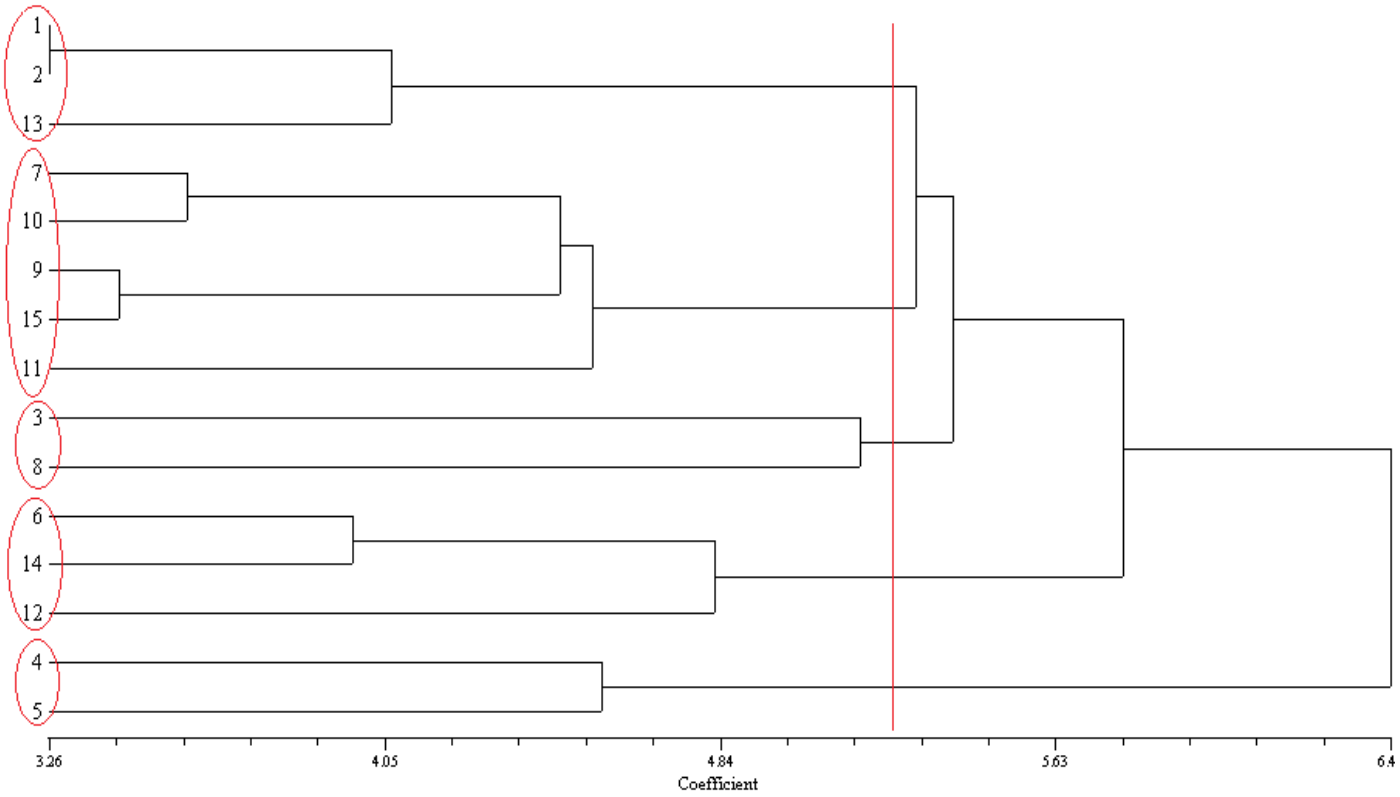

Figure 2. Cluster analysis dendrogram by Euclidian distance and UPGMA algorithm using all traits in 15 *B. persicum* ecotypes (the ecotypes name are similar to Table 1).

Table 6. Factor loading, communality, proportional and cumulative variances of four factors using principal component method and varimax rotation for all traits in 15 *B. persicum* ecotypes.

Traits	Factor loading				Communality	Specific variance
	1	2	3	4		
Number of primary umbels	0.62	-0.08	0.05	-0.33	0.51	0.49
Number of secondary umbels	0.59	-0.33	0.34	0.13	0.60	0.40
Number of stems	0.38	0.70	-0.31	-0.32	0.84	0.16
Plant height (cm)	0.68	-0.30	-0.27	0.30	0.73	0.27
First stem height from field surface (cm)	0.52	0.20	0.15	0.71	0.86	0.24
Diameter of stem (mm)	0.75	-0.39	-0.37	0.02	0.86	0.14
Thousand grain weight (g)	0.52	0.01	0.60	-0.02	0.64	0.36
Earliness	-0.53	0.34	0.53	-0.07	0.69	0.31
Oil yield (%)	0.56	-0.11	0.72	0.15	0.88	0.12
Cuminaldehyde (%)	0.21	0.82	0.04	0.19	0.76	0.24
α-Pinene (%)	0.66	0.15	0.14	-0.01	0.48	0.52
γ-Terpinene (%)	0.01	-0.90	-0.12	-0.11	0.84	0.16
Limonene (%)	0.49	0.37	0.09	-0.55	0.70	0.30
ρ-Cymene (%)	-0.24	0.32	-0.33	0.64	0.69	0.31
α-Terpinene-7-al (%)	0.40	0.28	-0.08	-0.06	0.25	0.75
γ-Terpinene-7-al (%)	-0.63	-0.19	0.58	0.06	0.77	0.23
Proportional variances (%)	27.8	18.2	13.4	10.3		
Cumulative variances (%)	27.8	46	59.4	69.8		

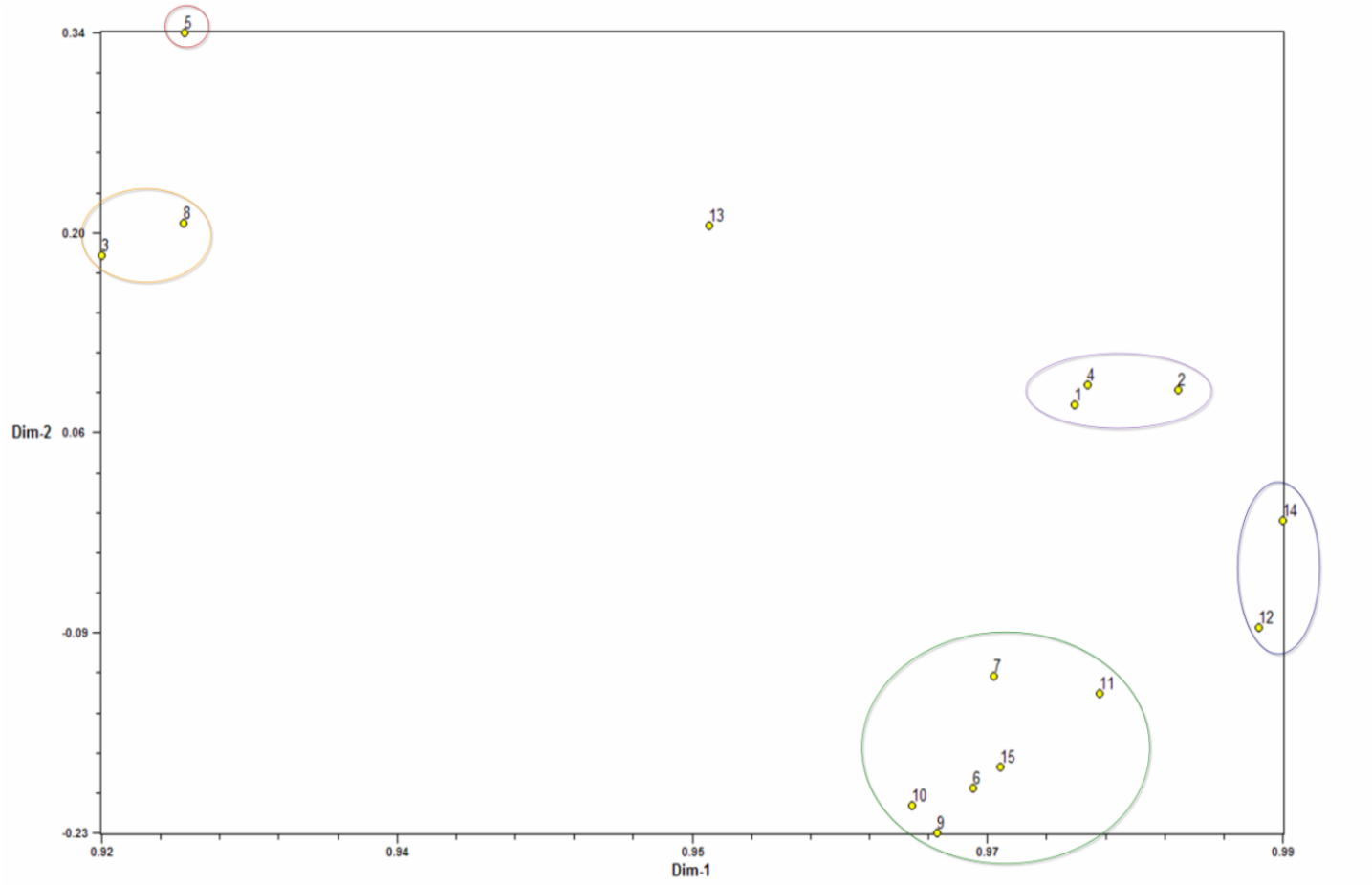

Figure 3. Biplot of PC1 and PC2 for the ecotypes (the ecotypes name are similar to Table 1).

previous study around PCA for comparing our results to this. As you see in the Table 6, some of the traits had great communality variance. These traits had the best accuracy and precision in factor analysis and more of their information was accounted in the analysis. There was not any previous study using factor analysis to compare with our results. Ecotypes exhibited the high genetic variation around coefficient of variations. The highest range of variation was for some biochemical characters such as Limonene,α-terpinene-7-al, γ-terpinene-7-al, ρ-cymene and cuminaldehyde. The coefficient of variations for morphological characters was too lower than biochemical characters. In the morphological characters the earliness and the first stem height from field surface showed high coefficient of variations. However, Kapila et al. (1997) reported that primary and secondary umbels per plant, tuber weight and seed yield per plant had high coefficient of variations. In the Devi (2004) report, number of seeds per plant had the highest coefficient of variations. In the Majeed (2006) results, high coefficients of variations were observed for plant height and number of seeds per plant. But in our study the plant height had the lowest coefficient of variations.

At the end, the wealth of medicinal and aromatic plants in Iran constitutes an inspiring fund of variation, because of the wide range of climatic and geographical conditions. From this colossal base of variability, desired genotypes characters can be selected to improve the efficiency and productivity of species and conserve the same for posterity as the total genetic diversity is being threatened by human notoriety. *B. persicum* is one of the most important medicinal and aromatic plants in Iran and the world, because of the numerous and important usages which we showed previously in this paper. This plant has great genetic sources in Iran and some other neighboring countries. By considering this great gene pool, native usages and low volume production of this plant, it is clear that this plant has great potential to improve in different breeding programs in the future.

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