Biosystematic study of annual species of *Persicaria* from Iran using SDS-PAGE

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Abstract. *Persicaria* (*Polygonaceae*), with some 12 species in Iran, has complicated taxonomic history. Seed storage protein patterns provide useful data for distinguishing the closely related species. Banding patterns of seed storage proteins in annual *Persicaria* species of Iran have been determined using SDS-PAGE. A total of 28 protein bands were observed in the studied species. Some bands are limited to *P. mitis, P. minor* and *P. lapathifolia* ssp. *nodosa*. Results have shown that three *P. lapathifolia* subspecies in Iran are closely related. A close relationship and high protein similarity were observed between *P. minor* and *P. mitis*. There are also hybrid populations nested between *P. hydropiper* and *P. minor*. SDS-PAGE results are in conformity with earlier findings.

Key words: Biosystematic, Iran, *Persicaria*, SDS-PAGE

Introduction

Persicaria (L.) Mill. (*Polygonaceae*) comprises about 12 annual and perennial species in Iran (Rechinger & Schiman-Czeika 1968). This genus has complicated taxonomic history and various studies have been undertaken for clarifying its relationship with other members of the family (Ronse Decraene & Akeroyd 1988). Most researches had focused on delimitation of *Polygonum* L. s.l. (Hong & al. 1998). *Persicaria* is well known for its weedy species occupying disturbed areas and crop fields. *Persicaria* plants are highly variable in morphology (Webb & Chater 1964). This has been attributed by some authors to hybridization. However, *Persicaria* plants usually self-fertilize, and some are even cleistogamous (Kim & al. 2008). These are aggressive species and the authors believe them to be allopolyploids. Both alloploidy and autoploidy are documented in the *Persicaria* species group (Kim & al. 2008). A number of these species have been dispersed by human activity. For example, *P. lapathifolia* (L.) Gray and *P. hydropiper* (L.) Spach, which are of Eurasian origin, are now cosmopolitan in the temperate regions (Kim & al. 2008).

Biosystematic studies of the *Persicaria* species are restricted to pollen grains study, leaf and tepal epidermis studies, and nut analysis (Ronse Decraene & Akeroyd 1988; Hong & al. 1998; Ronse Decraene & al. 2000; Amiri & Sharifnia 2007). Electrophoretic analysis has not been made in this genus. Owing to the importance of seed storage proteins in *Fagopyrum* Mill. for its nutritious and medicinal uses, electrophoretic analysis in this family has been mostly concentrated on this pseudocereal (Choi & Ma 2006). Zeller & al. (2004) in an attempt to identify seed storage proteins of *F. esculentum* Moench. Their study has revealed that that taxon had high intravarietal polymorphism. SDS-PAGE analysis of the extracted proteins of seed endosperm has shown complete distinction of *F. esculentum* from *F. tataricum* (L.) Gaertn. (Rout & Chrungoo 2007).

As the seed storage protein patterns do not reflect environmental influence, these patterns can be employed as a useful method for separating some closely

related species and genera. On the other hand, the results obtained by this method can be applied as an index for adaptation to different habitats in intra-population and intraspecific (inter-population) studies. Nevo & al. (1983) analyzed storage protein hordein in 123 individuals of *Hordeum spontaneum*. By gathering seeds along two transects and analyzing them electrophoretically, they detected 15 phenotypes in Hor1 and 16 phenotypes in Hor2. They interpreted this polymorphism due to the soil and topographic differences in the transects. By analyzing seed protein profiles of *Vicia sativa* populations, Ladizinsky & Waines (1982) found out variable profiles that could not be related to taxonomic division, chromosome number or specific karyotype, but rather to ecological flexibility.

Sheidai & al. (2008) analyzed seed protein in 17 *Bromus* L. species and varieties. Their studies resulted in the separation of species of two sections *Pnigma* Dumort. and *Bromus*. The present study reports the results of seed storage protein analysis in the annual species of *Persicaria* which can be efficiently used in identification and classification of species.

Material and methods

Plant material

Seed samples of 11 populations belonging to five species and three subspecies were obtained from the sources indicated in Table 1 and Fig. 1. Three individuals per each accession were separately identified and their mature seeds were collected. Identification of species and subspecies followed *Flora Iranica* (Rechinger & Schiman-Czeika 1968). The voucher specimens were deposited in Alzahra University Herbarium (AUH).

Pollen viability assay and identification of probable hybrids

Aniline blue (Lactophenol) staining was used to establish the pollen viability (Asghari 2000; Wang & al. 2004). Percentage of stainable pollen per 200 grains was determined on the basis of full staining from immersed florets of each population (3-5 individuals per each accession). Viable pollen grains were fully stained, while sterile ones remained uncolored. This method could be used to identify probable hybrids in the population (Sukno & al. 1999). To confirm the hybrid position of two populations in Dafchah and Chaparpord village, pollen fertility percentage, pollen morphological variation and multivariate morphometrics were determined in 40 populations (Mosaferi 2010).

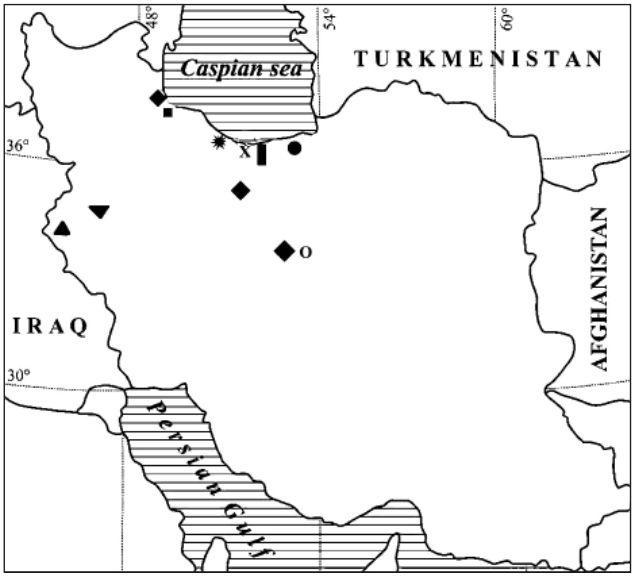
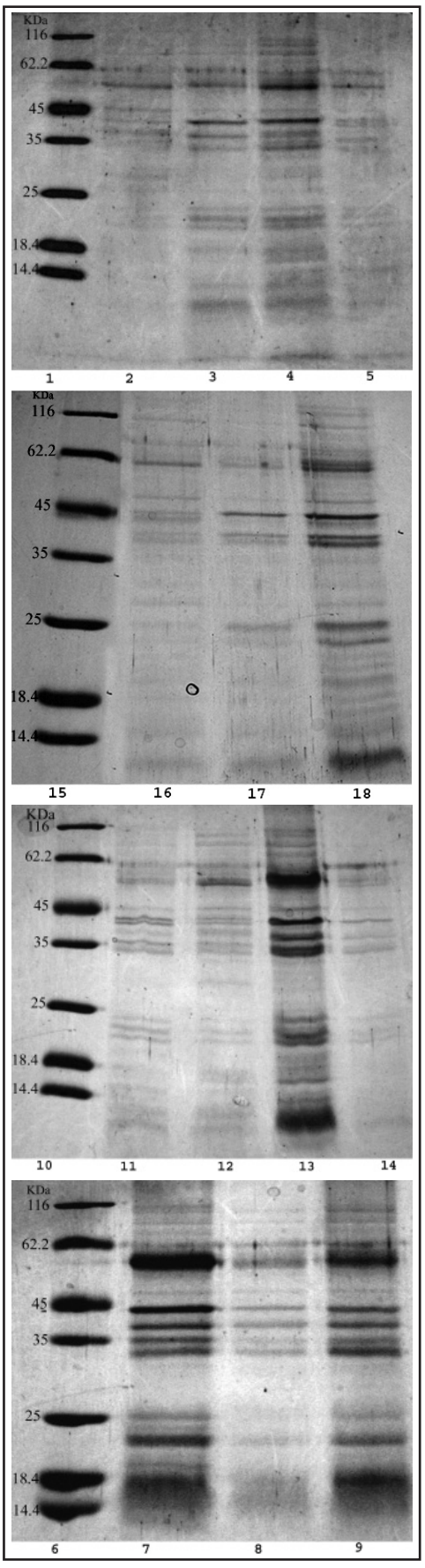


Fig. 1. Locations of the collected *Persicaria* species in this study. Legend: ■ – *P. hydropiper*, ● – *P. maculosa*, ▼ – *P. lapathifolia* ssp. *nodosa*, ▲ – *P. lapathifolia* ssp. *lapathifolia*, ✱ – *P. lapathifolia* ssp. *brittingeri*, **X** – *P. mitis*, ◆ – *P. minor*, ■ – hybrid (Dafchah population), **O** – hybrid (Chaparpord Village population).

Table 1. Collection data for populations used in this s tudy.

Species	Voucher number	Origin	Collector	Floristic region
Persicaria hydropiper (L.) Spach	500	Iran, Mazandaran province, Kelardasht, Gavitar village	*Mosaferi*	Hyrcanian
P. maculosa Gray	502	Iran, Mazandaran province, Zirab, Kechid village	*Keshavarzi*	Hyrcanian
P. lapathifolia ssp. *nodosa* (Pers.) Á. Löve	504	Iran, Hamadan province, Heydareh village	*Mosaferi*	Sahara-Arabian
P. lapathifolia L. ssp. *lapathifolia*	506	Iran, Kermanshah province, Kermanshah, Gharesoo river	*Gholami*	Sahara-Arabian
P. lapathifolia ssp. *brittingeri* (Opiz) Soják	513	Iran, Mazandaran province, Noushahr	*Amini*	Hyrcanian
P. mitis (Schrank) Holub	535	Iran, Mazandaran province, Abbas abad, Abbas abad forest	*Mosaferi*	Hyrcanian
P. minor (Huds.) Opiz	514	Iran, Isfahan province, Golpaygan, Saravar village	*Mosaferi*	Irano-Turanian
P.minor (Huds.) Opiz	515	Iran, Tehran province, Karaj, Jahanshahr	*Mosaferi*	Irano-Turanian
P.minor (Huds.) Opiz	519	Iran, Gilan province, Anzali to Lahidjan, Chaparpord village	*Keshavarzi*	Hyrcanian
Hybrid	542	Iran, Gilan, Lahidjan to Khamam, Dafchah village	*Keshavarzi*	Hyrcanian
Hybrid	540	Iran, Isfahan, Golpaygan, Zarangan village	*Mosaferi*	Irano-Turanian



SDS-PAGE of proteins

An amount of 0.5 g of mature achens were selected from each population and crushed in liquid nitrogen at low temperature. After obtaining a fine powder, proteins were extracted under cool conditions with 3 ml of Tris-Glycin buffer (pH 8.3). The resulting samples were centrifuged twice for 5 min at 11000 g. The protein electrophoresis was based on Laemmli's procedure (1970), using a discontinuous vertical slab gel. The separating gel comprises 5 ml of 30 % acrylamid stock solution, 2.5 ml Tris-HCl 1.5 M (pH 8.8), 100 µl SDS 10 %, 2.3 ml water, 7 µl TEMED, and 60 µl APS (Ammonium per Sulfate). After polymerization of the separating gel, the stacking gel with 530 µl of 30 % acrylamide stock solution, l ml Tris 0.5 M (pH 6.8), 40 ml SDS 10 %, 2.37 ml water, 5µl TEMED, and 40 µl APS was polymerized on the separating gel. The marker was a combinational of β-galactosidase (116 KDa), Bovine Serum Albumin (66.2 KDa), Ovalbumin (45 KDa), Lactate Dehydrogenase (35 KDa), Restriction Endonuclease B_{sp} 981(25 KDa), β- Lactoglobulin (18.4 KDa), and Lysozyme (14.4 KDa). The electrophoresis was carried out at a constant voltage of 100V for 2 h. Gels were stained in Coomasie Brilliant Blue for 3 h and overnight destained with acetic acid and methanol.

Protein banding profile analysis

Number and location of each protein band were identified and their R_F (relative factor) and molecular weight were estimated. In statistical analysis, each protein band was considered as a qualitative character and coded as 1 (presence) versus 0 (absence). Multivariate statistical analysis was performed using SPSS ver. 9 (1998) for cluster analysis and principle components analysis (PCA) (Ingrouille 1986).

Results

A total of 28 protein bands were observed in all studied species (Fig. 2 and Table 2). Bands 5, 11, 12, 14, and 24 were present in all population. Some bands occurred specifically, such as band 4 in Rasht-Tehran population of *P. mitis* and Zarangan population (hybrid), band 7 in Jahanshahr population of *P. minor*, band 26 only in *P.lapathifolia* ssp. *nodosa* (Mosaferi & al. 2010), band 17 in the populations of Rasht-Tehran, Zarangan and Dafchah (hybrid), and band 6 in Chaparpord population of *P. minor*, *P. maculosa* and Dafchah. As it is shown by the cluster analysis (Fig. 3), *P. lapathifolia* is placed in the first major cluster, and its subspecies in three subclusters. The hybrid population of Dafchah is placed in a distinct subcluster near to Jahanshahr population

Fig. 2. SDS-PAGE electrophoresis profiles of the studied species of *Persicaria*. Populations abbreviations: **1**, **6**, **10** and **15** – Marker, **2** – *P. maculosa*, **3** and **17** – *P. lapathifolia* ssp. *lapathifolia*, **4** – Chaparpord Village population of *P. minor*, **5** and **11** – *P. hydropiper*, **7** and **13** – *P. mitis*, **8** – Zarangan population (hybrid), **9** – Saravar population of *P. minor*, **12** – Dafchah population (hybrid), **14** – Jahanshahr population of *P. minor*, **16** – *P. lapathifolia* ssp. *brittingeri*, **18** – *P. lapathifolia* ssp. *nodosa.*

Table 2. Band number and molecular weight for each studied population of *Persicaria*.

Band No	MW	ssp. nodosa	ssp. lapathifolia	ssp. brittingeri	minor (5)	minor (6)	minor (7)	mitis	maculosa	hydropiper	hybrid (10)	hybrid (11)
1	88.86	1	1	1	0	1	1	1	0	1	0	1
2	86.73	1	1	1	0	1	1	1	1	0	1	0
3	79.69	1	1	1	0	1	1	1	1	1	1	1
4	75.83	0	0	0	0	0	0	1	0	0	0	1
5	67.82	1	1	1	1	1	1	1	1	1	1	1
6	64.89	0	0	0	0	0	1	0	1	0	1	0
7	63.27	0	0	0	1	0	0	0	0	0	0	0
8	62.29	1	0	0	1	0	0	0	0	1	1	0
9	60.57	1	1	1	0	0	1	0	1	1	0	1
10	57.46	1	1	1	0	0	0	0	0	0	1	0
11	50.13	1	1	1	1	1	1	1	1	1	1	1
12	44.93	1	1	1	1	1	1	1	1	1	1	1
13	43.65	1	0	1	0	1	0	1	0	1	1	1
14	40.67	1	1	1	1	1	1	1	1	1	1	1
15	38.12	1	1	1	1	0	1	0	1	1	1	0
16	35.18	1	1	1	0	1	1	1	0	1	1	1
17	34.31	0	0	0	0	0	0	1	0	0	1	1
18	31.95	0	0	0	0	0	1	0	1	0	0	0
19	30.79	0	0	0	0	1	0	1	0	0	0	0
20	29.75	1	0	1	0	0	0	0	0	1	1	0
21	28.76	0	0	0	0	0	1	1	1	0	0	0
22	26.71	1	1	1	1	1	1	1	0	1	1	1
23	22.74	1	1	1	1	0	1	1	1	1	1	0
24	20.84	1	1	1	1	1	1	1	1	1	1	1
25	19.22	1	0	1	0	0	0	0	0	0	0	0
26	17.71	1	0	0	0	0	0	0	0	0	0	0
27	16.31	1	1	1	0	0	1	0	0	0	0	0
28	15.44	1	1	1	0	0	1	0	0	0	0	0

Legend: **MW** – molecular weight, **1** – presence versus, **0** – absence (populations' abbreviation are based on Fig. 3).

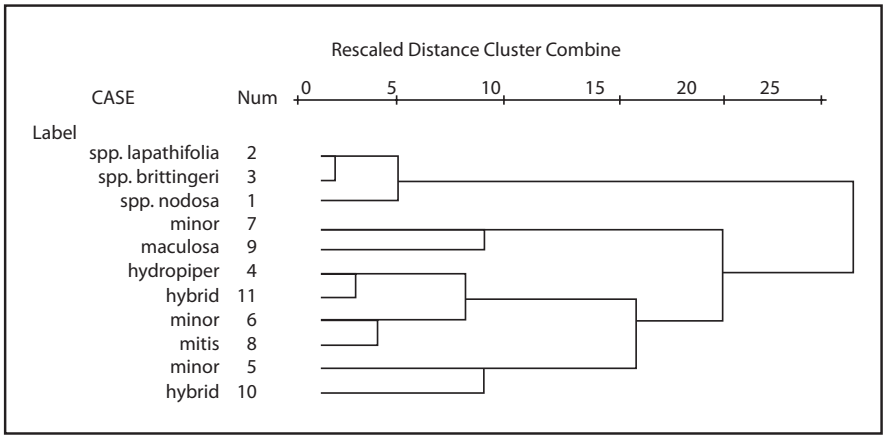


Fig. 3. Phenogram by WARD method based on SDS-PAGE electrophoresis characters in *Persicaria* species. Species abbreviations: **1** – *Persicaria lapathifolia* ssp. *nodosa*, **2** – *P. lapathifolia* ssp. *lapathifolia*, **3** – *P. lapathifolia* ssp. *brittingeri*, **4** – *P. hydropiper*, **5** – Jahanshahr population of *P. minor*, **6** – Saravar population of *P. minor*, **7** – Chaparpord village population of *P. minor*, **8** – *P. mitis*, **9** – *P. maculosa*, **10** – Dafchah population (hybrid), **11** – Zarangan population (hybrid).

Discussion

Different band numbers and varied densities for each population indicate their genetic variations. In comparison with other species, *P. minor* and *P. mitis* show greater similarity. This is in concordance with the earlier palynological, anatomical and morphological studies (Mosaferi 2010).

Britton (1933) recorded hybridization between *P. hydropiper* and *P. minor* in England. He believed that if there was chromosome doubling (4*n*=60), the hybrid would be viable. The hybrid was named *P.* ×*subglandulasum* Borbás (Dandy 1958). The hybrid population of Zarangan falls between *P. hydropiper* and *P. minor*, supporting its status in other taxonomic studies (Mosaferi 2010). Sterility of 75 % of the pollen grains testifies to hybridization in this population.

The hybrid population of Dafchah (with more than 75 % sterility of the pollen grains) is close to *P. minor* in the cluster analysis. In order to identify the proper position of hybrid populations, molecular surveys are needed.

At specific level and on the basis of SDS-PAGE analysis, protein profiles of the annual *Persicaria* species have clearly differed in the strength and positioning of bands. It was also evident that there was great similarity at the sub-specific level (in *P. lapathifolia*).

All SDS-PAGE findings though have not shed light on the nature of the great morphological variability of *P. minor*. However, Mosaferi (2010) has found that total variation among the natural populations of *P. minor* was mostly expressed in the shape and width of leaf, color of flowers and distribution of leaf spots which testify to the wide adaptability of this species. She concluded that the wide-ranging morphological differences between the plants of *P. minor* species collected in nature can be attributed to their plastic response to environmental differences of their habitats. The different populations of this taxon show differences in some habitat characteristics, including altitude (0–1800 m), soil texture (from clay to silty loam) and niche (from open sunny fields to shadowland). Phenotypic plasticity seems to be an important component for the wide-ranging adaptability of this weedy species complex.

One of the diploid species, *P. lapathifolia*, geographically and ecologically is widespread species, with plentiful and conspicuous flowers illustrating the ecological factors which could influence hybridization frequency in Iran. The allopolyploid species are also widespread, plastic and ecological generalists. The currently widespread, weedy allotetraploid *P. maculosa* seems to have originated from hybridization between *P. foliosa* (H.Lindb.) Kitag. and the widespread *P. lapathifolia* in a sympatric area (Kim & al. 2008).

The geographically widespread diploid, *P. hydropiper*, seems to have some features that may influence the hybridization potential, including the number of flowers and their longevity, floral attractiveness to potential pollen vectors, and duration of flowering period. Frequency of successful hybridizations involving *P. lapathifolia* may partly result from its high flower production, which enhances the opportunities for cross-pollination by generalist floral visitors.

The hexaploid *P. puritanorum* (Fernald) Soják originated from hybridization between tetraploid *P. hydropiperoides* Small and diploid *P. lapathifolia* (Kim & al. 2008). As *P. hydropiperoides* has been recently recorded from Iran (Mosaferi & Keshavarzi 2010), it should be found, if such hexaploids exist, as a sympatric species too. More samples of this genus would be desirable, especially in comparison with the perennial species of this group.

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