Ann. Bot. Fennici 50: 249–257 ISSN 0003-3847 (print) ISSN 1797-2442 (online)
Helsinki 14 June 2013

Molecular characterization of *Onobrychis altissima* (Fabaceae) populations from Iran, with the description of *O. chaldoranensis sp. nova*

Zeinab Toluei[1,2], Massoud Ranjbar[1,*], Michael Wink[3] & Morteza Atri[1]

[1)] *Department of Biology, Herbarium Division, Bu-Ali Sina University, P.O. Box 65175/4161, Hamedan, Iran (*corresponding author's e-mail: ranjbar@basu.ac.ir)*
[2)] *Department of Biotechnology, Faculty of Chemistry, University of Kashan, P.O. Box, 78317-51167, Kashan, Iran*
[3)] *Institute of Pharmacy and Molecular Biotechnology (IPMB), Department of Biology, Heidelberg University, Im Neuenheimer Feld 364, D-69120 Heidelberg, Germany*

Received 14 June 2012, final version received 16 May 2013, accepted 30 Jan. 2013

Toluei, Z., Ranjbar, M., Wink, M. & Atri, M. 2013: Molecular characterization of *Onobrychis altissima* (Fabaceae) populations from Iran, with the description of *O. chaldoranensis sp. nova*. — *Ann. Bot. Fennici* 50: 249–257.

Seventy specimens from 14 populations of *Onobrychis altissima* (Fabaceae) were collected from their natural habitats in Iran. In order to assess the genetic variation, we carried out genomic fingerprinting using inter-simple sequence repeat PCR (ISSR). We identified a population which is distinguishable from all other populations genetically as well as morphologically by several unique characters, most notably by having the keels longer than standards (rarely in other members of sect. *Onobrychis* in Iran), wings ± large and pods with four to five very short teeth on the crest. We describe this taxon as *Onobrychis chaldoranensis* Toluei, Ranjbar & Wink *sp. nova* (sect. *Onobrychis*).

Introduction

The genus *Onobrychis* (Fabaceae) comprises about 130 species (Mabberley 2008) and constitutes a major group within the tribe Hedysareae (Polhill 1981, Lock 2005). Its distribution ranges from the Mediterranean region to Caucasia, the Zagros Mountains and central Asia. Most species are concentrated in northwestern Asia, especially in Iran and Anatolia in Turkey (Ranjbar *et al.* 2012a, 2012b).

Rechinger (1984) treated 77 species under nine sections in *Flora Iranica*. Section *Onobrychis*, with nearly 15 species in Iran, is one of the largest sections. The species in this section are perennial herbs, rarely shrubs (unarmed) with many-flowered racemes, usually short wings, the ovary rarely more than 1-ovulate, the fruit not stipitate and with a straight suture, and the crest and disc often with spines or teeth, rarely unarmed; the length of the standard varies relative to the keel (Boissier 1872, Hedge 1970, Grossheim 1972, Rechinger 1984). *Onobrychis altissima*, which belongs in this section, occurs in Armenia, Azerbaijan, Georgia, Iran, and Turkey. It is closely related to the cultivated sainfoin (*O. viciifolia*) and may be its progenitor. *Onobrychis altissima* is of significant agricultural use as a perennial forage and fodder legume, for increasing the nutritive value of

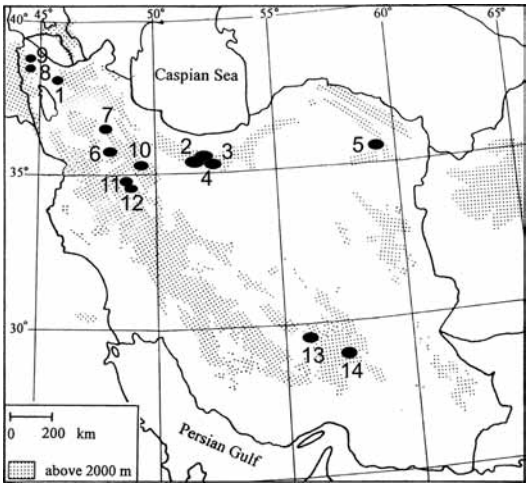

Fig. 1. Locations of the studied populations of *Onobrychis altissima*.

drought-resistant pastures through nitrogen fixation, and for soil conservation (Abou-El-Enain 2002, Elena 2006).

In conjunction with other taxonomic studies on *Onobrychis* in Iran (e.g., Ranjbar *et al*. 2007, 2009, 2010, Toluei *et al*. 2010, 2012, 2013), the present investigations were carried out to test the hypothesis that differences among populations of *O. altissima* result from environmental conditions of their habitats. When a species encounters novel environmental conditions, some phenotypic characters may be expressed differently than in the original environment (Grether 2005). Phenotypic plasticity is the ability of organisms to adapt in response to changes in the environment (West-Eberhard 2003). The extent of phenotypic plasticity in plants may be limited by both intrinsic and extrinsic factors (Valladares *et al*. 2007).

Some morphological variation may be associated with epigenetic responses to variations in environmental conditions but may also result from adaptive genetic differentiation within populations. Thus, in order to distinguish the amount of variation due to phenotypic plasticity as opposed to genetic differentiation, provenance testing is necessary. In the present study, the genetic variation and population differentiation were investigated by means of genomic fingerprints using inter-simple sequence repeat PCR (ISSR). ISSR analysis is a tool to assess genetic diversity among closely related species, and to detect similarities between and within species (Zietkiewics *et al*. 1994, Pasakinskiene *et al*. 2000, Ghariani *et al*. 2003, Treutlein *et al*. 2003a, 2003b, 2005, Chennaoui-Kourda *et al*. 2007). Because DNA sequences often have a slow mutation rate, mutations may not accumulate rapidly enough to track the evolutionary history of a young group, increasing the probability of discordance between gene-trees and the species tree (De Queiroz 2007). However, because population genetic markers evolve rapidly, patterns of genetic structure based on these markers can often discern genetically isolated population groups, even when other markers, such as DNA sequences, cannot. In this study, we used ISSR fingerprinting data to investigate among-population variation and delimit a new species in *Onobrychis*.

Material and methods

Sample collection

We extensively studied the distribution of *O. altissima* in Iran: 70 specimens (14 populations; Fig. 1) were collected from their natural habitats between 2008 and 2010 (during April–July each year). These populations are representative of the variation expressed throughout the distribution area of this species in Iran, and were collected from different geographical and ecological conditions. The specimens were prepared according to established herbarium techniques and voucher specimens (Table 1) were deposited in the herbarium of Bu-Ali Sina University (BASU). Identification of the specimens was done using *Flora Iranica* and other relevant floras (Boissier 1872, Hedge 1970, Grossheim 1972, Rechinger 1984) as well as the monograph by Širjaev (1926).

Molecular data

Our initial analysis of ITS sequences of the *O. altissima* complex did not reveal population differences (data and methods are available from the authors on request). Therefore, ISSR genomic fingerprints, which provide a better resolution than sequences, were used to analyze

the genetic variation and population differentiation in *O. altissima*. Total genomic DNA was isolated from dried leaf material using the standard CTAB (hexadecyl trimethyl ammonium bromide) extraction method (Doyle & Doyle 1987) and purified through QIAquick silica columns (Qiagen Inc., Hilden, Germany). Two individuals from each population were sampled based on a pilot study, which showed that there is no considerable genetic intrapopulation variation in this taxon. We used different primers for amplification in the pilot study. Finally the $(GACA)_4$ primer providing the most reliable, consistent results was chosen. PCR was performed in a final volume of 25 μl containing 30 to 60 ng of genomic DNA, 20 pmol of the primer $(GACA)_4$, 0.1 mmol l^{-1} each of dGTP, dCTP and dTTP, 0.045 mmol l^{-1} dATP, 0.1 μl (α-^{33}P)-dATP (Amersham Biosciences), 0.6 units of Taq DNA polymerase (Pharmacia Biotech) and 2.5 μl of 10× amplification buffer (100 mmol Tris-HCl pH 8.5, 500 mmol KCl and 15 mmol $MgCl_2$, 5% Triton ×100). PCR amplifications were performed in a DNA thermal cycler (Biometra, Göttingen, Germany). Initial denaturation was for 5 min at 94 °C; followed by 38 cycles of 45 s at 94 °C, 60 s at 48 °C, 120 s at 72 °C, and 10 min at 72 °C for final elongation. DNA fragments were separated by vertical polyacrylamide gel electrophoresis for 3 h at 65 watts using a Base Acer Sequencer (Stratagene, La Jolla, San Diego, CA, USA). After drying, the denaturizing gels were exposed for 24 h to X-ray film (BioMax MR Film, Kodak, Taufkirchen, Germany). To estimate the reproducibility of the ISSR data, DNA from five individuals (randomly selected from different populations) was extracted twice, and the replicates were scored independently. Each ISSR band was considered a character, and the presence or absence of a band was scored on a binary scale (present = 1, absent = 0). A data matrix was assembled and analyzed using PAUP* ver. 4.0b10 (Swofford 2002), and a pair-wise distance matrix was generated based on total character differences. An all-zero outgroup was included in the data matrix. The genetic relationships among the populations were analyzed using neighbor-joining

Table 1. Population data including their localities and voucher numbers.

Population number	Province	Locality	Lat. (N)	Long. (E)	Altitude (m a.s.l.)	Voucher number
1	Azerbaijan Sharghi	Sufian to Marand	38°20.528´	45°52.278´	1635	BASU 23100
2	Tehran	5 km past Polur-Firoozkooh crossroad	35°39.906´	52°05.487´	2262	BASU 23101
3	Tehran	5 km past Polur-Firoozkooh crossroad	35°50.255´	52°05.250´	2271	BASU 23102
4	Tehran	Tehran, before Firoozkooh, 5 km past Vazna village	35°50.441´	52°05.139´	2400	BASU 23103
5	Khorasan Shomali	Esfarayen to Bojnurd, Assadli neck	35°33.836´	58°33.038´	1718	BASU 23104
6	Zanjan	50 km to Mahneshan, past Andabad	36°02.841´	47°53.852´	1573	BASU 23106
7	Zanjan	Mahneshan to Halab	36°45.449´	47°53.189´	1772	BASU 23107
8	Azerbaijan Gharbi	before Chaldoran, Alimardan village	39°02.544´	44°40.613´	2005	BASU 23108
9	Azerbaijan Gharbi	Maku, Baduli village	39°02.516´	44°40.542´	1929	BASU 23109
10	Qazvin	Avaj	35°34.337´	49°12.922´	2037	BASU 23110
11	Hamedan	Heydareh-Ghazikhan village	34°49.659´	48°20.049´	2086	BASU 23111
12	Hamedan	Heydareh-Ghazikhan village	34°49.666´	48°20.009´	2089	BASU 23112
13	Kerman	Sarcheshmeh to Sirjan, Pariz, Pasoojan village	29°54.368´	55°43.840´	2193	BASU 23113
14	Kerman	Rabor to Jiroft, 23 km to Darb-e-Behesht	29°16.321´	57°11.827´	2513	BASU 23114

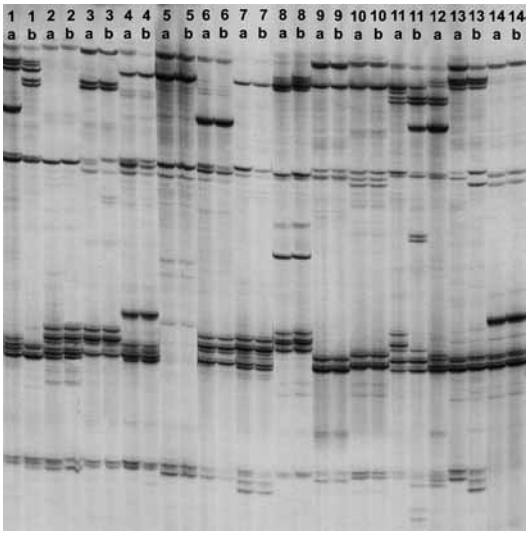

Fig. 2. ISSR-PCR profile of 14 populations generated with the $(GACA)_4$ primer. Lane numbers correspond to populations 1 to 14 (2 individuals, a and b, from each population).

based on total character difference. Confidence of each node of the trees was tested by bootstrapping (Felsenstein 1985) with 1000 replicates.

Results

Morphology

The study populations are distributed between 28°–40°N and 44°–59°E (Fig. 1). Population 8 is different from all other populations because of its long wings (5–6 mm *vs*. 2.7–5 mm), narrower leaflets (1.5–4 mm *vs*. (1)4–10.5 mm), keel longer than the standard (an unknown character in sect. *Onobrychis* in Iran), pods with 4–5 very short teeth on the crest and narrower pods (3.5–4 mm *vs*. 4–5.5 mm).

Population 9 is distinct by fewer leaflets (5–6 pairs *vs*. 4–11 pairs), maximum length × width of standard (13–15 × 8.5–10 mm *vs*. 8–12.5 × 5.5–9 mm), maximum length/width of keel (13–15 × 4.5–5.5 mm *vs*. 7–12.5 × 3–5 mm) and longer keel claw (4–5 mm *vs*. 2–4 mm).

Population 5 is distinct by a smaller range of the bract length (1.5–3.5 mm *vs*. 1.5–5 mm) and shorter hairs on the abaxial surface of leaflets (0.4–1 mm *vs*. 0.4–1.8 mm).

Populations 11 and 12 are distinct by the minimum length of keel (7–8.5 mm *vs*. 9–15 mm) and keel being shorter than standard.

Populations 6, 7 and 10 are distinct by glabrous and sparse hairs of the adaxial surface of leaflets (in the other populations they are glabrous).

Molecular data

The ISSR amplification generated a total of 43 distinguishable bands (Fig. 2), of which 36 (84%) are parsimony-informative characters, two characters are constant, and five variable characters are parsimony-uninformative. The size of the bands ranged from 247 to 1700 bp. For estimation reproducibility of the ISSR data, DNA from five individuals was extracted twice, and the replicates were scored independently but we saw the identical patterns for the same individuals.

A dendrogram was generated using NJ based on a cluster analysis of the distance matrix (Fig. 3). Population 8 from Chaldoran in Azerbaijan Gharbi Province clustered separately from the other populations with the bootstrap value of 100. The other populations can be divided into five groups: (1) population 5 (group C) in Khorasan Shomali Province, (2) population 9 (group D) in Azerbaijan Gharbi Province, (3) populations 13 and 14 (group A) in Kerman Province, (4) populations 11 and 12 (group B) in Hamedan Province, and (5) the last group (E) comprising populations 1, 2, 3, 4, 6, 7 and 10 in four neighbor provinces.

Taxonomic treatment

Morphological characters supported the separation of population 8 and revealed several unique characters for it. Because that population is genetically and morphologically distinguishable from all other populations, we consider it to be specifically distinct, and describe it as a new species here. A comparison of the most important diagnostic characters of the new species, *O. altissima*, *O. arenaria* and other *Onobrychis* species especially in East Anatolia is presented in Table 2.

Onobrychis chaldoranensis Toluei, Ranjbar & Wink, *sp. nova* (Fig. 4).

HOLOTYPE: Iran. Azerbaijan Gharbi Province, before Chaldoran, Alimardan village, 39°02.544´N, 44°40.613´E, 2005 m, 27 June 2009 *Ranjbar & Toluei 23108* (holotype BASU!).

ETYMOLOGY: The new species is named after its place of occurrence, near Chaldoran.

Perennial herbs, 65–75 cm tall; stems numerous, erect, usually branched above, glabrous to sparsely appressed hairy. Stipules lanceolate-acuminate, 5–10 × 2–4.5 mm, white, membranous-scarious, glabrous, sparse to ciliate hairy. Leaves 3.5–18 cm long, imparipinnate with 6–8 pairs of leaflets, petiolate, petioles 0.3–10 cm; leaflets oblong-elliptic, linear-oblong to oblanceolate, mucronulate, 7–19 × 1.5–4 mm, abaxial surface with loose to sparse appressed hairs, adaxially glabrous. Inflorescences racemose, many-flowered (12–35), somewhat elongating in fruit, 13–35 cm long; peduncles 10–23 cm, glabrous to sparsely or loosely to densely hairy, exceeding leaves; bracts membranous, lanceolate, 2.5–3 mm, with ciliate hairs or glabrous. Pedicel 1.5–2.5 mm long. Calyx 6–7 mm, teeth lanceolate-subulate, tube sparsely hairy, teeth with ciliate, loose to dense hairs, upper teeth 1.5–2.5 times as long as tube; bracteoles lanceolate-subulate, membranous minute. Corolla pink-purple, with darker veins; standard obovate or elliptic, retuse, 8.5–10.5 × 6–7 mm, glabrous, claw very short; wings ± large, 5–6 × 1.7–2 mm, lamina deltoid-oblong, with long claw (1.2–2.2 mm) and broad auricle (0.7–1 mm); keel longer than standard, 9.5–11 × 3.5–4.2 mm, deltoid-oblong, truncate, claw 2.5–3.5 mm. Ovary uniovulate. Pods suborbicular, 6–7 × 3.5–4 mm, loosely to densely appressed-hairy, upper suture almost straight, convex lower suture with 4–5 very short (0.2–0.5 mm) teeth on crest; disc spineless or with small spinules, foveolate, with 8–9 alveoles.

DISTRIBUTION AND HABITAT. So far, *O. chaldoranensis* is known only from the type locality where it grows on soil of sandy-clay-loam (SCL) texture and pH 7.43, at the elevation of 2005 m a.s.l. The average maximum and minimum annual temperatures at the location are 15.5 °C and 5.3 °C, respectively. The number of rainy days/year is 118.3 and the annual precipitation is 294.5 mm.

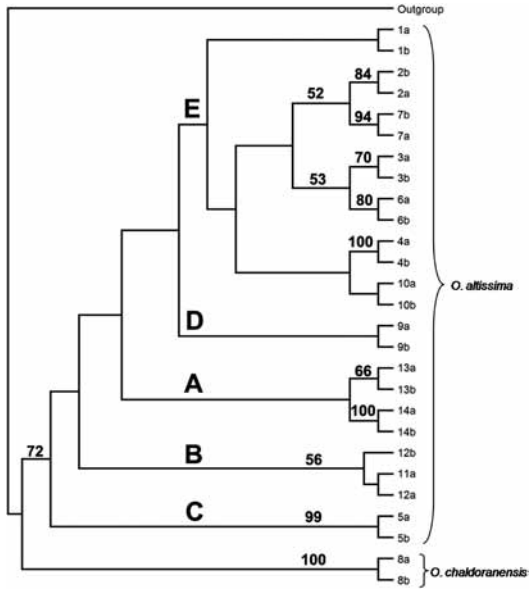

Fig. 3. Neighbor-joining tree generated from ISSR data of 14 Iranian populations. Population 8 is treated as a new species described in the present paper. Bootstrap values greater than 50% are shown above the branches (from 1000 replications) (maximum 2 individuals, a and b, from each population).

Discussion

Morphological characters are the external features of an organism and they are determined by both genetical and environmental factors. Intraspecific variation in plants is often regarded as an adaptive response to different environments (Mal & Doust 2005). The different populations of *O. altissima* show phenotypic differentiation. Some of the observed morphological variation may be associated with epigenetic responses to variation in environmental conditions. This differentiation could result from plastic responses to the diverse environments that populations of *O. altissima* occupy across its natural distribution in Iran, but it may also entail some degree of adaptive genetic differentiation among populations.

We used ISSR fingerprinting in order to distinguish the amount of phenotypic variation from the genetic background. The results showed that population 8 (Fig. 1) is genetically and morphologically different from all other populations. Therefore, we described it as a

Table 2. Comparison of *Onobrychis chaldoranensis* with the six morphologically similar species (based on Hedge 1970, Grossheim 1972, Rechinger 1984 and our data).

Characters	*O. chaldoranensis*	*O. altissima*	*O. arenaria*	*O. viciifolia*	*O. stenostachya*	*O. sulphurea*	*O. transcaucasica*
Plant height (cm)	65–75	38–125	30–50	30–70	25–50	30–60	20–60
Number of leaflets	6–8	4–11	6–12	7–10	7–11	7–9	6–10
Leaflet length (mm)	7–19	6–43	10–30	15–20(35)	7–20	10	(8)15–25(30)
Leaflet width (mm)	1.5–4	(1)4–10.5	2–5	5–6(7)	1.5–4	3	(1)2–3
Stipule length (mm)	5–10	3–16	–	2–15	ca. 8	–	5
Flower color	purple–pink	purple-pink	purple-pink	purple-pink	pink or creamy white	creamy white	purple–pink
Calyx length (mm)	6–7	5–9	–	5–7	5	7–9.5	6.5–9
Standard length (mm)	8.5–10.5	8–15	8–10	10.5–12	8–15	8–12	9.5–11
Keel length (mm)	9.5–11	7–15	–	10–12	7–13	9–11	10–12
Wing length (mm)	5–6	2.7–5	–	ca. 2	6–9	4–6.5	3.5–5
Comparison of standard and keel size	keel longer than standard	keel as long as or shorter than standard	standard as long as or shorter than keel	keel as long as or slightly shorter than standard	standard longer than keel	keel ± as long as standard	standard slightly longer than keel
Pod length (mm)	6–7	5–7(–8)	4–5	5–8	ca. 5	8	5–7
Pod width (mm)	3.5–4	4–5.5	–	4–6	3.5	6	4–5.5
Pod shape	crest with 4–5 very short teeth, disc spineless or with small spinules	unarmed or ± unarmed	crest and disc with short teeth	with very short or obsolete teeth on crest, scarcely toothed on disc	unarmed or with teeth on crest and short spin on disc	–	with 1–2 mm teeth on crest and disc

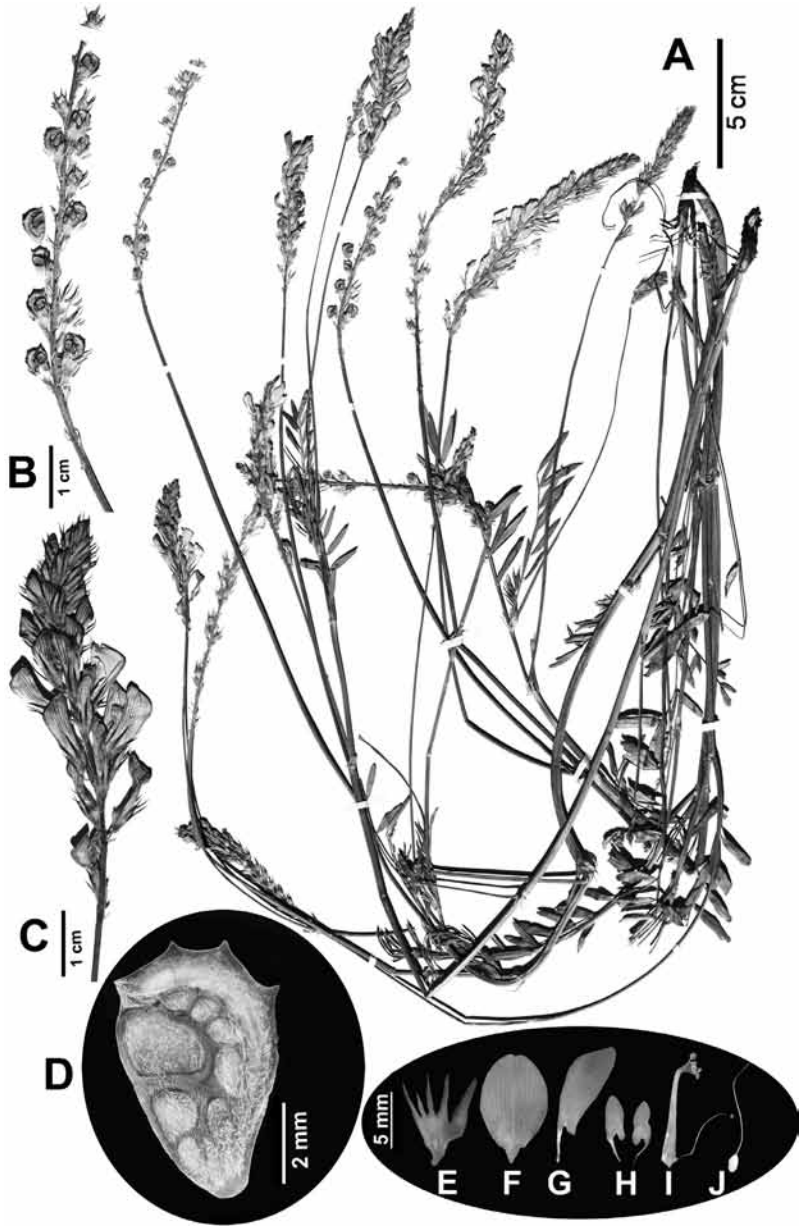

Fig. 4. *Onobrychis chaldoranensis* (from the holotype). — **A**: Habit. — **B**: Raceme in fruit. — **C**: Raceme in flower. — **D**: Pod. — **E**: Calyx. — **F**: Standard. — **G**: Keel. — **H**: Wings. — **I**: Androecium. — **J**: Gynoecium.

new species, *O. chaldoranensis*. In addition, we obtained new results about the infraspecific variation of *O. altissima* in Iran. A dendrogram from a cluster analysis of ISSR bands produced five groups (Fig. 3), which are also geographically separated in different provinces (Fig. 1). The genetic groups B, C and D are also morphologically distinct. So these three groups may reflect genetic and phenotypic responses to different habitats. The variation can be related to phenotypic plasticity and genetic assimilation, the latter a process by which non-heritable environmentally induced variation leads to adaptive heritable variation (Schlichting 1986, 2004, West-Eberhard 2003). It has the potential to explain a variety of evolutionary ecological processes (Pigliucci *et al.* 2006). Furthermore, populations 6, 7 and 10 are distinct morphologically, but not genetically based on ISSR data. That suggests that their morphological traits are not

yet genetically fixed, but could be attributed to epigenetic responses to variation in environmental conditions. Also populations 1, 2, 3, and 4 are genetically in the same group, and they are also distinguishable by their morphology, which suggests genetic assimilation.

A revised description of Iranian *Onobrychis altissima* populations

Perennial; 38–125 cm tall; stems numerous, erect, usually branched above, glabrous to sparsely hairy, rarely with loose to dense appressed or subappressed hairs. Stipules lanceolate or triangular-acuminate, 3–16 × 1.5–7 mm, white, scarious, membranous, glabrous, ciliate, sparse, loosely to densely hairy. Leaves 3.5–29 cm, imparipinnate with 4–11 pairs of leaflets, petiolate to sessile, petioles 0.2–12 cm; leaflets oblong-elliptic, obovate, linear-oblong to oblanceolate, mucronulate, 6–43 × (1)4–10.5 mm, abaxial surface with sparse to dense appressed hairs, adaxially glabrous to sparsely hairy. Inflorescences racemose, many-flowered (12–57), somewhat elongating in fruit, 8.5–50 cm long; peduncles 3–36 cm, glabrous to sparsely or densely hairy, exceeding leaves; bracts membranous, lanceolate or lanceolate-ovate, 1.5–5 mm, glabrous, ciliate, sparsely hairy. Pedicel 1–3 mm long. Calyx 5–9 mm, teeth lanceolate-subulate, tube sparsely hairy, teeth ciliate, loosely or densely hairy, upper teeth 1–3 times as long as tube; bracteoles lanceolate-subulate, membranous minute. Corolla pink-purple, with darker veins; standard obovate, retuse or emarginate, 8–15 × 5.5–10 mm, glabrous, claw very short; wings 2.7–5 × 1–2 mm, lamina deltoid-oblong, claw 0.7–2 mm, auricle 0.5–1 mm; keel as long as or shorter than standard, 7–15 × 3.5–5.5 mm, deltoid-oblong, truncate, claw 2–5 mm. Ovary uniovulate. Pods suborbicular, 5–7 × 4–5.5 mm, ± densely and shortly appressed-hairy, upper suture almost straight, lower suture convex, crest unarmed or rarely with short prickles when pod is immature; disc without teeth, foveolate, with 6–13 alveoles. Seeds reniform, 2.2–4.5 × 1.2–3.5 mm, brown, smooth.

Acknowledgements

This research was financially supported by Bu-Ali Sina University and in part by IPMB, Heidelberg University, Germany. The authors are grateful to Dr. Javier Gonzalez for fruitful discussions and help in the molecular laboratory, and to Hedwig Sauer-Gürth for technical help.

References

Abou-El-Enain, M. M. 2002: Chromosomal criteria and their phylogenetic implications in the genus *Onobrychis* Mill. sect. *Lophobrychis* (Leguminosae), with special reference to Egyptian species. — *Botanical Journal of the Linnean Society* 139: 409–414.

Boissier, P. E. 1872: *Onobrychis*. — In: Boissier, P. E. (ed.), *Flora Orientalis*, vol. 2: 525–553. Basileae & Lugundi, Genevae.

Chennaoui-Kourda, H., Marghali, S., Marrakchi, M. & Trifi-Farah, N. 2007: Genetic diversity of *Sulla* genus (Hedysareae) and related species using inter-simple sequence repeat (ISSR) markers. — *Biochemical Systematics and Ecology* 35: 682–688.

Hedge, I. C. 1970. *Onobrychis*. — In: Davis, P. H. (ed), *Flora of Turkey and the East Aegean Islands*, vol. 3: 560–589. Edinburgh University Press, Edinburgh.

De Queiroz, K. 2007: Species concepts and species delimitation. — *Systematic Biology* 56: 879–886.

Doyle, J. J. & Doyle, J. L. 1987: A rapid DNA isolation procedure for small quantities of fresh leaf tissue. — *Phytochemical Bulletin* 19: 11–15.

Elena, T. 2006: Cytological aspects of the *Onobrychis* genus. — *Bulletin USAMV* 62: 154–158.

Felsenstein, J. 1985: Confidence limits on phylogenies: An approach using the bootstrap. — *Evolution* 39: 783–791.

Ghariani, S., Trifi-Farah, N., Chakroun, M., Marghali, S. & Marrakchi, M. 2003: Genetic diversity in Tunisian perennial ryegrass revealed by ISSR markers. — *Genetic Resources and Crop Evolution* 50: 809–815.

Grether, G. F. 2005: Environmental change, phenotypic plasticity and genetic compensation. — *American Naturalist* 166: E115–E123.

Grossheim, A. A. 1972: *Onobrychis*. — In: Shishkin, B. K. & Bobrov, E. G. (eds.), *Flora URSS*, vol. 13: 244–281. Israel Program for Scientific Translation, Jerusalem.

Lock, J. M. 2005: Tribe Hedysareae. — In: Lewis, G., Schrire, B., Mackinder, B. & Lock, M. *(*eds.), *Legumes of the world*: 489–495. Royal Botanical Gardens, Kew, London.

Mabberley, D. J. 2008: *Mabberley's plant-book: a portable dictionary of plants, their classification and uses*, 3rd ed. — Cambridge University Press, Cambridge.

Mal, T. K. & Doust, J. L. 2005: Phenotypic plasticity in vegetative and reproductive traits in an invasive weed, *Lythrum salicaria* (Lythraceae), in response to soil moisture. — *American Journal of Botany* 92: 819–825.

Pasakinskiene, I., Griffiths, C. M., Bettanny, A. J. E., Paplauskiene, V. & Humphreys, M. W. 2000: Anchored

simple-sequence repeats as primers to generate specific DNA markers in *Lolium* and *Fetusca* grasses. — *Theoretical and Applied Genetics* 100: 384–390.

Pigliucci, M., Murren, C. J. & Schlichting, C. D. 2006: Phenotypic plasticity and evolution by genetic assimilation. — *The Journal of Experimental Biology* 209: 2362–2367.

Polhill, R. M. 1981: Hedysareae DC. — In: Polhill, R. M. & Raven, P. H. (eds.), *Advances in legume systematics*, I: 367–370. Royal Botanic Gardens, Kew, London.

Ranjbar, M., Karamian, R. & Hadadi, A. 2010: Cytosystematics of three *Onobrychis* species (Fabaceae) in Iran. — *Caryologia* 63: 237–249.

Ranjbar, M., Hajmoradi, F. & Karamian, R. 2012a: An overview on cytogenetics of the genus *Onobrychis* (Fabaceae) with special reference to *O*. sect. *Hymenobrychis* from Iran. — *Caryologia* 65: 187–198.

Ranjbar, M., Karamian, R. & Hadadi, A. 2009: Biosystematic study of *Onobrychis viciifolia* Scop. and *Onobrychis altissima* Grossh. (Fabaceae) in Iran. — *Iranian Journal of Botany* 15: 85–95.

Ranjbar, M., Karamian, R., Hadadi, A. & Joharchi, M. R. 2012b: Taxonomic notes on *Onobrychis* sect. *Onobrychis* subsect. *Macropterae* (Fabaceae) from Iran. — *Phytotaxa* 39: 51–60.

Ranjbar, M., Karamian, R., Toluei, Z. & Amirabadizadeh, H. 2007: *Onobrychis assadii* (Fabaceae), a new species from Iran. — *Annales Botanici Fennici* 44: 481–484.

Rechinger, K. H. 1984: *Onobrychis*. — In: Rechinger, K. H. (ed.), *Flora Iranica*, vol. 157: 387–464. Akademische Druck- u. Verlagsanstalt, Graz and Wien.

Schlichting, C. D. 1986: The evolution of phenotypic plasticity in plants. — *Annual Review of Ecology and Systematics* 17: 667–693.

Schlichting, C. D. 2004: The role of phenotypic plasticity in diversification. — In: DeWitt, T. J. & Scheiner, S. M. (eds.), *Phenotypic plasticity: functional and conceptual approaches*: 191–200. Oxford University Press, Oxford.

Širjaev, G. 1926: *Onobrychis generis revisio critica*. — Publication de la faculte' des sciences des L'Universite Masaryk, Brno.

Swofford, D. L. 2002: *PAUP*: phylogenetic analysis using parsimony (*and other methods)*, version 4.10b. — Sinauer Associates, Sunderland.

Toluei, Z., Atri, M. & Ranjbar, M. 2010: Taxonomic study of *Onobrychis transcaspica* V. Nikitin (Fabaceae) in northeastern of Iran with emphasis on altitudinal effect on morphological characters using floristic marker. — *Taxonomy and Biosystematics* 2: 25–38.

Toluei, Z., Atri, M., Ranjbar, M. & Wink, M. 2012: Morphological, genetical and ecogeographical characterization of long-winged species of *Onobrychis* sect. *Onobrychis* (Fabaceae) in Iran. — *Iranian Journal of Botany* 18 : 31–41.

Toluei, Z., Atri, M., Ranjbar, M. & Wink, M. 2013: Iranian *Onobrychis carduchorum* (Fabaceae) populations: morphology, ecology and phylogeography. — *Plant Ecology and Evolution* 146: 1–15.

Treutlein, J., Vorster, P. & Wink, M. 2005: Molecular relationships in *Encephalartos* (Zamiaceae, Cycadales) based on nucleotide sequences of nuclear ITS 1 & 2, *rbc*L, and genomic ISSR fingerprinting. — *Plant Biology* 7: 79–90.

Treutlein, J., Smith, G. F., van Wyk, B.-E. & Wink, M. 2003a: Evidence for the polyphyly of *Haworthia* (Asphodelaceae, subfamily Alooideae; Asparagales) inferred from nucleotide sequences of *rbc*L, *mat*K, ITS1 and genomic fingerprinting with ISSR-PCR. — *Plant Biology* 5: 513–521.

Treutlein, J., Smith, G. F., van Wyk, B.-E. & Wink, M. 2003b: Phylogenetic relationships in the Asphodelaceae (subfamily Alooideae) inferred from chloroplast DNA sequences (*rbc*L, *mat*K) and from genomic fingerprinting (ISSR). — *Taxon* 52: 193–207.

Valladares, F., Gianoli, E. & Gómez, G. M. 2007: Ecological limits to plant phenotypic plasticity. — *New Phytologist* 176: 749–763.

West-Eberhard, M. J. 2003: *Developmental plasticity and evolution*. — Oxford University Press, Oxford & New York.

Zietkiewics, E., Rafalski, A. & Labuda, D. 1994: Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. — *Genomics* 20: 176–183.