Cytotaxonomy of some *Onobrychis* (Fabaceae) species and populations in Iran

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Abstract — A karyological study of 20 taxa (45 populations) of the genus *Onobrychis* Adans. from different geographic origins is presented. We found the two usual basic chromosome numbers in the genus, $x = 7$ and $x = 8$. In the group with $x = 7$, six diploid ($2n = 14$), 22 tetraploid ($2n = 28$) populations and in the group with $x = 8$, 17 diploid populations were found. Detailed karyotype analysis allows us to group the different populations and to postulate relationships among them.

Keywords: chromosome, evolution, Fabaceae, karyology, *Onobrychis*

INTRODUCTION

The genus *Onobrychis* belongs to Fabaceae family, tribe Hedysareae, subfamily Faboideae. The genus is composed of 342 perennial and annual species which are well-distributed in south Europe and in temperate western Asia Nixone (2006). About 60 species exist in the grazing areas of Iran Rechinger (1984) and are distributed in various regions of the country. The discrepancy in the number of *Onobrychis* species is due to problems in taxonomy. The *Onobrychis* species are usually confused with *Hedysarum* during identification. The most important difference between *Onobrychis* and *Hedysarum* is number of lobes in their fruits. The fruits in *Onobrychis* have one lobe but fruits of *Hedysarum* have two lobes. It thrives on calcareous, dry and barren soils. It is useful non bloat legume pasture or a hay crop. This genus is subdivided into two subgenus namely *Onobrychis* with four sections and *Sisyrosema* with five sections distinguished by different karyotype morphological features and geographical origins Rechinger (1984). 45 populations that used in this study belong to five sections of two subgenus and also eight species were endemic in Iran. Although the available literature dealing with systematic, biosystematic and cytogenetic of *Onobrychis* species indicates the importance of these taxa (Astanova & Abdusaljamova 1981; Diaz Lifante *et al.* 1992; Oberprieler & Vogt 1996; Vogt & Aparicio 1999; Mohamed 1997; Magulaev 1995; Garnatje & Cardona 1988; Diosdado *et al.* 1993; Abou-el-Enain 2002), however no report is available on the cytogenetic of *Onobrychis* populations and endemic species from Iran. Therefore the present studies consider a mitosis analysis of 45 populations of 20 *Onobrychis* species and try to reveal the chromosome numbers and basic cytogenetic informations of these species for the first time.

MATERIALS AND METHODS

In this study, we used root tip meristems from seedling obtained by the germination of ripe seeds collected from natural populations (45 populations, representing 20 species) on wet filter paper in Petri dishes and left at 22°C temperature. The studied populations are listed in (Tab. 1). Vouchers are deposited in RIFR gene

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bank (Research Institute of Forest and Rangelands in Iran). Root tip meristems obtained from seedlings were pretreated with % 0.5 saturated α-Bromo naphthalene at 4°C for 4 h, fixed in % 10 formaldehyde and % 1 chromic acid (1:1) for at least 16 h at room temperature, then root tips were rinsed for 3 h in distilled water.

Hydrolysis was carried out with 1 N NaOH at 60°C for 7min, dyed with hematoxylin-iron for 3-4 h and squashed in a droplet of % 45 acetic acid and lactic acid (10:1) (WITTMANN 1965; HESAMZADEH HEJAZI & RASULI 2006).

The preparations were observed with an optical microscope (BH_2 Olympus supplemented digital color video camera) at a magnification of 1908 ×.

The best metaphasical plates were selected and measured by Micro measure 3.3 software (REEVES *et al.* 2000). In each mitotic metaphase (at least 5 plates) the arm's length of each chromosome was measured, according to the previous studies.

The following parameters were estimated in each metaphase plate to characterize the karyotypes numerically: long arm (LA), short arm (SA), total length (TL) [LA+SA], relative length percentage (RL %), arm ratio (AR) [LA/SA], centromeric index (CI) [SA/ (LA+SA)], value of relative chromatin (VRC) [ΣTL/n] (HESAMZADEH & ZIAIE 2009), BAZZICHELLI (1967), (MARTINOLI & OGLIOTTI 1970). Karyotype asymmetry was estimated by three different methods namely, total form percentage (TF %) $[(\Sigma SA/\Sigma TL)\times 100]$ HUZIWARA (1962); difference of relative length (DRL) $[Max_{RL\%} - Min_{RL\%}]$; intrachromosomal asymmetry index (A_1) $[1\text{-}\Sigma\ (\overline{SA}/\overline{LA})/n]$ and interchromosomal asymmetry index (A_2) $[\overline{Sd}/\overline{X}]$ ROMERO ZARCO (1986). Both indices (A_1 and A_2) are independent to chromosome number and size.

Also karyotypic evolution has been determined using the symmetry classes of Stebbins (SC) STEBBINS (1971). Karyotype formula was determined by chromosome morphology based on centromere position according to classification of Levan (LEVAN *et al.* 1964). For each population, karyograms were drawn based on length of chromosome size (arranged large to small).

In order to determine the variation between populations, one-way unbalanced ANOVA was performed on normal data and parameter means were compared by Duncan's test. The principal components analysis (PCA) was performed to evaluate the contribution of each karytypic parameter to the ordination of species. Clustering was performed using the unweighted pair group method with arithmetic (UPGMA) after calculattion of Cophenetic correlation coefficient (r) to examine karyotype similarity among populations. Numerical analysis were performed using SAS ver. 6.12 (1996), JMP ver. 3.1.2 (1995) and StatistiXL ver. 1.7 (2007) softwares.

RESULTS

The results showed that the basic chromosome number was varied between $x=7$ and $x=8$ and there was a high rate of chromosomal variations. The somatic chromosome numbers ($2n$), karyotype formulae and parameters for the studied populations are summarized in Table 1. Most of the populations belong to taxa with the basic number $x=7$, the most common one is in the genus. In the group with $x=7$, six diploid ($2n=14$), 22 tetraploid ($2n=28$) populations and in the group with $x=8$, 17 diploid populations exist (Tab.1). The karyotypes of diploid and tetraploid populations are illustrated in Figure 1.

The mean value of chromosome's long arm was varied from 1.404 µm in *O. crista-galli* (2520) to 2.373 µm in *O. persica* (6012). Averages of chromosome's short arm were different from 0.922 µm in *O. crista-galli* (2520) to 1.660 µm in *O. amoena* (5786). The mean value of chromosome's total length was varied from 2.33 µm in *O. crista-galli*(2520) to 4.02 µm in *O. amoena* (5786) and finally the mean value of chromosome's arm ratio was changing from 1.21 in *O. aucheri* (2900) to 1.78 in *O. crista-galli* (2543) (Tab. 2).

The chromosomes were mostly metacentric (m) or sub-metacentric (sm) in all populations except for *O. sativa* (325) and *O. viciaefolia* (3026) had one pair sub-telocentric (st) chromosomes (Tab. 1).

Symmetry type of STEBBINS (1971) and asymmetry indices of ROMERO-ZARCO (1986) are given in (Tab. 1) and the latter are represented graphically in (Fig. 3).

In terms of the Stebbins' system, the karyotype of populations mostly seizes 1A and 2A classes, which are considered majorly primitive classes in this system. 23 populations are classified as 1A group, 16 populations lodge in 2A class, 3 populations are stand as 1B group and 3 populations' namely *O. gypsicola* (1111), *O. hohenackeriana* (6013) from diploid and *O. altissima* (3501) from tetraploid populations are

TAB. 1 — Karyotype characteristics of 45 populations of *Onobrychis*. 2*n*- somatic chromosome number, *x* - Basic chromosome number, DRL - Difference of relative length, VRC - Value of relative chromatin, %T - Total form percentage, A_1 - intrachromosome asymmetry index, A_2 - interchromosome asymmetry index, SC - symmetry classes of Stebbins, K.F. - Karyotype formula, m - metacentric, sm - submetacentric, st - subtelocentric.

Taxon	Section	Gene bank code(RIFR)	Origin sites	Endemic	2n	x	DRL	VRC	%TF	A1	A2	SC	K.F.
O. cornuta	*Dendrobrychis*	2270	Zanjan		16	8	5,81	2,98	36,64	0,422	0,153	2A	6m+10sm
O. aucheri	*Heliobrychis*	2900	Azarbayjan-e Sharqi-jolfa	*	16	8	5,27	2,81	45,18	0,183	0,143	1A	16m
O. buhseana	*Heliobrychis*	5790	Azarbayjan-e Sharqi-Tabriz	*	16	8	8,09	2,5	41,57	0,277	0,226	1B	14m+2sm
O. gaubae	*Heliobrychis*	3422	Chahar Mahal va Bakhtiari	*	16	8	6,36	3,29	37,96	0,383	0,166	1A	6m+10sm
O. gaubae	*Heliobrychis*	4181	Chahar Mahal va Bakhtiari	*	16	8	7,18	3,34	36,82	0,404	0,204	2A	6m+10sm
O. gypsicola	*Heliobrychis*	1111	Bushehr-kangan	*	16	8	8,5	2,86	36,85	0,42	0,22	2B	6m+10sm
O. gypsicola	*Heliobrychis*	2569	Khuzestan-Haftgol	*	16	8	6,88	2,75	41,42	0,293	0,209	1A	12m+4sm
O. melanotricha	*Heliobrychis*	2863	Arak-khosbijan	*	16	8	7,14	2,94	39,78	0,314	0,178	2A	12m+4sm
O. plantago	*Heliobrychis*	5787	Yazd	*	16	8	6,83	3,26	40,18	0,321	0,186	1A	12m+4sm
O. tehranica	*Heliobrychis*	72	Tehran		16	8	9,53	2,76	44,19	0,2	0,274	1B	14m+2sm
O. amoena	*Hymenobrychis*	5786	Khorasan-Torbat-e eydariyeh		14	7	8,01	4,06	42,32	0,274	0,171	1A	14m
O. hohenackeriana	*Hymenobrychis*	1646	Azarbayjan-e Sharqi-Khalkhal		16	8	10,23	2,81	43,94	0,204	0,271	1B	14m+2sm
O. hohenackeriana	*Hymenobrychis*	6013	Azarbayjan-e Sharqi-Tabriz		16	8	10,5	2,6	43,41	0,239	0,254	2B	14m+2sm
O. radiata	*Hymenobrychis*	2721	Golastan-Gorgan		14	7	5,36	3,61	39,88	0,333	0,125	1A	14m
O. sintenisii	*Hymenobrychis*	1183	Zanjan	*	14	7	8,65	3,4	40,18	0,33	0,209	1A	14m
O. sintenisii	*Hymenobrychis*	4384	E fahan-faridan	*	14	7	7,44	3,4	39,22	0,349	0,199	2A	12m+2sm
O. crista- galli	*Lophobrychis*	2520	Khuzestan-Masjed-e Soleyman		16	8	4,34	2,33	39,63	0,337	0,127	1A	14m+2sm
O. crista- galli	*Lophobrychis*	2543	Khuzestan-Haftgol		16	8	4,18	2,6	35,95	0,424	0,104	2A	4m+12sm
O. crista- galli	*Lophobrychis*	2551	Khuzestan-Ramhormoz		16	8	4,93	2,68	38,63	0,362	0,135	2A	10m+6sm
O. crista- galli	*Lophobrychis*	3346	Ilam-Dehloran		16	8	6,84	2,6	43,01	0,241	0,166	1A	16m
O. altissima	*Onobrychis*	2260	Zanjan		28	7	3,97	3,01	38,98	0,349	0,16	2A	24m+4sm
O. altissima	*Onobrychis*	3501	Kerman		28	7	5,24	3,07	39,54	0,328	0,212	2B	20m+8sm
O. major	*Onobrychis*	242	Azarbayjan-e Sharqi-Marand		28	7	4,01	3,65	40,39	0,314	0,156	2A	24m+4sm
O. persica	*Onobrychis*	2759	Hamadan	*	28	7	4,84	3,39	39,09	0,333	0,197	2A	18m+10sm
O. persica	*Onobrychis*	6012	Azarbayjan-e Sharqi-Tabriz	*	28	7	2,81	4,04	38,71	0,354	0,201	2A	20m+8sm
O. sativa	*Onobrychis*	1586	Golastan-Gorgan		28	7	2,97	2,94	41,66	0,275	0,115	1A	24m+4sm
O. sativa	*Onobrychis*	1601	Golastan-Gorgan		28	7	3,39	3,17	40,31	0,307	0,141	2A	24m+4sm
O. sativa	*Onobrychis*	1763	Azarbayjan-e Sharqi-Urumieh		28	7	2,51	3,09	40,28	0,324	0,097	1A	22m+6sm
O. sativa	*Onobrychis*	182	Tehran-Karaj		28	7	4,28	2,98	40,8	0,298	0,165	1A	24m+4sm
O. sativa	*Onobrychis*	232	Qazvin		28	7	2,21	2,67	43,17	0,233	0,103	1A	28m
O. sativa	*Onobrychis*	281	Hamadan		28	7	3,54	3,85	39,1	0,348	0,174	2A	24m+4sm
O. sativa	*Onobrychis*	2985	Azarbayjan-e Sharqi-Tabriz		28	7	3,33	2,68	41,59	0,282	0,128	1A	28m
O. sativa	*Onobrychis*	3001	Tehran-Karaj		28	7	4,64	3,09	40,16	0,323	0,172	1A	26m+2sm
O. sativa	*Onobrychis*	3002	Tehran-Karaj		28	7	3,74	2,68	41,62	0,279	0,138	1A	24m+4sm

TAB. 1 Contd.

Taxon	Section	Gene bank code(RIFR)	Origin sites	Endemic	2n	x	DRL	VRC	%TF	A1	A2	SC	K.F.
O. sativa	*Onobrychis*	305	Tehran-Karaj		28	7	2,64	3,59	41,4	0,29	0,13	2A	26m+2sm
O. sativa	*Onobrychis*	325	Tehran-Karaj		28	7	2,36	3,22	40,12	0,32	0,105	2A	26m+2st
O. sativa	*Onobrychis*	3396	Chahar Mahal va Bakhtiari		28	7	3,03	2,71	42,91	0,242	0,117	1A	28m
O. sativa	*Onobrychis*	3981	Tehran-Karaj		28	7	2,78	3,09	42,29	0,264	0,113	1A	28m
O. tomentosa	*Onobrychis*	5	Boyer Ahmadi va Kohkiluyeh		16	8	3,31	2,56	38,83	0,362	0,083	1A	14m+2sm
O. transcaspica	Onobrychis	2475	Mazandaran		14	7	7,64	3,16	38,66	0,352	0,186	2A	12m+2sm
O. transcaspica	Onobrychis	5708	Golastan		14	7	7,81	2,85	42,49	0,258	0,189	1A	12m+2sm
O. viciaefolia	Onobrychis	3013	Tehran-Karaj		28	7	3,37	3,08	40,85	0,302	0,123	1A	28m
O. viciaefolia	Onobrychis	3026	Tehran-Karaj		28	7	3,58	3,85	37,18	0,396	0,159	2A	16m+10sm+2st
O. viciaefolia	Onobrychis	6014	Azarbayjan-e Sharqi-Tabriz		28	7	4,23	2,48	41,41	0,277	0,185	1A	28m

classified as 2B category (Tab. 1).

Romero's intrachromosomal asymmetry index (A_1) expresses the arm ratio of each pair of homologous chromosomes. The interchromosomal asymmetry index (A_2) corresponds to Pearson's coefficient of dispersion and gives an idea of the asymmetry caused by the different length of the chromosomes.

By using the Romero-Zarco asymmetry indices of A_1 and A_2 we can determine the more asymmetric karyotype among the populations which have the similar Stebbins classes of symmetry. For example in the populations with 2A class, *O. crista-galli* (2543) possesses the highest A_1 value (0.424) and almost the lowest DRL value (4.18), therefore has a more asymmetric karyotype. Similarly in the populations with 2B symmetry class, *O. gypsicola*(1111) possessed the highest value for A_1(0.420) and the highest asymmetric karyotype. Also amongst populations with 1B symmetry class, *O. teheranica* (72) had the highest value for A_2 (0.274) and nearly the highest DRL value (9.53) (Tab. 1).

The populations which are classified as 1A group also showed the lowest value of A_2 in range of 0.083 - 0.209 and the highest value of % TF ranged from 37.96 to 45.18.

Figure (2) clearly shows the analyzing patterns of karyotype asymmetry with respect to Stebbins' classification. Regarding to (Fig. 2) some 1A and 1B karyotypes are in fact more asymmetrical than some 2A and 2B ones respectively.

The total karyotype length, recorded from at least five cells, that roughly indicates the chromatin content amongst the studied diploid taxa with $x = 7$ was in range of 19.95 µm in *O. transcaspica* (5708) ($2n = 14$; Tab. 1; Fig. 1) to 28.42 µm in *O. amoena* ($2n = 14$; Tab. 1; Fig. 1). Also, the total karyotype length among the diploid taxa tested with $x = 8$, had a range from 18.64 µm in *O. crista-galli* (2520) ($2n = 16$; Tab. 1; Fig. 1) to 26.72 µm in *O. gaubae* (4181) ($2n = 16$; Tab. 1; Fig. 1). Variation among tetraploid populations, based on mean length of the haploid chromosome complement, was ranging from 34.72 µm in *O. viciaefolia* (6014) ($2n = 28$; Tab. 1; Fig. 1) to 56.56 µm in *O. persica* (6012) species ($2n = 28$; Tab. 1; Fig. 1).

The highest VRC (value of relative chromatin) amongst all populations was obtained for *O. amoena* (5786) and the lowest was obtained for *O. crista-galli* (2520) (Tab. 1).

In general, based on intrachromosomal asymmetry (A_1 and %TF), *O. crista-galli* (2543) had the most asymmetrical and evolutionary karyo-

TAB. 2 — Mean of chromosomes analysis of *Onobrychis* populations. LA- long arm, SA-short arm, TL- total length, AR- arm ratio, CI- centromic index and Sat- presence (+) or absence (–) of satellites. * indicated Mean within each column followed by different lowercase letters are significantly different at the 5% level according to the Duncan's test.

Populations and gene bank code (see Tab. 1).	LA	SA	TL	AR	CI	Sat
O. altissima (2260)	1.80±0.01 defghijk*	1.15±0.02 efghijkl	3.01±0.00 fghijklmn	1.57±0.04 bcdefgh	0.38±0.01 efghijklm	+
O. altissima(3501)	1.77±0.03 defghijk	1.16±0.03 efghijkl	3.00±0.06 fghijklmn	1.53±0.04 cdefghij	0.39±0.01 defghijklm	+
O. amoena (5786)	2.26±0.13 ab	1.66±0.08 a	4.02±0.17 a	1.37±0.07 ghijklmn	0.41±0.01 abcdefg	+
O. aucheri (2900)	1.51±0.06 ijk	1.24±0.03 defghi	2.76±0.09 ijklmno	1.21±0.03 n	0.45±0.01 a	+
O. buhseana(5790)	1.41±0.02 k	1.00±0.03 ijkl	2.47±0.03 no	1.41±0.06 fghijklmn	0.41±0.01 bcdefghi	+
O. cornuta (2270)	1.89±0.01 bcdefghi	1.09±0.10 fghijkl	2.98±0.09 fghijklmn	1.76±0.18 ab	0.37±0.02 jklm	-
O. crista- galli (2551)	1.57±0.16 ghijk	0.99±0.05 jkl	2.68±0.19 klmno	1.59±0.13 abcdefg	0.37±0.02 hijklm	+
O. crista- galli (3346)	1.48±0.04 ijk	1.12±0.01 fghijkl	2.60±0.04 lmno	1.33±0.04 jklmn	0.43±0.01 ab	-
O. crista -galli(2520)	1.40±0.07 k	0.92±0.02 l	2.33±0.08 o	1.52±0.07 cdefghijk	0.40±0.01 bcdefghijkl	-
O. crista -galli(2543)	1.67±0.02 fghijk	0.94±0.02 kl	2.60±0.01 lmno	1.78±0.06 a	0.36±0.01 lm	-
O. gaubae (3422)	1.95±0.12 bcdefg	1.19±0.06 defghij	3.26±0.20 cdefghijk	1.63±0.04 abcdef	0.37±0.01 ijklm	+
O. gaubae (4181)	2.03±0.20 abcdef	1.18±0.12 defghij	3.32±0.33 cdefghij	1.72±0.03 abcd	0.36±0.00 m	+
O. gypsicola (2569)	1.52±0.03 ijk	1.07±0.06 ghijkl	2.64±0.09 klmno	1.42±0.08 fghijklmn	0.41±0.02 bcdefghi	+
O. gypsicola(1111)	1.75±0.12 efghijk	1.02±0.09 hijkl	2.86±0.21 hijklmno	1.73±0.06 abc	0.36±0.01 m	+
O. hohenackeriana(1646)	1.52±0.04 ijk	1.19±0.09 defghij	2.81±0.05 hijklmno	1.28±0.13 lmn	0.42±0.02 abcd	+
O. hohenackeriana (6013)	1.41±0.08 k	1.08±0.04 fghijkl	2.60±0.13 lmno	1.30±0.04 klmn	0.42±0.01 abcdef	+
O. major (242)	2.09±0.15 abcde	1.42±0.07 bcd	3.65±0.21 abcde	1.47±0.06 efghijklm	0.39±0.01 defghijklm	+
O. melanotricha (2863)	1.57±0.05 ghijk	1.04±0.05 hijkl	2.83±0.09 hijklmno	1.52±0.06 cdefghijk	0.37±0.02 ijklm	+
O. persica (2759)	2.07±0.00 abcdef	1.33±0.01 bcdef	3.39±0.01 bcdefghi	1.56±0.01 bcdefgh	0.39±0.00 cdefghijklm	-
O. persica (6012)	2.37±0.05 a	1.50±0.03 ab	3.99±0.11 ab	1.58±0.03 abcdefg	0.38±0.00 ghijklm	+
O. plantago (5787)	1.95±0.16 bcdefg	1.31±0.06 bcdefg	3.26±0.21 cdefghijk	1.48±0.06 efghijklm	0.40±0.01 bcdefghij	-
O. radiata (2721)	2.17±0.09 abcd	1.44±0.11 bc	3.61±0.17 abcdef	1.52±0.11 cdefghijk	0.40±0.02 bcdefghijk	-
O. sativa (1586)	1.68±0.15 fghijk	1.20±0.17 defghij	2.94±0.31 ghijklmno	1.41±0.07 fghijklmn	0.41±0.01 bcdefghi	+
O. sativa (1601)	1.86±0.07 cdefghij	1.26±0.07 defgh	3.17±0.14 efghijklm	1.48±0.04 efghijklm	0.40±0.01 bcdefghijkl	+
O. sativa (1763)	1.76±0.03 efghijk	1.19±0.02 defghij	3.09±0.04 efghijklmn	1.48±0.05 efghijklm	0.38±0.01 defghijklm	+
O. sativa (182)	1.73±0.00 efghijk	1.20±0.05 defghij	2.98±0.09 fghijklmn	1.45±0.06 fghijklm	0.40±0.00 bcdefghij	+
O. sativa (281)	2.26±0.23 abc	1.45±0.12 bc	3.81±0.37 abcd	1.55±0.03 bcdefghi	0.38±0.01 efghijklm	+
O. sativa (2979)	1.94±0.03 bcdefg	1.41±0.01 bcd	3.43±0.04 abcdefgh	1.38±0.01ghijklmn	0.41±0.00 abcdefg	+
O. sativa (2985)	1.54±0.01 hijk	1.09±0.02 fghijkl	2.65±0.03 klmno	1.41±0.03 fghijklmn	0.41±0.00 abcdefg	+
O. sativa (3001)	1.81±0.12 defghijk	1.21±0.10 cdefghij	3.04±0.21 efghijklmn	1.50±0.06 defghijkl	0.40±0.01 bcdefghijk	+
O. sativa (3002)	1.53±0.14 hijk	1.09±0.10 fghijkl	2.64±0.24 klmno	1.40±0.00 ghijklmn	0.41±0.00 abcdefg	+
O. sativa (3396)	1.55±0.08 ghijk	1.16±0.03 efghijk	2.71±0.10 jklmno	1.33±0.06 ijklmn	0.43±0.01 ab	-
O. sativa (3981)	1.75±0.12 efghijk	1.29±0.07 bcdefg	3.07±0.19 efghijklmn	1.36±0.03 ghijklmn	0.42±0.00 abcde	+

TAB. 2 — Contd.

Populations and gene bank code (see Tab. 1).	LA	SA	TL	AR	CI	Sat
O. sativa(232)	1.50±0.04 ijk	1.14±0.02 fghijkl	2.65±0.06 klmno	1.32±0.02 jklmn	0.43±0.00 ab	-
O. sativa(305)	2.05±0.05 abcdef	1.42±0.05 bcd	3.54±0.10 abcdefg	1.44±0.03 fghijklm	0.40±0.00 bcdefghij	+
O. sativa(325)	1.89±0.06 bcdefghi	1.26±0.02 bcdefgh	3.22±0.08 defghijkl	1.49±0.03 efghijkl	0.39±0.00 bcdefghijklm	-
O. sintenisii (4384)	1.99±0.07 abcdef	1.29±0.04 bcdefg	3.40±0.10 bcdefghi	1.55±0.06 bcdefghi	0.38±0.01 fghijklm	+
O. sintensii(1183)	1.95±0.23 bcdefg	1.31±0.05 bcdefg	3.40±0.29 bcdefghi	1.48±0.12 efghijklm	0.39±0.02 defghijklm	+
O.teheranica(72)	1.48±0.09 ijk	1.17±0.06 defghijk	2.74±0.12 jklmno	1.26±0.02 mn	0.43±0.00 abc	+
O. tomentosa (5)	1.57±0.06 ghijk	0.99±0.05 ijkl	2.56±0.11 mno	1.58±0.05 abcdefgh	0.39±0.01 defghijklm	-
O. transcaspica (5708)	1.57±0.05 ghijk	1.16±0.03 efghijkl	2.81±0.08 hijklmno	1.36±0.05 hijklmn	0.41±0.01 abcdefg	+
O. transcaspica(2475)	1.94±0.09 bcdefgh	1.22±0.07 cdefghij	3.16±0.16 efghijklm	1.59±0.01 abcdefg	0.39±0.00 defghijklm	-
O. viciaefolia (3013)	1.82±0.22 defghij	1.26±0.11 cdefgh	3.08±0.33 efghijklmn	1.44±0.07 fghijklm	0.41±0.01 bcdefgh	-
O. viciaefolia (3026)	2.35±0.03 a	1.39±0.03 bcde	3.85±0.08 abc	1.69±0.01 abcde	0.36±0.00 klm	+
O. viciaefolia (6014)	1.46±0.01 jk	1.03±0.02 hijkl	2.48±0.01 no	1.42±0.03 fghijklmn	0.41±0.01 abcdefg	-

type and *O. aucheri* (2900) had the most symmetrical karyotype in all of the populations. According to interchromosomal asymmetry (A_2 and DRL), *O. hohenackeriana* (6013) had the most asymmetrical karyotype in all of the populations (Tab. 1). Asymmetry index %TF ranged from 35.95 to 45.18 and the intrachromosomal asymmetry index (A_1) varied from 0.183 to 0.424, while the interchromosomal asymmetry index (A_2) ranged from 0.083 to 0.271.

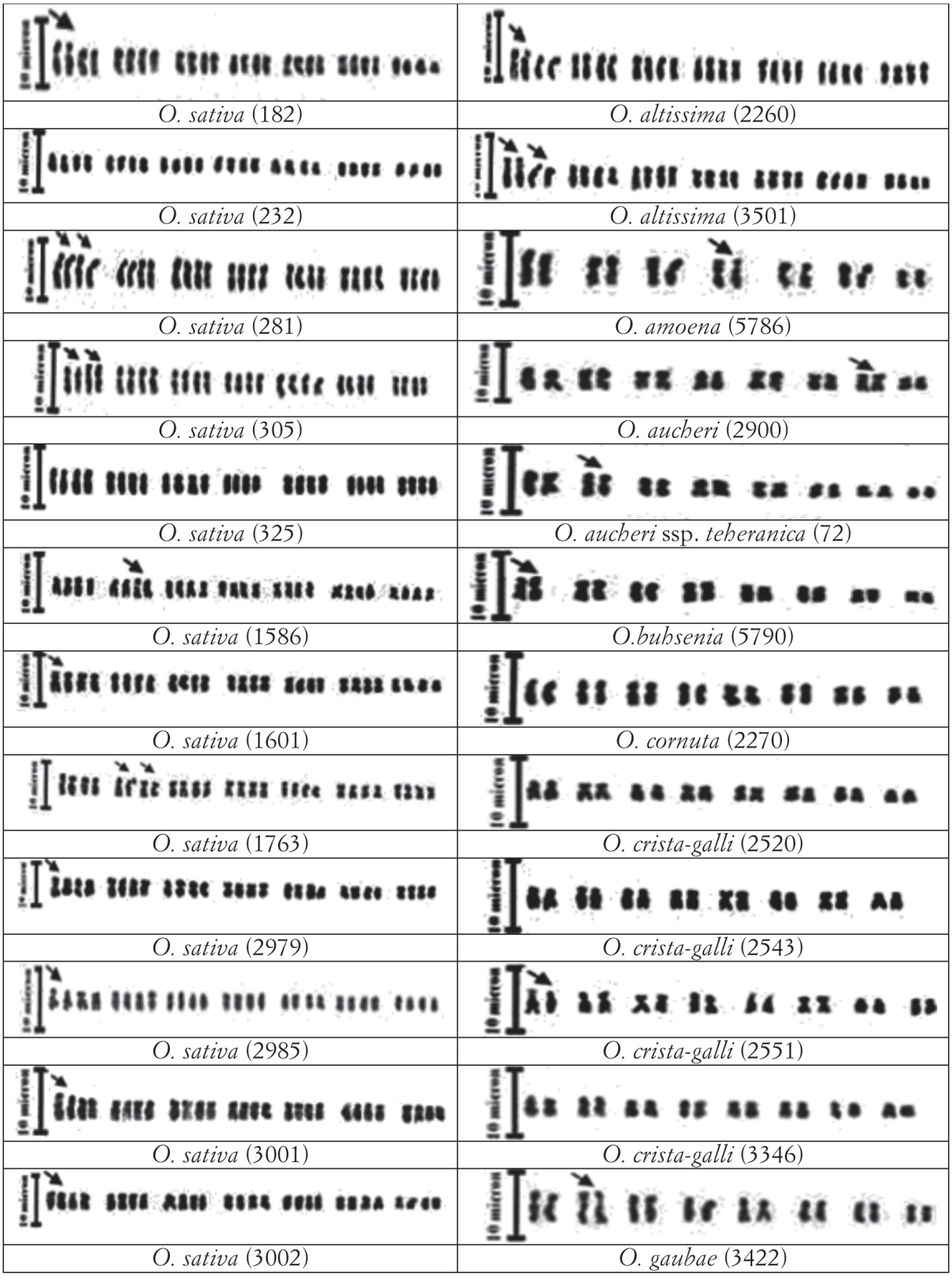
Most of the populations had one or two pairs of visible small satellites which were connected to the short or long arms of chromosomes (Fig. 1; Tab. 2).

A statistical comparison based on unbalanced completely randomized design demonstrates that there are significant differences among the populations for all the measured traits (P<%1) (Tab. 3). The principal component analysis (PCA), of the karyotypic parameter shows that the first two principal components account for % 98.70 of total variance. component one (% 62.18) put emphasized on the chromosome total length and long arm length which had the highest coefficients of eigen vectors, while component two (% 36.52) accentuates short arm length, arm ratio and centromer index (Tab. 4).

Grouping of the populations are studied based on their relative karyotypic as well as mitotic characteristics (Tab. 2, Fig. 5). By cutting dendrogram resulted from cluster analysis UPGMA methods with Cophenetic correlation coefficient ($r = 0.78$) in metric distance 2.04, the populations classified under seven groups which certainly the first and the second components had the most significant role in separated classes. The highest metric distance was obtained between *O. amoena* (5786) and *O. melanotricha* (2863) and the lowest metric distance was obtained between two populations of *O. sativa* (2985) and *O. sativa* (3002) (Fig. 5).

The diagram of the populations' dispersion, based on two first components showed the populations separated in seven groups, which completely fits with the results obtained through the UPGMA grouping analysis (Fig. 4).

By cutting dendrogram produced from cluster analysis UPGMA with the $r = 0.76$ in metric distance 1.22 based on two indices (A_1 and A_2) the populations were classified under eight groups (Fig. 6). The highest metric distance (3.21) was obtained between *O. amoena* (5786) and *O. teheranica* (72). The lowest metric distance (0.07) was obtained between *O. sintenisii* (1183) and *O. altissima* (3501).



O. sativa (182)	*O. altissima* (2260)
O. sativa (232)	*O. altissima* (3501)
O. sativa (281)	*O. amoena* (5786)
O. sativa (305)	*O. aucheri* (2900)
O. sativa (325)	*O. aucheri* ssp. *teheranica* (72)
O. sativa (1586)	*O.buhsenia* (5790)
O. sativa (1601)	*O. cornuta* (2270)
O. sativa (1763)	*O. crista-galli* (2520)
O. sativa (2979)	*O. crista-galli* (2543)
O. sativa (2985)	*O. crista-galli* (2551)
O. sativa (3001)	*O. crista-galli* (3346)
O. sativa (3002)	*O. gaubae* (3422)

Fig. 1 — Karyotypes of 45 diploid and tetraploid *Onobrychis* populations. Gene bank code in parenthesis (see Tab. 1). Arrows indicate the chromosome pair(s) with secondary constriction. Bar = 10µm.

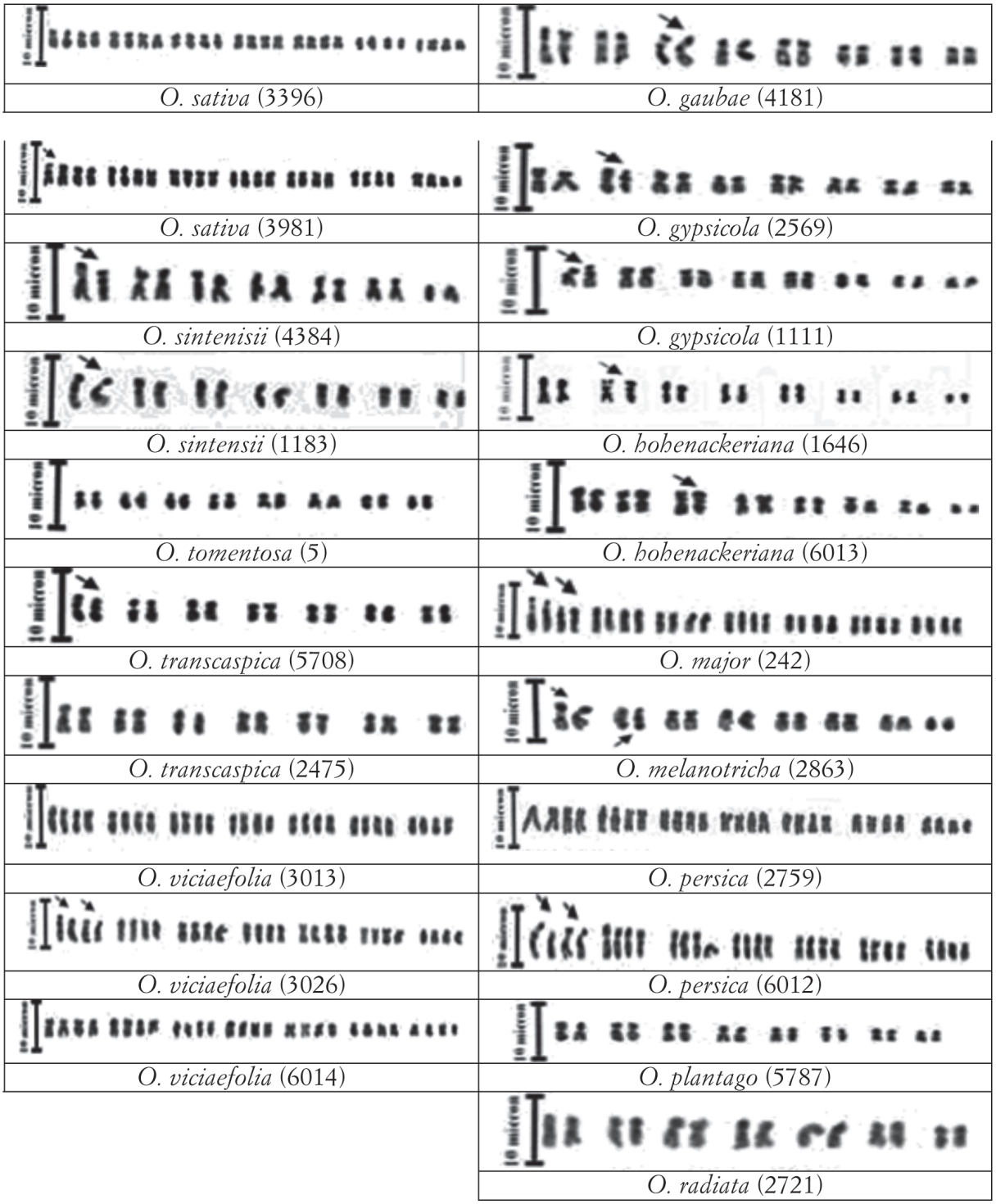


Fig. 1 — Continued.

DISCUSSION

The results of this study reveal a detailed picture of the chromosome features in *Onobrychis* species. While the DNA sequence can provide valuable data, the knowledge of chromosome numbers, karyotype evoulution, ploidy level and genome size can provide additional information

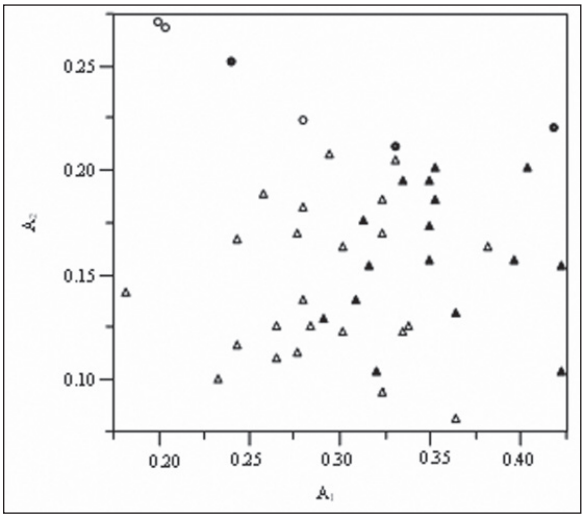


Fig. 2 — Graphic representation of the asymmetry indices of Romero-Zarco with symbols to indicate Stebbins' symmetry types: 1A (△); 2A (▲); 1B (○); 2B (●). Note: some "1A" karyotypes are in fact more asymmetrical than some "2A".

that not only gives further insight in to the functioning of the genome, but also have considerable predictive powers.

Numerous reports, including those of (ROMANO *et al.* 1987), (SEMERENKO & SHVETS 1989), BALTISBERGER (1991), MAGULAEV (1995), (SLAVIK *et al.* 1993), MOHAMED (1997) and (OBERPRIELER & VOGT 1996) have shown that the most frequent basic chromosome numbers for *Onobrychis* genus are $x=7$ and $x=8$ and ploidy levels are varied.

In this study, the basic chromosome numbers were $x=7$ and $x=8$ for diploid populations and only $x=7$ was for tetraploid populations. The chromosome number of *O. sativa, O. viciaefolia* and *O. crista-galli* are supported by previous studies (DIOSDADO *et al.* 1993; MOHAMED 1997; VOGT and APARICIO 1999), while the others specially 12 endemic Iranian populations which are reported for the first time (Tab. 1).

Results obtained from this research allow us to compare for the first time the karyotypes of

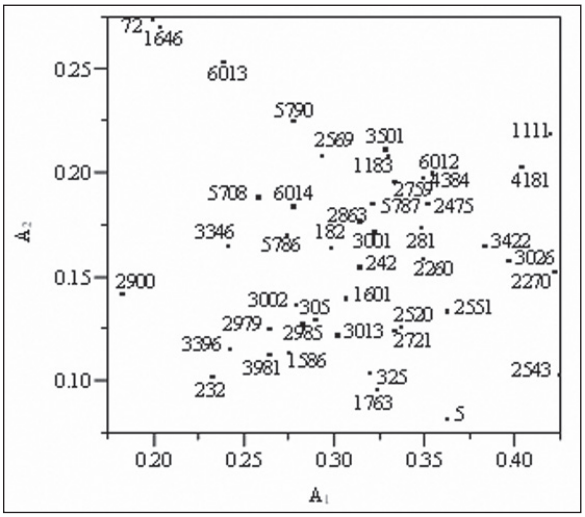


Fig. 3 — Scatter diagram of the Romero-Zarco asymmetry indices. Value of A_1 and A_2 are summarized in Tab. 1.

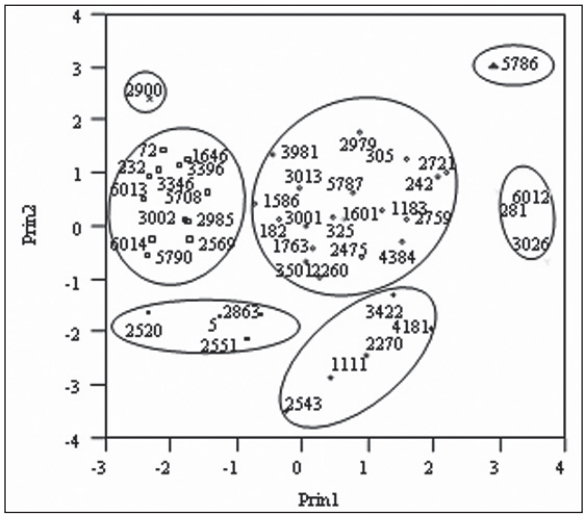


Fig. 4 — Scatter plot of 45 populations for the first two principal components.

TAB. 3 — The results of analysis of variance for karyotypic data based on unbalanced CRD design.

Source of variation	Degrees of freedom	Mean of squares				
		TL	LA	SA	AR	CI
Genotype	44	0.59**	0.23**	0.08**	0.06**	0.01**
Error	184	0.10	0.04	0.01	0.01	0.01
CV%		10.33	11.33	10.03	7.46	4.82

several diploid and tetraploid species of *Onobrychis* genus. Analysis of karyotype formulae showed that, generally in all diploid and tetraploid species, the number of "m" chromosomes is more than "sm" chromosomes except for *O. cornuta*, *O. crista-galli* (2543), *O. gaubae* and *O. gypsicola* (1111) species. Presence of two "st" chromosomes observed just in *O. sativa* (325) and *O. viciaefolia* (6014) tetraploid species.

At the interspecific level, quantitative and qualitative data allowed us the differentiation of several of the taxa studied. Among species of all sections, the most variable characters were the number of "m" and "sm" chromosomes, as well as the number and position of satellites. In 31 populations some chromosome pairs carried secondary construction on their short or long arms (Fig. 1; Tab. 1). As a result, the species also could be differentiated by the number, type and position of satellites.

Difference in the karyotypic formula of the same species especially in *O. sativa* may indicate the occurrence of chromosomes structural changes like translocations as evidenced by quadrivalent formation in metaphase of meiosis-I.

Grouping of the populations based on taxonomic sections, showed that the recorded variation in basic chromosome number is sighted only in section Hymenobrychis (Tab. 1). Therefore, we can suggest that section Hymenobrychis has a comparatively highly derived organization and can be considered as a heterogenous unit in the *Onobrychis* genus.

(EMRE *et al.* 2007) indicated that the eight species of *Onobrychis* genus belongs to sections Lophobrychis, Onobrychis and Hymenobrychis cluster together on the basis of seed protein similarities as designed by previous morphological classification. The formed dendrogram from SDS-PAGE analysis showed that all species constituted two clasters with 36% similarity. The sections Onobrychis and Lophobrychis occure in the same groups. Two species from section Lophobrychis, it can be concluded that the recorded variation in chromosome numbers in each of them can be referred to the differences in their taxonomic delimitation, had similar total band profiles. (ARSALAN & ERTUGRUL 2010) also investigated seven species of *Onobrychis*, collected from Turkey. The variability of seed storage proteins was analyzed by SDS-PAGE. The results showed that the sections Onobrychis and Lophobrychis occure in 2 different groups with the similarity rate of 77%. Therefore seed proteins electrophoresis is insufficient for investigation of phylogenetic relationships between species.

According to AHANGARIAN *et al.* (2007), within *Onobrychis* clade, the *Onobrychis* subgenus *Sisyrosema* forms a monophyletic group, while the *Onobrychis* subgenus *Onobrychis* is not monophyletic. In contrary to section *Heliobrychis*, sections *Dendrobrychis* and *Onobrychis* appear not to be monophyletic. The results from our present study are agreed with this grouping.

Karyotype asymmetry, applied in the comparative analysis of diploid and tetraploid *Onobrychis*, was used for species discrimination. The ratio of long arm /short arm chromosomes (AR) showed a high significant difference among some species belongs to same or different sections, while other species are not clearly distinct (Tab. 2). Diploid species of *O. crista-galli* (2543) for instance, had the largest AR value (1.784), the lowest % CI (35.9) or % TF value(35.95) and the highest A_1 value (0.424), exhibiting the most asymmetrically and intrachromosomally derived karyotypes, while *O. aucheri* (2900) with the lowest AR value (1.213), the highest % CI (45.0) or % TF value (45.18) and the lowest A_1 value (0.183) was introduced as the most symmetrical karyotypes (Tab. 1 and Tab. 2).

The diagram based on two parameters of A_1 and A_2, shows the state of symmetry and evolu-

TAB. 4 — Eigenvectors from the first two Principal components for 5 karyotype parameters to classify 45 populations of *Onobrychis*.

Parameters	Prin1	Prin2
TL	0.54	0.24
LA	0.56	0.10
SA	0.42	0.49
AR	0.32	-0.60
CI	-0.34	0.58
Eigenvalue	3.11	1.83
Percentage of variance	62.18	36.52
Cum.Percentage of variance	62.18	98.70

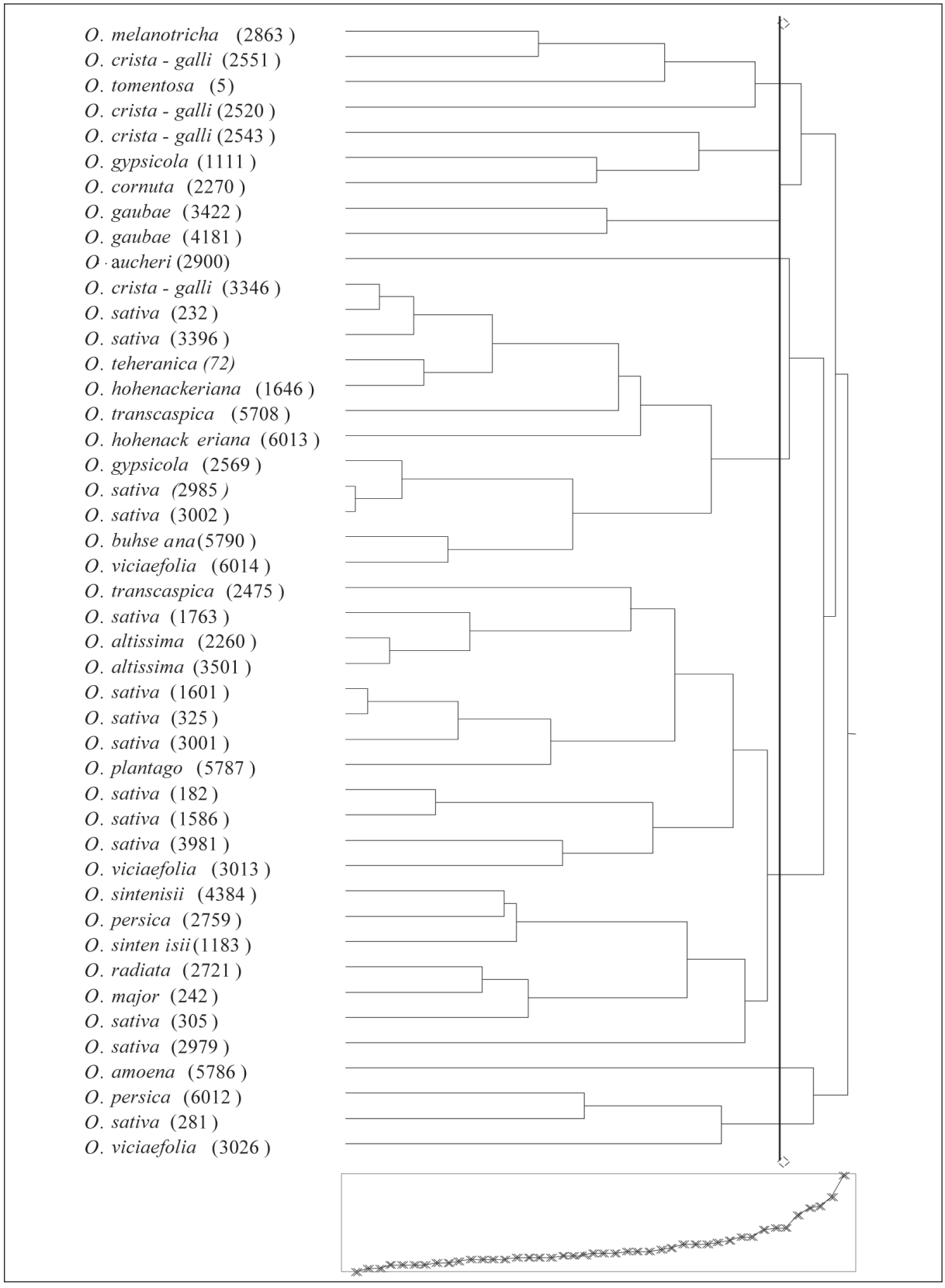


Fig. 5 — Dendrogram of 45 populations of *Onobrychis* by analyzing five karyotypic parameters using UPGMA cluster analysis method. Cophenetic correlation $r = 0.78$.

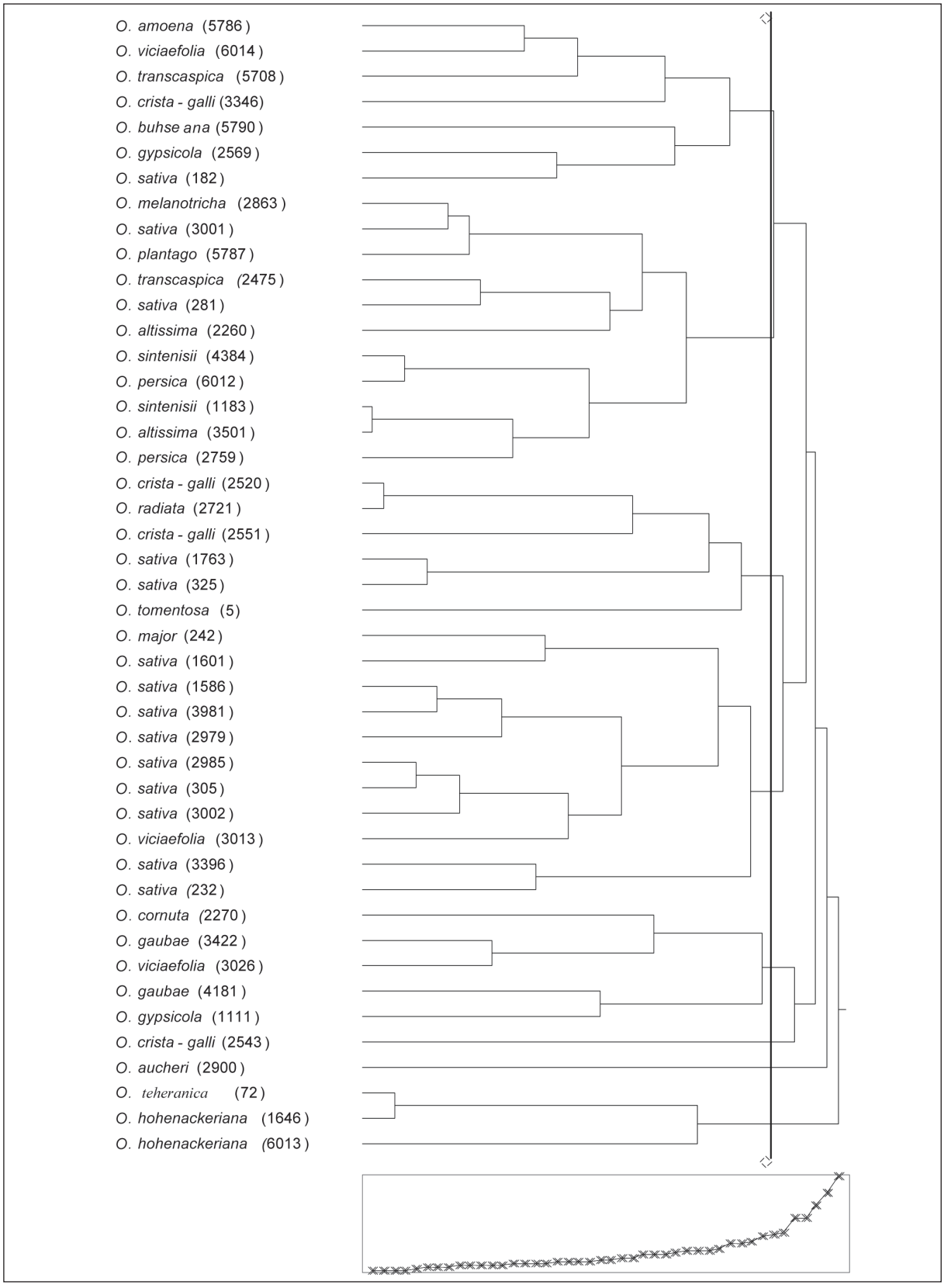


Fig. 6 — Dendrogram of cluster analysis (UPGMA) based on two parameters A_1 and A_2 on 45 populations. Cophenetic correlation $r = 0.76$.

tion in the karyotypes of different populations (Fig. 3). Regarding to Figure 3, *O. gypsicola* (1111), *O. hohenackeriana* (6013) from diploid species and *O. altissima* (3501) from tetraploid species had the most derived karyotypes. The variance of different populations according to A_1 and A_2 values in addition to various symmetrical states by Stebbins is presented in Figure 2. With regard to Figure 2, some "1A" karyotypes are in fact more asymmetrical than some "2A" ones. Therefore the pattern of variation of A_1 and A_2 values is not completely similar to the pattern of Stebbins' system in this study.

In view of the fact that, fewer DRL value illustrated more symmetry of karyotype, *O. hohenackeriana* and *O.sativa* (232) respectively with DRL 10.5 and 2.21 values had the most symmetric and asymmetric karyotypes. Similarly, high DRL value leads to more changes in the construction of chromosomes.

Different populations of several *Onobrychis* species show numerical chromosome polymorphism. For example (DARLINGTON & WYLIE 1955) and (GOLDBLATT & JOHNSON 1993) respectively reported a diploid ($2n = 14$) and a diploid ($2n = 16$) chromosome number for *O. crista-galli* species, while (GOLDBLATT & JOHNSON 1998) reported a tetraploid ($2n = 32$) for *O. crista-galli* species. However the present study reports the existence of $2n = 2x = 16$ for different populations of *O. crista-galli* in Iran.

The Duncan's test applied to the chromosome morphometric traits (LA, SA, TL, AR and CI) showed a highly significant difference among all examined populations belongs to different sections (Tab. 2).

ANOVA test showed the presence of significant difference ($P<\%1$) in the size of chromosomes as well as the ratio of long arms to short arms among diploid and tetraploid populations. So these results indicate a significant quantitative change in amount of chromatin in *Onobrychis* species diversification (Tab. 3).

Considering the changes of interchromosomal asymmetry index (A_2) among diploid and tetraploid species, the lowest value exists in the diploid species with $x = 8$ (*O. tomentosa*) and the highest value also exists in the diploid species with $x = 8$ (*O. teheranica*) (Tab. 1).

Generally it seems the variation in the size of chromosomes depends on the basic chromosomal number in species.

Cluster analysis based on cytological data showed the populations with the lowest metric distance may lead us to use populations in crosses for inducing the highest genetic variations (Fig. 5). However, grouping of the *Onobrychis* populations based on karyotypic data and (A_1 and A_2) indices, partly agrees with either the taxonomic treatment of the genus *Onobrychis* (Rechinger 1984) or phylogenetic analysis of the same species based on morphological characters.

Grouping based on karyotypic data indicated *O. melanotricha* (2863) stands far from *O. plantago* (5787) and *O. aucheri* (2900) and grouping based on A_1 and A_2 indices showed *O. melanotricha* (2863) stands just far from *O. aucheri* (2900). This may be due to either some missing available data in cytological analysis or different evolutionary history of cytological features and morphological characters in the species. However, the results based on A_1 and A_2 indices, for grouping of populations based on taxonomic sections was better than clustering by karyotipic data. The resulting arrangement from these tests can be interesting and noticeable depending on the researchers' aims.

Different populations of *O. sativa* are classified as different group majorly because of their different chromosome length than their arm ratio. For example *O. sativa* (281) that is significantly different from the other populations of this species through the difference of traits such as LA, SA, and TL, is separately classified as another group. In fact an explanation is that this genus is an open pollinated plant and presumably is strongly affected by environmental factors and variations of growing sites and specially the *Onobrychis* subgenus *Onobrychis* is not monophyletic.

The present study shows the change in the chromosomal traits as one of the mechanism of inter and intraspecies diversification in the *Onobrychis* genus as well as the earlier cytological reports. The differences in karyotype formulae and asymmetric indices found among the species suggest that structural changes of chromosomes may contribute to the diversification of the genus. These genomic differences could be used for breeding purposes.

In general, cytological studies of the *Onobrychis* species growing in Iran indicate the importance of polyploidy, chromosome structural changes, presumably quantitative changes in the amount of DNA and probably the role of growing sites in species diversification and suggest that such data may be used in the taxonomy and phylogenetic consideration of the genus.

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