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Chromosome Number and Karyotype Analysis of some Taxa of *Vicia* Genus (Fabaceae): Revision and Description

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Abstract

Karyotypes of 15 accessions belonging to five species of *Vicia* were determined. Chromosomes number varied between species and subspecies. *V. cordata* had chromosome count of 2n=10, *V. angustifolia* had 2n=12, *V. narbonensis*, *V. monantha ssp. calcarata* and *ssp. cinerea* presented 2n=14. Both *V. sativa ssp. amphicarpa* accessions with aerial and underground pods showed 2n=14 and were first reported. Chromosome numbers of *V. sativa ssp. sativa* were verified and revised as 2n=10, 12. Therefore, our karyological data provided information about phylogenetic position of the analysed species. *Vicia narbonensis* had the most symmetrical karyotype and could be considered as the primitive among the studied species. *Vicia monantha* had metacentric chromosomes with high differences in relative size between the chromosomes of the complement showing an asymmetrical karyotype. *V. sativa ssp. sativa* was the only subspecies having a metacentric chromosome marker in its complement within the species. Both *V. cordata* and *V. angustifolia* had all subtelocentric chromosomes showing an evolutionary tendency. Principal component analysis and Cluster analysis revealed that the studied species were grouped regarding to their evolutionary tendency. Our results support that the karyotypes analysis showed distinction between the taxa.

Keywords: Karyotype; Asymmetry index; Chromosome evolution; *Vicia ssp*

Introduction

The genus *Vicia* (Leguminosae, *Vicieae*) has long been a subject of active research because it contains several species of economic importance and many of which have been domesticated since the origin of agriculture (Gil and Cubero, 1993). It comprises about 166 species, chiefly located in Europe, Asia and North America, extending to the temperate regions of South America and Tropical Africa (Bisht *et al*., 1998) out of which 40 species are of considerable economic importance (Hanelt and Mettin, 1989). The seed is traditionally used as an additive to voluminous feeds for ruminants (Enneking, 1995), while the forage is often grown for hay in mixtures with cereal tutor crops, such as barley and oats (Van de Wouw *et al*., 2001). Though, *Vicia sativa ssp.amphicarpa* which produces two pod types: aerial and underground has an ability to survive in marginal areas with low rainfall (about 250 mm year^{-1}) and to produce nutritious herbage and pods which help address rehabilitation of degraded rangelands and increase feed production for small ruminants (Abd El Moneim and Elias, 2003).

The basic chromosome numbers in the genus are n = 5, 6 and (Maxted *et al*., 1991) and the great majority of the species have complements with 2n = 10, 12 and 14 respectively (Hanelt and Mettin, 1989; Murti et al., 2012). However, plants with these three basic chromosome numbers can exist even within one species as is the case for *Vicia sativa* (Hollings and Stace, 1974). Nevertheless, some representatives of the section *Cracca* are polyploidy (Yamamoto, 1973). Cytological studies have tended to focus on the two most important economic species: *V. faba* L. and *V. sativ*a (Maxted *et al*., 1991; Maxted, 1993). The study of rare and new species of *Vicia* has encouraged the simultaneous examination of their cytology. To obtain a better point of view regarding this subject, we will consider the chromosomal information, which gives us the possibility of classifying the species (Ayaz and Ertekin, 2008). Karyotype characteristics played a vital role in improvement and comprehension of the phylogenetic relationships between the related species (Lavia *et al*., 2009; Murti *et al*., 2012). In this study, the chromosomes reveal the full range of cytogenetical possibilities for understanding the delimitation, affinities and evolution of taxa (Moore, 1978). Karyotype asymmetry is a good expression of the general morphology of plant karyotypes (Zarco, 1986; Zuo and Yuan, 2011). Changes in morphology of the chromosomes

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have been frequently related to evolution in higher plants. A symmetrical karyotype is characterized by the predominance of m and sm chromosomes of approximately the same size (Zuo and Yuan, 2011). In Tunisia, research works on taxonomy of *Vicia* species and subspecies are still rare and not developed enough. However the karyotypes of 15 species and subspecies of *Vicia* (most of them are Tunisian) are analysed in this paper.

The objectives are: (i) to chromosome counts of various sub species, (ii) to revise the taxonomy of some *Vicia* species and subspecies by the use of cytological analysis and (iii) to evaluate the karyotype evolution and phylogenetic relationships of the studied species and subspecies.

Materials and Methods

In this study, we examined 15 accessions from 5 species (*Vicia narbonensis*, *V. sativa*, *V. monantha, V. angustifolia* and *V. cordata*). *V. sativa* is represented by two subspecies, *V. sativa sativa* and *V. sativa amphicarpa*. *V. monantha* is represented by two subspecies i.e., *V. monantha calcarata* and *V. monantha cinerea*. The list of the studied material and its origin is given in Table 1. Somatic chromosomes were studied in root meristems of germinating seeds, which were pre-treated with 0.1% colchicines at room temperature for 2 h, then fixed in ethanol chloroform acetic acid (6:3:1) during 24 h at 4°C and stored in 70% ethanol. Root tips were hydrolysed with 1N HCL at 60°C during 15 mn and stained according to the Feulgen technique. After that, root tips were washed briefly with distilled water. Meristematic regions with 1 mm of length excised and squashed in a drop of 1% acetic orcein mixed with a drop of 45% acetic water (Jahier, 1992). The slides were examined under an optical Microscope type Hund (H 600) and photomicrographs were taken with the same microscope fitted with a BenQ camera using an oil immersion objective (100 x). At least 10 metaphases were drawn for each accession (including 3–10 individuals) selecting the five best for measurements. The well spread chromosomes were photographed and arm length were measured on prints enlarged to a total magnification of 2000 (100 × 20). Each chromosome was identified on the basis of its total chromosome length. The nomenclature used for the description of the chromosome morphology is that proposed by Levan *et al.* (1964), where the abbreviations m, sm, and st designate metacentric, submetacentric, and subtelocentric chromosomes, respectively. Idiograms were drawn based on mean centromeric index and arranged in order of decreasing size.

Data Analysis

For the numerical characterization of the karyotypes the following parameters were calculated according to Yamamoto (1973); Seijo and Fernandez (2003); Sevimay *et al.* (2005) and Gaffazardeh Namazi *et al.* (2008): (i) total chromosome length of the haploid complement (TCL); (ii) mean chromosome length (MCL); (iii) mean centromeric index (MCI); (iv) intrachromosomal asymmetry index (A1)=1-[$\sum(b/B)/n$] (Zarco, 1986); and (v) interchromosomal asymmetry index (A2)= s/x , where b and B are the mean length of short and long arms of each pair of homologues, respectively, n is the number of homologues, s is the standard deviation, and x the mean chromosome length (Zarco, 1986; Seijo and Fernandez, 2003; Zuo and Yuan, 2011). Karyotype asymmetry has been determined using the A1 and A2 indices (Zarco, 1986), the categories of Stebbins, (1971), the asymmetry index (AI) according to following equation: $AI=CV_{CL*}\ CV_{CI}/100$; where CV_{CL} is the relative variation in chromosome length and CV_{CI} is the relative variation in centromeric index (Paszko, 2006).

Means were compared by using one-way analysis of variance (ANOVA) after Bartlett's test homogeneity. *Post hoc* Duncan test was used following ANOVA and was performed to compare the chromosomes pair's in each accession. A significant difference was considered when $P \leq 0.05$.

Clustering of the karyotype was performed using the average linkage method to examine karyotype similarity among species and subspecies. A data matrix of 15 OTus (operational taxonomic unit) x 10 variables was constructed. The TCL, MCL, MCI, A1, and A2 indices, number of m, sm, and st chromosomes as well as the position of satellites were considered. Correlation coefficients (r) between A1 and A2 indices and between TCL and MCI were also measured. Additionally, a multivariate analysis (PCA) was performed based on data matrix of 15 OTUs times the 5 mentioned quantitative variables (Seijo and Fernandez, 2003).

Results

General Karyotype Characteristics

Of 15 accessions analysed in this paper, three karyotypes were found: 2n=10 is present in three accessions of *V. cordata* and one of *V. sativa ssp. sativa*, 2n = 12 is present in two accessions of *V. sativa ssp. sativa* and one of *V. angustifolia* and 2n = 14 is present in four accessions of *V. narbonensis* var *narbonensis*, in two accessions of *V. sativa ssp. amphicarap* and in two accessions of *V. monantha ssp. calcarata* and *ssp. cinerea*. For all analysed species and subspecies, satellites were observed in one chromosomes pair except for two species, *Vicia sativa ssp. sativa* (acc. C3) and *V sativa ssp. sativa* var. Mghila (acc. T2), which have two chromosomes pairs having satellites (Table 2).

The karyotype formula among the studied species and subspecies of *Vicia* is 10st for *V. cordata* (acc. Sr, acc. 304 and acc. C1), followed in frequency by 14m for both of *V. monanatha* subspecies (acc. 157 and acc. 140), 14sm for Libanon and Syrian *V. narbonensis* accessions (acc. 13 and acc. 12), 10sm + 4st for Tunisian *V. narbonensis* accessions

Table 1: Species, subspecies, code accessions and origin of material analysed cytologically

Species/sub species/code accession	Origin
V. narbonensis acc. 856	Tunisia (Bizerte)
V. narbonensis acc. *488*	Tunisia (INRAT))
V. narbonensis acc. 13	Lebanon (ICARDA)†
V. narbonensis acc. 12	Syria (ICARDA)†
V. sativa ssp. sativa acc. C3	Tunisia (Tunis)
V. sativa ssp. sativa acc. 12	Bangladesh (ICARDA)†
V. sativa ssp. sativa var. Mghila acc. T2*	Tunisia (Siliana)
V. sativa ssp. amphicarpa upground seeds acc. 139	Tunisia (Thala)
V.sativa ssp. amphicarpa underground seeds acc. 139	Tunisia (Thala)
V .angustifolia acc. C2	Tunisia (Tunis)
V. cordata acc. 304	Tunisia (Ouslatia/Kairouan)
V. cordata acc. Sr	Tunisia (Seriya/Sejnane)
V. cordata acc. C1	Tunisia (Tunis)
V. monantha ssp. calcarata acc. 157	Tunisia (Makthar)
V. monantha ssp. cinerea acc. 140	Tunisia (Thala)

*: registred variety in Tunisia; † : received from ICARDA/Syria

Table 2: Names of species, code accessions, karyotype formula (KF), chromosomes number (2N), satellite position (SAT), total length of the haploid complement (TCL), Mean chromosome length (MCL), range of chromosome length (range), mean centromeric index (MCI), A1 and A2: the intrachromosome and interchromosome asymmetry index respectively (Romero zarco, 1986); Stebbins' types: intervals of Stebbins (1971) for karyotype asymmetry of *some Vicia specie*s and subspecies

Species/subspecies/var	Code accession	KF	2N	SAT	TCL ± SE	MCL	Range	MCI ± SE	A1	A2	Stebbins' Types
1-V. narbonensis	*acc. 856*	10sm+4st	14	7l	42.77 ± 0.191	6.11^{b}	4,07 -8,27	33.64 ±0.72	0.49	0.18	2A
2-V. narbonensis	acc. *488*	10sm+4st	14	6l	45.57 ±0.14	6.51^{b}	5-8,33	33.39 ±0.67	0.49	0.13	2A
3-V. narbonensis	*acc. 13*	14sm	14	7l	47.39 ± 0. 24	6.77^{a}	4,25-10	55.59 ± 2.38	0.43	0.22	2A
4-V. narbonensis	*acc. 12*	14sm	14	6l	49.77 ± 0.22	7.11^{a}	4,75-12,6	56.22 ±1.64	0.44	0.19	1A
5-V. monantha ssp. cinerea	*acc. 140*	14m	14	2s	26.62 ± 0.23	3.80^{efg}	2-8	43,69 ± 0,77	0.21	0.35	1A
6-V. monantha ssp. calcarata	*acc. 157*	14m	14	2s	25.27 ± 0.19	3.61^{fg}	2,14 - 6,9	43.67 ± 0.81	0.18	0.34	1A
7-V. sativa ssp. sativa	*acc. C3*	2m+2sm +6st	10	3l+5l	22.9 ± 0.24	4.58^{d}	2,86-7,83	27.53 ±2.36	0.58	0.27	3A
8-V. sativa ssp. sativa	*acc. 12*	2m +10st	12	4l	21.9 ±0.19	3.65^{fg}	1,71-5	30.23 ±3.85	0.69	0.27	3B
9-*V sativa ssp. sativa*	var Mghila*	2m +10st	12	3l+4s	19.92 ± 0.17	3.32^{g}	1,71-6	22.54 ±1.47	0.69	0.28	3B
10-V. sativa ssp. amphicarpa (upgs.)	*acc. 139*	14sm	14	1l	29.33 ± 0.19	4.19^{de}	2,43-8	30.45 ± 1.23	0.58	0.27	3A
11-V. sativa ssp. amphicarpa (undgs.)	*acc. 139*	6sm + 8st	14	2l	23.73 ± 0.13	3.38^{g}	1,86-5,25	25.79 ±1.37	0.65	0.23	4A
12-V. cordata	*acc. C1*	10st	10	5l	25.3 ±0.18	5.06^{c}	3,83-6,75	16.31 ±0.73	0.8	0.18	4A
13-V. cordata	*acc. 304*	10st	10	5l	17.77 ± 0.09	3.55^{fg}	2,86 -4,3	19.67 ± 1.12	0.72	0.14	4A
14-V .angustifolia	*acc. C2*	12st	12	5l	23.64 ± 0.13	3.94^{f}	2,46 -5,38	21.21 ± 0.55	0.73	0.19	4A
15-V. cordata	*acc. Sr*	10st	10	5l	19.6 ± 0.12	3.92^{ef}	2,75 -5	20.25 ± 0.75	0.75	0.15	4A

*: registered variety in Tunisia; upgs. = upground seeds; undgs. = underground seeds; values of MCL covered by the same letter are not significantly different at 5% level; sm = submetacentric chromosome; m = metacentric chromosome; st = telocentric chromosome

(acc. 856 and acc. 488), 2m +10st for both *V. sativa ssp. sativa* (acc. 12 and var. Mghila), 14sm for upground *V. sativa amphicarpa seeds* (acc. 139 upgs.), 4st + 3sm for underground *V. sativa amphicarpa* seeds (acc. 139 undgs.), 2m+2sm+6st for *V. sativa ssp. sativa* (acc. C3) and finally 12st for *V. angustifolia* (acc. C2). Fig. 1 illustrates the mitotic metaphasis, Fig. 2 the respective karyotypes and Fig. 3 the respective idiograms. In general, karyotypes of the analysed species and subspecies have a predominance of subteloncentric chromosomes (st).

Vicia narbonensis

The total chromosome length was considerably long; it ranged from 42.77 to 49.8 µm. There were two types of karyotypes: one of ten submetacentric and four subtelocentric chromosomes (10sm+4st) for Tunisian accessions and one of 14 submetacentric chromosomes (14sm) for Libanon and Syrian accessions that have MCI around 50. All accessions posses' satellites (SAT chromosome), a secondary constriction, connected to the long arm near centromeric region (Table 2; Fig. 2, 3).

Vicia monantha

The somatic chromosome number was 14 and the chromosomes were median (14 m) for both subspecies, which have MCI about 43.7 (Table 2). All the chromosomes have approximately the same length except the first pair which has the longest chromosomes (Fig. 3).

Vicia sativa

***V. sativa ssp. sativa* (2n=10 and 2n= 12):** The somatic

chromosome number was 2n=2x=10 for acc. C3 and 2n=2x=12 for both acc. 12 and var. Mghila (Fig. 1). For accessions with 2n=2x=12 chromosomes, there is one marker metacentric chromosome pair and six subtelocentric chromosomes. For the acc. 12, the secondary constriction is connected to the long arm (chromosome 4), while for the var. Mghila, there are two secondary constrictions, one connected to the long arm (chromosome 3) and one connected to the short arm (chromosome 4). For the acc. C3 with 2n=2x=10, the karyotype is compound of one marker metacentric chromosome pair, one submedian chromosomes pair and six subtelocentric chromosomes, two pairs with secondary constriction connected to the long arm (chromosome III and chromosome V). ***V. sativa ssp. amphicarpa* (2n= 14):** The somatic chromosome number was 2n=2x=14 for both accessions (acc. 139 upgs. and acc. 139 undgs.). For the former, all the chromosomes are submedian, one (chromosome 1) with a secondary connected to the long arm.

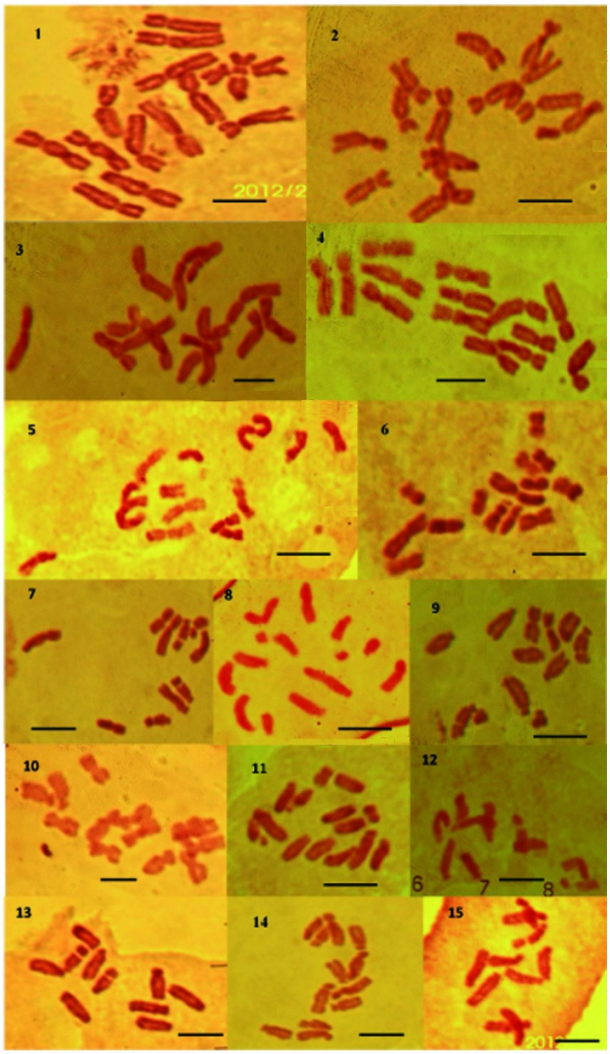

Fig. 1: Somatic chromosomes of *Vicia* species: **1**. *V. narbonensis var nar*bonensis (acc. 856); **2**. *V. narbonensis var nar*bonensis (acc. 488). **3**. *V. narbonensis var nar*bonensis (acc. 13). **4**. *V. narbonensis var nar*bonensis (acc. 12). **5**. *V. monantha cinerea* (acc. 140). **6**. *V. monantha calcarata* (acc. 157).**7**. *V. sativa ssp. sativa (C3)* **8**. *V sativa ssp. sativa (*acc. 12). **9**. *V. sativa ssp. sativa* (var. Mghila). **10**.*V. sativa ssp. amphicarpa* (acc. 139 upgs.). **11**. *V. sativa ssp. amphicarpa* (acc. 139 undgs.). **12**. *V. cordata* (acc. C1). **13**. *V. cordata* (acc. 304). **14**. *V. angustifolia* (acc. C2). **15**. *V. cordata* (acc. Sr). Scale bar= 5µm

V. cordata

The somatic chromosome number was 10 for all the accessions and only one type of karyotype formula was observed (10st). The total chromosome length was considered short; it ranged from 17.77 to 25.3 µm. However, the accession 304 has the shortest chromosome length (Table 2). The satellite (SAT chromosome) was connected to the long arm of the shortest chromosome of observed pairs.

V. angustifolia

The somatic chromosome number was 12 for the acc. C2.

Fig. 2: Karyotypes of *Vicia* species: **1**. *V. narbonensis var nar*bonensis (acc. 856); **2**. *V. narbonensis var nar*bonensis (acc. 488). **3**. *V. narbonensis var nar*bonensis (acc. 13). **4**. *V. narbonensis var nar*bonensis (acc. 12). **5**. *V. monantha cinerea* (acc. 140). **6**. *V. monantha calcarata* (acc. 157).**7**. *V. sativa ssp. sativa (C3)* **8**. *V sativa ssp. sativa (*acc. 12). **9**. *V. sativa ssp. sativa* (var. Mghila). **10**.*V. sativa ssp. amphicarpa* (acc. 139 upgs.). **11**. *V. sativa ssp. amphicarpa* (acc. 139 undgs.). **12**. *V. cordata* (acc. C1). **13**. *V. cordata* (acc. 304). **14**. *V. angustifolia* (acc. C2). **15**. *V. cordata* (acc. Sr). Scale bar= 5µm

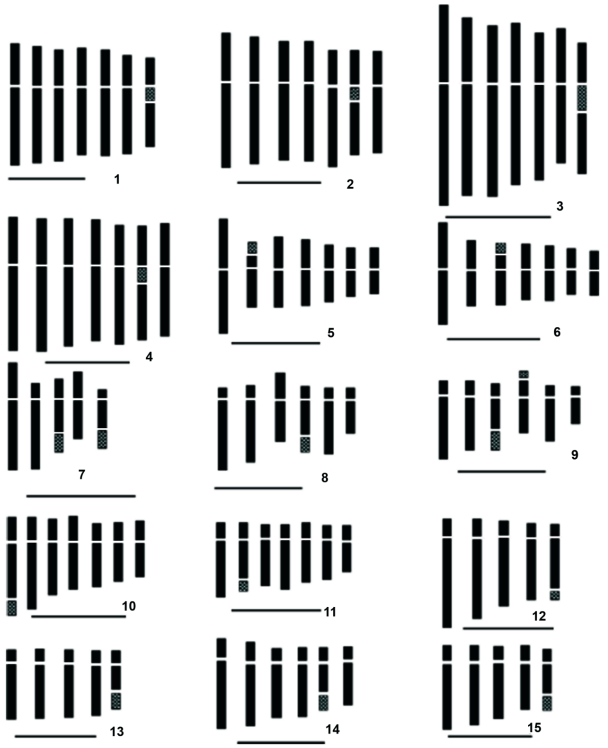

Fig. 3: Idiograms of *Vicia* species: **1**. *V. narbonensis var nar*bonensis (acc. 856); **2**. *V. narbonensis var nar*bonensis (acc. 488). **3**. *V. narbonensis var nar*bonensis (acc. 13). **4**. *V. narbonensis var nar*bonensis (acc. 12). **5**. *V. monantha cinerea* (acc. 140). **6**. *V. monantha calcarata* (acc. 157).**7**. *V. sativa ssp. sativa (C3)* **8**. *V sativa ssp. sativa (*acc. 12). **9**. *V. sativa ssp. sativa* (var. Mghila). **10**.*V. sativa ssp. amphicarpa* (acc. 139 upgs.). **11**. *V. sativa ssp. amphicarpa* (acc. 139 undgs.). **12**. *V. cordata* (acc. C1). **13**. *V. cordata* (acc. 304). **14**. *V. angustifolia* (acc. C2). **15**. *V. cordata* (acc. Sr). Scale bar= 5µm

The total chromosome length was relatively short (23.64 µm). All the chromosomes were subtelocentric (Fig. 2 and Fig. 3) with a satellite connected to the long arm of the fifth chromosome pair.

Karyotype asymmetry

All the indices proposed to evaluate intra-chromosomal asymmetry of the species and subspecies of *Vicia* karyotypes (Table 3). Among these methods, one qualitative classification and five different quantitative indices can be market out. According to Stebbins category, species having 2A and 3A such as *Vicia narbonensis* acc. 856, acc 488 and acc. 13 as well as *V. sativa ssp sativa* acc. C3 and *V. sativa ssp. amphicarpa* acc. 139 upgds., have karyotypes moderately symmetrical. While *V. narbonensis* acc. 12 falls in Stebbins 1A category and has a moderately symmetrical karyotype. The most symmetrical karyotype was observed in both *V. monantha ssp calcarata* and *ssp cinerea* which fall in Stebbins 1A category while the most asymmetrical

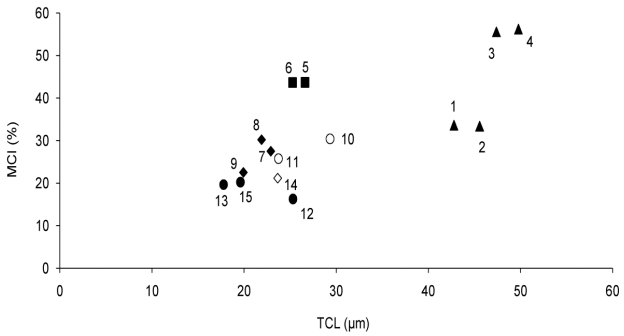

Fig. 4: Relationships between the total lengths of the haploid complement (TCL) and the mean centromeric index (MCI). Values of TCL and MCI are summarised in Table 2. **1**.*V. narbonensis var nar*bonensis (acc.856); **2**. *V. narbonensis var nar*bonensis (acc. 488). **3**. *V. narbonensis var nar*bonensis (acc. 13). **4**. *V. narbonensis var nar*bonensis (acc. 12). **5**. *V. monantha cinerea* (acc 140). **6**. *V. monantha calcarata* (acc. 157).**7**. *V. sativa ssp. sativa (C3)* **8**. *V sativa ssp. sativa (*acc. 12). **9**. *V. sativa ssp. sativa* (var. Mghila). **10**.*V. sativa ssp. amphicarpa* (acc. 139 upgs.). **11**. *V. sativa ssp. amphicarpa* (acc. 139 undgs.). **12**. *V. cordata* (acc. C1). **13**. *V. cordata* (acc. 304). **14**. *V. angustifolia* (acc. C2). **15**. *V. cordata* (acc. Sr)

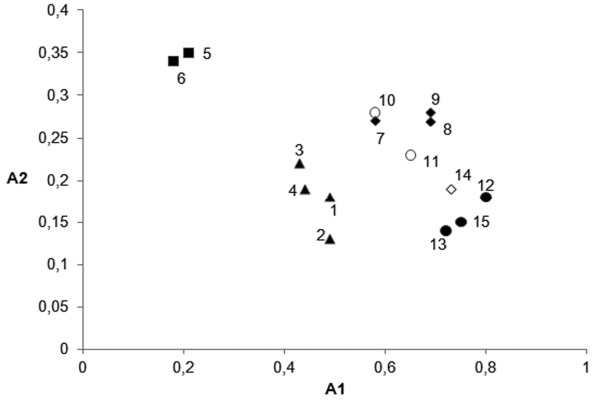

Fig. 5: Scatter diagram of the Romero Zarco (1986) asymmetry indices. Values of A1 and A2 are summarised in Table 2. **1**. *V. narbonensis var nar*bonensis (acc. 856); **2**. *V. narbonensis var nar*bonensis (acc. 488). **3**. *V. narbonensis var nar*bonensis (acc. 13). **4**. *V. narbonensis var nar*bonensis (acc. 12). **5**. *V. monantha cinerea* (acc. 140). **6**. *V. monantha calcarata* (acc. 157).**7**. *V. sativa ssp. sativa (C3)* **8**. *V sativa ssp. sativa (*acc. 12). **9**. *V. sativa ssp. sativa* (var. Mghila). **10**.*V. sativa ssp. amphicarpa* (acc. 139 upgs.). **11**. *V. sativa ssp. amphicarpa* (acc. 139 undgs.). **12**. *V. cordata* (acc. C1). **13**. *V. cordata* (acc. 304). **14**. *V. angustifolia* (acc. C2). **15**. *V. cordata* (acc. Sr)

karyotype was observed in acc. 12 and var. Mghila of *V. sativa ssp sativa* (3B) as well as the other accessions with Stebbins 4A category of asymmetry (Table 3).

ANOVA showed significant differences between the studied species and subspecies concerning MCL and MCI

(P<0.0001).The highest values of MCL are observed in *V. narbonensis* accessions, while the lowest MLC values are obtained in *V. sativa ssp. sativa* (var. Mghila) and *V. sativa ssp. amphicarpa* (acc. undgs.). The remaining accessions have intermediate values (Table 2). The two accessions of *V. monantha* (acc. 140 and acc. 157) have a high MCI but a medium TCL. The remaining accessions possess the intermediate TCL and MCI (Fig. 4).

The scatter diagram of A1 and A2 asymmetry indices (Fig. 5) presents three groups of species: (1) the two accessions of *V. monantha* (acc. 140 and acc. 157), which have the highest inter-chromosomal asymmetry index (average of A2=0.345); (2): the four accessions of *V. narbonensis,* which have intermediate values of A1 and A2; (3): the three accessions of *V. cordata* with the highest values of A1 and (4): the two accessions of *V. sativa ssp. sativa* (acc C3, acc. 12 and var. Mghila), the two accessions of *V. sativa ssp. amphicarpa* (acc. 139 upgs. and acc. 139 undgs.) and *V. angustifolia* (acc. C2) which have intermediate A2 but high values of A1.

Cluster analysis constructed on the basis of karyotype similarities (Fig. 6) using the average linkage method shows three major groups. The first group includes the four accessions of *V. narbonensis*, the second is composed by the two accessions of *V. monantha* and the third gathers both accessions of *V. sativa ssp. sativa* and *V. sativa ssp. amphicarpa*, the three accessions of *V. cordata* and *V. angustifolia* which is closed to *V. cordata*.

The principal component analysis (PCA) of the karyotypical traits shows that the first two components account for 93.91% of the total variation (Fig. 7). The first component (54.14%) is positively correlated to TCL, MCL and MCI and negatively correlated to A1, while the second component (35.76%) is defined positively by A1 and negatively by A2. The arrangement of the species and subspecies obtained by this analysis is similar to that obtained by the cluster analysis.

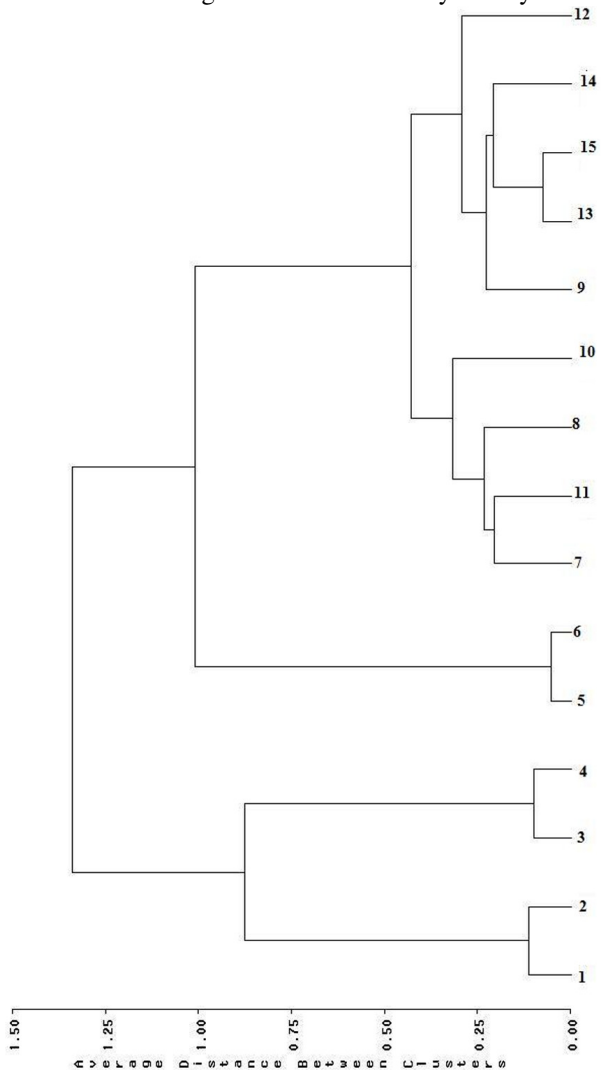

Fig. 6: Dendrogram showing the phonetic relationships among the studies species and sub species of *Vicia* constructed using the average linkage method. **1**. *V. narbonensis var nar*bonensis (acc.856); **2**. *V. narbonensis var nar*bonensis (acc. 488). **3**. *V. narbonensis var nar*bonensis (acc. 13). **4**. *V. narbonensis var nar*bonensis (acc. 12). **5**. *V. monantha cinerea* (acc. 140). **6**. *V. monantha calcarata* (acc. 157).**7**. *V. sativa ssp. sativa (C3)* **8**. *V sativa ssp. sativa (*acc. 12). **9**. *V. sativa ssp. sativa* (var. Mghila). **10**.*V. sativa ssp. amphicarpa* (acc. 139 upgs.). **11**. *V. sativa ssp. amphicarpa* (acc. 139 undgs.). **12**. *V. cordata* (acc. C1). **13**. *V. cordata* (acc. 304). **14**. *V. angustifolia* (acc. C2). **15**. *V. cordata* (acc. Sr)

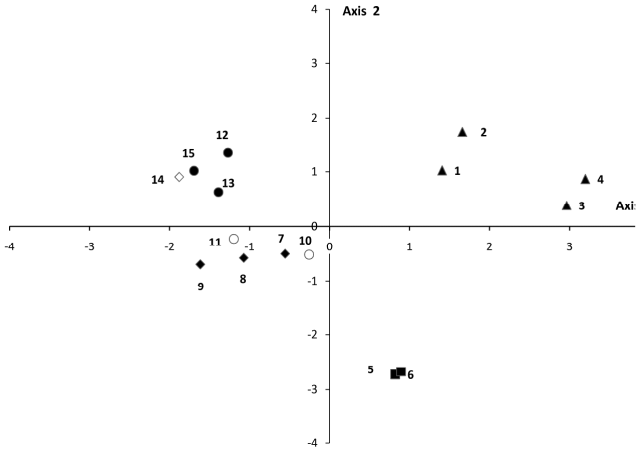

Fig. 7: Diagram of the principal component analysis (axis 1: highly correlated with TCL, MCL and MCI; axis 2: highly correlated with A1 and A2) of *Vicia* species and sub species. **1**. *V. narbonensis var nar*bonensis (acc.856); **2**. *V. narbonensis var nar*bonensis (acc. 488). **3**. *V. narbonensis var nar*bonensis (acc. 13). **4**. *V. narbonensis var nar*bonensis (acc. 12). **5**. *V. monantha cinerea* (acc. 140). **6**. *V. monantha calcarata* (acc. 157).**7**. *V. sativa ssp. sativa (C3)* **8**. *V sativa ssp. sativa (*acc. 12). **9**. *V. sativa ssp. sativa* (var. Mghila). **10**.*V. sativa ssp. amphicarpa* (acc. 139 upgs.). **11**. *V. sativa ssp. amphicarpa* (acc. 139 undgs.). **12**. *V. cordata* (acc. C1). **13**. *V. cordata* (acc. 304). **14**. *V. angustifolia* (acc. C2). **15**. *V. cordata* (acc. Sr)

Discussion

Our results showed a wide range of chromosome numbers and karyotype morphology in *Vicia* species. Chromosome count and their morphological features have been frequently recorded for cytological characterization of germplasm (Sharma and Sharma, 2013; Weiss-Schneeweiss and Schneeweiss, 2013). Studies revealed that all analysed accessions of *Vicia* were diploid. The chromosome number of *V. narbonensis* accessions was 2n=14 (Cremonini *et al*., 1998; Kamel, 1999; Venora *et al.,* 2009). Furthermore, we have found similar chromosome count in the two *V. monantha* accessions and the two *V. sativa ssp. amphicarpa accessions*. In *V. sativa ssp. sativa*, the chromosome number was 2n=10 for acc. C3 and 2n=12 for respectively acc. 12 and var. Mghila. *V. cordata* had chromosome number of 2n=10 for all the accessions and finally *V. angustifolia* had chromosome count of 2n=12. These results were in agreement with Kamel (1999); Weber and Shifino-Wittman (1999); Yamamoto (1973) and Javier *et al.* (1998).

Karyotype formula and quantitative analysis were variable among the studied species and subspecies except those that correspond to the different accessions of *V. monantha* and *V. cordata*. The chromosomes observed in the study were mainly subtelocentric or submetacentric types as mentioned by Jalilian and Rahiminejad (2012). The presence of two different formulas in the four *V. narbonensis* accessions may be due to the differences in their geographic origins (Bakatoushi and Ashour, 2009).

Primitive wild species had symmetrical chromosomes in their karyotypes (Stebbins, 1971). Therefore, the predominance of sm chromosomes in our *V. narbonensis* accessions of approximately similar size and the presence of only one pair with a secondary constriction revealed that these accessions might have retained some of their primitive wild traits as suggested by Zuo and Yuan (2011). In *V. sativa ssp. sativa*, two different formulas were found in three Tunisian accessions with a metacentric chromosome marker in their chromosome complements. This chromosome was noticeably absent from most of the other *Vicia sativa* aggregate (Maxted *et al.,* 1991; Kamel, 1999; Weber and Shifino-Wittman, 1999). Both acc. 12 and var. Mghila have 2m+10st while acc. C3 had 2m+2sm+6st. These differences in karyotype formula may be due to their different geographic origin and/or to the difference in their status. We also noticed the presence of two secondary constrictions in both the var. Mghila and the acc. C3. The predominance of st chromosomes revealed that these accessions transformed from their primitive wild form. In *V. sativa ssp. amphicarpa,* two different formulas were found within same accession. Aerial seed had 14 sm while underground seed had 6sm+8st. Ladizinsky and Temkin (1978) and Javier *et al.* (1998) also noticed similar change in the morphology of chromosome due pod disposition and environment could also impact on the morphology of chromosome. All the chromosomes in above aerial seed were sub median. Thus, karyotype of aerial seed was considered symmetrical while the underground seeds have a predominance of subtelocentric chromosomes showing an asymmetry tendency. In *V. monantha* and *V. cordata*, there was only one formula for each, 14m and 10st, respectively. According to Paszko (2006), the former has a symmetrical karyotype while the latest one has a karyotype in which asymmetry was increased due to the centromere position. Karyotype formula of the acc. C2 of *V. angustifolia* was 12st which was close to those of *V. cordata*. Its karyotype was considered as asymmetrical due to predominance of st chromosomes (Ruffini Castiglione *et al.,* 2012). Generally plant material showed two types of karyotype, symmetrical and asymmetrical. The symmetrical karyotype was represented by *V. narbonensis* and *V. monantha* with a predominance of sm and m chromosomes, while the asymmetrical karyotype includes *V. sativa, V. cordata* and *V. angustifolia* with a predominance of st chromosomes.

On the basis of our results, we revised the taxonomy of few accessions. The taxonomy of some species i.e. *V. cordata* and *V. angustifolia* was revised due to centromere position of the chromosomes, these species were considered previously as *V. sativa.* The acc. C1 and the acc. Sr were classified as *V. cordata* due to the presence of st chromosomes in all the complement and the presence of a secondary constriction in the shortest chromosome. C2 was also classified in *V. angustifolia* on the basis of st chromosomes in all the complement (2n=12) with a satellite connected to the long arm of the fifth chromosome pair as reported by Weber and Shifino-Wittman (1999). Several studies showed that *V. sativa* was taxonomically complicated, due to low morphological distinction between subspecies but little effort was done to describe relationships between each subspecies.

The species were grouped regarding to their evolutionary tendency. The comparison of the chromosomes and mechanisms of karytoype evolution helps to understand the process of diversification within various taxa (Sharma and Sharma, 2013; Weiss-Schneeweiss and Schneeweiss, 2013). *V. narbonensis.* species (2n=14) was considered the most primitive which further gave rise to 2n=12 and 10 through chromosomes rearrangement. The chromosome number of 2n=14 observed in *V. monantha* and *V. sativa ssp. amphicarpa* and the centromere position of median/submedian argues the primitive tendency of these two taxa. Nevertheless, differences in relative size between the chromosomes of the complement showed asymmetrical karyotypes and suggest that this structure led to the diversification of these taxa.

V. sativa ssp. sativa, *V. angustifolia* and *V. cordata,* had lower chromosome number of 2n=10 and 2n=12 may have arisen through Robertsonian translocation between two chromosomes in the complement (Maxted *et al.* (1991). These species also showed low TCL values, a predominance of st chromosomes and differences in relative size between the chromosomes of the complements suggesting asymmetry

indices for the establishment of the evolutionary changes in *Vicia* genus. This group of species could be considered as the most evolved in our study. Based on Stebbins' system the studied *Vicia* species were placed in five classes: 1A, 2A, 3A, 4A and 3B whose classes 1A and 2A were considered as primitives (Hejazi *et al.,* 2010). During the speciation and divergence of *Vicia* genus, cycles toward symmetry and asymmetry may have occurred as reported for different groups (Jones, 1970; Stebbins, 1971). The differences in the asymmetry of the karyotypes were great, for which it may be assumed that diversity of the genus has been accompanied by very small changes in the structure of the chromosomes. This study can be a model for genetic improvement programs and diversity studies for others *Vicia* species.

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