Pak. J. Agri. Sci., Vol. 52(2), 279-283; 2015
ISSN (Print) 0552-9034, ISSN (Online) 2076-0906
http://www.pakjas.com.pk

VICIA SATIVA SUBSP. *SATIVA* (*FABACEAE*):NEW TAXONOMIC DIVISION IN TUNISIA BASED ON KARYOLOGICAL DATA

Safia El-Bok[1,*], Aziza Zoghlami-Khelil[2], Rim Dougari[1], Cheima Jabri[2,3],Oussama Lamine[2] and Mohamed El-Gazzah[1]

[1]Laboratoire de Biodiversité, Biotechnologie et Changements climatiques, Faculté des Sciences de Tunis, Université El Manar, 2092 Tunis, Tunisie; [2]Laboratoire des Productions Animales et Fourragères, Institut National de la Recherche Agronomique de Tunisie, 2080 Ariana, Université de Carthage, Tunis; [3]Laboratoire de Génétique Moléculaire, Immunologie et Biotechnologie, Faculté des Sciences de Tunis, Université El Manar, 2092 Tunis, Tunisie.
[*]Corresponding author's e-mail: safia1elbok@yahoo.fr

Vicia sativa has a confused taxonomy and it is considered to be in active evolution. This species is morphologically and karyologically variable which makes its taxonomy difficult. In this paper we reported, for the first time in Tunisia, the presence of the subsp. *sativa* based on cytological characterization. This new identification was based on the presence of one metacentric chromosome marker pair in all the complements. All the accessions are diploids with 2n=2x=12 chromosomes. The karyotype revealed one metacentric (m) and five subtelocentric (s) chromosome pairs and in one a SAT-chromosome was observed. The identification of the subspecies of *Vicia sativa* based on cytological characterization has a great importance in Tunisian flora. Therefore, to complete this pertinent idea the research of correlation between karyological marker and morphological traits is our future aim which serves as important data base for further hybridization programs.

Keywords: Legume, mitotic chromosomes, variation, taxonomy, karyotyping

---

INTRODUCTION

The genus *Vicia* L. includes approximately 166-200 species found mainly in Europe, Asia and North America, extending to the temperate regions of South America and tropical Africa (Gaffarzadeh-Namazi *et al*., 2008; Heneidak and Hassan, 2007; Kamel, 1999; Mabberley, 2008; Maxted 1993, Meriç and Dane 1999; Raveendar *et al.*,, 2015; Roze and Rūrāne, 2013; Smykal *et al.,* 2015). In Tunisia, the species of *Vicia* genusare well spread. They grow in various bioclimatic zones on variable soil conditions and are eaten by animals (Hassen, 2000). The common vetch (*Vicia sativa* L) is a forage plant of economic importance. It is a species with variable ploidy level with basic chromosome number ranging 5-7 (Culot and Rabaute, 2009; Çceliktas *et al.,* 2010). Common vetch is polymorphic and includes confusion and taxonomic uncertainty. In Tunisian flora, Pottier-Alapetite (1979) and recently Nabli (2011) have reported only three subspecies of *Vicia sativa (amphicarpa, angustifolia* and *obovata)*. However, the subsp. *sativa* has been ignored. In the present paper, we studied four accessions of *Vicia sativa* for karyological traits in order to determine their taxonomical classification.

MATERIALS AND METHODS

The study focused on two accessions of common vetch namely, VS15 and VS8 (received from International Center For Agricultural Research In Dry Areas, ICARDA) and two varieties (Mghila, a local variety selected at the National Institute For Agronomic Research of Tunisia, INRAT, and registered in the official catalog in 2004) and CV (commercial variety introduced from France many years ago to be used by farmers for forage and hay purpose) (Table 1).

Table 1. Origins and karyological parameters of the varieties accessions and of *Vicia sativa* L. and ranking according to Duncan's test at 5%.

Varieties / Accessions	Origin	TL	LA	SA	CI	KF
VS T2 (Mghila)	Central Tunisia	3.58c	2.80b	0.78b	23.40a	4st+2st$_s$+ 2m+4st
CV	France	4.17a	3.36a	0.81b	19.48b	4st+2st$_s$+ 2m+4st
VS 8	Cyprus	4.24a	3.34a	0.90a	23.60a	4st+2st$_s$+ 2m+4st
VS 15	Afghanistan	3.78b	3.08a	0.70b	20.40b	4st+2st$_s$+ 2m+4st

Values accompanied by the same letter in the same column are not significantly different at the 5% level. TL: total length; L: long arm; S: short arm; CI: centromeric index; KF: karyotype formula

Mitosis was performed on actively growing root tips. Roots tips were obtained from seeds germinated in Petri dishes on filter paper moistened with distilled water and placed at room temperature (25°C) water. The root tips were treated with 0.1% colchicine solution for 1-2h and fixed in Carnoy's solution II (Jahier, 1992) for 24 hours at 5°C. The roots fixed were hydrolyzed in 1N HCl at 60°C for 20 minutes and stained with Feulgen for 30 minutes. The meristematic area was isolated and excised (about 1 cm long, zone of active division located between region of cell elongation and protective root cap) and crushed between slides in a drop of 1% acetic orcein. Finally, suitable mitotic plates were observed and photographed under an optical microscope type Hund (H 600) attached digital camera (12 MP BenQ type) with 3X Zoom. For each accession, a karyogram was constructed by rearranging the chromosomes in pairs, ordered by size and position of centromere for chromosomes of the same size according to Levan *et al.* (1964). Analysis of variance was applied on karyological data using the SAS program (SAS 2000) followed by Duncan's test at 5% for means comparison.

RESULTS AND DISCUSSIONS

Mitotic studies revealed that the two accessions and the two varieties are diploid with 2n = 2x = 12. The chromosomes complements of *Vicia sativa* accessions and varieties are shown in Figures 1, 2, 3 and 4. All of them have a pair of metacentric chromosomes marker.

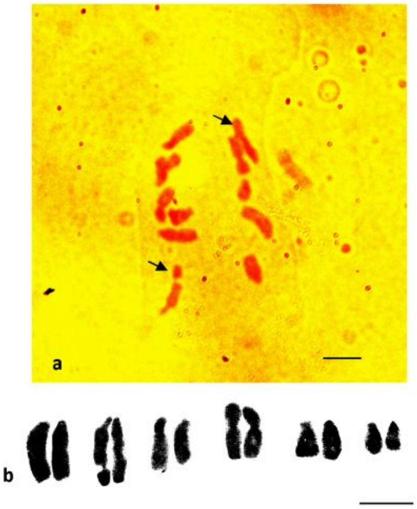

Figure 1.Somatic chromosomes of *Vicia sativa*Mghila (VST2)(a) 2n=2x=12 (Scale bar = 5µm), arrows indicate satellite chromosomes (b) karyotype.

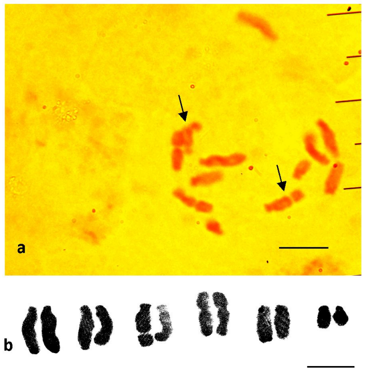

Figure 2. Somatic chromosomes of *Vicia sativa* commercial variety (CV) (a) 2n=2x=12 (Scale bar=5µm), arrows indicate satellite chromosomes (b) karyotype.

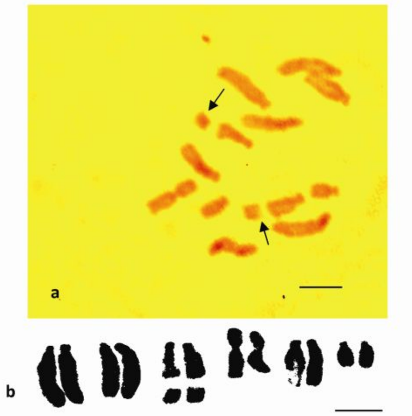

Figure 3. Somatic chromosomes of *Vicia sativa* (VS8 accession) (a) 2n=2x=12 (Scale bar = 5µm), arrows indicate satellite chromosomes (b) karyotype.

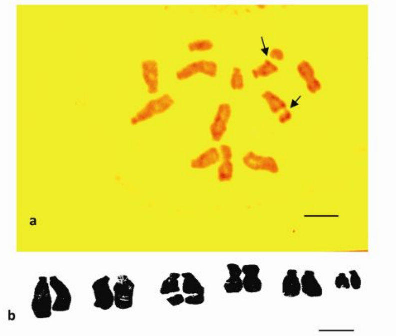

Figure 4. Somatic chromosomes of *Vicia sativa* (VS15 accession) (a) 2n=2x=12 (Scale bar = 5µm), arrows indicate satellite chromosomes (b) karyotype.

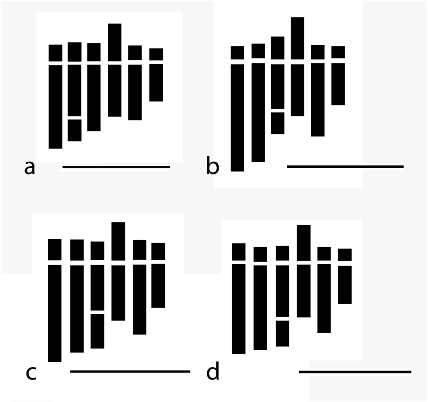

Figure 5. Idiograms of *Vicia sativa*: (a): Mgilla, (b): CV, (c): VS8, (d): VS15, (Scale bar = 5µm).

Figure 1a shows the somatic chromosomes of Mghila variety. One pair of satellite chromosomes is attached to the long arm of pair II (Fig. 1a,b). Chromosome length varied from 2.07 to 4.78 µm (Table 2). Figure 2a shows the somatic chromosomes of accession CV. One pair of satellite chromosomes is attached to the long arm of pair III (Fig. 2a,b). Chromosome length varied from 2.4 to 5.2 µm (Table 3). For each of accessions VS8 and VS15, one pair of satellite is linked to the long arm of pair III (Fig. 3a,b; Fig. 4a,b, respectively). Chromosome length varied from 2.51 to 4.98 µm and 2.04 to 4.52 µm for VS8 and VS15, respectively (Tables 4 and 5). For each accession and variety, an ideogram is given in Figure 5.

For all accessions the complement contained in majority subtelocentric chromosomes. The classification of accession and variety based on the total chromosome length, long arms, short arms as well as the centromeric index is shown in Table 1. Accessions VS8 (from Cyprus) and CV (commercial variety) have the longest chromosomes whereas Mghila accession (local variety) the shortest chromosomes (Table 1).

Karyological data for each accession and variety is given in

Table 2. Mean and standard deviation of karyological parameters (in µm) measured on the chromosomes pairs of Mghila (VST2).

Pair	TL	LA	SA	Sat	CI	CP
1	4.78±0.90	3.99±0.78	0.79±0.18		15.74±2.58	St
2	4.06±0.43	3.28±0.40	0.78±0.37	0.90±0.47	20.43±8.52	St
3	3.60±0.50	2.86±0.45	0.73±0.15		20.49±4.03	St
4	3.40±0.46	2.03±0.23	1.36±0.30		38.77±4.71	m
5	3.02±0.42	2.40±0.35	0.62±0.11		20.68±2.47	St
6	2.07±0.38	1.57±0.23	0.49±0.16		23.61±1.92	St

TL: total length; L: long arm; S: short arm; Sat: satellite; CI: centromeric index; CP: centromere position

Table 3. Mean and standard deviation of karyological parameters (in µm) measured on the chromosomes pairs of commercial variety (CV) varieties.

Pair	TL	LA	SA	Sat	CI	CP
1	5.20±0.59	4.65±0.48	0.55±0.11		10.50±0.87	St
2	4.85±0.51	4.26±0.43	0.60±0.13		12.32±2.16	St
3	4.55±1.26	3.60±0.95	0.95±0.48	0.90±0.47	28.67±15.3	St
4	3.95±0.34	2.25±0.17	1.70±0.20		41.80±8.50	m
5	3.75±0.35	3.20±0.44	0.55±0.11		14.94±4.58	St
6	2.40±0.20	1.90±0.13	0.50±0.00		21.90±2.10	St

TL: total length; L: long arm; S: short arm; Sat: satellite; CI: centromeric index; CP: centromere position

Table 4. Mean and standard deviation of karyological parameters in µm measured on the chromosomes pairs of accession VS 8.

Pair	TL	LA	SA	Sat	CI	CP
1	4.98±0.58	4.14±0.59	0.83±0.11		16.92 ±2.79	St
2	4.53 ±0.37	3.68 ±0.37	0.85 ±0.10		18.76 ±2.46	St
3	4.32±0.30	3.57±0.23	0.75 ±0.08	1.43±0.86	17.44 ±0.92	St
4	3.94 ±0.26	2.35 ±0.13	1.58 ±0.13		40.24 ±0.96	m
5	3.75 ±0.28	2.94 ±0.31	0.81 ±0.31		21.44 ±7.21	St
6	2.51 ±0.43	1.84 ±0.38	0.67 ±0.12		26.98 ±4.73	St

TL: total length; L: long arm; S: short arm; Sat: satellite; CI: centromeric index; CP: centromere position

Table 5. The average and standard deviation of karyological parameters in µm measured on the chromosomes of accession VS15.

Pair	TL	LA	SA	Sat	CI	CP
1	4.52±0.82	3.82±0.76	0.70±0.28		15.64±5.36	St
2	4.18±0.73	3.62±0.65	0.57±0.13		13.78±1.98	St
3	3.80±0.60	3.24±0.61	0.56±0.04	1.10±0.26	15.23±2.98	St
4	3.68±0.63	2.21±0.36	1.47±0.27		38.89±1.22	m
5	3.45±0.65	2.92±0.56	0.53±0.10		15.35±2.01	St
6	2.04±0.41	1.58±0.31	0.46±0.11		22.63±1.92	St

TL: total length; L: long arm; S: short arm; Sat: satellite; CI: centromeric index; CP: centromere position

Tables 2, 3, 4 and 5. The presence of metacentric chromosomes marker in our studied material is reported for the first time showing a relation between karyotype and taxonomy.

Furthermore, our results are in accordance with those of Gaffarzadeh-Namazi *et al*. (2008), Sevimay *et al*. (2005) and Çceliktas *et al.* (2010) who found that the chromosomes number of *Vicia sativa* subsp. *sativa* included ten subacrocentric (two of which with satellites) and two metacentrics. Maxted *et al.* (1991) also reported that the karyotype of *V. sativa* subsp. *sativa* has one pair of metacentric chromosomes and five pairs of acrocentric ones (the third one carried a satellite on the long arm). Moreover, Navratilova *et al*. (2003) reported four pairs of subtelocentric chromosomes, one pair of acrocentric and one pair of metacentric. These authors demonstrated that *Vicia sativa* subsp. *sativa* is the only subspecies that has a metacentric pair in its karyotype. However, this pair is absent in the other subspecies of the group *sativa*. According to Ruffini-Castiglione *et al.* (2012), the predominance of st chromosomes in all the complements indicated an asymmetrical karyotype.

Conclusions: The karyological study of the *Vicia sativa* accessions brought new information about the taxonomic position of these accessions which were unknown before this finding in Tunisian flora. Now these accessions belong to the subspecies *sativa*. We suggest that the examination of karyotypes such as conducted in this study is useful for a better taxonomic classification of undetermined species. This characterization is considered as a priority in order to preserve and enhance the local feed and forages genetic resources.

Acknowledgments: Acknowledgement and thanks to the Director of the Institute of Agronomic Research of Tunisia (INRAT) for his support. We also wish to thank all the members of the Laboratory of Animal and Forage Production for their thoughtful suggestions and help.

REFERENCES

Çceliktaşa, N., E. Cana, R. Hatipoğlub and S. Avci. 2010. Comparison between a wild population and cultivar of common vetch (*Vicia sativa* L., *Fabaceae*) on cytological and agronomic characteristics. New Zeal. J. Agr. Res. 49:389-393.

Coulot, P. and P. Rabaute. 2009. Découverte de deux vesces (*Vicia* L., *Leguminosae*) nouvelles pour le Languedoc-Roussillon et situation de ces espèces en France. Available online at http://herbier.philippe.rabaute.pagesperso-orange.fr/vicia.html/

Gaffarzadeh-Namazi, L., M. Badrzadeh and R. Asghari-Zakaria. 2008. Karyotypes of several *Vicia* species from Iran. Asian J. Plant Sci. 7:417-420.

Hassen, H. 2000. Induction de la tétraploïdie chez *Vicia narbonensis* L. (population locale): analyse de la variabilité et incidence sur l'amélioration agronomique de l'espèce. Ph.D. Dissertation, Académie de Bordeaux, Université de Pau et des Pays de l'Adour, 124 pp.

Heneidak, S. and A.E. Hassan. 2007. Morphological and anatomical studies of floral and extrafloral nectaries in some *Vicia* taxa (*Fabaceae*). Int. J. Bot. 3:329-341.

Jahier, J. 1992. Techniques de cytogénétique végétale, collections techniques et pratiques, Edition INRA, Paris, 183 pp.

Kamel, E.A. 1999. Karyological studies on some taxa of the genus *Vicia* L. (Fabaceae). Cytologia 64:441-448.

Levan, A., K. Fredga and A. Sandberg. 1964. Nomenclature for centromeric position on chromosomes. Hereditas 52:201-220.

Mabberley, D.J. 2008. Mabberley's Plant Book: A Portable Dictionary of Plants, their Classifications, and Uses, 3rd Ed., Cambridge University Press; p.1021.

Maxted, N. 1993. A phenectic investigation of *Vicia* L. subgenus *Vicia* (*Leguminosae*, *Viciae*). Bot. J. Linn. Soc. 111:155-182.

Maxted, N., M.A. Gallimassia and M.D. Bennett. 1991. Cytotaxonomic studies of Eastern Mediterranean *Vicia* species (*Leguminosae*). Plant Syst. Evol. 177:221-234.

Meriç, Ç. and F. Dane. 1999. Karyological studies on *Vicia sativa* subsp. *incisa* (Bieb.) arc. var. *incisa*. Turk. J. Bot. 23:63-67.

Nabli, M.A. 2011. Mise à jour de la Flore de la Tunisie, fascicule 1, Programme Flore et végétation tunisienne, Laboratoire de Botanique Fondamentale et Appliquée, Edit. Faculté des Sciences de Tunis, Université Tunis El Manar, Tunis ; p.487.

Navratilova, A. and J.M. Pneumann. 2003. Karyotype analysis of four *Vicia* species using *in situ* hybridization with repetitive sequences. Ann. Bot. 91:921-926.

Pottier-Alapetite, G. 1979. Flore de la Tunisie: Angiosperme-Dicotylédons-Apétales-Dialypétales, Première Partie, Edit. Imprimerie Officielle de la République Tunisienne, Tunis ; p. 651.

Raveendar, S., G.A. Lee, Y.A. Jeon, Y.J. Lee, J.R. Lee, G.T. Cho, J.H. Cho, J.H. Park, K.H. Ma and J.W. Chung. 2015. Cross-Amplification of *Vicia sativa* subsp. sativa microsatellites across 22 other *Vicia* species. Molecules 20:1543-1550.

Roze I. and I. Rurane 2013. Revision of genus *Vicia* L. in the flora of Latvia. Acta Biol. Univ. Daugavp. 13:85-93.

Ruffini Castiglione, M., M. Frediane, M.T. Gelati, G. Venora, L. Giorgetti, P. Caputo and R. Cremonini. 2012. Cytological and molecular characterization of *Vicia barbazitae* Ten. & Guss. Protoplasma 249:779-788.

SAS Institute Inc. 2000. SAS/STAT ^{TM} Guide for Person Computers, 6th Ed., Cary, North Carolina; p.534.

Sevimay, C.S., D. Guloglu and K.M. Khawar. 2005. Karyotype analysis of eight Turkish vetch *(Vicia sativa* L.) cultivars. Pak. J. Bot. 37:313-317.

Smykal, P., C.J. Coyne, M.J. Ambrose, N. Maxted, H. Schaefer, M.W. Blair, J. Berger, S.L. Greene, M.N. Nelson, N. Besharat, T. Vymyslicky, C. Toker, R.K. Saxena, M. Roorkiwal, M.K. Pandey, J. Hu, Y.H. Li, L.X. Wang, Y. Guo, L.J. Qiu, R.J. Redden and R.K. Varshney. 2015. Legume crops phylogeny and genetic diversity for science and breeding. Crit. Rev. Plant Sci. 34:43-104.