Pak. J. Agri. Sci., Vol. 52(4), 1035-1043; 2015
ISSN (Print) 0552-9034, ISSN (Online) 2076-0906
http://www.pakjas.com.pk

KARYOLOGICAL STUDIES OF *Vicia articulata* HORNEM

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This study reported a new counting of chromosomes number and karyotypes of ten introduced accessions of *V. articulata.* This forage species, native of the Mediterranean area, is widespread in south Europe, West Asia and Australia. Results showed that all accessions are diploid with a somatic chromosome numbers equal to 14. Furthermore, four karyotypic formulas were found among the studied accessions: $2sm+1m_s+4st$ for accessions Va-38; Va-2 and Va-4; $1m+1m_s+1sm+4st$ for accessions Va-57 and Va-91; $1m+1m_s+1sm +2st+2t$ for accessions Va-75 and Va-66 and $2m+1m_s+4st$ for accessions Va-85, Va-67 and Va-103. There was a predominance of subtelocentric pairs compared to metacentric and submetacentric pairs. Karyomorphological parameters based on Rec index (ratio between the length of each chromosome and the length of the longest one), SYi index (ratio between the average length of the short arms and the average length of the long arms) and TF% (ratio between the sum of the lengths of the short arms of individual chromosome and the total length of the complement) have been determined. They evidenced that the karyotype of accession Va-2 was the most asymmetric one having the lowest value of SYi index (27.95). Cluster analysis using thirteen karyological parameters has been carried out. The corresponding dendrogram based on Euclidian distance showed two main groups of accessions. Accession Va-2 is the farthest one and had the most karyotype in evolutionary trend.

Keywords:*Vicia* species, karyotype, cytogenetics, evolution, forage legume.

INTRODUCTION

The genus *Vicia* includes about 166 species located primarily in Europe, Asia and North America. It also extends to South America and East Africa on high altitudes (Ildis, 1999; Meric and Dane, 1999). Species are majorly exploited in organic farming for green manure and strong root system for tillage. In addition to their biological role for fixing atmospheric nitrogen, vetches also contributed for the development of arid and semi-arid areas which were dominated by monoculture barley, offering a practical solution to the replacement of fallow and reseeding of low fertility lands. Vetches were used in swards along with oats to provide a mix high proteins and grass. Vetches were also used for seed production which contained high protein contents (Lopez-Bellido *et al*., 2005). *Vicia articulata* was a single-flowered vetch, a crop native to the Mediterranean region (Laghetti *et al*., 2000). The species has been known for its drought and cold tolerance (Van de Wouw *et al*., 2001; Maxted and Bennett 2001). *V. articulata* has been an interesting prospect due to its role in soil conservation and rescue marginal areas. The species has been a useful source of genes in breeding programs to achieve diverse objectives (Laghetti *et al*., 2000). There was an increasing interest in Crop Wild Relatives (CWR) conservation and use in recent years. Therefore, there was increased focus on their systematic and cytogenetic conservation to ensure their continue availability (Maxted *et al*., 2012). In fact, targeted collections as *Vicia* species are required which provide an invaluable gene source for the improvement of food and forage legume cultivars (Maxted and Bennett 2001). Only few studies and information on karyology were available on *V. articulata.* Therefore, chromosomes number and karyomorphometry of some introduced accessions of *V. articulata* was determined. These results would provide information for the evolution of this species as well as its potential use for hybridization between *Vicia* species.

MATERIALS AND METHODS

The plant material involved in this study concerned ten accessions of *Vicia articulata* introduced from Spain for experimentation and breeding programs. Seeds were initially sown in the field of INRAT for multiplication and characterization. The main characteristics of these accessions are shown in Figure 1. The plant has a prostrate growth habit, thin stems, narrow and oblong leaflets, racemes one-small flowered with white-violet corolla (1-14 mm), glabrous pods (20-30mm x 7mm), 2-3 seeds/pod. Flowers were hermaphrodites and pollinated by insects. However, the plants were self-compatible. The main characteristics as code, country, region, coordinates and

Table 1.Main characteristics and origin of the studied accessions of *V. articulata* H.

Code accession	Country	Region	Altitude (m)	Latitude N	Longitude W
Va-2	Spain	Canarias	60	2847	01745
Va-4	Spain	Castilla y Leon	633	404203	0063951
Va-38	Portugal	Bragança	-	-	-
Va-57	Spain	Leon	845	422125	0051233
Va-66	Spain	Toledo	548	400042	0045250
Va-67	Spain	Toledo	537	401420	0041140
Va-75	Spain	Guadalajara	1041	410610	0030129
Va-85	Spain	Almeria	1090	370340	0024331
Va-91	Spain	Almeria	997	3717	00222
Va-103	Italy	-	-	-	-

Figure 1. *Vicia articulata* accessions growing in the field of INRAT with one of them carrying large amounts of anthocyanin pigments due to cold.

origins of *Vicia articulata*studied accessions are shown in Table 1. The chromosomes number as well as the karyotype analysis was investigated in the root tips of the seeds. The germination of seeds was done in Petri dishes at 22°C temperature. The root tips of 1-1.5 cm length were cut off and pre-treated with 0.1% colchicine at room temperature for 1h 30 mn, fixed in ethanol acetic acid (3v:1v) for 24 h and stored in 70% alcohol at 4°C. After that, root tips were hydrolysed with 1N HCL during 20 min. at 60°C. The Feulgen technique was used (Jahier et al., 1992). Slides were then prepared and captured using optical Microscope (type OPTIKA B-600Ti) supplemented with digital color video camera at a magnification of 2000x. At least five metaphases of five seedlings were selected and used for karyotype analysis. Chromosomal nomenclature was carried out according to Levan *et al*. (1964) for each cell. Calculations were made to determine long arm (LA) and short arm (SA) lengths, mean chromosome length (MCL), centromeric index (CI), total haploid chromosome length (THCL), TF% index (Sinha and Roy, 1979) and Rec and SYi indices (Castiglione *et al.,* 2010; 2012). The centromeric index is the ratio between short arm and long arm; The TF% (ratio between the sum of the lengths of the short arms of individual chromosome and the total length of the complement) index is expressed by the ratio between the sum of the lengths of the short arms of individual chromosome and the total length of the complement; the Rec index expresses the average of the ratios between the length of each chromosome and that of the longest one; the SYi value indicates the ratio between the average length of the short arms and the average length of the long arms. Statistical analysis of numerical data was carried out by a computer program (SAS, 2000) based on analysis of variance (ANOVA) and followed by ad-hoc Duncan test (Duncan, 1955) if the null hypothesis is rejected. A Total of fourteen karyological traits (Table 2) were analyzed using Cluster analysis Euclidean distance method in SAS program.

Table 2. **Parameters used in cluster analysis.**

Code	Description
TL	Total length
MCL	Mean chromosome length
THCL	Total haploid chromosome length
SA	Short arm
LA	Long arm
SAT	Satellite
CI	Centromeric index
Nb. sm	Number of sub median chromosomes
Nb. m	Number of median chromosomes
Nb. st	Number of sub telocentric chromosomes
Nb. t	Number of telocentric chromosomes
TF %	Total form (%)
Rec	Resemblance among chromosomes
SYi	Symmetry index (%)

RESULTS

The chromosome number of all the studied accessions is 2n = 2x = 14 (Fig. 2). MCL, SA, SAT, LA and THCL are reported in Table 3. The Rec, SYi as well as TF% indices and are summarized in Table 4. Significant differences between accessions were observed for the chromosomes size (Table 3).

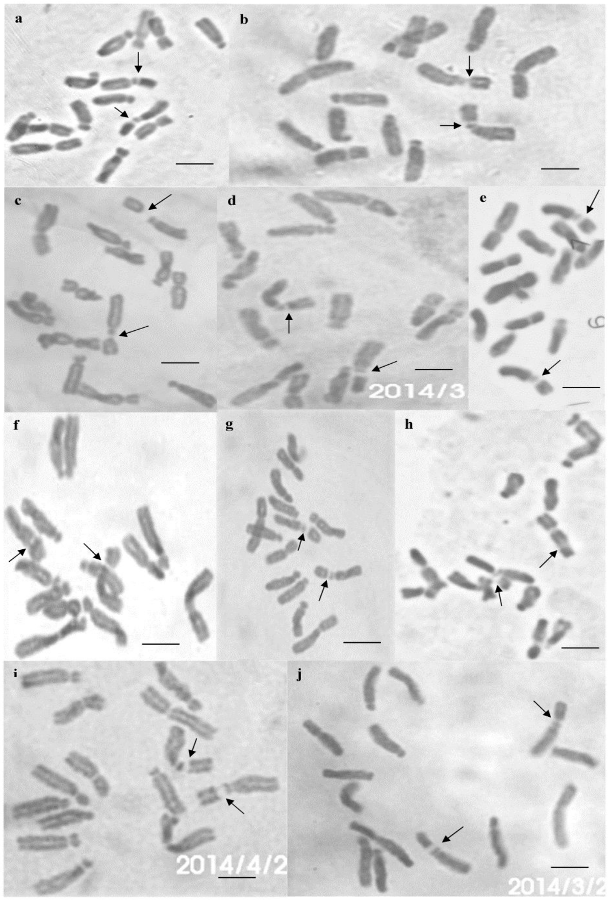

Figure 2.Somatic metaphases of the analyzed accessions of *Vicia articulata*; 2n=2x=14 (Scale bars = 5µm) arrows indicate satellite chromosomes (a=Va-4; b=Va-75; c=Va-57; d=Va-103; e=Va-38; f=Va-85; g=Va-67; h=Va-2; i=Va-91; j=Va-66).

Table 4. Chromosome morphometric data, karyotype formulas, symmetry indices (TF%, Rec and SYi) of *Vicia articulata* H. accessions analyzed.

Accession and chromosome nr.	Chromosome length (µm. mean ±SD)	Satellite length (µm. mean ± SD)	Long/Short arm ratio
Va-4			
1	6.94±0.59		1.96
2	6.24±0.45	2.1±0.3	1.13
3	5.18±0.36		3.69
4	4.57±0.33		5.45
5	4.57±0.14		2.37
6	4.39±0.33		4.67
7	3.89±0.51		3.92
2sm+1m_s+4st	TF %=22.13 ; Rec=78.91;	SYi=30.85	
2sm+1m_s+4st			
Va-75			
1	9.85±1.3		1.63
2	9.12±1.03	2.6±0.4	1.41
3	7.45±0.69		3.97
4	6.62±0.84		1.75
5	6.35±0.74		5.44
6	6.22±0.89		9.20
7	6.2±0.78		7.79
1m+1m_s+1sm+2st+2t	TF %=26.68 ; Rec=75.16;	SYi=36.48	
Va-57			
1	8.4±1.46		1.62
2	8.22±0.44	2.2±0.1	1.23
3	6.58±0.53		3.77
4	5.7±0.32		6.26
5	5.63±0.47		1.69
6	5.6±0.45		5.62
7	5.52±0.49		5.17
1m+1m_s+1sm+4st	TF %=28.46 ; Rec=77.57	SYi=39.81	
Va-103			
1	9.92±0.53		1.64
2	9.2±0.78	2.6±0.2	1.24

Table 3. Average karyological parameters (in µm) of the ten accessions of *V. articulata* H. and their classification using Duncan test at the 5% probability level.

Accession/Parameter	MCL	SA	LA	SAT	THCL
Va-4	5.11e	1.13d	3.67e	0.31d	35.8±1.1
Va-75	7.4b	1.97ab	5.41ab	2.6a	51.8±1.5
Va-57	6.52c	1.855bc	4.66c	2.2b	45.6±1.27
Va-103	7.43b	2.05ab	5.37ab	2.6a	52±1.51
Va-38	4.65f	1.03d	3.38e	0.24d	32.5±0.68
Va-85	7.1b	2.01ab	5.14b	2.2b	49.7±1.3
Va-67	5.7d	1.61c	4.09d	1.8c	39.9±1.1
Va-2	4.88ef	1.02d	3.63e	0.22d	34.1±0.9
Va-91	7.86a	2.23a	5.64a	2.62a	55±1.5
Va-66	6.27c	1.75bc	4.49c	2.1b	43.9±1.24

Values followed by the same letter in the same column are not significantly different at the 5% level (SA: short arm, LA: long arm, SAT: satellite, MCL: mean chromosome length. THCL: total haploid chromosome length).

3	7.27±0.86		4.13
4	6.57±0.68		6.47
5	6.42±0.77		1.61
6	6.32±0.54		7.17
7	6.27±0.64		6.30
2m+1m$_s$+4st	TF%= 27.64; Rec=; 74.85	SYi=38.31	
Va-38			
1	5.69±0.59		1.96
2	5.26±0.35	1.7±0.3	1.38
3	4.78±0.49		5.01
4	4.66±0.51		1.88
5	4.42±0.34		4.86
6	4.02±0.36		4.48
7	3.72±0.29		4.95
2sm+1m$_s$+4st	TF %=28 ; Rec=74.42;	SYi=39.10	

cont... Table 4

Accession and chromosome nr.	Chromosme length (µm. mean ±SD)	Satellite length (µm. mean ± SD)	Long/Short arm ratio
Va-85			
1	9.52±1.95		1.64
2	8.37±1.33	2.2±0.3	1.45
3	7±1.1		3.44
4	6.55±1.19		1.57
5	6.2±1.18		4.9
6	6.05±0.76		6.26
7	6±0.86		5.22
2m+1m_s+4st	TF%=28.36 ; Rec=74.53;	SYi=39.18	
Va-67			
1	7.5±0.80		1.63
2	6.85±0.74	1.8±0.3	1.4
3	5.8±0.45		3.69
4	5.125±0.2		1.57
5	5.05±0.57		4.98
6	4.875±0.37		6.95
7	4.7±0.92		6.73
2m+1m_s+4st	TF %=28.32 ; Rec=76	SYi=39.41	
Va-2			
1	6.45±0.71		2.10
2	5.74±0.35	1.6±0.1	1.56
3	5.11±0.43		4.64
4	4.39±0.48		2.25
5	4.25±0.43		5.67
6	4.09±0.34		5.42
7	4.11±0.31		5.42
2sm+1m_s+4st	TF%=20.83 ; Rec=75.61;	SYi=27.95	
Va-91			
1	10.62±1.34		2.07
2	9.1±0.10	2.6±0.1	1.44
3	7.97±0.81		3.89
4	6.97±0.49		1.71
5	6.87±0.47		5.1
6	6.8±0.84		5.7
7	6.7±0.99		5.4
1m+1m_s+1sm+4st	TF%=28.34 ; Rec=74.02;	SYi=39.52	
Va-66			
1	8.42±1.05		1.53
2	7.52±0.75	2.1±0.2	1.36
3	6.3±0.83		3.45
4	5.65±0.80		1.7
5	5.46±0.7		7.3
6	5.32±0.80		5.85
7	5.2±0.78		10.96
1m+1m_s+1sm+2st+2t	TF %=22.13; Rec=78.91;	SYi=30.85	

sm: sub median chromosomes; st: sub telocentric chromosomes; t: telocentric chromosomes m_s: median chromosomes; m: median chromosomes which posses satellite (SAT), TF%: Total form (%); Rec: Resemblance among chromosomes; SYi: Symmetry index (%)

Figure 3. Karyograms of the analyzed accessions of *Vicia articulata;* 2n=2x=14 (Scale bars=5µm); (a=Va-4; b=Va-75; c=Va-57; d=Va-103; e=Va-38; f=Va-85; g=Va-67; h=Va-2; i=Va-91; j=Va-66).

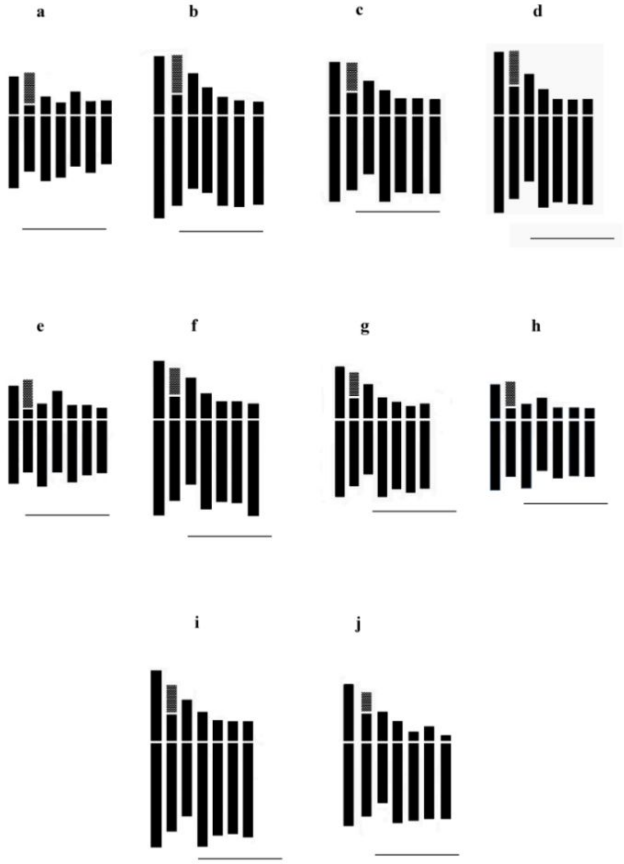

Figure 4. Idiograms of the haploid complement of *Vicia articulata* accessions: (scale bars = 5µm); (a=Va-4; b=Va-75; c=Va-57; d=Va-103; e=Va-38; f=Va-85 ; g=Va-67; h=Va-2; i=Va-91; j=Va-66).

The accession Va-91 has the longest chromosomes while the accession Va-38 has the shortest ones. Mean chromosome length (MCL) values vary between 4.88 to 7.86 µm for accessions Va-2 and Va-91, respectively. However, accessions Va-75, Va-103 and Va-91 had the longest satellite. In each karyotype, one satellite was observed and attached to the short arm of the second pair of each complement (Table 4). Four karyotypes were found among the studied accessions with a predominance of st chromosome: (i) $2sm+1m_s+4st$; (ii) $1m+1m_s+1sm+4st$; (iii) $1m+1m_s+1sm+2st+2t$ and (iv) $2m+1m_s+4st$. The TF index ranged from 20.83% to 28.46% while the SYi varied between 27.95% and 39.81% for respectively Va-2 and Va-57 (Table 4). Based on these parameters, Va-2 had the more asymmetric karyotype. It has also subtelocentric chromosomes with the smallest short arms and the lowest MCL (Fig. 4 and Table 2). The Rec index values are nearly similar between all the accessions. Figures 3 and 4 showed the karyograms and idiograms of haploid complement of the analyzed accessions of *V. articulata*. The obtained dendrogram of linkage distance showed two main groups of accessions (Fig. 5). Accessions Va-75 and Va-2 are used as out groups. Accession Va-2 is the farthest one (distance of 0.5).

DISCUSSION

The present study was undertaken to provide an accurate karyotype analysis of ten accessions of *Vicia articulata* and to investigate the relationships of karyotypic evolution and altitude of origin site. The karyotype of *V. articulata* was described for the first time by Blanco and Perino (1974) who pointed out that this forage legume has one pair of satellite, two pairs of submedian and four pairs of subterminal chromosomes. Besides this primary description there are no further reports of the characterization of the chromosomes of this species. In order to provide more karyological information, we analyzed the chromosomes of ten

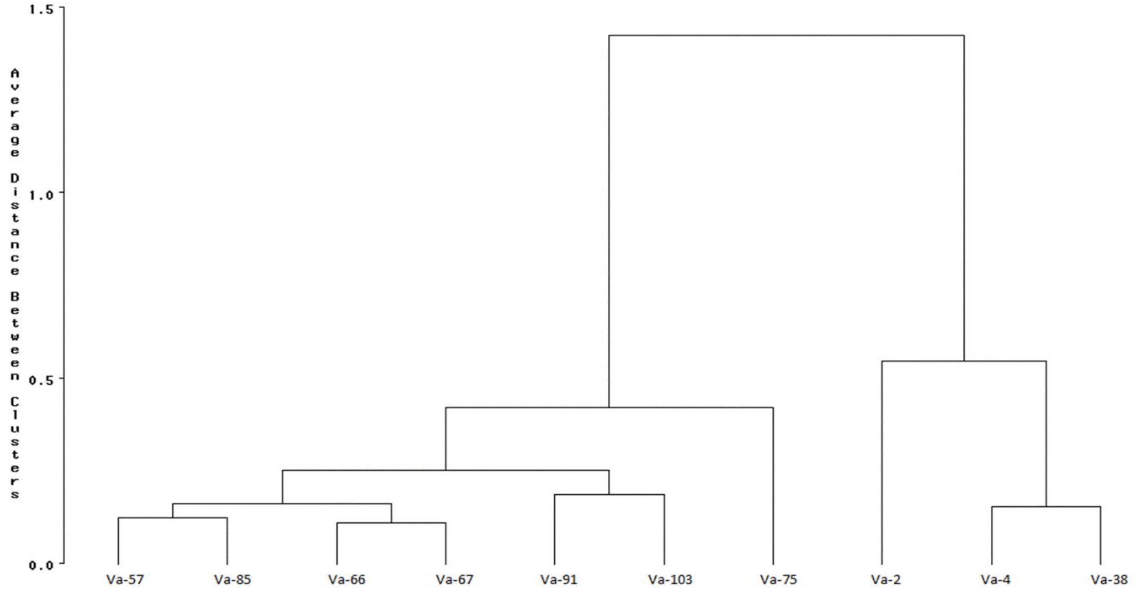

Figure 5. Hierarchical cluster analysis. Dendrogram of *Vicia articulata* accessions based on karyological data.

accessions of *V. articulata* of Mediterranean origins. The somatic chromosomes number observed in all accessions (2n=2x=14) agree with that of Laghetti *et al.* (2000). The chromosomes observed in this study were mainly subtelocentric types as indicated by their karyotypic formulas.

Among four karyotype formulas founded in our study, 2sm+1m_s+4st was reported by Blanco and Perino (1974) while others were reported for the first time in the study. However, accessions corresponding to each karyotype had karyological similarity. The predominance of st chromosomes revealed that these accessions transformed from their primitive wild form as reported by El Bok *et al.* (2014) in *V. sativa* accessions. The groups of accessions obtained by clustering indicated their evolutionary tendency. The variation in mean chromosome length between accessions may be associated with the altitude of their origin site. However, chromosomal analysis often provides a means of assessing direction of evolution. It seems that accession Va-2 with small and asymmetric chromosomes tend to be more evolved than Va-91 with larger chromosomes. The degree of variation of TF% in our accessions is in agreement with the previous reports of Sadeghian and Hejazi (2014).

Conclusion: Karyological analysis of ten accessions of *Vicia articulata* based on symmetry indices (TF%, Rec and SYi) and karyotype formula was undertaken in this paper. Data related on karyotype formula, symmetric indices and chromosomes length gave clear information on the evolutionary trend of this taxon. Differences for chromosome length and karyotype formulas were found. Accession Va-2 seems to be the most distant one due to its lowest SYi index and the most evolved one. However, *Vicia articulata* may definitively disappear because of years of intensive culture. For that, characterization using molecular traits should be used and more attention should be given to the conservation of this species in Mediterranean region.

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