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Karyological analyses in several Algerian populations of six species of the genus *Vicia* L. (*Fabaceae*)

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Abstract. As part of the evaluation and valorization of plant genetic resources of fodder and pastoral interest in Algeria, seventeen (17) natural populations belonging to six (06) species of the genus *Vicia* (*Vicia sativa*, *Vicia disperma*, *Vicia monardii*, *Vicia ohchroleuca*, *Vicia onobrychioides* and *Vicia lutea*), originated from different ecological regions in the North-Eastern of the country, were considered. The populations have been the subject of mitotic and meiotic studies. Haploid and diploid numbers and chromosome measurements were determined. Original results were observed for the first time in some species. In fact, chromosome counts have revealed some new chromosome numbers. The first number (2n=14, n=7) was observed in the endemic subspecies of Algeria, *V. ochroleuca subsp. atlantica* and in the species *Vicia onobrychioides*. The second number (n=6) was observed in the species *Vicia disperma*. Within *Vicia monardii*, the three new chromosome numbers, previously observed only in mitosis by our research team, were confirmed for the first time through the present meiosis study (n=6, 7 and 8), indicating that they are A type chromosomes. The base number x=7 is the most frequently observed number in the six *Vicia* species. The observed chromosome numbers would be related to some ecological factors (altitude, rainfall) of the origin environment of the populations. Chromosome measurements and established karyotypes were determined for the first time in *V. monardii*, *V. onobrychioides* and *V. ochroleuca subsp. atlantica*. Chromosome size and karyotype formula are variable among the studied species and subspecies. Karyotypes vary from symmetrical to asymmetrical and the intrachromosomal asymmetry is higher than interchromosomal one. The new cytogenetic data would contribute to a better understanding of the evolution mechanism of the species in the genus *Vicia* L.

Keywords: chromosomes, endemic, karyotype, plant genetic resources, *Vicia* L.

INTRODUCTION

The Leguminosae (*Fabaceae*) is the third largest angiosperm family in terms of species number after *Asteraceae* and *Orchidaceae,* comprising over 770 genera and 19500 species (Lewis et al. 2005, 2013). The family is morphologically, physiologically and ecologically diverse, representing one of the most spectacular examples of evolutionary diversification in plants (LPWG 2017).

The genus *Vicia* L. is a member of the legume tribe *Vicieae* of the subfamily *Papilionoideae* (Kupicha1976). The exact number of species in the genus *Vicia* L. is quite difficult to estimate, due to cytological and morphological differences (Kartal et al. 2020). It is including approximately 210 species that are widely distributed in temperate regions of Europe, Asia and America (Hanelt and Mettin 1989). Archeological evidence suggests that the main center of diversification of genus *Vicia* is the Mediterranean region (Raveendar et al. 2015). Most of species of the genus *Vicia* are annual but a few of them belonging to the section *Cracca* are perennial (Yamanoto 1973). Recently, the genus *Vicia* has been recognized for its vital role in sustainable agriculture (Han et al. 2021).

The most common classification is that of Kupicha (1976) revised by Maxted (1993). Kupicha (1976) divided the genus *Vicia* into two subgenera, *Vicilla* and *Vicia* with 17 and 5 sections, respectively. Subgenus *Vicia* is smaller than *Vicilla* but it is more coherent and includes the more agriculturally important species (Ruffini Castiglione et al. 2011). Maxted (1993) subdivided the subgenus *Vicia* into 9 series, 38 species, 14 subspecies and 22 varieties. The subgenus *Vicilla* is considered more primitive and diverse than subgenus *Vicia* (Kupicha 1976 and Maxed 1993).

According to Quezel and Santa (1962), the Algerian flora is represented by 26 species and 18 subspecies corresponding to the genus *Vicia* L. Two subspecies, *Vicia ochroleuca subsp. atlantica* and *Vicia ochroleuca subsp. baborensis* are respectively quite rare and very rare in Algeria. More recently, Dobignard and Chatelain (2012) report 39 taxa within the genus *Vicia* in Algeria, including two endemic subspecies, *V. ochroleuca subsp. atlantica* and *V. ochroleuca subsp. baborensis.* The prospecting and collection mission carried out, in 2016, in northeastern Algeria, showed the frequency of *Vicia sativa*, followed by *Vicia disperma* and the rarity recorded in some encountred species such as *Vicia monardii, Vicia narbonensis* and *Vicia ochroleuca* (*subsp. atlantica*) (Issolah et al. 2022).

Vicia sativa L. is a variable genus comprising of several subspecies; it is most commonly called *Vicia sativa* complex (Cvs) or *sativa* aggregate (Jauzein 1995; Kartal et al. 2020; Benlioglu 2021). Members of this complex are morphologically, karyologically and ecologically variables and were considered to be in active evolution (Potokina et al. 2000; Shiran and Raina 2001; El-Bok et al. 2015), making identification difficult and confusing.

Several karyological studies have been carried out in *Vicia*. They had an important role in improvement and solving of several taxonomic problems between the related species (Lavia et al. 2009; Murti et al. 2012). There is a considerable variability in haploid nuclear DNA content (1.8-13.3 pg) and basic chromosome number (2n=10, 12 or 14) between *Vicia* species (Raina and Narayan 1984; Maxed 1995). This makes the genus an interesting model for the study of plant genome and Karyotype evolution (Navratilova et al. 2003). Most of them are diploids with a basic number x =5, 6 or 7 (Maxted 1991), while only six of them are polyploids (Cremonini et al. 1992).

In Algeria, karyological studies on spontaneous populations of the genus *Vicia* L. are very rare. Several species are totally unknown. The main objectives of this study are to determine the number and size of chromosomes within some species of the genus *Vicia* in Algeria, in order to contribute to a better understanding of the mechanism of evolution of the different species in this genus.

This work follows previous studies conducted on the evaluation and valorization of diversity in spontaneous and local fodder legumes in Algeria (Issolah and Abdelguerfi 1999; Bouziane et al. 2019; Chabouni et al. 2019; Issolah et al. 2006, 2012, 2015, 2018, 2022).

MATERIAL AND METHODS

Plant material

Following a prospecting mission carried out by INRAA through the North-East of Algeria (Issolah et al. 2022), several species belonging to the genus *Vicia* were collected. Seventeen (17) populations belonging to six (06) species of the genus *Vicia* L. (*V. sativa subsp. sativa* (04), *V. sativa subsp. macrocarpa* (03), *Vicia disperma* (03), *Vicia monardii* (04), *V. ohchroleuca subsp. atlantica* (01), *Vicia onobrychioides* (01) and *Vicia lutea* (01) were the subject of the present study (Table 1).

Mitotic study

Diploid chromosome numbers were counted from the seedling root tips which were germinated in Petri dishes on filter paper at room temperature. The root tips meristems (about 1 cm long; zone of active division)

Table 1. Ecological characteristics of natural habitats of several spontaneous populations within some *Vicia* L. species in Algeria.

Populations	Species	Subspecies	Origin	Altitude (m)	Rainfall (mm)
11/14	*V. sativa* L.	*subsp. sativa*	Boumerdes	25	850
65/15	*V. sativa* L.	*subsp. sativa*	Bejaia	450	950
75/15	*V. sativa* L.	*subsp.sativa*	Bejaia	550	950
63/16	*V. sativa* L.	*subsp.sativa*	Bejaia	160	950
55/14	*V. sativa* L.	*subsp.macrocarpa*	Bejaia	1250	1100
73/15	*V. sativa* L.	*subsp. macrocarpa*	Bejaia	860	700
77/15	*V. sativa* L.	*subsp. macrocarpa*	Bejaia	410	800
49/14	*V. disperma* DC.	-	Bejaia	550	700
80/14	*V. disperma* DC.	-	Bejaia	90	750
79/15	*V. disperma* DC.	-	Tizi-Ouzou	1030	1100
40/16	*V. monardiii* Boiss	-	Bouira	800	500
48/16	*V. monardiii* Boiss	-	Bejaia	560	700
51/16	*V. monardiii* Boiss	-	Bejaia	595	700
66/16	*V. monardiii* Boiss	-	Bouira	730	500
52/16	*V. ochroleuca* Spreng.	*subsp. atlantica*	Bejaia	1140	1100
82/14	*V. onobrychioides* L.	-	Tizi-Ouzou	750	950
83/15	*V. lutea* L.	*subsp..vestita*	Blida	230	650

Source: (Issolah et al. 2022, completed).

were excised in the morning between 7.30 am – 8.30 am periods of active cell division. Firstly, root tips were pretreated for 02 h with α- bromonaphtalene (1%) at room temperature. Then, they were fixed in ethanol chloroform acetic acid (6:3:1) during 24 h at 4 °C. Root tips were hydolysed with 1 N HCL and were stained using lactopropionic orcein solution (Dyer 1963).

Chromosome counts in mitosis metaphase and karyotype analyses were obtained usually based on five best plates (metaphase cells) of chromosomes, for each population.

Each chromosome was identified on the basis of its total chromosome length. Chromosomal nomenclature was carried out according to Levan et al. (1964). Idiograms were constructed by arranging the chromosomes in homologous pairs by order of their length.

For the numerical characterization of the karyotypes, the following parameters were calculated: The length of long arm (L), short arm(S), total chromosome length (LT= L+S) and the relative length (RL ‰) = 1000x TL/∑ TL (Levan et al. 1964).

To determine chromosome type and centromere position, two parameters were calculed: arm ratio (r = L/S) and centromeric index (CI%=S/LT x 100) according to the nomenclature of Levan et al. (1964).

To determine the asymmetry of the karyotype, the interchromosomal asymmetry was determined by calculating the coefficient of variation of chromosome length $\mathbf{CV_{CL}}$ (Paszko 2006), **Rec index** (Venora et al. 2002) and the interchromosomal asymmetry index $\mathbf{A_2}$ (Zarco 1986). The intrachromosomal asymmetry was determined by calculating, the mean centromeric asymmetry $\mathbf{M_{CA}}$ (Peruzzi and Eroglu 2013), the percentage of karyotype asymmetry index **AsK%** (Arano 1963), total form percentage **TF%** (Huziwara 1962), the **Syi** index (Greilhuber and Speta 1976), the intrachromosomal asymmetry index $\mathbf{A_1}$ (Zarco1986) and the degree of asymmetry of karyotype **A** (Watanabe et al. 1999).

Meiotic study

A meiotic analysis was conducted in order to confirm the results obtained during mitosis and determine the chromosome type of the newly observed chromosome in the considered Algerian populations (*Vicia* L.). On this purpose, a trial was set-up at the INRAA experimental station. The protocol adopted is a total randomization. The sowing was carried out on 15 November 2018 at the rate of twenty seeds (individuals), for each population.

In April 2018, flower buds of different sizes were collected for each population and immediately fixed in a solution of Carnoy acetic ethanol (3:1 v/v). The hydrolysis was performed using 1N HCL for 1 to 3 min at 60°C. The staining was done with lactopropionic orcein at room temperature. The meiotic behavior was analyzed.

The observations of the mitotic plates were made using a Primo Zeiss Star microscope and photographed with a digital camera attached to this microscope. The analysis of cytogenetic data was made with the Axiovision software (1999-2009). The different karyotype calculations were made with Excel (2007).

RESULTS

Mitotic analyses

In this study, the chromosome numbers and detailed chromosome measurements were determined for the natural populations of *Vicia species* in Algeria. Mitotic studies revealed that all studied populations were diploid (figure 1).

Vicia sativa subsp. sativa

At the metaphase stage, chromosomal counts revealed the existence of a chromosomal number (2n=2x=12) for all analysed populations of *Vicia sativa subsp. sativa*. However, a second chromosome number (2n=2x=10) was found in population 11/14 with a high frequency (80%) in the cells of the same individual and in different individuals of this population (figure1). This indicates a chromosomic variation within and between the populations of *Vicia sativa* L.

The shortest chromosome length is 1.9 µm (population n° 11/14); the longest one is 4.84 µm (population n° 63/16). The mean value of the total length TLG of all the studied populations is 3.65 µm. The centromeric index varies between 23.4 to 38.53 and relative lengths vary from 87.51 to 222.97. The chromosomes observed in *Vicia sativa subsp. sativa* populations were mainly submetacentric or subtelocentric types (Table 3).

For the karyotype asymmetry, the lowest value of intrachromosomal asymmetry M_{CA} is 30.63, the highest one is 47.63. The lowest value of interchromosomal asymmetry CV_{CL} is 9 and the highest value is 30 (Table 7).

The idiograms were drawn based on centromeric index and arranged in the decreasing size order (Figure 2).

Vicia sativa subsp. macrocarpa

The present study showed that 2n=2x=12 is the chromosome number of all populations of *Vicia sativa subsp. macrocarpa* (Table 2). The shortest chromosome length is 2.25 µm (population n° 77/15), the longest one is 4.85µm (population n° 55/14). The mean value of the total length TLG of all studied population is 3.46 µm, the centromeric index varies between 21, 2 and 37.92 and the relative lengths vary from 111 to 206.7 (Table 4).

For karyotype asymmetry, the lowest value for intrachromosomal asymmetry M_{CA} is 36.68, the highest one is 49.0. The lowest and highest values of interchromosomal asymmetry CV_{CL} are 13 and 20, respectively (Table 7).

The karyotype formula is very variable between populations of the same subspecies in *Vicia sativa* (Table 3, 4).

The idiograms of *Vicia sativa subsp. sativa* and *Vicia sativa subsp. macrocarpa* were illustrated on the basis of their centromeric index and arranged in descending order of the chromosomal size (Figure 2).

Vicia monardii

Within this rare species (Quezel and Santa 1962), our observations showed that populations n°51/16 and n°40/16 have a stable number of chromosomes (2n=14), while populations n°48/16 and n°66/16 have two chromosomes numbers (2n=12 and 2n=14) and (2n=14 and 2n=16), respectively (Table 2).

In *Vicia monardii*, karyological measurements were determined for the first time in the present study. The shortest chromosome length is 2.76 µm (population n°48/16), the longest chromosome is 6.37 µm (population n° 66/16). The mean value of the total length TLG of all studied population is 4.40. The centromeric index varies between 23.07 and 37.9 and the relative lengths vary from 87.45 to 196.29 (Table 5).

For the karyotype asymmetry, the lowest value of M_{CA} intrachromosomal asymmetry is 36, the highest one is 44. The lowest value for interchromosomal asymmetry CV_{CL} is 15.4 and the highest value is 24.22 (Table 7).

The karyotype formula is variable between the populations of *Vicia monardii*. The observed chromosomes are mainly submetacentrics (Table 5).

The idiograms were illustrated on the basis of the centromeric index and arranged in descending order of chromosome size (Figure 2).

Vicia ochroleuca subsp. atlantica

The somatic chromosome number of *Vicia ochroleuca subsp. atlantica* is 2n =2x=14 (Figure1). It is a new number observed for the first time in this endemic species in Algeria.

Karyological measurements were made for the first time within this subspecies. It is characterized by chromosomes with lengths of 3.19 - 4.86 µm and an average value of the total length of 4.14 µm. The centromeric

Table 2. Chromosome number within 17 Algerian populations of the genus *Vicia* L.

Population	Species	Subspecies	Chromosomes numbers	
			2n	n
11/14	*V. sativa*	*subsp. sativa*	10 and 12	5 and 6
65/15	*V. sativa*	*subsp. sativa*	12	6
75/15	*V. sativa*	*subsp. sativa*	12	6
63/16	*V. sativa*	*subsp. sativa*	12	6
55/14	*V. sativa*	*subsp. macrocarpa*	12	6
73/15	*V. sativa*	*subsp. macrocarpa*	12	6
77/15	*V. sativa*	*subsp. macrocarpa*	12	6
49/14	*V. disperma*	-	14	7
80/14	*V. disperma*	-	14	7
79/15	*V. disperma*	-	-	6 and 7
40/16	*V. monardii*	-	14	-
48/16	*V. monardii*	-	12 and 14	6 and7
51/16	*V. monardii*	-	14	-
66/16	*V. monardii*	-	14 and 16	7 and 8
52/16	*V. ochroleuca*	*subsp. atlantica*	14	-
82/14	*V. onobrychioides*	-	14	7
83/15	*V. lutea*	*subsp. vestita*	-	7

index varies from 25.92 to 38.91 and the relative length varies from 109.96 to 167.52 µm. The karyotype formula is 2n=14=2m+12sm (Table 6).

For the intrachmosomal asymmetry M_{CA} and interchromosomal asymmetry CV_{CL}, the values are 39.61 and 14.5, respectively (Table 7).

The idiogram was illustrated on the basis of the centromeric index and arranged in descending order of chromosome size (Figure 2).

Vicia onorychioides

For the polymorphic species *Vicia onobrychioides,* the somatic chromosome number *is* 2n =2x=14. It is a new number observed in this species (figure 1).

The shortest chromosome length, the longest chromosome length, the mean chromosome length and the total haploid length are 2.91, 4.55, 3.67and 25.69 µm, respectively.

The centromeric index varies from 22.63 to 38.14 µm, respectively. The relative length varies from113.27 to 177.11. The karyotype formula is 2n =14= 10sm+2m+2st (Table 6).

The values of intrachromosomal asymmetry M_{CA} and interchromosomal asymmetry Cv_{CL} are 39 and 16, respectively.

The idiogram was illustrated on the basis of the centromeric index and arranged in descending order of chromosome size (Figure 2).

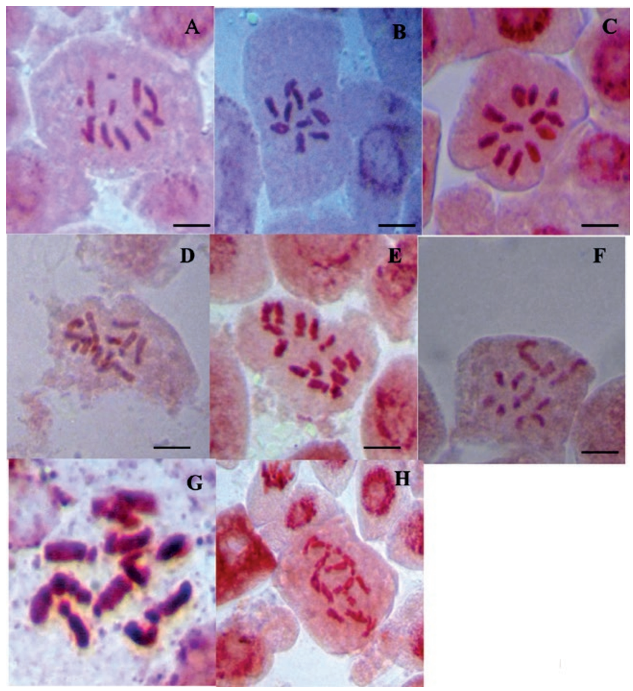

Figure 1. Mitosis observed in natural populations of the genus *Vicia* in Algeria: **A** - *Vicia sativa subsp. sativa* (population 11/14, 2n = 12) ; **B**- *Vicia sativa subsp. sativa* (population 11/14, 2n = 10); **C**- *Vicia sativa subsp. macrocarpa* (population 55/14, 2n = 12); **D**- *Vicia ochroleuca* (population 52/16, 2n = 14); **E**- *Vicia onobrychioides* (population 82/14, 2n = 14); **F**- *Vicia disperma* (population 49/15 , 2n= 14); **G** - *Vicia monardii* (population 48/16, 2n = 12); **H**- *Vicia monardii* (population 66/16, 2n = 16) . Scale bar 5 µm.

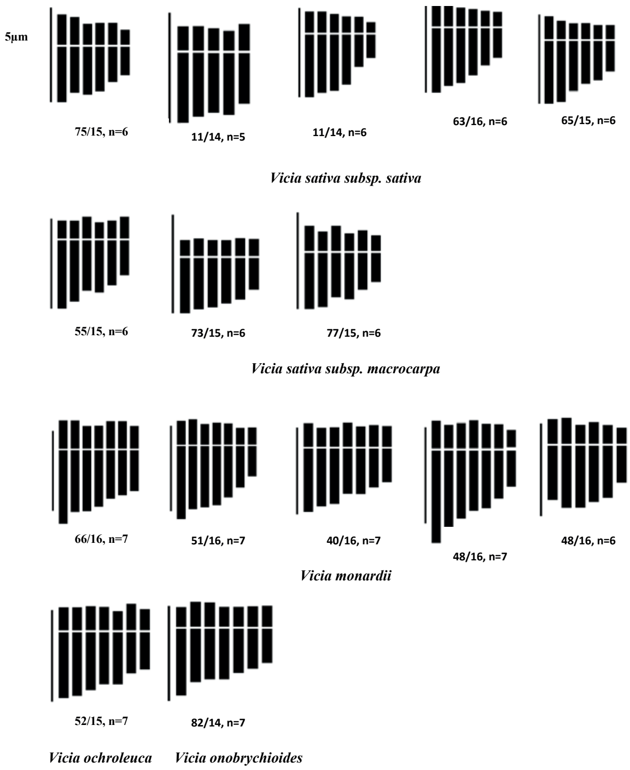

Figure 2. Idiograms of Algerian populations corresponding to four species in the genus *Vicia* L.

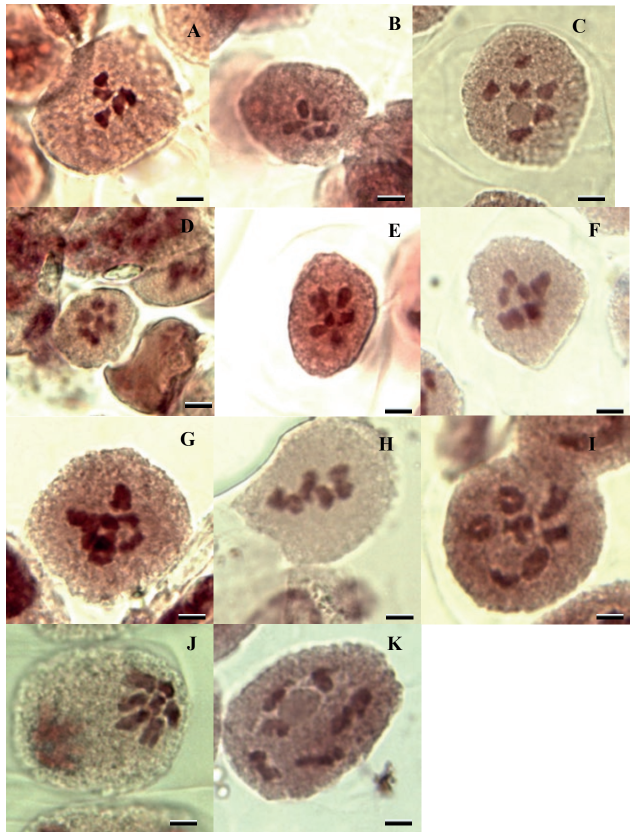

Figure 3. Meiosis observed in natural populations of the genus *Vicia* in Algeria. **A**- *Vicia sativa* subsp. *sativa* (population 11/14, n = 5); **B**- *Vicia sativa* subsp. *sativa* (population 11/14, n = 6); **C**- 55/14 *Vicia sativa* subsp. *macrocarpa* (population 55/14, n = 6); **D**- 48/16 *Vicia monardii* (population 48/16, n =7); **E**- *Vicia monardii* (population 48/16, n = 6); **F**- *Vicia monardii* (population 66/16, n = 7); **G**- *Vicia monardii* (population 66/16, n = 8); **H**- *Vicia disperma* (79/15 , n = 6); **I**- *Vicia disperma* (population 80/14, n = 7); **J**- *Vicia lutea* (population 83/15, n = 7); **K**- *Vicia onobrychioides* (population 82/14 , n = 7) . Scale bar 5 µ.

Vicia disperma

The somatic chromosome number of *Vicia disperma* is 2n =2x=14 for the populations 49/15 and 80/16 (Table 2).

DISCUSSION

The present study showed a high variation in the number, the asymmetry of chromosomes and the karyotype formula within Algerian populations of different species in the genus *Vicia* (*Vicia sativa, Vicia monardii, Vicia disperma, Vicia ochroleuca, Vicia onobrychioides and Vicia lutea).*

The chromosome number of *Vicia sativa. subsp. macrocapa* is 2n=12 for all Algerians populations. These results were in agreement with those of Raina and Rees (1983), Meriç and Dane (1999), Bisht et al. (1998), Raina et al. (2001), Basbag et al. (2013), Osman et al. (2020), Kartal et al. (2020) and Benlioglu (2021). However, Karyotype formula and quantitative analysis are variable among the populations, considered in the present study.

In *Vicia sativa subsp. sativa*, we recorded two chromosome numbers (2n=10 and 12). Similar results were reported by El-Bok et al. (2014) in Tunisian accessions of the same subspecies. According to Raina and Rees (1983), the multi basic chromosome number is a common phenomenon in the genus *Vicia* which was assigned as Rebertsonia translocation. The number (2n=12) has been reported by several authors in different ecotypes of *V. sativa subsp. sativa* (Meriç and Dane 1999; Navratilova et al. 2003; Gaffazardeh-Namazi et al. 2008; El-Bok et al. 2015; Martin et al. 2018; Osman et al. 2020). Ladizinsky and Shefer (1982) revealed that 2n=10 cytotypes were found in secondary and artificial habitats while 2n=12 cytotypes were found in natural vegetation among dwarf shrubs or in the maquis.

Within *Vicia sativa* aggregate, different experiments from several areas revealed that three chromo-

Table 3. The measurement data of chromosome pairs in Algerian populations of *V. sativa subsp. sativa.*

Pop	Pair	L(µm) (±SD)	S (µm) (±SD)	TL (µm)	ΣTL	TLG	RL$^0/_{00}$	r	Ci	Ct	Karyotype formula
	1	3.19(0.24)	1.08(0.26)	4.27			222.97	2.95	25.29	sm	
11/14	2	2.98(0.35)	1.1(0.26)	4.08			213.05	2.7	26.96	sm	
n=5	3	2.7(0.27)	1.07(0.16)	3.77	19.15	3.83	196.86	2.52	28.38	sm	4sm+1st
	4	2.74(0.29)	0.86(0.17)	3.6			188	3.18	23.88	st	
	5	2.29(0.24)	1.14(0.20)	3.43			179.11	2	33.32	sm	
11/14	1	3.5(0.37)	1.27(0.33)	4.77			219.71	2.75	26.62	sm	
n=6	2	3.24(0.11)	1.2(0.30)	4.44			204.51	2.7	27.02	sm	
	3	3.13(0.24)	1.05(0.23)	4.18	21.71	3.62	192.53	2.98	25.11	sm	
	4	2.8(0.61)	0.92(0.21)	3.72			171.34	3.04	24.73	st	5sm+1st
	5	1.8(0.66)	0.9(0.31)	2.7			124.36	2	33.33	sm	
	6	1.3(0.59)	0.6(0.06)	1.9			87.51	2.16	31.57	sm	
63/16	1	3.63(0.53)	1.21(0.11)	4.84			216.45	3	25	sm	
n=6	2	3.25(0.7)	1.18(0.22)	4.43			198.12	2.75	26.63	sm	
	3	3.08(0.32)	1.04(0.27)	4.12	22.36	3.73	184.25	2.96	25.24	sm	6sm
	4	2.65(0.50)	0.96(0.21)	3.61			161.44	2.76	26.59	sm	
	5	2.11(0.26)	0.86(0.17)	2.9			129.69	2.45	29.65	sm	
	6	1.68(0.30)	0.78(0.17)	2.46			110.01	2.15	31.7	sm	
65/15	1	3.49(0.62)	1.28(0.29)	4.77			220.53	2.72	26.83	sm	
n=6	2	3.39(0.69)	1.04(0.19)	4.43			204..8	3.25	23.47	st	
	3	2.83(0.65)	0.9(0.28)	3.73	21.63	3.61	172.44	3.14	24.12	st	4sm+2st
	4	2.37(0.62)	0.91(0.26)	3.28			151.64	2.6	27.74	sm	
	5	2.18(0.67)	0.77(0.12)	2.95			136.38	2.83	26.1	sm	
	6	1.69(0.54)	0.78(0.16)	2.47			114.19	2.16	31.57	sm	
75/15	1	2.92(0.51)	1.61(0.42)	4.53			217.68	1.81	35.54	sm	
n=6	2	2.43(0.21)	1.46(0.42)	3.89			186.92	1.66	37.53	m	
	3	2.53(0.36)	1.11(0.35)	3.64	20.81	3.47	174.91	2.27	30.49	sm	
	4	2.36(0.24)	1.16(0.26)	3.52			169.14	2.03	32.95	sm	4sm+2m
	5	1.85(0.11)	1.16(0.39)	3.01			144.64	1.59	38.53	m	
	6	1.47(0.20)	0.75(0.11)	2.22			106.68	1.96	33.78	sm	

Abreviations: long arm length (L), short arm length (S), total chromosome length (TL), mean value of total length (TLG), relative length (RL), arm ratio (r), centromeric index (Ci), chromosome type (Ct), median(m), submedian (sm), subterminal(st), standard deviation (SD).

some number (2n=10,12 and 14) were reported by several authors (Ladiznsky 1978; Yamanoto and Plitman 1980; Frediani et al. 2004; Arslan 2012; El -Bok et al. 2015; Martin et al. 2018; Kartal et al. 2020). However, the most reported chromosome number is 2n=12 (Ladizensky 1978; Ladizensky and Temkin 1978).

Very little work has been done on the species *Vicia monardii*. The present study follows and completes previous preliminary work (mitosis) carried out by our research team (Melzi 2018) on the same Algerian populations belonging to *Vicia monardii*. The preliminary results of mitosis (2n=12, 14 and 16) were confirmed through the present study and completed by the study of meiosis, indicating the effective presence of three chromosomes numbers (n=6, 7 and 8) in *Vicia monardii*, confirming that the observed chromosomes are indeed of type A.

Furthermore, in *Vicia sativa* and *Vicia monardii*, the chromosome numbers 2n=10 and 2n=12 were found only in populations originating from regions of low altitude and relatively high rainfall, whereas the number 2n=16 was found in populations originating from regions of high altitude and relatively low rainfall. Therefore, the variability of the chromosome number within and between the populations observed in *Vicia sativa* and *Vicia monardii*, would be linked to the ecological factors (altitude, rainfall) of the environment of origin of the considered populations.

In *Vicia onobrychioides* and the endemic subspecies *V. ochroleuca subsp. Atlantica*, the chromosome counts

Table 4. The measurement data of chromosome pairs in Algerian populations of *V. sativa subsp. macrocapa.*

Pop	Pair	L(µm) (±SD)	S(µm) (±SD)	TL (µm)	ΣTL	TLG	RL$^{0}/_{00}$	r	Ci	Ct	Karyotype formula
73/15	1	2.74(1.94)	0.78(0.13)	3.52	18.49	3.08	190.37	3.51	22.15	st	2sm+4st
n=6	2	2.6(0.62)	0.84(0.07)	3.44			186.04	3.09	24.41	st	
	3	2.44(0.52)	0.75(0.09)	3.19			172.52	3.25	23.51	st	
	4	2.26(0.31)	0.73(0.13)	2.99			161.7	3.09	24.41	st	
	5	2.1(0.57)	0.81(0.15)	2.91			157.38	2.59	27.83	sm	
	6	1.57(0.39)	0.87(0.23)	2.44			131.96	1.8	35.65	sm	
55/14	1	3.82(0.53)	1.03(0.33)	4.85	23.6	3.93	205.5	3.7	21.23	st	1m+2sm+3st
n=6	2	3.38(0.15)	1.02(0.28)	4.4			186.44	3.31	23.18	st	
	3	2.8(0.42)	1.16(0.30)	3.96			167.79	2.41	29.29	sm	
	4	2.9(0.31)	0.94(0.15)	3.84			162.71	3.08	24.47	st	
	5	2.45(0.38)	0.99(0.21)	3.44			145.76	2.47	28.77	sm	
	6	1.95(0.43)	1.16(0.38)	3.11			131.77	1.68	37.92	m	
77/15	1	2.94(0.50)	1.25(0.42)	4.19	20.27	3.38	206.7	2.35	29.83	sm	6sm
n=6	2	2.82(0.25)	1.03(0.25)	3.85			189.93	2.73	26.75	sm	
	3	2.32(0.41)	1.34(0.36)	3.66			180.56	1.73	36.61	sm	
	4	2.43(0.40)	0.94(0.16)	3.3			162.8	2.58	28.48	sm	
	5	1.96(0.41)	1.06(0.23)	3.02			148.99	1.84	35.1	sm	
	6	1.44(0.45)	0.81(0.11)	2.25			111	1.77	36	sm	

Abreviations: long arm length (L), short arm length (S), total chromosome length (TL), mean value of total length (TLG), relative length (RL), arm ratio (r), centromeric index (Ci), chromosome type (Ct), median(m), submedian (sm), subterminal(st), standard deviation (SD).

(mitoses) showed, for the first time, 2n=14. Bolkhoskik et al. (1974) reported a chromosome number of 2n =12 for *Vicia ochroleuca* and *Vicia onobrychioides*.

In the Algerian populations of *Vicia disperma* and *Vicia lutea*, the haploid chromosome number recorded is n= 7. This result is in agreement with the reports of Choii (1971) and El Allaoui- Faris (2011), concerning these same species. However, our study highlighted also a new number (n=6), reported for the first time in *Vicia disperma*.

The diploid number (2n =14) was recorded by several authors in *Vicia disperma* and *Vicia lutea* (Raina and Ress 1983; Jauzein1995; Venora et al. 2008; Bas Bag et al. 2013).

Through this study, we found that the number 2n=14 is more frequent in Algerian populations of the genus *Vicia* L. According to Holling and Satce (1974) and Tabour et al. (2002), 2n=14 is the most common chromosome number in the genus *Vicia* L. Thus, Raina and Ress (1983) indicated that the chromosome number 2n=14 is the most primitive in the genus *Vicia* and reported that the numbers 2n=10 and 2n=12 appeared later, by chromosomal rearrangement.

Concerning the chromosome size of the *Vicia sativa subspecies,* our results (1.9 - 4.84 µm) are very similar to those recorded by Benlioglu (2021) in Turkey (1.68-4.88 µm). However, they are relatively lower than the results reported by El-Bok et al. (2014) and Gaffazardeh-Namazi et al. (2008) on Tunisian (1.71 - 6 µm) and Iranian accessions (2.89 - 5. 69 µm) within the same subspecies.

Concerning the chromosome size observed in *V. sativa subsp. macrocarpa* (2.25 - 4.85 µm), it appears to be lower than that reported by Osman et al. (2020) (7.9 - 15.71 µm) and similar to that found by Benlioglu (2021), in some wild populations of the same species, in Turkey (2.54 - 4.98 µm).

Within the Algerian populations of the genus *Vicia* L., the chromosome size of the populations is relatively different from one species to another. According to their size, we can classify the chromosomes as follows: *Vicia sativa, Vicia onobrychioides, Vicia ochroleuca* and *Vicia monardii. Vicia sativa* presents the smallest chromosome size and *Vicia monardiii* is characterised by the largest one. Akpinar and Bilaloglu (1997) signalized that subspecies of *Vicia sativa* have smaller chromosomes and a lower DNA content than other species of the genus *Vicia*.

Exception made for *Vicia sativa*, detailed chromosome measurements and degrees of karyotype asymmetry, indicated through the present study, would be determined, for the first time, in some species (*Vicia monardii, Vicia ochroleuca and Vicia onobrychioides*).

Table 5. The measurement data of chromosome pairs in Algerian populations of *Vicia monardii.*

Pop	Pair	L(µm) (±SD)	S(µm) (±SD)	TL (µm)	ΣTL	TLG	$RL^{0}/_{00}$	r	Ci	Ct	Karyotype formula
66/16	1	4.60(1.00)	1.77(0.5)	6.37			179.43	2.59	27.78	sm	
n=7	2	3.86(0.89)	1.76(0.40)	5.62			185.3	2.19	31.31	sm	
	3	3.82(0.62)	1.42(0.26)	5.24			123.18	2.69	27.1	sm	
	4	3.54(0.81)	1.44(0.44)	4.98	35.5	5.07	144.68	2.45	28.91	sm	6sm+1m
	5	3.04(0.54)	1.72(0.30)	4.76			138.29	1.76	36.13	sm	
	6	2.82(0.70)	1.72(0.74)	4.54			131.9	1.63	37.88	m	
	7	2.56(0.53)	1.42(0.34)	3.98			115.63	1.8	35.67	sm	
51/16	1	4.26(0.86)	1.38(0.36)	5.64			184.07	3.08	24.46	st	
n=7	2	3.69(0.41)	1.48(0.32)	5.17			68.73	2.49	28.62	sm	
	3	3.56(0.47)	1.2 (0.25)	4.76			155.35	2.96	25.21	sm	
	4	3.44(0.46)	1.27(0.54)	4.71	30.6	4.37	153.72	2.70	26.96	sm	6sm+1st
	5	2.98(0.17)	1.23(0.22)	4.21			137.40.	2.42	29.21	sm	
	6	2.41(0.54)	0.98(0.27)	3.39			110.63	2.45	28.9	sm	
	7	1.76(0.31)	1.00(0.09)	2.76			90.08	1.76	36.23	sm	
40/16	1	3.66(0.54)	1.35(0.49)	5.01			177.91	2.71	26.94	sm	
n=7	2	3.34(0.78)	1.08(0.20)	4.42			156.69	3.09	24.43	st	
	3	3.20(0.56)	1.11(0.15)	4.31			153.05	2.88	25.75	sm	
	4	2.62(0.6)	1.38(0.60)	4.00	28.2	4.02	142.04	1.89	34.5	sm	5sm+1m+1st
	5	2.63(0.32)	1.16(0.34)	3.79			134.58	2.26	30.6	sm	
	6	2.25(0.68)	1.24(0.13)	3.49			123.93	1.81	35.53	sm	
	7	1.95(0.28)	1.19(0.14)	3.14			111.5	1.63	37.9	m	
48/16	1	4.80(0.61)	1.44(0.04)	6.24			196.29	3.33	23.07	st	
n=7	2	4.01(0.56)	1.23(0.03)	5.24			164.83	3.26	23.47	st	
	3	3.53(0.47)	1.33(0.28)	4.86			152.88	2.65	27.36	sm	
	4	3.13(0.06)	1.46(0.12)	4.59	31.8	4.54	144.38	2.14	31.8	sm	5sm+2st
	5	2.99(0.34)	1.29(0.23)	4.28			134.63	2.31	30.14	sm	
	6	2.51(0.33)	1.27(0.13)	3.80			119.53	1.97	33.42	sm	
	7	1.88(0.87)	0.90(0.28)	2.78			87.45	2.08	32.37	sm	
48/16	1	2.92(0.25)	1.33(0.25)	4.23			174.36	2.18	31.44	sm	
n=6	2	3.35(0.37)	1.41(0.21)	4.76			196.20	2.37	29.62	sm	
	3	3.34(0.27)	1.03(0,13)	4.37	24.3	4.04	180.13	3.24	23.56	st	5sm+1st
	4	3.03(0.15)	1.17(0.05)	4.2			173.12	2.58	27.85	sm	
	5	2.82(0.08)	1.02(0.18)	3.84			158.28	2.76	26.56	sm	
	6	2.00(0.50)	0.86(0.07)	2.86			117.88	2.32	30.07	sm	

Abreviations: long arm length (L), short arm length (S), total chromosome length (TL), mean value of total length (TLG), relative length (RL), arm ratio (r), centromeric index (Ci), chromosome type (Ct), median(m), submedian (sm), subterminal (st), standard deviation (SD).

The presence of different karyotypes formulas within Algerians populations of the genus *Vicia* may be due to the ecological differences characterizing their geographic origins. According to Benlioglu (2021), the changes in the structure of chromosome morphology can be explained as a gradual alteration which occurred through the evolution of the karyotype during natural or manual selection.

In *Vicia sativa subsp. sativa*, the karyotype formulas reported in the present study, are different from those reported by other authors: 1m +5 st (Namazi et al. 2008; El- Bok et al. 2014), 1m+1sm+3st (El -Bok et al. 2014), 3 m +3st (Osman et al. 2020), 3m+3sm, 2m+3sm and 2sm+4st (Benlioglu 2021), but similar (5sm+1st) to that reported by Martin et al. (2018).

The chromosomes observed in Algerian populations of *genus Vicia* were mainly submetacentric. According to Zuo and Yuan (2011), the predominance of submetacentric chromosomes indicated that these populations might have retained some of their primitive wild traits.

Table 6. The measurement data of chromosomes pairs in Algerian populations of *Vicia onobrychioides* and *Vicia ochroleuca subsp. atlantica.*

Pop	Species		BL(µm) (±SD)	BC(µm) (±SD)	LT	ΣLT	TLG	RL$^0/_{00}$	R	Ci	Ct	Karyotype formula
52/16		1	3.60(0.58)	1.26(0.19)	4.86			167.52	2.85	25.92	sm	
n=7		2	3.48(0.63)	1.26(0.05)	4.74			163.39	2.76	26.58	sm	
		3	3.17(0.69)	1.32(0.27)	4.49			154.77	2.4	29.39	sm	
	V. ochroleuca subsp. atlantica	4	2.85(0.38)	1.27(0.24)	4.12	29.01	4.14	142.01	2.24	30.82	sm	6sm+1m
		5	2.86(0.51)	1.05(0.20)	3.91			134.78	2.72	26.85	sm	
		6	2.26(0.43)	1.44(0.17)	3.70			127.54	1.56	38.91	m	
		7	2.03(0.27)	1.6(0.09)	3.19			109.96	1.75	36.36	sm	
82/14		1	3.52(0.82)	1.03(0.28)	4.55			177.11	3.41	22.63	st	
n=7		2	2.81(0.42)	1.3(0.30)	4.11			159.98	2.16	31.63	sm	
		3	2.66(0.34)	1.28(0.17)	3.94			153.36	2.07	32.48	sm	
	V. onobrychioides	4	2.66(0.38)	1.04(0.21)	3.70	25.69	3.67	144.02	2.55	28.10	sm	5sm+1m+1st
		5	2.33(0.21)	1.04(0.20)	3.30			128.45	2.24	31.51	sm	
		6	2.10(0.21)	1.08(0.11)	3.18			123.97	1.94	33.96	sm	
		7	1.8(0.18)	1.11(0.15)	2.91			113.27	1.62	38.14	m	

Abreviations: long arm length (L), short arm length (S), total chromosome length (TL), mean value of total length (TLG), relative length (RL), arm ratio (r), centromeric index (Ci), chromosome type (Ct), median(m), submedian (sm), subterminal (st), standard deviation (SD).

Table 7. The asymmetry index values in natural populations of *Vicia* species in Algeria.

Pop	Species	2N	Ask	TF	Syi	Rec	A_1	A_2	A	CV_{cl}	M_{CA}
11/14	*V. sativa subsp. sativa*	10	72.58	27.41	37.77	89.69	0.47	0.09	0.45	9	45.16
11/14	*V. sativa subsp. sativa*	12	72.63	27,36	37.8	75.85	0.15	0.3	0.45	30	45.02
63/16	*V. sativa subsp. sativa*	12	73.34	26.97	36.81	76.99	0.14	0.24	0.46	24	46.38
65/15	*V. sativa subsp. sativa*	12	73.74	26.25	35.47	75.57	0.53	0.24	0.47	24	47.63
75/15	*V. sativa subsp. sativa*	12	65.16	34.83	53.09	76.56	0.4	0.22	0.3	22	30.63
55/14	*V. sativa subsp. macrocarpa*	12	73.3	26.69	36.45	81.09	0.47	0.16	0.46	16	45.56
73/15	*V. sativa subsp. macrocarpa*	12	74.14	21.14	34.21	87.55	0.61	0.13	0.49	13	49.01
77/15	*V. sativa subsp. macrocarpa*	12	68.62	31.72	46.32	80.62	0.46	0.2	0.36	20	36.68
40/16	*V. monardii*	14	69.77	30.22	43.21	78.88	0.39	0.15	0.39	15.40	39
48/16	*V. monardii*	12	71.88	28.11	38.96	84.94	0.43	0.16	0.44	16.00	44
48/16	*V. monardii*	14	71.87	28.05	38.95	72.77	0.86	0.24	0.44	24.22	44
51/16	*V. monardii*	14	72.12	27.87	38.73	77.60	0.39	0.23	0.44	23.00	44
66/16	*V. monardii*	14	68.44	31.7	46.1	79.61	0.20	0.16	0.36	16.40	36
82/14	*V. onobrychioides*	14	69.59	30.67	43.92	80.65	0.44	0.16	0.39	16	39
52/16	*V. ochroleuca subsp. atlantica*	14	69.8	30.19	43.25	85.27	0.37	0.14	0.39	14.5	39.61

Abreviations: karyotype asymmtry index(Ask), total form percentage (TF), the index of karyotype symmetry (Syi), the symmetric index (Rec), the intrachromosomal asymmetry index (A_1), interchromosomal asymmetry index(A_2), degree of asymmetry of karyotype (A), coefficient of variation of the centromeric index (CV_{CL}), mean centromeric asymmetry (M_{CA}).

The predominance of subtelocentric chromosomes in *V. sativa subsp. macrocarpa* populations (73/15 and 55/14) indicated asymmetrical Karyotypes. According to Hanelt and Mettin (1989), the subtelocentric chromosomes are predominant in the subgenus *Vicia* and it may have an evolutionary significance.

All the chromosomes of the population 77/15 coresponding to *V.sativa subsp. macrocarpa* are sub median. According to Paszko (2006), the karyotype of the last population *V. sativa subsp. macrocarpa* is considered as symmetrical.

Several authors (Maxed et al. 1991; Kamel 1999; Weber and Shifino-Wittman 1999; Navratilova et al. 2003) demonstrated that *Vicia sativa subsp. sativa* is the only subspecies of *Vicia sativa* that has a metacentric pair in its Karyotype. However, our study showed the presence of one pair of metacentric chromosomes in *Vicia sativa subsp. macrocarpa* (population n° 55/14). This type of chromosome is also indicated by El Bok et al (2014), Osman et al. (2020) with 3m+2sm+1st and Benlioghlu (2021) with 4sm+2st and 3 m +3sm.

In *Vicia monardii*, the symmetrical karyotype is represented by the population 66/16, while asymmetrical karyotypes characterize the populations 40/16, 48/16 and 51/16 with a predominance of subtelocentric chromosomes.

The study of karyotype asymmetry is one of the most important parameters in the karyomorphology (Astuti et al. 2017; Shamsolshoara et al. 2020; Martin et al. 2018). The intrachromosomal asymmetry gradually increases with centromere shift from median point to terminal point, while the interchromosomal asymmetry increases with more chromosome size heterogeneity (Martin et al. 2018).

In the present study, karyotype asymmetry was assessed on the basis of quantitative indices. The values of some indices (M_{CA}, $CV_{cl,}$ Ask %, A_1, A_2 and A) increase with increasing asymmetry while the values of some indices (TF%, Syi and Rec) decrease with increasing asymmetry (Zuo and Yuan 2011; Eroglu et al. 2013; Atlay et al. 2017).

Based on the following asymmetry indices: M_{CA}, AsK, TF, Syi and A, the population 75/15 (*V. sativa ssp. sativa*) presents the most symmetrical karyotype, while the population 73/15 (*V. sativa subsp. macrocarpa*) is characterized by the most asymmetrical one. However, the asymmetric karyotypes are different in interchromosomal asymmetries. The population 11/14 (2n=10) (*V. sativa subsp. sativa*) is the most symmetrical karyotype with respect to three indices (CV_{CL}, Rec and A2), whereas the population 48/16 (2n=14) corresponding to *Vicia monardii* species, is the most asymmetrical karyotype, with respect to only two indices (Rec and A_2).

The results of the karyotype asymmetry index analysis showed that the chromosomes of the studied species in the genus *Vicia* vary from median to subterminal. The karyotypes vary from symmetrical to asymmetrical and the intrachromosomal asymmetry was higher than interchromosomal one. These results were in agreement with the reports of Martin et al. (2018) and Benlioglu (2021), within the genus *Vicia*.

According to Kamel et al. (1999), the evolution of Karyotype might be inferred from symmetry to asymmetry as a result of pericentric inversion or unequal translocation.

The differences in the asymmetry of karyotype were great in the genus *Vicia*, for which it may be assumed that diversity of the genus has been accompanied by very small changes in the structure of the chromosome (El-Bok et al. 2014). According to Altay et al. (2007), the difference in chromosome morphologies may contribute to the variation of the genera, sections and species.

In addition, a number of studies have been carried out, using different methodological approaches to gain a better understanding of the complex phylogenetic relationships between the different species of the *Vicia* genus.

Thus, our results indicated that x = 7 is the most frequently observed number in the six *Vicia* species. Previous work has confirmed that x = 7 is the ancestral number of the genus *Vicia* (Shiran et al. 2014). For their part, Metin and Hanelt (1964) hypothesised that x = 7 is the most likely chromosome number in the genus *Vicia* and the numbers x= 6 and x= 5, observed in some species, are derived base numbers. Other authors think that x = 5 is the basic number and x = 6 and 7 are derived numbers (Schubert et al. 1986).

From a phylogenetic point of view, the results of Schaefer et al. (2012) showed that the phylogenetic relationships between species in the genus *Vicia* are as follows: In section *Cracca*, *Vicia disperma* is closely related to *Vicia ochroleuca* and *Vicia monardii*, but the degree of relationship between *Vicia disperma* and *Vicia ochroleuca* is less than that found between *Vicia disperma* and *Vicia monardii*; these two species (*Vicia disperma* and *Vicia monardi*) are the most closely related species.

In the *Sativa* section, *Vicia sativa* is distant from *Vicia lutea*, but it is closely related to *Vicia angustifolia*. On the other hand, *Vicia onobrychioides* in the *Pedunculatea* section is distant from all the other studied species in the *Cracca* and *Vicia* sections (Schaefer et al. 2012).

According to Shiran et al. (2014), *V. sativa subsp. sativa* is distant from *V. sativa subsp. macrocarpa* but it is closely related to *V. sativa subsp. angustifolia* and these two subspecies (*subsp. sativa and subsp. macrocarpa*) are distant from *Vicia lutea* and *Vicia disperma*.

Shiran and Raina (2001) and Shiran et al. (2014) revealed that within the *Vicia sativa* complex two lineages are evident in all phylograms. Lineage 1 consists of *V. sativa subsp. macrocarpa* and *V. sativa subsp. angustifolia*, while lineage 2 includes *V. sativa subsp. sativa*, *V. sativa subsp. cordata, V. sativa subsp. amphicarpa, V. sativa subsp. incisa and V. sativa subp. nigra* (Shiran and Raina 2001; Shiran et al. 2014).

More recently, results have described a close relationship between *Vicia macrocarpa* and *Vicia narbonensis* (Osman et al. 2020).

CONCLUSION

The present study highlighted the characteristics of the chromosomes in the natural populations belonging to six (06) species (*Vicia sativa*, *Vicia disperma*, *Vicia monardii*, *Vicia ohchroleuca*, *Vicia onobrychioides* and *Vicia lutea*) of the genus *Vicia,* coming from different eco-geographical zones of North-Eastern Algeria, and the relationships which would be exist with some ecological factors of the environment of origin (altitude and rainfall).

The results carried out in mitosis and meiosis, showed the presence of some new chromosomal numbers in Algerian populations of the genus *Vicia* L. The first number (2n=14, n=7) was observed for the first time in the endemic subspecies *Vicia ochroleuca subsp. atlantica* (Population n° 52/16) and the species *Vicia onobrychioides* (Population n° 82/14). The second number (n=6) was observed in *Vicia disperma* (Population n° 79/15).

In *Vicia monardii*, the three new numbers of chromosomes previously observed in mitosis (2n=12, 14 and 16), were confirmed through the study of meiosis (n=6, 7 and 8), indicating that they are indeed A-type chromosomes.

This study has shown that the base number x=7 is the most frequently observed number in the six *Vicia* species.

The observations showed that chromosome numbers 2n=10 and 2n=12 are more frequently encountered within populations located in regions of relatively low altitude and relatively high rainfall, respectively in the two species *Vicia sativa* and *Vicia monardii*. The number 2n =16 is only found in populations of *Vicia monardii* originating from high altitude and relatively low rainfall regions.

Exeption made for *Vicia sativa*, detailed chromosome measurements and degrees of karyotype asymmetry would be determined for the first time in the following species: *Vicia monardii, Vicia ochroleuca and Vicia onobrychioides.*

The karyological variations observed in Algerian populations corresponding to some species of the genus V*icia* are clearly detectable in the chromosomal morphologies. The chromosomes vary from median to subterminal and the karyotype varies from symmetrical to asymmetrical. Ecological conditions, in particular the altitude factor of the geographical origin of the populations, would have an effect on the changes in chromosome structure.

This research is a contribution to the evaluation and valorization of plant genetic resources in Algeria, particularly in the genus *Vicia* L. The analysis of chromosomal diversity, based on new data, allowed to answer some questions related to the mechanism of evolution of the species belonging to the genus *Vicia* L. The characterization carried out could play an important role in the conservation and use of these genetic resources through a plant breeding programme.

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