

Full Length Research Paper

The morphology, chromosome number and nuclear DNA content of Tunisian populations of three *Vicia* species

Samiha Kahlaoui^{1*}, David J. Walker², Enrique Correal², Pedro Martínez-Gómez³, Hamadi Hassen⁴ and Sadok Bouzid¹

¹Laboratoire de Biologie végétale, Faculté des Sciences de Tunis, Campus Universitaire, 1060 Tunis, Tunisia.

²Departamento de Recursos Naturales: Instituto Murciano de Investigación y Desarrollo Agrario y Alimentario (IMIDA), Estación Sericícola, Calle Mayor s/n, La Alberca, 30150 Murcia, Spain

³Departamento de Mejora Vegetal, Centro de Edafología y Biología del Segura, CSIC, Apartado 4195, 30150 Murcia, Spain.

⁴Institut National de la Recherche Agronomique de Tunisie. Rue Hédi Karray, 2049-Ariana, Tunisia.

Accepted 5 June, 2009

The aim of this work was to determine, for Tunisian populations (wild and cultivated) of *Vicia sativa*, *V. villosa* and *V. narbonensis*, whether differences in chromosome number, nuclear DNA content or morphology exist among the populations; in the case of *V. sativa*, with respect to a commercial cultivar from Spain and, in the case of *V. villosa*, with respect to two accessions from Aleppo (Syria). The idea was to identify variation that could be exploited for agronomic purposes. For the study, nine populations (3 per species) were compared regarding 12 morphological characters. The three species differed significantly with respect to the majority of the characters such as leaf area, node number, ramification, length of the most developed axis, leaf length, and the number of leaflets and inflorescences per plant. The comparison of these characters within each species revealed an intra-population polymorphism especially for *V. sativa* and *V. narbonensis*. For *V. villosa*, the three populations appeared homogeneous for the majority of studied phenotypical characters, independent of their origin. The polymorphism detected in the species seems to depend on altitude and pedoclimatic factors. All the studied populations were diploid, with $2n = 2x = 12$ for *V. sativa* and $2n = 2x = 14$ for *V. villosa* and *V. narbonensis*. The mean 2C DNA contents were 3.67 - 3.79 pg for *V. sativa*, and 12.83-13.17 pg for *V. narbonensis*: significant differences ($P < 0.001$) among the populations were observed only for *V. villosa*, the mean 2C nuclear amounts being 3.72 pg and 3.80 for the 2 Syrian populations and 4.17 pg for the population from Tunisia. The DNA content correlated significantly and negatively with the parameters related to the growth rate (number of branches and nodes at 10, 20 or 30 days after germination), indicating that some populations, of smaller genome size and faster growth, are adapted to sites having shorter growing seasons.

Key words: *Vicia*, Tunisia, morphology, nuclear DNA, populations, ploidy.

INTRODUCTION

The genus *Vicia* comprises about 160 species (Allkin et al., 1986) which are widely distributed in the temperate zone of both hemispheres. It has considerable economic importance (Akpınar and Bilaloğlu, 1997); many *Vicia*

species are cultivated for food and fodder (Cremonini et al., 1998). The three species studied in this report, *V. sativa* L., *V. villosa* Roth and *V. narbonensis* L., are cultivated in many countries like Spain, Turkey, Jordan, Syria and Iraq (Siddique et al., 1996; Caballero et al., 2001) for their high-quality fodder and protein-rich seeds (28 to 32%). These species are cultivated in Tunisia in different bioclimatic areas and on different substrata they are very

*Corresponding author. E-mail: Sameh_kahlaoui@yahoo.fr.

Table 1. Features of the original sites of the selected plant material.

Species	Code	1000 seed weight (g)	Country	Rain (mm)	Altitude (m a.s.l.)	Co-ordinates
<i>V. sativa</i> Var commune	Vs1	71.5	Tunisia	468	210	36°49' N, 10°11' E
<i>V. sativa</i> Var vereda	Vs2	70.1	Spain	nd	nd	37°11' N, 3°36' W
<i>V. sativa</i> Var INRAT 303	Vs3	66	Tunisia	855	10	36°57' N, 8°45' E
<i>V. villosa</i> Acc 2565	Vv1	36.9	Syria	342	362	35°55' N, 36°55' E
<i>V. villosa</i> Var Sejnene	Vv2	24.8	Tunisia	1030	5	37°04' N, 9°13' E
<i>V. villosa</i> Acc 3615	Vv3	52.6	Syria	342	362	35°55' N, 36°55' E
<i>V. narbonensis</i> Var P1	Vn1	251.2	Tunisia	470	100	37°03' N, 10°01' E
<i>V. narbonensis</i> Var P3	Vn2	124.3	Tunisia	378	50	37°03' N, 9°06' E
<i>V. narbonensis</i> Acc 545	Vv3	114.8	Tunisia	600	10	36°43' N, 9°11' E

Table 2. Morphological characteristics used to describe the variability of the three *Vicia* species.

Measured characters	Symbol
Leaf surface area (cm ²)	SF
Number of nodes 10 days after germination	N1
Number of nodes 20 days after germination	N2
Number of nodes 30 days after germination	N3
Number of branches 10 days after germination	R1
Number of branches 20 days after germination	R2
Number of branches 30 days after germination	R3
Length of the main stem (cm)	LT
Length of the leaf (cm)	LF
Number of leaflets per leaf	NF
Number of flowers per plant	IN
Dry matter (g)	DM

appreciated by animals (Hassen et al., 1994). They represent, in association with oats (*Avena sativa* L), the most important fodder cultivated in northern Tunisia (170000 ha).

Genetic diversity studies of *Vicia* species, based, in general, on morphological and physiological characters, provide information about variability between species and populations (Kupicha, 1976; Van de Wouw et al., 2003a) and form the basis of breeding programmes. Many species of *Vicia* have been the object of karyological, cytogenetic and molecular genetic studies (Chooi, 1971; Sinha and Das, 1985; Narayan et al., 1985; Navrátilová et al., 2003). For the three vetch species of interest to us, only diploid plants have been reported previously: $2n = 2x = 10$, 12 or 14 for *V. sativa* (Kawakami, 1930; Yamamoto, 1959; Chooi, 1971; Raina and Bisht, 1988; Akpinar and Bilaloğlu, 1997; Navrátilová et al., 2003), $2n = 2x = 14$ for *V. villosa* (Senn, 1938; Chooi, 1971; Raina and Bisht, 1988; Yeater et al., 2004) and $2n = 2x = 14$ for *V. narbonensis* (Chooi, 1971; Raina and Bisht, 1988; Navrátilová et al., 2003), but none of these reports included Tunisian populations. There seems to be considerable intra-specific variation in the 2C nuclear DNA contents of the *Vicia* species

studied here. The ranges of reported values are 3.8-6.0 pg for *V. sativa* (Chooi, 1971, Raina and Bisht, 1988; Akpinar and Bilaloğlu, 1997; Navrátilová et al., 2003), 4.1 - 6.3 pg for *V. villosa* (Chooi, 1971, Raina and Bisht, 1988) and 14.3-16.1 pg for *V. narbonensis* (Chooi, 1971, Raina and Bisht, 1988; Frediani et al., 1992).

In spite of their domestic economic importance, Tunisian species of vetch have not been the object of real breeding programmes, because the necessary studies of morphological and genetic variation have not been performed. The aim of this work was to compare the morphology, genome size and the ploidy level of *V. sativa*, *V. villosa* and *V. narbonensis* from different sites in Tunisia. These parameters would also be compared with commercial cultivars from Spain (*V. villosa*) or Syria (*V. sativa*), to allow elucidation of the relationships between ploidy, morphology and edapho-climatic conditions and of any outstanding characteristics possessed by the Tunisian populations which could be exploited for agronomic purposes.

MATERIALS AND METHODS

Plants from three *Vicia* species were analysed: *V. sativa* (sub-species *sativa*), *V. villosa* (sub-species *villosa*) and *V. narbonensis* (sub-species *villosa*). The study involved nine populations, three for each species; Tunisian populations were obtained from the gene bank of INRAT (Tunis, Tunisia), and the study included also one Spanish cultivar of *V. sativa* (cv. Vereda), and 2 accessions of *V. villosa* from ICARDA (Syria). The main features of this plant material are shown in Table 1.

The seeds were scarified mechanically and put to germinate in Petri dishes (30 per dish), in a growth chamber at 25°C in the dark. On October, 15 2004 seedlings were transplanted to an experimental field of the INRAT, in plastic pots, as a randomised block of three replicates. For each population belonging to a particular species, each repetition contained 20 plants. The maximum and minimum temperatures at this site (altitude = 210 m a.s.l.) were 30.1°C and 5.4°C, respectively. The soil has a pH of 7.8. Twelve characters were chosen to study the genetic variability within the three species and their respective populations. These characters are shown in Table 2. At harvest, plants were 6 months-old.

For chromosome counting, seeds were sown on moist filter paper in Petri dishes at a temperature of 25°C and a photoperiod of 16 h.

These studies were carried out in meristematic cells of root tips (1 cm in length). Root tips were collected and incubated in water for 4 h at 0°C, pre-treated with colchicines (0.2%) for 3 h at 5°C and fixed in 3:1 ethanol/acetic acid for 24 h at 4°C. Root tips were hydrolysed in 1N HCl at 60°C, for 20 min. For the staining, root tips were immersed in Schiff's reagent for 2 h.

Observations were achieved using a Nikon® Eclipse EG00 microscope. Image-processing was performed with the aid of the Sut-hot® programme. At least five slides were observed for each seedling.

For flow cytometry, seeds of the nine populations were sown in a controlled-environment chamber with a photoperiod of 12 h, a day/night temperature of 27/22 °C and a relative humidity of 50-80%. For all populations, six plants grown under these conditions were analysed: one measurement was conducted on each of six days of analysis. Soya (*Glycine max* L.) (2C nuclear DNA content = 2.50 pg; Doležel et al., 1994) was chosen as the internal standard for the determination of DNA content for *V. sativa* and *V. villosa*, whereas for *V. narbonensis* the internal standard was rye (*Secale cereale*) (2C nuclear DNA content = 16.19 pg; Lysák and Doležel, 1998). Samples of vetch leaf tissue were chopped (at 4°C) with the appropriate standard (*Vicia*: standard, 3:1 by area) for 30 - 60 s in a 60-mm-diameter, plastic Petri dish containing 0.4 ml extraction buffer (Partec cystain PI Absolute P Nuclei Extraction Buffer; Partec GmbH, Münster, Germany). To the extraction buffer were added ascorbic acid, dithiothreitol, polyvinylpyrrolidone-10 and Triton X-100, to give final concentrations of 6 mM, 5 mM, 0.1% and 12.5 g l⁻¹, respectively. The resulting extract was through a 30 µm filter into a 3.5 ml tube (on ice), to which was added 1.6 ml of Partec Cystain PI Absolute P staining buffer, to give final propidium iodide (PI) (the fluorescent stain) and RNase concentrations of 50 µg ml⁻¹ and 17.5 µg ml⁻¹ respectively. Samples were then kept for 20 min at room temperature (37°C) prior to analysis. Flow cytometric estimation for nuclear DNA content was performed with a Partec PA II flow cytometer, employing a 20 mW argon ion laser light source (488 nm wavelength) (Model PS9600, LG-Laser Technologies GmbH, Kleinostheim, Germany) and RG 590 long pass filter. At least 5000 nuclei were analysed in each sample. *Vicia* nuclear DNA was estimated by the internal standard method, using the ratio of the *Vicia*: standard G₀/G₁ peak positions (Doležel, 1997). The equivalent number of base pairs was calculated assuming that 1 pg DNA = 965 Mbp (Bennett et al., 2000).

The morphology and nuclear DNA data were first subjected to ANOVA, followed by a comparison of means via the Student-Newman-Keuls test ($P < 0.05$). The calculations were performed using the programme SAS (Statistical Analysis System, version 6.12)

RESULTS

Morphological analyses

The statistical analysis of the data (Table 3) showed that the three species differ significantly with respect to the majority of the studied characters such as leaf area, number of nodes, branching, length of the most-developed axis, length of the leaves, number of leaflets and number of inflorescences per plant. The comparison of these characters within each species revealed an inter-population polymorphism especially for *V. sativa* and *V. narbonensis*. The polymorphism detected in the species included in this study seems independent of the geographic origin of the population, but does seem to depend on altitude and the geographical conditions.

The difference starts to be expressed from a young age. Ten days after sowing, the majority of the seedlings had started to form leaves and branches: *V. sativa* is classified first followed by *V. villosa*, and *V. narbonensis*. With time these differences were maintained and led to the formation of 2 groups: *V. sativa* and *V. villosa* on the one hand and *V. narbonensis* on the other. This latter species always had the lowest values. According to the Student-Newman-Keuls test, the length of the leaf is comparable for *V. sativa* and *V. narbonensis*. These two species have a leaf length greater than that of *V. villosa*. For characters such as leaf area, length of the most developed stem and the number of leaflets and inflorescences per plant, the three species present significant differences, showing different vegetative and productive performance. Only the dry weight did not show any variability between the studied species.

For the different populations of *V. sativa*, the ANOVA revealed significant differences between the three populations for the studied characters (Table 4). Ten days after sowing, the majority of the seedlings had more than 5 leaves, but populations Vs2 and Vs3 had significantly more than Vs1, whilst the greatest ramification was observed for populations Vs2 and Vs3 with, respectively, 1.44 and 1.49 branches per plant. The difference between the three populations for the number of nodes and of branches per plant was maintained with time, with always a clear superiority of Vs2 and Vs3 compared to Vs1. With regard to the most developed axis, a parameter highly correlated with dry matter production (Dobias and Sestrienka, 1976), the averages calculated for Vs1, Vs2 and Vs3 are, respectively, 49.31, 45.44 and 36.32 cm. The superiority of population Vs1 was also noted for the leaf area; 22.56 cm² against 14.45 and 14.14 cm² for Vs2 and Vs3. These data and the equivalent number of leaves of the three populations (Table 4) make it possible to say that the three populations of *V. sativa* present significant morphological differences with regard to leaf architecture and growth rate. For population Vs1, the plants develop longer branches on which larger leaves grow, compared with the two other populations. Indeed, the length of the leaf measured on adult plants was of 5.4, 4.5, and 3.4 cm, respectively for Vs1, Vs2 and Vs3.

Regarding *V. villosa*, for the majority of the studied characters, such as leaf area, the number of nodes and branches per plant, the length of the most developed axis and the leaf dimensions, the ANOVA did not reveal significant difference between the populations (Table 5). The three populations showed very similar growth rate and development. They are populations belonging to different geographical environments, but one can explain this similarity of vegetative behaviour through the effect of the culture conditions which were possibly unfavourable to this species and did not allow, consequently, the populations to show differences. However, Table 5 shows the large differences in the number of inflorescences per plant; for example, population Vv1 had about double the number of

Table 3. Mean values for the morphological parameters listed in Table 2, for all three species considered together, together with the ANOVA.

Species	SF (cm ²)	N1	N2	N3	R1	R2	R3	LT (cm)	LF (cm)	NF	IN	DM (g)
<i>V. sativa</i>	17.05b	5.99a	10.90a	14.84a	1.10a	2.32a	2.97a	43.69b	4.47b	13.81b	55.18a	28.72a
<i>V. villosa</i>	6.88c	4.73b	9.49a	14.88a	0.47b	2.08a	2.88a	55.71a	5.35a	15.36a	40.23b	27.084a
<i>V. narbonensis</i>	28.19a	2.92c	5.2b	7.84b	0.09c	1.03b	1.99b	30.06c	4.17b	6.68c	16.33c	17.93a
ANOVA												
F value	31.91	106.27	25.10	20.63	30.87	18.22	6.75	21.29	4.61	80.67	27.7	0.93
P	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0027	0.0001	0.0151	0.0001	0.0001	0.4032

Values in the same column followed by different letters differ significantly according to the Student-Newman-Keuls test ($P < 0.05$).

Table 4. Mean values for the morphological parameters listed in Table 2, for *Vicia sativa*, together with the ANOVA.

Population	SF (cm ²)	N1	N2	N3	R1	R2	R3	LT (cm)	LF (cm)	NF	IN	DM (g)
Vs1	22.56a	5.49b	7.71b	12.09b	0.38b	1.66c	2.16b	49.31a	5.43a	13.94a	42.5a	33.93a
Vs2	14.45b	6.27a	13.16a	16.38a	1.44a	2.94a	3.44a	45.44a	4.57ab	13.88a	63.66a	17.184a
Vs3	14.136b	6.21a	11.82a	16.10a	1.49a	2.38b	3.33a	36.32b	3.42b	13.60a	59.39a	27.10a
ANOVA												
F value	5.11	4.25	36.71	45.25	16.25	16.91	27.52	8.98	3.3	0.36	2.35	0.79
P	0.0203	0.0345	0.0001	0.0001	0.0002	0.0001	0.0001	0.0027	0.0649	0.7048	0.1296	0.4794

Values in the same column followed by different letters differ significantly according to the Student-Newman-Keuls test ($P < 0.05$).

Table 5. Mean values for the morphological parameters listed in Table 2, for *Vicia villosa*, together with the ANOVA.

Population	SF (cm ²)	N1	N2	N3	R1	R2	R3	LT (cm)	LF (cm)	NF	IN	DM (g)
Test Duncan												
Vv1	6.88	5.16a	10.55a	16.55a	0.55a	2.27a	3.38a	43.70a	4.34a	14.10a	58.66a	20.72a
Vv2	.	4.71a	8.38a	13.49a	0.44a	1.83a	2.55a	64.47a	6.18a	16.21a	29.83b	21.88a
Vv3	.	4.33a	9.55a	14.60a	0.44a	2.16a	2.72a	58.96a	5.52a	15.77a	31.21b	38.66a
ANOVA												
F value	.	1.79	0.62	0.55	0.10	0.41	0.75	2.42	2.46	1.23	5.33	0.79
P	.	0.2003	0.5517	0.5886	0.9084	0.6680	0.4876	0.1232	0.1195	0.3213	0.0178	0.4698

Values in the same column followed by different letters differ significantly according to the Student-Newman-Keuls test ($P < 0.05$).

Table 6. Mean values for the morphological parameters listed in Table 2, for *Vicia narbonensis*, together with the ANOVA.

Population	SF (cm ²)	N1	N2	N3	R1	R2	R3	LT (cm)	LF (cm)	NF	IN	DM (g)
Vn1	30.49a	3.38a	7.77a	12.22a	0.16a	1.94a	2.38a	42.40a	5.07a	8.90a	25.94a	17.31a
Vn2	31.52 a	2.72a	3.99b	5.38b	0.00a	0.88b	1.60b	26.49b	4.12b	5.55a	9.55b	29.05a
Vn3	22.55a	2.66a	3.83b	5.94b	0.11a	0.27b	1.55b	21.30b	3.32c	5.49a	13.49b	7.45a
ANOVA												
F value	2.46	2.64	4.74	5.39	0.66	8.64	4.52	7.23	11.02	3.11	2.83	1.06
P	0.1188	0.1039	0.0254	0.0172	0.5310	0.0032	0.0291	0.0063	0.0011	0.074	0.09	0.37

Values in the same column followed by different letters differ significantly according to the Student-Newman-Keuls test ($P < 0.05$).

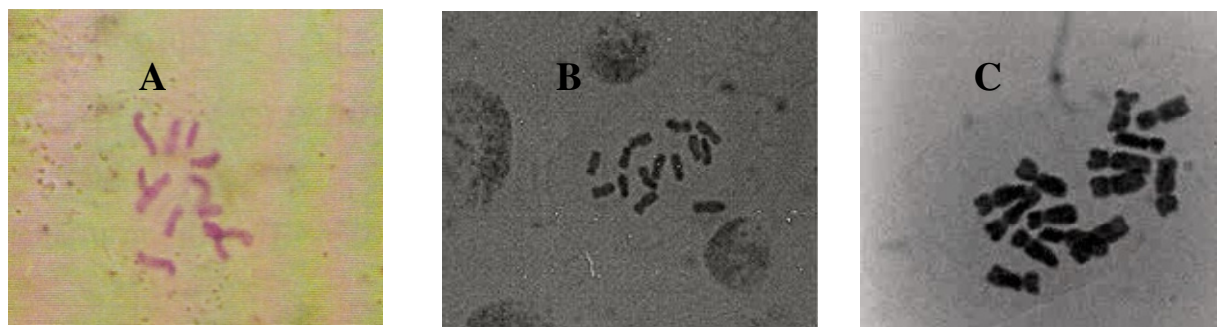


Figure 1. Somatic chromosomes of (A) *Vicia sativa*, $2n = 12$, (B) *Vicia villosa*, $2n = 14$ and (C) *Vicia narbonensis*, $2n = 14$ (magnification = $\times 400$). Chromosome lengths are 7 - 9 μm for *V. sativa*, 5 - 7 μm for *V. villosa* and 12 - 14 μm for *V. narbonensis*.

the other populations.

For *V. narbonensis*, the ANOVA was significant for several characters and highlights an interesting variability between the populations (Table 6). At the seedling stage, the morphological differences are statistically negligible. Whatever the studied population, the 10 day-old seedlings had the same number of nodes and ramifications. But the difference becomes clear at the advanced stages of morphogenesis (Table 6). The Vn1 population had formed more leaves and branches 20 and 30

days after sowing that the other populations (Vn2 and Vn3), and thus had a faster growth. Rapid growth is synonymous with good establishment (especially in cold zones) and better competition against weeds. This strength of the Vn1 population is confirmed by the measurement of the most developed orthotropic axis. The average length of this axis was about 42 cm for Vn1, significantly higher than for the two other populations, 26 and 21 cm, respectively, for Vn2 and Vn3. The same superiority was also observed for the number of

inflorescences per plant.

The chromosome counts demonstrated that all populations of *V. sativa* had 12 chromosomes, whilst populations of the two other species had 14. Figure 1 show photographs of typical root cell metaphase chromosome preparations of *V. sativa* (A), *V. villosa* (B) and *V. narbonensis* (C). A representative histogram of the flow cytometric analyses of *V. sativa* is shown in Figure 2. The nuclear DNA content ranges were 3.67-3.79 pg for *V. sativa*, 3.72-4.17 pg for *V. villosa* and 12.83-13.17

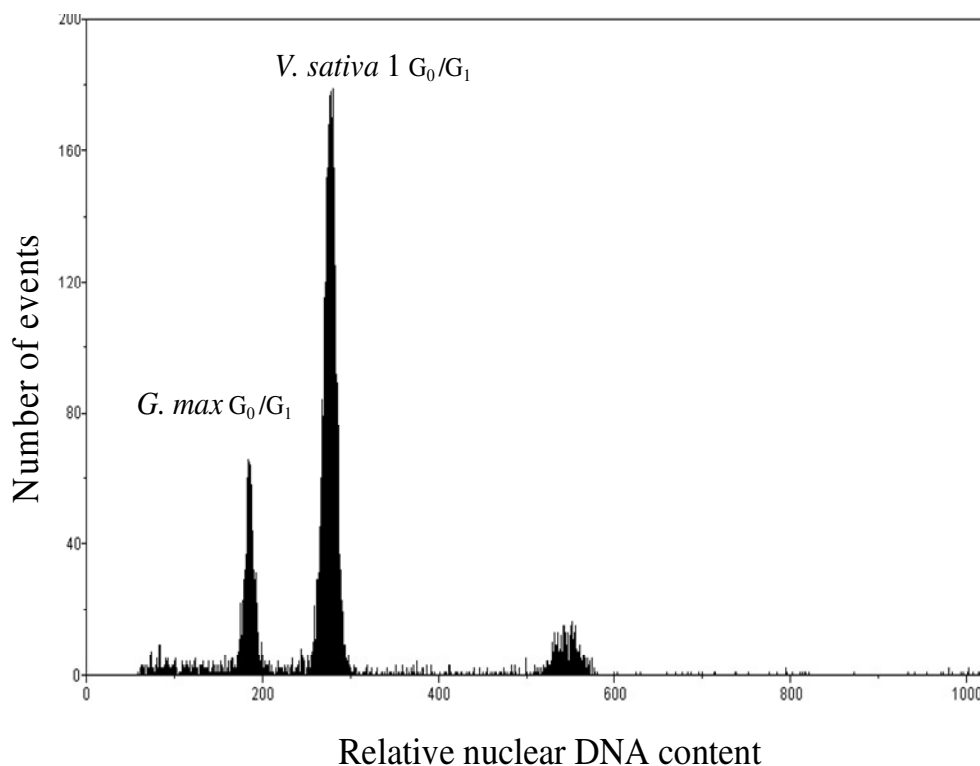


Figure 2. Flow cytometric analysis of *Vicia sativa* population 1. The first peak is the G_0/G_1 peak of soya (*Glycine max*) and the second peak is that of *V. sativa*.

Table 7. Somatic chromosome number and mean nuclear DNA (2C) amounts (pg) for the studied species.

Population	Chromosome number (2n)	2C nuclear DNA content (pg)	1C genome size (Mbp)	DNA/chromosome (pg)
Vs1	12	3.686	1778	0.307
Vs2	12	3.732	1800	0.311
Vs3	12	3.786	1826	0.315
Vv1	14	3.797b	1832b	0.271b
Vv2	14	4.172a	2012a	0.298a
Vv3	14	3.719c	1794c	0.265c
Vn1	14	13.170	6354	0.940
Vn2	14	12.830	6190	0.916
Vn3	14	12.853	6201	0.918

For each species analysed separately, different letters indicate statistically-significant differences ($P < 0.05$) according to the Student-Newman-Keuls test.

pg for *V. narbonensis* (Table 7). Significant differences between species were found with respect to their mean 2C nuclear DNA content ($P < 0.001$). There were no significant differences among populations within species except for *V. villosa*: population Vv2 had a greater mean DNA content (4.17 pg) than Vv1 and Vv3 (3.79 and 3.71 pg, respectively). The amount of DNA per chromosome was much greater for *V. narbonensis*.

DISCUSSION

Morphology

Regarding phenotypic variability, a strong interspecific variation and also an inter-population variation were observed for the majority of the measured parameters. Other morphological studies of variability in *Vicia* showed

that the vegetative parameters, like leaf length, number of leaflets and the length of the most-developed axis, do not have a significant heterogeneity between taxa studied (Wouw et al., 2003b). The characters related to the flowers can, on the other hand, define a phenotypical polymorphism (Wouw et al., 2003b; Choi et al., 2006). In our study, the number of inflorescences appeared a powerful discriminating factor since the studied species are easily distinguished from each other by this parameter. *V. sativa* has the highest number of inflorescences per plant (55) followed by *V. villosa* (40) and *V. narbonensis* with 16. Within the same species, the populations of *V. narbonensis* and *V. villosa* showed differing numbers of inflorescences.

Wouw et al. (2003a) found that the variation between the species of vetch that they studied was due mainly to their geographical distribution, which is not in agreement with our results. Morphological variability that we saw seems independent of the geographic origin since the two Tunisian populations of *V. sativa* (Vs1 and Vs2) show relatively distinct morphological and floral characteristics. For *V. narbonensis*, the 3 populations studied are of Tunisian origin. The populations showed a significant variability for the majority of the studied morphological characters, separating Vn1 from the Vn2 and Vn3. In addition, no significant morphological difference was observed between populations of *V. villosa* during the first 20 days after sowing. This was foreseeable since the species *V. villosa* is a spring type; the morphological differences are observed especially at an advanced stage of morphogenesis, in particular the floral characters (Wouw et al., 2003a). In addition, the altitude of the stations of origin could have an effect on phenotypical polymorphism. For *V. sativa* and *V. narbonensis*, the variability of the characters seems to follow a gradient of altitude. Thus, the populations Vs1 and Vn1 collected, respectively, at altitudes of 210 and 100 m, are characterised by a greater and more rapid vegetative development than that observed for the populations of the same species from lower altitudes. The phenotypical differences observed in the species and populations of local and foreign vetches would result from genotypic variability since the plants are cultivated under the same experimental conditions. This variability constitutes a genetic base to facilitate the improvement of these species for different uses (fodder, seeds, novel uses) in Tunisia and beyond.

Chromosome number

The process of speciation and evolution within the genus *Vicia* has involved large changes in chromosome size and in nuclear DNA amount (Martin and Shank, 1966; Raina and Rees, 1983). One aim of this work was to determine the variation in nuclear DNA content and compare the chromosome numbers of three *Vicia* species of agronomic interest in Tunisia and other Mediterranean areas (Abd El Moneim, 1992; Hassen et al., 1994;

Akpınar and Bilaloğlu, 1997; Siddique et al., 1996; Cremonini et al., 1998; Caballero et al., 2001).

The ploidy level can affect the growth rate, plant size, stress tolerance and other agronomically-important characteristics (Walker et al., 2005; El Ferchichi et al., 2006). The base chromosome number is $x = 5, 6$ or 7 for *V. sativa* and 7 for *V. villosa* and *V. narbonensis* (Hanelt and Mettin, 1989). For all three species, only diploid plants have been reported. The chromosome numbers are $2n = 2x = 14$ for *V. villosa* populations, of unknown origin (Chooi, 1971; Raina and Bisht, 1988), and $2n = 2x = 14$ for *V. narbonensis*, of unknown origin (Chooi, 1971; Raina and Bisht, 1988) or from Greece (Navrátilová et al., 2003). Our results for *V. villosa* and *V. narbonensis* show that all the Tunisian and Syrian populations studied are diploid with 14 chromosomes. The two Tunisian populations and the Spanish cultivar of *V. sativa* had 12 chromosomes, as found previously for *V. sativa* populations from the Czech Republic (Navrátilová et al., 2003), Turkey (Akpınar and Bilaloğlu, 1997) or of unknown origin (Chooi, 1971; Raina and Bisht, 1988). Populations of unknown origin having 10 or 14 chromosomes have been described also (Chooi, 1971).

Nuclear DNA content and its relationship with plant morphology

Previous work has shown that the 2C nuclear DNA contents of the *Vicia* species studied here vary widely, being 3.8-6.0 pg for *V. sativa* (Chooi, 1971; Raina and Bisht, 1988; Akpınar and Bilaloğlu, 1997; Navrátilová et al., 2003), 4.1-6.3 for *V. villosa* (Chooi, 1971; Raina and Bisht, 1988; Yeater et al., 2004) and 14.3-16.1 pg for *V. narbonensis* (Chooi, 1971; Raina and Bisht, 1988; Frediani et al., 1992). Differences between DNA amount of *V. sativa* and *V. villosa* on the one hand and *V. narbonensis* on the other are hypothesised to be due to differences in chromosome size. We found chromosome lengths of 7-9 μm for *V. sativa*, 5-7 μm for *V. villosa* and 12-14 μm for *V. narbonensis* (Figure 1). Our corresponding values for the 2C nuclear DNA contents were 3.7-3.8, 3.7-4.2 and 12.8-13.2 pg for *V. sativa*, *V. villosa* and *V. narbonensis*, respectively. These values are lower than those found before, or in the lower part of the reported ranges. This could reflect true differences or simply differences related to the methods and equipment used (Doležel et al., 1998).

Genome size can affect cellular parameters, such as cell size, and these nucleotypic effects can impact on important characteristics such as growth and yield (Turpeinen et al., 1999; Walker et al., 2006). Taking all three species together, we obtained significant, negative Pearson correlation coefficients for the nuclear DNA content and the parameters related to the growth rate (number of nodes and branches at 10, 20 or 30 days) (data not shown), in line with previous suggestions that in

plants with smaller genome sizes cell division and thus growth are more rapid, an advantage in environments of limited growing season (Bennett, 1972; Turpeinen et al., 1999). *V. narbonensis*, with relatively much higher content of nuclear DNA compared to the two other species, was characterised by a slower vegetative growth compared to *V. sativa* and *V. villosa*, containing approximately the same quantity of nuclear DNA. A lack of information regarding the edapho-climatic conditions of the populations studied by other authors prevents the determination of possible relationships between nuclear DNA content and factors such as maximum and minimum temperatures and rainfall, which determine the growing season at each site. For *V. villosa*, population Vv2 had a significantly greater mean DNA content (4.17 pg) than Vv1 and Vv3 (3.79 and 3.71 pg, respectively). This could be due to the higher rainfall at the Vv2 site permitting a longer growing season (Turpeinen et al., 1999; Walker et al., 2006) and thus a larger genome size. Interestingly, population Vv2 also required a greater time before commencement of flowering (139 days) than Vv1 (118 days) or Vv3 (117 days).

REFERENCES

- Abd El Moneim AMJ (1993). Narbon vetch (*Vicia narbonensis* L), a potential feed legume crop for dry areas in West Asia. *J. Argon. Crop Sci.* 169: 347-53.
- Akpınar N, Bilaloglu R (1997). Cytological investigations of certain species of *vicia* L. *Trend J. Biol.* 21: 197-207.
- Allkin R, Goydir DJ, Bisby FA, white RJ (1986). Names and synonyms of species and subspecies in the Viciaeae. Issue 3, *Viciaeae* Data base Project, publication No 7 (ISSN 0263-8517), University of Southampton, UK.
- Bennet MD, Bhandol P, Leitch IJ (2000). Nuclear DNA amounts in Angiosperms and their modern uses-807 new estimates. *Ann. Bot.* 86: 859-909.
- Caballero R, Alzueta C, Ortiz LT, Rodriguez ML, Rebole A (2001). Carbohydrate and protein fractions of fresh and dried common vetch at three maturity stages. *Agron. J.* 93: 1006-1013.
- Choi BH, Seok DI, Endo Y, Ohashi H (2006). Phylogenetic significance of stylar features in genus *Vicia* (Leguminosae): an analysis with molecular phylogeny. *J. Plant Res.* 119: 523-523.
- Chooi WY (1971). Variation in nuclear DNA content in the genus *vicia*. *Genetics*, 68:195-211.
- Cremonini R, Miotto D, Ngu MA, Tota D, Pignone D, Blangiforti S, Venora G (1998). Cytology of *vicia* species 5. Nuclear chromatin structure, Karyomorphological analysis and DNA content in newly discovered relatives of *Vicia faba* L: *Vicia kalakhensis* Khattab, Maxted et Bisby and *Vicia eristaloides* Maxted. *Cytologia*, 63: 371-379.
- Dobias A, Sestrienka A (1976). Genetic, variability and correlations of productivity characters of lucerne. *Vedecke Prace. Vyskumneho Ustavu Rastlinej Vyrob. V. Piestanoch*, 13: 301-307.
- Dolezel J (1997). Application of flow cytometry for study of plant genomes. *J. Appl. Genet.* 38: 285-302.
- Dolezel J, Dolezelova M, Novak FJ (1994). Flow cytometric analysis of nuclear DNA amount in diploid Bananas (*Musa acuminata* and *M. balbisiana*). *Biol. Plant*, 36: 351-357.
- Dolezel J, Greilhuber J, Lucretti S, Lysak MA, Nardi L, Obermayer R (1998). Plant genome size estimation by flow cytometry: inter-laboratory comparison. *Ann. Bot.* 82: 17-26.
- El ferchichi OH, H'cini K, Bouzid S (2006). Chromosome number in Tunisia populations of *Atriplex halimus* L. (Chenopodiaceae). *Afr. J. Biotechnol.* 5 : 1190-1193.
- Frediani M, Sassoli O, Cremonini R (1992). Nuclear DNA characterization of two species of *Vicia* : *Vicia bithynica* L and *Vicia narbonensis* L. *Biologia Plantarum*, 34(3-4): 335-344.
- Hanelt P, Mettin D (1989). Biosystematics of the genus *vicia* L (Leguminosae). *Ann. Rev. Ecol. Syst.* 20: 199-223.
- Hassen H, Zoghalmi A, Sassi S (1994). Contribution a l'étude de quelques espèces spontanées de légumineuses pastorales en Tunisie centrale : répartition Géographique et relation avec le milieu environnant. *Ann. De l' INRAT*, 67(1-2): 203-222.
- Kawakami I (1930). Chromosome numbers in Leguminosae. *Botanical Magazine of Tokyo*, 44: p. 319.
- Kupicha FK (1976). The infrageneric structure of *vicia*. *Notes Roy. Bot. gard Edinburgh*, 34: 287-326.
- Lysak MA, Dolezel J (1998). Estimation of nuclear DNA content in *Sesleria* (Poaceae). *Caryologia*, 52: 123-132.
- Martin PG, Shank R (1966). Does *vicia faba* have multistandarded chromosomes. *Nature*, 211: 650-651.
- Narayan RKJ, Ramachandran C, Raina SN (1985). The distribution of satellite DNA in the chromosome complements of *vicia* species. *Genetica*, 66: 115-121.
- Navratilova A, Neumann P, Macas J (2003). Karyotype Analysis of four *Vicia* species using *In situ* Hybridisation with repetitive Sequences. *Ann. Bot.* 91: 921-926.
- Raina SN, Bisht MS (1988). DNA amounts and chromatin compactness in *Vicia*. *Genetica*, 77: 65-77.
- Raina SN, Rees H (1983). DNA variation between and within chromosome complements of *vicia* species. *Heredity*, 51: 335-346.
- Senn HA (1938). Chromosome number relationships in the leguminosae. *Bibliographia Genetica*, pp. 175-345.
- Siddique KHM, Loss SP, Enneking D (1996). Narbon bean (*vicia narbonensis* L), a promising grain legume of south western Australia. *Aust. J. Exp. Agric.* 36: 53-62.
- Sinha SSN, Das IN (1985). Karyotypic analysis in some European species of *vicia*. *Genetica Iberia*, 37(3-4): 229-246.
- Turpeinen T, Kulmala J, Nevo E (1999). Genome size variation in *Hordeum spontaneum* populations. *Genome*, 42: 1094-1099.
- Van de Wouw M, Maxted N, Bria Fordlioyd BV (2003a). Agromorphological characterisation of common Vetch and its close relatives. *Euphytica*, 130(2): 381-292.
- Van de Wouw M, Maxted N, Bria Fordlioyd BV (2003b). A multivariate and cladistic study of *Vicia* L. ser. *Vicia* (Fabaceae), based on analysis of morphological characters. *Plant Syst. Evol.* 237: 19-39.
- Walker DJ, Monino I, Gonzalez E, Frayssinet N, Correal E (2005). Determination of ploidy and nuclear DNA content in populations of *Atriplex halimus* (Chenopodiaceae). *Bot. J. Linn. Soc.* 147: 441-448.
- Walker DJ, Monino I, Correal E (2006). Genome size in *Bituminaria bituminosa* (L) C.H. Stirton (Fabaceae) populations: separation of true differences from environmental effects on DNA determination. *Environ. Exp. Bot.* 55: 258-265.
- Yamamoto K (1959). On various characteristics of hybrids between a Moroccan strain of *Vicia sativa* and the common strain. *Technical Bulletin of the Faculty of Agriculture of Kagawa University*, 2: p. 28.
- Yeater KM, Bollero GA, Bullock DG, Rayburn AL (2004). Flow cytometric analyses for ploidy level differentiation of 45 hairy vetch accessions. *Ann. Appl. Biol.* 145: 123-127.