Flow cytometry DNA assay of Mediterranean lupins

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RÉSUMÉ

GHRABI GAMMAR, Z., S. PUECH & M. ZOUAGHI (1999). Essai de cytométrie en flux d'ADN pour les lupins méditerranéens. *Candollea* 54: 45-56. En anglais, résumés français et anglais.

La quantité individuelle d'ADN par noyau dans plusieurs populations appartenant à trois espèces du genre Lupinus se développant en Tunisie (*L. luteus* L., *L. angustifolius* L. et *L. cosentinii* Guss.) et de cinq autres espèces se développant dans le Bassin Méditérranéen (*L. micranthus* Guss., *L. pilosus* Murr., *L. palaestinus* Boiss., *L. digitatus* Forssk. et *L. atlanticus* Gladst.) est dosée par cytométrie en flux. Pour les espèces à graines lisses, ce dosage révèle: (1) une régularité de la quantité d'ADN des individus de deux populations de *L. luteus* L. (Tabarka 1 et 3) et de *L. angustifolius* L. (M'raïssa et Tabarka 2); (2) une fluctuation non significative dans la quantité d'ADN des individus des autres populations de ces deux espèces et entre les individus de populations différentes appartenant à une même espèce; (3) une quantité d'ADN pour *L. micranthus* Guss. ($2n$ = 52) significativement inférieure à celle de *L. luteus* L. ($2n$ = 52) et de *L. angustifolius* L. ($2n$ = 42). Pour les espèces à graines rugueuses, le dosage révèle: (1) une régularité ou une fluctuation non significative de la quantité d'ADN des individus de *L. cosentinii* Guss. appartenant à une même population ou à des populations différentes; (2) une même quantité d'ADN pour *L. pilosus* Murr. et *L. palaestinus* Boiss., tout deux à $2n$ = 42; (3) une variation significative de la quantité d'ADN entre *L. pilosus* Murr., *L. palaestinus* Boiss. et *L. cosentinii* Guss., à $2n$ = 32, d'un coté, *L. digitatus* Forssk., à $2n$ = 36 chromosomes, de l'autre. Cette variation n'est pas corrélée avec le nombre chromosomique de ces espèces; (4) *Lupinus atlanticus* Gladst., endémique du Maroc à $2n$ = 38, s'est distingué par une quantité d'ADN nettement supérieure à celle des autres espèces analysées. Il constitue à lui seul un sous-groupe au sein des lupins à graines rugueuses du Bassin méditerranéen.

ABSTRACT

GHRABI GAMMAR, Z., S. PUECH & M. ZOUAGHI (1999). Flow cytometry DNA assay of Mediterranean lupins. *Candollea* 54: 45-56. In English, French and English abstracts.

The individual amounts of DNA per nucleus in several populations of three species of the genus *Lupinus* in Tunisia (*L. luteus* L., *L. angustifolius* L. and *L. cosentinii* Guss.) and five other species in the Mediterranean basin (*L. micranthus* Guss. *L. pilosus* Murr., *L. palaestinus* Boiss., *L. digitatus* Forssk. et *L. atlanticus* Gladst.) were assayed using flow cytometry. This assay revealed the following features in smooth seed species: (1) steady DNA quantity in specimens in two populations of *L. luteus* L. (Tabarka 1 and 3) and *L. angustifolius* L. (M'raissa and Tabarka 2); (2) non-significant fluctuation of the quantity of DNA in specimens of the other populations of these two species and between individuals of different populations belonging to the same species; (3) a significantly smaller quantity of DNA in *L. micranthus* Guss. ($2n$ = 52) than in *L. luteus* L. ($2n$ = 52) and *L. angustifolius* L. ($2n$ = 42). The assay revealed the following features in rough-seed species: (1) steadiness or non-significant fluctuation in the quantity of DNA in specimens of *L. cosentinii* Guss. belonging to the same population or to different populations; (2) the same quantity of DNA in *L. pilosus* Murr. and *L. palaestinus* Boiss., both at $2n$ = 42; (3) a significant variation in the quantity of DNA between *L. pilosus* Murr., *L. palaestinus* Boiss. and *L. cosentinii* Guss., from $2n$ = 32, *L. digitatus* Forssk. to $2n$ = 36 chromosomes. This variation is not correlated with the chromosome number in this species; (4) *Lupinus atlanticus* Gladst., endemic in Morocco at $2n$ =

CODEN: CNDLAR
ISSN: 0373-2967
54(1) 45 (1999)

38, displayed a distinctly larger quantity of DNA than in the other species. It may alone form a sub-group among the rough-seeded lupins of the Mediterranean basin.

KEY-WORDS: Mediterranean region – Tunisia – Lupins – Flow cytometry – DNA – Chromosome numbers.

1. Introduction

The Genisteae tribe of which the genus *Lupinus* L. is a member, is characterised by intense polyploidy with the base numbers $x = 8$, $x = 9$ and $x = 12$ (GILOT, 1995). The genus comprises some 200 species around the world (WILLIS, 1973) with particular distribution. Only ten species grow in the Mediterranean basin, only two species are found in East Africa (Kenya, Tanzania and Ethiopia) (GLADSTONES, 1958, 1974; GREUTER & al., 1989) and more than 180 species are American. The two groups differ in their geographical distribution, morphological characters and chromosome numbers.

The karyological study of lupins can help in the understanding of their current distribution and possibly in the reconstruction of their past. Various work has been carried out with this aim. One can mention that of PAZY & al. (1977), GLADSTONES (1974), CRISTOFOLINI (1988) and CASTAIRS & al. (1991) who show that the American species mainly possess $n = 24$, only three taxa possess $n = 18$ or $n = 48$ and a single species possesses $n = 12$ (PAZY & al., 1977). The basic number is constant at $x = 6$. The stability and uniformity of the basic number and the rare changes in ploïdy in the large number of American species contrast with those found in the small number of species in the Mediterranean basin. The latter display heterogeneity in the chromosome numbers and intense ploïdy (PAZY & al., 1977). It can therefore be affirmed that lupins have two centres of diversification with different speciation and chromosome evolution modes. The Mediterranean basin species are annuals and form a group which, according to PAZY & al. (1977), have one or more common ancestors and currently occupy scattered distribution areas.

The Mediterranean species with rough seeds display high haploid numbers (16,18,19 and 21) and form a polyploïd complex (ROY & al., 1985, 1988; CASTAIRS & al., 1991). The rough seeds of *L. cosentinii* Guss. collected in Tunisia have a steady chromosome number ($2n = 32$) and a stable microtextural model (protuberances with 160 µm base diameter; the upper face is narrower, bare and with regular folds on the sides). This stability is also observed in the other Mediterranean cytotypes with rough seeds. These species do not include diploid. They form a changing polyploid group (GLADSTONES, 1974; PLITMANN & al., 1984; CARSTAIRS & al., 1991).Their cytoevolution process has not yet been settled in a convincing manner. Many hypotheses concerning their origin have been put forward and discussed.

For GILOT (1965), PAZY & al. (1981) and CARSTAIRS & al. (1991) the mediterranean smooth seed species display heterogeneous chromosome numbers and a tendency towards aneuploidy, especially in *L. luteus, L. angustifolius* L. and *L. micranthus* Guss. Seeds of *L. angustifolius* L. and *L. luteus* L. collected in Tunisia from individuals in the same population displayed differences in size, shape and ornamentation (GHRABI GAMMAR & al., 1997a). A variation in chromosome number was also observed: $2n = 52$, 54 and 56 in *L. luteus* L. and $2n = 40$, 42 and 44 in *L. angustifolius* L. (GHRABI GAMMAR & al., 1997b). This diversity led us to measure the quantity of DNA using flow cytometry. This techniques gave an average value for DNA per nucleus and per individual and enabled the analysis of a large number of individuals fairly rapidly. It also made it possible to know whether there was a significant fluctuation within each population, to compare different populations of the same species and to gain an idea of their dynamics and adaptation to the ecological conditions of their habitat (BROWN, 1993). The purpose is also to verify whether there is also a good correlation between variation of DNA content and variation of chromosome number (BENNETT, 1987).

The variation of DNA content between plants from populations of the same species has been reported by SGORBATI & al. (1989), BINO & al. (1993) and BROWN (1993). WAKAMIYA (1993) has revealed with this technique, a relation between the genome size of eighteen species of *Pinus,* the climatic conditions and growth factors. EL OUALIDI (1991) explain the variation of DNA content of three *Teucrium mairei* Senn. by variation of chromosomes size, difficult to detect when chromosomes are very small.

The aim of this study was to compare, by flow cytometry, DNA content of eight mediterranean species of Lupins (five rough seed species, one from Tunisia, and three smooth seed species from Tunisia) and to verify whether there is a correlation between the DNA content and their chromosome numbers.

2. Material and methods

DNA assays were performed on fresh plant material. This consisted of the plantlets grown from seeds of *L. luteus*, *L. angustifolius* and *L. cosentinii* in Tunisia. Collection stations for the seeds of these three species and their ecological characteristics are shown in Table 1. The seeds of the other species were from the INRA collection, Lusignan, France. It should be noted that *L. micranthus* has been reported in Tunisia (POTTIER ALAPETITE, 1979; GLADSTONES, 1974), but we did not find any samples of these species during our various prospection operations. For this reason, we made up for the lack of material by using seeds from the collection at the *Institut national de recherche agronomique*, Lusignan (INRAL), near Poitiers (France).

The plantlets grown from seeds planted in Petri dishes were transplanted in soil and installed in the culture room at the Institut de Botanique in Montpellier. The plantlets were processed at the Service de cytométrie en flux, INSERM Unité 291, Montpellier.

The nuclei were extracted from the leaves of the young plantlets using the method of GALBRAITH & al. (1983), METEZEAU & al. (1988), that consists of extracting the nuclei from the cells using a mother osmotic solution of macroelements. The nuclei were permeabilised with Triton and stained with 0.002% propidium iodide. There is a linear relationship between the quantity of fluorescence emitted by the fluorochrome, the DNA marker (propidium iodide) and the quantity of DNA in the nuclei of the cells analysed.

The quantity of DNA was determined with a 50 H orthocytofluorograph equipped with a 250 mw 488 nm Informatique MCA 3000 argon laser (Bruker W 15 Jernbourg). The number of individuals analysed in the Tunisian species is shown in Table 1. Three individuals of each of the other species were analysed. We analysed in each individual the quantity of DNA in 500 nuclei in three different leaves and with three repetitions for each leaf.

This technique gives curves whose *y*-axis (number of cells) is the number of cells analysed, and the *x*-axis (Fl. area) is the fluorescence emitted by the nucleus of each cell analysed. The peak of the curve would therefore be point Pk (x) on the *x*-axis where the fluorescence corresponds to the largest number of nuclei. The area of the region for which the calculation is made (Reg.), the percentage of nuclei analysed (% Tot.), the fluorescence for which there is the largest number of nuclei (Fl. area, Pk (x)), the average quantity of DNA per nucleus (Mn (x)), the standard deviation (SD) and the coefficient of variation (CV) are shown for each curve. The curves have only one major peak when the great part of cells analysed are in $2n$ phase at the time of tests but have two peaks when a part of cells are in 2n phase, the other in $4n$ phase at the time of tests. Only the $2n$ phase is considered.

SPECIES	STATION	ECOLOGICAL CHARACTERISTICS	BATCH	SEED ORNAMENTATION	PLANTS ANALYSED
Lupinus luteus L.	TABARKA 1	NW Tunisia, sandy soil, lower humid Mediterranean bioclimate with mild winters.		White ground strongly speckled with dark brown, dark brown arc beneath the hilum.	3
	TABARKA 2	NW Tunisia, sandy soil, lower humid Mediterranean bioclimate with mild winters.	A	White ground strongly speckled with dark brown, dark brown arc beneath the hilum.	3
			B	White ground weakly speckled with dark brown, dark brown arc beneath the hilum.	3
			C	White ground strongly speckled with dark brown, yellow arc beneath the hilum.	3
			D	White ground weakly speckled with dark brown, yellow arc beneath the hilum.	3
	TABARKA 3	NW Tunisia, sandy soil, lower humid Mediterranean bioclimate with mild winters.		White ground strongly speckled with dark brown, dark brown arc beneath the hilum.	3
Lupinus angustifolius L.				White ground strongly speckled with black.	3
	M'RAISSA	Cape Bon, sandy soil, upper semi-arid Mediterranean bioclimate with mild winters.		White ground strongly speckled with black.	3
	BORJ H'FAIEDH	Borj F'faiedh, Cape Bon, sandy soil upper semi-arid Mediterranean bioclimate	A	White ground with small brown speckles.	10
			B	White ground speckled with greyish brown.	10
			C	Greyish ground speckled with black.	10
			D	Brown ground speckled with black.	10
			E	White ground strongly speckled with black	10
Lupinus cosentinii Guss.				White ground strongly speckled with black, black arc beneath the hilum.	6
	M'RAISSA	Cape Bon, sandy soil, upper semi-arid Mediterranean bioclimate with mild winters.			10
	SOUSSE	Sahel, sandy soil, upper semi-arid			10

Table 1. – The Tunisian species of the genus *Lupinus* for which individuals were analysed by flow cytometry: origin of the populations, ecological characteristics of the stations, type of seed ornamentation and the number of individuals analysed.

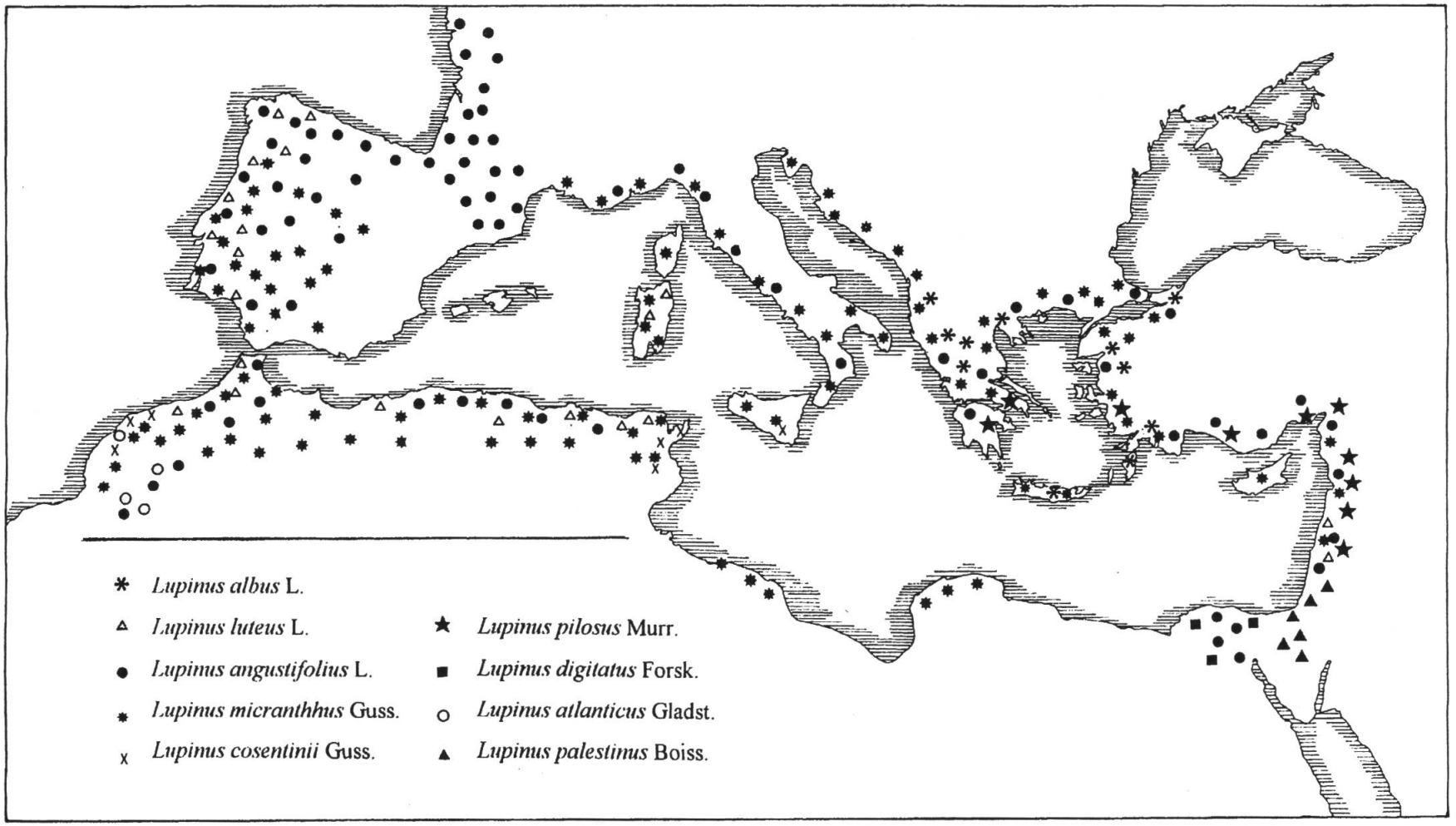


Fig. 1. – The geographical distribution of Mediterranean *Lupinus* (after GLADSTONES, 1974).

Smooth seed species: *L. luteus* L., *L. angustifolius* L., *L. micranthus* Guss. and *L. albus* L.; Rough seed species: *L. cosentinii* Guss., *L. pilosus* Murr., *L. digitatus* Forssk., *L. atlanticus* Glad. and *L. palaestinus* Boiss.

3. Results

3.1. Smooth seed species

The assay of *L. luteus* ($2n$ = 52, 54, 56) showed evenness in the amount of DNA in individuals in the populations from Tabarka 1 and Tabarka 3. Those of the population from Tabarka 2 displayed non-significant fluctuation (4 to 5.5%) in DNA quantity. Individuals from Tabarka 3 displayed an average quantity of DNA that was non-significantly greater than that of individuals from Tabarka 1 and Tabarka 2, attaining 3.4 and 9.6 % respectively (Fig. 2 A).

The average quantity of DNA in individuals of the *L. angustifolius* population ($2n$ = 38, 42, 44) from Borj H'faiedh was unstable between individuals in the same batches or in two different batches. This fluctuation is not significant. It reaches up to 6% in the first case and does not exceed 3% in the second. The smallest quantity of DNA was found in batch E. In contrast, individuals in the M'raissa and Tabarka 3 populations displayed always a stable, identical average and constant quantity of DNA. This quantity is greater (by 5.7 to 19%) than that of individuals in the Borj H'faiedh populations (Fig. 2 B).

The small number of individuals of *L. micranthus* analysed displayed remarkable stability in DNA quantity. This was much smaller than that observed in *L. angustifolius* and *L. luteus* (Fig. 2 B).

3. 2. Rough seed species

Lupinus cosentinii ($2n$ = 32) – The different populations displayed a steady average DNA quantity per individual. The Sousse and Borj H'faiedh populations had the same average quantity. The nuclei of individuals of the M'raissa population emitted slightly less fluorescence (3.1%) than these of the two other populations, but the difference is not significant. The average quantity of DNA per individual in each of the 3 populations is shown in Figure 3.

Lupinus pilosus ($2n$ = 42) and *L. palaestinus* ($2n$ = 42) possessed the same amount of DNA (Fig. 4). It was significantly greater by 2% than that of *L. cosentinii* significantly lower by 5% than that of *L. digitatus* and by 15% than that of *L. atlanticus* (Fig. 4). The latter species stands out clearly from the other species analysed in that it has the highest average quantity of DNA.

4. Discussion

The remarkable inter-individual regularity of the average DNA content of smooth-seeded species in two of the populations of *L. luteus* L. (Tabarka 1 and 3) and *L. angustifolius* L. (M'raissa and Tabarka 3) would appear to indicate the stabilisation of the quantity of DNA at these four sites. In contrast, in the *L. angustifolius* L. population of Borj H'faiedh, the quantity of DNA in the individuals grown from seeds of batch E is greater than that in individuals of batch A. This difference is not significant, but we consider that it might be correlated with the chromosome counts of individuals from these two batches: $2n$ = 42 chromosomes for batch A and $2n$ = 44 for batch E (GHRABI GAMMAR & al., 1997b). In this study chromosome counts and DNA assays are performed in parallel and cover a larger number of individuals in all the populations of the species, taking into account the ecological conditions in which the specimens grow. This should make it possible to verify whether there are correlations or no between the substantial variation in the quantity of DNA, chromosome size and number and biogeographical origin.

In the Tabarka 2 population of *L. luteus* L., the non-significant intra-individual variation in the average quantity of DNA also corresponds to a variation in the number of chromosomes in

individuals of the population, i.e. $2n = 52$, 54 and 56 chromosomes (GHRABI GAMMAR & al., 1997b). The most frequent number is $2n = 52$. A more detailed investigation of the karyograms of individuals of the population and the measurement of DNA quantity in a large number of individuals could revealed the existence of a correlation between size, shape, and chromosome numbers of these individuals and the quantity of DNA in their nuclei.

Lupinus micranthus Guss. ($2n = 52$) possesses a significantly smaller quantity of DNA than *L. angustifolius* L. ($2n = 42$) although the number of chromosomes is higher. DNA quantity is also different to that of *L. luteus* L., which also possesses $2n = 52$ chromosomes. Is this sufficient to classify *L. angustifolius* L. as an intermediate species of *L. micranthus* Guss. and *L. luteus* L.? GHRABI GAMMAR & al. (1997a) observed the ultrastructure of the seed integument of the three species using scanning electron microscopy. Their results showed that the ultrastructures of *L. angustifolius* L. and *L. micranthus* Guss. were identical but different from that of *L. luteus* L. AINOUCHE (1988) performed morphological and biochemical analyses of population of *L. micranthus* Guss., *L. luteus* L. and *L. angustifolius* L. of various geographical origins in Algeria. His results revealed inter and intra-specific phenotypic variability and showed 40 to 50% similarity between *L. micranthus* Guss. and *L. luteus* L. and 30 to 40% similarity between *L. micranthus* Guss. and *L. angustifolius* L. The dendrogram plotted also shows a grouping of the *L. luteus* L. and *L. micranthus* Guss. populations.

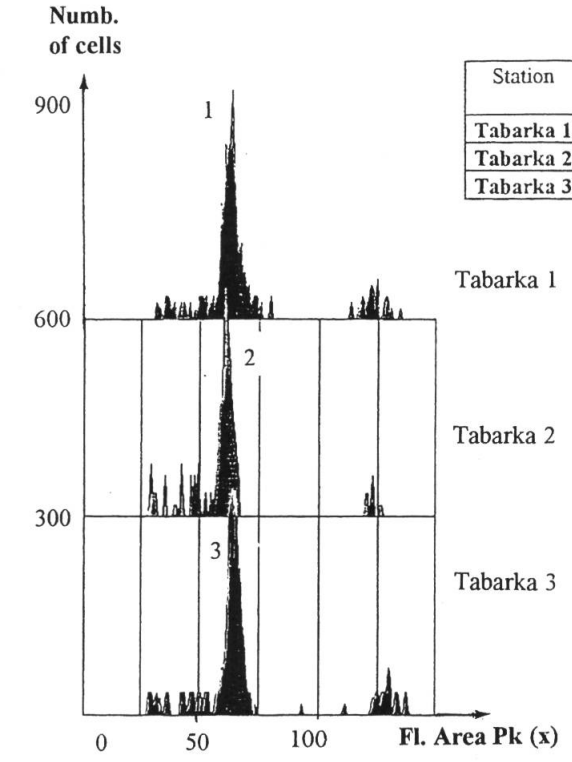
The heterogeneity of the number of chromosomes and the tendency for aneuploidy have also been reported for smooth-seeded lupins (GILOT, 1965; PAZY & al., 1988; CARSTAIRS & al., 1991, etc.). This is the case in particular for *L. luteus* L., whose geographical distribution is limited to the western Mediterranean basin, and for *L. angustifolius* L. and *L. micranthus* Guss. that are also found in the *L. luteus* L. distribution area but grow around almost the whole of the Mediterranean (GLADSTONES, 1974). Comparisons of the detailed karyograms of these three species are necessary to see whether the chromosomes of *L. micranthus* Guss. are smaller than those of *L. angustifolius* L. and whether the variation in the quantity of DNA observed between the latter species and *L. luteus* also corresponds to a variation in chromosome size.

Among the rough-seeded species, the quantity of DNA in *Lupinus cosentinii* Guss. individuals of the M'raissa population was slightly smaller that that of the two other populations. This difference is not significant and the result shows the genetic stability of the populations of this species. We have always counted $2n = 32$ chromosomes in individuals of these populations (GHRABI GAMMAR & al., 1997b).

Lupinus digitatus Forssk. ($2n = 36$, Egypt) displayed only 2% more DNA than *Lupinus cosentinii* Guss. ($2n = 32$, Tunisia) even though there is a difference of two pairs of chromosomes. This small variation might be explained by the difference in chromosome size. Indeed, *L. cosentinii* Guss. is the only rough-seeded lupin species with long chromosomes. Those of all the other species are shorter than 1 µm (PAZY & al., 1977).

Lupinus pilosus Murr. and *L. palaestinus* Boiss. are annual species that grow in the eastern Mediterranean basin but in two different habitats (Fig. 1). They have considerable morphological resemblance and possess the same number of chromosomes, $2n = 42$ (GLADSTONES, 1974; PAZY & al., 1977, 1981). This leads to supposing that one evolved from the other following eco-geographical speciation without any change in the number of chromosomes (PLITMANN, 1981 and PAZY & al., 1981). The results for these two species confirm the hypothesis that their isolation is accompanied by the shift of certain characters, such as ecological preferences and genetic incompatibility. Crossing of the two species gave a sterile F_1 (PLITMANN, 1981).

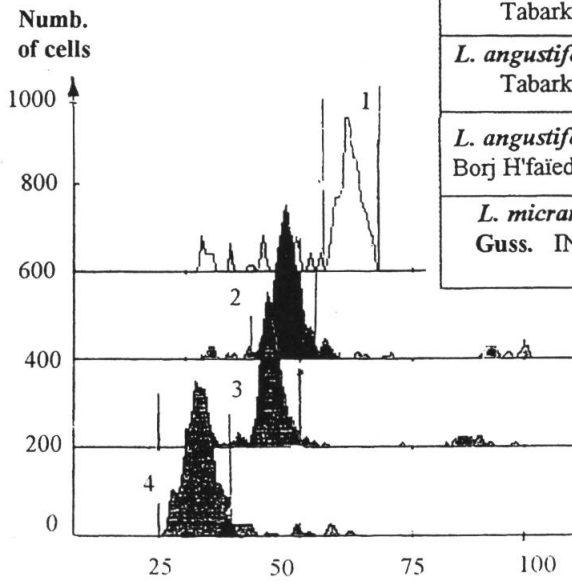
Lupinus pilosus Mur. and *L. palaestinus* Boiss. both have $2n = 42$ chromosomes and possessed a slightly larger quantity of DNA (3%) than *L. cosentinii* Guss., $2n = 32$, and slightly less (5%) than *L. digitatus* Forssk., $2n = 36$. It is difficult to correlate the variation in genome size observed between these species and their chromosome numbers (difference of five and three pairs of chromosomes). In contrast, there may be a correlation with chromosome size. According to this hypothesis, *L. pilosus* Mur. and *L. palaestinus* Boiss. should possess the smallest chromosomes.



***Lupinus luteus* L.**

Station	Reg	Cell	Pk (x)	Mn (x)	% Tot	SD	C V
Tabarka 1	1	347	62,1	60,4	72	3.6	2,3
Tabarka 2	2	350	58,1	58,4	82	3.1	4,6
Tabarka 3	3	342	64,0	63,8	70	3.4	3,2

A



Species & Stations	Reg	Cell	Pk (x)	Mn (x)	% Tot	SD	CV
L. luteus **L.** Tabarka 2	1	300	60,3	58,4	93	3,4	6,9
L. angustifolius **L.** Tabarka 3	2	300	47,6	45,6	85	2,6	3,3
L. angustifolius **L.** Borj H'faïedh lot E	3	300	42,9	42,1	82	4,7	5,8
L. micranthus **Guss.** INRAL	4	300	27	26,9	86	3,1	5,5

B

Fig. 2. – The average quantity of DNA, in G1, in three *L. luteus* L. specimens from three different populations: Tabarka 1, 2 et 3: **A;** in an *L. luteus* individual from the Tabarka 2 population, in two *L. angustifolius* L. individuals (Tabarka 3 and Borj H'faiedh) and in an *L. micranthus* Guss. individual: **B**.

Reg. = region in which the calculation was performed; % Tot. = percentage of nuclei analysed; Pk (x), Fl. area = fluorescence for which there is the highest number of nuclei; Mn (x) = average quantity of DNA per nucleus; SD = standard deviation and CV = coefficient of variation.

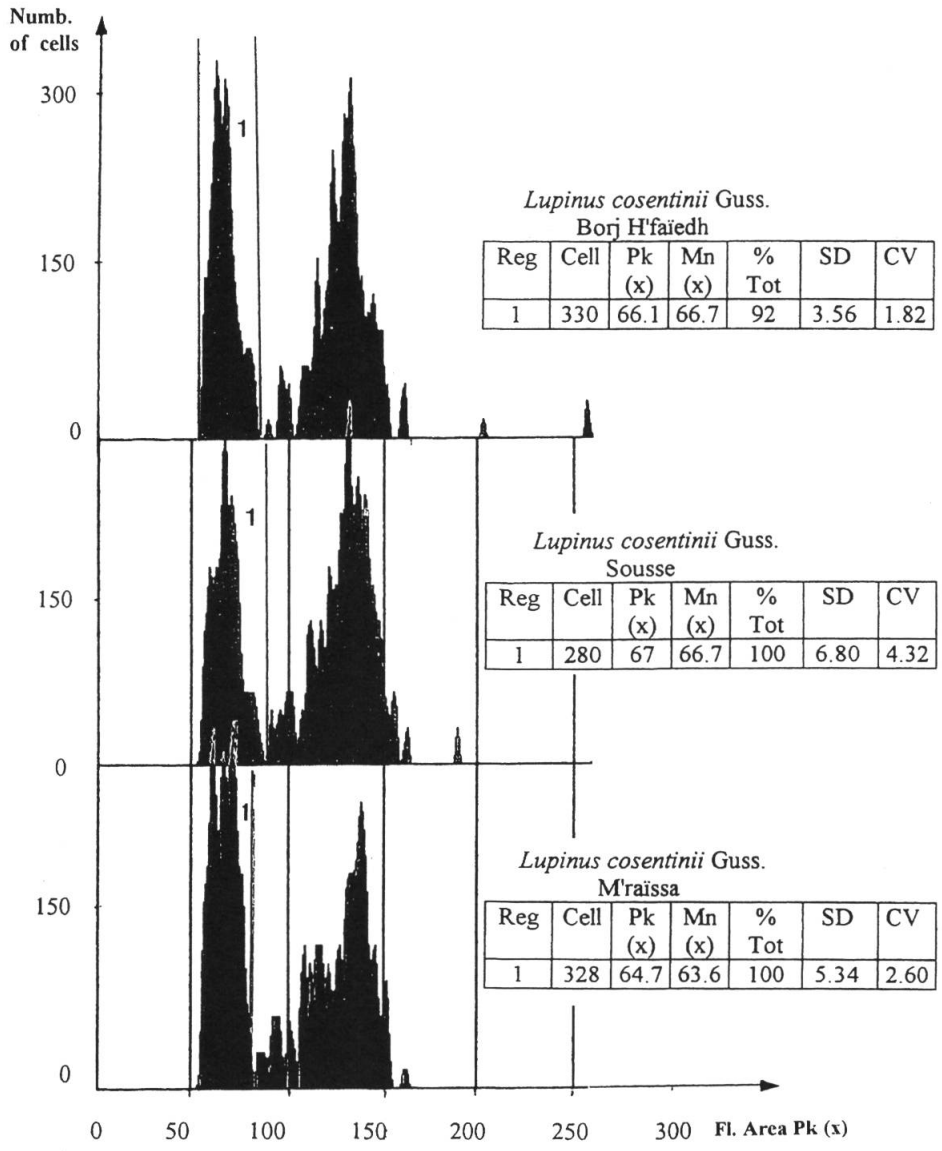
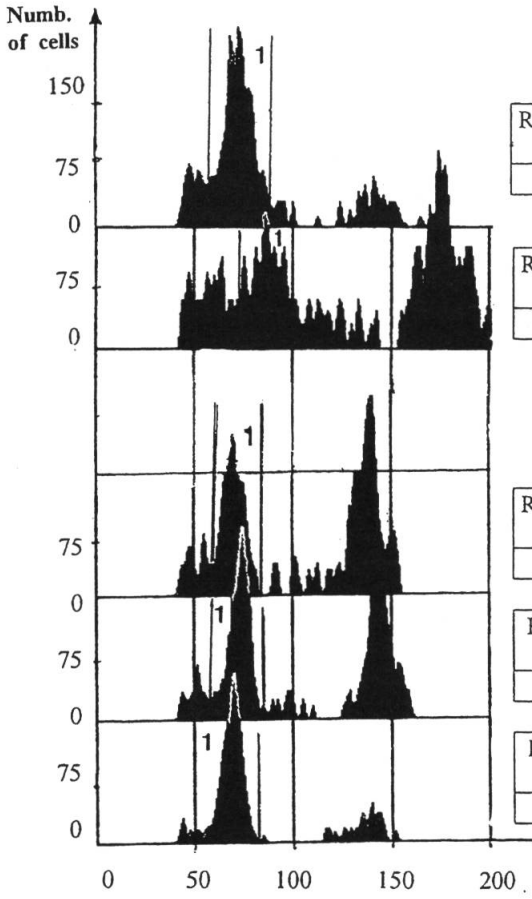


Fig. 3. – Superposition of the fluorescence peaks, in G1, in three individuals of *L. cosentinii* Guss. from spontaneous populations at Borj H'faiedh, Sousse and M'raissa, Tunisia.

Reg. = area of the region where the quantity of NDA was assayed; cell. = number of cells analysed; Pk (x) = point with the maximum number of nuclei on the *x*-axis (fluorescence for which there are the most nuclei); Mn (x) = average quantity of DNA per nucleus; % Tot = percentage of nuclei analysed; SD = standard deviation; CV = coefficient of variation. The curves have two peaks because a part of cells are in 2*n* phase, the other in 4*n* phase at the time of tests. Only the 2*n* phase is considered.



Lupinus pilosus Murr.

Reg	Cell	Pk (x)	Mn (x)	% Tot	SD	CV
1	238	69.1	70	81	2.45	1.23

Lupinus atlanticus Gladst.

Reg	Cell	Pk (x)	Mn (x)	% Tot	SD	CV
1	180	82.3	85	44	8.76	5,79

Lupinus palaestinus Boiss.

Reg	Cell	Pk (x)	Mn (x)	% Tot	SD	CV
1	200	70.2	70	58	2.10	1.06

Lupinus digitatus Forsk.

Reg	Cell	Pk (x)	Mn (x)	% Tot	SD	CV
1	230	74.3	75	63	3.64	2.31

Lupinus cosentinii Guss.

Reg	Cell	Pk (x)	Mn (x)	% Tot	SD	CV
1	280	67	66.7	100	3.56	1.82

Fig. 4. – Comparison of the average quantities of DNA, in G1, in *L. pilosus* Murr., *L. atlanticus* Glad., *L. palaestinus* Boiss., *L. digitatus* Forssk. and *L. cosentinii* Guss.

Reg. = area of the region where the quantity of NDA was assayed; cell. = number of cells analysed; Pk (x) = point with the maximum number of nuclei on the *x*-axis (fluorescence for which there are the most nuclei); Mn (x) = average quantity of DNA per nucleus; % Tot = percentage of nuclei analysed; SD = standard deviation; CV = coefficient of variation. The curves have two peaks because a part of cells are in 2*n* phase, the other in 4n phase at the time of tests. Only the 2*n* phase is considered.

Lupinus atlanticus Gladst. is the only species that possesses $2n = 38$ chromosomes (PAZY & al., 1981; ROY & al., 1988; CARSTAIRS & al., 1991). It stands out from all the other species analysed in the size of its genome. It has the largest quantity of DNA. It may alone form a subgroup among the rough-seeded lupins of the Mediterranean basin. This confirms the findings of SALMANOWICZ & al. (1994), who used an electrophoretic technique based on albumin variation in Mediterranean lupin seeds.

ACKNOWLEDGEMENTS

This work was supported by Action Intégré franco-tunisienne, "*Ressources biologiques, stratégies adaptatives et systématique d'espèces de la flore tunisienne*", No. 903, Ministère des Affaires étrangères. The authors are grateful for the help given by C. Duferray of *Service de cytométrie en flux*, INSERM U.291, Montpellier. Translation into English by Simon Barnard.

This is publication ISEM No. 98-159 de l'Institut des Sciences de l'Evolution de Montpellier, Unité Mixte de Recherche (UMR) 5554 CNRS-Université Montpellier II.

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