CARYOLOGIA Vol. 55, no. 2: 105-110, 2002

Karyological study of *Melilotus alba* Med. (Fabaceae) populations in Bulgaria

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Abstract - Karyological study was conducted on 10 populations of *Melilotus alba* Med. collected from different floristic regions in Bulgaria. The karyotypes of the investigated populations appear quite similar in their chromosomal morphology. Two cytotypes are delimited on the base of differences in the composition of the karyotypes and marked chromosomal differences as to length and morphological features. The results are compared with previous investigations on *M. alba*. The minor differences in the karyotypes established give reasons to consider these cytotypes as infraspecific taxa (varieties or forms) from a taxonomic point of view. The chromosomal polymorphism testify the susceptibility of the karyotype to the environmental factors resulting in genome modifications.

Key words: Bulgaria, chromosome number, karyotype, *Melilotus alba*, populations.

INTRODUCTION

Genus *Melilotus* Mill. belongs to tribe Trifolieae and comprises about 20 species distributed mainly in the temperate and subtropical regions of Europe, Asia and North Africa (Polhill and Raven 1981). Part of the species are synanthrops and cultivars.

In the bulgarian flora are found 5 species (Kozuharov 1976, 1992) related to sections Coelorytis Ser. (*Melilotus alba* Med., *M. officinalis* (L.) Pall., *M. dentata* (W.K.) Pers.) and Laccocarpus Ser. (*M. neapolitana* Ten. and *M. indica* (L.) All.) (Schulz 1901).

The objective of this karyological study is the species *M. alba* taking also into account its agricultural importance. Meusel *et al*. (1965) determined it as an european-caucasian-centralasiatic-anatolian-siberian-westnorthafrican geoelement. The species is distributed in Bulgaria up to 1500 m a. s. l. (Kozuharov 1976, 1992).

A great deal of cytological investigations has been conducted on populations of *M. alba* in Western and Central Europe, and in Russia (Lesins 1952; Magulaev 1980; Raghuvanshi *et al*. 1980). Less information is available from Asia, predominantly from the western part of the continent. Except for Bulgaria (Kozuharov *et al*. 1975), chromosome numbers and composition of karyotypes of populations from the Balkan peninsula were reported from from F. Y. R. of Macedonia (Sopova and Secovski 1982).

The aim of the present study was to analyse the karyotype variation of bulgarian populations of *M. alba* in order to make comparison with other ecotypes previously studied from Bulgaria (Kozuharov *et al*. 1975), F. Y. R. of Macedonia (Sopova and Secovski 1982), Japan (Kita 1966), India (Bairiganjan and Patnaik 1983) considering evolutionary aspects.

MATERIALS AND METHODS

The material is collected from 10 populations of *M. alba* distributed in different floristic regions and altitudes indicated on UTM Grid map of Bulgaria (Scale 1:1500000). The voucher specimens are deposited in the Herbarium of Sofia University "St. Kliment Ohridski", Department of Botany (SO). (Fig. 1; Table 1)

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Table 1 – Material source of the populations of *Melilotus alba* studied and data on the chromosomes.

N°	Population	Pair N°	S+L, (μm)	L/S	Ic, %	Chromosome type	Relative length, %	Xmin: Xmax
A	Balchik SO 100288 10 m a.s.l., NJ-90	1	2.4+2.4=4.8	1.00	50.0	M	100	1.6:1
		2	2.4+2.4=4.8	1.00	50.0	M	100	
		3	2.0+2.0=4.0	1.00	50.0	M	83.3	
		4	1.6+2.0=3.6	1.25	44.0	M	83.3	
		5	1.2+1.6=2.8	1.30	42.9	M	58.3	
		6	1.6+2.8=4.4	1.75	36.4	SM	91.7	
		7	1.6+2.8=4.4	1.75	36.4	SM	91.7	
		8(sat)	0.8+1.6=2.4	2.00	33.3	SM	50.0	
B	Trigrad SO 101150, 1200 m a.s.l., KG-80	1	2.0+2.4=4.4	1.20	45.0	M	100	1.4:1
		2	1.6+2.0=3.6	1.25	44.0	M	81.8	
		3	1.6+1.6=3.2	1.00	50.0	M	72.7	
		4	1.6+1.6=3.2	1.00	50.0	M	72.7	
		5	1.6+1.6=3.2	1.00	50.0	M	72.7	
		6	1.6+2.4=4.0	1.50	40.0	SM	90.9	
		7	1.2+2.4=3.6	2.00	33.0	SM	81.8	
		8(sat)	1.2+2.4=3.6	2.00	33.0	SM	81.8	
C	Pirin Mt. SO 101416, 1650 m a.s.l., GM-03	1	2.4+2.4=4.8	1.00	50.0	M	100	1.5:1
		2	2.4+2.4=4.8	1.00	50.0	M	100	
		3	2.0+2.4=4,4	1.20	45.5	M	91.7	
		4	1.6+2.0=3.6	1.25	44.4	M	75.0	
		5	1.6+1.6=3.2	1.00	50.0	M	66.7	
		6	1.6+2.8=4.4	1.76	36.4	SM	91.7	
		7	1.2+2.4=3.6	2.00	33.3	SM	75.0	
		8(sat)	1.2+2.4=3.6	2.00	33.3	SM	75.0	
D	Samokov SO 101152, 700 m a.s.l., GM-19	1	2.0+2.4=4.4	1.20	45.5	M	100	1.6:1
		2	1.6+2.0=3.6	1.25	44.4	M	81.8	
		3	1.6+2.0=3.6	1.25	44.4	M	81.8	
		4	1.6+1.6=3.2	1.00	50.0	M	72.7	
		5	1.2+1.6=2.8	1.33	42.9	M	63.6	
		6	1.6+2.8=4.4	1.75	36.4	SM	100	
		7	1.6+2.4=4.0	1.50	40.0	SM	90.9	
		8(sat)	1.2+2.0=3.2	1.67	37.5	SM	72.7	
E	Vitosha Mt. SO 101417, 1810 m a.s.l., FN-92	1	2.4+2.4=4.8	1.00	50.0	M	100	2.0:1
		2	2.0+2.4=4.4	1.20	45.5	M	91.7	
		3	1.6+2.0=3.6	1.25	44.4	M	75.0	
		4	1.6+1.6=3.2	1.00	50.0	M	66.7	
		5	1.2+1.6=2.8	1.33	42.9	M	58.3	
		6	1.2+2.4=3.6	2.00	33.3	SM	75.0	
		7	1.2+2.4=3.6	2.00	33.3	SM	75.0	
		8(sat)	0.8+1.6=2.4	2.00	33.3	SM	50.0	
F	Vakarel SO 101151, 800 m a.s.l., GN-21	1	2.4+2.8=5.2	1.67	46.2	M	100	1.9:1
		2	2.4+2.4=4.8	1.00	50.0	M	95.6	
		3	2.0+2.4=4.4	1.20	45.5	M	84.6	
		4	1.2+1.6=2.8	1.33	42.9	M	53.9	
		5	1.2+1.6=2.8	1.33	42.9	M	53.9	
		6	1.6+2.8=4.4	1.75	36.4	SM	84.6	
		7	1.2+2.4=3.6	2.00	33.3	SM	69.2	
		8(sat)	1.2+2.4=3.6	2.00	33.3	SM	69.2	
G	Galabetz SO 101149, 600 m a.s.l., GN-33	1	2.4+2.4=4.8	1.00	50.0	M	100	2.0:1
		2	1.6+2.0=3.6	1.26	44.4	M	75.0	
		3	1.6+2.0=3.6	1.26	44.4	M	75.0	
		4	1.2+1.6=2.8	1.33	42.9	M	58.3	
		5	1.2+1.2=2.4	1.00	50.0	M	50.0	
		6	1.6+2.4=4.0	1.63	40.0	SM	83.3	
		7	1.2+2.6=3.8	2.17	31.6	SM	79.2	
		8(sat)	1.2+2.0=3.2	1.67	37.5	SM	66.7	

N°	Population	Pair N°	S+L, (µm)	L/S	Ic, %	Chromosome type	Relative length, %	Xmin: Xmax
H	Pirdop SO 101415, 550 m a.s.l., KH-63	1	2.4+2.4=4.8	1.00	50.0	M	100	1.5:1
		2	2.0+2.4=4.4	1.20	45.5	M	91.7	
		3	1.6+2.0=3.6	1.25	44.4	M	75.0	
		4	1.6+1.6=3.2	1.00	50.0	M	66.7	
		5	1.6+1.6=3.2	1.00	50.0	M	66.7	
		6	1.6+2.8=4.4	1.75	36.4	SM	91.67	
		7	1.2+2.4=3.6	2.00	33.3	SM	75.0	
		8(sat)	1.2+2.4=3.6	2.00	33.3	SM	75.0	
I	Sofia SO 100289, 550 m a.s.l., FN-93	1	2.4+2.4=4.8	1.00	50.0	M	100	1.2:1
		2	2.0+2.4=4.4	1.20	45.5	M	91.7	
		3	20.+2.0=4.0	1.00	50.0	M	83.3	
		4	20.+2.0=4.0	1.00	50.0	M	83.3	
		5	20.+2.0=4.0	1.00	50.0	M	83.3	
		6	1.6+2.8=4.4	1.75	36.4	SM	91.7	
		7	1.2+2.8=4.0	2.33	30.0	SM	83.3	
		8(sat)	1.2+2.8=4.0	2.33	30.0	SM	83.3	
J	Kavarna SO 101414, 10 m a.s.l., PJ-00	1	2.0+2.4=4.4	1.2	45.5	M	100	1.6:1
		2	1.6+2.0=3.6	1.25	44.4	M	81.8	
		3	1.6+1.6=3.2	1.00	50.0	M	72.7	
		4	1.6+1.6=3.2	1.00	50.0	M	72.7	
		5	1.2+1.6=2.8	1.33	42.9	M	63.6	
		6	1.6+2.8=4.4	1.76	36.4	SM	100	
		7	1.6+2.8=4.4	2.33	30.0	SM	90.9	
		8(sat)	1.2+2.4=3.6	2.00	33.3	SM	81.8	

The karyotypes are investigated on metaphase plates prepared from root meristem of germinating seeds, stained in chematoxilin after Gomory (PEARSE 1960). The idiograms are constructed after measurements were performed on three metaphase plates from each population (Fig. 2). The chromosomal type is determined after the centromere index $I^c = s/s+l$, according to the classification proposed by GRIF and AGAPOVA (1986). The composition of the karyotypes is shown on Table 1.

RESULTS

The analysis of literature data and the results of the present study reveal that the basic chromosome number in *Melilotus* Mill. is x=8. All investigated bulgarian species are diploids (2*n*=2x=16). Tetraploid and aneuploid cytotypes are not established for *M. alba* in contrast to the results of ATWOOD (1936), LESINS (1952) and RAGHUVANSHI *et al.* (1980).

Comparing the karyotype constitution to that of already studied populations (KOZUHAROV *et al.* 1975; SOPOVA and SECOVSKI 1982) the following structural differences were established: 1) Two cytotypes are observed differing mainly by the presence of a pair of intercentric chromosomes in the karyotypes of populations I and J. 2) The number of metacentric chromosomes is constant whereas the number of submetacentric chromosome pairs varies. The composition of the karyotype for 8 of the populations is identical with the results of KITA (1966) for annual varieties of *M. alba*. 3) In all populations the satellites are always attached to the short arm of the smallest pair of submetacentric chromosomes. 4) The ratio between the longest and the shortest chromosome (X_{max}:X_{min}) ranges between 1.2:1 and 2:1. This ratio is insignificant for the karyotype of population B where the difference in the length of the longest and the shortest chromosomes is about 1µm. The highest ratio 2.5 is observed in populations E and G. 5) Breakage in the centromere part is observed for the second in length pair of metacentric chromosomes in populations D and F.

DISCUSSION

The symmetry of the karyotype of *M. alba* with its constant composition and insignificant differences in the dimensions of the chromosomes is characteristic for slightly evolved karyotypes.

The karyotypes of the investigated populations are quite similar in their chromosomal morphology. Most of the chromosomes are of M

(63%) and SM type. A pair of intercentric chromosomes is observed only in populations J and I that gives ground to consider them as a separate cytotype.

Karyologically most advanced karyotypes are observed in population J followed by I, with marked chromosomal differences as to length and morphological features.

The karyotype of population J shows substantial length difference between the largest and the smallest chromosomes. On the other hand, measures of large chromosomes differ substantially for population I while the difference between these and the small ones is less marked than for J (Table 1; Fig. 2).

The composition of the karyotype in 8 of the populations studied ($2n$=10m + 4 sm + 2sm^{sat}) is identical with the results of Kita (1966) whereas the observations by Sopova and Secovski (1982) evidence a more heterogeneous composition ($2n$=6m + 6sm + 2a + 2a^{sat}). The most symmetrical karyotype of *M. alba* ($2n$=12m + 4sm) was observed by Bairiganjan and Patnaik (1989).

Satellite chromosomes of SM type are clearly distinguished in all karyotypes (Kita 1966; Fernandes and Santos 1971; Kozuharov *et al.* 1975) except for the results provided by Sopova and Secovski (1982).

The last authors observed satellites attached to one of the chromosomes of the shorter pair of acrocentric type. Bairiganjan and Patnaik (1989) are the only who do not report satellite chromosomes for the karyotype of *M. alba* from India.

Satellites attached to the short arm of the shortest SM chromosome pair are found like the observation of Kita (1966). The satellites vary in their form and size. Large spherical satellites (0.8µm) prevail with the exception in populations B, C and E (0.3µm). In karyotypes A, D and H the secondary constrictions cut off long segments of chromatids.

The differences in the composition of the karyotypes of the investigated populations I and J might be linked with variation of the vegetative and reproductive plant parts, their shrubly habit, and probably a biennial life cycle. Similar data are presented by Kita (1966) after investigation of annual and biennial cultivated varieties of *M. alba*.

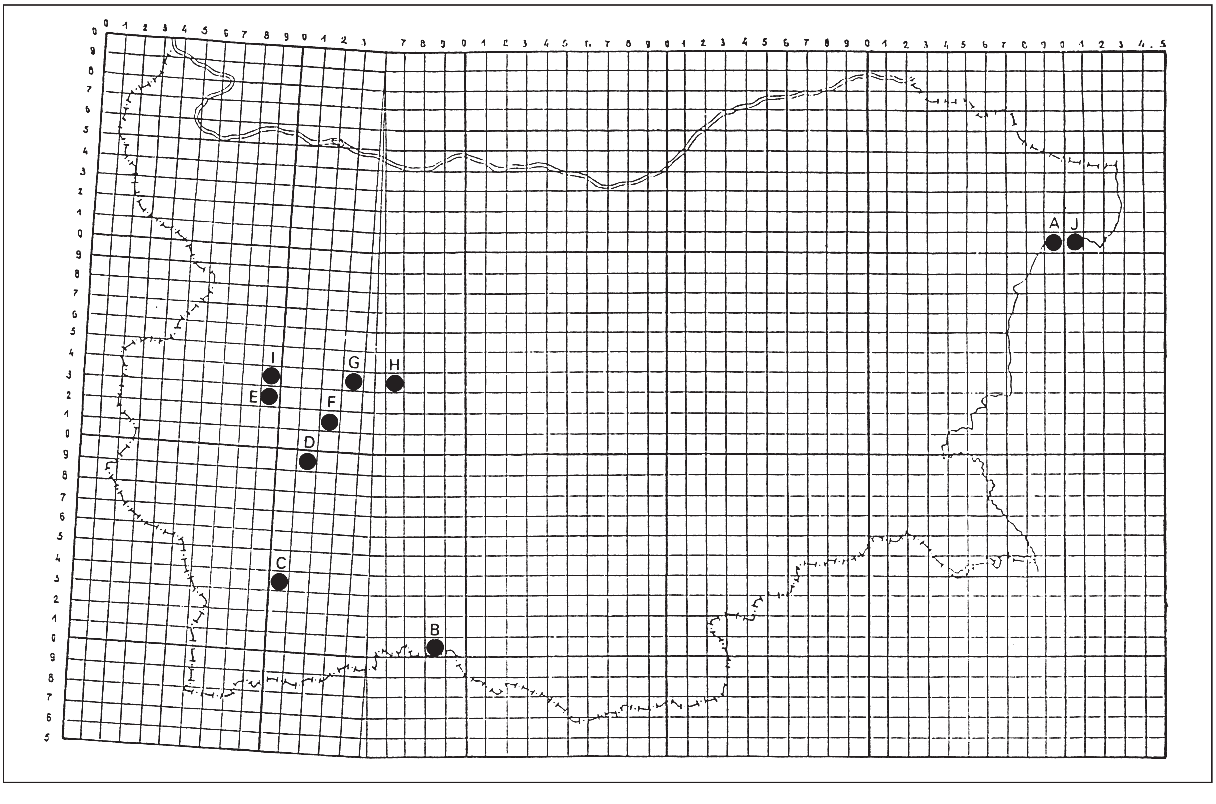

Fig. 1 – An UTM grid map of Bulgaria showing the location of investigated populations mentioned in the text and Table 1 (Scale 1: 1500000). A - Balchik; B - Trigrad; C - Pirin Mt.; D - Samokov; E - Vitosha Mt.; F - Vakarel; G - Galabetz; H - Pirdop; I - Sofia; J - Kavarna.

On the other hand, the ecological conditions of the plant habitats exposed to a pronounced anthropogenic impact, appear as stress factors provoking mutation variation.

However, the insignificant karyological differences established give reason to interpret such variation from a taxonomic point of view by delimitation of infraspecific taxa (varieties or forms) as considered also by STEVENSON (1969).

Our results from the cytological investigation of 10 populations of *M. alba* show that no correlation exists between karyotype variation and altitude. The chromosomal polymorphism observed in separate species or in populations of a given species within a genus range, as well as the structural chromosomal type in the course of the evolutionary process, testify to the susceptibility of the karyotype to the environmental factors resulting in genome modifications.

Differences in minor details of the karyotypes in each species suggest that structural chromosomal rearrangements as translocation, inversion and duplication, or deficiency, play an important role in the evolution of *Melilotus*.

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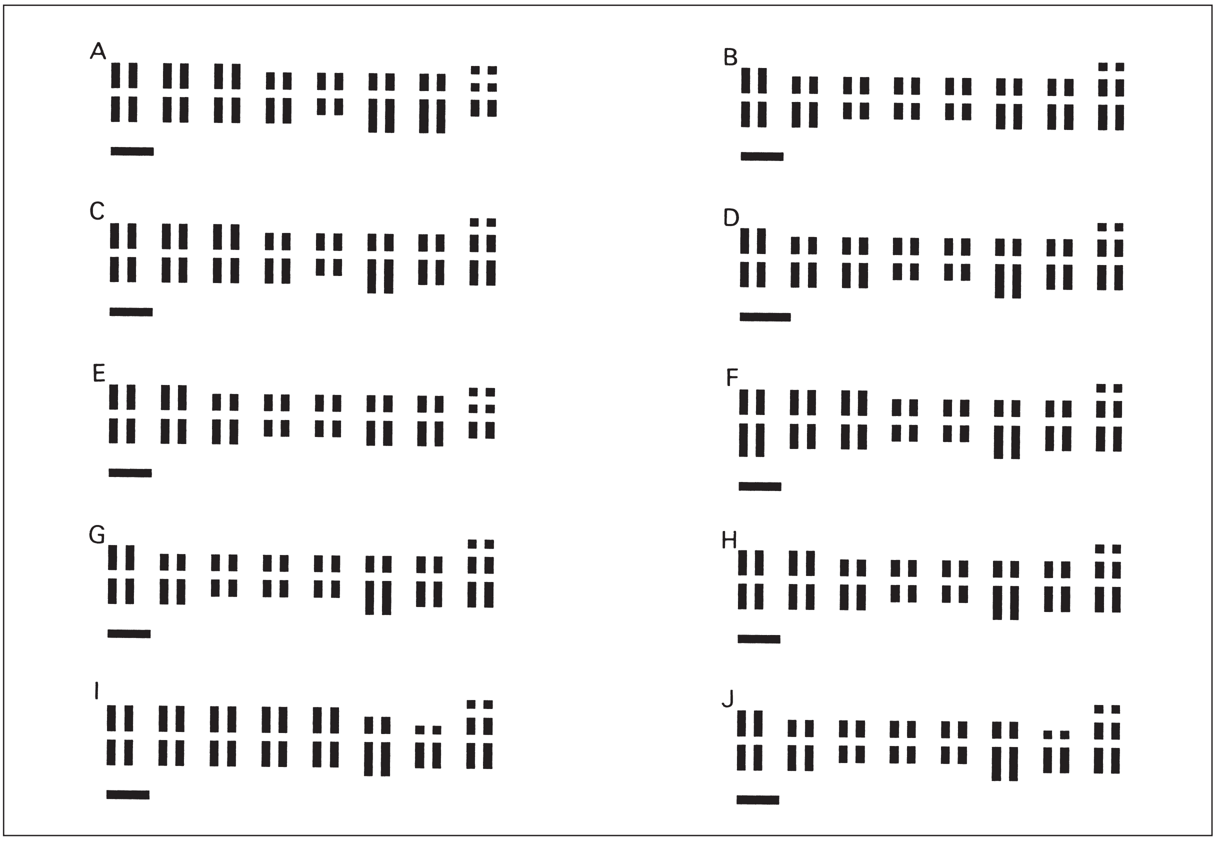

Fig. 2 – Idiograms of investigated populations (scale bar 4µm).

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Received October 9, 2001; accepted November 29, 2001