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KARYOTYPING, C- AND NOR BANDING OF *ALLIUM SATIVUM* L. (LILIACEAE) CULTIVATED IN TURKEY

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Abstract

The cytological features including chromosome number, karyotypic characteristics, C-banding and silver NOR-banding were investigated in a local cultivar of *Allium sativum* L., from Turkey. Actual lengths, relative lengths, L/S arm ratios of mitotic chromosomes were calculated from best six metaphase plates. Only Chromosome 5 was submedian, all the others were median. C-banding was observed on 4 chromosomes. Bands were centromeric on Chromosome 1 and 4, located to the neighbourhood of the secondary constrictions on Chromosome 5 and were interstitial close to centromere on Chromosome 8. Darkly stained silver nitrate bandings were present in the NORs of Chromosome 5 and 7, on Chromosome 1 and 8 in some cells.

Introduction

Allium sativum L., (garlic) a member of the Liliaceae family and a popular condiment is cultivated all over the world. The chromosome number of *Allium* sp., is reported as 2n=16 (Levan, 1935; Mensinkai, 1939) and 2n=18 in two varieties of garlic (Sharma & Bal, 1959). Intraspecific variations in the size and type of chromosomes, positions of the secondary constrictions and positions and numbers of the heterochromatic bands occur frequently in most of plant species. This study was conducted to observe karyotype features, heterochromatic C-bands and silver NOR (nucleolar organizing regions) bands present in a common cultivar of *Allium sativum* L., commonly cultivated in Turkey.

Materials and Methods

Root tips of a local cultivar of *A. sativum* pretreated with 0.05 % colchicine for 3h at room temperature and fixed in 3:1 absolute alcohol: acetic acid were hydrolysed in 1N HCI at 60ºC for 12 min., and stained with Feulgen. Squashes were made in 45% acetic acid. For Giemsa C-banding, root tips hydrolysed in 45% acetic acid for 25 min., at 60ºC were squashed in 45% acetic acid. Slides treated in 5% $Ba(OH)_2$ for 5 min., at 55ºC, were washed in tap water and submerged in 2XSSC at 55-60ºC for 1 hr. Slides stained in 5% Giemsa at pH 6.8 for 10-30 min., were rinsed and air-dried (Ünal *et al*., 1995). Silver nitrate NOR staining was done according to the method of Hsu (1981) with some modification. For Ag-NOR staining, root tips hydrolysed in 1N HCI for 14 min., at 60ºC were squashed in 45% acetic acid. Slides treated in 50% silver nitrate for 20 min., at 60ºC were washed in tap water and air dried. All slides were mounted in depex and examined using Prior microscope. Photographs were taken with a Centon DF-300 camera on Agfa ST 8 film sat at 12 ASA.

Measurements are based on 6 unbanded mitotic metaphases. C- and Ag-NOR banding patterns were determined from Giemsa and silver nitrate stained slides,

respectively. Chromosomes were classified according to the nomenclature of Levan *et al*., (1964). The satellites of nucleolar chromosomes were also measured and considered in determining the total chromosome length, relative length and long to short arm ratio. In the karyotype, the chromosomes were arranged in order of decreasing length.

Results and Discussion

Feulgen Staining and Giemsa C-banding

Similar to earlier reports (Levan, 1935; Mensinkai, 1939; Cortes *et al*., 1983; Wajahatullah & Vahidy, 1990) somatic metaphases showed the chromosome number of *A. sativum* as 2n=16 (Fig. 1A). However, Sharma & Bal (1959) observed 2n=18 chromosomes in 2 garlic varieties while Banerjee (1980) and Etoh (1986) have also reported 2n=12 and 2n=18, respectively. The length of chromosomes in *Allium sativum* varied from 12.20 μm to 7.32 μm. Chromosome 5 has the highest L/S arm ratio and is submedian. All the other chromosomes are median with arm ratio 1.03-1.62 (Table 1). However, Wajahatullah & Vahidy (1990) reported that Chromosome 1 and 2 as median and the rest of the chromosomes as submedian but the variation in chromosome sizes between 11 μm and 7.5 μm observed by them is quite close to our findings. In this study, secondary constrictions were present on Chromosome 5 and 7 while these were observed on Chromosome 6 and 7 in the studies of Wajahatullah & Vahidy (1990), Cortes *et al*., (1983) and Cortes & Escalza (1986). Secondary constrictions were larger in size than their respective short arms which is named as "sativum" type of nucleolar chromosome specific for section *Allium* of genus *Allium* (Mathew, 1996). An additional secondary constriction at the proximal end of the long arm of Chromosome 8 observed by Cortes *et al*., (1983) and Cortes & Escalza (1986) was neither present in this study nor that reported by Wajahatullah & Vahidy (1990).

C-banding analysis of *A. sativum* showed that only four chromosome pairs carry banding patterns (Fig. 1B). These patterns were centromeric on the short arm of Chromosome 1 and on the long arm of Chromosome 4. On Chromosome 5, there were located at the neighbourhood of the secondary constriction. These bands were often visible as thin spot or were totally absent. On Chromosome 8, an interstitial band close to centromere was present on the short arm (Fig. 2A). C-bands were not very prominent on photography while they were clearly observed under microscope, that is why they are indicated by arrows in Fig. 1B. Cortes *et al*., (1983) also reported C-bands at the secondary constrictions on Chromosome 6, 7 and 8 and an additional telomeric band on the long arm with an interstitial band on the short arm of the chromosome 7. They also determined a telomeric C-band on the short arm of Chromosome 4. They did not observe centromeric C-band as observed in this study. However, telomeric C-bands were not observed in this material. Cortes & Escalza (1986) used two modified C-banding techniques. In one of these, C-bands were present mainly on secondary constrictions as well as in some telomeres. The other procedure allowed a preferential staining of all centromeres and NORs. The differences between C-banding patterns obtained in two different methods used by Cortes & Escalza (1986) and in this study could be due to the differences on the procedures used for C-banding. The difference might also be due to some other reasons. Such a C-banding polymorphism is very common in chromosomes of several plant species. In *Scilla sibirica*, all 20 plants examined were unique in their

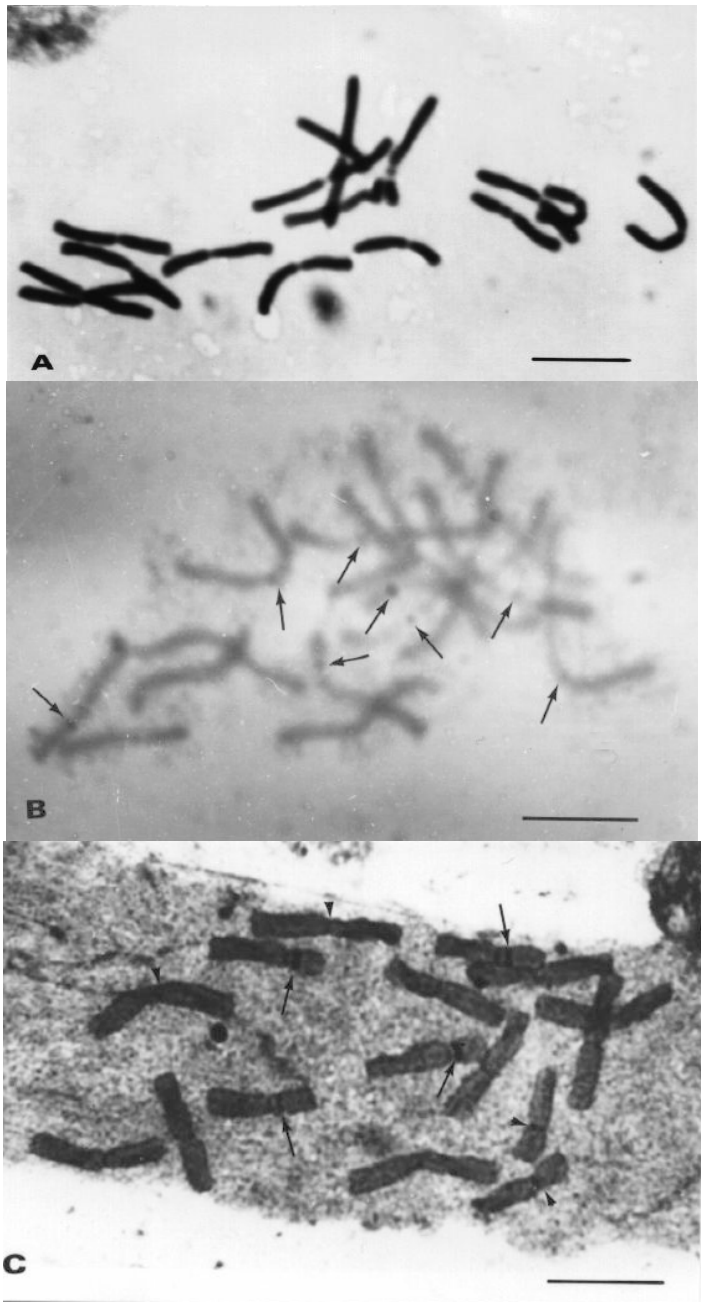

Fig. 1. A) Mitotic metaphase chromosomes of *Allium sativum*, B) C-banded metaphase chromosomes in *Allium sativum* (bands are shown by arrows), C) Chromosomes at mitotic metaphase showing NORs through silver nitrate staining (arrows show NORs on Chromosome 5 and 7, arrow heads show NORs on Chromosome 1 and 8).
Bar= 10 μm.

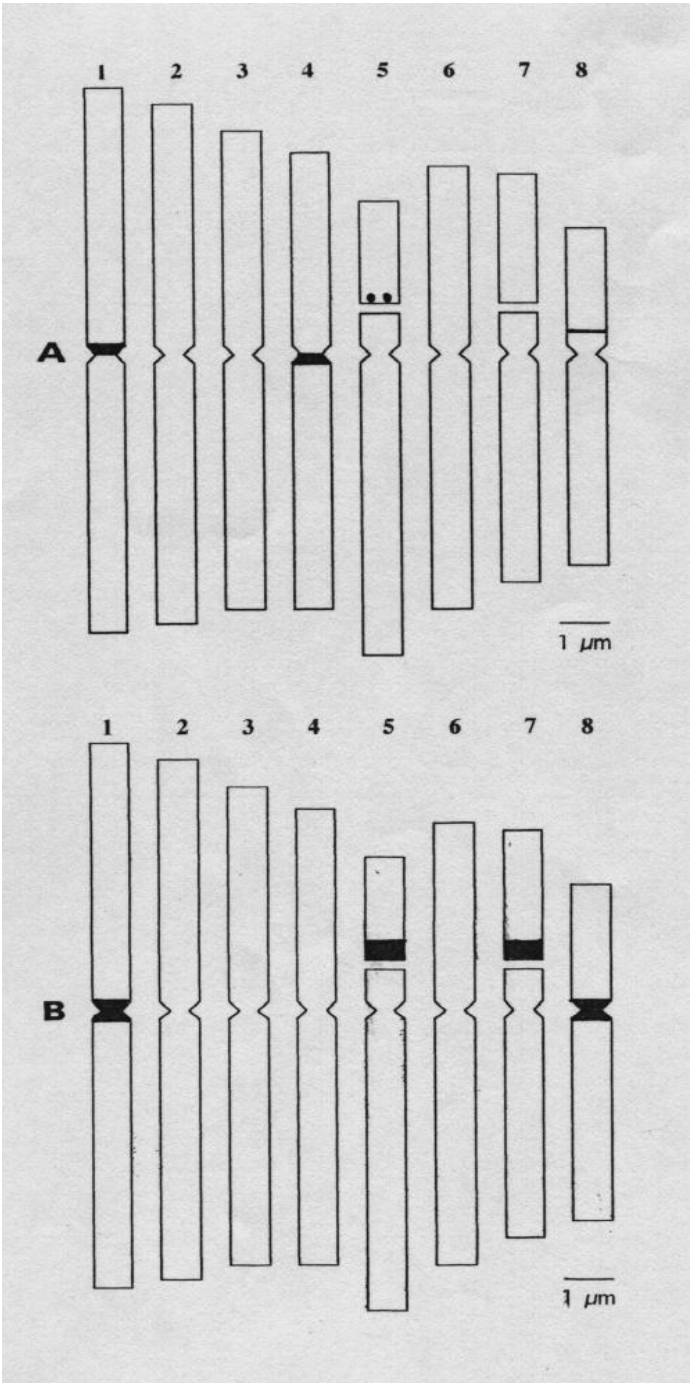

Fig. 2. A) Idiograms of C-banded chromosomes in *Allium sativum*, B) Idiograms of Ag-NOR banded chromosomes in *Allium sativum*.

Table 1. Karyotype characteristics of somatic metaphase chromosomes of *A. sativum*.

Chromosome pair no	Chromosome arms		Satellite length µm	Total length µm	Arm ratio (r) (L/S)	Relative length (%)	Centromeric position*
	Long arm (L) µm	Short arm (S) µm					
1.	6.19	6.01	-	12.20	1.03	15.17	m
2.	5.98	5.59	-	11.57	1.07	9.92	m
3.	5.70	5.00	-	10.70	1.14	9.18	m
4.	5.76	4.55	-	10.31	1.27	8.84	m
5.	6.73	0.90	2.31	9.94	2.10	8.53	sm
6.	5.53	4.16	-	9.69	1.33	8.32	m
7.	4.91	0.90	2.87	8.68	1.30	7.45	m
8.	4.53	2.79	-	7.32	1.62	6.28	m

Total length of haploid complement: 80.41 µm
* m = median; sm = submedian

band endowment and the polymorphism involved all chromosomes of the complement (Vosa, 1973). In *A. pulchellum*, a total of 30 plants from three populations investigated by Vosa (1976) were found unique in their banding patterns. In *A. flavum*, 15 plants analyzed from two populations were found to be unique in their banding patterns except three from population 1 which showed the same banding patterns and were obviously members of a clone. Polymorphism was also observed in *A. stamineum*, *A. paniculatum*, *A. fiscum* and *A. pallens* (Vosa, 1976). In *A. cepa*, Stack (1974) reported Giemsa bands occuring preferentially at centromeres and secondary constrictions. Cortes *et al*., (1983) showed C-bands at telomeres and secondary constrictions. The results show that homologous chromosomes between individuals and populations may be polymorphic and differ in the position, number and size of the heterochromatic segments that would replace normal euchromatic segments (Vosa, 1973, 1976). Differences in banding patterns might have resulted from different treatments because different storage times of the slides was found to affect the banding patterns (Fiskesjö, 1974; Cortes & Escalza, 1986). Moreover, the banding patterns differences among populations might be an indicative of their probable adaptive value because of their growing habitat (Vosa, 1976).

Silver nitrate stained NORs

In our material, Chromosome 5 and 7 showed darkly stained NORs after silver nitrate staining. In addition, silver nitrate stained bands were detected in some cells on Chromosome 1 and 8 (Fig. 1C, 2B). Wajahatullah & Vahidy (1990) reported that while N-banding was successfully used for identifying the bands at 4 NORs of chromosomes, only two of them were darkly stained after silver nitrate staining. At the cellular level, Ag-staining methods demonstrate gene activity at rDNA sites (Nucleolus Organizing Regions) (Hubbell, 1985). Although it is recognized that secondary constrictions on chromosomes arise from the activity of nucleolus organizing regions (NORs) during the preceding interphase, some of them could not be detected by conventional staining. Furthermore, it has been demonstrated that not all secondary constrictions are actual NORs (Pijnacker & Ferwerda, 1984; Jamilena *et al*., 1990). Though at least 4 NORs are present in *A. sativum* as detected in different studies by Feulgen staining, two of them could not be shown by Wajahatullah & Vahidy (1990). However, all of these sites were prominent as well as some additional ones in our material stained with silver nitrate. This might have resulted either from the varietal differences or from the procedure used.

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