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Comparative Study of Chromosome Morphology and C-banding Patterns in Several Genotypes of *Lens culinaris*

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Abstract: Karyotype and C-banding patterns of mitotic chromosomes in 10 cultivars and landraces of lentil were studied. Aceto-iron-hematoxilin staining and Giemsa C-banding techniques were used to staining choromosomes and banding patterns analysis, respectively. Chromosome characteristics including long arm, short arm and chromosome lengths, total length of chromosome set, arm ratio index, relative chromosome length, the width and position of each band, heterochromatin percent per chromosome and per chromosome set were measured using Micromeasure software. The results of this study revealed that the genome of this species consisted of four pairs of metacentric and three pairs of submetacentric chromosomes. Chromosome 4 had a secondary constriction near centromeric region of its long arm. Arm ratio index of chromosomes ranged from 1.24 in chromosome 3 to 2.38 in chromosomes 6. Each chromosome, having a distinctive banding pattern, was recognizable. Karyological characteristics and banding patterns of all materials studied were similar to each other, however, some polymorph C-bands were observed on chromosome arms.

Key words: Aceto-iron-hematoxilin staining, lentil, karyotype, C-banding

INTRODUCTION

Lens culinaris Medik is a diploid (2n = 2x = 14), self-pollinating, annual crop and one of the oldest domesticated cool-season grain legumes. Remains of this species were found in archaecological sites dated 6 to 7 thousand years ago (Cubero, 1981). Originated in West Asia, its cultivation rapidly spread to the Mediterranean and Africa (Zohary, 1999). Lentils are a major source of proteins. There are several reports on chromosome number, karyotypes and idiograms for *L. culinaris* (Ladizinsky, 1979; Mehra *et al*., 1986; Ahmad *et al*., 1992; Ramesh and Salimuddin, 1992; Kumar and Gupta, 1997; Kumar *et al*., 2001). However, we are still far from an unambiguous identification of each chromosome pair and some disagreement still exists on the exact karyotype. To establish an accurate karyotype of *L. culinaris* is becoming important for plant breeding programs, providing the assignment of genetic linkage groups to chromosomes and the integration of physical and genetic recombination maps. Moreover, phylogenetic relationships and chromosome evolution in the genus can be studied if the *Lens* chromosomes can be distinguished using a well described karyotype as reference. In species with highly symmetrical karyotypes and very similar chromosome morphology, in fact, chromosome length, arm length and ratio and position or number of secondary constrictions may not be sufficient criteria to differentiate individual chromosome pairs. Galasso *et al*. (2001) proposed the karyotype of *Lens* with three pairs of metacentric or submetacentric chromosomes, three pairs of acrocentric chromosomes and one satellited pair of chromosomes.

Giemsa C-banding technique, which stains constitutive heterochromatin (Arrighi and Hsu, 1971), is a powerful technique that can be used to identify individual chromosomes. In this study we attempt to identify individual chromosomes of lentil with C-banding procedure and chromosome arm length ratio, to develop more detailed karyotypes than those previously available and to evaluate the polymorphism of C-bands among this crop cultivars.

MATERIALS AND METHODS

This study was conducted at cytogenetic laboratory of Mohaghegh Ardabili University of Iran in 2006. Seeds of ten lines and landraces of lentil including (Flip 92-15L, Ill-6002, Ill-6468, Ill-7523, Ill-6206 and Khalkhal, Koraeim, Ardebil, Moghan and Ahar landraces from northwest of Iran) were germinated on moist blotting paper and the root tips were obtained. Staining method and C-banding technique has been used as described earlier by Asghari-Zakaria *et al*. (2002).

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Chromosome measurements including long arm, short arm and chromosome lengths, total length of chromosome set, arm ratio index, relative chromosome length, the width and position of each band, heterochromatin percent per chromosome and per chromosome set were made from 10 enlarged well-spread metaphase cells, using Micromeasure software developed by the Biology department of Colorado State University, available on internet at http: //www. colostate. edu/Depts/ Biology/Micromeasure. Homologous chromosomes were identified based on similarities of C-banding patterns and the position of centeromers. The nomenclature followed Levan *et al.* (1964) and chromosomes were named as 1, 2, 3, 4, 5, 6 and 7 in descending order of length.

RESULTS AND DISCUSSION

Mitotic chromosomes and their C-banding patterns are shown in Fig. 1 and 2, respectively. Karyotypic and C-banding characters of the seven mitotic chromosomes are shown in Table 1 and 2 and the mean position (the distance of the band center from the centeromer in % of the chromosome arm length on which the band is located) and width (in % of the chromosome arm length on which the band is located) of the C-bands in the 10 lentil lines and landraces are shown in Table 3.

The analysis of karyotype showed that this species has 2n = 2x = 14 chromosomes (Fig. 1). Chromosome length in lentil ranged from 5.59 μm in chromosome 7 to

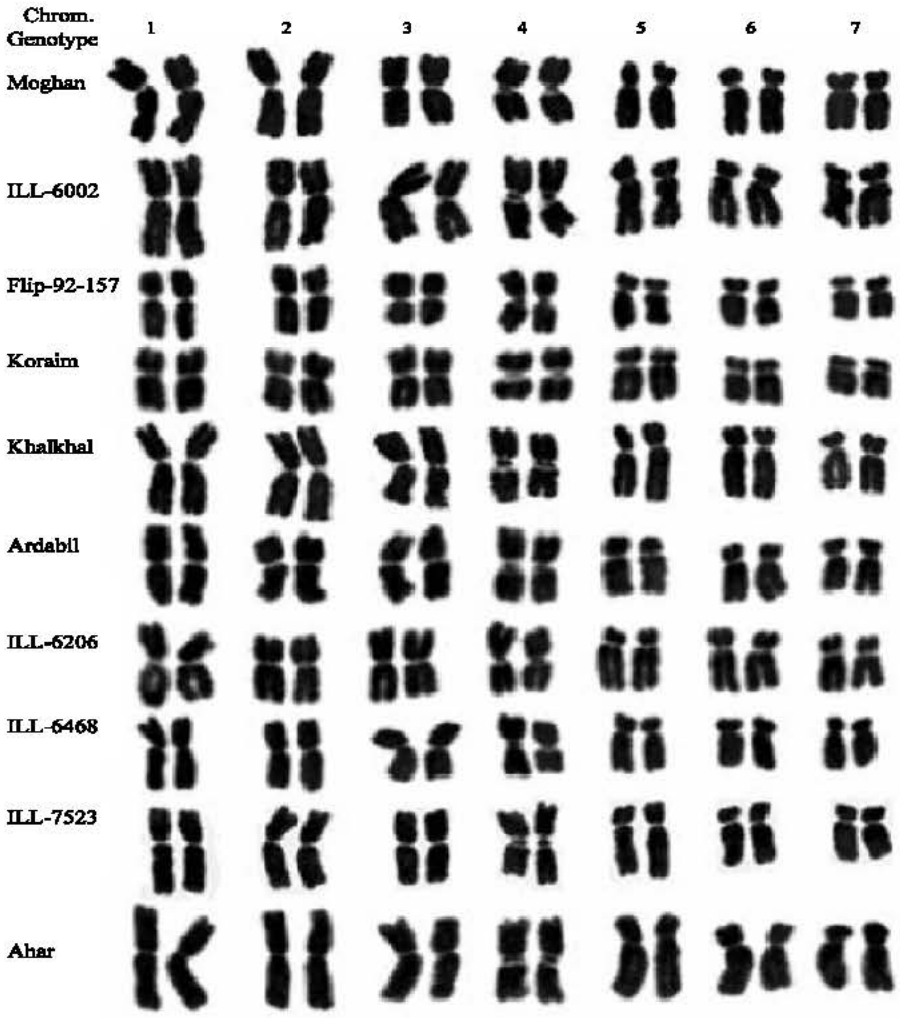

Fig. 1: Somatic metaphase chromosomes of *L. culinaris* stained with aceto-iron-hematoxilin. The chromosome 4 shows secondary constriction near its centromeric region

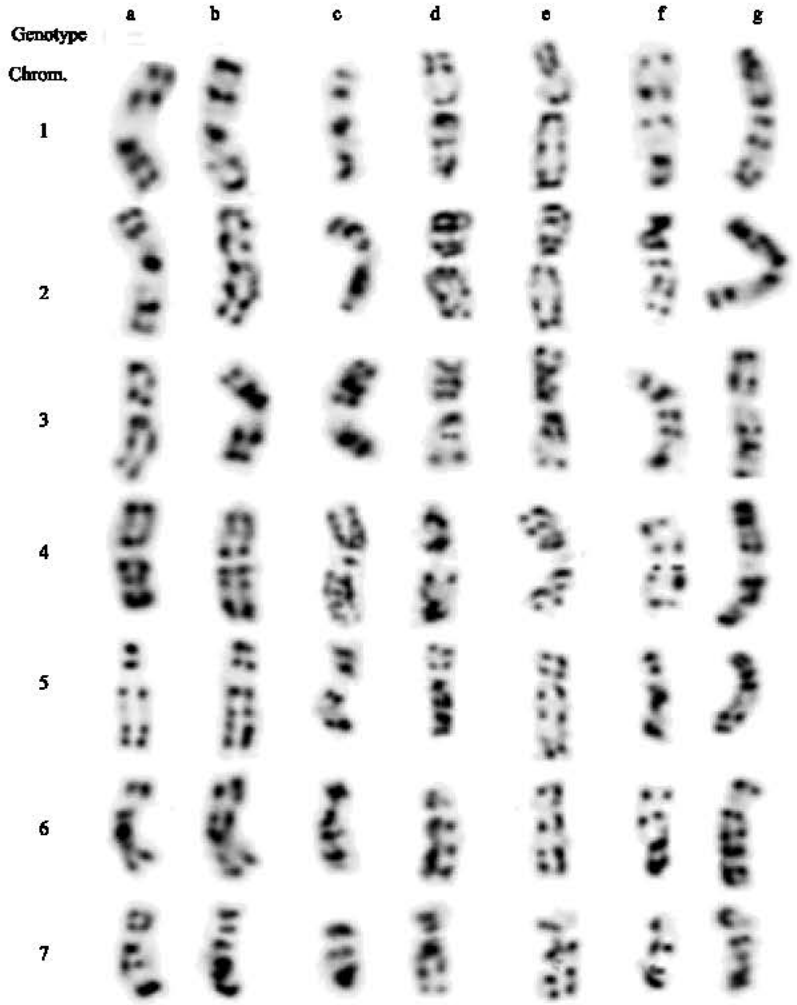

Fig. 2: Giemsa C-banded chromosomes in some genotypes of *L. culinaris*: a) Moghan, b) ILL-6002, c) Flip-92-157, d) Koraim, e) ILL-6468, f) ILL-7523, g) ILL-6206

Table 1: The karyotypic characters of seven mitotic chromosomes in ten genotypes of lentil

		Lentil genotype									
Chr.	Character	Local	6002	6468	7523	6206	Flip	Koraim	Ahar	Moghan	Khalkal
	length	8.72±0.32	7.95±0.66	8.56±0.25	8.29±0.35	9.10±0.31	7.75±0.20	6.96±0.14	9.17±0.29	9.67±0.45	8.23±0.23
	RL	17.51±0.3	17.6±0.39	16.7±0.19	17.1±0.17	16.4±0.33	17.1±0.31	17.0±0.19	16.7±0.27	17.1±0.20	17.1±0.26
1	L	4.96±0.20	4.67±0.39	4.96±0.15	4.90±0.23	5.22±0.19	4.54±0.11	4.01±0.08	5.27±0.17	5.78±0.25	4.72±0.13
	S	3.76±0.14	3.28±0.28	3.61±0.12	3.39±0.14	3.88±0.15	3.21±0.11	2.94±0.07	3.90±0.16	3.89±0.21	3.52±0.12
	L/S	1.32±0.03	1.43±0.05	1.38±0.04	1.44±0.03	1.35±0.04	1.43±0.04	1.37±0.03	1.37±0.04	1.50±0.04	1.35±0.03
	length	7.47±0.27	7.19±0.56	8.38±0.27	7.74±0.33	8.60±0.30	7.22±0.18	6.71±0.18	8.67±0.28	9.15±0.37	7.66±0.32
	RL	15.0±0.28	15.9±0.30	16.3±0.19	16.0±0.24	15.5±0.23	15.9±0.25	16.4±0.23	15.7±0.19	16.2±0.31	15.9±0.44
2	L	4.39±0.16	4.10±0.33	4.76±0.17	4.32±0.17	4.98±0.17	4.05±0.10	3.83±0.10	4.94±0.15	5.12±0.22	4.35±0.17
	S	3.09±0.13	3.08±0.25	3.62±0.11	3.41±0.17	3.61±0.15	3.17±0.09	2.89±0.09	3.73±0.15	4.03±0.16	3.31±0.17
	L/S	1.43±0.04	1.36±0.07	1.32±0.02	1.28±0.03	1.39±0.04	1.28±0.02	1.33±0.03	1.34±0.03	1.28±0.03	1.34±0.05
	length	7.49±0.21	6.46±0.42	7.54±0.31	7.12±0.37	8.32±0.20	6.55±0.16	5.88±0.11	7.92±0.31	8.28±0.38	7.16±0.27
	RL	15.1±0.27	14.3±0.28	14.7±0.27	14.6±0.22	15.1±0.17	14.4±0.18	14.4±0.33	14.3±0.12	14.6±0.18	14.9±0.34
3	L	4.17±0.14	3.66±0.25	4.07±0.15	3.99±0.19	4.52±0.11	3.64±0.11	3.25±0.07	4.41±0.17	4.67±0.25	3.86±0.16
	S	3.33±0.09	2.79±0.18	3.47±0.17	3.13±0.19	3.80±0.10	2.91±0.08	2.63±0.06	3.51±0.17	3.60±0.15	3.30±0.12
	L/S	1.26±0.04	1.32±0.04	1.19±0.03	1.29±0.04	1.19±0.02	1.26±0.04	1.24±0.03	1.27±0.05	1.29±0.04	1.17±0.03
	length	7.29±0.27	6.26±0.44	7.52±0.28	6.97±0.30	8.12±0.30	6.79±0.22	5.87±0.14	8.22±0.35	8.19±0.42	6.83±0.12
	RL	14.6±0.21	13.8±0.25	14.6±0.25	14.4±0.32	14.6±0.26	15.0±0.35	14.3±0.29	14.9±0.35	14.4±0.27	14.2±0.30

Table 1: Continued

		Lentil genotype									
Chr.	Character	Local	6002	6468	7523	6206	Flip	Koraim	Ahar	Moghan	Khalkal
4	L	4.17±0.15	3.78±0.26	4.31±0.18	4.01±0.17	4.59±0.18	3.97±0.12	3.43±0.09	4.66±0.21	4.80±0.23	4.00±0.10
	S	3.12±0.13	2.48±0.20	3.22±0.11	2.95±0.14	3.54±0.13	2.83±0.11	2.44±0.06	3.56±0.16	3.40±0.20	2.83±0.05
	L/S	1.34±0.03	1.56±0.05	1.34±0.03	1.37±0.03	1.30±0.02	1.42±0.04	1.41±0.02	1.32±0.04	1.43±0.04	1.41±0.04
	length	6.91±0.22	6.47±0.46	6.83±0.17	6.59±0.33	7.51±0.22	6.40±0.20	5.55±0.09	7.72±0.33	7.68±0.35	6.45±0.22
	RL	13.9±0.23	14.3±0.32	13.4±0.25	13.5±0.20	13.6±0.17	14.1±0.27	13.5±0.17	13.9±0.24	13.6±0.30	13.4±0.27
5	L	4.85±0.17	4.60±0.35	4.73±0.13	4.63±0.25	5.34±0.14	4.54±0.13	3.77±0.07	5.42±0.25	5.43±0.29	4.38±0.17
	S	2.06±0.08	1.87±0.13	2.10±0.08	1.96±0.09	2.17±0.09	1.86±0.07	1.77±0.04	2.29±0.10	2.25±0.09	2.07±0.08
	L/S	2.38±0.08	2.48±0.11	2.29±0.09	2.35±0.07	2.49±0.07	2.45±0.03	2.14±0.06	2.37±0.07	2.42±0.11	2.13±0.08
	length	6.16±0.22	5.71±0.37	6.38±0.19	6.10±0.28	7.13±0.20	5.56±0.16	5.18±0.08	6.97±0.24	7.06±0.27	6.01±0.19
	RL	12.3±0.15	12.6±0.28	12.4±0.15	12.5±0.20	12.9±0.23	12.2±0.18	12.6±0.09	12.6±0.15	12.5±0.19	12.5±0.20
6	L	4.34±0.15	4.03±0.27	4.54±0.15	4.35±0.23	4.98±0.16	3.92±0.13	3.55±0.06	4.93±0.20	4.97±0.18	4.18±0.14
	S	1.82±0.09	1.69±0.11	1.84±0.06	1.75±0.07	2.15±0.08	1.64±0.04	1.63±0.04	2.04±0.07	2.08±0.11	1.83±0.06
	L/S	2.43±0.11	2.40±0.09	2.47±0.06	2.50±0.12	2.35±0.10	2.40±0.07	2.20±0.05	2.43±0.09	2.42±0.08	2.29±0.07
	length	5.69±0.17	5.12±0.33	5.90±0.16	5.65±0.29	6.45±0.17	4.99±0.10	4.70±0.12	6.32±0.19	6.44±0.29	5.55±0.18
	RL	11.4±0.16	11.3±0.29	11.5±0.13	11.6±0.16	11.6±0.16	11.0±0.13	11.5±0.20	11.5±0.18	11.4±0.15	11.6±0.17
7	L	3.98±0.14	3.52±0.26	4.05±0.11	3.99±0.20	4.51±0.14	3.32±0.07	3.28±0.09	4.40±0.12	4.52±0.23	3.84±0.12
	S	1.71±0.06	1.61±0.08	1.85±0.07	1.66±0.10	1.94±0.06	1.67±0.05	1.42±0.05	1.92±0.09	1.92±0.08	1.71±0.07
	L/S	2.35±0.09	2.16±0.08	2.21±0.06	2.42±0.06	2.34±0.08	2.01±0.06	2.33±0.09	2.33±0.09	2.37±0.10	2.27±0.08

RL = chromosome relative length, L = Long arm length, S = Short arm length, L/S = Arm ratio Index

Table 2: The mean karyotypic characters of seven mitotic chromosomes in lentil

Chr.	Type	Chromosome (μm)	Relative length (%)	Long arm (μm)	Short arm (μm)	Arm ratio
1	m	8.36±0.25	17.10±0.11	4.86±0.15	3.50±0.10	1.40±0.02
2	m	7.76±0.25	15.88±0.13	4.41±0.14	3.35±0.11	1.33±0.02
3	m	7.17±0.25	14.64±0.09	3.98±0.14	3.19±0.12	1.26±0.02
4*	m	7.13±0.26	14.57±0.11	4.14±0.13	3.00±0.13	1.40±0.02
5	sm	6.77±0.21	13.83±0.10	4.75±0.16	2.02±0.05	2.37±0.04
6	sm	6.15±0.21	12.56±0.06	4.32±0.15	1.83±0.06	2.38±0.03
7	sm	5.59±0.19	11.42±0.06	3.87±0.15	1.72±0.05	2.26±0.04

* SAT-chromosome

Table 3: The mean position (the distance of the band center from the centeromer in % of the chromosome arm length on which the band is located) and width (in % of the chromosome arm length on which the band is located) of the C-bands in the 10 lentil lines and landraces

Chromosome	Band	Position	Width	Maximum heterochromatin (%)
1	$1L^5$	100.0±0.00	5.59±0.47	19.37±2.81
	$*1L^4$	71.00±3.47	4.82±0.37	
	$*1L^3$	43.90±2.48	4.78±0.26	
	$1L^1$	12.02±1.04	5.31±0.55	
	$*1S^1$	18.14±2.49	5.31±0.56	
	$1S^3$	62.67±8.35	6.91±0.88	
	$1S^4$	100.0±0.00	5.87±0.47	
2	$2L^4$	100.0±0.00	6.74±0.51	23.19±2.60
	$2L^3$	61.53±4.17	7.51±0.74	
	$*2L^2$	43.48±1.11	3.91±0.00	
	$2L^1$	21.93±2.01	6.69±0.42	
	$*2S^1$	43.69±4.61	8.75±0.66	
	$2S^2$	73.20±1.45	6.11±0.22	
	$2S^3$	100.0±0.00	6.16±0.92	
3	$3L^3$	100.0±0.00	6.71±0.62	19.23 ±2.00
	$3L^2$	61.44±2.97	7.07±0.54	
	$*3L^1$	20.91±3.07	6.02±0.89	
	$3S^1$	23.67±1.41	6.01±1.00	
	$3S^2$	59.36±4.05	6.39±1.01	
	$3S^3$	97.61±2.25	6.13±0.64	
4	$4L^3$	100.0±0.00	8.30±0.75	19.11±1.61
	$4L^2$	59.46±2.89	5.99±0.51	
	$4L^1$	31.63±2.77	5.31±0.66	
	$4S^1$	28.23±2.03	5.81±0.64	
	$*4S^2$	66.45±2.99	5.24±0.97	
	$4S^3$	100.0±0.00	7.00±0.64	
5	$5L^4$	100.0±0.00	8.01±0.48	23.18±2.12
	$*5L^3$	73.48±2.02	6.70±0.42	

Table 3: Continued

Chromosome	Band	Position	Width	Maximum heterochromatin (%)
	*5L^2	45.87±2.46	6.53±0.54	
	5L^1	23.14±1.86	6.09±0.56	
	5S^1	38.09±2.54	5.91±0.63	
	5S^2	100.0±0.00	7.88±0.82	
6	6L^5	100.0±0.00	7.84±0.51	28.38±1.37
	*6L^4	77.85±1.61	6.75±0.42	
	6L^3	55.62±2.78	8.14±0.45	
	*6L^2	43.07±1.11	6.24±0.21	
	6L^1	23.43±1.68	5.34±0.75	
	*6S^1	39.21±2.91	5.21±0.45	
	6S^2	100.0±0.00	10.0±0.76	
7	7L^3	100.0±0.00	8.39±0.67	19.85±1.77
	7L^2	71.97±1.54	6.86±0.28	
	7L^1	47.91±1.55	7.12±0.54	
	7S^1	23.60±1.55	7.01±0.45	
	7S^2	43.18±1.53	7.21±0.48	

* Polymorph bands

8.39 μm in chromosome 1. On the other hand, arm ratio index values ranged from 1.26 in chromosome 3 to 2.38 in chromosome 6 (Table 1). The ratio between the largest and the smallest chromosome was 1.49: 1. Since, Lentil has chromosomes with median or sub-median centeromers, it shows a symmetric karyotype. The distinct morphological characters and C-banding patterns for each chromosome are described as follows:

Chromosome 1: Was the largest chromosome among the chromosomes of lentil. There were one proximal and one telomeric band on its long arm and one and distal one telomeric band on its short arm. Another one interstitial and one distal band were also observed in some of genotypes in long arm and one proximal band on its short arm.

Chromosome 2: The banding pattern of this chromosome consisted of one proximal, one interstitial, one distal and one telomeric band on its long arm and one distal, one interstitial and one telomeric band on its short arm. The interstitial bands on its long and short arm showed polymorphism.

Chromosome 3: It showed three C-bands on proximal, interstitial and telomeric regions on its long and short arms. The proximal band of the long arm was not seen in some of genotypes and showed polymorphism.

Chromosome 4: (SAT chromosome), was distinguishable from other chromosomes through a secondary constriction located near the centromeric region of its long arm. Three sharp bands were observed at proximal, interstitial and telomeric regions on its long arm and two others at proximal and telomeric regions on its short arm. Another band was also observed in distal region of the short arm in some studied genotypes.

Chromosome 5: The banding pattern of this chromosome consisted of one proximal and one telomeric band on its long and short arms and a two other polymorphic bands on interstitial and distal regions of its long arm.

Chromosome 6: Had prominent C-bands at telomeric region of its short arm and three others at proximal, interstitial and telomeric regions of its long arm. Another distal or interstitial band on its long arm was also seen in some genotypes.

Chromosome 7: The smallest one in the chromosome set of lentil. It had one proximal and one telomeric band on its short arm and one proximal, one interstitial and one telomeric band on its long arm.

The results of this study revealed that the genome of this species consisted of four pairs of metacentric and three pairs of submetacentric chromosomes. Chromosome 4 had a secondary constriction near centromeric region of its long arm. Slinkard (1985), Raziuddin *et al.* (1990) and Ahmad *et al.* (1992) showed that the karyotype of lentil composed of four metacentric and three sub-metacentric chromosomes according to Levan *et al.* (1964). Whereas, according to Galasso *et al.* (2001) lentil has three submetacentric, three acrocentric and one pair metacentic chromosome with secondary constriction very close to its centromeric region. It seems that they used another criterion for naming chromosomes. Ahmad *et al.* (1992) suggested that unlike earlier works (Sinha and Singh, 1982; Sindhu *et al.*, 1984; Slinkard 1985; Raziuddin *et al.*, 1990) there is only one SAT chromosome in lentil which was the second chromosome in length and the secondary constriction of this chromosome is near centromeic region of its long arm. However, Kumar and Gupta (1997) reported that in pachytene chromosomes of lentil three NOR regions are seen on chromosomes 1, 2 and 3, but in mitotic chromosomes the secondary constriction is

located on the chromosome 4 near centromeic region of its long arm. Abbo *et al.* (1994) and Galasso *et al.* (2001) also, reported only one nucleolar organizing chromosome in lentil through *in situ* hybridization. Use of a ribosomal DNA probe (pTa71) for FISH, Kumar *et al.* (2001) revealed only one pair of nucleolar organizing chromosomes on mitotic metaphase chromosomes, but at spore quartet stage, they suggested the presence of two nucleolar organizing chromosomes.

The SAT chromosome of lentil in this study was the fourth chromosome in average length, which was in agreement with Kumar and Gupta (1997) and Galasso *et al.* (2001). In three lines of studied materials (Flip 92-15L, Ill-6468 and Ahar landrace) it was the third chromosome (Table 2). However, Ahmad *et al.* (1992) reported this chromosome is the second in length.

There are a few reports on chromosome banding patterns in lentil. Mehra *et al.* (1986) using N-banding technique reported N-bands near centromeric region of lentil chromosomes. In this study each chromosome had a distinct C-banding pattern and this technique provided adequate information to identify all of lentil chromosomes. The mean amount of heterochromatin percent in each metaphase cells of these lines were 22%. Besides of prominent monomorph bands a considerable amount of polymorphism was observed in banding pattern. These results can be used in chromosome engineering of lentil chromosomes for transferring of alien chromatin into this species.

CONCLUSIONS

The results of this study revealed that while there are minor differences among genotypes of lentil in chromosome morphology a considerable polymorphism are seen in C-banding patterns of chromosomes.

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