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Quantitative karyotypic analysis in five varieties of lentil (*Lens Culinaris* Medik.)

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Abstract

Lentil (*Lens culinaris* Medik.) is one of the common pulse crop worldwide belongs to the family Leguminosae bearing chromsome number 2n=14. Five varieties of lentil seeds i.e., BARI Musur-1, 2, 3, 4 and 8406-122 were selected for quantitative karyotypic analysis released by BARI. The identified chromosome pairs were four (I, II, IV, VI) in BARI Musur-1, five (I, II, III, V, VII) in BARI Musur-2, five (I, II, III, IV, VII) in BARI Musur-3, four (I, II, IV, VII) in BARI Musur-4 and three (I, IV, VI) in BARI Musur-8406-122. Unidentified chromosomes were allocated in different morphological classes based on probabilistic inferences. Total chromatin length (TCL) was found to be the largest in BARI masur-3 and smallest in BARI masur-2. Total fraction frequency (TF %) was observed highest in BARI masur-8406-122 and lowest in BARI masur-4. The proposed standard karyotype were $2L^{1m+1Sm}+2M^{1m+1st}+3S_1^{2sm+1st}$ for BARI Musur-1, $1L^m+2M^{1m+1sm}+3S_1^{1m+1sm+1st}+1S_2^{1st}$ for BARI Musur-2, $4L^{2m+2sm}+3M^{1sm+2st}$ for BARI Musur-3, $5L^{1m+3sm+1st}+1M^{1St}+1S_1^{1Sm}$ for BARI Musur-4 and $1L^{1m}+3M^{2m+1sm}+3S_1^{1Sm+2st}$ for BARI Musur-8406-122. All examined varieties of lentil showed assymetric karyotype with the presence of two sub-terminal chromosomes except BARI Musur-1 consist of only one. These research findings suggest their advanceness in nature and leads to the evolutionary trends which may be helpful for plant breeding program.

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Introduction

The legume lentil (*Lens culinaris* Medik) is a cool-season food that is high in protein and a good source of prebiotic carbohydrates, minerals, and vitamins (Choukri H et al., 2020). Legumes are rich in macro- and micronutrients such proteins, carbs, minerals, vitamins, and dietary fiber, which are crucial components of the human diet. They are also well known for acting as beneficial bioactive substances in the body by reducing the risk of cardiovascular disease, cancer and inflammation (Yeo J et al., 2020) Quantitative karyotype analysis, also known as cytogenetics, is a powerful technique used to study the structure, organization, behavior of chromosome in different organisms. It provides valuable insights into chromosomal abnormalities, gene mapping, genome evolution and overall genetic diversity within a species. In case of lentil, karyotype analysis can offer crucial information about the genomic constitution and chromosome characteristics of different examined varieties. The object of karyotype analysis is in part to compare the various karyotypes in allied groups and to consider the morphological resemblance to get phylogenetic relations of the karyotypes. Diploid number of chromosomes (2n=14) reported for first time by Sakamura (1920) in different species of *Lens*. Sharma and Mokhupadhayay (1944) studied the karyotype constancy in different varieties of *Lens esculenta* Moench. They also reported presence of supernaturally chromosome in one of the strain. Naithani and Sarbhoy (1983) found two pairs of satellite, two pairs of J or L shaped and three pairs of V shaped chromosomes. Symmetrical karyotypes are considered to be more primitive than asymmetrical ones.

Despite of good number of works on the karyotype of *Lens culinaris* there are still disputes regarding few cytological events such as satellite chromosomes, preponderance of metacentric chromosomes, karyotypic differences in macrosperma microsperma type of lentil etc. Thus, the present study in all examined varieties of *Lens culinaris* was under taken to find out the similarities and difference among karyotype of each one to provide diagnostic features of different chromosome in their haploid complement.

Materials and methods

The seeds of these five varieties were collected from Bangladesh Agricultural Research Institute, Jaydebpur, Gazipur. Fresh and dry seeds were spread over petridish with moistened filter paper and kept in laboratory at room temperature ($32\pm2^{o}C$) for germination and kept for 48-72 hours. When the root tips were grown 1-1.5 cm in length these were collected at about 09.00-09.30 am and pretreated in standard aqueous solution of para-dichlorobenzeen (PDB) for 4-5 hours at $5^{o}C$. The treated root tips were thoroughly washed by distilled water and were fixed in 1:3 aceto-alcohol for 48 hours at room temperature and then preserved in 70% ethanol at $4^{O}C$ in refrigerator. Staining of root tip chromosomes was done by haematoxylin method. Temporary slides (0.5% acetocarmine) were examined under a compound microscope with 40X16 magnification and chromosomes were measured from photomicrographs. Chromosomes were classified according to Kutarekar and Wanjari (1983) as 6.21μm and above = large chromosome (L), 5.37 μm $\leq$ X $\leq$ 6.20 = medium (M), 4.53μm $\leq$ X $\leq$ 5.36= relatively short (S_1) and 4.52μm and less = short chromosome (S_2).

Data on chromosome morphology (from three cells of each variety with similar degrees of chromosome contraction) were taken as described by Ahmad *et al*. (1983). The standard karyotypes proposed were derived on the basis of centromeric formula, range and average chromatin length per chromosome. The total frequency (TF%) was calculated using the formula of Huziwara (1962). In case of chromosome morphology, uniformity regarding degree of contraction of chromosomes in the studied three cells were determined by comparing haploid total length of all the chromosomes in each cell of each varieties and standardized haploid length for three cells and chromosomes distribution in both of them were determined. The process was repeated for each of the

three cells under study. The similarity and homogeneity of the distribution of chromosomal morphology in three cells were tested by using a contingency table incorporating chromosome length and arm ratio classes. The non-significant χ^2 values indicated the chromosome to be homogenous for the frequency of haploid length and arm ratio classes.

In case of chromosome identification, the three points representing the haploid homologues of each chromosome should cluster closely and such cluster must contain one point from each studied cells (A, B, C). For each of the identified groups (chromosomes) of different cells, the mean ($\overline{X}$), standard error (SE) and coefficient of variation percentage (CV%) were determined for length and arm ratio using the diploid

values. The chromosomal groups of three points indicated the numbers of distinct individually identifiable chromosomes in each variety. On the other hand the groups of seven points indicated two chromosomes so similar morphologically that they could not be distinguished from each other but identifiable from the rest.

Results

For this study, the quantitative method was adopted for identification of chromosome on the basis of scatter diagram of total chromatin length (TCL) and arm ratios (SA/LA=AR) of all the chromosomes in a number of cells. The cells with well spread metaphase chromosomes having more or less distinct morphology are presented in Figures 1-5.

Table 1. Haploid complement length and chromosome distribution in five varieties of *Lens culinaris* Medik.

Name of varieties	Haploid total complement length (µm) in five varieties of *Lens culinaris* Medik. of three different metaphase plates			Mean ($\overline{X}$)	Standard error (± SE)	CV%	χ^2 value
	A cell	B cell	C cell				
BARI Musur-1	39.56	40.17	39.55	33.76	0.167	0.729	0.002114
BARI Musur-2	37.53	37.52	37.52	37.52	2.721	0.012	0.00000086
BARI Musur-3	44.98	44.98	45.01	44.99	0.0081	0.031	0.0000044
BARI Musur-4	43.37	43.37	43.35	43.36	0.0054	0.021	0.0000023
BARI Musur-8406-122	39.40	39.32	39.35	39.36	0.0190	0.083	0.000027

Table 2. Combined mean length and arm ratio of the identified chromosome in five varieties of *Lens culinaris* Medik.

		I	II	III	IV	V	VI	VII
BARI Musur-1	Chromosome type	m	sm	st	sm			
	TCL	6.70±0.09	6.55±0.14	5.51±0.038	4.89±0.146			
	Arm ratio	0.81±0.036	0.62±0.030	0.48±0.007	0.61±0.024			
BARI Musur-2	Chromosome type	m	m	sm	sm	st		
	TCL	6.66±0.06	6.01±0.14	5.57±0.042	5.13±0.120	4.04±0.038		
	Arm ratio	0.76±0.035	0.97±0.007	0.69±0.036	0.62±0.023	0.48±0.005		
BARI Musur-3	Chromosome type	sm	m	sm	m	St		
	TCL	6.89±0.016	6.88±0.029	6.46±0.080	6.46±0.140	6.05±0.115		
	Arm ratio	0.58±0.002	0.84±0.009	0.63±0.030	0.92±0.023	0.46±0.009		
BARI Musur-4	Chromosome type	sm	m		st			sm
	TCL	6.71±0.147	6.81±0.002		6.40±0.136			5.22±0.038
	Arm ratio	0.63±0.032	0.84±0.005		0.39±0.021			0.61±0.019
BARI Musur-8406-122	Chromosome type	m			m			sm
	TCL	6.15±0.008			5.93±0.138			5.11±0.086
	Arm ratio	0.98±0.004			0.83±0.005			0.64±0.025

Chromosome morphology

The diploid chromosome numbers were found to be 2n=14 in five varieties of *Lens culinaris* Medik. The average values of total length and arm ratio for each pair of chromosomes in each cell of all the varieties were calculated constituting the haploid complement of those cells. The chromosomes of haploid complements were numbered in decreasing order of length and increasing order of arm ratio within the same length.

Table 3. The allocation of unidentified chromosomes in *Lens culinaris* Medik. (BARI Musur-1) karyotype to different morphological categories based on unstandarized length.

Types of chromosome	Length classes (X) μm	Arm ratio classes (Y) μm	Total no. of chromosomes in three haploid sets	Mean no. of chromosomes per haploid set	No. of identified chromosomes with names	Proposed no. of unidentified chromosomes	Total no. of chromosomes	Paired no. of identified chromosomes	Assigned no. of chromosomes
Large (L)	6.21≤X	0.76≤Y≤1.0	3	1	1m	0	1	Im	I, II
		0.51≤Y≤0.75	3	1	1sm	0	1	IIsm	
		0.05≤Y	0	0	0	0	0	0	
Medium (M)	5.37≤X≤6.2	0.76≤Y≤1.0	3	1	0	1m	1	0	III, IV
		0.51≤Y≤0.75	0	0	0	0	0	0	
		0.05≤Y	3	1	1st	0	1	IVst	
Relatively short (S₁)	4.53≤X≤5.36	0.76≤Y≤1.0	0	0	0	0	0	0	IV(st)
		0.51≤Y≤0.75	6	2	1sm	1sm	2	VIsm	
		0.05≤Y	3	1		1st	1		
Short (S₂)	3.69≤X≤4.52	0.76≤Y≤1.0	0	0	0	0	0	0	IV,V,VII
		0.51≤Y≤0.75	0	0	0	0	0	0	
		0.05≤Y	0	0	0	0	0	0	
			21	7.0		3.0	7.0		

Table 4. The allocation of unidentified chromosomes in *Lens culinaris* Medik. (BARI Musur-2) karyotype to different morphological categories based on unstandarized length.

Types of chromosome	Length classes (X) μm	Arm ratio classes (Y) μm	Total no. of chromosomes in three haploid sets	Mean no. of chromosomes per haploid set	No. of identified chromosomes with names	Proposed no. of unidentified chromosomes	Total no. of chromosomes	Paired no. of identified chromosomes	Assigned no. of chromosomes
Large (L)	6.21≤X	0.76≤Y≤1.0	3	1	1m	0	1	Im	I
		0.51≤Y≤0.75	0	0	0	0	0	0	
		0.05≤Y	0	0	0	0	0	0	
Medium (M)	5.37≤X≤6.2	0.76≤Y≤1.0	3	1	1m	0	1	IIm	II,III
		0.51≤Y≤0.75	3	1	1sm	0	1	IIIsm	
		0.05≤Y	0	0	0	0	0	0	
Relatively short (S₁)	4.53≤X≤5.36	0.76≤Y≤1.0	3	1	0	1m	1	IVm	IV, V, VI
		0.51≤Y≤0.75	3	1	1sm	0	1	Vsm	
		0.05≤Y	3	1	0	1st	1	VIst	
Short (S₂)	3.69≤X≤4.52	0.76≤Y≤1.0	0	0	0	0	0	0	VII
		0.51≤Y≤0.75	0	0	0	0	0	0	
		0.05≤Y	3	1	1st	0	1	VIIst	
			21	7.0		2	7.0		

Chromosome identification

Corresponding chromosomes in different haploid complements of each variety were determined through a grouping technique applying to a combined scatter diagram of the arm ratios and standardized haploid length of chromosomes in all three cells. Thus the numbers of identified chromosomes were found to be only 4 pairs in BARI Musur-1, 5 pairs in BARI Musur-2, 5 pairs in BARI Musur- 3, 4 pairs in BARI Musur-4 and 3 pairs in BARI Musur-8406-122.

The mean of identified chromosomes among five varieties were found to highest (6.89 μm) in BARI Musur-3 and lowest was (4.04 μm) in BARI Musur-2.

Table 5. The allocation of unidentified chromosomes in *Lens culinaris* Medik. (BARI Musur-3) karyotype to different morphological categories based on unstandarized length.

Types of chromosome	Length classes (X) μm	Arm ratio classes (Y) μm	Total no. of chromosomes in three haploid sets	Mean no. of chromosomes per haploid set	No. of identified chromosomes with names	Proposed no. of unidentified chromosomes	Total no. of chromosomes	Paired no. of identified chromosomes	Assigned no. of chromosomes
Large (L)	$6.21 \leq X$	$0.76 \leq Y \leq 1.0$	6	1	2m	0	2	IIm	I, II,III,IV
		$0.51 \leq Y \leq 0.75$	6	2	2sm	0	2	Ism,IVsm	
		$0.05 \leq Y$	0	0	0	0	0	0	
Medium (M)	$5.37 \leq X \leq 6.2$	$0.76 \leq Y \leq 1.0$	0	0	0	0	0	0	V,VI,VII
		$0.51 \leq Y \leq 0.75$	3	1	0	0	1	Vm	
		$0.05 \leq Y$	6	2	0	2st	2	VIst, VIIst	
Relatively short (S$_1$)	$4.53 \leq X \leq 5.36$	$0.76 \leq Y \leq 1.0$	0	0	0	0	0	0	
		$0.51 \leq Y \leq 0.75$	0	0	0	0	0	0	
		$0.05 \leq Y$	0	0	0	0	0	0	
Short (S$_2$)	$3.69 \leq X \leq 4.52$	$0.76 \leq Y \leq 1.0$	0	0	0	0	0	0	
		$0.51 \leq Y \leq 0.75$	0	0	0	0	0	0	
		$0.05 \leq Y$	0	0	0	0	0	0	
			21	7.0		2	7.0		

Table 6. The allocation of unidentified chromosomes in *Lens culinaris* Medik. (BARI Musur-4) karyotype to different morphological categories based on unstandarized length.

Types of chromosome	Length classes (X) μm	Arm ratio classes (Y) μm	Total no. of chromosomes in three haploid sets	Mean no. of chromosomes per haploid set	No. of identified chromosomes with names	Proposed no. of unidentified chromosomes	Total no. of chromosomes	Paired no. of identified chromosomes	Assigned no. of chromosomes
Large (L)	$6.21 \leq X$	$0.76 \leq Y \leq 1.0$	1	1	0	1m	1	IIIm	I, II,III,IV,V
		$0.51 \leq Y \leq 0.75$	9	3	2sm	1sm	3	Ism,IIsm	
		$0.05 \leq Y$	3	1	1st	0	1	IVst	
Medium (M)	$5.37 \leq X \leq 6.2$	$0.76 \leq Y \leq 1.0$	0	0	0	0	0	0	VI
		$0.51 \leq Y \leq 0.75$	0	0	0	0	0	0	
		$0.05 \leq Y$	3	1	0	1st	1	VIst	
Relatively short (S$_1$)	$4.53 \leq X \leq 5.36$	$0.76 \leq Y \leq 1.0$	0	0	0	0	0	0	VII
		$0.51 \leq Y \leq 0.75$	3	1	1sm	0	1	VIIsm	
		$0.05 \leq Y$	0	0	0	0	0	0	
Short (S$_2$)	$3.69 \leq X \leq 4.52$	$0.76 \leq Y \leq 1.0$	0	0	0	0	0	0	
		$0.51 \leq Y \leq 0.75$	0	0	0	0	0	0	
		$0.05 \leq Y$	0	0	0	0	0	0	
			21	7.0		3	7.0		

Allocation of unidentified chromosomes

The unidentified chromosomes in five varieties were allocated to different morphological categories based on their total chromosome length (TCL) and arm ratio (SA/LA) classes following Ahmad et al. (1983). The allocation of unidentified chromosomes of each variety was given in Tables 3-7.

The unidentified chromosomes were distributed to the various morphological categories using probabilistic inferences, especially on the chromosome frequency in a given class per haploid set (L=Large, M=Medium, S$_1$=Relatively short and S$_2$=Short).

Proposed standard karyotype

The standard karyotype proposed in all examined varieties of *Lens culinaris* Medik. were derived on the basis of centromeric formula, and range and average chromatin length per chromosome.

Data recording chromosome morphology, i.e. length, arm ratio, relative length, TCL%, TF% and chromosome types are given in Table 8.

Table 8. Analysis of lengths, arm ratios, relative lengths, TCL%, TF%, centromeric position and chromosome type in five varieties of *Lens culinaris* Medik.

Taxa	Characters	I	II	III	IV	V	VI	VII
Lens culinaris Medik. (BARI Musur-1) TF% = 39.05%, Total chromatin length =39.54	Long arm length (µm)	3.69	4.05	2.99	3.73	3.16	3.04	3.43
	Short arm length (µm)	3.01	2.50	2.69	1.78	2.15	1.85 1.46	
	Total arm length (µm)	6.70	6.55	5.69	5.51	5.31	4.89	4.89
	Arm ratio	0.81	0.62	0.91	0.48	0.69	0.61	0.42
	Relative chromosome length (µm)	100	97.76	84.92	82.24	79.25	72.99	72.99
	TCL%	16.94	16.57	14.39	13.94	13.43	12.37	12.37
	Centromeric position	m	sm	m	st	sm	sm	st
	Chromosome type	L	L	M	M	S$_1$	S$_1$	S$_1$
Lens culinaris Medik. (BARI Musur-2) TF% = 39.90%, Total chromatin length = 37.52	Long arm length (µm)	3.78	3.04	3.43	2.91	3.15	3.50	2.74
	Short arm length (µm)	2.87	2.97	2.14	2.25	1.97	1.47	1.30
	Total arm length (µm)	6.01	6.66	5.57	5.16	5.13	4.95	4.04
	Arm ratio	0.97	0.76	0.63	0.81	0.62	0.41	0.48
	Relative chromosome length (µm)	100	90.24	83.63	77.48	77.03	74.32	60.66
	TCL%	17.75	16.02	14.85	13.75	13.67	13.19	10.77
	Centromeric position	m	m	sm	m	sm	st	st
	Chromosome type	L	M	M	S$_1$	S$_1$	S$_1$	S$_2$
Lens culinaris Medik. (BARI Musur-3) TF% = 38.95%, Total chromatin length =44.98	Long arm length (µm)	4.37	3.74	3.83	3.33	3.97	3.80	4.16
	Short arm length (µm)	2.52	3.15	2.63	3.13	2.18	2.00.1.91	
	Total arm length (µm)	6.89	6.88	6.46	6.46	6.18	6.06	6.05
	Arm ratio	0.58	0.84	0.92	0.69	0.55	0.47	0.46
	Relative chromosome length (µm)	100	99.85	93.76	93.75	89.70	87.95	87.81
	TCL%	15.32	15.29	14.36	14.36	13.74	13.47	13.45
	Centromeric position	sm	m	m	sm	sm	st	st
	Chromosome type	L	L	L	L	M	M	M
Lens culinaris Medik. (BARI Musur-4) TF% = 38.22%, Total chromatin length = 43.46	Long arm length (µm)	3.71	4.13	3.46	4.62	4.12	3.74	3.23
	Short arm length (µm)	3.10	2.58	3.10	1.77	2.43	1.65	1.98
	Total arm length (µm)	6.81	6.71	6.56	6.40	6.37	5.39	5.22
	Arm ratio	0.84	0.63	0.90	0.39	0.65	0.44	0.61
	Relative chromosome length (µm)	100	98.53	96.33	93.98	93.54	79.15	76.65
	TCL%	15.67	15.44	15.09	14.73	14.66	12.40	12.01
	Centromeric position	St	sm	st	sm	sm	sm	m
	Chromosome type	L	L	L	L	L	M	S$_1$
Lens culinaris Medik. (BARI Musur-8406-122) TF% = 42.16%, Total chromatin length =39.37	Long arm length (µm)	3.89	3.11	3.44	3.24	3.50	3.11	2.87
	Short arm length (µm)	2.92	3.04	2.51	2.70	1.74	2.00	1.69
	Total arm length (µm)	6.15	5.95	5.93	5.24	5.11	4.56	
	Arm ratio	0.76	0.98	0.73	0.83	0.48	0.64	0.60
	Relative chromosome length (µm)	100	95.65	92.53	92.22	81.49	79.47	70.92
	TCL%	16.33	15.62	15.11	15.06	13.31	12.98	11.58
	Centromeric position	m	m	sm	m	st	sm	st
	Chromosome type	M	L	M	M	S$_1$	S$_1$	S$_1$

Discussion

Karyotype is an important field of investigation for understanding generic or specific intra or interrelationships and evolutionary trends (Feden, 1980) and also for indicating the genomic difference. In the present study it was observed that for somatic karyotype in root tip cells, germinating root tips collected between 9.00 am to 9.30 am showed maximum numbers of dividing stages particularly of metaphase. It was also observed that 1.5 mm length of germinating root tips were appropriate for metaphase stages. Similar findings were reported by Datta and Neogi (1970). The morphology of chromosomes was found to vary and differences were observed regarding chromosome length, total chromatin length (TCL), centromeric position and chromosome type in five varieties of *Lens culinaris*.
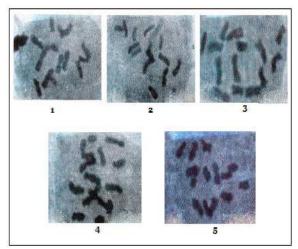

Fig. 1-5. Metaphase plates used for quantitative karyotype analysis of five varieties of *Lens culinaris* Medik. 1. BARI Musur-1, 2. BARI Musur-2, 3. BARI Musur-3, 4. BARI Musur-4 and 5. BARI Musur-8406-122.

However, quantitative method used by Ahmad et al. (1983 and 1984) for *Glycine soya*, and *Glycine max* was adopted for karyotypic analysis in the present study. Quantitative method was applied to identify the individual chromosomes and their homologues.

The values for total length and arm ratios of the chromosomes were also used for this study and thereby allocated (both identified and unidentified) chromosomes helped to predict the composition of their genome.
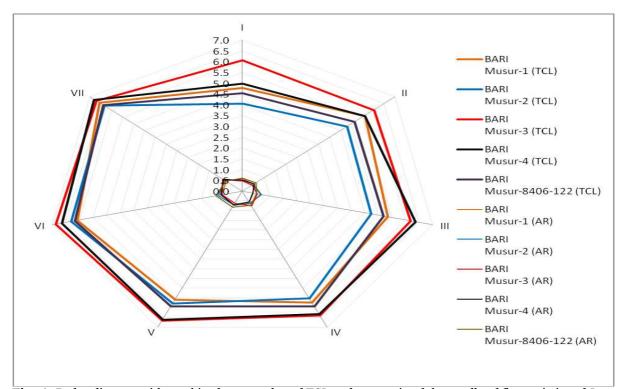

Fig. 6. Radar diagram with combined mean value of TCL and arm ratio of three cells of five varieties of *Lens culinaris* Medik.

The chromosomes may not always have the same total length (Shindhu et al. 1984) because of variation from cell to cell and differences in fixation. The morphology of the chromosomes varies from cell to cell and major changes are associated with the cell division process (Wilson and Morrison, 1973). However, the identified chromosomes were found to indicate the possible changes in genomic construction of different varieties of *Lens culinaris* Medik. The chromosomal structural change is considered as the factor of evolution.

Jha et al. (2016) revealed that all the cultivars under investigation had a consistent karyotype formula (3m+1m (sat)+2sm+1st), one pair of interstitial sat in either chromosomal number 3 or 4, and intriguing differences in total chromatin length (53.6–121.2μm). In this study, two large, two medium and three relatively short chromosomes with median, sub-median and sub- telocentric position were found in *Lens culinaris* (BARI Musur-1). There were one large, two medium, three relatively short and one short chromosome with median, sub-median and sub-telocentric position were present in *Lens culinaris* (BARI Musur-2). In *Lens culinaris* (BARI Musur-3), there were four large and three medium chromosomes with metacentric, sub-metacentric and sub-telocentric position were present. There were five large, one median and one relatively short chromosome with metacentric, sub-metacentric and sub-telocentric position were clear in *Lens culinaris* (BARI Musur-4). Again there were one large, three medium and three relatively short chromosomes with metacentric, sub-metacentric and sub-telocentric chromosomes were found in *Lens culinaris* (BARI Musur- 8406-122).
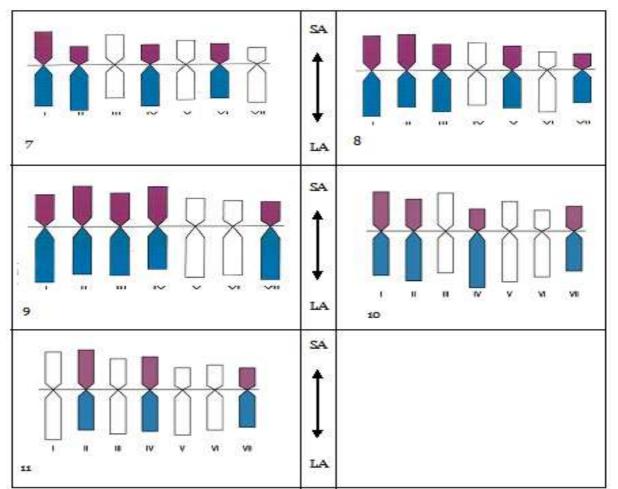
Fig. 7-11. Ideogram of identified chromosome of five varieties of *Lens culinaris* Medik. 7. BARI Musur-1, 8. BARI Musur-2, 9. BARI Musur-3, 10. BARI Musur-4 and 11. BARI Musur-8406-122.

In the present findings no satellite chromosome was found in any *Lens culinaris* varieties. But it was clear in this study that its chromosomes were present in all the varieties in to a good number, which indicates most advance nature of all the varieties.

Khandaker el al., 2007 observed that the total chromatin length of Bari masur-3 (97.83 μm) and Bari masur-2 (107.05 μm). In this present study, longest chromosome (6.89μm) was observed in BARI Musur-3 and shortest (4.04 μm) in BARI Musur-2. Maximum chromatin length of haploid complement (44.98μm) was found in BARI Musur-3 and minimum (37.52μm) in BARI Musur-2. The highest range of arm ratio (0.98 to 1.0) was observed in BARI musur-8406-122 and the smallest range (0.42 to 1.0) was observed in BARI Musur-1. As it has been mentioned earlier cytological study is very much related to the size of chromosome and staining and

spreading them, lentil chromosomes in the present study showed sometimes inadequate staining. It caused barrier for identifying few small chromosomes an all the five varieties of *Lens culinaris* Medik. It might be related to the DNA content of the small chromosomes of lentil (Sparrow and Miksche, 1961), in general a positive correlation is found between DNA content and size and volume of the chromosomes (Rees et al., 1966).

However according to combined mean value of TCL three chromosomes (IV, VI and VII) founds similar among five varieties. On the other hand according to combined mean value of AR two chromosomes (I and VII) founds similar among five varieties also may shows some evolutionary trends of this five varieties. But presence of sub-terminal chromosomes in all the varieties indicates that there may be fewer opportunities in breeding program.

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