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ORCID
TBJ: 0000-0003-0900-8167
BKB: 0000-0001-6029-1098

Analysis of CMA-DAPI bands and preparation of fluorescent karyotypes in thirty Indian cultivars of *Lens culinaris*

Timir Baran Jha[1,*], Biplab Kumar Bhowmick[2], Partha Roy[1]

[1] *Department of Botany, Maulana Azad College, Kolkata -700013, West Bengal, India*
[2] *Department of Botany, Scottish Church College, 1&3, Urquhart Square, Kolkata-700006, West Bengal, India*
*Corresponding Author. E-mail: tbjha2000@yahoo.co.in

Abstract. India holds a significant rank in production and consumption of the age old protein rich crop Lentil with only one cultivated species and a large number of phenotypically similar cultivars. The need for a reliable and cost effective method of genetic characterization to unravel differences within the Lentil cultivars was felt. The present paper adopted EMA based chromosome preparation followed by staining with two contrasting fluorochrome dyes CMA and DAPI that bind directly to GC and AT rich heterochromatic segments on chromosomes. Analysis of fluorochrome banding pattern furnished a comparative account of genetic diversity within the cultivars that could not be achieved by traditional karyotyping. The marker pair of nucleolar chromosomes (4th and 3rd, majorly) occupied a pivotal position to intensify differences between cultivars in terms of banding patterns around secondary constrictions, suggestive of yet unknown variation in heterochromatin composition. Our study has strengthened genetic background and relationships of Lentil cultivars. We observed certain types of unusual fluorochrome bands that put forward the exclusivity of Indian germplasm and have questioned the mainstream heterochromatin elements of plant chromosomes captured by CMA-DAPI stains. The comprehensive fluorescent karyotypes of 30 *L. culinaris* Medik. cultivars prepared for the first time, serve as an archetype for the benefit of future breeding programmes.

Keywords: lentils, CMA-DAPI, chromosomal bands, fluorescent karyotype, heterochromatin.

INTRODUCTION

Lentil is one of the richest protein containing domesticated ancient crop with only one globally cultivated species *Lens culinaris* Medik. India is the second highest producer and biggest consumer of Lentils. The genus belongs to the largest subfamily (Papilionoideae) of Fabaceae (Azani et al. 2017), along with many economically important genera producing pulses and beans. Being the single cultivated species, large number of cultivars is in cultivation in our country. The characterization of Indian germplasm is

needed to sustain conservation and programmable utilization of resources. Chromosomal characterization is a cost effective method to provide foundational information on the genome and genetic conservation for any future breeding program of particular crop plants. Cytogenetic studies of Indian Lentils through conventional method failed to provide uniformity on chromosome morphometric parameters (Bhattacharjee 1953; Sharma and Mukhopadhyay 1963; Sinha and Acharia 1972; Naithani and Sarbhoy 1973; Lavania and Lavania 1983; Nandanwar and Narkhede 1991). On the other hand, we have published detailed karyotype analysis of more than thirty *L. culinaris* cultivars obtained from the Indian Institute of Pulses (Jha et al. 2015, 2017; Jha and Halder 2016) through EMA based Giemsa staining method. Our results were found to have near similarities with the results obtained by Ladizinsky (1979). However, *Lens* chromosomes (2n=14) are nearly similar in morphology. Considering the status of research, we question i) is there any karyotype variability across cultivars beyond chromosome number, morphology and ploidy? ii) is it possible to find visible chromosomal landmarks in accordance with the germplasm diversity? and iii) whether we can step forward towards molecular kayotype database for Indian Lentils. As EMA based chromosome analysis (Fukui 1996) is the basis of molecular cytogenetics, we decided to carry forward our work with two contrasting fluorescent stains DAPI and CMA on the same cultivars. Having affinity towards specific base pairs of DNA, these fluorescent dyes reliably identify heterochromatin rich sectors on chromosomes, differentiate morphologically alike chromosomes and improve karyotype characterization (Schweizer 1976; Guerra et al. 2000; Yamamoto 2012; Weiss-Schneeweiss and Schneeweiss 2013). So, our objective is to address chromosomal behavior after application of base specific fluorochromes and compile cultivar specific fluorescent banding profiles. The present paper considers a fluorescent karyotype dataset of 30 Indian *L. culinaris* cultivars for the first time, as an important kit for Lentil breeders and genome researchers.

MATERIALS AND METHODS

Chromosome preparation and fluorochrome staining

The fluorescent karyotype analysis was carried out on 30 cultivars of *Lens culinaris* presented in Table 1. Except for two (Barasat, Micro type and Barasat, Macro type, Table 1), all the cultivars of Lentil were obtained from the Indian Institute of Pulse Research (IIPR), Kanpur. Germination of seeds and chromosome processing through enzymatic maceration and air drying (EMA) was carried out as per our earlier protocol (Jha and Yamamoto 2012; Jha et al. 2015, 2017, 2020). For fluorescent staining with DAPI and CMA, we followed our protocol (Jha 2019) with required modifications. For DAPI staining, slides were kept for 30 min in McIlvaine buffer, stained with 0.1µg ml^{-1} solution of DAPI for 10 min, counterstained with 0.25mg/ml of Actinomycin D (AMD) for 15min and then mounted in non-fluorescent glycerol and observed under Carl Zeiss Axio Lab A1 fluorescence microscope using Carl Zeiss DAPI filter cassette. Chromosome images were captured with CCD camera attached with microscope. The slides were destained and air-dried. The same slides were placed in McIlvaine buffer for 30 min followed by incubation in McIlvaine buffer with 5mM $MgCl_2$ for 10 mins and then stained with 0.1mg ml^{-1} CMA solution for 45-50 mins. The slides were again washed in McIlvaine buffer with 5mM $MgCl_2$ and finally mounted with non-fluorescent glycerol and kept for maturation at 4^0C for 48-72 hrs. CMA stained slides were observed under the above-mentioned fluorescence microscope fitted with Carl Zeiss FITC filter cassette, images captured with attached CCD camera and signals were analyzed using the software Prog Res 2.3.3.

Statistical analysis of karyotype relations

Karyotype relations among the cultivars was evaluated with the help of cluster analysis for data matrix normalization by unweighted pair group method with arithmetic averages (UPGMA) based on Euclidean distance using Info Stat 2017d (free version). Here, only the fluorochrome banding pattern of the cultivars *viz*. types and numbers of CMA and DAPI bands were utilized to draw the phenogram.

RESULTS

Fluorochrome banding pattern in cultivars of L. culinaris Medik.

Somatic chromosome analysis of the 30 Lentil cultivars based on fluorescence banding patterns has provided an interesting catalogue of chromosome diversity. The chromosomes took up DAPI stain within 10 minutes of incubation while the incubation time for CMA staining was about 45-50mins. The same CMA and DAPI staining protocol was followed for all the 30 cultivars of *L. culinaris*. Interestingly, we have obtained different types of DAPI and CMA banding patterns within the studied

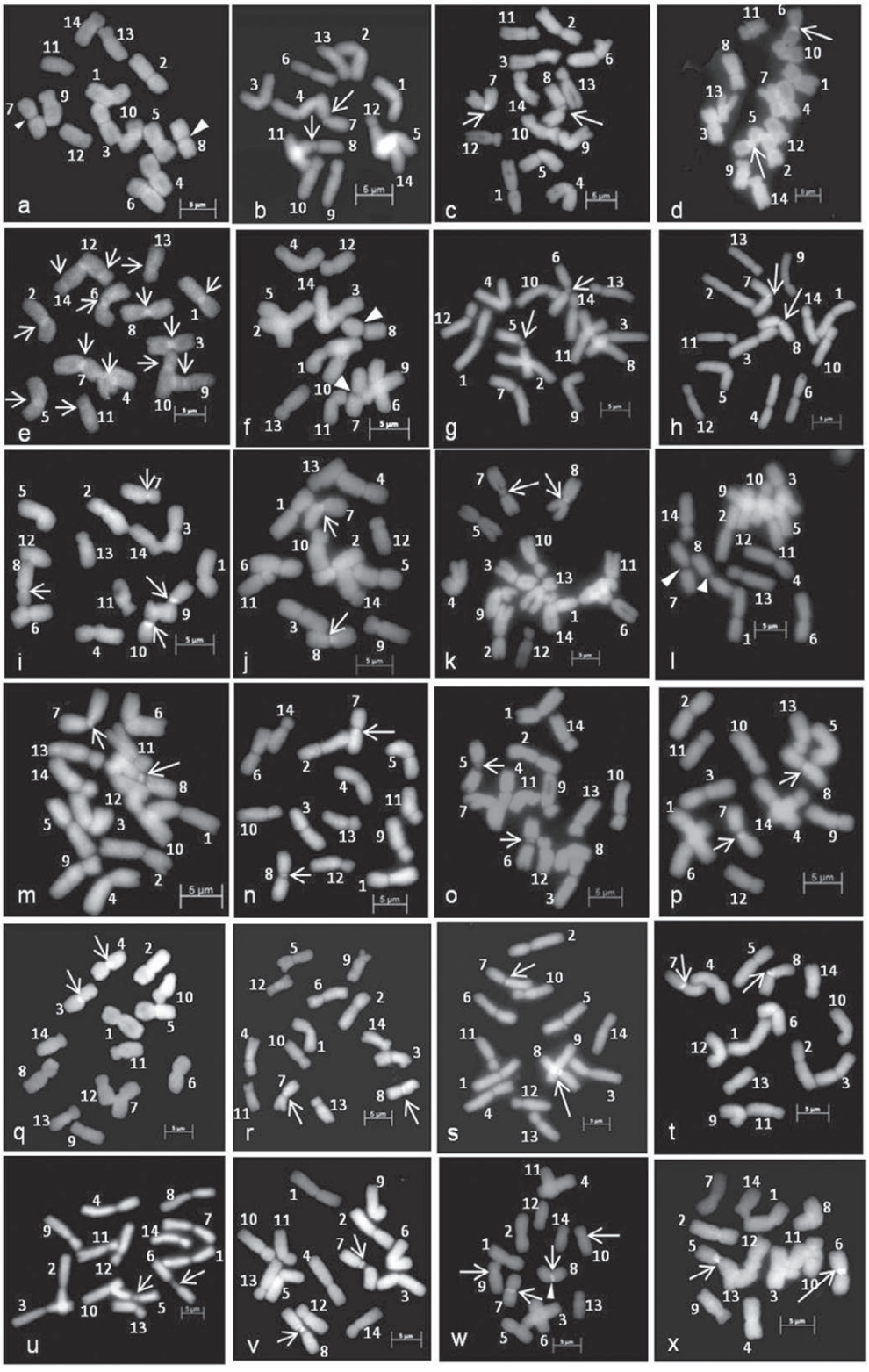

Figure 1. Somatic metaphase chromosomes of *Lens culinaris* cultivars stained with CMA: (a) DPL-15, (b) DPL-62, (c) IPL-81, (d) IPL-406, (e) IPL-316, (f) JL-1, (g) HUL-57, (h) KLS-210, (i) EC-70394, (j) EC-70403, (k) EC-70404, (l) EC-78452, (m) EC-78455, (n) EC-78461, (o) EC-78475, (p) EC-78498, (q) EC-78542-A, (r) EC–223188, (s) EC – 255491, (t) EC–267526, (u) EC-267569-A, (v) EC–267590, (w) EC–267877, (x) Barasat Micro type. White arrows indicate CMA^+ bands and arrowheads indicate CMA^0 bands. Bars 5µm.

cultivars. At least 10 plates stained with DAPI and CMA for each cultivar was considered for analysis of banding types. Secondary constriction marked the nucleolar organizing region (NOR) of most of the cultivars, showing CMA^+ bands with different intensities while some NORs remained neutral and termed CMA^0 as per Barros e Silva and Guerra (2010). DAPI staining in most of the cultivars resulted a clear gap ($DAPI^-$) corresponding to CMA^+ band. However, few exceptional cultivars yielded $DAPI^+$ band in the NOR regions. Based on the CMA and DAPI fluorescent banding, we have categorized following types of somatic chromosomes. The chromosomes with $CMA^+/DAPI^-$ band in the nucleolar region is termed type 'A'. Type 'B' has $CMA^+/DAPI^-$ nucleolar constriction followed by a $DAPI^+/CMA^0$ band below centromere. The 'C' type nucleolar chromosome has a distinct $CMA^+/DAPI^+$ secondary constriction. The fourth type 'D' has neutral CMA band in secondary constriction. Chromosomes with centromeric $CMA^+/DAPI^0$ bands are termed type 'E' while those with centromeric $DAPI^+/CMA^0$ bands are termed type 'F'. Type 'G' chromosome contains intercalary $DAPI^+/CMA^0$ band. The chromosomes having no detectable bands were termed as type 'H'. Distribution of different types of fluorochrome bands among the cultivars is summarized in Table 1. A detailed analysis of the fluorochrome stained metaphase plates (Figures 1-3) was carried out to formulate the diagrammatic fluorescent karyotypes of the 30 cultivars under study (Figures 4 and 5).

CMA-DAPI banding patterns have revealed that in majority of *L. culinaris* cultivars (Table 1), the marker secondary constrictions with CMA^+ signals are present in the 4th pair of chromosomes. However, the same in some cultivars are present in the 3rd and exceptionally in the 5th and 2nd pairs, as in two cultivars (EC-70394, EC-78542-A). The most abundant CMA^+ satellites (type A chromosomes) are found among 50% of the presently studied cultivars. In addition to CMA^+ satellites, existence of type B chromosomes is found in 8 different cultivars (HUL-57, EC-70403, EC-78542-A, EC-267526, EC – 267877, Barasat Micro type, PL -1406, EC -78410, Table 1) and type D chromosomes in 5 different cultivars (DPL15, JL-1, EC-78452, EC – 70306, EC – 78473, Table 1). Of special mention, are the two cultivars (EC-70404, EC-267569-A, Table 1) with $CMA^+/DAPI^+$ satellite (type C chromosome). Three cultivars (IPL -316, EC-70394, EC – 267877) had centromeric CMA^+ bands (type E) (Table 1). One of them (IPL -316) shows centromeric CMA^+ bands (type E) in every chromosome except the nucleolar pair (Table 1). On the other hand, EC -78410 shows intense centromeric $DAPI^+$ bands (type F) in all non-nucleolar chromosome pairs (Table 1). Centromeric $DAPI^+$ bands are consistently found in the 2nd or the 3rd pair of chromosomes in 5 cultivars (HUL-57, EC-70403, EC-267526, Barasat, Macro type, PL -1406, Table 1). Intercalary $DAPI^+$ band (type G) is seen only in IPL-406 (Table 1).

Comparative statistical assessment of fluorochrome banding pattern

Statistical evaluation of karyotype relations among the 30 Lentil cultivars was carried out using Euclidean distance matrix on the basis of CMA and DAPI bands. The UPGMA phenogram presented relative karyotype affinities and distances with a cophenetic correlation of 0.986 as a good fit between the cophenetic value matrix and the average Euclidean distance matrix (Figure 6). There are three separate groups in the UPGMA phenogram of which Group I consisted of cultivars that do not have close affinity with each other (Figure 6). Within this group, EC -78410 and IPL -316 have fluorescent banding pattern that are in contrast to each other. Also, existence of intercalary $DAPI^+$ band makes IPL-406 distinct, placed at the extreme end of the phenogram. The next noticeable cultivars are EC-70404 and EC-267569-A with $CMA^+/DAPI^+$ secondary constriction (Table 1) (Figure 6). The Group II is large, composed of three subgroups mainly differentiated by nucleolar banding pattern in their marker chromosomes. The first subgroup comprised of 5 cultivars with neutral CMA-DAPI bands in their satellites (type D) (Table 1, Figure 6). The second subgroup is largest, comprising of 13 cultivars with $CMA^+/DAPI^-$ satellite (type A). Here, two cultivars (EC-70394 and Barasat, Macro type) show little distance from rest of the cultivars, because of different types of centromeric bands (Table 1, Figure 6). The third subgroup comprises of 7 cultivars with 'B' type nucleolar chromosomes. This subgroup shows heterogeneity because of variations in centromeric bands (Table 1, Figure 6).

DISCUSSION

Cytogenetics of *L.culinaris* is traditionally acknowledged for species delimitations, crossing behavior, conservation and utilization of plant genetic resources (Ladizinsky 1979; Tadmor et al. 1987; Ladizinsky et al. 1990; Ladizinsky 1999; Mishra et al. 2007). With the present approach, we have entered the modern karyotyping system to study chromosomal specialization in Indian Lentils. The diversity of fluorescent karyotypes can be indisputably attributed to the differences in underlying chromosomal heterochromatin of the samples since i)

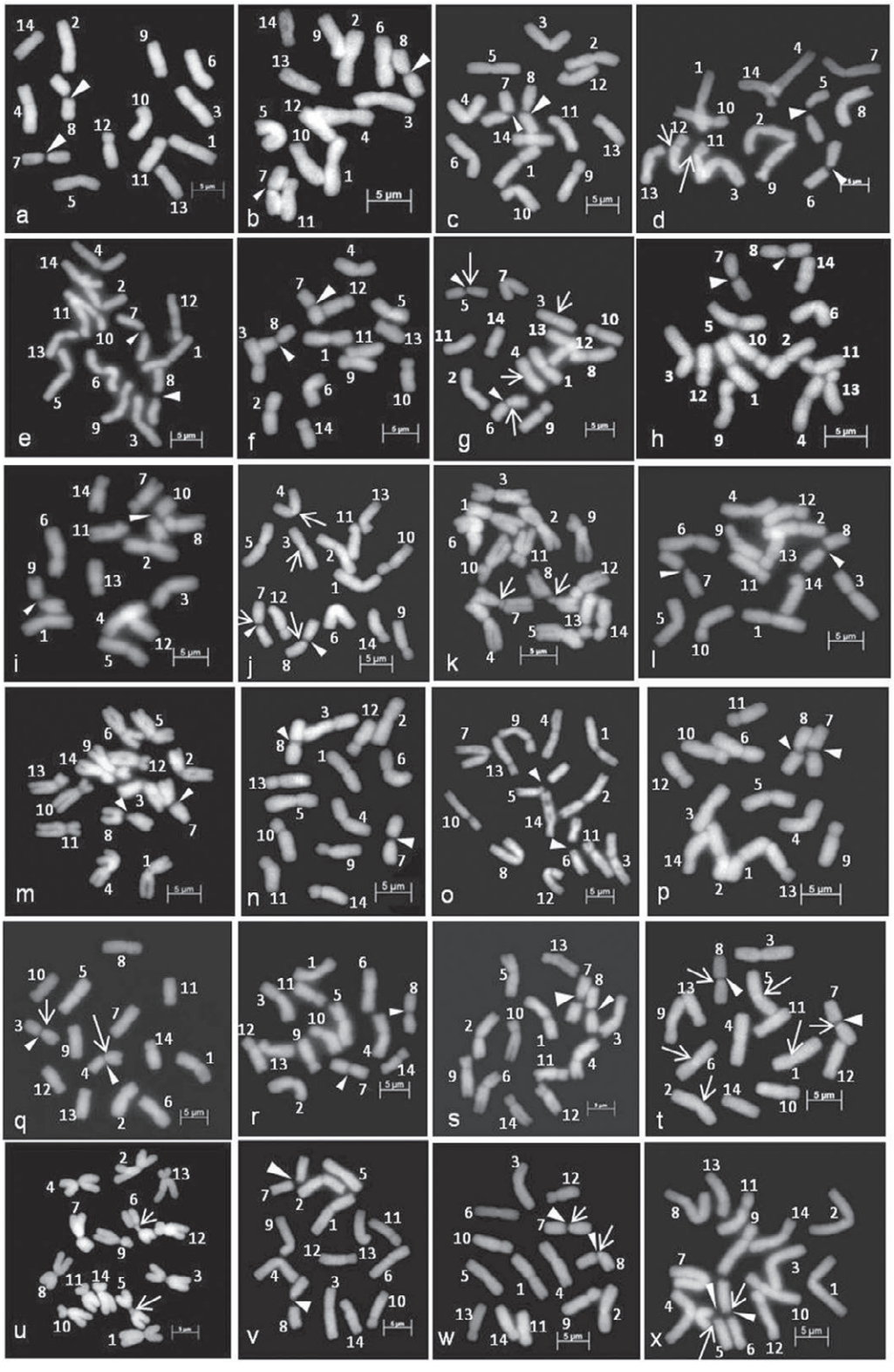

Figure 2. Somatic metaphase chromosomes of *Lens culinaris* cultivars stained with DAPI: (a) DPL-15, (b) DPL-62, (c) IPL-81, (d) IPL-406, (e) IPL-316, (f) JL-1, (g) HUL-57, (h) KLS-210, (i) EC-70394, (j) EC-70403, (k) EC-70404, (l) EC-78452, (m) EC-78455, (n) EC-78461, (o) EC-78475, (p) EC-78498, (q) EC-78542-A, (r) EC–223188, (s) EC – 255491, (t) EC–267526, (u) EC-267569-A, (v) EC–267590, (w) EC–267877, (x) Barasat Micro type. White arrows indicate $DAPI^+$ bands and arrowheads indicate $DAPI^-$ and $DAPI^0$ bands. Bars 5µm.

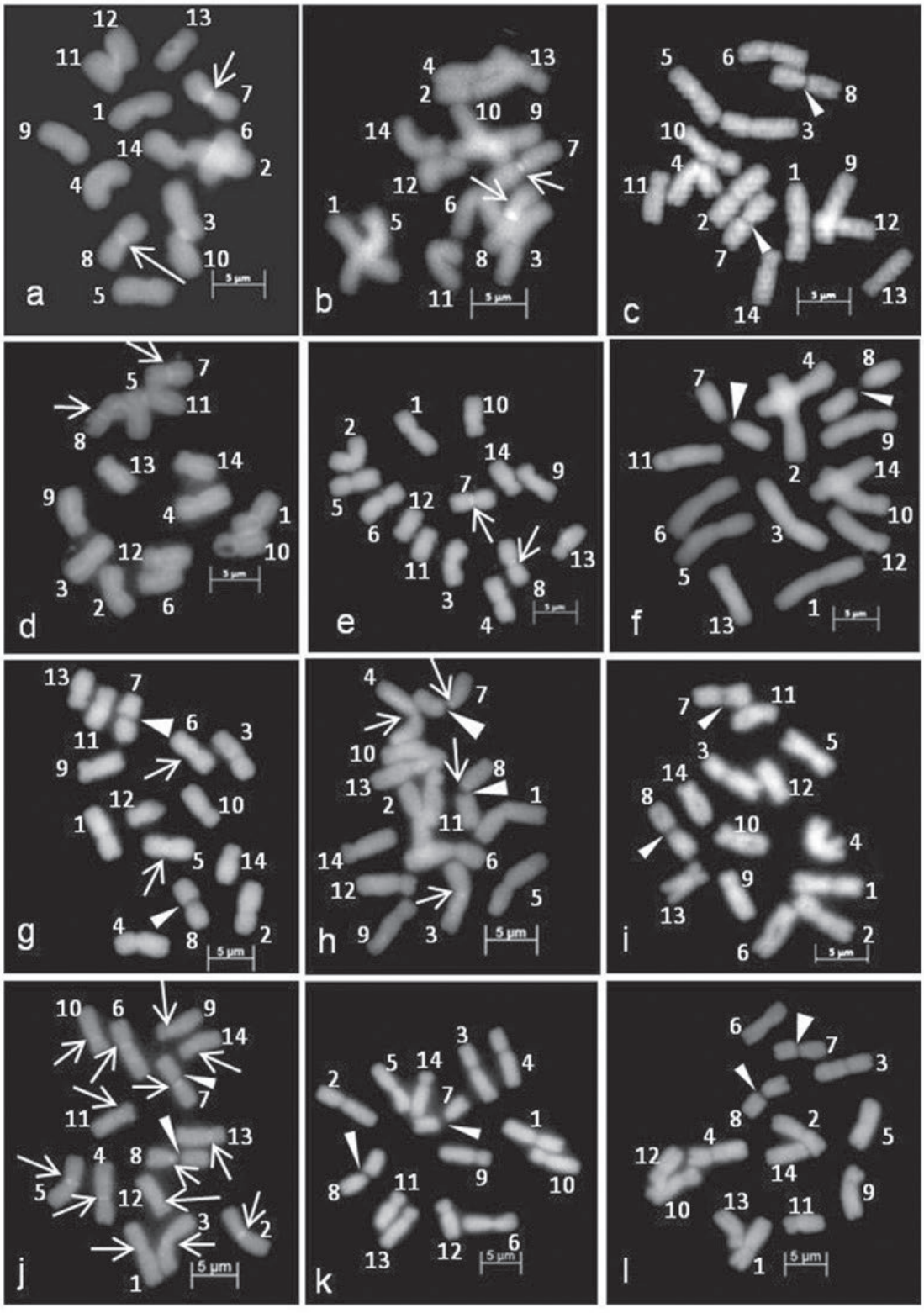

Figure 3. Somatic metaphase chromosomes of *Lens culinaris* cultivars. CMA stained plates: (a) Barasat Macro type, (b) PL-1406, (c) EC-70306, (d) EC -78410, (e) EC-78451-A, (f) EC-78473. DAPI stained plates: (g) Barasat Macro, (h) PL-1406, (i) EC-70306, (j) EC -78410, (k) EC-78451-A, (l) EC-78473. White arrows indicate CMA^{+} or $DAPI^{+}$ bands and arrowheads indicate CMA^{0} or $DAPI^{0}$ and $DAPI^{-}$ bands. Bars 5µm.

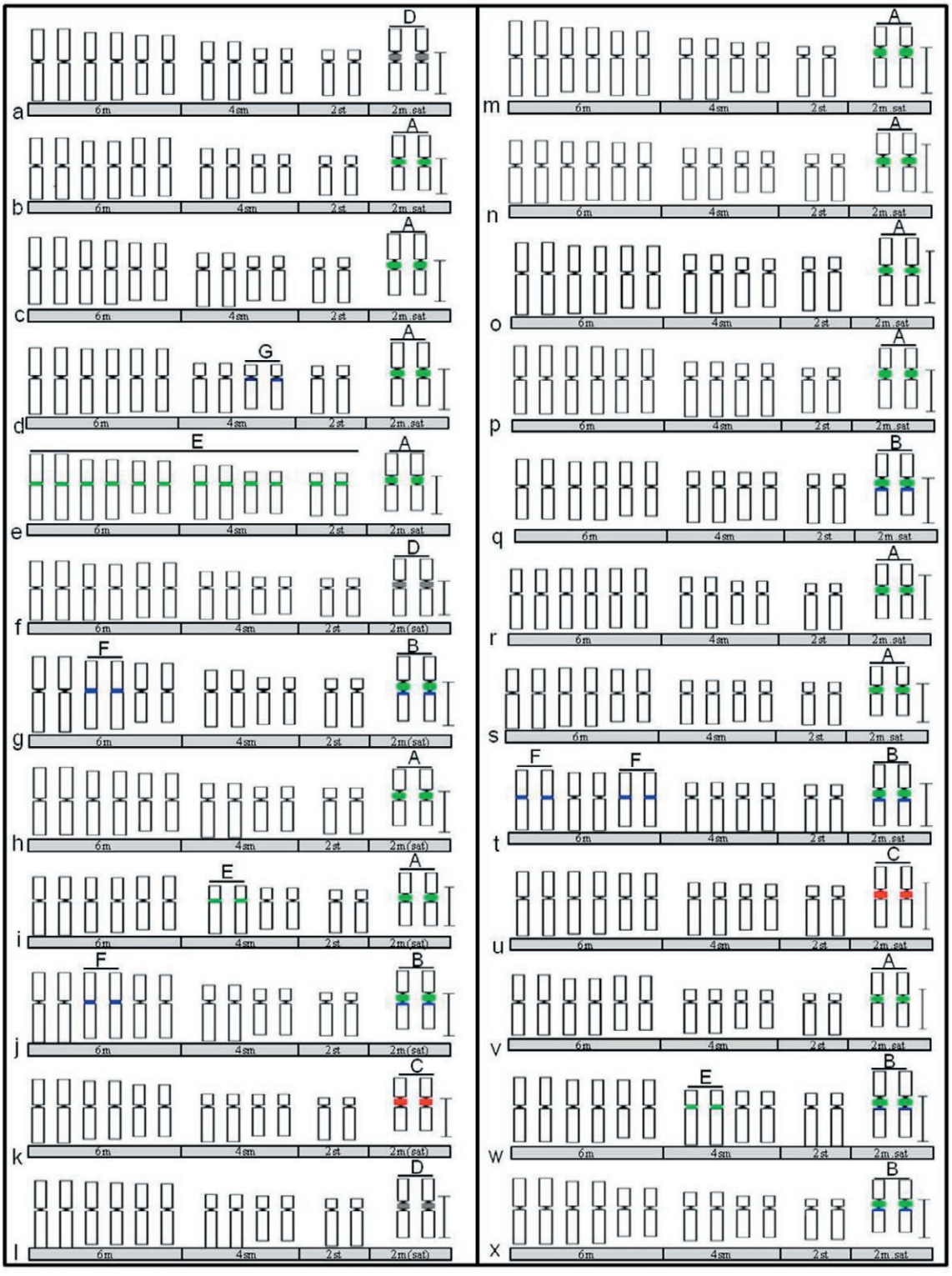

Figure 4. Fluorescent ideograms of *Lens culinaris* cultivars based on CMA/DAPI banding pattern: (a) DPL-15, (b) DPL-62, (c) IPL-81, (d) IPL-406, (e) IPL-316, (f) JL-1, (g) HUL-57, (h) KLS-210, (i) EC-70394, (j) EC-70403, (k) EC-70404, (l) EC-78452, (m) EC-78455, (n) EC-78461, (o) EC-78475, (p) EC-78498, (q) EC-78542-A, (r) EC–223188, (s) EC – 255491, (t) EC–267526, (u) EC-267569-A, (v) EC–267590, (w) EC–267877, (x) Barasat Micro type. CMA^+, $DAPI^+$, $CMA^+/DAPI^+$ and CMA^0 bands are highlighted with green, blue, red and grey colors on the chromosomes, respectively and the types are indicated above the chromosome diagrams. Bars 5μm

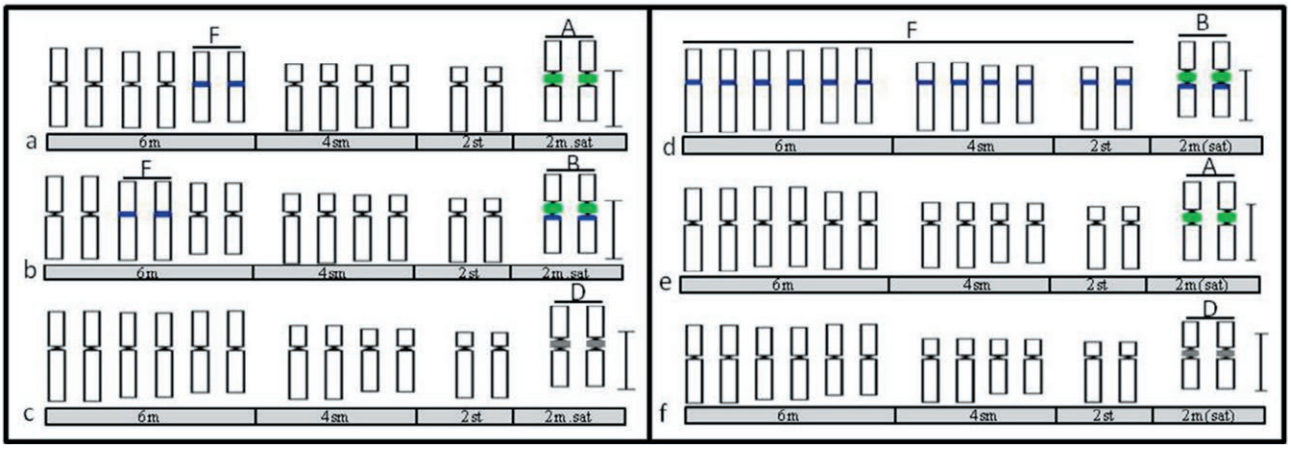

Figure 5. Fluorescent ideograms of *Lens culinaris* cultivars based on CMA/DAPI banding pattern: (a) Barasat Macro type, (b) PL-1406, (c) EC-70306, (d) EC -78410, (e) EC-78451-A, (f) EC-78473. CMA^+, $DAPI^+$, $CMA^+/DAPI^+$ and CMA^0 bands are highlighted with green, blue, red and grey colors on the chromosomes, respectively and the types are indicated above the chromosome diagrams. Bars 5μm.

we have applied the same fluorochrome staining protocol for every cultivar, ii) the method is repeated a number of times before ascertaining banding pattern in a cultivar and iii) at least 5 best metaphase plates of each cultivar with scorable signals were considered for establishing the fluorescent karyotype.

Considering the nature of nucleolar chromosomes, molecular banding technique has shed light on chromosomal landmarks and possible differences in NORs that were previously found to be similar in *Lens* (Mehra et al. 1986; Jha et al. 2015, 2017; Jha and Halder 2016). The marker nucleolar chromosomes (4^{th}, along with the 3^{rd}, 2^{nd} and 5^{th} in few cases) have been confirmed with characteristic CMA-DAPI signals, corroborating to our previous report (Jha et al. 2017). The CMA^+ signals are generally accepted as the GC heterochromatic elements of the NORs in plant groups (Guerra et al. 2000; Barros e Silva and Guerra 2010; Yamamoto 2012; Olanj et al. 2015) and so in Papilionoids such as *Vicia* (Fuchs et al. 1998), *Cicer* (Galasso et al. 1996) and *Crotalaria* (Mondin and Aguiar-Perecin 2011). Previously, 18S-5.8S-25S rDNA probes had been localised in a single pair of *L. culinaris*, near the centromere (Balyan et al. 2002), corroborating to the observation of CMA^+ signals in our present study. However, we found that the intensity of the nucleolar CMA signals (type A) varies in certain cultivars, suggesting differences in NORs that influence affinity towards the stain. Intraspecific rDNA variation has been thoroughly worked out in *Phaseolus* (Moscone et al. 1999; Pedrosa-Harand et al. 2006) and *Vigna* (Bortoleti et al. 2012; She et al. 2015, 2020) of Papilionoideae. A number of factors such as transposition, unequal crossing over, inversion or locus duplication, had been suggested to drive NOR variation in plant groups, including Papilionoideae (Moscone et al. 1999; Chung et al. 2008; Raskina et al. 2008). We consider similar possibilities in the Indian Lentils, subject to future confirmation by AgNOR staining or rDNA FISH.

The type D chromosomes have satellites that respond indifferently to the CMA stain. The CMA^0 satellites indicate GC neutral nature of heterochromatin (Barros e Silva and Guerra 2010). The type D satellites are in sharp contrast to type A bands, marking cultivar distinction. The other unusual type was the $CMA^+/DAPI^+$ satellites (type C). Previously, the $CMA^+/DAPI^+$ satellites were suggested to be a 'less common' or 'rare' type of heterochromatin (Barros e Silva and Guerra 2010), breaking the generality of GC rich composition of plant NORs (Schweizer 1976; Guerra et al. 2000). We document the occurrence of $CMA^+/DAPI^+$ satellites for the first time in *Lens* of Papilionoideae. Co-localized $CMA^+/DAPI^+$ satellites are so far reported in *Allium nigrum* (Maragheh et al. 2019) and *Cestrum* (Fernandes et al. 2009). It is difficult to ascertain the heterochromatin composition of this type. There is a possibility of having AT and GC rich segments to be placed so close that the different chromatin bands cannot be distinguished in condensed mitotic chromosomes (Maragheh et al. 2019). However, nucleolar heterochromatin composition of Indian *Lens culinaris* displays considerable variation, perhaps due to enormous cultivation practice and artificial hybridization, which is a yet unaddressed field of study.

Cultivar specific differences were also accentuated by the non-nucleolar $DAPI^+$ and CMA^+ bands. The type E centromeric CMA bands are unique type

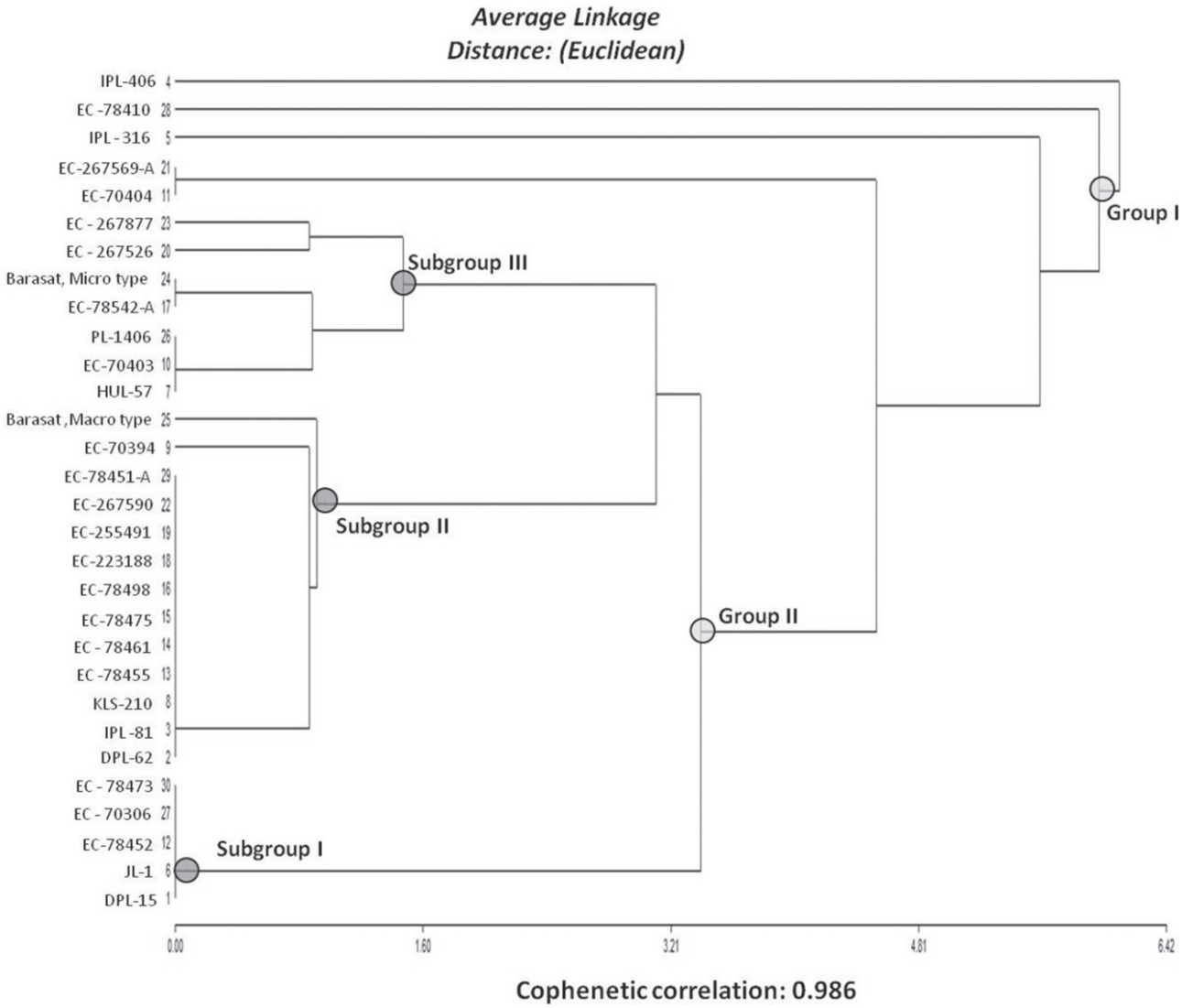

Figure 6. UPGMA dendrogram derived from average Euclidean distance based on fluorochrome banding pattern of 30 Indian Lentil cultivars, cultivar names in the left of their serial numbers.

of heterochromatin rarely reported in plants. However, non-nucleolar GC-rich heterochromatin was previously characterized in centromeric as well as pericentromeric regions of Papilionoid species belonging to *Dioclea* (Souza and Benko-Iseppon 2004), *Psophocarpus* (Chaowen et al. 2004), *Crotalaria* (Mondin and Aguiar-Perecin 2011), *Vigna* (Bortoleti et al. 2012; She et al. 2015, 2020), *Phaseolus* (Bonifácio et al. 2012), *Lablab* (She and Jiang 2015), and *Canavalia* (She et al. 2017). She et al. (2020) suggested the centromeric or pericentromeric GC- heterochromatin to be a relic of genomic evolution in the subfamily Papilionoideae. Other even rare heterochromatin blocks were the centromeric (type F), pericentromeric (type B) and intercalary (type G) DAPI bands, constituting landmarks to differentiate karyotypes of certain Lentil cultivars. Terminal or intercalary $DAPI^+$ bands were documented in few plants (Vanzela and Guerra 2000; Divashuk et al. 2014), including few species of Cucurbitaceae (Bhowmick and Jha 2015, 2019). Terminal DAPI bands are found in *Crotalaria* (Mondin and Aguiar-Perecin 2011) of Papilionoideae. Centromeric DAPI bands are yet rare to encounter. However, in case of *L. culinaris* and related species, AT heterochromatic regions were mapped by repetitive sequence probe FISH (Galasso et al. 2001; Galasso 2003). Also in Papilionoideae, AT rich heterochromatin at centromere and pericentromeric regions are reported in *Vigna* (Bortoleti et al. 2012; She et al. 2020), *Lablab* (She and Jiang 2015) and

Table 1. Analysis of CMA and DAPI fluorescent bands in thirty Indian cultivars of *Lens culinaris* (2n = 14).

Sl. No.	Cultivars	Order of nucleolar pair	CMA bands				DAPI bands				Total no. of bands/ 2n	Fluorescent Karyotype formula (2n)	Figure no.
			No.	Chromosome pair/s	Type*/ intensity	Figure no.	No.	Chromosome pair/s	Type*/ intensity	Figure no.			
1	DPL15	4th	2	4th	D/ neutral	1a	0	-	-	2a	2	2D+12H	4a
2	DPL-62	4th	2	4th	A/low	1b	0	-	-	2b	2	2A+12H	4b
3	IPL -81	4th	2	4th	A/low	1c	0	-	-	2c	2	2A+12H	4c
4	IPL-406	3rd	2	3rd	A/high	1d	2	6th	G/high	2d	4	2A+2G+10H	4d
5	IPL -316	4th	2 12	4th 1st-3rd, 5th-7th	A/high E/high	1e	0	-	-	2e	14	2A+12E	4e
6	JL-1	4th	2	4th	D/ neutral	1f	0	-	-	2f	2	2D+12H	4f
7	HUL-57	3rd	2	3rd	B/ low	1g	2 2	3rd 2nd	B/low F/high	2g	4	2B+2F+10H	4g
8	KLS-210	4th	2	4th	A/high	1h	0	-	-	2h	2	2A+12H	4h
9	EC-70394	5th	2 2	5th 4th	A/high E/high	1i	0	-	-	2i	4	2A+2E+10H	4i
10	EC-70403	4th	2	4th	B/high	1j	2 2	4th 2nd	B/high F/high	2j	4	2B+2F+10H	4j
11	EC-70404	4th	2	4th	C/high	1k	2	4th	C/low	2k	2	2C+12H	4k
12	EC-78452	4th	2	4th	D/ neutral	1l	0	-	-	2l	2	2D+12H	4l
13	EC-78455	4th	2	4th	A/high	1m	0	-	-	2m	2	2A+12H	4m
14	EC- 78461	4th	2	4th	A/high	1n	0	-	-	2n	2	2A+12H	4n
15	EC-78475	3rd	2	3rd	A/low	1o	0	-	-	2o	2	2A+12H	4o
16	EC– 78498	4th	2	4th	A/high	1p	0	-	-	2p	2	2A+12H	4p
17	EC-78542-A	2nd	2	2nd	B/high	1q	2	2nd	B/low	2q	2	2B+12H	4q
18	EC-223188	4th	2	4th	A/high	1r	0	-	-	2r	2	2A+12H	4r
19	EC-255491	4th	2	4th	A/high	1s	0	-	-	2s	2	2A+12H	4s
20	EC-267526	4th	2	4th	B/high	1t	2 4	4th 1st , 3rd	B/low F/low	2t	6	2B+4F+8H	4t
21	EC-267569-A	3rd	2	3rd	C/low	1u	2	3rd	C/low	2u	2	2C+12H	4u
22	EC-267590	4th	2	4th	A/high	1v	0	-	-	2v	2	2A+12H	4v
23	EC - 267877	4th	2 2	4th 5th	B/high E/low	1w	2	4th	B/high	2w	4	2B+2E+10H	4w
24	Barasat, Micro type	3rd	2	3rd	B/high	1x	2	3rd	B/low	2x	2	2B+12H	4x
25	Barasat, Macro type	4th	2	4th	A/high	3a	2	3rd	F/high	3g	4	2A+2F+10H	5a
26	PL -1406	4th	2	4th	B/high	3b	2 2	4th 2nd	B/low F/low	3h	4	2B+2F+10H	5b
27	EC - 70306	4th	2	4th	D/ neutral	3c	0	-	-	3i	2	2D+12H	5c
28	EC -78410	4th	2	4th	B/high	3d	2 12	4th 1st -3rd, 5th-7th	B/high F/high	3j	14	2B+12F	5d
29	EC – 78451-A	4th	2	4th	A/high	3e	0	-	-	3k	2	2A+12H	5e
30	EC - 78473	4th	2	4th	D/ neutral	3f	0	-	-	3l	2	2D+12H	5f

*Types of nucleolar bands- A: $CMA^+/DAPI^-$ satellite, B: $CMA^+/DAPI^-$ satellite and $DAPI^+/CMA^0$ band in long arm, C: $CMA^+/DAPI^+$ satellite, D: CMA^0 satellite; centromeric bands- E: $CMA^+/DAPI^0$, F: $DAPI^+/CMA^0$; intercalary band G: $DAPI^+/CMA^0$; H: no bands.

Arachis (Silvestri et al. 2020). Nonetheless, occurrence of centromeric CMA^+ or $DAPI^+$ bands along with nucleolar $CMA^+/DAPI^+$ or CMA^0 bands certainly advocate atypical heterochromatin composition in *Lens*. The non-uniform composition and rearrangements of heterochromatin had been observed repeatedly in Papilionoideae species (Moscone et al. 1999; Souza and Benko-Iseppon 2004; Pedrosa-Harand et al. 2006; Mondin and Aguiar-Perecin 2011; She et al. 2020), which becomes apparent in our study once again.

In view of the diversity in fluorochrome banding pattern, we attempted to resolve karyotype relationships by the UPGMA method. Identification of distinct subgroups has opened further scopes to complement marker assisted analysis of genetic diversity across varied range of Indian cultivars with valuable agronomic traits. Application of fluorochrome banding method has therefore helped to i) break the perception of an overall similar karyotype of cultivated Lentils as observed in Giemsa plates (Jha et al. 2015, 2017; Jha and Halder 2016) ii) serve as the chromosomal blueprint for cultivar discrimination, ii) statistically represent the status of chromosomal relationships, iii) highlight the uniqueness of certain Indian cultivars by means of unconventional banding pattern, and v) construct a fluorescent karyotype dataset of Indian Lentil cultivars.

CONCLUSION

Being a crop 'as old as agriculture' (Sandhu and Singh 2007), an exclusive chromosomal database of Lentils is essential to complement genomic research databases like Legume Information System (Dash et al. 2016) and KnowPulse (Sanderson et al. 2019). As an extension of our study involving Lentil cytogenetics, we have delved into the first molecular karyotypes of the country's native cultivars. Notably, the cultivars are hosted by world's second largest *ex situ* Lentil germplasm stock *i.e.* IIPR of NBPGR, the first being ICARDA (Muehlbauer and McPhee 2005; Coyne and McGee 2013). In future, molecular cytogenetic study of wild *Lens* species of India can be expected to strengthen the base of chromosomal evolution in Papilionoideae. In face of stern climatic changes that affect future cultivation, the Indian cultivars with interesting karyotype features and relationships can be fluently tested for performance and productivity. Thus, our findings complement traditional or marker assisted breeding and would undoubtedly bridge up the lacuna for a systematic chromosomal database of Indian Lentils.

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