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

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**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.

**Key words**

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 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 karyotype 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 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). 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 0.1 µg ml⁻¹ solution of DAPI for 10 min, counterstained with 0.25 mg/ml of Actinomycin D (AMD) for 15 min 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 5 mM MgCl₂ for 10 mins and then stained with 0.1 mg ml⁻¹ CMA solution for 45-50 mins. The slides were again washed in McIlvaine buffer with 5 mM MgCl₂ and finally mounted with non-fluorescent glycerol and kept for maturation at 4°C 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 45-50 mins. 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 cultivars. At least 10 plates stained with DAPI and CMA for each cultivar was considered for analysis of banding types. Secondary constriction marks the nucleolar organizing region (NOR) of most of the cultivars showed 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 pattern have revealed that in majority *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 5th and 2nd pairs 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 DPL-62 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) 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 (4th, along with the 3rd, 2nd and 5th 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 CMA0 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, maybe 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 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 *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 Pailionoideae 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 have 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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**Disclosure statement**

The authors declare that they have no conflict of interest.

**References**


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Sharma AK, Muhkopadhyay S. 1963. Karyotype constancy in different strains of *Lens esculenta* Moench as worked out through recent techniques. Indian Agric. 7:103-111.


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

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Cultivars</th>
<th>Order of nucleolar pair</th>
<th>CMA bands</th>
<th>DAPI bands</th>
<th>Total no. of bands/2n</th>
<th>Fluorescent Karyotype formula (2n)</th>
<th>Figure no.</th>
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### Types of nucleolar bands

- **A**: CMA+/DAPI satellite
- **B**: CMA+/DAPI satellite and DAPI+/CMA₀ band in long arm
- **C**: CMA+/DAPI satellite
- **D**: CMA₀ satellite
- **E**: CMA⁺/DAPI₀
- **F**: DAPI⁺/CMA₀
- **G**: DAPI⁺/CMA₀
- **H**: no bands

### Table: Fluorescent Karyotype

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<th>Sl. No.</th>
<th>Cultivars</th>
<th>Order of nucleolar pair</th>
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<th>DAPI bands</th>
<th>Total no. of bands/2n</th>
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<td>4th</td>
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<td>D/ neutral</td>
<td>3c</td>
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<td>5c</td>
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<td>B/high</td>
<td>3d</td>
<td>2B+12F</td>
<td>5d</td>
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<td>4th</td>
<td>4th</td>
<td>A/high</td>
<td>3e</td>
<td>2A+12H</td>
<td>5e</td>
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<td>4th</td>
<td>D/ neutral</td>
<td>3f</td>
<td>2D+12H</td>
<td>5f</td>
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</tbody>
</table>

*Types of nucleolar bands: A: CMA⁺/DAPI⁻ satellite, B: CMA⁺/DAPI⁻ satellite and DAPI⁺/CMA₀ band in long arm, C: CMA⁺/DAPI⁺ satellite; D: CMA₀ satellite; centromeric bands: E: CMA⁺/DAPI₀, F: DAPI⁺/CMA₀; intercalary band G: DAPI⁺/CMA₀; H: no bands.
Figure 1
Figure 2
Figure 3
Figure 5
Figure 6
Figure Captions

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 5\(\mu\)m

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 5\(\mu\)m

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\(^0\) bands. Bars 5\(\mu\)m

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 5\(\mu\)m

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

**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.