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ORIGINAL ARTICLE

Extended centromere and chromosomal mosaicism in some varieties of grass pea, *Lathyrus sativus* L.

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Abstract
An abnormal mitotic behavior revealing chromosomal mosaicism was observed in two among the three studied cultivars (cv. Nirmal, cv. Prateek and cv. Ratan) of *Lathyrus sativus*. Several numerical variants of chromosome complement were detected in the different cells coexisted with normal diploid cells in the somatic tissue of the same root-tip. Somatic chromosome number 2n = 14 were found with the greatest frequency (67%); however, a spectrum of quite low percentage of discordant and variable chromosome numbers especially of aneuploidy mode, ranging from 2n = 11 (9.6%), 2n = 12 (8.7%), 2n = 13 (4.7%) and 2n = 15 (10.3%) in Nirmal cultivar were observed. In Prateek cultivar, the most interesting cytological character is that karyotype though symmetrical but indicating transition between symmetrical to asymmetrical is slightly bimodal due to presence of one pair of very small chromosomes. In general, most of the chromosomes of all cultivars were nearly median centromered. Although the cv. Ratan shows the normal chromosome number, but in 4 pairs of chromosomes, the centromere region appeared unusually 'extended'. In Nirmal and Prateek cultivars karyotype, the secondary constriction is proximal on the short arm whereas, in some other metaphase plates, the same constriction bearing macrosatellite is on the long arm of bigger nearly submedian chromosome of pair one. We propose that the differences in satellite position arose by karyotype rearrangements; probably involving translocations. At the same time, extended centromeres may be due to the activities of retrotransposons at the centromere region of the said chromosome of cv.Nirmal.

Keywords Chromosomal mosaicism · *Lathyrus sativus* · Karyotype analysis

Abbreviations
pDB *Para-dichlorobenzene*
F% Form/centromeric percentage
cv. *Cultivar*
LTR Long-terminal repeat

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Introduction

The genus *Lathyrus* belongs to the family Leguminosae (Fabaceae), subfamily Papilionoideae, tribe Vicieae. It contains more than 200 taxa and shows broad diffusion throughout the world [1]. Cytologically, most of the species of *Lathyrus* have 2n = 14 chromosomes, with the basic number being x = 7 [14]. Comparative study of karyotype analysis within a group, variability of chromosome structure, instability of chromosome number and chromosomal mosaicism in higher plants has been studied and reported by several workers [10, 11, 18, 48–51, 54, 57]. In case of chromosomal mosaicism, the somatic cells have the different number of chromosomes that may be caused by somatic mutation or chromosomal non-disjunction. There are numerous reports of karyotype analysis of *Lathyrus* species [2, 3, 5, 8, 12, 13, 16, 17, 19–22, 26, 27, 35, 38, 39, 41, 42, 44–46, 56, 61]. From the available information on chromosome study of *Lathyrus*, some contradictory observations have emerged. Some workers reported that, besides the numerical constancy (2n = 14), *Lathyrus* species exhibit morphological similarity of chromosomes and heterogeneous karyotype arrangement [24, 43]. Although there is no change in chromosome number, there are deviations in centromere location, in chromosome size and in the number, size and position of secondary constrictions [4, 6, 14, 15, 37, 47]. Others have observed enough karyotype dissimilarities among different species to permit species characterization [4]. In contrast, several researchers also noted that chromosome numbers were not identical in most species of *Lathyrus*. Such discrepancies i.e. variation of chromosome number and heterogeneous karyotype arrangement were also observed at the infraspecific level. But chromosomal mosaicism was not observed by any workers in grass pea (*Lathyrus sativus*). So, the present work is an attempt to compare the chromosomal characteristics, mosaicism and karyotype analysis among three elite cultivars of *Lathyrus sativus* L.

Materials and methods

The seeds of three cultivars of *Lathyrus sativus* L. namely cv. RATAN (BIO L 212), cv. PRATEEK and cv. NIRMAL (B-1) used in the present study were collected from Pulses and Oilseeds Research Station, Berhampore, West Bengal, India. Root tips at the time of peak mitotic activity, were collected from young healthy roots of germinating seeds of different cultivars and pretreated with mixture of saturated solution of pDB and 0.5% colchicine (3:1) for 5 h at 12 °C under refrigeration was found suitable and potentially effective [52]. Root tips were then fixed in glacial acetic acid and ethanol (1:3) at room temperature and kept for overnight. The fixed root tips were then kept in 45% acetic acid for 20 min. subsequently stained in 2% acetic-orcein: HCl (1N) mixture (9:1) with initial warming over flame and then kept at room temperature for 3 h. Stained root-tips were squashed in 45% acetic acid. Chromosome number in normal and variant cells and their frequency were recorded from several mitotic metaphase plates with well scattered chromosomes. Pollen grains were studied following the aceto-carmine stain technique.

The position of centromere of each chromosome [25, 29] i.e. F% (the ratio of the short arm to the total length of the chromosome expressed in percentage), was calculated using the following formula:

$$\text{Centromeric Index } (F\%) = \frac{\text{Short arm length}}{\text{Total length of the chromosome}} \times 100$$

Results

In general, the normal somatic metaphase plate of three cultivars studied were diploid, with 2n = 14 chromosomes. This agrees with previous cytological studies on *Lathyrus* species. However, they diverged in the number and size of nearly metacentric, nearly sub-metacentric chromosomes and in the number(s) and position(s) of the secondary constrictions and macrosatellite. For the convenience of understanding, metaphase chromosomal characteristics of three cultivars are described separately.

cv. Nirmal (B1) The karyotype of this cultivar contains some numerical variations of chromosomes (Table 1). So, chromosomal mosaicism or chimerism is found. Since in most of the mosaic conditions, diploid chromosome number is odd, it is also termed as aneusomaty in which, in addition to mitosis with the diploid chromosome number (2n = 14), there occurred aneuploid mitoses with 11–15 chromosomes. On the basis of variation of chromosome number, karyotypes were subdivided as follows:

1. *Normal type* The karyotype (Fig. 2a) appears normal (2n = 14) with five pairs of nearly median chromosomes and one pair of nearly submedian chromosomes and one pair median (Fig. 1b). Chromosome size ranges from 8.66 to 13.15 µm (Table 2). The karyotypes of this cultivar have a predominance of nearly median chromosomes. Karyotype is moderately symmetrical as seen in idiogram. This type is observed in nearly 67% of the total observation. On the basis of position of satellites on chromosome arm, karyotypes are of two subtypes: (a) A pair of nearly median chromosome has a satellite (macrosatellite) attached to the long arm (Fig. 3a), and (b) A pair of nearly median chromosome has a satellite (macrosatellite) attached to the short arm (Fig. 3b).
2. *Karyotype with different chromosome numbers—I* The metaphase plate shows 2n − 3 = 11 chromosomes (Fig. 1c). The karyotype (Fig. 2b) consists of five pairs of nearly median chromosomes among them four pairs are comparatively long and one small (10.62 µm) and one extra unpaired very long nearly median chromo-

Table 1 Percentage of cells with mosaic chromosome number in the examined cultivars of *Lathyrus sativus*

Name of cultivar	Mosaic chromosome number	% of cells
cv. Nirmal	2n = 11	9.6
	2n = 12	8.7
	2n = 13	4.7
	2n = 15	10.3
cv. Prateek	Variant type I	12 + 8.6 = 20.6
	Variant type II	8.0
cv. Ratan	Nil	Nil

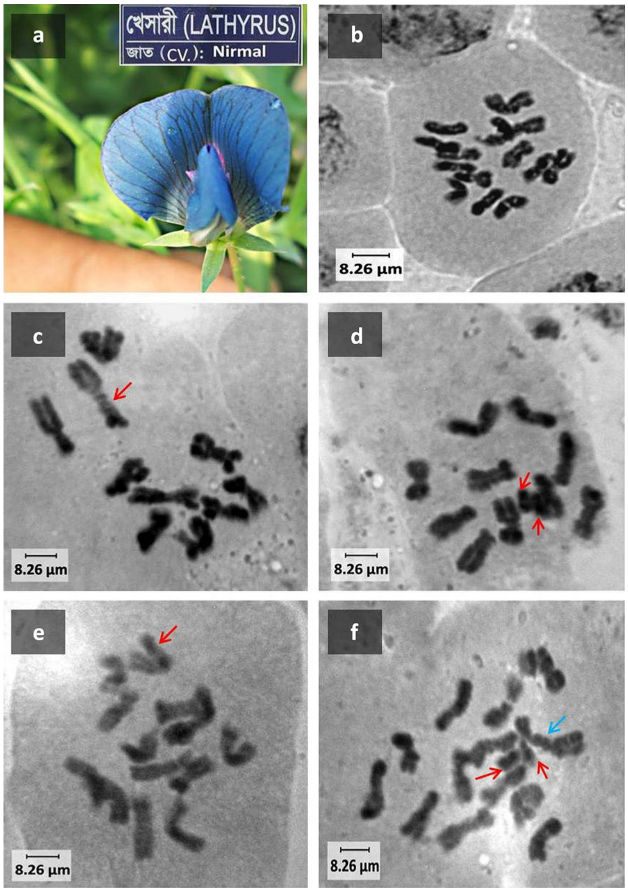

Fig. 1 Flower and somatic chromosomes of cultivar Nirmal: **a** Flower, **b** photomicrograph of normal somatic metaphase chromosomes (2n = 14), **c** 11 chromosomes with one unpaired long chromosome (red arrow marked), **d** 12 chromosomes with one pair small chromosome (red arrow marked), **e** 13 chromosomes with one unpaired long chromosome (red arrow marked), **f** 15 chromosomes with one pair small chromosome and one unpaired long chromosome (blue arrow marked). *Scale Bar = 8.26 µm

some (21.91 µm). Chromosome size ranges from 10.62 to 21.91 µm (Table 3). The karyotype of this cultivar has a predominance of nearly median chromosomes, and it is symmetrical (Fig. 3c). This is observed in nearly 9.6% of total observation.

3. *Karyotype with different chromosome numbers—II* The metaphase plate shows 2n − 2 = 12 chromosomes (Fig. 1d). The karyotype (Fig. 2c) consists of six pairs of nearly median chromosomes of which one pair is very small chromosome (6.42 µm). Satellites were not visible. Chromosome size ranges from 6.42 to 17.10 µm (Table 4). The karyotype and idiogram (Fig. 3d) is symmetrical. This is observed nearly 8.7% of total observation.
4. *Karyotype with different chromosome numbers—III* The metaphase plate shows 2n − 1 = 13 chromosomes (Fig. 1e). The karyotype (Fig. 2d) contains five pairs of nearly median chromosomes, one pair of nearly submedian and one extra unpaired long (19.24 µm) nearly median chromosome. Satellites were not visible. Chromosome size ranges from 12.17 to 19.24 µm (Table 5). The karyotype is fairly **s**ymmetrical which is also reflected in idiogram (Fig. 3e). This is nearly 4.7% of total observation.
5. *Karyotype with different chromosome numbers—IV* The metaphase plate shows 2n + 1 = 15 chromosomes (Fig. 1f). The karyotype (Fig. 2e) consists of six pairs of nearly median chromosomes, one pair of nearly median very small chromosome (6.18 µm) and one extra unpaired long (21.75 µm) nearly median chromosome. Satellites were not visible. Chromosome size ranges from 6.18 to 21.75 µm (Table 6). The karyotype is moderately symmetric as also seen in ideogram (Fig. 3f). It is nearly 10.3% of the total observation.

cv. Prateek The karyotype of this cultivar contains some structural variations of chromosomes. Such variations of karyotype were subdivided as follows:

1. *Normal type* The chromosomes (Fig. 4b) appear normal (2n = 14) with six pairs of nearly median chromosomes and one pair of nearly submedian chromosomes. Chromosome size ranges from 10.52 to 16.02 µm (Table 7). Satellites were not visible. This karyotype (Fig. 5a) has a predominance of nearly median chromosome. Karyotype is fairly symmetrical (Fig. 6a). This type has been observed nearly 71.4% of total observation.
2. *Variation type I* The chromosomes (Fig. 4c) contain five pairs of nearly median chromosomes, one pair small nearly median chromosome and one pair of nearly submedian chromosomes. Satellites were not visible. Chromosome size ranges from 6.33 to 16.59 µm (Table 8).

Table 2 Chromosome types, chromosome length and karyotype formula of *Lathyrus sativus L.* cv. Nirmal (2n = 14)

Chromosome pairs	Long arm (µm)	Short arm (µm)	Total chromosome length (µm)	Centromeric Index	Centromeric Position	Karyotype formula
I	6.86	6.29	13.15	47.83 and 13.67	nm^{Sat}	$2^{sat}_{nm} + 8_{nm} + 2_{nsm} + 2_{m}$
II	6.46	4.41	10.87	40.60	nm	
III	6.70	3.92	10.62	36.92	nsm	
IV	5.39	4.66	10.05	46.34	nm	
V	6.05	3.68	9.73	37.81	nm	
VI	4.74	4.00	8.74	45.76	nm	
VII	4.33	4.33	8.66	50	m	

nm nearly median, *nsm* nearly sub median, nm^{Sat} nearly median with satellite, *m* median

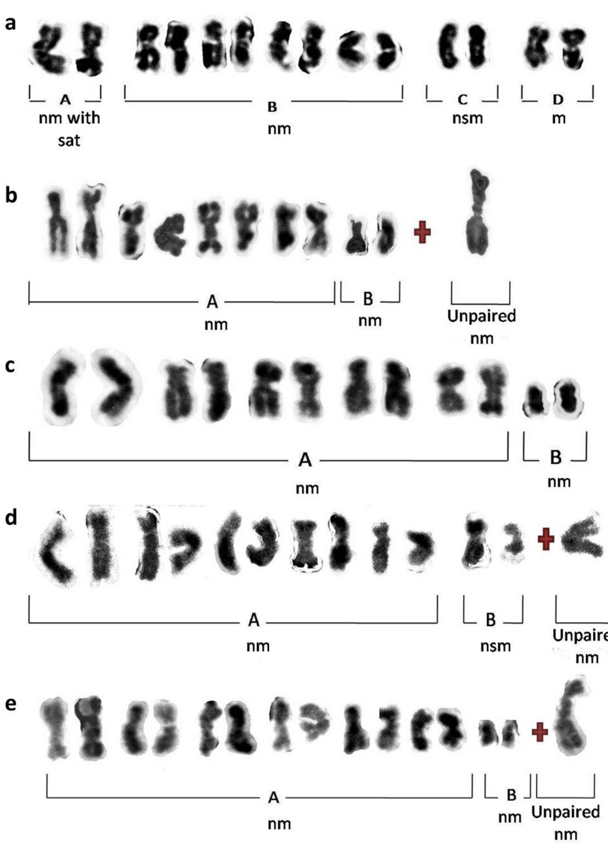

Fig. 2 Karyotypes of cv. Nirmal: **a** normal karyotype (2n = 14), **b** karyotype of 11 chromosomes with one unpaired long chromosome, **c** karyotype of 12 chromosomes with one pair small chromosome, **d** karyotype of 13 chromosomes with one unpaired long chromosome, and **e** karyotype of 15 chromosomes with one pair of small chromosome and one extra unpaired long chromosome. *Scale Bar = 8.26 µm

The most interesting character is that karyotype though symmetrical but indicating transition between symmetrical to asymmetrical and is slightly bimodal (Fig. 5b). This type has been observed nearly 12% of total metaphase plate studied. Out of six nearly median pair of chromosome, 5 pairs are comparatively long chromosomes and 1 pair is very small chromosome (6.33 µm). Figure 6b shows the ideogram. This form of karyotype is observed in 8.6% of metaphase plate studied.

3. *Variation type II* The chromosomes (Fig. 4d) of this cultivar consist of seven pairs of nearly median chromosomes. So, karyotypes (Fig. 5c) have a predominance of nearly median chromosomes. Chromosome size ranges from 11.30 to 15.44 µm (Table 9). One long pair of nearly median chromosome (15.44 µm) has a satellite connected either to long arm (Fig. 6c) or short arm (Fig. 6d). Karyotype is symmetrical. This type has been observed on 8% of total observation.

cv. Ratan (Bio L 212) The chromosomes (Fig. 7b) of this cultivar consist of four pairs of nearly median chromosomes, two pairs of nearly submedian chromosomes and one pair nearly sub terminal. Chromosome size ranges from 7.74 to 15.48 µm (Table 10). Satellites were not visible properly. The karyotypes (Fig. 7c) of this variety have a predominance of nearly median chromosomes. No chromosomal mosaicism is found. Centromeric region of four pairs of chromosomes is unusually extended. In general, karyotype is symmetrical which is reflected in the idiogram (Fig. 7d).

Table 3 Chromosome types, chromosome length and karyotype formula of *Lathyrus sativus L.* cv. Nirmal (2n = 11)

Chromosome pairs	Long arm (μm)	Short arm (μm)	Total chromosome length (μm)	Centromeric index	Centromeric position	Karyotype formula
I	10.30	8.09	18.39	43.99	nm	$10_{nm} + 1_{nm}$ (unpaired and extra)
II	8.75	8.66	17.41	49.74	nm	
III	7.60	5.88	13.48	43.62	nm	
IV	5.96	5.80	11.76	49.31	nm	
V	5.80	4.82	10.62	45.38	nm	
VI (unpaired)	12.43	9.48	21.91	43.26	nm	

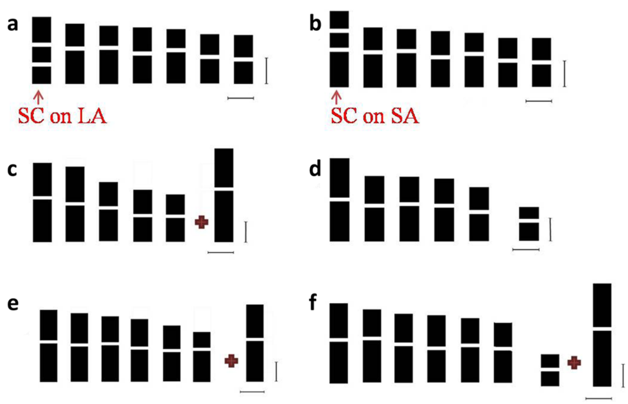

Fig. 3 Idiograms of cv. Nirmal: **a** secondary constriction (SC) on the long arm (LA), **b** secondary constriction (SC) on the short arm (SA), **c** presence of one unpaired long chromosome, **d** presence of one pair of small chromosome, **e** presence of one unpaired long chromosome, **f** presence of one pair of small chromosome and one extra unpaired long chromosome

Discussion

The chromosome number of three cultivars studied was found as $2n = 14$ which is coincident with the number published in previous reports. In general, the karyotypes of three cultivars of *Lathyrus* are apparently symmetrical, with predominance of nearly metacentric chromosomes. In spite of that, several interesting observations in different cultivars emerged. In cv. Nirmal (B1) chromosomal mosaicism were observed. There are several evidences and reports on chromosomal mosaicism in plants [31, 59, 60]. Such reports have pointed out [40] that sub diploid variations are not only caused by environmentally controlled mitotic abnormalities, but also certain gene combinations may induce and bring about this behaviour in somatic tissues. Later on, it was well established that this numerical mosaicism of chromosomes reported in some important crop plants such as wheat and tobacco is genetically controlled in some biotypes [31, 33, 34, 56]. Since the chromosomal mosaicism was transmitted to progeny plants, the mosaicism were considered definitely to be genetically controlled [33]. It is also stated that variation in chromosome number in somatic cells within individual plants is possibly controlled by genetic factors [28], which result in spindle abnormalities, chromosome degradation and minute chromosomes. The mosaicism is an important trait for long term survival of some species and enables a population to adapt to new conditions brought by environmental change [34]. Slight pollen polymorphism (16.12%) for pollen shape and size has been identified in cv.

Table 4 Chromosome types, chromosome length and karyotype formula of *Lathyrus sativus L.* cv. Nirmal (2n = 12)

Chromosome pairs	Long arm (μm)	Short arm (μm)	Total chromosome length (μm)	Centromeric index	Centromeric position	Karyotype formula
I	8.59	8.51	17.10	49.76	nm	6_{nm}
II	7.25	6.00	13.25	45.28	nm	
III	7.59	5.50	13.09	42.01	nm	
IV	7.25	5.42	12.67	42.77	nm	
V	5.84	4.92	10.76	45.72	nm	
VI	3.92	2.50	6.42	38.94	nm	

Table 5 Chromosome types, chromosome length and karyotype formula of *Lathyrus sativus L.* cv. Nirmal (2n = 13)

Chromosome no.	Long arm (μm)	Short arm (μm)	Total chromosome length (μm)	Centromeric index	Centromeric position	Karyotype formula
I	10.02	7.93	17.95	44.17	nm	$5_{nm} + 1_{nsm} + 1_{nm}$ (unpaired and extra)
II	9.14	7.85	16.99	46.20	nm	
III	9.46	6.73	16.19	41.56	nm	
IV	8.34	7.13	15.47	46.08	nm	
V	7.61	6.09	13.70	44.45	nm	
VI	8.09	4.08	12.17	33.52	nsm	
VII (unpaired)	10.58	8.66	19.24	45.01	nm	

Table 6 Chromosome types, chromosome length and karyotype formula of *Lathyrus sativus* L. cv. Nirmal (2n = 15)

Chromosome pairs	Long arm (μm)	Short arm (μm)	Total chromosome length (μm)	Centromeric index	Centromeric position	Karyotype formula
I	9.05	7.70	16.75	45.97	nm	1_{nm} (unpaired and extra)
II	9.21	5.95	15.16	39.24	nm	
III	7.94	6.35	14.29	44.43	nm	
IV	7.06	6.83	13.89	49.17	nm	
V	7.62	5.63	13.25	42.49	nm	
VI	6.98	5.32	12.30	43.25	nm	
VII	3.17	3.01	6.18	48.71	nm	
VIII (unpaired)	12.15	9.60	21.75	44.14	nm	

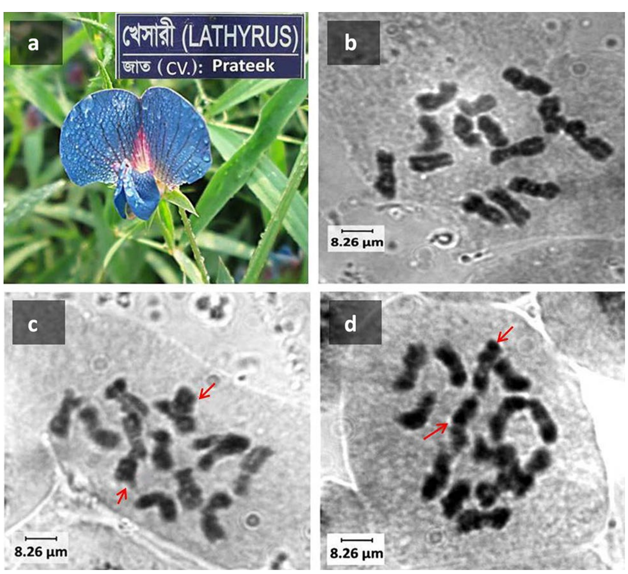

Fig. 4 Flower and somatic chromosomes of cv. Prateek: **a** Flower, **b** photomicrograph of normal somatic metaphase chromosomes (2n = 14), **c** normal type (2n = 14) with one pair of small chromosome (red arrow marked), **d** 2n = 14 chromosomes with one pair of satellite chromosome (red arrow marked). *Scale Bar = 8.26 μm

Nirmal which exhibits aneusomaty. Several numerical variations of chromosome may be the possible reason for pollen polymorphism [28].

The cv. Ratan (Bio L 212) shows that the region of centromere of 4 pairs of chromosomes appeared unusually 'extended'. The presence of extended primary constriction of some chromosomes clearly seen in metaphase plate is in agreement with the work of molecular cyto-geneticists [32] on some legume genera. The nature of the 'extended' centromeres may be retrotransposon effect which is predominant in centromeric region. However, it needs further investigation with improved staining techniques. These were not referred in other studies on these species [27, 31, 58]. All such previous studies showed that an extensive band of heterochromatin is closely associated with the centromere of all or some chromosomes in these species. Centromeres located at the primary constriction of the chromosome typically contain tandem repeat sequences. Long-terminal repeat (LTR) retrotransposons, also known as centromeric retrotransposons (CRs), are often intermingled with tandem repeats and are enriched in plant centromeric regions. Indeed, *ogre* LTR retrotransposons are widespread in legume

Table 7 Chromosome types, chromosome length and karyotype formula of *Lathyrus sativus* L. cv. Prateek (2n = 14)

Chromosome pairs	Long arm (µm)	Short arm (µm)	Total chromosome length (µm)	Centromeric index	Centromeric position	Karyotype formula
I	8.50	7.52	16.02	46.94	nm	$12_{nm}+2_{nsm}$
II	7.85	7.44	15.29	48.66	nm	
III	7.44	6.31	13.75	45.89	nm	
IV	6.55	5.42	11.97	45.28	nm	
V	7.20	4.61	11.81	39.03	nm	
VI	5.99	5.18	11.17	46.37	nm	
VII	7.69	2.83	10.52	26.90	nsm	

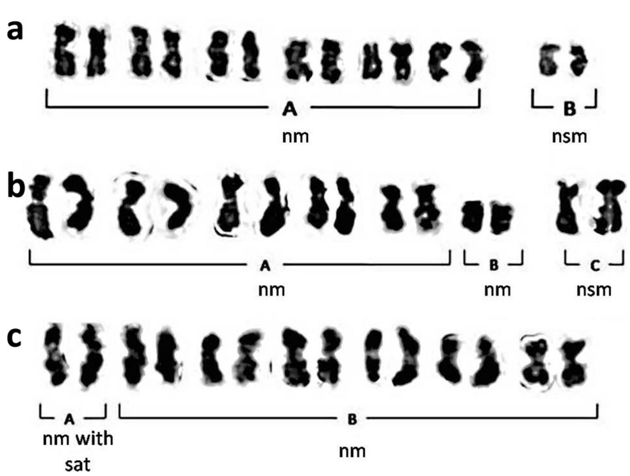

Fig. 5 Karyotypes of cv. Prateek: **a** normal (2n = 14) karyotype, **b** karyotype with five pairs of nearly median chromosomes, one pair small nearly median chromosome and one pair of nearly submedian chromosomes, **c** karyotype with seven pairs of nearly median chromosomes and one pair of satellite chromosome

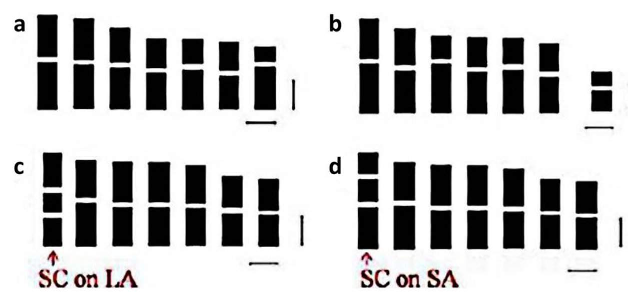

Fig. 6 Idiograms of cv. Prateek: **a** normal ideogram, **b** ideogram with one small chromosome, **c**, **d** idiogram showing one long pair of chromosome with a satellite connected either to long arm or short arm (red arrow marked)

plants [7]. Heterochromatic centromeric regions accumulate significantly more transposable elements than the euchromatic regions [30]. In spite of their abundance, the majority of them is silenced and rarely transposed. However, most of the LTR retrotransposons in plant genomes are accumulated in gene-poor centromeric regions, resulting in proliferation of these elements with less harmful effects to the gene-rich euchromatin regions [36, 47, 48]. Proliferation and transposition of some centromeric retrotransposons in the centromeric region may be the one of the causes of formation of chromosome with extended centromere which could be considered as marker chromosome of the cultivars such as Ratan.

Lathyrus is characterized by symmetrical karyotypes [61] in general. Transition towards asymmetry, bimodality as well as presence of a pair of small chromosomes was

Table 8 Chromosome types, chromosome length and karyotype formula of *Lathyrus sativus L.* cv. Prateek (2n = 14)

Chromosome pairs	Long arm (µm)	Short arm (µm)	Total chromosome length (µm)	Centromeric index	Centromeric position	Karyotype formula
I	9.17	7.42	16.59	44.73	nm	$12_{nm} + 2_{nsm}$
II	7.92	6.84	14.76	46.34	nm	
III	9.09	4.33	13.42	32.27	nsm	
IV	7.67	5.58	13.25	42.11	nm	
V	8.17	5.00	13.17	37.97	nm	
VI	6.75	5.25	12.00	43.75	nm	
VII	3.83	2.50	6.33	39.49	nm	

Table 9 Chromosome types, chromosome length and karyotype formula of *Lathyrus sativus* L. cv. Prateek (2n = 14)

Chromosome pairs	Long arm (µm)	Short arm (µm)	Total chromosome length (µm)	Centromeric index	Centromeric position	Karyotype formula
I	7.76	7.68	15.44	49.74 and 08.57	nm^{Sat}	$2^{sat}_{nm} + 12_{nm}$
II	8.01	6.60	14.61	45.17	nm	
III	7.10	7.02	14.12	49.72	nm	
IV	7.10	6.93	14.03	49.39	nm	
V	6.85	6.60	13.45	49.07	nm	
VI	6.11	5.61	11.72	47.87	nm	
VII	5.69	5.61	11.30	49.65	nm	

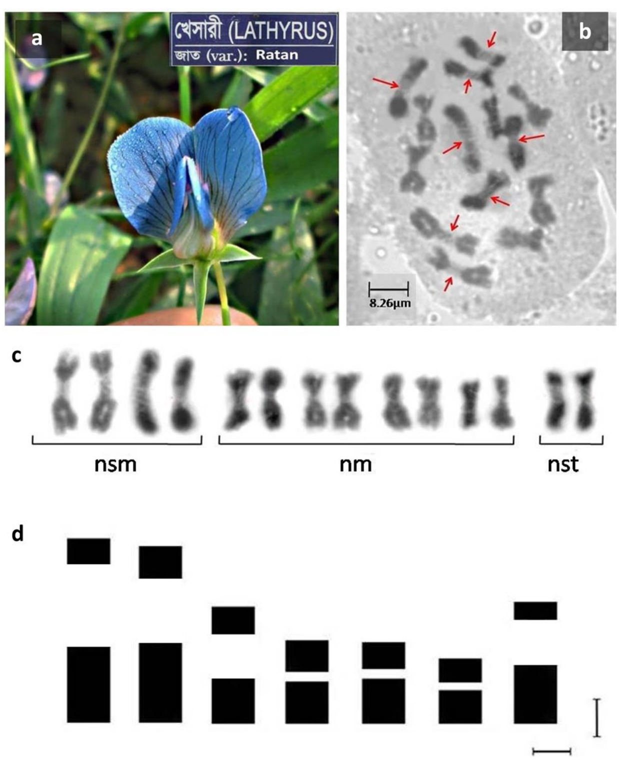

Fig. 7 Flower and somatic chromosomes of cv. Ratan: **a** flower, **b** somatic chromosomes (2n = 14) with extended centromeres (red arrow marked). **c** normal karyotype, **d** idiogram showing 4 chromosomes with extended centromere. *Scale Bar = 8.26 µm

observed in the cv. Prateek and cv. Nirmal (B1). Differences in karyotypes found among two cultivars of *Lathyrus* reveal that structural alterations of chromosomes may have contributed exclusively to the karyotypic divergence of such cultivars. Our results suggest that during this diversification of these cultivars, a recurring series of changes toward symmetry and asymmetry may have happened as has been found out for different groups of angiosperms [23, 53, 55].

One of the more useful markers for karyotype analysis is the secondary constriction [9]. In the karyotype of cv. Nirmal (B1) and cv. Prateek, the secondary constriction carrying macrosatellite is proximal on the short arm; whereas in some other metaphase plates, the same constriction with terminal macrosatellite is on the long arm of bigger nearly submedian chromosome of pair one. We suggest that the differences in satellite position arose by karyotype rearrangements, probably involving translocations. Our observations on the presence of one pair of macrosatellited chromosomes are in agreement with the karyotype of this species reported by Sahin et al. [41]. Furthermore, the presence of one large chromosome in cv. Nirmal with aneuploid number e.g. 2n − 3 (Fig. 2b), 2n − 2 (Fig. 2c), 2n − 1(Fig. 2d) and 2n + 1 (Fig. 2e) may be due to structural changes of chromosomes or the effect of retrotransposons in these same chromosome at early developmental stage.

Table 10 Chromosome types, chromosome length and karyotype formula of *Lathyrus sativus* L. cv. Ratan (2n = 14)

Chromosome pairs total	Long arm (μm)	Short arm (μm)	chromosome length (μm)	Centromeric index	Centromeric position	Karyotype formula
I	10.97	4.51	15.48	27.27	nsm	$8_{nm} + 4_{nsm} + 2_{nst}$
II	10.32	3.87	14.19	29.16	nsm	
III	6.45	4.00	10.45	38.27	nm	
IV	8.00	2.45	10.45	23.45	nst	
V	5.67	4.25	9.93	37.33	nm	
VI	6.06	3.61	9.67	42.85	nm	
VII	4.51	3.22	7.74	41.66	nm	

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Author contributions All authors contributed equally to this paper. TP, AM and MM performed the cytological work; AG performed the karyotype analysis and their interpretations; KKD contributed in design, analyses of the results and writing the paper.

Compliance with ethical standards

Conflict of interest The authors declare they have no conflict of interest.

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