



NUCLEAR PHENOTYPE AND KARYOTYPE ANALYSIS IN TWO VARIETIES OF *LATHYRUS SATIVUS* L.

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Abstract

Lathyrus sativus known as grass pea belongs to the family of Fabaceae commonly grown for human consumption and livestock feed throughout the world. This study based on the morphological features of the basic chromosome numbers and numerical characterization of the karyotypes using total chromosome length (TCL), arm ratio and total fraction frequency. Outcome of BARI Khesari-2 is more effective than BARI Khesari-3 on the basis of mitotic index, nuclear volume and interphase chromosome volume. Whereas, mitotic index directly relevant to cell division; when MI high than cell division rate will be high. The two varieties (BARI Khesari-2 and BARI Khesari-3) were found to possess $2n = 14$ chromosomes. Karyotype analysis is an essential tool for studying the genetic constitution of any organism. In this investigation, we found the karyotype formula of BARI Khesari-2 is $7L^{4m+2sm+1st}$ and BARI Khesari-3 is $7L^{2m+3sm+2st}$. Chromosome morphology showed a variation in the chromosome length, chromosome structure and centromere position of the accessions. Presence of sub-terminal chromosome in both varieties shows asymmetric karyotype which indicates advancedness. The variation in *L. sativus* may be regarded as having evolution and adaptive significance.

Key words: Karyotype analysis, *Lathyrus sativus*, mitotic index, nuclear phenotype.

Introduction

Lathyrus sativus L. belongs to the family Fabaceae consisting about 187 annual and perennial species Basaran et al. (2010). It is one of the most important food legumes and known as Grass pea, Blue Sweet pea, Chickling vetch or white vetch in English. It is known as Khesari in Bangladesh. In the Indian sub-continent, Nezamuddin (1970) reported that two types of grass pea one of which is poisonous (dark colored seeds) and another one is non-poisonous (yellow-colored seeds). *L. sativus* can fix atmospheric nitrogen as it is a legume crop and it fits very well into a lengthy sustainable farming system Campbell (1997). The over consumption of this crop causes constant paralyze of lower limbs which is known as neurolathyrisms because of the existence of neurotoxin called ODAP (β -N-oxalyl-L- α , β -diaminopropionic acid). As an important crop scientist are paying attention to improve this plant and that's why knowledge about its genetic constituents should be known. Different cytological observations have shown that the basic chromosome number of $x = 7$ constantly throughout the genus and that the most of the species are diploid with rare polyploids (Senn 1938, Yamamoto et al. 1984, Battistin and Fernandez 1994, Klamt and Schifino-Wittmann 2000). Akter et al. (2005) characterized three varieties of *Lathyrus sativus* L. released by BARI by fluorescent karyotype and RAPD

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analysis. However, comparison of nuclear phenotype was not done before. Therefore, the present study deals the determination of nuclear phenotype and to determine the similarities and differences between karyotype of two varieties of *Lathyrus sativus* L. released by BARI in Bangladesh.

Materials and Methods

The experiment was conducted with moisture free seeds of the two varieties of *Lathyrus sativus* L. Those two varieties (BARI Khesari-2 and BARI Khesari-3) were collected from Pulse Research Center, Ishwardi, Pabna, Bangladesh. Seeds were germinated in petri dishes with moist filter paper at room temperature. When the root tips were grown up to 1-1.5 cm in length, these were collected by fine forceps and pre-treated in saturated solution of para-di-chlorobenzene (PDB) for 4-4.30 hours at 11:00 am to 12.30 pm. Then the root tips were fixed in 1:3 aceto-alcohols at room temperature. After 48 hours of fixation root tips were preserved in 70% ethanol and stored in the refrigerator until they were used in the laboratory. Root tip cells of *Lathyrus sativus* L. were examined and the temporary slides were prepared by haematoxylin method following the

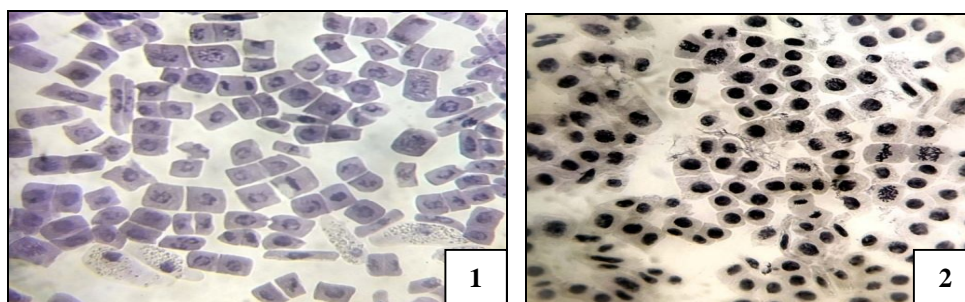
Haque et al. (1976). The mitotic index, $MI = \frac{\text{No. of dividing cells}}{\text{No. of total cells}} \times 100$, Nuclear Volume (NV) =

$\frac{4}{3} [\pi r^3]$ Interphase chromosome volume (ICV = $\frac{NV}{2n \text{ no. of chromosome in somatic cell}}$ were calculated as

described by Fiskesjo (1993), Nayar et al. (1971). Two karyotype analysis were either measured as long arm (L) and short arm (S) lengths, or calculated as total length of chromosomes (TL), arm ratio (AR: L/S) r-value (S/L). Idiograms were drawn from mean values, and chromosome types were determined, using Levan et al. (1964) formula. To make karyotype analysis conventionally centromeric position and chromatin length of chromosomes were determined as followed by Kutarekar and Wanjari (1983).

Results

Determination of mitotic index, nuclear volume and interphase chromosome volume of two varieties of *Lathyrus sativus* L. in the present study represents nuclear phenotype. In this study, two varieties are showed mean values for as mitotic index 17.93% (BARI Khesari-2) and 15.56% (BARI Khesari-3) and these are shown in Table 1, Figs. 1 and 2. These two varieties revealed nuclear volume with mean values of $92.23 \pm 0.704 \mu^3$ (BARI Khesari-2) and $82.40 \pm 0.415 \mu^3$, (BARI Khesari-3). Interphase Chromosome Volume (ICV) was found with mean values of $6.58 \pm 0.050 \mu^3$, (BARI Khesari-2) and $5.88 \pm 0.030 \mu^3$ (BARI Khesari-3).



Figs. 1 and 2: Mitotic index of *Lathyrus sativus* L. (1 = BARI Khesari-2 and 2 = BARI Khesari-3).

Table 1. Mean values of mitotic index, nuclear volume and interphase chromosome volume in two varieties of *Lathyrus sativus*.

Types	Chromosome number (2n)	Mitotic index ($\bar{X} \pm SE$) %	Nuclear volume ($\bar{X} \pm SE$) μ^3	Interphase chromosome volume ($\bar{X} \pm SE$) μ^3
BARI Khesari-2	14	17.93±0.287	92.23±0.704	6.58±0.050
BARI Khesari-3	14	15.56±0.254	82.40±0.415	5.88±0.030

Karyotype analysis

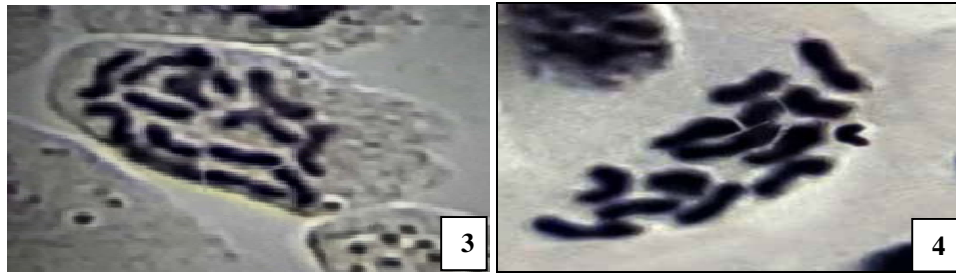
Chromosomes number in BARI Khesari-2 was found to be $2n = 14$ (Figs. 3 and 4). The chromosomes complement was made up of four pairs of metacentric (I, II, VI, VII), two pairs of sub-metacentric (IV, V), one pair of sub-terminal (III) chromosomes. The largest chromosomes were $10.31 \pm 0.28 \mu\text{m}$ and the shortest chromosome was $6.58 \pm 0.58 \mu\text{m}$ in length with a TCL of $57.87 \mu\text{m}$. TF% was found to be 41.57 (Table 2). The karyotype formula (K.F) was $7L^{4m+2sm+1st}$.

Chromosomes number in BARI Khesari-3 was found to be $2n = 14$. The chromosomes complement was made up of two pairs of metacentric (IV, VI), three pairs of sub-metacentric (II, III, V), two pair of sub-terminal (I, VII) chromosomes. The largest chromosomes were $10.39 \pm 0.35 \mu\text{m}$ and the shortest chromosome was $6.80 \pm 0.57 \mu\text{m}$ in length with a TCL of $61.19 \mu\text{m}$. TF% was found to be 39.61 (Table 2). The karyotype formula (K.F) was $7L^{2m+3sm+2st}$.

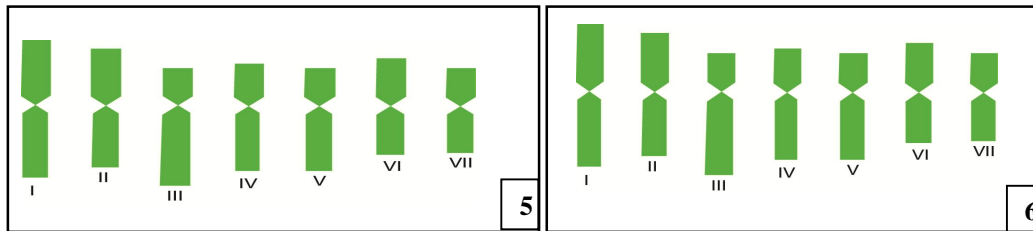
Table 2. Range of chromosome length, total chromatin length (TCL), TF% karyotype formula (K.F) in two varieties of *Lathyrus sativus* L.

Name of species	Number of chromosome (2n)	Range of chromosome Length (μm)	Total chromatin length (TCL) (μm)	TF%	Karyotype formula (K.F)
BARI Khesari-2	14	6.58-10.31	57.44	41.57	$7L^{4m+2sm+1st}$
BARI Khesari-3	14	6.80-10.39	61.19	39.61	$7L^{2m+3sm+2st}$

L = large, m= metacentric, sm= sub-metacentric, st= sub-terminal.



Figs. 3 & 4: Mitotic metaphase chromosomes of two varieties. 3 = BARI Khesari-2 and 4 = BARI Khesari-3.



Figs. 5 & 6: Idiogram of mitotic metaphase haploid chromosomes of *Lathyrus sativus* L. 5 = BARI Khesari-2 and 6 = BARI Khesari-3.

Discussion

Mitotic index refers to the ratio of no of cells in the dividing phase to the total number of cells observed. In this study, the highest mitotic index was observed in BARI Khesari-2 which indicates the high division rate compared to that of another one. It can be said that mitotic index is directly proportional to high division rate of meristematic cell. In this present investigation mitotic index is higher BARI Khesari-2 than in BARI Khesari-3. The size of nucleus was found to be relatively compared two varieties, in this present investigation the nuclear volume and interphase chromosome volume mean values of BARI Khesari-2 larger than BARI Khesari-3. Since, the nucleolar size is a reliable refector of nuclear activity and RNA Sen and Bhattacharya, (1979) such decrease may be correlated to the mitostatic activity of the cells. Interphase nuclear phenotype and chromosomal characterization are very useful cytogenetic parameters for distinguishing cytotypes, accessions and even germplasm of a plant species Huang et al. (2014).

The somatic chromosome numbers of the two varieties of *Lathyrus sativus* L. were found to possess $2n = 14$ Figs. 3 and 4. Similar $2n$ chromosome number was reported earlier by Akter et al. (2005) and Ozcan et al. (2006). The diploid chromosome number of two varieties of *L. sativus* L. (BARI Khesari-2, BARI Khesari-3) which released by BARI correlated with the earlier reports. Somatic chromosome number of *Lathyrus pratensis* L. and *L. palustris* L. subsp. *Palustris* was $2n = 28$ and $2n = 42$, respectively which was reported by Darlington and Wylie (1995). This result indicated tetraploidy and hexaploidy of two *Lathyrus* sp., respectively. Nevertheless, there was no tetraploid or hexaploid in Khesari varieties released by BARI.

The karyotype analysis was made from three metaphase plates of two varieties. Differences were observed in chromosome length, Total Chromatin Length (TCL) between two varieties of *L. sativus* L. (BARI Khesari-2, BARI Khesari-3). Longest chromosome ($10.39 \mu\text{m}$) was observed in BARI Khesari-3 and shortest ($6.58 \mu\text{m}$)

chromosome in BARI Khesari-2. Maximum chromatin length of the diploid complement (61.19 μm) was found in BARI Khesari-3 and minimum (57.44 μm) in the BARI Khesari-2. The widest range of arm ratio (0.43-0.91) was found in the BARI Khesari-2 and the smallest range (0.46-0.81) was found in the BARI Khesari-3 (Figs. 3 and 4; and Table 2).

Significant differences in TCL within varieties of *Lathyrus sativus* L. were found by Verma and Ohri (1979) considering different age of root meristem, which is an important factor for variation. They stated that difference was happened because TCL were affected by age of the root, variation of treatments and other unidentified experimental conditions. Nevertheless, in this present investigation we don't consider such factors to ascertain the varietal differences. Many researchers found secondary constriction and satellite chromosomes in different varieties of *Lathyrus sativus* L. For example, Bhattacharjee (1954) found two pairs of chromosomes with secondary constriction and a pair with satellite, on the contrary Srivastava and Naithani (1964) indicated four pair of chromosomes with secondary constrictions in *Lathyrus sativus*. Roy and Singh (1967) indicated only one pair of chromosomes with secondary constrictions in 6 strains of *L. sativus* L., and Fouzder and Tandon (1975) observed one SAT pair in only one of the five strains studied by them. But in our investigation, we do not find any secondary constriction or SAT chromosome. In this present investigation, we found the karyotype formula of BARI Khesari-2 is $4L^m + 2L^{sm} + L^{st}$ and BARI Khesari-3 is $2L^m + 3L^{sm} + 2L^{st}$. We found metacentric chromosomes (m), sub-metacentric chromosomes (sm) and also sub-telocentric chromosomes (st) in both varieties of BARI Khesari-2 and BARI Khesari-3. This result agreed with Ghasem et al. (2011). He also found sub-telocentric chromosome (st) chromosomes in *L. sativus* accessions. This type of karyotype is called asymmetrical karyotype. Badr et al. (1987) found symmetrical karyotype in *L. sativus* due to the evolution of this species that kind differences might be caused. Chandola and Jain (1979) Start that as the evolution proceeds a species chromosome length may decreased and turn from symmetrical to asymmetrical form during the course of evolution. In these two BARI varieties there was a progressive decrease in the length of individual chromosome.

The above discussion indicated the occurrence of asymmetric karyotype in these two varieties. According to Stebbins (1950) the asymmetrical karyotypes are supposed to be more advanced than the symmetrical ones. As the karyotype of two varieties of *Lathyrus sativus* L. were found to be asymmetric so these varieties may be regarded as advanced. Due to the presence of 1 and 2 sub-terminal chromosomes, respectively in BARI Khesari-2 and BARI khesari-3 this might be showing advances.

Conclusion

The study based on the morphological features of the basic chromosome numbers and numerical characterization of the karyotypes using total chromosome length, arm ratio and total fraction frequency. Outcome of BARI Khesari-2 is more effective than BARI Khesari-3 on the basis of mitotic, nuclear volume and interphase chromosome volume. From this study it may be concluded that the mitotic index directly relevant to cell division; when MI high than cell division rate will be high.

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Conflict of interest: The authors declare that there is no competing interest.

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