J. Bio-Sci. 32(1): 13-19, 2024 ISSN 1023-8654
http://www.banglajol.info/index.php/JBS/index
DOI: doi.org/10.3329/jbs.v32i1.74984

INTERPHASE NUCLEAR STRUCTURE AND HETEROCHROMATIN PERCENTAGES IN SOMATIC CELLS ON TEN CULTIVARS OF *LABLAB PURPUREUS* L.

Md. Saifur Rahman[1,2], Ummay Salma Pritha[1], Priya Khatun[1], Md. Abdul Bari[1], Md. Mamunur Rashid Sarkar[1], Mosleh Ud-deen[2*] and Golam Kabir[1]

[1]*Professor Sultanul Alam Cytogenetics Laboratory, Department of Botany, University of Rajshahi, Rajshahi-6205, Bangladesh*
[2]*Department of Crop Science and Technology, University of Rajshahi, Rajshahi-6205 Bangladesh*

Abstract

Mitotic index of *Lablab purpureus* was found highest in BARI Sheem-6 and lowest in BARI Sheem-1. A high mitotic index value shows the higher growth patterns of plants than others. The interphase nuclear structure analysis showed differences in the organization and distribution of chromatin within the nuclei of the cultivars. Percentages of heterochromatin were found highest in BARI Sheem-5 and lowest in BARI Sheem-3 which may be considered as more primitive and more advance in nature, respectively. Some cultivars exhibited a more condensed and organized nuclear structure, while others displayed a more dispersed and irregular arrangement of chromatin. Furthermore, the measurement of heterochromatin percentage indicated significant variations among the cultivars. Heterochromatin, which is associated with the gene silencing and genome stability, ranged from low to high percentages in different cultivars. This suggests potential differences in gene expression and genomic stability among the 10 cultivars of *L. purpureus*. Overall, the study highlights the existence of genetic diversity in *L. purpureus* cultivars, as evidenced by variations in interphase nuclear structure and heterochromatin percentage. These findings contribute to a better understanding of the genetic and epigenetic factors that influence the phenotypic characteristics agronomic performance of *L. purpureus*.

Key words: Chromocenter, heterochromatin, *Lablab purpureus*, mitotic index, nuclear structure.

Introduction

Lablab purpureus L. known as country Bean are members of Fabaceae family (Rai et al. 2010). Plants of this species have some medicinal and ethno-botanical importance. The plant is anticholesterolemic, antidotal, antivinous, carminative, and hypoglycaemic. It has been widely distributed in many tropical and subtropical countries covering South and Central America, the East and West Indies, Africa, Asia, China, India, and Bangladesh as an annual or a short-lived perennial (Whyte et al. 1953). This species is rich in minerals and vitamins (Basu et al. 2002). It is one of the most diversified legume crops domesticated to use as pulse, vegetable (green bean, pod, and leaf part), green manure, and ornamental crops (Maass et al. 2010). The crop also appears to be used for medicinal use in pharmaceutical or nutraceutical sectors (Morris 2009).

*Author for correspondence: uddeenm@yahoo.com

Country bean is one of the most popular vegetables in Bangladesh. It is one of the diverse annual legume crops in tropical and subtropical regions worldwide (Smykal et al. 2015).

It is well known that, heterochromatic and euchromatic domain, each with a unique set of nuclear function, are organized in a complicated way in the eukaryotic nucleus. In multicellular organisms somatic cells are genetically identical but they might have radically different nuclear structures and gene expression profiles. The interphase nuclear structure refers to the organization and arrangement of chromatin within the nucleus during the non-diving phase of the cell cycle. It plays a crucial role in gene expression, DNA replication and overall cellular function. The interphase nucleus consists of euchromatin, which is loosely packed and transcriptionally active, heterochromatin, which is densely packed and transcriptionally inactive. Heterochromatin percentage refers to the proportion of the nucleus occupied by heterochromatin. It can vary among different cell types, developmental stages and even within different cultivars of the same species. The nuclear structure and heterochromatin percentages among ten cultivars of *L. purpureus* L. can provide valuable insights into their genetic diversity and potential for future breeding efforts.

The present study deals the determination of mitotic index, interphase nuclear structure and percentage of heterochromatin (%) on ten cultivars of *L. purpureus* L. The major objectives of the study are- i) to observe the mitotic index, nuclear volume and interphase chromosome volume on ten cultivars of *L. purpureus* L., and ii) to observe the chromocenter number and heterochromatin estimation on ten cultivars of *L. purpureus* L.

Material and Methods

Seeds from ten different cultivars *L. purpureus* BARI Sheem-1, BARI Sheem-2, BARI Sheem-3, BARI Sheem-4, BARI Sheem-5, BARI Sheem-6, BARI Sheem-7, BARI Sheem-8, BARI Sheem-9 and BARI Sheem-10) were placed on petri dish with damp tissue paper to germinate, and it took about two to three days. About 1.5 to 2 cm long root tips were collected, preserved in 1:3 aceto-alcohol for 48 hours, and then stored in 70% ethanol in the refrigerator for later use. According to the Haque et al. (1976) method, temporary slides were created for all parameters. Mitotic index was recorded from the meristematic cells of the root tips of different cultivars of *L. purpureus.* Using temporary slides, an oculometer measured the nuclear volume of meristematic and developed cells. To determine the nuclear volume and interphase chromosome volume of ten cultivars (BARI Sheem-1, BARI Sheem-2, BARI Sheem-3, BARI Sheem-4, BARI Sheem-5, BARI Sheem-6, BARI Sheem-7, BARI Sheem-8, BARI Sheem-9 and BARI Sheem-10) of *L. purpureus* L. we followed different methods. Using the formulas provided by Fiskesjo (1993) and Nayar et al. (1971), the mitotic index, nuclear volume, and interphase chromosomal volume were determined. To observe chromocenter number and estimate the percentage of heterochromatin, various methods were used. Ten nuclei of each kind were viewed under a microscope using the planimetry approach, and their predicted chromocenters were taken from the prepared slide. According to Nagl and Fusenig (1979), chromocenters combine to form heterochromatin.

Results

The highest (12.05±0.23%) and lowest (7.64±0.23%) mitotic index was found in BARI Sheem-6 and BARI Sheem-1, respectively (Table 1).

Table 1: Mean values of Mitotic Index, Nuclear Volume and Interphase Chromosome Volume on ten cultivars of *L. purpureus* L.

Variety	Mitotic index (%) (X±S.E)	Nuclear volume (μ^3) (X±S.E)	Interphase chromosome volume (μ^3) (X±S.E)
BARI Sheem-1	7.64±0.23	12.39±0.20	0.56±0.27
BARI Sheem-2	9.85±0.59	13.25±0.27	0.60±0.13
BARI Sheem-3	9.36±0.73	10.75±0.23	0.49±0.35
BARI Sheem-4	9.02±0.31	13.72±0.33	0.62±0.22
BARI Sheem-5	8.47±0.27	13.90±0.46	0.63±0.28
BARI Sheem-6	12.05±0.23	14.50±0.17	0.66±0.23
BARI Sheem-7	10.09±0.17	11.90±0.36	0.54±0.35
BARI Sheem-8	12.02±0.29	12.55±0.38	0.57±0.35
BARI Sheem-9	10.45±0.13	15.70±0.72	0.71±0.34
BARI Sheem-10	9.12±0.21	13.75±0.81	0.63±0.19

Nuclear volume

Data were recorded for nuclear volume from interphase stage of mitotic cell division in root tips. Among the ten cultivars of *L. purpureus*, the highest (15.70±0.72μ^3) and lowest (10.75±0.23μ^3) nuclear volume was found in BARI Sheem-9 and BARI Sheem-3 respectively (Table 1).

Interphase chromosome volume

The values for interphase chromosome volume in the meristematic cells in ten cultivars of *L. purpureus* were found to range from 0.470±0.35 μ^3-0.695±0.34 μ^3. Among the ten cultivars of *L. purpureus* the highest (0.71±0.34 μ^3) and lowest (0.49±0.35μ^3) interphase chromosome volume was found in BARI Sheem- 9 and BARI Sheem- 3, respectively (Table 1).

Chromocenter number and heterochromatin percentages

Chromosome number in each of the ten cultivars of *L. purpureus* L. species were reported to be 2n = 22 which was confirmed in the present investigation. In the present study all cultivars of *L. purpureus* shown a lower number of chromocenters compared to their respective diploid chromosome number. The root tips of ten cultivars exhibited different number of chromocenter in meristematic cells were found in the range from 13.50±0.14 to 16.50±0.41. It was observed that the chromocenter number in root tip of ten cultivars of *L.*

purpureus were less than their total chromosome number 2n = 22 (Table 2). As chromocenter corresponds to heterochromatin, the value for nuclear area and chromocenter area were used to determine the heterochromatin percentage. It was observed that in meristematic cell the highest (24.13±0.14%) and lowest (17.19±0.13%) heterochromatin content per nuclear area were found in BARI Sheem-5 and BARI Sheem-3 (Table 2, Fig. 1).

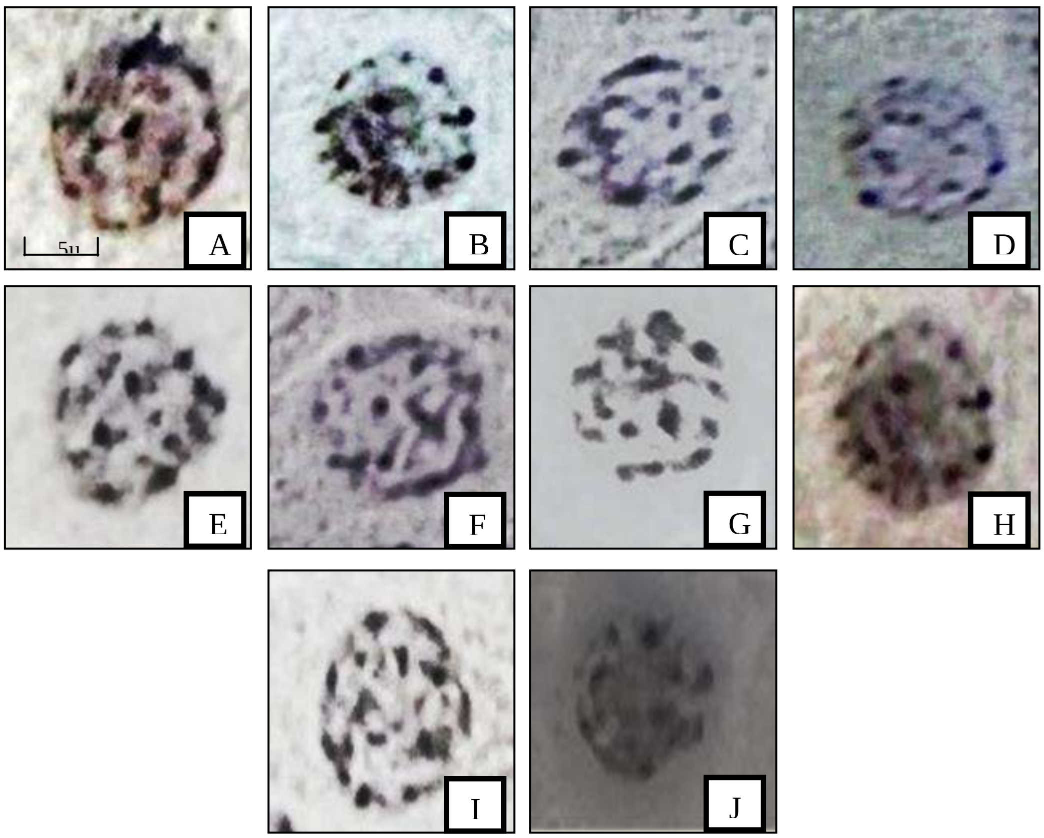

Fig. 1 (A-J). Photomicrograph of interphase nuclear structure and heterochromatin in meristematic cells on ten cultivars of *L. purpureus* L. (A: BARI Sheem-1, B: BARI Sheem-2, C: BARI Sheem-3, D: BARI Sheem-4, E: BARI Sheem-5, F: BARI Sheem-6, G: BARI Sheem-7, H: BARI Sheem-8, I: BARI Sheem-9 and J: BARI Sheem-10.

Table 2. Number of chromocenters and heterochromatin percentage per nuclear area in ten cultivars of *L. purpureus* L.

Variety	Chromosome number (2n)	Number of chromocenters (Mean±SE)	Hetrochromatin per nuclear area (%) (Mean±SE)
BARI Sheem-1	22	15.75±0.37	22.57±0.75
BARI Sheem-2		14.66±0.24	23.03±0.15
BARI Sheem-3		13.50±0.14	17.19±0.13
BARI Sheem-4		13.75±0.27	19.33±0.29
BARI Sheem-5		15.66±0.43	24.13±0.14
BARI Sheem-6		16.50±0.41	22.31±0.18
BARI Sheem-7		15.33±0.87	20.45±0.21
BARI Sheem-8		14.66±0.32	19.50±0.31
BARI Sheem-9		15.33±0.14	22.71±0.18
BARI Sheem-10		13.75±0.61	23.17±0.51

Discussion

Mitotic index was found highest 12.05±0.23% in BARI Sheem-6 and lowest 7.64±0.23% in BARI Sheem-1. A high mitotic index value shows the higher growth patterns of plants than others. According to Rahman et al. (2023) high mitotic index value indicates that the division rate is high and low mitotic index value indicates relatively lower divisional rate. The variation of those values could also vary on root collection time, environmental factors or their cellular activity.

In the present investigation, among the ten cultivars of *L. purpureus* the highest (15.70±0.72 μ^3) and lowest (10.75±0.23 μ^3) nuclear volume was found in BARI Sheem-9 and BARI Sheem-3, respectively. The values for interphase chromosome volume in the meristematic cells in ten cultivars of *L. purpureus* were found to range from 0.49±0.35 μ^3- 0.71±0.34 μ^3. Among the ten cultivars of *L. purpureus* the highest (0.71±0.34 μ^3) and lowest (0.49±0.35 μ^3) interphase chromosome volume was found in BARI Sheem- 9 and BARI Sheem-3, respectively (Table 2). The research findings suggest that, the nuclear volume and interphase chromosome volume do not correlate with the somatic chromosome number but may be related with environmental condition. More or less same result was examined by Yamakawa and Sparrow (1966) and Sarkar et al. (2022).

The chromocenter number on ten cultivars of *L. purpureus* L. seed i.e. BARI Sheem-1, BARI Sheem-2, BARI Sheem-3, BARI Sheem-4, BARI Sheem-5, BARI Sheem-6, BARI Sheem-7, BARI Sheem-8, BARI Sheem-9 and BARI Sheem-10 become more clear and distinct after disruption of euchromatin by 25% HCl, indicating their heterochromatin nature. In meristematic cells, heterochromatin percentage was highest in BARI Sheem-5 (24.13±0.14%) and lowest in BARI Sheem-3 (17.19±0.13%), respectively. The heterochromatin percentage is influenced by various factors including genetic variations, environmental conditions and epigenetic modifications. It was observed that chromocenter number in all cultivars were less than their total chromosome number. In the present study, the reduction of the chromocenter number in the ten cultivars might be due to fusion or overlapping of chomocenter; indicating the somatic association of chromosome. Dayal and Prasad (1983) reported that the number of chromocenters was considered to be genotypically controlled and there was therefore considered as species specific character.

Lavania and Sharma (1983) suggested that there is a tendency to shed heterochromatin segment during the course of evolution in plant. Heterochromatin percentage has been a key indicator in plant evolution (Sarkar et al. 2022). From this point of view, the older plants should have more heterochromatin than the recent one. BARI Sheem-3 which has the lowest heterochromatin percentage may therefore be considered to be the advanced type and BARI Sheem-5 which has the highest heterochromatin percentage, may therefore consider as primitive type in nature.

Conclusion

Mitotic index was observed higher in BARI Sheem-6 and lower in BARI Sheem-1. The interphase chromosome volume was found to be highest in BARI Sheem-9 and lowest in BARI Sheem-3 which do not correlated with the somatic chromosome number but may be related with environmental condition. The nuclear organizations were observed chromocentric in all examined varieties. According to the interphase nuclear structure and heterochromatin percentage in ten cultivars of *L. purpureus*, BARI Sheem-3 can be considered as primitive and BARI Sheem-5 as advance. This research can have implications for breeding programs, gene expression regulations and understanding of nuclear organizations on different cultivars of *Lablab purpureus*.

Acknowledgement

The authors also are gratefully acknowledging to Professor Sultanul Alam Cytogenetics Laboratory, Department of Botany, University of Rajshahi, Rajshahi-6205, Bangladesh for providing laboratory and related other facilities.

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

Contribution: The first author completed all the research works and other authors contributed to taking photomicrographs and data analysis. All authors read and revised the article.

References

Basu N, Todgham AE, Ackerman PA, Bibeau MR, Nakano K, Schulte PM and Iwama GK (2002). Heat shock protein genes and their functional significance in fish. Gene 295(2): 173-183.

Dayal N and Prasad C (1983). Genetic regulation of chromocentres in radish, *Raphanus sativus* L. Cytologia 48: 245-252.

Fiskesjo G (1993). A 2-3 day plant test for toxicity assessment by measuring the mean root growth of onion (*Allium cepa* L.). Environmental Toxicology and Water Quality 8: 461-470.

Haque A, Ali MA, Waxuddin M and Khan MK (1976). Squash method for the mitotic choromosomes *Lathyrus* of grasses. Current Science 45: 382-383.

Lavania UC and Sharma AK (1983). Chromosome banding in evolutionary plant cytogenetics. Proceedings: Plant Sciences 92: 51-79.

Maass BL, Knox MR, Venkatesha SC, Angessa TT, Ramme S and Pengelly BC (2010). *Lablab purpureus*- A crop lost for Africa. Tropical Plant Biology 3: 123-135.

Morris JB (2009). Morphological and reproductive characterization in hyacinth bean, *Lablab purpureus* (L.) sweet germplasm with clinically proven nutraceutical and pharmaceutical traits for use as a medicinal food. Journal of Dietary Supplements 6(3): 263-279.

Nagl W and Fusenig HP (1979). Types of chromatin organization in plant nuclei. *Plant Systematics and Evolution* 2: 221-233.

Nayar GG, George KP, and Gopal-Ayengar AR (1971). Relation between cytological abnormalities and interphase chromosome volume in plants growing in a high radiation area. Bhabha Atomic Research Centre, Bombay. Radiat Biology 11(2):175-8. https://doi.org/10.1016/S0033-7560(71)90713-7.

Rahman MS, Pritha US, Ferdous S, Sarkar MMR and Kabir G (2023). Nuclear phenotype and karyotype analysis in two varieties of *Lathyrus sativus* L. Journal of Bio-Science 30(2): 59-65.

Rahman Z, Habib M A, Begum R and Alam SS (2013). Fluorescent banding and RAPD analysis of four varieties of *Lablab purpureus* L. Cytologia 78(4): 391-397.

Rai SK, Rai RK and Jha S (2010). Cyanobacteria of Nepal: A checklist with distribution. Our Nature 8(1). doi:10.3126/on.v8i1.4342.

Sarkar MMR, Rahman MS, Karim R and Kabir G (2023). Nuclear phenotype and heterochromatin percentages in somatic cells on five species of *Crotalaria* L. Journal of Bio-Science 30(2): 79-88.

Smýkal P, Coyne CJ, Ambrose MJ, Maxted N, Schaefer H, Blair MW and Varshney RK (2015). Legume crops phylogeny and genetic diversity for science and breeding. Critical Reviews in Plant Sciences 34(1-3): 43-104.

Whyte RO, Nilsson-Leissner G and Trumble HC (1953). Legumes in Agriculture 76(5): 403.

Yamakawa K and Sparrow AH (1966). The correlation of interphase chromosome volume with pollen abortion induced by chronic gamma irradiation. Radiation Botany 6(1): 21-38.

(Manuscript received on 7th December 2023 and revised on 24th January 2024)