## Executive summary of the UGC major research project awarded to

**Prof. Dr. Timir baran Jha (PI) and Dr. Partha Roy (Co.P.I.)**

- **The major research project entitled "Karyosystematic analysis of Indian Lentils through Fluorescent chromosome banding**" was awarded to Prof. Dr. Timir baran Jha (Retd. Prof. of Botany, West Bengal Senior Education Service (WBSES and P.I.), & Dr. Partha Roy, Associate Prof. & Head. Dept. of Botany. Maulana Azad College, Kolkata ( Co.PI ) vide UGC's letter no. : F. No. 43-113 / 2014 (SR) dated 18.01.2016.
- The project was implemented in the Dept. of Botany. Maulana Azad College (under the University of Calcutta), 8, Rafi Ahmed Kidwai Road, Kolkata-700013, West Bengal on and from 01,07.2015 – 30.06.2018

**Executive summary**

- **Introduction :**

  Lentil ( *Lens culinaris*, Medik) is an ancient domesticated food crop popularly known as "poor man's meat" due to its high protein content. Though India is the second highest producer of Lentil it can meet the domestic demand through import. Further improvement of this crop is necessitated. Knowledge and conservation of available plant genetic resources plays the pivotal role in crop improvement. The genus Lentil comprises only one cultivated species (*Lens culinaris* Medik ) with large number of cultivars and six wild species with huge genetic potentials. Chromosomal studies have enormously benefitted plant breeders in their crop improvement programmes. However, it transpires that a detailed chromosomal data base is not available in this important crop. Keeping in mind we intended to carry

out detailed Karyosystematic Analysis of Indian Lentils through Fluorescent Chromosome Banding.

Limitations of conventional method of chromosome analysis have been upgraded by the process of Enzymatic maceration and air drying (EMA) method. The method is the basics of molecular cytogenetic studies. Slides once prepared through EMA method can be repeatedly used for different experiments. Chromosomal analysis can be done precisely as they are free from cytoplasms. Fluorescent banding studies with different flurochromes are possible with the slides prepared through EMA method. The method allows the use of base specific fluorochromes like Chromomycin $A_3$ (CMA) and 4- 6-diamidino-2-phenyl-indole (DAPI)which are very specific for enriched GC and AT regions on chromosomes. The method helps identification of chromosomes with morphological similarities. Depending on the laboratory facilities the same slides can be used for FISH and GISH for locating particular genes on chromosomes.

The present project considered 34 Indian cultivated cultivars of *L.culinaris* and five wild species; *L.orientalis, L.odemensis, L.nigricans, L.lamottei* and *L.ervoides* ,primarily obtained from the Indian Institute of Pulses, Kanpur India to prepare comparative chromosomal database for the first time in India as an aid to the lentil breeders and genome researchers for their future crop improvement programmes.

- **Summary of the findings**

- We have successfully standardized EMA method of chromosome preparation in this crop and developed a reproducible protocol for non-fluorescent Giemsa as well as fluorescent CMA and DAPI staining.

- Correlations of morphological (seed morphology and tendril formation) and chromosomal (position of secondary constriction) parameters have helped us to divide six species in two distinct groups. Group one comprises the lone

cultivated species *L. Culinaris* and two wild species *L. orientalis* and *L. odemensis* , where we have noted almost uniform seed morphology, no tendril formation and intercalary sat chromosome. On the other hand second group comprising three wild species *L. nigricans, L.lamottei and L. ervoides* shows different seed morphology, prominent tendril formation and terminal secondary constriction.

- Documentation of terminal sat in three wild species was not reported earlier. We consider these findings as unique for Indian lentils as they are distinct chromosomal markers and can be utilized in future crop improvement programmes. Our observation was further confirmed by CMA and DAPI fluorescent banding.

- We have carried out fluorescent staining with DAPI and CMA to locate AT & GC rich heterochromatic(HC) regions on chromosomes for the first time . Our results revealed additional GC,AT CMA and DAPI signals on chromosomes that will help to differentiate the cultivars

- Following the same procedural steps of chromosome preparation and giemsa staining, our results demonstrated that though cultivated and wild species contains 2n=14 chromosomes, they differ widely in the total chromatin length (TCL) that ranged from 53.6 - 137.24 µm. We have also observed that secondary constriction may be present on $2^{nd}$ , $3^{rd}$ , $4^{th}$ , $5^{th}$ and $6^{th}$ pairs of Lentil chromosomes

- Our results revealed similar karyotypic formula 3m + 1m (sat) + 2 Sm + $1^{st}$ in all the cultivated cultivars of Lens culinaris and two wild species namely *L. orientalis* and *L. odemensis*. However we have obtained different karyotype formula in *L. nigricans* [1M+4 m+1 m (sat)+1St], *L. lamottei* [5 m+1St (sat)+1St] and *L.ervoides*. [5m+1St+1St.Sat]

- Based on the karyotypic formula we have prepared comparative idiograms for all the species and cultivars.

- With our detailed karyo morphological analysis using Giemsa, DAPI and CMA and Ideograms we have prepared comprehensive chromosomal database in Indian Lentils, a valuable protein rich crop of India .

- The progress of the project was evaluated as **"Excellent"** by the UGC expert committee members during midterm evaluation in Feb 2017. (UGC Web site)

- We have published three full length papers in reputed international journals and presented our findings in an International Botanical conference held at Dhaka, Bangladesh in Feb 2018. We hope to publish more papers based on our fluorescent banding work.

- **Publications**

**i)** **Jha T B**. and Halder M Searching chromosomal landmarks in Indian Lentils through EMA based Giemsa staining method. **Protoplasma**, DOI 10.1007/s00709-015-0873-7**, 252:283–299**, Published online in **2015**

**ii)** **Jha T.B.,** Mahanti A and Ghorai A: Karyotype Analysis of Indian Lentils Through EMA Based Giemsa Staining Caryologia **68 (4) 280-288,** Published online: **06 Jan 2016.**

**iii)** **Jha T.B**., Saha P.S., Adak M., Jha S. and Roy P. Chromosome morphometric analysis of Indian cultivars of *Lens culinaris* Medik. using EMA based Giemsa staining method. Caryologia: International Journal of Cytology, Cytosystematics and Cytogenetics**, Vol. 70, No. 3, 270–283, 2017.**

**iv)** **Jha T.B**., Roy P and Jha S. **"**Chromosomal database in Indian Lentils through EMA based giemsa staining method" **P**resented in the 7$^{th}$ International Botanical Conference held in the University of Dhaka, Bangladesh in Feb **2018.**

**Prof. Dr. Timir baran Jha (PI)** **&** **Dr. Partha Roy (Co.PI)**