

KARYOTYPE STUDY OF *CICER ARIETINUM* H2K VARIETY

Richa Sinha
AMITY University Jharkhand
Ranchi, India

Abstract:

The detailed karyotypic analysis of *Cicer arietinum* H2K variety collected from Ranchi, Jharkhand was carried out to develop the karyotype of this variety to understand the possibilities of its use in genetic improvement of chickpea. It was observed to have diploid chromosome set ($2n=16$) with symmetrical karyotype.

Such karyotype of the observed variety shows primitive tendency which is considered to give rise to advanced members with asymmetrical karyotype.

Key words: *Cicer arietinum* H2K, Karyotype, symmetrical karyotype, diploids.

I. Introduction:

Cicer arietinum, commonly called chickpea or desi chana is a small herbaceous annual plant of Fabaceae family. It has been cultivated since the Roman era and is believed to be a native of South East Europe, from where it was transported to Egypt. *Cicer arietinum* is of great economic importance due to its high nutrient content. It is the source of proteins, folate and Zinc, having a very high dietary fiber making it the healthy source of carbohydrates for persons with insulin sensitivity or diabetes. It is the most important pulse grown extensively in India. Moreover, India is the world's leader in chickpea production followed by Pakistan and Turkey.

Since chickpea is an herbaceous plant, cultivars with greater resistance to biotic and abiotic stresses are needed to be grown which in turn has led to the increased attention of the chickpea researchers towards wild *Cicer* species as a potential source of high and multiple resistances.^[1-3]

The morphologically similar or uniform groups having same chromosome number is reported to have separate genetic system. Therefore, to understand the evolutionary development and genetic relationship of the morphologically distinguished varieties of plants, chromosome study is an absolute device. Therefore, the objective of this research is to develop the karyotype of *Cicer arietinum* H2K variety collected from Ranchi, Jharkhand and to understand possibilities of its use in genetic improvement of chickpea.

II. Research Methodology

Seeds of *Cicer arietinum* H2K variety were collected from the Birsa Agricultural University, Ranchi. Seeds were germinated on moist filter paper in Petri dishes. For the karyotypic analysis, primary roots were cut off and pretreated in a saturated aqueous solution of 1,4 Para dichlorobenzene for 5 hours. They were then fixed in ethanol-acetic acid 3:1 for 24 hours. The roots were then thoroughly washed and preserved in 70% alcohol. Preserved root tips were hydrolyzed in 1N HCL for five to ten minutes depending upon the thickness of the roots. Washed and hydrolyzed root tips were then stained with 2 per cent aceto-carmin and slides were prepared by squash technique. Well spread metaphase plate was selected and length and width of long arms and short arms of chromosomes were measured for karyotype analysis. The plate was then photographed in digital SLR Nikon camera. Visible chromosomes at metaphase plates were scored under 450x magnifications. Types of chromosome were identified and classified according to Abraham and Prasad (1983)^[4].

Data obtained were analyzed statistically using the following formulae. The idiograms and graphs were prepared from the statistically analyzed data.

1. **Relative Length:** The relative length of each chromosome was calculated by dividing the length of chromosome pair with the length of longest chromosome pair and multiplying it by 100.

$$\text{Relative Length} = \frac{\text{Length of chromosome pairs}}{\text{Length of the longest chromosome}} \times 100$$

2. The Form Percentage (F%), Arm Ratio (L/S) and Total Chromatin Index (TCI) were calculated as follows:

$$F\% = \frac{\text{Length of short arms}}{\text{Length of the chromosome pair}} \times 100$$

$$L/S = \frac{\text{Length of long arm}}{\text{Length of the short arm}} \times 100$$

$$\text{TCL}\% = \frac{\text{Length of the chromosome pair}}{\text{Total Chromatin length}} \times 100$$

3. Total chromatin length (TCL) was the total sum of haploid set of chromosomes. The Total Form Percentage (TF%), Gradient Index (GI%), Symmetry Index (SI%) and Disparity Index (DI%) were calculated [170,171] as:

$$\text{TF}\% = \frac{\text{Total sum of the short arm Length}}{\text{Total sum of the chromosome Length}} \times 100$$

$$\text{GI} = \frac{\text{Length of shortest chromosome of the complement}}{\text{Length of the longest chromosome of the complement}} \times 100$$

$$\text{SI} = \frac{\text{Total Length of all the short arms}}{\text{Total Length of all the long arms}} \times 100$$

$$\text{DI} = \frac{\text{Longest chromosome} - \text{shortest chromosome}}{\text{Longest chromosome} + \text{shortest chromosome}} \times 100$$

III. Results and Discussion:

The statistical data of Karyotype are presented in tables (table 1 to 2; fig. 1 to 4). The analysis of *Cicer arietinum* H2K variety showed $2n = 16$ chromosomes (Figure 1), showing normal mitotic division in all the examined cells. The size of chromosomes in cicer species is comparatively smaller, which makes the identification of most of the chromosomes difficult affecting the clear discrimination of the arm ratios and the total chromosome length. Individual chromosome length ranged from 2.1 to 4.52 (Table 1). Seven chromosomes presented nearly median chromosomes and one nearly submedian. The total chromatin length was 25.32. Neither secondary constriction nor satellites were observed.

In table 2 summarized chromosome parameters of the examined species are presented. The chromosome numbers were named from I to VIII in accordance with their length. The chromosomes were observed to fall into two groups: nearly median and nearly submedian (-) with the karyotype formulae: $2n = 2 = 7m + 1sm$. Total Chromatin Length (TCL), Total Form Percentage (TF%), Gradient Index (G.I.), Symmetry Index (S.I.) and Disparity Index (D.I.) are depicted in table 2. Symmetry Index (S.I.) and Disparity Index (D.I.) was comparatively high which was recorded to be 75.589 and 36.611 respectively (table 2).

The results obtained through karyotypic studies of different species are helpful in the development of new and improved forms of the economically important plants. The chromosome study also explains the cause of variations among different cytotypes of the same species found in different geographical regions of the country. Chromosome study is also used to correlate differences in chromosome number or morphology with morphological differentiation of the species [5].

The chromosome numbers of *Cicer arietinum* H2K have been reported $2n = 2x = 16$. The early findings also reports diploids with $2n = 16$ chromosomes in *Cicer arietinum* H2K. In the present investigation the nearly median and nearly submedian chromosomes with negligible variation in their size were observed.

When the karyotype asymmetry is taken into consideration, the asymmetrical karyotypes are supposed to be more advanced than the symmetrical ones (Stebbins 1950). In the present study maximum number of nearly median chromosomes was observed which may be considered as the primitive type showing the symmetrical karyotype. Also, sub-terminal chromosomes were completely absent which is the characteristic of advancedness. Asymmetrical karyotypes possess many chromosomes with sub terminal centromeres or great differences in size between the largest and the smallest chromosomes or both [6].

Apart from analyzing the chromosome number and size, Total Form percentage (TF%), Form percentage (F%), Gradient Index (GI), Symmetrical Index (SI) and Disparity Index (DI) were also calculated. These parameters were observed for analytical studies of the karyotype symmetry, which offers appropriate basis for indication of the nature of evolutionary process occurring or occurred among the species. This also shows the trends in which the evolution has taken place in the cytotypes [7]. It has also been reported that the study of chromosome of plants reveals that primitive members with symmetrical karyotype gives rise to advance members with asymmetrical karyotype [8].

The Total Form percentage (TF%) were comparatively lower. The lower value of Total Form Percentage shows the tendency of taxon towards asymmetry which means that lower the value of Total Form Percentage the higher will be the level of asymmetry [9]. The Symmetrical Indices (SI) was also recorded. The Gradient Index value was noticed to be comparatively lower showing their

higher level of asymmetry. As the Gradient Index value less than 30 are considered to be highly asymmetrical. The Gradient Index value was less than 30. It has also been reported that higher degree of asymmetry shows the greater degree of chromosome variation and evolution. On the other hand, higher degree of symmetry shows the smaller degree of chromosome variation and evolution^[10].

Thus, the present finding is justified from the cytological stand point, as chromosome numbers observed in *Cicer arietinum* H2K was very similar to the earlier findings. The number of chromosomes in *Cicer arietinum* H2K was reported to be $2n=2x=16$. The result showed the symmetrical tendency of the variety.

Table 1: Cytotaxonomical data of *Cicer arietinum* H2K

Chrom. number	Arm length		Chrom. Length (μ)	Arm ratio	R. L. (μ)	F%	TCI	Classification
	Long arm (μ)	Short arm (μ)						
1	2.52	2.0	4.52	1.26	100	44.25	17.85	nm
2	2.4	1.8	4.2	1.33	92.20	42.86	16.59	nm
3	2.1	1.6	3.7	1.31	81.86	43.24	14.61	nm
4	2.0	1.2	3.2	1.67	70.80	37.50	12.64	nsm(-)
5	1.6	1.2	2.8	1.33	61.95	42.86	11.06	nm
6	1.4	1.1	2.5	1.27	55.31	44.00	9.87	nm
7	1.3	1.0	2.3	1.30	50.89	43.48	9.08	nm
8	1.1	1.0	2.1	1.10	46.46	47.62	8.293	nm

Table 2: Data related to karyotype of *Cicer arietinum* H2K

Total form percentage (TF%)	Gradient index (GI)	Symmetry index (SI)	Disparity index (DI)
43.049	46.460	75.589	36.611

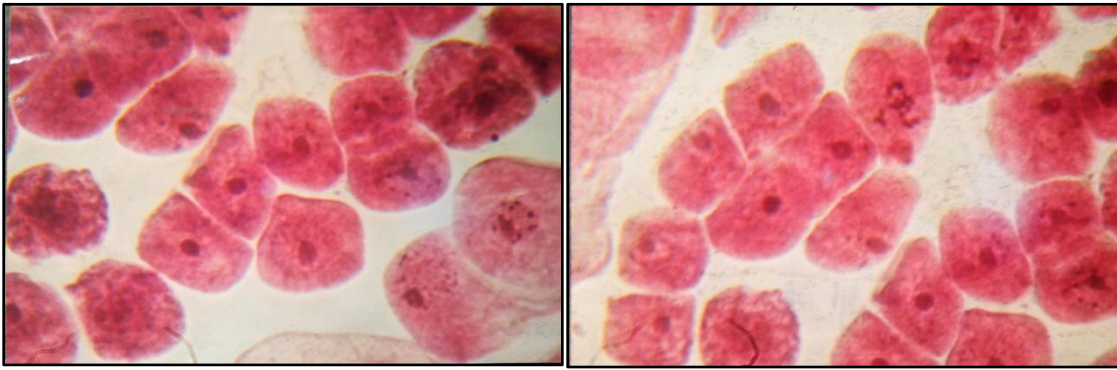


Fig. 1 Fig. 2

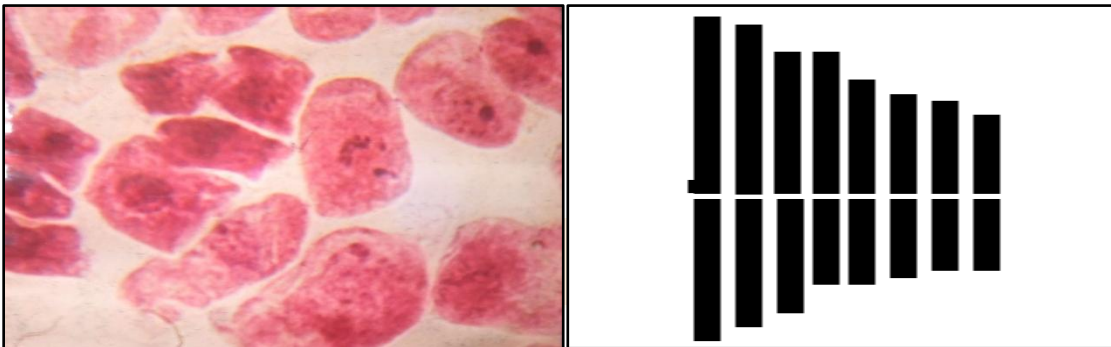


Fig. 3 Fig. 4

Fig.1, 2, 3: Mitotic (1) prophase, (2) pre-metaphase and (3) Metaphase Chromosomes of *Cicer arietinum* H2K variety

Fig. 4: Idiogram of Mitotic Metaphase Chromosomes of *Cicer arietinum* H2K variety

IV. Acknowledgement:

Special thanks to the lab facility provided by Ranchi Woman's college, Ranchi.

V. References:

1. Van Der Maesen, L.J.G., and Pundir, R.P.S., 1984. Availability and use of wild *Cicer* germplasm. *Plant Genetic Resources' Newsletter*, 57: 19-24.
2. Malhotra, R.S., Pundir, R.P.S., and Slikard, A.E., 1987. Genetic Resources of Chickpea. In: M.C. Saxena and K.B. Singh eds., *The Chickpea* CAB International, pp. 67-81.
3. ICARDA (International Centre for Agricultural Research in Dry Areas). 1989. Food Legume Improvement Program. In: *Icarda Annual report*, 1989, pp. 59-60.
4. Abraham, Z. and Prasad, P.N. 1983. A system of chromosome classification and nomenclature. *Cytologia*. 48 : 95-101.
5. Bocher, T.W. 1959. A cytotaxonomic study in the tetraploid and hexaploid *Trisetum spicatum* Coll. *Tidsskrift*, 55 : 23-29.
6. Stebbins, G.L. 1950. Variation and evolution in plants. Columbia University Press, New York
7. Kumar, Kamini and Kumar, Jyoti. 2014. Studies on the cytotaxonomy among different species of *Aloe* collected from Ranchi, Jharkhand. *International Journal of Bioassays*. 3 (3) : 1846-1850.
8. Levitsky, G.A. 1931. The Karyotype in Systematic Bulletin of Applied Botany, Genetics and plant breeding. 27 : 19-174.
9. Huziwaru, Y. 1962. Karyotype analysis in some genera of Compositae. VIII. Further studies on the chromosomes of Aster. *Amer. J. Bot.* 49 : 116-119.
10. Stebbins, G.L. 1971. Chromosomal evolution in higher plants. Edward Arnold Ltd., London.