Karyotype Analysis in Some Varieties of *Lens culinaris*

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Introduction

The different varieties of *Lens culinaris* show a significant variation in their morphological characters specially seed size. On the basis of such differences *L. culinaris* has been classified into two major varieties.

i) *Microsperma*—constituting the varieties having small (3–6 mm) round, convex seeds.

ii) *Macrosperma*—constituting the varieties having bigger (6–9 mm), flattened seeds (Shaw and Bose 1928, Barulina 1931).

But, even the members belonging to the same varieties do not possess uniformity in their seed size and morphological characters. And as such, no hard and fast line can be drawn in between those two major varieties. Because of the above fact, now a days, varieties differing in their morphological characters and seed size have been given independent status by the taxonomists.

It appears that such an artificial division may not hold good on the basis of cytological findings. Very little work has been done on the finer details of the cytology of the different varieties, only a few workers like Bhattacharjee (1951, 1953) and Sharma and Mukhopadhyaya (1963) have done some work in this direction.

The present investigation is based on the study of fifteen varieties of *L. culinaris* having bigger seed size. Out of these fifteen varieties, three (K 1895, K 1995, K 1289) have been supplied by All Union Plant Industry, U.S.S.R. and rest of the varieties have been obtained from Plant Introduction Division, I.A.R.I. New Delhi.

The detailed study of the chromosomes of these varieties shows that they differ from each other in the number of metacentric and sub-metacentric chromosomes and in their total chromatin length. The other difference lies in the presence or absence of the SAT-chromosome. In each variety, under discussion, the chromosome number is 14 (2n), which is in accordance with the earlier findings of Bhattacharjee (1951, 1953) and Sharma and Mukhopadhyaya (1963), (Figs. 1, 2). Although some of the cytological differences in these varieties are quite clear, still for much detailed studies *General Distance* D²-statistics (Mahalanobis 1936, 1949, Rao 1948, Bansal *et al.* 1965, Jagathesan and Ratnambal 1967, 1969) has been applied.

Materials and methods

The seeds were soaked in water for 4 to 5 hrs at room temperature (25°C ± 2°C) and then were transferred to petridishes lined with moist seed-testing paper.

They were allowed to germinate under laboratory conditions (light 40 W, Phillips incandescent lamp, temperature $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$, humidity 73%). Root tips 2–3 mm long, were taken off and they were pretreated with saturated solution of p-dichlorobenzene at the temperature of $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$, for two hours. The pre-treated root tips were fixed in acetoalcohol (1:3) with a little amount of ferric chloride, for 3 hrs. The photomicrographs and the cytological studies were made from temporary acetocarmine (1%) squash preparations. For statistical analysis, the cytological findings from an average of ten plates were used.

For D^2 analysis, following data were taken for individual chromosome in each variety (i) length of the individual chromosome transformed into angular scale = p and (ii) length of long arm/short arm transformed to logarithmic scale = q . Chromosomes were regarded as 1, 2, 3, 4, 5, 6 and 7 according to the value of their absolute length (Table 1). As in all the varieties under discussion, except N P varieties, SAT-chromosomes were not present, so for the convenience of grouping and calculations, they have not been placed separately. The SAT-chromosomes have been grouped along with others, according to their absolute length.

Treating ' p ' as abscissa and ' q ' as ordinate each chromosome can be plotted as point p, q . The graph will be a straight line, p/q will represent the gradient of the line ($= \tan \phi$), length of the line being $= (p^2 + q^2)^{\frac{1}{2}} = Z$ (say). The ratio $\tan \phi / Z = K$ (say) will give a good key to all the quantitative characters of an individual chromosome. Let Ki^x and Ki^y be the K value in varieties X and Y , respectively. Then for the 7 chromosomes, which are independent variables, the degree of divergence between varieties X and Y will be $D^2 = \sum_j^7 (Ki^x - Ki^y)^2$.

Observations

All the varieties under discussion, differed in the morphology of the chromosome, total chromatin length, presence or absence of SAT-chromosomes and also in D^2 values.

All the varieties possess only two types of chromosomes except N P varieties, in which a pair of SAT-chromosomes were also recorded. The EC varieties had the highest number of metacentric chromosomes (4 pairs), coming next to them were Russian varieties, with 3 pairs only. But in N P varieties the number of metacentric chromosomes ranged from 2–3 pairs. The lowest number of metacentric chromosome was recorded in N P 1, N P 8, N P 10 and N P 45 (2 pairs) (Fig. 3).

The maximum total chromatin length was recorded in N P 45 (72.5μ) and it was minimum in N P 4 (28.2μ). In all other varieties, the value of total chromatin length was intermediate between these two figures.

All N P varieties appeared to possess one pair of SAT-chromosome in each case. They were sub-metacentric in nature and their size differed from variety to variety. The position of secondary constriction was very near to the primary constriction. But the distance between the two was not found to be constant amongst all these varieties. The maximum and minimum distances between primary and secondary constrictions in SAT-chromosome were 0.71μ (N P 45) and 0.16μ (N P 4),

respectively. For more critical analysis, D^2 statistics was applied. On the basis of the value obtained for generalized statistical distance D^2 (Table 2), the varieties could be grouped into 9 clusters (Fig. 5).

The cluster 'A' consisted of four varieties namely N P 1, N P 4, N P 10 and N P 16. Due to very little difference in their D^2 values, they were put in the same cluster. Cluster 'B' comprised of N P 3 and EC 36676 and K 1995, cluster 'C' incorporated

Fig. 1. Somatic chromosomes of *L. culinaris* (A-N.P. 1, B-N.P. 2, C-N.P. 3, D-N.P. 4, E-N.P. 7, F-N.P. 8, G-N.P. 10, H-N.P. 16).

N P 2 and EC 27161. Remaining varieties showed greater differences in their D^2 values, so they were treated as independent units. The values of generalised distance = D^2 have been given in Table 2. It was observed by treating ' p ' as abscissa and ' q ' as ordinate, that the points (p and q) formed a straight line, but because of fifteen varieties included in this case, the graph became much complex and it was

Table 1. Morphological characteristics of karyotype of *Lens culinaris*

Variety	No.	Chromosome-1	Chromosome-2	Chromosome-3	Chromosome-4	Chromosome-5	Chromosome-6	Chromosome-7
N.P. 1.	1	18.14±0.811 (25.20)	15.30±0.256 (23.00)	13.90±0.377 (21.88)	13.90±0.377 (21.88)	13.22±0.045 (21.32)	13.10±0.030 (21.22)	12.44±0.439 (20.66)
N.P. 2.	2	1.28±0.061 (0.11)	1.12±0.095 (0.035)	1.36±0.264 (0.11)	1.30±0.273 (0.09)	1.59±0.163 (0.198)	1.84±0.151 (0.265)	1.50±0.075 (0.178)
N.P. 3.	1	17.35±0.272 (24.60)	17.33±0.254 (24.59)	17.06±0.223 (24.34)	15.34±0.679 (23.04)	13.06±0.545 (21.15)	10.23±0.459 (18.65)	9.73±0.251 (18.18)
N.P. 3.	2	1.45±0.184 (0.144)	1.30±0.184 (0.096)	1.21±0.066 (0.078)	1.71±0.656 (0.146)	1.86±0.210 (0.258)	1.53±0.209 (0.168)	1.60±0.245 (0.180)
N.P. 3.	1	20.38±0.467 (26.83)	17.68±0.139 (24.84)	13.93±0.764 (21.91)	12.73±0.166 (20.90)	12.26±0.129 (20.46)	11.75±0.100 (20.04)	11.26±0.226 (19.62)
N.P. 4.	2	1.44±0.080 (0.15)	1.60±0.015 (0.20)	1.40±0.246 (0.13)	1.48±0.188 (0.16)	2.00±0.264 (0.295)	1.83±0.168 (0.205)	1.67±0.280 (0.205)
N.P. 4.	1	17.00±0.268 (24.37)	15.12±0.199 (23.36)	15.71±0.199 (23.36)	14.91±0.631 (22.71)	13.27±0.303 (21.355)	12.34±0.156 (20.57)	11.04±0.236 (19.40)
N.P. 7.	2	1.12±0.039 (0.047)	1.09±0.048 (0.035)	1.28±0.446 (0.10)	3.10±1.753 (0.32)	1.48±0.303 (0.15)	1.64±0.108 (0.21)	3.00±0.408 (0.46)
N.P. 7.	1	17.14±0.300 (24.48)	15.58±0.384 (23.24)	15.07±0.156 (22.84)	14.63±0.237 (22.49)	13.90±0.147 (21.89)	12.13±0.432 (20.38)	11.54±0.275 (19.85)
N.P. 8.	2	1.03±0.030 (0.01)	1.67±0.667 (0.16)	1.05±0.047 (0.02)	1.69±0.654 (0.17)	1.06±0.056 (0.02)	4.36±1.986 (0.557)	2.61±0.360 (0.407)
N.P. 8.	1	18.95±0.658 (25.795)	17.27±0.055 (24.36)	16.06±0.668 (23.60)	13.79±0.166 (21.79)	11.65±0.234 (19.93)	11.13±0.115 (19.48)	11.13±0.115 (19.48)
N.P. 10.	2	1.60±0.136 (0.20)	1.21±0.169 (0.07)	1.04±0.035 (0.015)	1.21±0.125 (0.075)	1.34±0.704 (0.115)	1.77±0.228 (0.235)	1.77±0.228 (0.235)
N.P. 10.	1	16.34±0.469 (23.84)	16.28±0.481 (23.78)	15.59±0.512 (23.24)	14.47±0.198 (22.36)	13.595±0.575 (21.63)	12.48±0.726 (20.66)	11.24±0.456 (19.56)
N.P. 16.	2	1.535±0.411 (0.145)	1.08±0.082 (0.03)	1.75±0.144 (0.24)	2.86±1.712 (0.27)	1.66±0.318 (0.198)	2.96±1.682 (0.305)	1.58±0.212 (0.155)
N.P. 16.	1	17.95±0.503 (25.08)	15.98±0.354 (23.58)	15.38±0.216 (23.11)	14.48±0.410 (22.38)	12.38±0.498 (20.58)	11.93±0.191 (20.21)	11.91±0.175 (20.18)
N.P. 16.	2	1.21±0.103 (0.077)	1.07±0.073 (0.03)	1.16±0.163 (0.057)	1.83±0.601 (0.22)	1.88±0.453 (0.24)	3.00±0.500 (0.467)	2.50±0.000 (0.40)

Variety	No.	Chromosome-1	Chromosome-2	Chromosome-3	Chromosome-4	Chromosome-5	Chromosome-6	Chromosome-7
N.P. 45	1	17.24±0.257 (24.54)	15.58±0.020 (23.26)	15.58±0.020 (23.26)	13.77±0.160 (21.76)	13.63±0.020 (21.64)	12.52±0.016 (20.70)	11.68±0.016 (20.00)
	2	1.34±0.95 (0.13)	2.00±1.00 (0.24)	2.00±1.00 (0.24)	1.75±0.750 (0.20)	2.50±0.000 (0.40)	3.50±0.000 (0.54)	1.02±0.020 (0.01)
K. 1995	1	18.45±1.264 (25.40)	16.41±0.153 (23.91)	16.22±0.214 (23.77)	14.93±0.482 (22.74)	13.10±0.433 (21.23)	10.94±0.100 (19.30)	9.96±0.720 (18.33)
	2	1.33±0.236 (0.105)	1.50±0.289 (0.15)	1.59±0.223 (0.188)	2.07±0.649 (0.262)	2.11±0.634 (0.278)	2.50±0.0500 (0.36)	2.00±0.577 (0.24)
K. 1895	1	18.63±0.572 (25.56)	16.98±0.851 (24.295)	15.04±0.730 (22.81)	14.15±0.099 (22.12)	13.35±0.648 (21.41)	11.80±0.91 (20.05)	10.03±0.488 (18.42)
	2	2.88±1.885 (0.232)	1.43±0.245 (0.13)	1.50±0.289 (0.15)	2.38±0.898 (0.295)	1.01±0.013 (0.005)	2.77±0.684 (0.405)	2.00±0.577
K. 1289	1	20.33±0.431 (26.80)	16.11±0.449 (23.65)	15.65±0.667 (23.28)	14.61±0.418 (22.46)	12.10±0.444 (20.35)	11.25±0.560 (19.60)	9.94±1.005 (18.36)
	2	1.02±0.017 (0.007)	1.52±0.290 (0.167)	1.67±0.333 (0.20)	1.83±0.883 (0.18)	2.00±0.500 (0.28)	2.00±1.000 (0.20)	2.11±0.450 (0.31)
E.C. 36676	1	17.79±1.023 (24.95)	17.79±1.023 (24.95)	15.27±0.458 (23.00)	13.47±0.616 (21.52)	12.64±0.493 (20.795)	10.93±0.770 (19.27)	9.01±0.400 (17.47)
	2	2.00±1.000 (0.175)	1.75±0.250 (0.225)	1.71±0.208 (0.2275)	1.95±0.375 (0.270)	2.10±0.412 (0.290)	2.50±0.500 (0.360)	1.96±0.375 (0.270)
E.C. 27161	1	17.97±0.663 (25.075)	17.28±0.489 (24.53)	15.07±0.476 (22.84)	14.10±0.840 (22.03)	12.89±0.478 (21.02)	11.52±0.327 (19.82)	11.91±0.321 (19.52)
	2	1.33±0.236 (0.105)	1.25±0.250 (0.075)	1.88±0.375 (0.250)	1.66±0.454 (0.1775)	2.38±0.688 (0.315)	2.50±0.500 (0.360)	2.13±0.516 (0.255)
E.C. 27656	1	18.55±0.276 (25.53)	17.53±0.454 (24.74)	15.46±0.231 (23.17)	12.37±0.184 (20.575)	12.37±0.184 (20.575)	12.37±0.184 (20.575)	11.34±0.576 (19.65)
	2	2.00±1.000 (0.175)	1.38±0.239 (0.120)	2.12±0.625 (0.255)	2.00±0.577 (0.240)	1.50±0.500 (0.120)	1.50±0.500 (0.120)	1.15±0.150 (0.050)

1. = Relative length ± S.E.

() = Angular transformation value from ° to degrees.

2. = Long arm/short arm ± S.E.

() = Log (long arm/short arm).

Table 2. Generalised distances (D²) between 15 varieties of *L. culinaris*

Cluster	Varieties	v1, v4, v7, v8 A	v3, v10, v13 B	v2, v14 C	v6 D	v5 E	v15 F	v9 G	v12 H	v11 I
A	v1, v4, v7, v8	194.8	786.3	489.56	5011.9	11921.15	970.15	10091.875	17705.55	38647.35
B	v3, v10, v13	786.3	22.1	85.4	5718.2	12725.9	322.4	9182.5	18390.3	38643.2
C	v2, v14	489.56	85.4	100.4	5275.0	12400.9	348.05	9224.05	18197.5	38678.8
D	v6	5011.9	57108.2	5275.0	—	4615.6	6333.2	15078.4	12587.0	42434.4
E	v5	11921.15	12725.9	12400.9	4615.6	—	13146.0	22376.9	6028.8	33545.0
F	v15	970.15	322.4	348.05	6333.2	13146.0	—	6498.3	19135.0	37035.7
G	v9	10091.875	9182.5	9224.05	15078.4	22396.9	6498.3	—	27652.7	48220.8
H	v12	17705.55	18390.3	18197.5	12587.0	6028.8	19135.0	27652.7	—	57797.6
I	v11	38647.35	38643.2	38678.8	42434.4	33545.0	37035.7	48220.8	57797.6	—

Abbreviation: v1 = N P 1, v2 = N P 2, v3 = N P 3, v4 = N P 4, v5 = N P 7, v6 = N P 8, v7 = N P 10, v8 = N P 16,
v9 = N P 45, v10 = K 1995, v11 = K 1895, v12 = K 1289, v13 = EC 36676, v14 = EC 27161, v15 = EC 27656.

not possible to give a representation of this graph in the present paper.

Discussions

While discussing the differences in chromosome morphology of different varieties, it can be noted from Fig. 3 that the number of metacentric chromosomes in EC varieties are highest (4 pairs). The rest of the chromosomes, in these varieties are submetacentric in nature. The SAT-chromosomes are absent. In Russian (K 1289, K 1895 and K 1995) varieties, the number of metacentric chromosomes

Fig. 2. Somatic chromosomes of *L. culinaris* (A-N.P. 45, B-Ec 36676, C-Ec. 27161, D-Ec. 27656, E-K 1289, F-K 1895, G- K 1995).

are 3 pairs only in each case. The remaining chromosomes are sub-metacentric. There are no SAT-chromosome in these varieties. In some of the N P varieties (N P 2, N P 3, N P 4, N P 7 and N P 16) there are 3 pairs of metacentric chromosomes and one pair of SAT-chromosome, accompanied by 3 pairs of submetacentric chromosomes. But in rest of N P varieties (N P 1, N P 8, N P 10 and N P 45), there are only 2 pairs of metacentric chromosomes, in addition to a pair of SAT-chromosome and 4 pairs of submetacentric chromosomes.

According to Stebbins (1951), the species or varieties having greater number of submetacentric chromosomes should be treated as more evolved, than those in

VARIETIES	M	SAT	S
NP 1			
NP 2			
NP 3			
NP 4			
NP 7			
NP 8			
NP 10			
NP 16			
NP 45			
EC 36676			
EC 27161			
EC 27656			
K 1289			
K 1895			
K 1995			

Fig. 3. Haploid karyotype of fifteen varieties of *L. culinaris* showing SAT-chromosomes in pair (M—Metacentric, SAT—SAT-chromosome, S—submetacentric).

which there are lesser number of submetacentric chromosomes. So, some of the NP varieties (NP 1, NP 8, NP 10 and NP 45) should be treated as more evolved than Russian or EC varieties.

When total chromatin length is taken into consideration, we find that Russian varieties show a small variation in their chromatin length. It is highest in K 1289 (57.5μ) and the lowest in K 1995 (52.5μ). The chromatin length in EC varieties appear to be more or less the same (49.0μ in EC 36676, 48.5μ in EC 27161 and 48.0μ in EC 27656). Further, it is noted that in NP varieties chromatin length is highest in NP 45 (72.5μ) and the lowest value is recorded in NP 4 (28.2μ), Fig. 4. Although the range of chromatin length in NP varieties appears to be wide, (72.5μ in NP 45 to 28.2μ in NP 4), but except NP 45, all the other NP varieties can be grouped together because of lesser differences in their total chromatin lengths. The variation in total chromatin length in different varieties might have been caused due to the loss of translocated segments formed during asymmetrical exchanges.

One of the most interesting observation in the present investigation is the nature of SAT-chromosomes. The SAT-chromosomes are recorded in NP varieties only, while other varieties lack in this. The chromosome pair which carried

satellite are sub-median in nature, in all cases. This is in accordance with the previous observations of Bhattacharjee (1951, 1953). It has also been noted that there is a variation in the distance between the two constrictions (primary and secondary) of SAT-chromosome in different NP varieties. In NP 45 the length of the segment between the two constrictions in SAT-chromosome is 0.71μ , but this value decreases to 0.16μ in NP 4. In rest of the NP varieties, the length of the segment lies

in between these two values.

From the above findings, it appears that there might have been a gradual reduction in the distance between the two constrictions due to translocation and hybridization. This might have lead to their total loss, thus, giving rise to the varieties without SAT-chromosomes. The process of elimination of satellite by translocation and hybridization has also been supported by Delauney (1926) and Sato (1937).

It appears that the variability in chromosome morphology along with the change in chromatin length has also influenced SAT-chromosomes and the length of the segment between the two constrictions. The decrease in the distances between primary and secondary constrictions of SAT-chromosomes appears to go hand in hand, with the decrease in total chromatin length in NP varieties. As the length of the segment shows a definite tendency towards decrease, it may be presumed that such a change is bound to influence the genetic characters due to the loss in genetic

Fig. 4. Histogram showing total chromatin length in fifteen varieties of *L. culinaris*.

Fig. 5. Graphic representation of D^2 (distance) values between fifteen varieties of *L. culinaris*.

materials. Thus, it may be concluded that the variation in the distance between the primary and the secondary constrictions in SAT-chromosomes along with the other changes discussed above, might have lead to variety formation in *Lens culinaris*.

To deal with much more minor differences in chromosome morphology the D^2 statistics which represents the general divergences among biological population, has been applied (Mahalanobis 1936, Mahalanobis *et al.* 1949, Murty and Pavate 1962). The degree of divergences have been calculated by squaring the distances for seven chromosomes between a pair of varieties, which could be utilised to group them tentatively, so that D^2 values within a group are much smaller than those belonging to different groups.

On the basis of D^2 value relationship, the varieties can be placed under '9' clusters (Fig. 5). Cluster 'A' consists of four N P varieties (N P 1, N P 4, N P 10 and N P 16), cluster 'B' consists of (N P 3, EC 36676 and K 1995), cluster 'C' consists of (N P 2 and EC 27161). Rest of the varieties have been placed at six different points, because they record much difference in thier D^2 values (Table 2). Clusters, D, E, F, G, H and I consist of varieties N P 8, N P 7, EC 17656, N P 45, K 1289 and K 1895, respectively.

The maximum D^2 value is found between cluster E and I, that is, between N P 7 and K 1895 and minimum D^2 value is between N P 8 and EC 27656 (Fig. 5).

From the earlier discussion on the basis of number of metacentric and sub-metacentric chromosomes and total chromatin length, it seems probable that some of the N P varieties are nearer to EC or Russian varieties. This fact can also be traced out in the grouping pattern (cluster B and C) according to D^2 values, at least to some extent. In cluster 'A' there are four N P varieties (N P 1, NP 4, N P 10 and N P 16). In addition to D^2 values these varieties show a clear relationship in total chromatin length and in the presence of SAT-chromosomes. The cluster 'B' consist of N P, 3 and EC-36676 and a Russian variety K 1995. These three varieties have other similarities except in SAT-chromosomes which are absent in K 1995 and in EC 36676. In cluster 'C' we find that N P 2 and EC 27161 are together.

All these facts also indicate that the N P varieties might be closer to EC and Russian varieties. But keeping in view, the differences in cytological characters and much difference in D^2 values of other varieties, it may be said that variety formation in *Lens culinaris* has been due to the change in the nature and morphology of the chromosomes. Although, the role of gene mutation along with changes induced by climatic and physiological factors, can not be ruled out completely at this stage.

Summary

Karyotype analysis in fifteen varieties of *Lens culinaris* was carried out. The varieties differ from each other in chromosome morphology and in total chromatin length. For much detailed studies D^2 statistics was applied. According to D^2 values the varieties were placed at nine different clusters.

From the analysis of chromosome morphology and D^2 values, it was revealed that variety formation in *Lens culinaris* has taken place due to structural changes in

the chromosomes. It has also been accompanied by change in total chromatin length in different varieties. The SAT-chromosomes appear to have undergone changes as regard the position of secondary constriction, (N P varieties). In Russian and EC varieties they have not been recorded at all.

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