Cryptic Structural Differences at the Interstrain Level of *Pisum sativum* L.

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Pisum sativum L., the cultivated species of the family Leguminosae, includes a large number of strains with distinct and wide geographical distribution. The genus is one of the earliest genera in which controlled breeding work has been carried out for the production of improved types and it is the work on this genus that led to the foundation of the modern science of genetics. All the cultivated strains studied so far have $2n=14$ chromosomes.

Notwithstanding the fact, that analysis of chromosomes of *Pisum sativum* has been studied earlier, no systematic analysis of the structure and behaviour of chromosomes of indigenous strains specially with a view to explore cryptic differences, if any, has been undertaken, excepting a few earlier records (Yarnell 1962, Sen and Tiwari 1966, Fauzdar and Tandem 1976, Sharma and Gupta 1982). In view of the vast potential of improved techniques of chromosome analysis, the present investigation was undertaken to analyse the minute structural differences of chromosomes at intraspecific level. The present study includes a detailed analysis of chromosome length and volume, chiasma frequency per cell and nucleus and classification of the finer details at the microscopic level.

Materials and methods

Seeds of eleven strains of *Pisum sativum* were obtained through the courtesy of different agricultural centres in India. The seeds were germinated at 25°C, on moist filter paper, in petri-dishes and sown during the months of October and November in the Experimental Garden of the Department of Botany, University of Calcutta.

Somatic chromosomes were observed from root-tip cells. Young root-tips, cut into 2 mm segments, were pretreated in a saturated mixture of para-dichlorobenzene and aesculin at 12°C for 2 h followed by fixation in Newcomer's fluid for 1 h. The root-tips were then slightly warmed and stained in 9:1 mixture of 2% propionic-orcin and (N)HCl.

Chromosome volume was measured using the formula:

$$V = \pi r^2 h$$

where, V is the chromosome volume, r is the radius and h is the length of the chromosome.

Meiotic chromosomes were observed from pollen mother cells (PMCs). Flower buds were fixed in Newcomer's fluid for 1 h and the PMCs stained in 2% propionic-carmin along with a drop of ferric-acetate solution. All the stages of meiotic divisions, chiasma frequency and meiotic abnormalities were scanned from at least 100 PMCs of each strain.

Observations

The chromosome complements revealed gross morphological similarities. The chromosomes were classified into 6 types according to the position of the centromeres and secondary

CHROMOSOME TYPES

Fig. 1. Diagrammatic representation of chromosome types.

constrictions (Fig. 1). Somatic chromosome number, $2n=14$ is constant for all the strains (Figs. 2–12). The total chromosome length ranges from 53.4 to 70.8 μm (Table 1). There are one to three pairs of chromosomes with secondary constrictions (Fig. 13). Total chromosome volume ranges within 38.9 to 89.9 cu. μm (Table 1). Total TF% varies from 29.21 to 37.83% (Table 1). No somatic irregularities were noticed in any of the strains.

Figs. 2–12. Somatic metaphase plates of *Pisum sativum* L. showing $2n=14$ chromosomes. 2, PG-3. 3, T-163. 4, B22. 5, PLP-371. 6, EC-33866. 7, Bonneville. 8, PLP-401. 9, J-1. 10, Arkel. 11, PLP-525. 12, IC-775. (Bar = 10 μm).

Meiotic metaphase I manifested 7 regular bivalents (Figs. 14–24). Chiasma frequency per nucleus and per bivalent ranges from 7.84 to 12.9 and 1.1 to 1.8 respectively (Table 2). Despite regular behaviour of chromosomes, cytotoxicity was observed in the strains PLP-371 (Fig.

Fig. 13. Comparative karyograms of the different strains in *Pisum sativum* L.

Table 1. Details of karyotype of eleven strains of *Pisum sativum* L.
($2n=14$)

Name of the strains	Karyotype formula	Nos. of chromosomes with secondary constriction	TF%	Range of chromosome length (μm)	Total chromosome length (μm)	Range of chromosome volume (cu. μm)	Total chromosome volume (cu. μm)
PG-3	$C_2D_8E_2F_2$	2	35.52	5.0–3.3	55.4	7.1–4.5	80.8
T-163	$C_4D_8E_2F_2$	4	33.81	4.5–3.6	55.9	7.5–4.5	89.9
B-22	$A_2B_2E_{10}$	4	36.19	4.7–3.4	57.4	6.4–2.3	57.6
PLP-371	$B_2D_8F_8$	2	29.26	5.0–3.5	57.4	3.8–2.2	40.7
EC-33866	$B_4D_4E_4F_2$	4	37.83	5.4–4.0	60.6	4.9–1.6	40.0
Bonneville	$B_2C_4D_4E_4$	6	31.81	5.4–3.9	62.8	4.2–2.0	39.5
PLP-401	$A_4D_8F_4$	4	29.21	5.2–3.6	64.4	4.0–2.3	44.7
J-1	$A_2D_8E_4$	2	30.61	6.3–2.9	64.5	8.3–2.7	75.6
Arkel	$A_2D_8E_4$	2	31.96	5.6–3.6	65.7	8.0–3.1	81.3
PLP-525	$A_4D_4E_8$	4	31.42	5.4–4.5	68.8	3.2–2.3	40.1
IC-775	$A_2D_8E_4$	2	32.11	5.5–4.5	70.8	3.5–1.7	38.9

Figs. 14-24. Meiotic metaphase I plates of *Pisum sativum* L. showing 7 bivalents, cytomixis, sticky chromatin connection and late separation of bivalents. 14, 14a, PG-3. 15, T-163. 16, 16a, B-12. 17, 17a, PLP-371. 18, 18a, EC-33886. 19, Bonneville. 20, PLP-401. 21, J-1. 22, Arkel. 23, PLP-525. 24, IC-775. Bar = 10 μ m.

17a) and EC-33866 (Fig. 18a) and sticky chromatin connection in anaphase I in PG-3 (Fig. 14a), and B-22 (Fig. 16a).

Discussion

Chromosome characteristics

Karyotype analysis in 11 strains of *Pisum sativum* shows gross uniformity in morphology. Somatic chromosome number, $2n=14$ is constant for all the strains which agrees with earlier reports (Darlington and Janaki Ammal 1945, Yarnell 1962, Sharma and Chatterjee 1963, Sen and Tiwari 1966, Fauzdar and Tandem 1976, Sharma and Gupta 1982).

Chromosomes are mostly long to medium in length with secondary constrictions in one to three pairs. 5 of the 11 strains possess a single pair of chromosomes with secondary constrictions or satellites. Another 5 of the strains show 2 pairs of such chromosomes while in a single strain, Bonneville 3 pairs of chromosomes with secondary constrictions are present (Fig. 13). Previous

Table 2. Details of chromosome behaviour in eleven strains of *Pisum sativum* L.

Strains of <i>Pisum sativum</i>	No. of bivalents	Chiasma frequency per nucleus	Chiasma frequency per bivalent	Meiotic irregularities
PG-3	7	10.6	1.51	Sticky chromatin connection in anaphase I
T-163	7	9.5	1.35	
B-22	7	12.9	1.88	Late separation of bivalent at anaphase I
PLP-371	7	11.3	1.61	Cytomixis during prophase I
EC-33866	7	11.6	1.65	Cytomixis during metaphase I
Bonneville	7	19.2	2.70	
PLP-401	7	8.4	1.20	
J-1	7	7.91	1.13	
Arkel	7	11.0	1.57	
PLP-525	7	7.84	1.12	
IC-775	7	9.1	1.30	

reports record either a single pair of chromosomes with secondary constrictions (Sen and Tiwari 1966, Sharma and Gupta 1982) or 2 pairs of such chromosomes (Morrison and Lin 1955, Gopinath and Subramaniam 1963). Primary constrictions in chromosomes range from median to submedian, showing gross morphological similarities in this taxa despite the evolution of different strains. On this basis the homogeneity of the species is undoubtedly clear.

Notwithstanding this overall homogeneity, the detailed karyotype analysis reveals minute differences. Each strain is characterised by its own karyotype, which may serve as one of the identifying criteria. Therefore, based on this parameter, the strain Bonneville can be distinguished from the rest of the strains by the presence of 3 pairs of chromosomes, B_2 and C_4 with secondary constrictions and 2 pairs of chromosome each D_4 and E_4 with primary constrictions (Fig. 13). The strain B-22 can also be identified from the rest of the strains by the presence of four pairs of chromosomes F_8 with nearly median constrictions besides A_2 and B_2 types of chromosomes with secondary constrictions. The strains J-1, Arkel and IC-775, unlike the rest have similar karyotype formula of $A_2D_8E_4$, though IC-775 strain can be distinguished from the

two by its very low total chromosome volume and the strain J-1 by its wide range of chromosome length (Table 1). As these data have been found constant in all the replicate preparations, the validity of the observations is ensured. This is an evidence to show that minute structural alterations have taken place in evolution of different strains of *Pisum sativum* leading to their diversification.

Total chromosome length and its range

Despite minor karyotypic differences, absolute chromosome lengths do not show marked variation, excepting certain strains like PLP-525 and IC-775 where the total chromosome lengths are very high, 68.8 and 70.8 μm respectively, yet the ranges of their chromosome lengths within the complement are not very wide, 5.4 to 4.5 μm and 5.5 to 4.5 μm , respectively. Maximum variation in the range of chromosome length within a complement has been observed in the strain J-1, where the longest (6.3 μm) as well as the shortest (2.9 μm) chromosome pairs are present together, while their total chromosome length is quite close to the other strains. The presence of such unusually long or short chromosomes may be due to differential spiralization. Such differences in the range of chromosome size can be explained on the basis of structural alterations which have been adapted for the strains and are more or less universal for this genus (Sutton 1937, Sen and Tiwari 1966).

Total chromosome volume and its range

As compared to total chromosome length, absolute chromosome volume shows a wide difference. The lowest chromosome volume, 38.9 cu. μm is found in the strain IC-775, though its total chromosome length is the highest among the strains. In sharp contrast, the strain T-163 shows the maximum total chromosome volume, 89.9 cu. μm , despite its low absolute chromosome length (Table 1). These differences have been consistently noted, indicating distinct genetic control. The volume of a chromosome is dependent on DNA and protein components as well. The extent to which these components differ as noted in several species (Jensen 1958, Sau *et al.* 1980, Sharma and Mukhopadhyay 1984, Mukherjee and Sharma 1984, 1986) in the organs tested, is yet to be worked out.

All these features of the chromosome morphology clearly indicate that in the karyotype, the parameters tested to a great extent serve as identifying characters for the different strains of the species. These evidences also indicate the role of structural alterations of chromosomes in the evolution of the strains (vide Sharma and Sharma 1959). The genetic control of these parameters is quite evident.

Chromosome behaviour

Meiotic studies, however, reveal regular 7 bivalents in metaphase I. Anaphasic separation too is regular as also observed earlier by Cooper (1938). Chiasma frequency per nucleus and per bivalent shows little variation, ranging from 12.9–7.84 and 1.88–1.12 respectively. Irregularities in meiotic behaviour are low in this species. Besides cytotoxicity, during prophase and metaphase I in the strains PLP-371 and EC-33866 respectively, sticky chromatin connection in anaphase I in PG-3 and late separation of bivalents in B-22, no other irregularities were found. The importance of the irregularities in stable cultivated strains is yet to be ascertained. Earlier workers (Richardson 1929, Håkansson and Levan 1942, Morrison and Lin 1955, Gopinath and Subramaniam 1964, Gottschalk and Pietrini 1965, Gottschalk 1970, Gottschalk and Milutinovic 1970, Klein 1971, Klášterská and Ramel 1981, Medina and Risuno 1981) have observed meiotic irregularities like ring-chromosomes, extra-chromosomal connections and multiple nucleolar formation in *Pisum sativum*.

Conclusion

The discrepant data on chromosome behaviour may be due to varietal differences as well as manifestations of climatic or other conditions. Karyotype has revealed the importance of structural alterations in the evolution of different strains. Meiotic analysis does not reveal any distinct manifestation of structural hybridity in these strains. The formation of clear bivalents may imply that the individuals represent homozygosity for structural alterations in the chromosomes, the heterozygosity being eliminated in the course of evolution. Since the species is a widely cultivated crop, judicious selection has evidently resulted in this homozygosity, playing a very important role in stabilization of these structural alterations.

Summary

Karyotype analysis of 11 strains of *Pisum sativum* L. shows gross uniformity in morphology. Somatic chromosome number, $2n=14$, is constant for all the strains. Chromosomes are long to medium in length. Primary constrictions range from median to submedian. Secondary constrictions are present in 1 to 3 pairs of chromosomes. Each strain is characterised by its own karyotype, which may serve as an identifying criterion. Absolute chromosome length does not show marked variation, whereas absolute chromosome volume reveals a wide difference. Meiotic analysis discloses regular seven bivalents with a low frequency of irregularities. Karyotype data have revealed the importance of structural alterations in the evolution of different strains. Meiotic behaviour does not reveal any distinct manifestation of structural hybridity in these strains which has been suggested as due to the attainment of homozygosity for structural alterations through selection.

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