Chiasma Formation in Induced Autotetraploids of Pea (*Pisum sativum* L.)—An efficient method of scoring

H. Kumar, V. C. Mercykutty¹ and C. P. Srivastava

Department of Genetics and Plant Breeding Institute of Agricultural Sciences,
Banaras Hindu University, Varanasi 221 005, India

Accepted December 8, 1989

Chiasma formation plays an important role in the process of recombination leading to the production of diverse types of gametes. In diploids, the nature and frequency of chiasma formation have been resolved in a variety of crop plants (Darlington 1932, Fedak 1973, David 1974, Jones 1974, Parker 1975, Srivastava and Balyan 1977, Srivastava 1980 a, b, and others). However, induced autotetraploids, which have varying levels of gametic fertility, have not received adequate attention (Kaul 1964, Hazarika and Rees 1967, Dynansagar and Sudhakaran 1970, Hossain and Moore 1975, and others). Moreover, pea has inherent drawback of abnormal meiosis even in diploid (Gottschalk 1968, Blixt 1972, Muller 1976) leading to poor resolution of chromosomes until metaphase I and hence, it is difficult to resolve the chiasma formation. This paper examines the attempt of scoring chiasma formation at metaphase I and its subsequent partitioning in autotetraploids of four diverse genotypes of pea (*Pisum sativum* L.).

Materials and methods

Autotetraploids of four diverse pea genotypes, namely, T 163 (indigenous), 68 C (West Germany), 5064-S (Sweden) and PI 280064 (Canada) induced at this laboratory and their selfed progeny (C₂ and C₃ generations) alongwith their diploid counterparts were subjected to meiotic studies. PMCs were fixed in acetic-alcohol (1:3) and squeezed in a drop of 2% propiono-carmin for chromosome analysis. Ten PMCs from each sample plant in C₁, C₂ and C₃ generations were analysed.

Based on varying appearance of chromosome configurations at metaphase I, the number and relative position of chiasma were recorded in all possible types of quadrivalent and bivalent configurations.

Results and discussion

In pea, prior to metaphase I, chromosomes are highly deformed and shapeless (Gottschalk 1968, Blixt 1972 and muller, 1976) and thus chiasma is difficult to score. Further, usual method of scoring chiasma at Metaphase I resolved only a part of the chiasmata actually formed and also did not permit partitioning of total chiasmata into proximal (P) and distal (D). Therefore, an attempt was made to score the chiasma frequency and its distribution (Plates 1–3) more precisely on the line of Ward 1976 and as also favoured by Darlington 1937.

Data presented in Table I showed significant variation in the chiasma frequency scored by the revised method and the conventional method. On an average, chiasma score was more (34.5 per cell) in revised method as compared to conventional method (24.5 per cell); the increase in former over the latter was over 40 per cent.

¹ Present address: Rubber Research Institute of India, Kottayam, Kerala, India

The chiasma frequency scored on autotetraploids of the four cultivars in all the three generations (C_1 , C_2 and C_3) was statistically analysed. Variances due to treatment and cultivar were highly significant for proximal (P), distal (D) and total chiasmata (P+D). The generation effect was significant only for proximal and total chiasmata, while the cultivar and generation interaction was significant for total chiasmata only.

Plate 1. Representative drawings of quadrivalent morphology.

Figs. 1-10. Quadrivalents at metaphase I indicating the proximal (P) and distal (D) chiasma following two methods of chiasma scoring: revised and conventional (values in parentheses relate to conventional).

		P	D	P+D
Fig. 1.	Ring	4	4	8 (4)
Fig. 2.	"	4	3	7 (4)
Fig. 3.	"	3	4	7 (4)
Fig. 4.	"	3	3	6 (4)
Fig. 5.	"	3	3	6 (4)
Fig. 6.	"	4	2	6 (4)
Fig. 7.	"	2	4	6 (4)
Fig. 8.	"	3	2	5 (4)
Fig. 9.	"	3	2	5 (4)
Fig. 10.	"	2	3	5 (4)

Chiasma frequency in relation to cultivar

Autotetraploids of the cultivars 5064-S recorded maximum proximal as well as total chiasmata per cell (Table 2). However, the mean difference between proximal and distal chiasmata was minimum in the tetraploids of cultivar T 163 (0.87 per cell) as compared to maximum in the cultivar 68 C (3.00 per cell). Evidently each genotype had its own level of chiasma formation and distribution thereof.

Chiasma frequency in relation to generation

Both the proximal and total chiasmata frequencies per cell showed significant increase with the advance of generation (Table 2). The rate of increase was more in C_2 as compared to C_3 . The positive shift in means of proximal and total chiasmata from C_1 to C_3 generation with no remarkable variation in the distal chiasma suggested that the process of chiasma formation and distribution was under different genetic systems.

Plate 2. Representative drawings of quadrivalent morphology.
Figs. 11–20.

		P	D	P+D
Fig. 11.	Chain	2	3	5 (3)
Fig. 12.	Ring	4	1	5 (4)
Fig. 13.	"	1	4	5 (4)
Fig. 14.	"	2	2	4 (4)
Fig. 15.	Chain	2	2	4 (4)
Fig. 16.	Ring	3	1	4 (4)
Fig. 17.	Chain	3	1	4 (3)
Fig. 18.	Ring	1	3	4 (4)
Fig. 19.	Chain	1	3	4 (3)
Fig. 20.	Ring	4	—	4 (4)

Non-participation of distal chiasma in increasing the frequency of total chiasmata further demonstrated the existence of differences between the systems operating in the control of proximal and distal chiasmata. Rees 1961, Hazarika and Rees 1967, Jones 1967 and others recognized the genetic control of chiasma formation. Jones 1967 suggested that two independent fundamental different genetic systems maintained the efficient formation of chiasmata in rye; one system controls level of chiasma formation and the other regulates the distribution pattern. Crowley and Rees 1968 also evidenced redistribution of chiasmata in autotetraploids of rye in different generations (C_2 through C_7). Contrary to above findings,

Venkateswarlu and Rao 1976 could not get significant variation in chiasma frequency between raw and evolved tetraploids in *Coix lacryma jobi*, which might be attributed to differences in the materials under investigation or inefficient method of chiasma scoring.

Summary

Autotetraploids of four diverse cultivars, namely, T 163, 68 C, 5064-S and PI 280064

Plate 3. Representative drawings of quadrivalent and bivalent morphology.

Figs. 21-25. Quadrivalents at metaphase-I indicating the proximal (P) and distal (D) chiasmata following two methods of chiasma scoring: revised and conventional (values given in parentheses relate to conventional).

	P	D	P+D
Fig. 21. Ring	—	4	4 (4)
Fig. 22. Chain	2	1	3 (3)
Fig. 23. "	1	2	3 (3)
Fig. 24. "	3	—	3 (3)
Fig. 25. "	—	3	3 (3)

Figs. 26-35. Bivalents at metaphase-I indicating the proximal (P) and distal (D) chiasmata.

	P	D	P+D
Fig. 26.	—	1	1 (1)
Fig. 27.	1	—	1 (1)
Fig. 28.	2	—	2 (1)
Fig. 29.	—	2	2 (1)
Fig. 30.	—	2	2 (1)
Fig. 31.	1	1	2 (1)
Fig. 32.	1	1	2 (1)
Fig. 33.	2	1	3 (2)
Fig. 34.	1	2	3 (2)
Fig. 35.	2	2	4 (2)

and their selfed progeny (C_2 and C_3) were examined for nature and frequency of chiasma formation. The proposed revised method of chiasma scoring, considering the varied appearance of chromosome configurations, was found to be 41 per cent more efficient than the conventional method. Further, it also helped in partitioning chiasma frequency in to proximal and distal.

Table 1. Mean, range and SE of chiasma frequency in autotetraploids of four diverse cultivars of pea. Scored by conventional and revised methods

Particular	Chiasma frequency (per cell)			
	Conventional method	Revised method		
	Total chiasmata	Proximal (P)	Distal (D)	Total (P+D)
Cultivar T 163				
Mean	23.9	18.5	16.4	34.9*
SE	± 0.458	± 0.586	± 0.664	± 0.794
Range	21–26	16–23	14–21	32–39
Cultivar 68 C				
Mean	24.1	18.9	14.1	33.0*
SE	± 0.639	± 0.727	± 0.715	± 0.078
Range	21–27	16–24	11–18	27–38
Cultivar 5064-S				
Mean	25.5	21.0	14.6	35.6*
SE	± 0.496	± 0.721	± 0.816	± 0.920
Range	23–28	18–25	10–18	31–40
Cultivar PI 280064				
Mean	24.4	18.0	16.6	34.6*
SE	± 0.829	± 1.278	± 0.961	± 1.318
Range	19–28	10–24	12–21	28–41
Pooled over cultivar (mean)	24.48	19.10	15.43	34.53

* Significant at $P=0.05$ from the chiasma scored by conventional method.

Table 2. Mean proximal (P), distal (D) and total chiasmata (P+D) in autotetraploids of pea. Cultivar- and generation-wise

Particular	Chiasma frequency per cell		
	P	D	P+D
Cultivar wise			
T 163	18.00	17.13	35.13
68 C	16.27	13.27	29.53
5064-S	20.20	17.73	37.95
PI 280064	16.13	13.20	29.33
CD at 5%	1.59	1.80	0.73
Generation-wise			
C_1	15.20	15.45	30.65
C_2	18.20	15.45	33.15
C_3	19.65	15.50	35.15
CD at 5%	1.38	NS	0.634

Chiasma formation and its distribution varied with the genotype. Autotetraploid of the cultivar 5064-S recorded maximum proximal and total chiasmata per cell. Both proximal and total chiasmata showed significant increase with the advance of generation; distal chiasma did not contribute in enhancing the frequency of total chiasmata suggesting different control mechanisms for proximal and distal chiasmata formation and distribution.

Acknowledgments

Authors are grateful to Dr. W. Gottschalk (W. Germany), Dr. S. Blixt (Sweden) and Dr. A. E. Slinkard (Canada) for providing the diploid materials of pea.

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