Pachytene Analysis in Two Varieties of *Oryza sativa L**

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The limited size variation and the smallness of the absolute length of the somatic chromosomes have stood in the way of progress in the karyotypic analysis in the genus *Oryza.* Pachytene analysis has been recognised in recent times as a very useful method for the study of rice karyomorphology.

Results of pachytene analysis in two varieties of *0. sativa* belonging to the *javanica* sub-species are presented in this paper.

Review of Literature

Pachytene analysis in the genus *Oryza* was initiated for the first time by Yao *et a!* (1958) for demonstration of cryptic structural hybridity as a cause for inter-varietal sterility.

Shastry *et al* (1960) employed this technique for analysis of the karyotype of a strain of 0. *sativa* and observed that the longest chomosome was four times the size of the shortest as in the mitotic complement.

The usefulness of pachytene stage for karyotypic analysis in *Oryza* has been demonstrated by Shastry (1962, 1964) wherein he has reviewed the work done on this aspect at the Indian Agricultural Research Institute, New Delhi, in recent years. It has been concluded that the cultivated species of *Oryza* possess more asymmetric karyotypes than the wild species and that a diversity between the sub-species *indica* and *japonica* is also apparent.

Shastry *et al* (1962) studied the pairing at pacbytene in four forms of *0. spontanea* and found various abnormalities like differential segments, loosely paired regions and heteromorphic terminal ends. These were cited as evidence for the hybrid origin of these forms as postulated by Sampath and Rao (1951) although it was not possible to exclude the possibility of their origin as a result of high frequency of out pollination in a stable pure species.

Materials and Methods

Studies were limited to the following two varieties of *Oryza sativa* L. selected from

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the germplasm maintained at the Paddy Breeding Station, Coimbatore:

1. W. 108 - a *bulu* type introduced from Indonesia,
2. T. 2357 - a *tjereh* type introduced from Indonesia

Young panicles of proper stage were fixed in Farmer's fluid (1:3 acetic alcohol) using acetic acid saturated with ferric acetate. Fixing was done between 9.30 and 10.00 a. m., material kept in fixative overnight, washed and stored in 70 per cent alcohol.

Temporary slides made with 2 per cent propiono-carmine stain were used for analysis and for camera lucida drawings.

The measurements of absolute length, length of arms, and unpaired segments of the chromosomes were made from the drawings by using a vernier calipers and these were then converted into microns. The chromosomes were numbered from 1 to 12 in a descending order of their lengths. The relative lengths and the arm ratios of of the chromosomes were used for erecting idiograms and in comparing the karyotypes.

Results

AU UK* twelve chromosomes could be traced out from end to end in two cells of the *bulu* type and three cells of the *tjereh* type. Two nucleoli were observed in all the cells, one comparatively smaller than the other; but the nucleolar chromosomes could not be indentified.

The average lengths and arm ratios of the individual chromosomes, the cell to cell variation for the total chromatin length, and the absolute lengths, relative lengths and arm ratios of the individual chromosomes for both the types are presented in Tables I to IV.

In one of the cells in the *bulu* type the chromosome number 3 on its short arm, and 9, 10, 11 and 12 on their long arms showed interstitial unpaired regions 3.41 μ, 4·01 μ, 0.48 μ, 6·21 μ, and 3·21 μ long respectively and the chromosome number 10 showed a terminal loosely paired region 0 19 μ long (vide cell No. 5 in Plate I and Fig. 1). The "Differential Index" worked out to 6·14 per cent.

Discussion

Low degree of condensation of chromosomes is the main advantage of the panchytene stage which allows a higher degree of refinement in karyotypic analysis. Though the low degree of the condensation of chromosomes is of advantage, the absolute lengths of chromosomes at this stage cannot be much relied upon, especially for comparison of karyotypes mainly due to the difficulty of identifying and utilising PMC.s at "mid-pachytene" for analysis. Even among cells considered to belong to mid-pachytene based on visual observation, Gopakumar (1961) recorded cell to cell variation in the total chromatin length in the same line of *O. spontanea* up to about 130 *ft*,

Tjio and Hagberg (1951) introduced the usage of "relative value" for comparison of individual chromosomes in different species or cultures as the best way of obviating the errors of absolute measurements. This method of comparison has been made use of by many workers in different crops (Venkateswarlu and Reddi., 1956 in *Sorghum subglabrescens*; Misra, 1960 in *Oryza sativa*). Comparing the absolute values and the relative values of individual chromosome lengths in four

TABLE I

Mean values of absolute length, relative length, and arm ratio of individual chromosomes in the karyotypes of two varieties of *Oryza satiya* L. by pachytene

Variety	Chromosome No.	Length in μ			Relative length	Arm ratio Short/Long	Classification
		Long	Short	Total			
W. 108	1	46.49	15.80	62.29	15.31	0.35	SM
	2	36.25	12.93	49.18	12.09	0.37	SM
	3	29.86	12.25	42.11	10.36	0.41	SM
	4	21.88	19.11	40.99	10.08	0.87	M
	5	22.25	J6.11	38.36	9.43	0.74	M
	6	21.26	11.81	33.07	8.13	0.59	SM
	7	19.86	10.14	30.00	7.38	0.53	SM
	8	16.38	10.78	27.16	6.68	0.69	SM
	9	16.66	5.77	22.42	5.51	0.40	SM
	10	11.40	9.76	21.16	5.20	0.86	M
	11	15.22	4.03	19.25	4.73	0.29	ST
	12	9.83	7.82	17.65	4.34	0.84	M
T. 2357	1	34.19	13.58	47.77	• 13.70	• 0.39	SM
	2	28.15	10.54	38.69	11.29	0.43	SM
	3	22.11	13.86	35.97	10.43	0.62	SM
	4	19.68	11.13	30.81	8.98	0.56	SM
	5	22.47	6.83	29.30	8.57	0.35	SM
	6	16.26	11.48	27.74	8.13	0.72	M
	7	15.77	10.55	26.32	7.67	0.68	M
	8	16.33	7.91	24.24	7.10	0.51	SM
	9	15.75	7.62	23.37	6.81	0.49	SM
	10	13.76	8.16	21.92	6.36	0.55	SM
	11	13.61	6.65	20.26	5.90	0.60	SM
	12	13.10	4.43	17.53	5.06	0.32	ST

M : Median, arm ratio greater than 0.66

SM : Submedian, arm ratio between 0.33 and 0.66

ST : Sub-telocentric, arm ratio less than 0.33

TABLE II

Cell to cell variation in absolute lengths of individual chromosomes and total chromatin length in two varieties of *Oryza sativa* L.

Chromosome No.	Length in μ								
	Variety W. 108				Variety T. 2357				
	Cell No. 1	Cell No. 2	Mean	S. D.	Cell No. 1	Cell No. 2	Cell No. 3	Mean	S. D.
1	54.67	69.90	62.29	10.76	32.75	59.03	51.54	47.77	13.54
2	47.29	51.06	49.18	2.26	32.48	38.76	44.83	38.69	6.18
3	45.52	38.70	42.11	4.82	28.11	38.76	41.03	35.97	6.90
4	43.61	38.36	40.99	3.71	25.93	33.37	33.13	30.81	4.23
5	40.40	36.31	38.36	2.89	25.52	30.23	32.15	29.30	3.41
6	35.90	30.24	33.07	4.00	24.63	29.55	29.06	27.75	2.71
7	31.32	28.67	30.00	1.87	22.11	28.59	28.26	26.32	3.65
8	28.45	25.87	27.16	1.52	21.50	26.68	24.54	24.24	2.60
9	19.59	25.26	22.43	4.01	19.52	26.55	24.03	23.37	3.59
10	17.33	24.99	21.16	5.41	17.40	24.43	23.93	21.92	3.92
11	15.42	23.07	19.25	5.40	16.78	23.74	20.27	20.26	3.48
12	14.53	20.75	17.64	4.40	13.64	22.93	16.00	17.52	4.83
Total	394.03	413.18	403.61	1.35	280.37	382.62	368.77	343.92	55.48

TABLE III

Cell to cell variation in relative lengths of individual chromosomes in two varieties of *Oryza sativa* L.

Chromosome No.	Length in p								
	Variety W. 108				Variety T. 2357				
	Cell No. 1	Cell No. 2	Mean	S. D.	Cell No. 1	Cell No. 2	Cell No. 3.	Mean	S. D.
1	13.88	16.92	15.40	2.15	11.69	15.43	13.98	13.70	1.89
2	12.00	12.36	12.18	0.24	11.58	10.13	12.15	11.29	1.04
3	11.55	9.37	10.46	1.54	10.03	10.13	11.13	10.43	0.61
4	11.07	9.28	10.18	1.26	9.25	8.72	8.98	8.98	0.26
5	10.25	8.79	9.52	1.04	9.10	7.90	8.72	8.57	0.60
6	9.11	7.32	8.22	1.26	8.78	7.72	7.88	8.13	0.57
7	7.95	6.94	7.45	0.71	7.89	7.47	7.66	7.67	0.22
8	7.22	6.26	6.74	0.68	7.67	6.97	6.65	7.10	0.52
9	4.97	6.11	5.54	0.81	6.96	6.94	6.52	6.81	0.26
10	4.40	6.05	5.23	1.17	6.21	6.39	6.49	6.36	0.14
11	3.91	5.58	4.75	1.18	5.98	6.21	5.50	5.90	0.36
12	3.69	5.02	4.36	0.94	4.86	5.99	4.34	506	0.85

TABLE IV

Cell to cell variation in arm ratios of individual chromosomes in two varieties of *Oryza sativa L.* (Short arm/Long arm)

Chromosome No.	Arm ratio								
	Variety W. 108				Variety T. 2357				
	Cell No. 1	Cell No. 2	Mean	S, D.	Cell No. 1	Cell No. 2	Cell No 3	Mean	S. D.
1	0.14	0.56	0.35	0.29	0.05	0.82	0.31	0.39	0.62
2	0.43	0.30	0.37	0.09	0.42	0.72	0.14	0.43	0.29
3	0.46	0.36	0.41	0.07	0.28	0.80	0.78	0.62	0 29
4	0 96	0.78	0 87	0.13	0,44	0.49	0.76	0.56	0.27
5	0.91	0.56	0.74	0.24	0.66	0.20	0.18	0.35	0.27
6	0.42	0.76	0.59	0.24	041	0.75	0.99	0.72	0.32
7	0.42	0.63	0.53	0.15	0 68	090	0.47	0 68	0.22
8	0.51	0.87	0.69	0.25	0.17	057	0.79	0.51	0.31
9	0.61	0.19	0.40	0.29	0 42	0.38	0.66	0.49	0.15
10	0.87	0.85	0.86	0.01	0.32	051	0.82	0.55	0.25
11	0.36	0.21	0.29	0.10	0 13	0.62	0.94	0.60	0.33
12	0.99	0.68	0.84	0.22	0.30	0.44	0.23	0.32	0.17

TABLE V

Classification of karyotypes of the genus *Oryza* (Based on the scheme of Stebbins, 1958) (After Shastry, 1962)

Ratio Largest/ Smallest	Proportion of chromosomes with arm ratio > 2:1			
	0.00	0,.01 - 0.50	0.51 0.99	1.00
< 2:1	1.a	2,a	3.a	4.a
2:1 - 4:1	1.b 1. *0. perennis* (var. *barthi)* *2. 0. perennis* (Assam)	2.b L 0. *perennis* (var. *balunga)* 2. *0. spontanea* 3. *0. australiensis* 4. *0. officinalis* 5. *0. stapfii* 6. *o< sativa* Sub Sp *indica* 7. *0. sativa* sub. sp. *javanica* (var. feu/.u)*	3.b 1. *0. Glaberrima* 2 0. *sativa* sub. Sp. *javanica* (var *tjereh*) *	4.b
>4:1	1.c	2.c	3.c 1. *O. saliva* (sub sp. *japonic* a	4.c

* Based on the present study

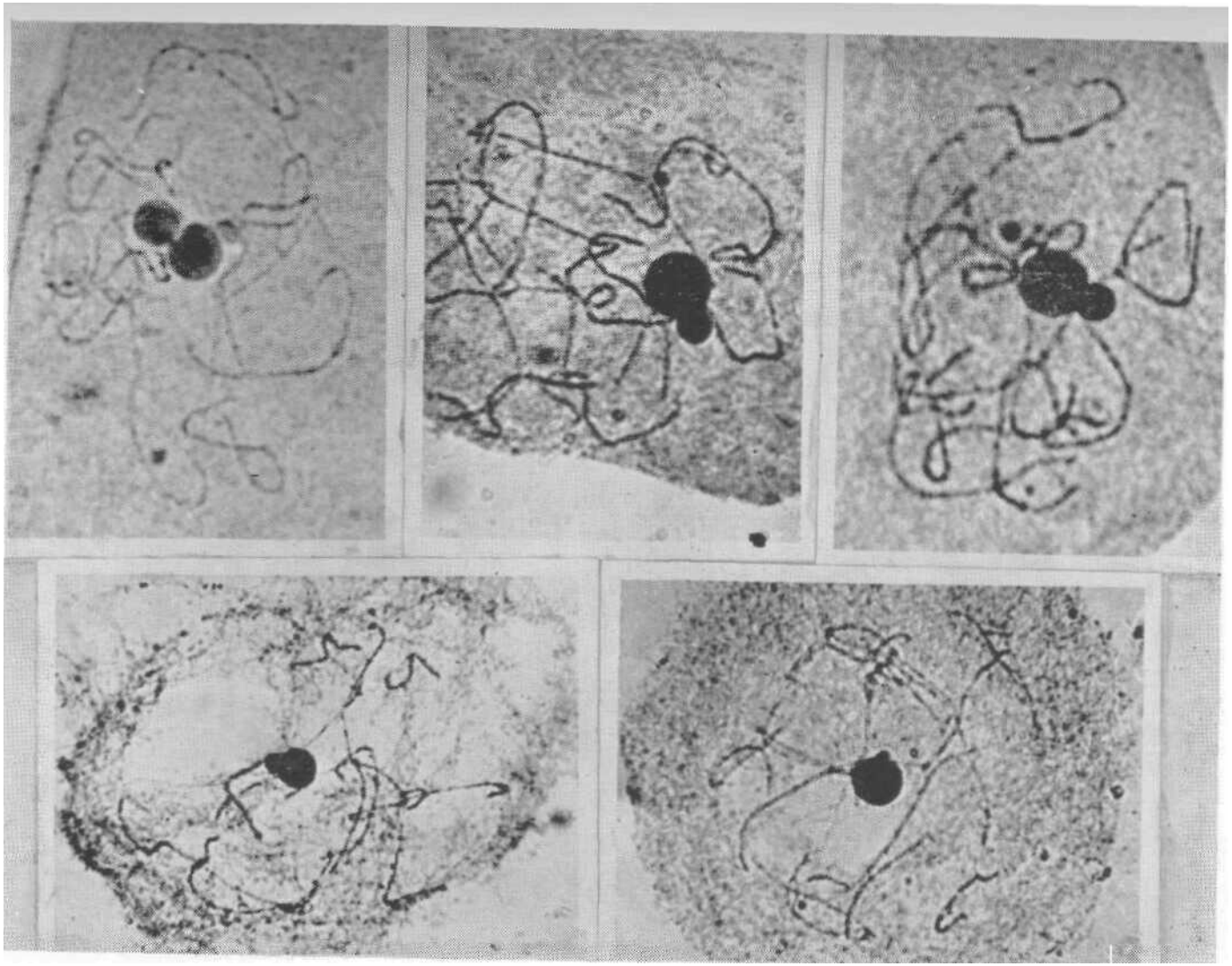

Plate 1. Photomicrographs of PMCs. at pachytene
1—3 (Top row) Variety T 2357 (Tjereh)
4—5 (Bottom) Variety W. 108 (Bulu)

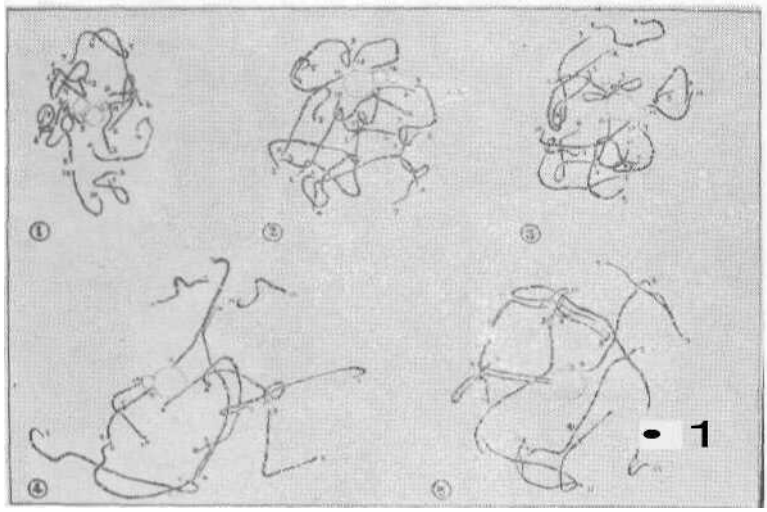

Fig. 1. Camera–lucida drawings of pachytene chromosomes
1—3 (Top row) Variety T. 2357 (Tjereh)
4—5 (Bottom) Variety W. 108 (Bulu)

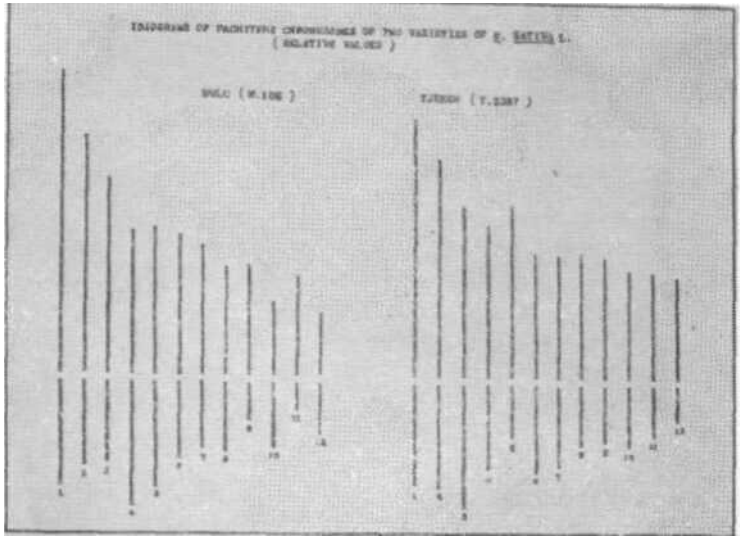

Fig. 2. Idiograms of pachytene chromosomes of two varieties of *o. Sativa* L.

lines of *0. spontanea*, Gopakumar (1961) found that the former showed a wide divergence in the different PMC.s while in the latter the variation was negligible. Data presented in Tables II and III allow a comparison of the cell to cell variation in the absolute length and relative length of individual chromosomes in the two varieties of *0. sativa* studied in the present investigation and point out that compared to the absolute lengths, the relative lengths show lesser variation from cell to cell.

Next to relative length, the arm ratios have been considered important in comparing the individual chromosomes. Fair degree of uniformity for arm ratios in different PMC.s for the chromosome complements of four lines of *0 spontanea* has been recorded by Gopakumar (1961). In the present study, however, the cell to cell variation of the arm ratios individual chromosomes in both the varieties was fairly high compared to the relative lengths (Table IV). In a study on the variability in length and arm ratios of pachytene chromosomes in 271 micro-sporocytes of corn, Maguire (1962) found that longer chromosome arms have greater variances but smaller co-efficients of variability and that chromosomes with larger arm ratios have inherently more variable arm ratios.

The scheme of classification of karvotypes evolved by Stebbins (1958) employing variation in size between the extreme members of the complement and the preponderance of sub-terminal chromosomes as two criteria for asymmetry has been utilised by Shastry (1962 & 1964) for comparison of karyotypes of different species of *Oryza* using data from pachytene. According to this classification the karyotypes belonging to group 1. a are most symmetric while those belonging to group 4. c are most asymmetric. Among other observations Shastry *(loc. cit.)* found that the *indiea* and *japonica* subspecies of *0. sativa* were included in groups 2.b and 3 c respectively. In the present study *bulu* and *tjereh* varieties could be included under groups 2. b and 3. b respectively (Table V and Fig. II). Intensive studies of more varieties appear to be warranted for confirmation of these findings.

The occurrence of differential segments etc., in *0. spontanea* pachytene bivalents has been explained to be either due to the hybrid origin of the complex species or due to its out pollinated nature (Shastry *et al.*, 1962). In the present study one of the the two PMC.s analysed in the *bulu* type showed a differential index of 6.41 per cent. The *bulu* types have been recorded to be characterised by a high degree of natural cross pollination upto 15 per cent (Chandraratna, 1951), and hence the occurrence of the differntial segments may be as a result of this.

Summary

Studies on the pachytene karyotypes of two varieties of *Oryza sativa* indicated that:

(i) Relative lengths showed lesser cell to cell variation compared to absolute lengths of chromosomes at pachytene stage, and

(ii) There is possible inter-varietal karyotypic diversity in *0. Saliva.*

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References

1. Chandraratna, M. F. (1951) Rice in Java *Trop. Agriculturist, 107:* 103-109

2. Gopakumar, K. (1961) Pachytene analysis *(in Oryza spontanea* M. Sc. **Thesis,** 1. A. R. I. (Unpublished)

3. Maguire, M. R. (1962) Variability in length and arm ratio of pachytene chromosomes of corn ***Cytologia,*** 27: 248-57

4. Misra, R. N. (1960) Pachytene analysis in *Oryza sativa* L. M. Sc. Thesis I. A. R. I. (Unpublished)

5. Shastry, S. V. S. (1962) Pachytene analysis in the genus *Oryza, Bull. Bot. Surv. India* 4: 241–247

6. Shastry, S. V. S, (1964) A new approach to the study of rice karyomorphology *Symp. Rice Genetics and Cytogenetics*, Elssiver Pub. Co. Amsterdam, pp. 62-67

7. Shastry, S. V. S., Das, D. C. and Gopakumar, K. (1962) Karyotypic studies in *Oryza* by pachytene analysis *Proc.* 49th. *Ind Sc. Cong* Part III, Section VI Abst. No. 217

8 Shastri, S. V. S , Ranga Rao, D. R. and Misra, R. N. (1960) Pachytene analysis in *Oryza,* I - Chromsosome morphology in *O.sativa. Indian J. Genet* **20** : 15-21

9. Stebbins, G. L. (Jr) (1958) The inviability, weakness and sterility in interspecific hybrids *Adv. Genet* 9: 147-215

10. Tjio, J. H. and Hagberg, A. (1951) Cytological studies on some X-ray mutants of barley *Ann- Est; Exp. Aula. Dei,* 2: 149-64 (Quoted by Gopakumar, 1961)

11. Venkateswarlu, T., and Ramakrishna Reddi V. (1956) Morphology of the pachytene chromosomes and meiosis in *Sorghum subglabrascens J. Indian. Bot. Soc.,* 35: 344-56