Indian J. Genet., **56** (1): 94–99 (1996)

A PARTIAL MALE STERILE RICE PLANT (*ORYZA SATIVA* L.) — CYTOGENETICS OF MULTIVALENT CHROMOSOMAL ASSOCIATIONS

T. RAM AND N. D. MAJUMDER

Central Agricultural Research Institute, Port Blair
Andaman & Nicobar Islands 744101

(Received: May 3, 1993; accepted: June 10, 1995)

ABSTRACT

A partial male sterile plant of spontaneous origin was isolated from a traditional tall rice variety Singhawar. The cytological observations of male sterile plant suggested that chromosomal irregularities such as abnormal chromosome number, chromosomes not arranged properly at metaphase plate, chromosomal fragments at pollen mitosis, and high frequency of quadrivalents ($1_{IV} + 10_{II}$, $2_{IV} + 8_{II}$ and $3_{IV} + 6_{II}$) and trivalent associations in meiocytes probably caused male sterility. The multivalent associations combined with aneuploidy in the pollen mother cells of selfed progenies caused high pollen sterility.

Key words: Rice male sterility, multivalents, cytogenetics.

Male sterility could be found in nature originating from spontaneous mutations or occasional hybridization with closely related wild relatives. The information on male sterility whether it is genic, cytoplasmic or chromosomal, could find useful applications. Cytological studies related to male sterility in plants were reported earlier [1–4]. Here we report cytological studies on partial male sterile plants isolated in a field crop of rice variety Singhawar.

MATERIALS AND METHODS

A partial male sterile dwarf rice plant was identified in a field of traditional tall cultivar Singhawar during germplasm collection from Uttar Pradesh where abundant spread of a wild species of *O. rufipogan* Griff was also found. The male sterile plant was dwarf (46 cm) with profuse tillering and semispread plant habit. Pollen fertility and cytological observations were recorded on the male sterile plant. Pollen germination and pollen tube

---

*Present address: Directorate of Rice Research, Rajendra Nagar, Hyderabad 500030.

growth were studied in normal (cv. Singhawar) and male sterile plants in pollen media. Panicles containing florets at appropriate stage were fixed in aceto-alcohol (1:3) and stained in with aceto-carmine for meiotic studies. Observations on chromosomal associations were recorded in diplotene, diakinesis, and first metaphase of meiotic cell division. In addition, this plant was multiplied vegetatively by separating the tillers and several panicles were covered with butter paper bags to obtain selfed seed. Out of several panicles bagged only 31 seeds were set. All the seeds were germinated in laboratory. Out of 31 seeds only 17 plants developed, 8 seeds did not germinate and 6 plants died after germination. After 20 days each seedling was transplanted separately in cement pots of uniform size. Pollen meiosis (in 50 PMCs each) and pollen fertility were studied in the selfed progenies (17 plants) of male sterile plants. Observations were also recorded on plant height, number of tillers/plant, spikelet sterility, pollen sterility, growth habit and grain shattering at the time of maturity.

RESULTS AND DISCUSSION

Pollen stained with aceto-carmine showed that 90 per cent pollens were sterile. The pollen grains of sterile and normal plants were germinated in media for one and two hours, the data recorded as germination percentage (Table 1) revealed that the sterile plant had poor germination in both the periods (51.7% and 61.8%, respectively) as compared to the normal plant (80.6 and 87.1%). Similarly, poor pollen tube growths were also recorded in the sterile plant in comparison to the normal one. Pollen mitosis showed chromosomal irregularities (Table 2), such as, irregular number of chromosomes (34.1%), chromosomes not lying on metaphase plate (24.1%), chromosome fragments (32.9%) and stickiness (0.9%). Normal pollen mitosis was observed only in 8 per cent of cells. Such abnormalities in pollen mitotic cell division might be affecting the pollen grains fertility. In normal plant (Singhawar), no such abnormalities were observed. The observation on meiotic cell division showed the formation of multivalents, such as, quadrivalents and trivalents

Table 1. In vitro pollen germination of the sterile rice plant

Incubation period (h)	Pollen grain germination (%)		Pollen tube growth (μm)	
	sterile	normal	sterile	normal
1	51.7	80.6	99.2	369.0
2	61.8	87.1	237.8	384.9

Table 2. Chromosomal abnormalities in pollen mitosis of the sterile rice plant

Type of cell division	Cells in different categories	
	total	%
Irregular chromosome number	85	34.1
Irregular metaphase	60	24.1
Fragments	82	32.9
Stickiness	2	0.9
Normal	20	8.0
Total	249	100.0

which may give rise to irregular distribution of chromosomes in the anaphase. Among the chromosomal association observed in diplotene, diakinesis and metaphase I, the frequency of $1_{IV} + 10_{II}$ was highest (30.9, 66.1 and 42.3%, respectively) followed by $2_{IV} + 8_{II}$ besides occasional trivalent formation were also observed in diplotene and metaphase I (Table 3). Disturbances in spindle fibre formation, chromosomes not lying on metaphase plate, unequal distribution of chromosomes, formation of laggards and bridges at anaphase I, followed by chromosomal elimination, and formation of micronuclei at anaphase II were observed in meiocyte cells. Observations on multivalent formation, occurrence of laggards, chromosomal elimination, spindle disruption in meiocytes have been reported [5] earlier in an interspecific hybrid (*O. rufipogan* x *O. sativa* var IR 50). The accession of *O. rufipogan* used in interspecific hybrid was collected from the same locality where the present male sterile plant was identified. In the light of these observations as well as similarity in plant habits like semispreading growth habit, grain shattering, profuse tillering and greater height, it is assumed that the sterile plant probably originated through outcrossing of the rice cultivar with *O. rufipogan* in the natural habitat. High frequency of quadrivalent formation accompanied with occasional fragments indicates structural changes like translocational heterozygote and inversion. This may have affected the genetic balance and gametophytic viability which ultimately caused male sterility [4, 6, 7]. Nondisjunction, failure of proper coorientation and mispairing may cause aneuploidy [2]. Seed formation and its viability in a selfed hybrid is possibly of segregational nature where the selfed progenies could be heterozygous for the interchanges. However, the exclusive formation of bivalents is unexpected.

Table 3. Chromosomal associations in meiocytes of the sterile rice plant

Stage	Chromosomal associations			Distribution of PMCs	
	IV	III	II	total No.	%
Diplotene	1	—	10	17	30.9
	—	2	9	10	18.2
	2	2	5	3	5.5
	1	2	7	2	3.5
	2	—	8	10	18.2
	3	—	6	3	5.5
	—	—	12	10	18.2
Diakinesis	1	—	10	41	66.1
	—	—	12	4	6.5
	3	—	6	3	4.8
	2	—	8	14	22.6
Metaphase	1	—	10	22	42.3
	—	—	12	8	15.4
	2	—	8	14	26.9
	—	2	9	4	7.7
	1	2	7	4	7.7

The phenotypic characters of selfed progenies of the sterile plant revealed plant height ranging from 30.1 to 58.6 cm, profuse tillering in most of the plants, very high spikelet (50–96%) and pollen sterility (39.9–100%). Six out of 17 plants were erect, 6 semierect, and 5 spreading (Table 4). Five plants showed grain shattering and 12 nonshattering. All the plants were awnless with pigmented apiculus and stigma.

Table 4. Phenotypic characteristics of the selfed progenies of the sterile rice plant

Plant No.	Plant height (cm)	Tillers per plant	Spikelet sterility (%)	Pollen sterility (%)	Growth habit	Grain shattering
1	47.2	17	62.5	77.5	Semierect	Nonshattering
2	46.5	12	88.2	82.8	Semierect	Nonshattering
3	58.6	7	76.9	81.6	Erect	Shattering
4	43.0	19	83.8	90.7	Semierect	Nonshattering
5	36.3	23	50.0	51.6	Erect	Nonshattering
6	35.2	15	66.7	61.6	Spreading	Nonshattering
7	45.8	14	84.4	88.4	Erect	Nonshattering
8	35.1	24	86.7	83.2	Semierect	Shattering
9	32.0	26	85.0	91.7	Spreading	Nonshattering
10	30.5	15	55.0	39.9	Spreading	Nonshattering
11	34.5	23	96.0	100.0	Erect	Shattering
12	36.4	13	91.3	97.2	Erect	Nonshattering
13	44.6	24	92.1	87.8	Semierect	Nonshattering
14	30.1	15	61.9	63.4	Spreading	Shattering
15	52.0	16	68.6	60.9	Erect	Nonshattering
16	38.2	23	64.7	77.3	Spreading	Shattering
17	41.2	13	68.0	59.3	Semierect	Nonshattering

The tendency of multivalent formation in the meiotic cells was inherited by the selfed progenies also (Table 5). All the selfed plants had very high frequency of quadrivalent formation, except in one plant (10.0 to 83.4%). In addition, variety of multivalent associations, such as, $1_{IV} + 10_{II}$, $2_{IV} + 8_{II}$, $3_{IV} + 6_{II}$ and $4_{IV} + 4_{II}$, were observed in these plants. Out of the 17 plants studied, 13 had aneuploid chromosome numbers in 3.3 to 86.8% metaphase I cells (Table 5) and 4 plants had only multivalents. One plant, although free from multivalent associations, had high frequency of aneuploid chromosome number in the meiocytes. It is interesting to note that the aneuploidy up to $2n = 10_{II}$ was also observed in the meiocytes of the progeny of the sterile plant. The plants with multivalent associations and/or aneuploid chromosome number in their meiocytes had very high pollen sterility (Tables 4, 5). The quadrivalent formation was perhaps due to translocation and inversion [1]. The structural heterozygosity transmitted to the progeny by the sterile plant is evident from the formation of multivalents during meiosis in the selfed progenies.

Table 5. Frequency of different chromosomal associations in the selfed progenies of the sterile rice plant at metaphase I

Plant No.	Chromosomal associations (%)									Total multi-valent associ-ations	Cells with aneuploid chromo-some numbers
	12_{II}	10_{II} + 1_{IV}	8_{II} + 2_{IV}	6_{II} + 3_{IV}	4_{II} + 4_{IV}	10_{II}	11_{II}	13_{II}	14_{II}		
1	42.8	28.6	28.6	—	—	—	—	—	—	57.2	—
2	26.6	14.3	18.4	16.3	2.0	6.1	6.1	10.2	—	51.0	22.4
3	30.5	27.7	19.4	5.6	—	5.6	5.6	5.6	—	52.7	16.8
4	24.4	28.9	22.3	6.7	—	4.4	13.3	—	—	57.9	17.7
5	44.4	22.3	33.3	—	—	—	—	—	—	55.6	—
6	30.6	36.1	33.3	—	—	—	—	—	—	69.4	—
7	12.2	19.5	26.8	—	—	6.3	9.8	19.6	5.8	46.3	41.5
8	13.3	16.7	36.7	20.0	10.0	—	—	3.3	—	83.4	3.3
9	20.8	14.0	30.2	4.7	—	9.3	14.0	7.0	—	48.9	30.3
10	59.3	9.4	6.3	—	—	—	18.7	6.3	—	15.7	25.0
11	7.5	2.5	5.0	2.5	—	25.0	42.5	12.5	2.5	10.0	82.5
12	13.2	—	—	—	—	30.2	30.2	18.8	7.6	—	86.8
13	8.3	14.6	12.5	—	—	12.5	16.7	27.1	8.3	27.1	64.6
14	14.8	16.4	23.0	18.0	—	4.8	19.7	3.3	—	57.4	27.8
15	59.2	18.4	22.4	—	—	—	—	—	—	40.8	—
16	38.7	16.6	25.8	—	—	—	—	19.4	—	41.9	19.4
17	38.7	16.6	12.9	3.2	—	—	22.6	6.5	—	32.2	29.1
Mean	28.8	17.8	21.0	4.5	0.7	6.1	11.7	8.3	1.4	44.0	27.5

As the male sterility is due to the multivalent associations following the formation of laggards, bridge, chromosomal elimination at anaphase, and high frequency of segregational aneuploidy, the chromosome numbers in the meiocytes of the selfed progenies may produce aneuploid plants which can be utilized for genetic investigations, gene mapping, and in breeding programme.

REFERENCES

1. C. D. Darlington. 1982. Chromosome behaviour and structural hybridity in *Tradescentia*. J. Genet., **21**: 207–286.

2. M. S. Brown, M. Y. Menzel, C. A. Hasenkampf and S. Nagi. 1981. Chromosome configurations and orientations in 58 heterozygous translocations in *Gossypium hirsutum*. J. Hered., **72**: 161–168.

3. T. D. Ray. 1984. Metaphase I configurations of the reciprocal translocation in a cytogenetic tests of *Gossypium hirsutum*. J. Hered., **75**: 371–377.

4. D. H. Ling, M. F. Chen, W. Y. Chen and Z. R. Ma. 1988. The cytogenetic research on desynaptic variation of somaclone from somatic cell culture of *indica* rice. Acta Genet. Sinica, **15**: 86–88.

5. N. D. Majumder and T. Ram. 1992. Chromosomal behaviour in an interspecific rice hybrid (*O. rufipogan* Griff. x O. Sativa L.). Exp. Genet., **8**: 58–61.

6. C. R. Burnham. 1956. Chromosomal interchange in plants. Bot. Rev., **22**: 419–552.

7. W. Gottschalk. 1978. Open problems in polyploid research. Nucleus, **21**: 91–112.