Indian J. Genet., **60**(4): 441-450 (2000)

GENETIC AND CYTOGENETIC ANALYSIS OF SPIKELET STERILITY IN *INDICA* × *JAPONICA* CROSSES IN *ORYZA SATIVA* L.

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(Received: December, 1998; accepted: September, 2000)

ABSTRACT

Expression of variable degree of spikelet sterility in *indica* × *japonica* inter-subspecific crosses is one of the barriers in the exploitation of higher degree of heterosis manifested in such crosses. Twenty six inter-subspecific crosses derived from four *japonicas* and seven *indicas* (including two wide compatible varieties) and 12 F_2 progenies were evaluated for spikelet fertility and sterility expression. Structural heterozygosity in highly sterile cross was not detected at various stages of meiosis. Therefore, chromosomal differentiation has not been attributed to spikelet sterility in the present *indica* × *japonica* cross. Based on erratic mode of segregation for fertility and sterility, expression of wide compatible gene has been suggested to be highly cross specific, which may be due to the presence of different set(s) of modifier genes and epistasis. Trigenic complementary ratio with one basic gene for wide compatible trait obtained during the present study in four crosses may partly account for complex genetics of spikelet sterility in inter-subspecific crosses.

Key Words : *Oryza sativa* L., *indica*, *japonica*, inter-subspecific, wide compatible gene

Exploitation of higher degree of heterosis manifested in *indica* × *japonica* inter-subspecific crosses, is one of the current trends in hybrid rice breeding. However, low seed setting with poor filling of spikelet and obvious transgression of plant height and growth duration[1] are the major barriers in the commercial exploitation of heterosis from such crosses. Among these barriers, expression of variable degree of spikelet sterility in *indica* × *japonica* crosses is most significant. Most of the initial reports interpreted the spikelet sterility in *indica* × *japonica* crosses due to the small structural differences between the chromosomes of two subspecies[2, 3]. However, no correlation between hybrid sterility and meiotic abnormalities was observed, since some of the most sterile plants showed 95% normal cells at all stages of meiosis[4]. In past, several genetic models have been proposed to explain the spikelet sterility

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in inter-subspecific crosses of rice[5]. Among these models, one locus gameto-sporophytic multiple allelic (wide compatible) is most important as this wide compatible model provides the way to overcome sterility barrier in *indica* × *japonica* crosses. Ikehashi and Araki[6-8] discovered and described wide compatible varieties (WCVs) possessing wide compatible gene (*Wc*), which are able to produce normal fertile hybrids when crossed to *indica* or *japonica* lines. Subsequently, several WCVs (some with non- allelic *Wc* gene) have been identified at global level[9, 10]. In several WCVs including that of Indian origin, wide compatibility (WC) trait has been reported to be controlled by single dominant gene[9-11]. However, involvement of a pair of major genes in the expression of WC trait[1] and non-allelic interaction conditioning spikelet sterility[12] have also been reported. Further, several results on genetics of spikelet sterility in *indica* × *japonica* crosses showed complex genetic basis and several mechanisms leading to spikelet sterility have been proposed[13-15].

In view of these results, it is important to study the cytogenetic and genetic behavior of spikelet sterility in *indica* × *japonica* crosses derived from the parents of Indian origin. Therefore, a study was conducted to examine the possible existence and role of small structural chromosomal changes between *indica* and *japonica* subspecies in the light of expression of spikelet sterility and also to study the inheritance of the WC trait (i.e. spikelet fertility vs. spikelet sterility) in inter-subspecific crosses.

MATERIALS AND METHODS

Development and raising of F_1 and F_2 generations

During the dry season of 1995 at Central Rice Research Institute (CRRI), Cuttack, 28 inter-subspecific crosses were attempted utilising four early *japonica* and seven *indica* lines (Table 1) in line × tester fashion on individual plant basis. Among the *indicas*, two lines, *viz.*, Dular and Nagina-22 (N-22) were WCVs[9]. Two improved lines, *viz.*, JD-6 and JD-8 were deliberately included in crossing programme with the expectation of having *Wc* gene, since these lines are derivatives of cross between induced mutants of Dular and N-22. During the wet season of 1995 at IARI, New Delhi, seeds of 25 F_1s and parents were finally grown and evaluated for spikelet fertility. In the same season, based on preliminary results on spikelet fertility, four F_1s were also developed using JD-8 as common parent and evaluated during wet season of 1996 at IARI. Thus, in both the wet seasons at IARI, 26 cross combinations were evaluated. For obtaining F_2 seeds, plants of F_1s were bagged in order to eliminate any possibility of out crossing. A total of twelve F_1s representing highly fertile and highly sterile groups were advanced to study the segregation of spikelet

Table 1. Parental lines and spikelet fertility, wet season 1995

S.No.	Parents	Sub.sp.	Source of availability	Spikelet fertility % Mean ± SE
1	Kagalikai	*japonica*	CRRI	85.72 ± 2.90
2	Fukumishi	*japonica*	CRRI	88.32 ± 1.83
3	Siam-2	*japonica*	CRRI	95.74 ± 1.20
4	Toride	*japonica*	CRRI	92.06 ± 3.71
5	JD-5	*indica*	IARI	96.74 ± 0.44
6	JD-6	*indica*	IARI	87.23 ± 2.10
7	JD-8	*indica*	IARI	88.79 ± 2.96
8	Heera	*indica*	CRRI	73.76 ± 5.10
9	PNR-381	*indica*	IARI	92.84 ± 1.42
10	Dular	*indica*	CRRI	94.99 ± 1.01
11	N-22	*indica*	CRRI	93.53 ± 2.01

sterility in F_2 generation. F_2 families of five F_1 plants from each cross were raised and individual plants were evaluated during dry season of 1996 at CRRI. Since it was not possible to harvest sufficient quantity of F_3 seeds from F_2 individual plant due to the heavy rain followed by flood at CRRI, Cuttack during wet season of 1996, eight F_2 families were again raised from remaining F_2 seeds of the wet season, 1995, at IARI, New Delhi.

Scoring of data and classification of fertility groups

For the estimation of spikelet fertility two panicles of primary tillers from five plants of each F_1s, parents and individual F_2 plants were collected and spikelet fertility was determined by counting relative proportion of filled and empty spikelets. For F_2 progenies, maximum number of available plants from each family were screened for spikelet fertility. Initially data were recorded on each cross developed on individual plant basis but results are presented on pooled data, since there were no significant differences between the same cross combination developed from different pollen and seed plants. For parents, F1s and segregating progenies, plants with spikelet fertility percentage of 75% or above between 50% and 75% and less than 50% were classified as highly fertile, intermediate fertile and highly sterile respectively (Table 2). For the analysis of F_2 segregation test, intermediate fertility class was divided into high fertility and high sterility groups in equal proportion according to Kumar and Virmani[9].

Cytological studies

For meiotic analysis, young panicles of appropriate spikelet size from five plants of each F_1s and parents were collected in freshly prepared Carnoy's fixative (6 parts of absolute alcohol, 3 parts of chloroform, 1 part of glacial acetic acid) to which few crystals of ferric chloride were added in order to facilitate proper staining. After 24 hrs. material was transferred to 70% ethyl alcohol. For meiotic slide preparation, anthers were directly squashed in 2% acetocarmine solution and freshly prepared slides were screened for meiotic behaviour at different stages. Besides scoring meiotic configurations at metaphase I (MI), varying number of PMCs of both crosses and parents were screened at different stages (pachytene, diakinesis/metaphase I and anaphase I).

RESULTS AND DISCUSSION

Although 28 inter-subspecific cross combinations were attempted, finally plants from 26 crosses and their parents were grown and evaluated for spikelet fertility.

Spikelet fertility of parents and hybrids

Mean spikelet fertility for all parents except Heera (73.76%) was more than 75.0% (Table 1). The mean values for spikelet fertility percentage of 26 crosses and their fertility classes are given in Table 2. Results on spikelet fertility percentage of crosses revealed that like Dular and N-22, JD-8 also possesses *Wc* gene because most of the hybrids derived from JD-8 were highly fertile (Table 2). On the contrary, use of PNR-381 or Heera as a parent produced highly sterile F_1s (Table 2) and hence, PNR-381 and Heera can be used as tester parent in future study. The mean performance of JD-8 in terms of producing fertile F_1s was comparable to that of two WCVs (N-22 and Dular) and JD-8 has already been reported as new WCV[16]. However, initially

Table 2. Fertility percentage and fertility groups (in bracket) of 26 *indica* × *japonica* crosses, wet season, 1995

Parents	JD-5	JD-6	JD-8	Heera	PNR-381	Dular	N-22
Kagalikai	68.6 (IF)	61.3 (IF)	78.2 (HF)	66.1 (IF)	52.7 (IF)	83.6 (HF)	86.0 (HF)
Fukumishi	57.1 (IF)	43.1 (HS)	77.7 (HF)	55.9 (IF)	47.1 (HS)	87.7 (HF)	N
Siam-2	73.8 (IF)	59.4 (IF)	74.05 (IF)	N	54.6 (IS)	89.9 (HF)	83.4 (HF)
Toride	55.9 (IF)	42.3 (HS)	78.3 (HF)*	47.6 (HS)	41.6 (HS)	90.7 (HF)	83.8 (HF)

*data from wet season, 1996; HF - Highly fertile; IF - Intermediate fertile; HS - Highly sterile; N - cross not analysed

it was anticipated that JD-6, being a derivative from same cross combination like that of JD-8, would behave like JD-8 (WCV) but all the F_1s derived from JD-6 were of intermediate fertility group (Table 2). Hence, probable existence of non-allelic *Wc* gene in Dular and N-22 is indicated. Kumar and Virmani[9] also reported probable existence of non-allelic *Wc* genes in Dular and N-22. Earlier findings pertaining to the wide compatibility has led to the identification of several WCVs, which are being utilised in inter-subspecific hybrid breeding [9, 10].

Meiosis in F_1s and parents

Meiotic study was initiated on twelve crosses representing different fertility groups. However, based on preliminary results, study was concentrated only on two hybrids representing one each from highly fertile and sterile groups from Fukumishi × Dular and Fukumishi × PNR-381, respectively and their parents. The frequency of ring bivalents at M I was 9.84 and 9.89 per cell while those of chain bivalents was 2.16 and 2.11 in crosses Fukumishi × Dular and Fukumishi × PNR-381, respectively (Table 3). The chiasmata frequency was 23.10 in PNR-381 and 23.40 in Dular. Among

Table 3. Meiotic configurations (range) and chiasmata frequency at MI

S. No.	F_1s/Parents	R II	C II	Chiasmata/cell
1	Fukumishi × Dular	9.84 (8-12)	2.16 (0-4)	23.16 ± 0.29
2	Fukumishi × PNR-381	9.89 (8-12)	2.11 (0-4)	23.37 ± 0.31
3	Dular	9.80 (8-12)	2.20 (0-4)	23.40 ± 0.35
4	Fukumishi	9.70 (7-12)	2.30 (0-5)	23.15 ± 0.33
5	PNR-381	9.60 (8-12)	2.40 (0-4)	23.10 ± 0.30

R II - Ring bivalent; C II - Chain bivalent

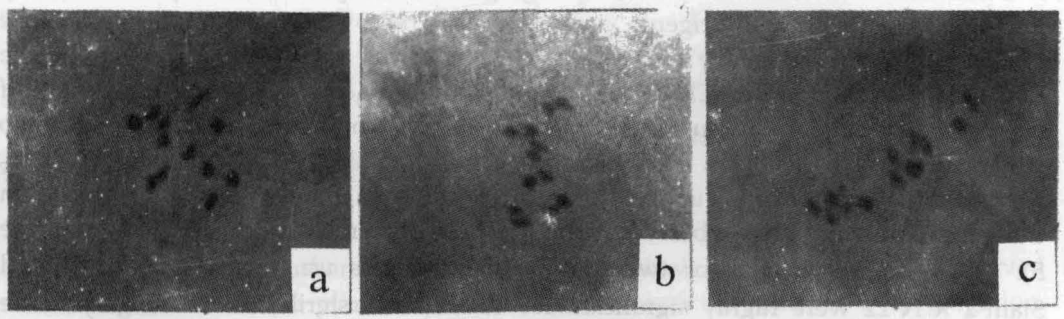

Fig. 1. Metaphase I showing 12 normal bivatents in (a) PNR-381, (b) PNR-381 × Siam-2 and (c) Siam-2

the crosses, Fukumishi × Dular and Fukumishi × PNR-381 were having 23.16 and 23.37 chiasmata per cell respectively. Hence, there were no significant differences in the meiotic configurations and chiasmata frequency at M I between highly sterile F_1 (Fukumishi × PNR-381), their parents and highly fertile F_1 (Fukumishi × Dular) (Table 3 and Fig. 1). Any kind of abnormality was not detected in highly sterile cross at several active stages of meiosis (Table 4). Furthermore, high pollen fertility associated with high spikelet sterility in cross Fukumishi × PNR-381 [16] also suggest absence of chromosomal structural heterozygosity in the cross Fukumishi × PNR- 381. These findings are in contradiction with those of earlier reports where existence of structural heterozygosity has been suggested as the cause of hybrid sterility [2, 3] and in agreement with those which attributed genetic control of spikelet sterility in *indica* × *japonica* cross [4, 17].

Table 4. Number of PMCs screened at different stages of meiosis

S. No.	F_1s/Parents	Pachytene	Dia./MI	AI	Total
1	Fukumishi × Dular	20	29	25	74
2	Fukumishi × PNR-381	17	27	20	64
3	Dular	15	20	20	55
4	Fukumishi	10	21	24	55
5	PNR-381	15	20	22	57

Genetics of spikelet sterility/fertility

In four highly fertile crosses *viz.*, Kagalikai × JD-8, Fukumishi × JD-8, Kagalikai × Dular and Fukumishi × Dular, F_2 segregation for spikelet fertility and sterility were in agreement with trigenic complimentary ratio (45:19) with one basic gene (Table 5). Since during dry season of 1996, crop was extensively damaged by the rain followed by flood at CRRI, it was not possible to harvest sufficient quantity of F_3 seeds from the individual F_2 plants for further confirmation of obtained ratio (45:19) through F_3 analysis. However, with the remaining F_2 seeds, analyses for F_2 segregation were repeated in wet season, 1996 at IARI for the aforesaid crosses. In the repeated study also, observed ratio showed agreement with 45:19 ratio (Table 5). χ^2 values, however, for two highly fertile crosses *viz.*, Kagalikai × N-22 and Siam-2 × N-22 were highly significant for 45:19 (fertile:sterile). In two highly fertile crosses viz., Siam-2 × JD-8 and Siam-2 × Dular, there were no sterile plant in the population of 45 and 144 fertile F_2 plants, respectively (Table 5). Highly sterile F_1s were also studied for segregation in F_2 generation in four crosses, derived from

Table 5. F_2 Segregation of fertility and sterility in eight highly fertile F_1s

S. No.	F_1s	Season	O/E	F_2 segregation		Total	χ^2 45:19
				Fertile	Sterile		
1	Kagalikai × JD-8	Dry,96	O	138	50	188	0.860
			E	132.19	55.81	188	
		Wet,96	O	80	37	117	0.210
			E	82.27	34.37	117	
2	Kagalikai × Dular	Dry,96	O	110	54	164	0.824
			E	115.31	48.69	164	
		Wet,96	O	58	19	77	1.06
			E	54.14	22.86	77	
3	Fukumishi × JD-8	Dry,96	O	68	30	98	0.039
			E	68.98	29.09	98	
		Wet,96	O	58	22	80	0.183
			E	56.25	23.75	80	
4	Fukumishi × Dular	Dry,96	O	86	41	127	0.410
			E	89.30	37.7	127	
		Wet,96	O	64	20	84	1.60
			E	59.06	24.94	84	
5	Siam-2 × JD-8	Dry,96	O	45	0	-	-
		Wet,96	O	103	0	-	-
6	Siam-2 × Dular	Dry,96	O	144	0	-	-
		Wet,96	O	93	0	-	-
7	Kagalikai × N-22	Dry,96	O	212	47	259	16.34*
			E	189.11	76.89	259	
8	Siam-2 × N-22	Dry,96	O	131	15	146	26.36*
			E	102.66	43.34	146	

*Significant at 1% level of significance; O - Observed ratio; E - Expected ratio

PNR- 381 as pollen parent and all four *japonicas* as female parents (Table 6). Observed ratio of fertile and sterile plants was tested against expected ratio for 1:1 segregation. The χ^2 values for only one cross (Siam-2 × PNR-381) was non-significant while in crosses Fukumishi × PNR-381, Kagalikai × PNR-381 and Toride × PNR-381, χ^2 values were significant (Table 6).

Table 6. F_2 segregation of fertility and sterility in four highly sterile F_1s, dry season, 1996

S. No.	F_1's	O/E	F_2 segregation			
			Fertile	Sterile	Total	χ^2 (1:1)
1	Kagalikai × PNR-381	O	80	62	142	
		E	71	71	142	4.00*
2	Fukumishi × PNR-381	O	82	112	194	
		E	97	97	194	4.64*
3	Siam-2 × PNR-381	O	73	57	130	
		E	65	65	130	1.97
4	Toride × PNR-381	O	81	53	134	
		E	67	67	134	5.85*

*Significant at 5% level; O - Observed ratio; E - Expected ratio

Based on results on inheritance of WC trait (spikelet fertility restoration in F_1), it may be suggested that in Dular and JD-8, WC trait is controlled by three genes (one basic and two complementary genes) at least in four crosses (Table 5) rather than single gene as according to multiple allelic model, there should not have been segregation for sterility in F_2 generation, as observed during this study (45:19 ratio). Earlier workers suggested control of WC trait by single dominant gene in Dular [9, 11] and several other WCVs[10]. However, present results on genetics of spikelet sterility are in close agreement with that of Wu[12] who suggested involvement of non-allelic interaction in the expression of WC trait. Involvement of one basic dominant gene and two complementary genes in the expression of WC trait, as obtained in this investigation, is further supported by the fact that expression of WC trait has been reported to be unstable over environment [6, 10]. But at genetic level such unstable expression of environmental sensitive characters are mostly explained in the light of presence of modifiers or epistasis. Recently, through Restriction Fragment Length Polymorphism (RFLP) analysis, three loci (one major and two minor) conferring significant effects on hybrid fertility have been identified[18].

As progenies from crosses Siam-2 × JD-8 and Siam-2 × Dular showed no segregation for sterility (Table 5), this finding could be explained either on the basis of control of WC trait by the single dominant gene in Dular and JD-8 or on the basis of the presence of different genetic constitutions of modifier gene in Siam-2 than that in Kagalikai and Fukumishi. The first explanation could be ruled out, because in other set of four crosses derived from Dular or JD-8 with Kagalikai and Fukumishi, segregation (45 fertile: 19 sterile) was observed (Table 5).

After assigning the genotypes for Dular, JD-8 and Siam-2 based on trigenic complementary ratio with one basic gene, it was expected that Siam-2 should have behaved like WCV with other *indicas* such as PNR-381, JD-5, JD-6 etc. but cross Siam-2 × PNR-381 was highly sterile. The observation on this cross really complicated the explanation for results on inheritance of sterility and fertility restoration (WC trait). Even if an inhibitory gene was assumed to be present in PNR-381, then also the F_2 segregation was not in agreement with the inhibitory ratio in this cross. Observation on other three crosses derived from PNR-381 also did not show segregation according to inhibitory ratio (Table 6). Moreover, there was unexpected mode of segregation in crosses-Kagalikai × N-22 and Siam-2 × N-22 (Table 5). It was not possible to explain all these complicated erratic segregation ratios. Hence, erratic and cross specific segregation pattern observed during present study could be explained only in the light of existence of modifier genes which influence the major gene for WC trait. Therefore, considerable variation in the fertility level of hybrids derived from the crossing of same WCV with different varieties may be observed, depending upon the kind of modifier combination(s) existing in these varieties. Such variable expression of same WCV with different lines has been observed earlier[8].

The present study indicated complex genetic basis of spikelet sterility in *indica* × *japonica* crosses. The expression of *Wc* gene seems to be highly cross specific due to the genetic differentiation for modifier genes between WCVs and normal variety (non-WCVs) and epistasis (non-allelic interaction). Trigenic complementary ratio with one basic gene for WC trait obtained during the present study in four crosses may partly account for complicated genetics of sterility in inter-subspecific crosses. Several recent reports also suggest the complex genetics and possible presence of several genetic systems influencing the manifestation of spikelet sterility in *indica* × *japonica* crosses [13-15].

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