

RESEARCH NOTE

Pollen and Spikelet Analysis in F₁ Rice Hybrids and their Parents

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ABSTRACT

Pollen analysis can be used to discriminate between different species, identify possible interspecies hybrids, identify restorer and maintainer lines, useful to study genetics of restorer gene, interaction between chromosome and cytoplasm and relationship between parents. Pollen abortion system of male sterility is an important tool in hybrid rice production and spikelet is the major yield components. Nine improved cultivars, six landraces and three wild aborted cytoplasmic-genetic male sterile (CMS) lines were used to analyze pollen and spikelet in F₁ rice hybrids and their parents. The frequency of pollen categories and its relationship to spikelet fertility were investigated. Pollen sterility of the F₁s was determined by staining pollen grains in 1% potassium iodide-iodine (I-KI) solution. Spikelet fertility was determined by counting the total number of seed set in proportion to the total number of spikelets. Correlation and regression coefficients for some traits were computed. In hybrids, pollen fertility ranged from 0.5 to 82% and spikelet fertility from 0 to 87%. Pollen fertility varied from 28 to 97%, while spikelet fertility from 73 to 91% in pollen parents. The highest and the lowest percentages of pollen fertility were found in Chaite-6 and Chiunde cultivars respectively. Spikelet fertility percentage varied widely among hybrids and many hybrids had lower spikelet fertility percentage than their parents. Therefore, it is of practical importance to understand the causes of high spikelet sterility in hybrids for possible increase in spikelet fertility. Highly significant positive correlation was found between stained round fertile (SRF) pollen and spikelet fertility. The positive value of correlation and regression coefficient on SRF and spikelet fertility were found between F₁ and mid parent, and F₁ and male parent. High fertility of cross may be due to the presence of a wide compatibility gene or restorer genes in the cultivar. Lower pollen and spikelet fertility of the crosses was attributed to effect of the genetic background of the tester parent.

Key words: Correlation, F₁ rice, pollen category, regression coefficient

INTRODUCTION

Many scientists had studied the sterility in the inter-varietal hybrids of cultivated rice ($2n = 2x = 24$). Oka (1974) proposed a duplicate gametophytic lethal model and assumed that the sterility in the inter-varietal hybrids is controlled by multiple sets of duplicate genetic lethal genes. Ikehashi and Araki (1986) adopted the one locus sporo-gametophytic interaction model and postulated that the spikelet sterility in Hsien Keng hybrids is caused by the allelic interaction at the S-5 locus (Ikehashi 1991). The degrees of pollen sterility in the F₁ hybrids depend both on the number of heterozygous locus for F₁ pollen sterility and on the heterozygous degree between alleles at the locus. The sterility genes determined by allelic interaction seem to be of wide occurrence (Sano 1990) between distantly related taxa and serve as one of the genetic mechanism for hybrid sterility (Sano 1983, Scoles and Kibirige-Sebunya 1983). Two out of three different genes causing F₁ hybrids sterility between *O. sativa* and *O. glaberrima* (Sano 1983) acted as gamete eliminator and the other acted as pollen killer, which suggests that gametic abortion due to allelic interaction frequently occurs between them.

Pollen analysis can be used to discriminate between different species, identify possible interspecies hybrids (Mariani and Tavoletti 1993), identify restorer and maintainer lines (Ikehashi and Araki 1984), useful to study genetics of restorer gene, interaction between chromosome and cytoplasm, and relationship between parents. Cultivated rices are grouped into two types Indica and Japonica based on character associations and hybrid sterility (Kato 1930). Ikehashi and Araki (1984) subdivided the Indonesian varieties into four types based on spikelet fertility in F_1 s. The characters of pollen grains had been used as taxonomic traits (Small et al 1981). Due to the haploidy nature of pollen, it has many advantages in genetic and breeding studies. Male sterility in rice results due to abnormality at any stage from microsporogenesis to pollen maturation. Pollen abortion system of male sterility is an important tool in hybrid rice production. Many factors affect the pollen abortion system. Pollen can be categorized either on the basis of number of nuclei or on the basis of shape and staining behavior (Chaudhary et al 1981). Shape and staining behavior system of classifying pollen is simple, which have been used widely for identifying restorers and maintainers and to evaluate CMS lines (Joshi 2001). Here, pollen and spikelet were analysed in F_1 rice hybrids and their parents. The frequency of pollen categories and its relationship to spikelet fertility were investigated.

MATERIALS AND METHODS

Nine improved cultivars, six landraces and three wild aborted cytoplasmic-genetic male sterile (CMS) lines (Table 1) were used in this experiment, which was conducted at the Institute of Agriculture and Animal Sciences (IAAS), Rampur, Nepal during the dry and wet seasons of 1999. The improved cultivars and landraces were obtained from National Rice Research Program, Hardinath, and IAAS, Rampur, respectively. The CMS lines were obtained from International Rice Research Institute (IRRI), the Philippines.

Approach method (Erickson 1970) was used for pollinating the seed parents. F_1 seeds were produced in greenhouse. Field, consisting of 14 F_1 s, 14 pollen parents, three CMS lines and one check cultivar, Masuli was divided into 32 plots of 0.8 m² size each. The pollen parent was planted beside their F_1 and CMS planted after the pollen parent. Masuli was planted in the middle plot. Field was fertilized as recommended. Twenty-one-day old seedlings were transplanted in the field in two rows with 10 hills per row at spacing of 20- × 20-cm. Single seedling was planted to each hill. Each plot had 20 plants.

Pollen sterility

Pollen sterility of the F_1 s was determined by staining pollen grains in 1% potassium iodide-iodine (I-KI) solution (Dalmacio et al 1995, Virmani et al 1997, Chaudhary et al 1981, Sohu and Phul 1995). Pollen sterility was determined in F_1 s and their parents. At heading about 15 spikelets from each experimental unit were collected in the morning just before their blooming and fixed in 70% alcohol. All the anthers from six spikelets were taken out and placed in the stain. Pollen grains were released using a needle. After removing the debris these were observed in microscope (10x). The pollen grains were counted in 3 random microscopic fields and classified based on their shape, size and extent of staining (Virmani et al 1997, Chaudhary et al 1981) as follows:

CATEGORIES OF RICE POLLEN AND THEIR FEATURES

Categories of pollen	Shape and staining behavior	Classification
Unstained withered sterile (UWS)	Withered and undeveloped, unstained	Sterile
Unstained spherical sterile (USS)	Spherical and smaller, unstained	Sterile
Stained round sterile (SRS)	Round and small, lightly or incompletely stained, rough surface	Sterile
Stained round fertile (SRF)	Round and large, darkly stained, smooth surface	Fertile

In case of CMS lines and some hybrids, pattern of pollen abortion was classified as follows (Chaudhary et al 1981):

Type 1: Almost all pollen grains appear as UWS and USS

Type 2: Majority of the pollen grains appears as USS (51%) followed by SRS (36%) and UWS (14%).

Type 3: Majority of the pollen grains are SRS (52%) however UWS and USS are 20-25%.

Spikelets fertility

Five panicles from each experimental unit were bagged before flowering for spikelet fertility analysis. At maturity, the bagged panicles were examined for seed set. Spikelet fertility was determined by counting the total number of seed set in proportion to the total number of spikelets.

F₁s were also classified on the visual basis as seed set as male parent or weaker than male parent and anthers whether plumpy yellow or white shriveled. Data from the field were analyzed and correlation and regression coefficients for some traits were computed using MS Excel (2000) and MINITAB (12.32) software.

RESULTS AND DISCUSSION

In hybrids, pollen fertility ranged from 0.5 to 82% and spikelet fertility from 0 to 87% (Table 1a and b). Pollen fertility varied from 28 to 97%, while spikelet fertility from 73 to 91% in pollen parents. The highest and the lowest percentages of pollen fertility were found in Chaite-6 and Chiunde cultivars (Table 1a) respectively. Chaite-6 had the lowest spikelet per panicle and IAR-97-34 had the highest number of spikelet and percentage of filled grain. IR58025A/Sabitri had the highest percentage of pollen and filled grains. Most of the hybrids had higher spikelet number than their parents. The highest spikelet number was found in R58025A/IAR-97-34 (Table 1b). Half of the hybrids had set the seed as their pollen parents. Only a hybrid, IR68888A/Khumal-7 had white shriveled anthers on visual basis. Bindesowari had the highest frequency of SRF and Masuli had lowest among the pollen parents (Table 2). The highest and the lowest frequency of SRF was found in IR58025A/Sabitri and IR68888A/Chiunde among hybrids respectively.

Table 1a. Pollen and spikelet fertility of the pollen parents and CMS lines, Rampur, Nepal 1999

SN	Line/cultivar	Pollen		Spikelet		r†
		Counted, n	Fertility, %	No. panicle ⁻¹	Fertility, %	
CMS line						
1	IR58025A	385	1	178	0	-0.49**
2	IR62829A	268	12	150	0	-0.71**
3	IR68888A	238	0	134	0	0
Cultivar						
1	Bindesowari	468	97	162	83	-0.26*
2	Chiunde	403	56	211	73	-0.31*
3	Chaite-6	283	95	141	86	0.98**
4	Deharadune	338	70	181	82	-0.79**
5	Gogi	440	81	194	67	-0.42**
6	IAR-97-34	496	67	234	91	-0.06
7	Janaki	521	80	145	75	0.65**
8	Kanchan	427	84	176	73	0.77**
9	Khumal-4	553	79	179	89	0.92**
10	Khumal-7	457	77	146	79	0.97**
11	Kature	547	63	154	87	-0.3
12	Masuli	266	28	172	84	1
13	Ratodhan	455	83	156	78	-0.78**
14	Radha-11	493	74	188	84	-0.73**
15	Sabitri	587	67	148	86	-0.61**
Range		266-587	28-97	141-234	67-91	-0.79-1
Mean		423.61	61.89	169.39	67.61	-0.01
SE		107.12	30.63	26.25	31.72	0.69

*, **, Significant at 5% and 1% level respectively. † Simple correlation coefficient between fertile pollens and fertile spikelets.

Table 1b. Pollen and spikelet fertility of hybrids, Rampur, Nepal, 1999

SN	Hybrid	Pollen		Spikelet		r†	Seed set as‡	F/S§
		Counted, n	Fertility, %	No panicle ⁻¹	Fertility, %			
1	IR58025A/Janaki	374	49	165	33	0.86**	W	F
2	IR58025A/Kanchan	426	65	201	75	0.75**	MP	F
3	IR58025A/Khumal-4	289	30	243	57	-0.84**	MP	F
4	IR58025A/Sabitri	440	70	202	84	0.97**	MP	F
5	IR58025A/Chaite-6	304	55	195	58	-0.06	W	F
6	IR58025A/IAR-97-34	451	56	256	49	0.03	MP	F
7	IR62829A/Deharadune	401	1	192	0	0.38**	W	F
8	IR62829A/Ratodhan	462	78	174	79	0.19	MP	F
9	IR62829A/Kature	352	56	198	76	-0.32*	MP	F
10	IR68888A/Radha-11	455	58	246	87	0.56**	MP	F
11	IR68888A/Bindesowri	288	1	181	0	0.36**	W	F
12	IR68888A/Khumal-7	193	1	177	0	-0.62**	W	S
13	IR68888A/Gogi	394	59	213	26	-0.99**	W	F
14	IR68888A/Chiunde	228	1	251	0	1	W	F
	Range	193-462	1-78	165-256	0-87	-0.99-1		
	Mean	361.21	41.43	206.71	44.57	0.16		
	SE	87.80	28.59	30.54	34.23	0.18		

*, **, Significant at 5% and 1% level respectively. † Simple correlation coefficient between fertile pollens and fertile spikelet. ‡ MP- male parent, W- weaker than MP. § F- plumpy yellow anthers, S-white shriveled anthers on visual basis.

The higher percentage of SRS in hybrids: IR68888A/Bindesowri, IR68888A/Kumal, IR62829A/Dehardune and IR68888A/Chiunde was associated with on an average with 1% SRF. The hybrids having higher SRS were associated with high frequency of SRF as in IR68888A/Radha-11, IR58025A/Janaki, IR58025A/Kanchan, IR58025A/Khumal-4, IR58025A/Sabitri, IR58025A/Chaite-6, IR62829A/Ratodhan, IR62829A/Kature and IR58025A/IAR-97-34. Table 2 showed that hybrids with some SRF pollen had fewer filled grains in the panicles. It indicates that hybrids having higher UWS and USS will be better in developing new CMS lines from their sterile hybrids. The hybrids were classified as semi-sterile on the basis of spikelet fertility of 40-80%. The male parents of these hybrids were designated as partial restorer. In these hybrids, SRS had dominated the other pollen categories. The partial restorer, IAR-97-34 had more spikelet sterility than the other two partial restorers, Khumal-4 and Chaite-6. Spikelet fertility percentage varied widely among hybrids and many hybrids had lower spikelet fertility percentage than their parents. Therefore, it is of practical importance to understand the causes of high spikelet sterility in hybrids for possible increase in spikelet fertility.

IR58025A and IR68888A had Type I pollen abortion and IR58025A/Khumal-4, IR62829A/Deharadune, IR68888A/Bindesowari and IR68888A/Khumal-7 had Type III. Different pollen abortion pattern were found in hybrids, which indicate the different degree of interaction between nuclear and cytoplasm. Also this suggests that sterility in rice depends upon the genetic diversity between the cytoplasmic donor parent and the nuclear donor parent. Chaudhary et al (1981) suggested that morphology and staining pattern of pollen depend on the developmental stage at which it aborts. Further study on pollen staining system is necessary for better understanding the relationship between staining and viability of pollen. Data in some cases showed that pollen stainability with I-KI solution is not strong evidence to have generalization about spikelet fertility. Results of pollen test were underestimates of spikelet fertility. This may be due to the ability of single fertile pollen to fertilize a spikelet. Therefore even low number of fertile pollen counted in study can give higher seed set. In most of the cultivars and F₁ positive relationship was found between fertile pollen and filled grain. Hybrid having less fertile pollen showed the lowest percentage of filled grain. The sterilities of the inter-varietal rice hybrids are mainly the pollen sterilities and the spikelet sterilities are mainly caused by the pollen sterilities. Guiquen et al (1994)

reported that the sterility in the inter-varietal hybrids of cultivated rice is caused by the allelic interaction at the F₁ pollen sterility loci. Six loci of genes controlling F₁ pollen sterility in rice have been reported (Guiquen et al 1994). The finding of the more the F₁ hybrids had the heterozygote Sⁱ/S^j at the six loci, the higher they showed the pollen sterility and the spikelet sterility (Guiquen et al 1994) is supported by this study.

Table 2. Pollen categories and types of male sterility in male sterile lines, hybrids and pollen parents, Rampur, Nepal, 1999

SN	Hybrid/parent	Total pollen examined, n	Frequency, %†				Type‡
			UWS	USS	SRS	SRF	
1	IR58025A	385.33	29.67	68.25	0.69	1.38	I
2	IR62829A	267.67	27.77	38.85	21.67	11.7	I
3	IR68888A	238.33	47.27	50.77	1.96	0	I
4	IR58025A/Janaki	373.67	12.13	18.11	20.79	48.97	
5	Janaki	520.67	3.78	2.18	14.08	79.96	
6	IR58025A/Kanchan	426.33	2.35	4.77	27.91	64.97	
7	Kanchan	427.33	0.86	0.86	14.74	83.54	
8	IR58025A/Khumal-4	289	3.92	15.11	51.1	29.87	III
9	Khumal-4	552.91	0	0	20.51	79.26	
10	IR58025A/Sabitri	440.33	1.29	7.65	21.05	70.02	
11	Sabitri	587.33	0.57	2.44	29.51	67.48	
12	IR58025A/Chaite-6	304	4.93	8.88	31.36	54.83	
13	Chaite-6	283.33	1.18	0.71	3.53	94.59	
14	IR58025A/IAR-97-34	451.33	6.35	13.52	23.71	56.43	
15	IAR-97-34	496.33	0.6	6.51	25.52	67.36	
16	IR62829A/Deharadune	401	4.91	21.36	73.15	1	III
17	Deharadune	338.33	4.14	17.24	9.07	69.56	
18	IR62829A/Ratodhan	462	0.22	2.74	19.48	77.56	
19	Ratodhan	454.67	0.37	3.59	12.68	83.36	
20	IR62829A/Kature	352	7.76	10.89	25	56.34	
21	Kature	547	0.43	4.2	32.66	62.71	
22	IR68888A/Radha-11	454.91	4.94	5.49	31.14	58.43	
23	Radha-11	492.67	0.14	1.49	24.36	74.02	
24	IR68888A/Bindesoawri	288.33	12.49	38.27	48.25	1	III
25	Bindesoawri	468.33	0	2.99	0.36	96.66	
26	Masuli	265.67	0.63	16.44	54.96	27.98	III
27	IR68888A/Khumal-7	193	10.02	28.67	60.32	1	III
28	Khumal-7	457.33	0.22	1.31	21.79	76.68	
29	IR68888A/Gogi	394	4.82	22.51	13.96	58.71	
30	Gogi	439.67	0.3	2.2	16	81.5	
31	IR68888A/Chiunde	228.33	26.28	48.18	25.5	0.5	II
32	Chiunde	403	0.99	12.33	31.02	55.67	
	Range	193-587.33	0-47.27	0-68.25	0.36-73.15	0-96.66	
	Mean	396.38	6.92	14.95	25.24	52.91	
	SE	102.53	10.93	17.07	17.05	31.04	

† UWS, unstained withered sterile, USS, unstained spherical sterile, SRS, stained round sterile, SRF, stained round fertile, ‡ Type I- almost all pollen appears as UWS and USS, II-majority of pollen as USS (51%) followed by SRS (36%) and UWS (14%), III-majority of pollen SRS followed by USS and UWS.

With respect to maintaining ability, Bindesoawri, Khumal-7 and Deharadune, all appeared to be good source of maintaining the sterility. All F₁ of this pollen parents with CMS showed zeros spikelet fertility rate with 0.5 to 1% pollen fertility. Virmani (1996) found the frequency of restorer lines less among Northern China, Eastern Europe, Japan, and Korea. The restoring ability of rice cultivars has been found to be related to their origin to some extent (Govinda Raj and Virmani 1988). Among indica rice cultivars the frequency of R gene is higher in late maturing cultivars than in early maturing ones (Ahmed 1996). The restorer frequency is very low in typical japonica rice cultivars (Lin and Yuan 1980, Virmani et al 1981). It suggests that origin and pedigree of test lines are important characters to be considered in analyzing the pollen and spikelet in rice.

Highly significant positive correlation was found between SRF and spikelet fertility (Table 3). The regression coefficient of 0.96 indicates the strong evidence that SRF is the most important pollen to make spikelet fertile. These regression equations (Table 3) support the basis of pollen categorization system based on the shape and staining behavior. The positive value of correlation and regression coefficient on SRF and spikelet fertility were found between F_1 and mid parent, and F_1 and male parent. It is observed that spikelet fertility is significantly correlated between F_1 and their parents. Table 3 showed that SRF and spikelet fertility on F_1 could be predicted on the basis of pollen and spikelet of their parents. Many factors affect the pollen and spikelet development even though these characters are found inherited.

Table 3. Simple correlation (r), regression coefficient (b) and intercept (a) between F_1 and their parents for 12 characters, Rampur, Nepal, 1999

Pollen category	Spikelet fertility			Mid parent			Male parent		
	r	a	b	r	a	b	r	a	b
Unstained withered sterile	-0.77**	74.76	-2.43	0.56**	15.56	0.37	0.12	0.79	0.02
Unstained spherical sterile	-0.87**	84.0	-1.74	-0.04	30.23	-0.02	0.43**	1.36	0.16
Stained round sterile	-0.22*	09.14	-0.44	-0.12	13.49	-0.05	-0.31**	24.35	-0.18
Stained round fertile	0.86**	7.04	0.96	0.15*	38.61	0.03	0.09	75.0	0.04
Spikelet fertility	-	-	-	0.33**	39.0	0.03	0.33**	-84.85	1.59

*, **, Significant at 5% and 1% level respectively.

Environmental factors eg stresses can induce nuclear abnormalities and then affect pollen development. Anther morphology, pollen dispersal and viability in rice (Satake 1991) are affected by chilling. Darkness causes pollen sterility in sorghum (Alami et al, cited by Demotes-Mainard et al 1996). Tian et al (1998) reported that anomalies in the distribution of calcium accumulation correlate with the failure of pollen development and pollen abortion. Phytochrome also involve in regulation of pollen fertility (Oard and Hu 1995). Genetic male sterile system could be utilized in the production of backcross (Fujimaki 1975), interspecific and recurrent selection breeding population (Brim and Stuber 1973). It is assumed that genes at specific loci controlled both pollen and seed fertility. The thermo sensitive genic male sterility (TGMS) trait was controlled by a single recessive gene (Borkakati and Virmani 1996). At certain temperatures occurring after panicle initiation, the male sterility is altered into partial to complete fertility. Yabuno (1977) reported that spikelet sterility is caused by indehiscenced anthers in rice. Jennings (1966) surveyed pollen and spikelet fertility in F_1 in many crosses of Indica with japonica. He found that there were not any F_1 s showing spikelet sterility with normal pollen fertility and spikelet fertility was correlated with pollen fertility. Variability for fertility restoration exists among these hybrids. The low pollen fertility of the inter varietal hybrids may be due to chromosome imbalance caused by incompatibility barriers. High fertility of cross may be due to the presence of a wide compatibility gene or restorer gene in the cultivar.

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