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Pollen Sterility in wide crosses derivatives of rice *(Oryza sativa L.)*

Abdul Waheed[1], Habib Ahmad[1], Fida M. Abbasi[1], F.S.Hamid[2,] A.H.Shah[1], Fayaz Ahmad Safi[1] , Hamid Ali[1]

[1]*Genetics Department Hazara University (KPK) Pakistan*
[2] *National Tea Research Institute (PARC) Shinkiari Mansehra, Pakistan*
[c]*Genetics Department Hazara University (KPK) Pakistan*

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* Corresponding author. E mail: abdulw900@gmail.com; Tel + 92 997 531125

Abstract
Study was conducted at Genetics department Hazara University, Pakistan for the determination of pollen sterility and fertility. *Bas-385, Oryza rufipogon, Bas-385 X Oryza rufipogon* were used for experiment. Pollen sterility was determined from >1000 pollen grains from parents and F_1 hybrids. The spikelet was collected near anthesis in 70 % ethanol. The pollen grains were stained in 1 % I_2 KI solution. Darkly stained and round pollen grains were counted as fertile while lightly stained and shrivelled were counted as sterile. Out of over 1000 pollen grains, 851 were darkly stained and were counted as fertile, whereas 149 remain unstained and were considered as sterile. Pollen sterility of *Oryza rufipogon* was thus 4.6 %. The average spikelet sterility of *Oryza rufipogon* was 44.3 %, indicating that this specie was partly sterile although it showed high pollen fertility.

Keywords: Oryza sativa. Pollen. Sterility. Fertility. KPK. Pakistan.

Introduction
Rice varieties differ in numerous morphological and physiological traits and have been selected for adaptation to different growing conditions. Some mature in less than 80 days from sowing. Others, like variety Rayada of Bangladesh, have a growth cycle of about 280 days. These are photoperiod-sensitive deep-water rice and are planted with the onset of rains in March and harvested in December [1]. Sterility has been a major barrier for the utilization of strong heterosis between different species such as, *indica* and *japonica*, which are two subspecies of Asian cultivated rice (*Oryza sativa* L.). Cytological investigations have revealed that many factors contribute to sterility, including male gamete abortions, female gamete abortions, affinity between the uniting male and female gametes, and reduced dehiscence of anthers [2]. In addition, environmental conditions, especially low temperature, can also greatly reduce the fertility in interspecific hybrids [3]. The parent homozygous for *S5n* is called a wide-compatibility variety (WCV). The typical sterility shown in the F_1 rice hybrids between variety Ketan Nangka and variety Tuanguzao is a genetic barrier for the introduction of desirable genes in Tuanguzao [4]. Detection and when mapping of the locus (loci) controlling hybrid sterility and identification of the corresponding neutral alleles are key to pyramid WCGs by MAS and further to utilize desirable genes in Tuanguzao. An estimated 120,000 distinct rice varieties exists in the world [4]. Approximately 80,000 are preserved in the Gene Bank of the International Rice Research Institute (IRRI) Philippines. China 40,000 and India about 25,000 in their gene banks while other countries have smaller selections [4].
Rice varieties also differ in endosperm traits, which determine their acceptability to various consumer groups. While the vast majority of rice varieties are no glutinous, glutinous varieties form the everyday diet of the people of Laos and northeast Thailand. Most of the major rice-growing countries have a few aromatic varieties that are prized on the market. [5]. Varieties differ in the level of cold tolerance and tolerance to other abiotic stresses such as drought, submergence, and salinity. There are differences in resistance to diseases and insects [6]. Several biotic and abiotic stresses continue to limit rice productivity. There is, thus, an urgent need to broaden the gene pool of cultivated rice. Wild species of rice are an important source of variability for

resistance to major diseases and insects and offer an important source of novel resistance genes for rice improvement [7]. The main objectives of this study were to determine the cytological basis for sterility in the F_1 hybrid between *Oryza sativa Bas-385* and *Oryza rufipogon.*

2. Materials and methods

2.1. Plant material

Three parents were used in this study. *Bas-385, O. rufipogon, Bas-385 X O. rufipogon. Bas-385* belongs to a Pakistan. *O. rufipogon* belongs to Taiwan [8]. A backcross population (*Bas-385 X O. rufipogon*) consisting of >100 individuals was used for the sterility with lower than 50% spikelet fertility were selected to produce a BC_1F_2 population for further confirmation. The three parents plus their F1 the >200 individuals in the BC_1F_1 population were planted in the rice growing seasons of 2009-11 at the experimental field block of Genetics department at Hazara University, Mansehra (KPK) .

2.2. Pollen sterility determination procedure BC_1F_2 and F_1 hybrids

The parents and 1,050 individuals of the BC_1F_1 population were planted in 2010. Pollen sterility was determined from at least 1000 pollen grains from parents and F_1 hybrids. The spikelet was collected near anthesis in 70 % ethanol. The pollen grains were stained in 1 % l_2 KI solution. Darkly stained and round pollen grains was counted as fertile while lightly stained and shrivelled were counted as sterile. Pollen sterility was determined as reported by [9].
Pollen sterility below determined according to the formula:

$$\text{Pollen sterility (\%)} = \frac{\text{No. of sterile pollen grains}}{\text{Total no. of pollen grains}} \times 100$$

2.3. Fertility examination of F1hybrids

Fertility examination ten individual plants from each var .and their F_1 hybrids were examined for pollen fertility. Six florets from three panicles in each plant were collected 1–2 days before flowering. One anther per florets from the same plant was mixed and stained with 1% iodine potassium iodide (I_2–KI) solution, and views were observed per plant under microscope. Embryo sac fertility was evaluated by the ferrovanadium–haematoxylin method [4]. One hundred to one hundred and fifty mature florets from ten plants of both Var. and their F_1 hybrids were collected 1–2 days before flowering and immediately fixed in FAA solution (containing an 18:1:1 mixture of 70% ethanol, formalin and acetic acid).

2.4. Protocol applied

The standard protocol was used. In vitro pollen germination and fertilization status of embryo sacs were tested according to the methods of [10-11] respectively. The spikelet fertility of individual plants of the parents, F_1 hybrids, BC_1F_1 and Bast-385 population was determined by counting fertile and sterile spikelets on the upper half of three main panicles after maturity. The pollen fertility of normal plants was assumed to be 97%, which was a mean for 60 observations, and when the mean for a plant was x%, its sterility was shown by 97-x. The standard error for the mean of two measurements was about 2% in normal plants, though the lowest pollen fertility observed in a normal plant was 92%. For computing the variances of sterility among plants, the percentage data were converted into arc-sine values [1].

3. Results and discussion

3.1. Procedure for in crosses distantly species

In crosses between distantly related species, reproductive barriers are frequently observed. Cultivated var. *Bast- 385* crossed with one wild species originated from Taiwan and BC_1F_2 population were crossing consisted of > 100 plants.

3.2. Chemical solution and pollen fertility results

Pollen fertility of F_1 plants was 46.2 % in the G-4 .and 48.2 % in the cross. Frequently distribution of pollen fertility was continuous in both BC_1 F_2 population Pollen sterility of *O. rufipogon*, Bas-385 and their F_1 hybrids were determined by using 2 % acetocarmine solution. Darkly stained and round pollen grains were

counted as fertile, whereas lightly stained and shrivelled pollen grains were counted as sterile. A total of >1000 pollen grains of *O. rufipogon* were analyzed. Out of over 1000 pollen grains, 851 were darkly stained and were counted as fertile, whereas 149 remain unstained and were considered as sterile. Pollen sterility of *O. rufipogon* was thus 4.6 %. The average spikelet sterility of *O. rufipogon* was 44.3 %, indicating that this specie was partly sterile although it showed high pollen fertility.

Table 1: Pollen and spikelet sterility of *O. rufipogon, O. sativa* and their F_1 hybrids

Parents/Hybrids	Pollen grains			Florets		
	Stained	Unstained	Pollen sterility (%)	Filled	Unfilled	Spikelet sterility (%)
O. rufipogon	851	149	4.6	207	153	44.3
Bas-385	512	35	6.4	158	68	30.3
Bas-385 × O. rufipogon	600	465	74	65	558	95.5

Figure 1: *Oryza rufipogon* (wild species)

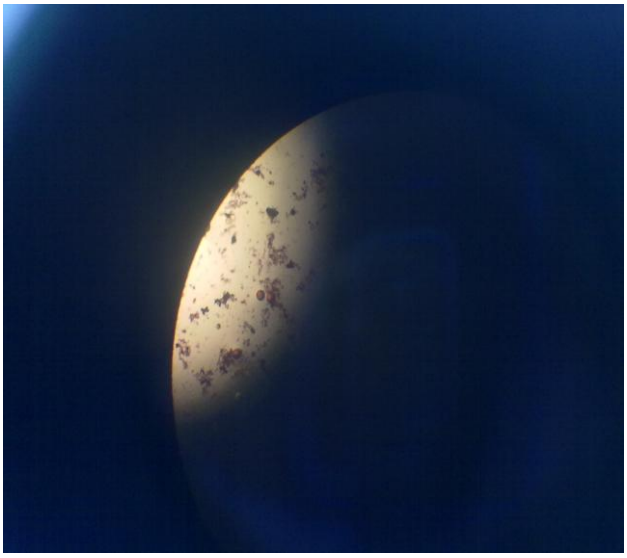

Figure 2: Darkly fertile pollen & lightly stained sterile pollen grains under microscopic view

Figure 3: *Oryza rufipogon* in field at booting stage

The F_1 hybrid Bas-385 × *O. rufipogon* was also highly pollen and spikelet sterile. Pollen sterility was 74 % and spikelet sterility was 95.5 %. A total of 600 pollen grains were analyzed, out of them 465 were fertile, whereas 600 were sterile (Table 1). Thus hybrids showed both high level of pollen and spikelet sterility. Relationship among meiotic abnormalities, pollen sterility and spikelet sterility meiotic abnormalities were correlated among themselves and also with pollen and spikelet sterility. A significant correlation was found among meiotic abnormalities, pollen and spikelet sterility. Univalent were highly but negatively correlated with bivalents and showed highly positive association with trivalent, quadrivalents, laggards, pollen sterility and spikelet sterility. Bivalents showed a significant negative correlation with trivalent, quadrivalents, laggards, pollen sterility and spikelet sterility. Obviously increase in bivalents will result decrease in meiotic abnormalities and pollen sterility. Pollen sterility showed significantly high correlation with spikelet sterility. Thus pollen sterility may be considered as the main cause of spikelet sterility (Table 2). and meiotic abnormalities such as univalent may be one of the patent causes of pollen sterility.

Table 2: Correlation among meiotic abnormalities, pollen and spikelet's sterility

	Bivalent	Trivalent	Quadrivalent	Laggards	Pollen sterility	Spikelet sterility
Univalent	-0.815**	0.692**	0.599**	0.0520**	0.839**	0.799**
Bivalent		-0.739**	-0.699**	-0.689**	-0.681**	-0.763**
Trivalent			0.539*	0.600**	0.627**	0.762**
Quadrivalent				0.769**	0.414	0.570**
Laggards					0.371	0.572**
Pollen sterility						0.821**

*, **=significant at P<0.05 and P<0.01 levels, respectively.

Pollen stain ability and seed fertility of interspecific hybrids of rice reported earlier was very low [12]. In the present study hybrids obtained by crossing *O. sativa cv.Bas-385* and wild rice *O. rufipogon* were highly sterile. Pollen sterility of the hybrids ranges from 74-64 and spikelet sterility ranges from 95.5-44 (Table-I) Meiotic abnormalities such as univalent showed significantly high correlation with both pollen and spikelet sterility. Previous study pointed out that the abnormal chromosome number and meiosis of the PMCs, which led to the microspores aborting, were the reasons of low fertility in rice [13]. The frequency of univalent at metaphase-1 had the most impact on pollen sterility and seed set, i.e., pollen sterility increases with the increase of univalent[14].Although meiotic abnormalities showed significant correlation with pollen sterility yet more than 90 % of the PMCs of the hybrids showed normal meiosis. Therefore it seems that the sterility has more likely occurred at the gene level. Artificial *O. meridionalis* and *O. nivara* interspecific hybrids, spikelet fertility was substantially lower (≤ 3%) than that of other interspecific hybrids [12]., but full chromosome pairing at the metaphase-I was still evident in these hybrids, suggesting that sterility of these hybrids was not caused by meiotic abnormality like in many other inter-population or interspecific hybrids [12]. Instead, it has more likely occurred at the gene level. It therefore seems that certain genetic mechanisms have been established at the gene level to isolate populations of the same species, as has occurred between species, where the interspecific hybrids presented low spikelet fertility [15- 16].

Moreover, pollination is an essential process for the fructification of rice. Thus, poor dehiscence of anthers causes a low seed-setting rate and affected its yield. Naredo, *et al* [12] reported that abnormal anther dehiscence was the main factor to lower rice yield when under weather stresses i.e., cool (< 20^{o}C) and high (< 35^{o}C) temperature at flowering time. Chu, *et al* [16] pointed out that the anther opening was an important factor affecting the fertility of indica × japonica hybrid. They found that thickened endothecium cells (TEC) and dehiscence chamber were two main factors controlling anther dehiscence through the observation on paraffin section of the male sterile lines. The anther opening would be failed if the dehiscence chambers did not exist, or TEC very weak [17-18].So the failure to anther opening may affect the fertility of the hybrids. The seed-setting rate was still low when the F_1 hybrid as female parent was hybridized with a normal cultivar *(Bas-385)*, in order to produce the BC_1. Therefore the female gametophytes aborting might be the main cause of the low fertility of these hybrids. The female gametophyte aborting could result in poor fertility of F_1

between indica and japonica rice. [19-3]. While the Fig 1 and Fig 3 showed their performance in pots and in the field under booting stage. Fig 2 cleared the darkly fertile pollen & lightly stained sterile pollen grains under microscopic view. On the other view of Fig 4 and Fig 5 exposed the view of cross was being made in between *Bas-385 and O. rufipogon* and single grain obtain which reflect the gain length and thickness of individual GT.

Figure 4: Back-cross *Oryza rufipogon* X Bas-385

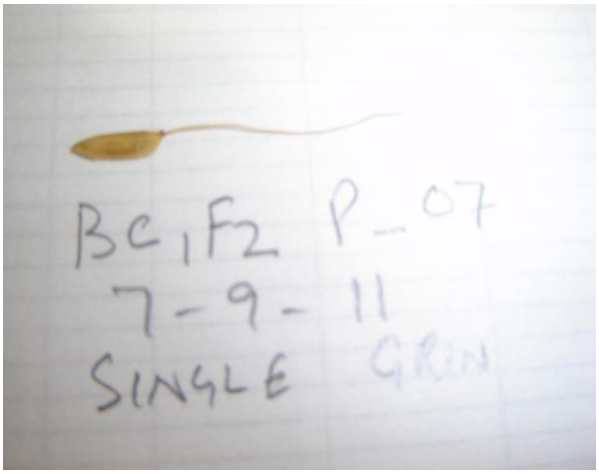

Figure 5: Single grain (BC1F2 P-07)

Table 3: Mean measurements with standard deviation with (correlation observed in *O. rufipogon, O. sativa* and their F_1 hybrids

Characters	*O. rufipogon*	*Bas-385*	*Bas-385 x O .rufipogon (F1)*
Mean			
Pollen fertility %	84.4 ± 13.2	83.3 ± 18.7	89.6 ± 15.6
Seed fertility %	67.5 ± 20.9	49.5 ± 24.6	52.8 ± 15.5
Correlation			
Pollen fertility %	.33* Ph c	-.11	.44**
seed fertility %	.53 G	-.10	.74

Ph c = phenotype correlation G= genotype correlation
*, **=significant at P<0.05 and P<0.01 levels, respectively.

The F_1 populations showed continuous fertility variations. In all the pollen and seed fertilities were correlated. The genetic correlations were estimated to be 0.53 and 0.74, respectively (Table 3). This suggests that the pollen and seed fertilities are controlled partly by the same genes probably .However, the pollen and seed fertilities were not correlated suggesting that they were controlled by different genes. In this cross, pollen fertility was strongly correlated with each other. These results similar to [12,3-2] that female gametophyte abortion occurred at one-nucleate or two-nucleate stage in F_1 hybrids of indica-japonica cross and three major QTLs regulating embryo-sac fertility were identified using its derived populations. In naked seed rice (NSR), the various female gametophyte abortions happened in the process of the female gametophyte formation and mainly occurred during mature embryo sac formation [17]. But the cause of female gametophytes aborted in NSR was not clear. These results reported are more or less similar to [16, 17-18].

Conclusion

It was concluded from the study that out of >1000 pollen grains, 851 were darkly stained and were counted as fertile, whereas 149 remain unstained and were considered as sterile. Pollen sterility of *O. rufipogon* was thus 4.6 %. The average spikelet sterility of *O. rufipogon* was 44.3 %, indicating that this specie was partly sterile although it showed high pollen fertility. While pollen & fertility showed .44 and .74 in F1 respectively.

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