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Interspecific crosses *Oryza rufipogon and Oryza longistaminata with Oryza Sativa* (Bas-385 and F. Malakand)

Abdul Waheed [1], Habib Ahmad [1], Fida M. Abassi [1], F.S.Hamid [2], A.H. Shah [1], Hamid Ali [1], Fayaz Ahmad [2], Naseer Ahmad [3]

[1]*Department of Genetics Hazara University Mansehra (KPK) Pakistan.*
[2]*National Tea Research Institute Shinkiari Mansehra (KPK) Pakistan.*
[3]*Crop Sci.Div,Pakistan Agricultural Research Council Islamabad.*
[C]*Department of Genetics Hazara University Mansehra (KPK) Pakistan.*

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* Corresponding Author: abdulw900@gmail.com

Abstract
Study was conducted at Genetics Department of Hazara University (KPK) Pakistan, to develop. Genetic Interspecific materials derived from successful crossing of the two cultivated rice species *Oryza sativa* and *Oryza rufipogon* were intercrossed to find out their level of cross-compatibility and the extent of sterility in the Bas- 385 and Fakhar Malakand. All hybrids were partially sterile and showed significant differences in their seed set (0.6 to 33.09%). Pollen fertility tests indicated reduced pollen viability in the hybrids. Seed set was improved (up to 65%) when hybrids were backcrossed to either parent. Within the selected *O. sativa* improvement could therefore be possible through conventional crosses and best through backcross breeding. However *O.longistaminata* was not synchronize completely with *Oryza sativa because of late booting and flowering. Only cross 1 & 2 (F. Malakand)* formed 2 grains but it was not sure to either it will be fertile or not.

Key words: Wild species. Interspecific crosses. Sterility. KPK.Hazara university.

Introduction
Rice has a special significance in Asia where about 90% of rice produced and consumed as a staple food. Considering the increasing requirements because of population increased on one hand and decreasingly land and water resources on the other hand, it is serious to use and develop rice technologies that will be resultant in higher yield **[1].** Experience in China and outside in different countries clearly indicates that hybrids rice has a viable option to meet this challenge and shown 1-1.15 T ha^{-1} in the farmers fields **[2].** Rice has become the most rapidly growing food source in Sub Saharan Africa **[3]**. The relative growth in demand for rice is faster in this region than anywhere in the world **[4]**. Africa's annual rice production represents only 3% of the global production **[5]** and accounts for 26.1% of global imports **[6].** Wild species of Oryza is a rich source of use full genes for the improvement of cultivated rice. Successful have been made to develop wide F_1 hybrids of rice to transfer desirable useful genes to cultivated varieties to broaden gene pool. **[7].**Genetic diversity is one of the important tools to quantify genetic variability in both cross and self pollinated crops. **[8-9]** made interspecific and intraspecific crosses using different wild species including *O.rufipogon* and other diploid accession from the new world Asian, Australian AA genome species. All interspecific and intraspecific crosses combination produced seeds and hybrids but at different level of success. F_1 hybrids were scored for selected morphological traits and compared with their parents. Conventional attempts to combine the desirable characteristics of the two species elsewhere proved futile because F_1 plants exhibit complete sterility. Many such self fertile lines, which differ in several characteristics, are available, giving a large gene pool from which desirable characteristics can be combined into a desirable plant type.

2. Materials and Methods

2.1. Experimental plan of interspecific crosses: Interspecific crosses were conducted between cultivated varieties comprising Bas-385, F. Malakand and wild rice *O. rufipogon having* "IRGC accession number `106516 ". Cultivated varieties were used as (♀) female parents, while *O. rufipogon* as a (♂) male parent. To prepare (♀) female parents, panicles of rice plants at appropriate stage i.e., ¼ the of panicle had emerged from the flag leaf, were emasculated and pollinated with pollen of *O. rufipogon* wild species on following day. Gibberellic acid (GA_3) 75 ppm was sprayed-on to pollinated panicles immediately and again one day after pollination. The pollinated panicles were also sprayed 2 time a day for 5 consecutive days with a mixture of growth hormones GA_3 + NAA "Nepthaline acetic acid" and kinetin with proportion of 100, 25 and 5 mg L^{-1}, accordingly . Beginning with the afternoon following pollination. The pollinated panicles were harvested 30 days after pollination, F_1 seeds were collected and % seed set was determined for each cross. For confirmation of interspecific crosses, the F_1 seeds were grown in buckets/pots. The F1 hybrid seeds were harvested at maturity. Hybridization block was set-out for the above selected material at experimental field block under high shade tunnel in buckets /pots of Genetics Department of Hazara University Mansehra (KPK). Possible reciprocal crosses were carried out at flowering by emasculating and transferring pollen.

2.2. F_1germination: For the germination of F1 seeds, the treatment for germination were followed as by **[10],** after germination nursed in buckets/pots for 25 days and transplanted as single plant per bucket. The hybrids were provided fertigation at tillering and panicle initiation stage and maintained its all cultural practice whenever necessary till maturity. The degree of self sterility/fertility was examined by counting the number of filled and unfilled spikelets per panicle and per plant. Fertile spikelets were identified by pressing the spikelets with fingers to note those that were filled and sometimes by de-husking. Fertile and sterile spikelets were counted manually.

2.3.Data observance: Data were taken on total number of spikelets per plant, number of filled and unfilled spikelets per plant, percentage fertility/sterility per plant and percentage fertility/sterility per cross. Percent fertility per plant was obtained by calculating the number of filled spikelets, as a percentage of the total number of spikelets (filled and unfilled) counted from that plant. To enable statistical comparison of F_1 hybrid seed fertility for the various crosses, six panicles per plant for four plants were used, in calculating the percentage seed fertility.

*2.4. Statistical analysis (F_1) seed fertility :*Analysis of variance (ANOVA) for seed fertility was first carried out using the arcsine transformed values, for each harvested group. When the results of the analysis of variance revealed significant differences, multiple mean comparisons were carried out using LSD at 0.05. **[11-9].**

2.5. Backcross: Some F1 hybrids were backcrossed to either parent, to find out the possibility of setting seeds and the extent to which sterility can be restored by conventional backcrossing **[12].** Backcrosses were done to find out the possibility of increasing seed set. The following in **Table 3** were obtained from backcrosses.

2.6. Fertility test of pollen: Twenty five spikelets were collected at random from the available hybrid plants for each cross at flowering but before anthesis. Ten anthers were sampled at random from the collected spikelets. Pollen grains were stained in 1% iodine-potassium iodide (IKI) solution and observed under a microscope at ×100 magnification. A total of 900 to 1000 pollen grains were counted on each slide and classified as sterile or fertile based on their staining behaviour **[13].** All dark and brown stained pollen were scored as fertile and irregularly-shaped, yellow or unstained pollen grains were scored as sterile. Sixteen Bas-384, Fakhar Malakand cross combinations with *O. rufipogon were* obtained. The crosses with their mean percent F1 spikelet fertility, as well as percent pollen fertility are summarized in Table 1. Hybrids from two crosses were not included because of insufficient data. Pollen fertility was tested for eleven hybrids

3. Results and discussion

The research work has been conducted at Hazara University of KPK(Pakistan) during 2010-11on Interspecific crosses in between *Oryza Sativa* and wide species. The detail results are given below:

3.1. Seed fertility: The International Network for Genetic Evaluation of Rice **[14]** has suggested that hybrid plants with percent seed fertility of < 50% to trace, be classified as highly sterile and that of 0%, as completely sterile. **Table 1 & 2** revealed that all the hybrids were therefore highly sterile because the highest percent spikelet fertility recorded was 33.09 (Bas-385 7/1). None of the hybrids was completely sterile because none recorded percent spikelet fertility of 0. The results are similar to that of **[15]** who reported that the average proportion of *O. sativa* genome in to NERICA7 is 87.4% while an average of 6.3% was covered by *O.glaberrima* genome based on 130 micro-satellite markers.

Table 1: Percent spikelet fertility, mean arc sine transformed of % spikelet fertility and % pollen fertility of F1 hybrids.

Cross combination *Bas-385 x O .rufipogon* FM x O. rufipogon	Mean percent F1 spikelet fertility	Mean, arc sine transformed	Percent pollen fertility
Bas 385 7/1	33.09	34.98 a	52.00
FM 13/1	31.26	33.92a	66.00
FM20 /1	28.87	32.75a	59
Bas-385 10/7 /15	19.43	25.02b	60.20
Bas-385 15 /10	9.26	12.43c	35.00
Bas-385 8 /3	2 5.91	14.02 c	**
FM 6 /1	5.74	13.42 c	37.50
FM 14/ 11	4.10	11.42 c	47.40
Bas-385 18 / 9	3.97	11.43 c	31.40
Bas-385 17 /10	3.31	9.41 c	**
Bas-385 19 /1	3.26	7.96 c	**
FM 12 /9	3.26	7.96 c	**
FM 4 /10	2.79	*	**
Bas-385 3 /19	2.54	9.03 c	**
Bas-385 16 /4	2.18	11.96 c	50.40
Bas-385 9 /10	1.87	8.85 c	51.30
Bas-385 1 / 9	0.6	*	42.00
CV			28.8
LSD(0.05)			28.8

* Were not included in the Anova and ** Pollen fertility analysis could not be determined for hybrids of that cross because no data were available .

Table 2: Number of emasculated & pollinated spikelet and their percent seed sets

Backcross	Number of spikelets emasculated and pollinated	Number of spikelets filled	Percent fertility
Bas11/FM1/O.r 3	548	360	65.70
Bas12/1/O.r 16	708	388	54.80
Bas13/2/ O.r 14	76	48	63.12
Bas14/1/O.r 14	438	132	30.14
Bas15/10/ O.r 14	180	96	53.33
Bas23/1/O.r 1	168	102	60.71

Table 3: No. of Cross *O.longistaminata X Fakhre Malakand and O. rufipogon x F. Malakand* at Genetic block

Cross No *		Pollinated Date*		Maturity Data*		Spike emasculated *		No of grain*	
O.l	*O.r*	*O.l*	*O.r*	*O.l*	*O.r*	*O.l*	*O.r*	*O.l*	*O.r*
1	7	12-10	02-05	12-11	25-10	84	83	2	72
2	13	13-10	01-10	13-11	03-11	134	72	2	70
3	20	14-10	06-10	14-11	06-11	150	264	0	200
4	10	15-10	20-9	15-11	29-10	130	76	0	3
5	15	17-10	3-11	17-11	3-11	165	164	0	8
6	8	18-10	28-09	18-11	28-10	140	176	0	10
7	6	18-10	25-09	18-11	25-10	120	89	0	5
8	14	18-10	02-10	18-11	02-11	155	177	0	3
9	18	18-10	06-10	18-11	06-11	140	54	0	0
10	17	18-10	03-10	18-11	03-11	130	220	0	7
11	19	22-10	06-10	22-11	06-11	145	51	0	1
12	12	22-10	08-09	22-11	08-10	165	58	0	5
13	4	24-10	22-09	24-11	22-10	220	150	0	36
14	3	27-10	21-09	27-11	21-10	120	143	0	22
15	16	29-10	00-10	29-11	03-11	133	167	0	15
16	9	02-10	28-09	02-12	28-10	223	45	0	9
17	1	03-10	20-09	03-12	20-11	167	128	0	6
18	2	02-10	21-09	02-12	21-11	132	114	0	3
19	0	03-10	0	03-12	0	178	0	0	0

*Data pertaining to all parameters on basis of Single plant
** *O.r*: Oryza rufipogon, *O.l, Oryza longistaminata*

*3.2. Findings: S*uggests that each Bas-385 cultivar, had a genetic background that was mainly *O. sativa*, so the gamete eliminator or pollen killer genes derived from both *O. rufipogon* and *O. sativa* **[16],** might be operating in the F1 hybrids of this current study. Molecular profiling of the F1 hybrids may be helpful to confirm the true cause. Significant differences in mean percent F1 spikelet fertility indicated that the F1 hybrids varied in their inherent fertility. This could be that the gene(s) implicated in hybrid sterility in the Bas-385 and FM, were expressing variably or were distributed differently within each other , during the initial breeding process. **Ikeda *et. al*., [17]** crossed upland NERICA cultivars with two accessions of *O. glaberrima* and two cultivars of *O. sativa* and reported percent F1 seed fertility of 0.4 to 91.7%, depending on the cross combination suggesting that the NERICA cultivars varied in their inherent fertility. Cross ability of the Bas-385 was irrespective of parentage. Compatibility would have been expected to be higher for cultivars from the same parentage and less for those from different parentages. However, the first three hybrids that ranked high, (Bas 385 7/1 FM 13/1 and FM20 /1 had female parents i.e., Bas-385 18 / 9 FM 12 /9 and FM20 /1) .Bas-385 1 / 9 which recorded the least mean F1 percent seed fertility, had their parental origin. Bas 385 7/1 FM 13/1 and FM20 /1, the female parents of the first three hybrids that ranked high, had *O. Rufipogon* cytoplasm. These were among the three Bas-385 , FM cultivars, classified compatible with both japonica and indica cultivars **[17]. Ikeda *et. al*., [17]** reported that though it does not deny the possibility that certain nuclear genes were involved in their compatibility, the cytoplasm of *O. rufipogon* , may have a suppressant effect on hybrid sterility. Cytoplasmic effect could also be implicated from this direction; percent spikelet fertility of FM 20/1 was significantly different from its reciprocal Bas-385 10/7 /15. Bas-385 10/7 /15 was derived from Bas -385 14/ *O.r* 1 and Bas-385 19 /1 **[18].** There was no significant difference between Bas-385 17 /10, Bas-385 19 /1 and their reciprocals. All four hybrids maintained the cytoplasm of *O. sativa.* Aside from cytoplasmic effect

accounting for part of the variability in the Bas-385 FM crosses, cytoplasmic nucleus interactions could also play a role. There were significant differences among crosses that maintained *O. rufipogon* cytoplasm. The difference could be accounted for by a possible interaction between the cytoplasm and the nucleus composition of the paternal parent. A similar difference was also observed in the crosses that maintained *O. sativa* cytoplasm.

3.3. ***Table 3*** comparison among crosses of *Oryza rufipogon & Oryza longistaminata that* percent of grain were formed between *F.Malakand X O.longistaminata* were only 2 cross has been made successful while two seed has been formed on the other hand O.rufipogon has > 90 % resulted in grains. Maximum 200 grains were formed in cross No.20 on October 2011 respectively. Pollination during the month Oct for O. *longistaminata* and May for O.rufipogon was also indication of non synchronization because all the local cultivars has almost were ready for harvesting. The flowering and booting stages were quite different in case of O. *Longistaminata* while in case of *O.rufipogon i*s was ok accordingly

3.4. Back cross: Transfer of pollen grains from either parent to some emasculated spikelets of some F1 hybrids, increased percent spikelet fertility. Hybrids of Bas-385 14/1 /14 increased percent seed set by about sixteen times (3.26 increased to 60.71) in the backcross (Bas-385 14/ 1/ 1); that of Bas385 11 /FM/O.r 3 more than doubled the percent spikelet fertility (27.87 to 65.70). This suggested reduced pollen fertility in the hybrids. **Sano, [16]** reported that the hybrids between *O. rufipogon* and *O. sativa* were male sterile but partially female fertile. The fact that backcrossing increased seed set in hybrids, suggested some pistil/ gynaecium remained functional. Though F2 seeds could be produced indefinitely by rooting the F1 hybrid plants, the hybrid that improved seed set 16 times in the backcross will require 16 cycles of rooting, to produce that same seed lot. Hence, genetic improvement within the selected varieties may best be done through backcross breeding.

3.5. Pollen fertility: Results of the pollen fertility test indicated reduced pollen viability in the hybrids from the study, indicating the inherent infertility problem in the intraspecific crosses. The differences could be due to variations in cytoplasmic effects as reported by **[19]**

Conclusion

Based on the observations and results of the study, it can be concluded that crossability of the first and second generation BC1F2 were variable, depending on the type of cytoplasm and cytoplasm nucleus interactions. Reduced pollen viability might be the cause of F1 hybrid sterility. Hybrid sterility within the selected material was not complete. Within the selected.Bas-385, improvement can therefore be possible through conventional crosses and best through backcross breeding. Raising a large F1 population through emasculation, as well as rooting the F1 plants, could help increase F2 populations.

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