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Development of F_1 and BC_1F_1 interspecific hybrids of *O. sativa* cv. Savitri / *O.brachyantha* to introgress yellow stem borer resistance genes into cultivated rice

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ABSTRACT

In the present study an attempt has been made to introgress yellow stem borer resistance genes from O. brachyantha (FF) to cultivated rice through development of F_1 and BC_1F_1 interspecific hybrids of O. sativa cv Savitri / O.brachyantha. The morpho-cytological characterization of the hybrids was done in order to know the phenotypic characters of the interspecific hybrids and cytological behavior of the chromosomes. The data revealed that in general morphologically, the F_1 was intermediate between the donor and recipient parents. In F_1 interspecific hybrid no chromosome pairing was observed, where as in BC_1F_1 presence of 0-2 trivalents and bivalents was observed.

Key words : *rice, genome, wild species, interspecific hybrids, yellow stem borer*

Rice, the most important cereal crop in the world is cultivated under diverse agro-ecosystems. The production and productivity of rice are affected by several biotic and abiotic stresses. Yellow stem borer (YSB) *Scirpophaga incertulas* (Walker) is one of the major insect pests that damage the crop in most of the agro-ecosystems. YSB is the most destructive widely occurring pest causing an yield loss of about 1-19% and 38-80% in early and late planted rice crop, respectively (Catindig and Heong, 2003). Genetic variability for resistance to YSB is either limited or lacking in the primary gene pool of rice. Wild species of secondary gene pool of rice are important reservoirs of many useful genes including resistance to diseases and insect pests. Among the wild rice germplasm, *O. officinalis, O. brachyantha, O. ridleyi* and *Porteresia coarctata* were found to be resistant / tolerant to YSB (Padhi and Sen, 2002). For the introgression of the YSB resistance genes, wide hybridization between wild species and cultivated rice is required which is difficult due to genomic incompatibility and chromosome non-homology that may cause pre-fertilization and post-fertilization barriers for genetic exchange. Pre-fertilization barriers can be overcome by applying growth hormones (Sitch and Romero, 1990). Post-fertilization barriers can be overcome by rescuing the embryo before abortion on a suitable nourishing medium under asceptic condition. Employing the embryo rescue technique, a number of wide cross hybrids in rice have been developed by several researchers (Jena and Khush, 1990; Sitch and Romero, 1990; Brar *et al.,* 1991; Panda 2006, Sen *et al.,* 2006). In the present study an attempt has been made to introgressYSB resistance genes from *O. brachyantha* to cultivated rice through development of F_1 and BC_1F_1 interspecific hybrids of *O. sativa* cv Savitri / *O.brachyantha*.

MATERIALS AND METHODS

For the introgression of YSB resistance genes to cultivated rice *O. sativa* cv Savitri was used as female parent and *O.brachyantha* was used as the donor and the crosses were made treating the spikelets of Savitri with phyto-hormones before and after the pollination. The expected fertilized embryos were rescued in ¼ MS media 10-12 days after pollination. The embryos were incubated in dark for germination and after germination they were grown in the illuminating chamber and subsequently subcultured in rooting medium. At 3-4 leave stage the seedlings were removed from the test tubes and roots were washed thoroughly under running tap water. Then they were transferred to pots with sterilized soil and were kept for 3-4 days in the room temperature in the laboratory for acclimatization and finally shifted to net house where they were allowed to grow with a good agronomic condition till maturity. The F_1 interspecific hybrid of *O. sativa* cv Savitri / *O.brachyantha* was backcrossed with the recurrent parent *O. sativa* cv Savitri for the development of BC_1F_1 hybrid employing embryo rescue technique.

The morphological characters of the embryo rescued plants of F_1 and BC_1F_1 interspecific hybrids were studied and both qualitative and quantitative characters were recorded. The cytological behavior of both was studied fixing the young spikelets in aceto alchol (1:3) and smearing the anthers at appropriate stages in 2% acetocarmine solution. The F_1 and BC_1F_1 interspecific hybrids were evaluated against YSB along with cv. Jaya (susceptible check) and cv. Ratna (tolerant check). The screening was made by implanting the freshly hatched YSB larvae @ 2 larvae per tiller into the plant in both wet and dry seasons in replicated trials. Scoring of the damage was done by following SES system developed by IRRI (1992).

RESULTS AND DISCUSSION

Out of 1302 spikelets of *O. sativa* cv. Savitri pollinated with *O. brachyantha*, only five hybrids were obtained indicating very low crossability (0.38%). Subsequently, the F_1 interspecific hybrid was backcrossed with the recurrent parent *O. sativa* cv Savitri for the development of BC_1F_1 hybrid employing embryo rescue technique. The crossability percentage was enhanced to 1.29% in BC_1F_1. Out of 1549 spikelets of F_1 hybrids

Table 1. Crossability between *O. sativa* and *O. brachyantha*

Cross combination	No. of spikelets pollinated	No. of embryos cultured	Percentage of germination	No. of embryos rescued	No. of hybrids obtained	Percentage of crossability	No. of hybrids survived up to maturity
O. sativa cv Savitri/ O. brachyantha(F_1)	1302	66	48.5	13	5	0.38	5
O. sativa cv Savitri / O. brachyantha // Savitri (BC_1F_1)	1549	91	43.96	30	20	1.29	1

Table 2. Morphological traits of F_1 and BC_1F_1 hybrids of cross *O. sativa* cv. Savitri / *O. brachyantha* and both the parents

Characters	F_1 hybrid	BC_1F_1	Donor parent	Recipient parent
Plant height (cm)	98.6±3.61	90.9 ±1.75	105.8 ±0.76	97.2±1.50
Ear bearing tillers	35.8±2.04	16.3±4.8	80.6±1.87	8.8±0.31
Leaf length(cm)	30.7±1.14	38.3±2.02	22.6±1.16	26.8±0.97
Leaf breadth (cm)	0.9±0.03	1.0±0.24	0.8±0.03	1.7±0.04
Panicle length(cm)	17.4±0.24	24.2±0.81	17.1±0.72	23.3±0.88
No. of spikelets per panicle	51.0±4.17	24.2±0.8	53.2±1.61	98.7±3.47
Spikelet length(cm)	0.74±0.01	0.8±0.08	0.8±0.0	0.6±0.0
Spikelet breadth (cm)	0.2±0.0	0.2±0.0	0.2±0.0	0.3±0.0
Awn length (cm)	9.3±0.20	6.2±0.33	16.8±0.42	0.0±0.0
Anther length(cm)	0.3±0.0	0.3±0.0	0.2±0.0	0.2±0.0
Stigma length(cm)	0.1±0.0	0.2±0.0	0.2±0.0	0.2±0.0
Pollen fertility (%)	0.0	2.8	91.4	98.2

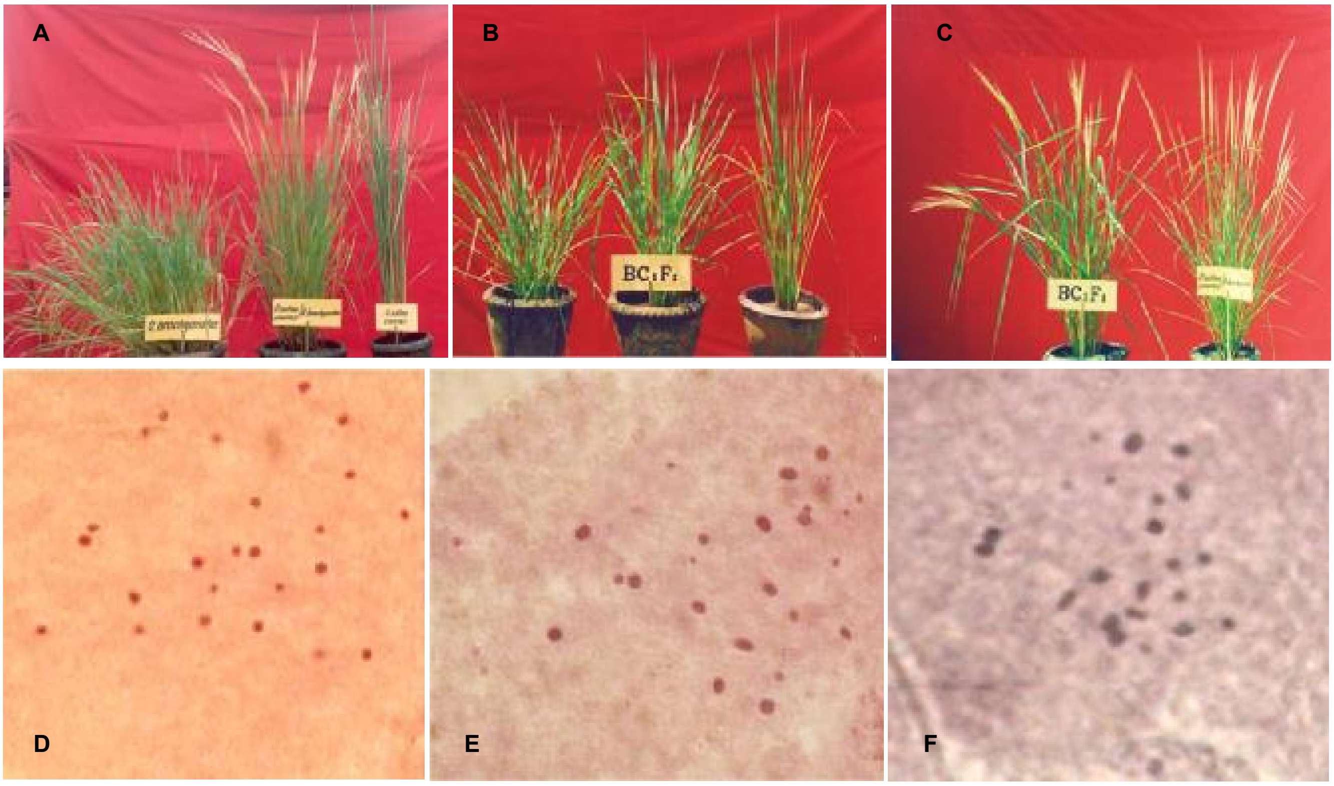

Fig 1. Morpho-cytological photographs of F_1 and BC_1F_1 hybrids of *O. sativa* cv Savitri / *O. brachyantha*

A. Morphology of F_1 hybrids of *O. sativa* (Savitri)/*O. brachyantha* with their parents *O. sativa* cv. Savitri and *O. brachyantha*,
B. Morphology of BC_1F_1 hybrid of Savitri/*O.brachyantha*//Savitri (centre), F_1 hybrid (left) and cv. Savitri (right) at vegetative stage,
C. Morphology of BC_1F_1 hybrid of Savitri/*O.brachyantha*//Savitri with F_1 hybrid of Savitri/*O. brachyanhta* at flowering stage,
D. PMC of F_1 hybrid of *O. sativa* cv Savitri /*O. brachyantha* at metaphage 1 showing 24 Is,
E. Chromosome association of BC_1F_1 of Savitri /*O. brachyantha*//Savitri showing 1 III, 12 IIs and 9 Is at metaphage I of Meiosis
F. Chromosome association of BC_1F_1 of Savitri/O. brachyantha // Savitri showing 2 IIIs + 11 IIs + 8 Is at metaphage I of meiosis

pollinated with recurrent parent Savitri, 20 hybrids were obtained from which only one BC_1F_1 hybrid could survive till maturity (Table 1). Similar observations were reported by Jena and Khush (1990), Brar *et al*. (1991) and Multani *et al*. (1994) using other interspecific hybrids.

In case of F_1 hybrids of *O. sativa* cv Savitri / *O. brachyantha* most of the characters were intermediate between the parents. The mean plant height of F_1 interspecific hybrid was about 99cm and reduced in BC_1F_1 (Savitri / *O. brachyantha* // Savitri) to 90 cm (Table 2 and Fig. 1A, B and C). Likewise, the number of ear bearing tillers was reduced from 35.8 in F_1 to 16.3 in BC_1F_1. Awn length showed similar trend. However, leaf length increased incase of BC_1F_1 (38.3 cm) as compared to F_1 hybrids (30.7 cm). Similar trend was observed with panicle length. Pollen fertility increased to 2.8 % in BC_1F_1. The characters like spikelet length and breadth and anther length in BC_1F_1 were found to be intermediate between F_1 hybrids and parents. The plant type was observed to be more towards the *sativa* parent. Similar results were obtained by Brar (1996).

The genomic constitution of F_1 interspecific hybrid is AF. At metaphage-I, 70, 65, 74, 80, 60 PMCs of hybrid 1 to 5, respectively were analyzed. The

Table 3. Frequency of PMCs with different chromosome association in BC_1F_1 hybrid of Savitri / *O. brachyantha* // Savitri

	No. of PMCs studied	Number of PMCs with different chromosome configuration										Frequency of PMCs with a range of 11-15 IIs per cell(%)	Frequency PMCs with with a range of 1-2 IIIs per cell (%)
		12IIs+12Is	13IIs+10Is	11IIs+14Is	14IIs+8Is	15IIs+6Is	1IIIs+10IIs+13Is	1III+11IIs+10Is	1III+12IIs+9Is	2IIIs+11IIs+8Is	2IIIs+12IIs+6Is		
BC_1F_1	127	48 (37.79)	23 (18.11)	13 (10.24)	10 (7.87)	5 (3.94)	9 (7.09)	5 (3.94)	6 (4.72)	4 (3.15)	4 (3.19)	59.84	22.05

Mean frequencies of PMCs are presented in parenthesis

frequency of univalents was 100 % (Fig. 1D). Brar *et al* (1996) reported 0.06 bivalents per PMC and Abbasi (1999) reported 0.05 bivalents per PMC between genome of these two species.

The cytological characterization of BC_1F_1 (Savitri / *O. brachyntha* // Savitri) and the pairing behavior of the chromosomes revealed as many as 12 IIs + 12 Is in most of the PMCs with a mean frequency of 37.79 % and 1-2 trivalents (III s) were observed with a mean frequency of 22.05 % per PMCs (Table 3). All the *sativa* chromosomes paired with one another and a single chromosome of *O. brachyantha* attached to that pair, thereby giving a trivalent shape. Thus, BC_1F_1 hybrids showed an improvement in the pairing of chromosomes than F_1 hybrids. Similar observations were reported by Jena and Khush (1990) in *O. officinalis*, Brar *et al*. (1991) in *O. brachyantha.*

For the screening of F_1 and BC_1F_1 interspecific hybrids against YSB, the dead heart and white ear head infestation were studied in both wet and dry seasons of 2005-06. The data revealed that in both the seasons, the F_1 interspecific hybrids were observed to be resistant (R) to YSB with the score 1 and 3 for dead heart and white ear head respectively in 0-9 scale. For BC_1F_1 registered a damage score of dead heart and white ear head infestation of YSB (Table 4). This may be due to the addition of the *sativa* genome when crossed with recurrent parent Savitri and similar observations were recorded by Jena and Khush (1990) for introgression of BPH resistance genes from *O. officinalis*.

The F_1 and BC_1F_1 interspecific hybrids of *O. sativa* cv Savitri / *O. brachyantha* were developed for the introgression of alien genes resistant to YSB available in the species belonging to the secondary gene pool. The study revealed that morphologically the F_1 hybrids were more or less intermediate between the parents with a few variations. In BC_1F_1, the presence of 0-2 trivalents and 10-15 bivalents at metaphase I was observed, which indicates addition of more *sativa* genome. The F_1 hybrid was resistant to YSB where as BC_1F_1 was moderately resistant. The BC_1F_1 interspecific hybrids will be further utilized in the backcrossing programme for the development of

Table 4. Reaction of the F_1 and BC_1F_1 hybrids against dead heart and white ear head

Genotypes	Mean DH percentage and reaction to YSB				Mean WEH percentage and reaction to YSB			
	Original Mean	Transferred mean	Score	Reaction to YSB	Original Mean	Transferred Mean	Score	Reaction to YSB
Savitri / *O. brachyantha* (F_1)	9.74	18.66	1	R	10.08	19.00	3	MR
Savitri / *O. brachyantha//* Savitri (BC_1F_1)	12.50	21.04	3	MR	10.50	19.32	3	MR
Ratna (Check)	22.42	34.95	3	MR	20.36	27.20	5	MS
Jaya (Check)	32.46	22.97	7	HS	39.81	39.41	7	S

monosomic alien addition lines (MAALs) which in turn will help to develop rice genotypes resistant to YSB, the deadly pest of rice.

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