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Introgression of yellow stem borer (*Scirpophaga incertulus*) resistance genes into cultivated rice (*Oryza* sp.) from wild species

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ABSTRACT

Wild rice germplasm has been screened against yellow stem borer (*Scirpophaga incertulas* L.) and *O. brachyantha*, *O. officinalis*, *O. ridleyi* and *Porteresia coarctata* were found to be resistant/tolerant. It is essential to introgress these resistance genes from wild species to cultivated rice for the development of rice varieties with in-built resistance against yellow stem borer. The BC_1F_1 inter-specific hybrid of *O. sativa* cv 'Savitri'/*O.brachyantha* was backcrossed with recurrent parent 'Savitri' employing embryo rescue technique. The morphological characters of the embryo rescued plants were studied and they were observed to be phenotypically different types, namely grassy, bushy, erect, dwarf, sterile and pseudonormal etc. The morphology of the chromosome variants was observed to be different due to addition of different chromosome numbers. The cytological analysis of the variants was done to identify the different chromosome variants for the development of monosomic alien addition lines to introgress yellow stem borer resistance genes to cultivated rice.

Key words: Embryo rescue, Introgressed lines, Monosomic alien addition lines, Rice, Yellow stem borer resistance

Rice (*Oryza sativa* L.) is one of the most important cereal crops in the world and the major food for almost half of the global population. The crop is cultivated under various diverse agro-ecosystems. However, the production and productivity of rice are affected by several biotic and abiotic stresses. Yellow stem borer (*Scirpophaga incertulas* L.) is one of the major insect pests, which damages the crop in all most all agro-ecosystems. The yield loss due to yellow stem borer is observed to be as high as 40% in an unprotected crop in the field condition (Manwan and Vega 1975). Though some high-yielding rice varieties were reported to be resistant against yellow stem borer by various researchers but not a single variety could yet be found perfectly resistant against yellow stem borer. Wild species of rice are important reservoirs of various desirable traits. Wild rice germplasm has been screened against yellow stem borer and *O. brachyantha*, *O. officinalis*, *O. ridleyi* and *Porteresia coarctata* were found to be resistant/tolerant against yellow stem borer (Padhi and Sen 2002). It is essential to introgress these resistance genes from wild species to cultivated rice for the development of rice varieties with in-built resistance against yellow stem borer. For the introgression of the yellow stem borer resistance genes, wide hybridization between wild species and cultivated rice is essential which is difficult due to genomic distance and chromosome non-homology. During wide hybridization between distant genomes and cultivated rice pre-fertilization and post-fertilization barriers occur. Pre-fertilization barriers can be overcome by applying growth hormones. Post-fertilization barriers can be overcome by rescuing the embryo before abortion on a suitable nourishing medium under asceptic condition to produce viable inter-specific hybrids (F_1s). Employing embryo rescue technique, a number of wide cross hybrids in rice have been developed by several researchers (Sen *et al.* 2006, Panda 2007). In the present study an attempt has been made to transfer yellow stem borer resistance genes from *O. brachyantha* to cultivated rice through repeated backcrossing employing embryo rescue technique and developing chromosome variants.

MATERIALS AND METHODS

The BC_1F_1 inter-specific hybrid of *O. sativa* cv Savitri/ *O. brachyantha* developed by Panda in 2006 at CRRI, Cuttack was backcrossed with recurrent parent 'Savitri'. The spikelets of BC_1F_1s were treated with hormonal solutions before and after pollination during hybridization. The expected fertilized back crossed embryos were rescued before abortion. The embryos of different ages (7, 10, 12, 14, 16, 18 and 20 days old) from the backcross were cultured in 1/4

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MS medium. The spikelets were washed with doubled distilled water and surface sterilized by treating them in 70% ethanol for 2 min. The excess alcohol was removed by absorbent cotton and then spikelets were transferred to 4% sodium hypoclorite for 15 min. inside the laminar air flow chamber. Spikelets were again washed two times in sterilized water. The lemma and palea of the spikelets were opened with the fine forceps under stereomicroscope (10×40 X). The endosperm sac with embryo was come out of the spikelet. Then the hard, globular embryo was excised from the watery endosperm by sterilized needle. The entire process was held under stereo-microscope in aseptic condition. The hard globular excised embryos were inoculated in culture tube which contains 1/4 MS medium and the cultured embryos were incubated in dark (25 ± 1°C) until germination. Subsequently they were transferred to illuminating incubation room. The percentage of embryo germination and embryo rescued were studied. After that the seedlings developed up to 4–5 leaves stage with well developed roots 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 room temperature in the laboratory for acclimatization and finally they were shifted to net house where they were grown till maturity. Percentage of crossability was calculated as follows.

$$\% \text{ of crossability} = \frac{\text{Number of inter-specific hybrids obtained}}{\text{Total number of spikelets pollinated}} \times 100$$

Subsequently the plants were grown through stubble multiplication to increase the population. The morphological characters of the embryo rescued plants were studied taking both qualitative and quantitative characters on five plants and data were recorded. The spikelets of BC_2F_1 plants were fixed with aceto-alcohol (1:3) for 48 hr and the fixed materials were squashed with 2% aceto-carmine solution for the cytological studies to find out the chromosome numbers of the BC_2F_1 plants.

RESULTS AND DISCUSSION

Rescue of back crossed hybrid embryos and development of chromosome variants

For the introgression of alien genes resistant to yellow stem borer into cultivated rice, the BC_1F_1 backcrossed hybrids were again backcrossed with recurrent parent 'Savitri' employing embryo rescue technique. The expected fertilized embryos of 7 to 20-days-old were aseptically excised and were cultured in ¼ MS medium to find out the suitable age of developing embryo for embryo rescue. The data revealed that 10-days-old developing embryos when cultured gave the maximum percentage of embryos rescued (50%), followed by 12-days-old developing embryos (39%). When the older embryos were cultured (14 days and above), the percentage of rescued embryos were reduced and finally embryos were aborted, which indicated that 10–12-days-old developing embryos were suitable for rescuing the expected backcrossed hybrids. Similar observations were recorded by Panda (2006) for developing backcrossed hybrids involving *O. officinalis* and *O. brachyantha*, respectively.

For developing introgression lines BC_1F_1 hybrid of *O. sativa* cv 'Savitri'/*O. brachyantha*//'Savitri' was backcrossed with recurrent parent 'Savitri' treating with hormonal

Table 1 Morphological characteristics of introgression lines

Introgression line	Plant height (cm)	Panicle length (cm)	EBT	Flag leaf		Ligule length(cm)	Auricle P*/A*	Awn length (cm)	Spikelet	
				L*	B*				L*	B*
1	58	33.0	28	21.0	1.5	1.7	A	10.7	1.6	0.3
2	65	19.0	37	23.0	0.3	0.6	A	0.5	0.7	0.2
3	56	13.0	45	19.0	1.0	1.7	A	1.0	0.5	0.2
4	87	23.0	25	28.0	1.0	1.5	A	2.5	1.0	0.5
5	83	23.0	40	32.0	0.5	1.0	A	4.0	1.0	0.2
6	86	19.0	20	32.0	1.5	1.2	A	1.5	0.6	0.2
7	39	20.0	41	32.0	0.7	1.0	A	0	0.5	0.2
8	100	25.0	15	22.0	1.0	1.0	A	4.0	1.0	0.2
9	58	19.0	35	18.5	0.6	0.8	A	2.3	0.7	0.3
10	31	9.5	16	14.5	0.5	0.5	P	0	0.5	0.3
11	84	23.0	27	24.0	1.0	1.0	A	2.5	0.6	0.3
12	45	12.0	25	13.0	1.0	0.9	A	0.3	0.3	0.2
13	96		19	21.0	1.5	1.2	A			
14	65	13.0	15	13.0	0.4	1.0	A	0.3	1.0	0.2
15	66	11.5	10	18.0	1.6	0	A	0	0.5	0.3
16	47	16.0	18	11.0	0.4	0.9	P	2.5	0.7	0.2
17	73		26	46.0	0.6	2.5	A			
18	70		18	45.0	1.0	2.5	P			

***** L, Length; B, breadth; P, present; A, absent

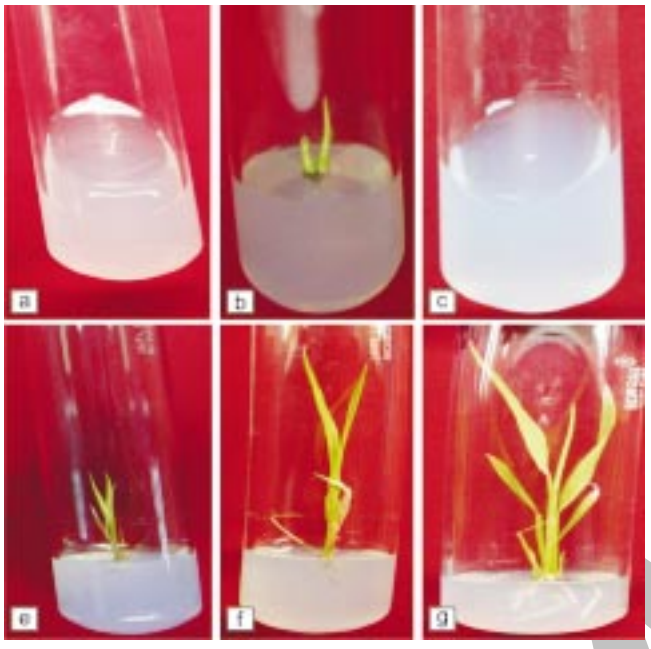

Fig 1 Different stages of embryo rescued plants, (*A*) Embryo inoculated, (*B*) Germinated after 48 hr (*C-E*) At growing stages (*F*) At rooting stage

solutions and 10–12-days-old developing embryos were rescued. Out of 4 984 pollinations on BC_1F_1 spikelets made, 1 381 expected fertilized spikelets were harvested and 169 expected fertilized embryos were rescued. Out of them 70 embryos were germinated a total of 32 BC_2F_1 plants/ chromosome variants were obtained and 19 were survived. The different stages of embryo rescued plants were shown in Fig 1(A-E). The plants at 4-5 leaves stage with proper root development in the tissue culture media were transferred to sterilized soil in the earthen pots for acclimatization in room temperature. Finally, they were shifted to earthen pots with good agronomic base in the net house and were allowed to grow up to maturity. The crossability percentage was observed to be 2.32. The crossability percentage was increased as compared to F_1 inter-specific hybrid due to hybridization of BC_1F_1 hybrid with recurrent *sativa* parent 'Savitri', addition of more doses of *sativa* genome. Similar results were obtained by Sen *et al*. (2010).

Morpho-cytological characterization of introgression lines

The morphological characters of the introgression lines were studied taking observations on 10 different qualitative and quantitative characters, ie plant height, panicle length, number of ear bearing tillers (Ebt), flag leaf length and breadth, ligule length, presence of auricle, awn length and spikelet length and breadth etc. (Table 1). The main morphological characters, like plant height of the variants observed ranging from 31 cm to 100 cm, panicle length varied from 9.5 cm to 33.0 cm, Ebt varied from 10 to 45 (bushy), flag leaf length varied from 11 cm to 46 cm and flag leaf breadth varied from 0.3 cm to 1.6 cm and awn length reduced to 10.7 cm (ranging from 0.3 to 10.7 cm) as compared to wild parent *O. brachyantha* (18.0 cm) and in some variants absence of awn was also observed which may be due to presence of more doses of *sativa* genome in the repeated backcrossing with the recurrent parents (Table 1).

According to the morphological characters the plants/ chromosome variants were grouped into grassy, bushy, erect, dwarf, sterile and pseudonormal etc. Morphological photographs of some of the chromosome variants were presented in Fig 2. Out of 19 plants, one plant could not flower through out its growth period. Taking the cytological observations, the chromosome variants with 2n (1), 2n+1 (8), 2n+1+1 (2), 2n+1+1+1 (2) and in five plants more than three addition chromosome numbers were identified. The chromosome variants were grouped according to the addition chromosome numbers. In similar method, Jena *et al*. (1990) identified MAALs/chromosome variants in rice, Shigyo *et al*. (1997) in onion (*Allium cepa* L.), Singh *et al*. (1998) in soybean (*Glycine max* L. Merr) and Chen *et al*. (2004) in cucumber (*Cucumis sativus* L.).

For the introgression of yellow stem borer resistant gene from *O. brachyantha* into cultivated rice the back crossing

Fig 2 Morphological grouping of chromosome variants

of BC_1F_1 with recurrent parent 'Savitri' was made rescuing 10–12-days-old embryos and BC_2F_1 plants or the chromosome variants with chromosome number 2n, 2n+1, 2n+1+1, 2n+1+1+1 and more than three additional chromosome numbers were obtained. Based on their morphological peculiarities they are grouped into grassy, dwarf, bushy, erect, sterile and pseudonormal.

The chromosome variants will help for the development of Monosomic Alien Addition Lines (MAALs) which in turn will help in developing introgressed lines with alien genes resistant to yellow stem borer. The introgressed lines can be easily crossed with cultivated rice and the rice varieties with in-built yellow stem borer resistance genes can be developed. As a result the major crop loss due to yellow stem borer infestation can be minimized and production and productivity of rice will be enhanced.

REFERENCES

Chen J F, Luo X D, Qian C T, Jahn M M, Staub J E, Zhuang F Y, Lou Q F and Ren G. 2004. *Cucumis* monosomic alien addition lines: morphological, cytological, and genotypic analyses. *Theoretical Applied Genetics* **108** (7): 1343–8.

Manwan I and Vega C R. 1975. Resistance of rice varieties to yellow stem borer. Paper presented at IRRI Saturday Seminar held during 10 May 1975 at International Rice Research Institute, Los Banos, Laguna, Philippines 37 pp.

Padhi G and Sen P. 2002. Evaluation of wild rice species against YELLOW STEM BORER (*Scripophaga incertulas*, Walk). *Journal of Applied Zoological Research* **11(2 & 3)**: 103–4.

Panda B. 2006. 'Wide hybridization for the introgression of yellow stem borer resistance genes from wild rice species to cultivated rice species (*Oryza sativa* L.)'. (unpublished thesis)

Panda B and Sen P. 2007. Application of embryo rescue technique for development of interspecific hybribs in rice resistant to yello stem borer. *Journal of Cytology and Genetics* **8**: 57–63.

Sen P, Panda B, Kar M K, Padhi G and Mohanty S K. 2006. Introgression of alien genes for resistance to bacterial blight (BB) and yellow stem borer in rice. (*in*) *Abstracts of 26th International Rice Research Conference*, pp 256–7, held during 9–13 October 2006 at New Delhi.

Sen P, Panda B, Kar M K and Mishra R N. 2006. Introgression of alien genes for genetic improvement of rice. *Centenary Volume of Full text papers on National Seminar on Emerging Trends in Plant Sciences: Morphology to Biotechnology*, pp 103–111, held during 29–30 October at 2006 at Department of Botany, Ravenshaw University, Cuttack.

Sen P, Kar M K, Panda B, Behura N, Bose L K. and Mishra R N. 2010. Exploitation of secondary and tertiary gene pool in genus *Oryza*. *Oryza* **47** (1):1–12.

Shigyo M, Iino M, Isshiki S and Tashiro Y. 1997. Morphological characteristics of a series of alien monosomic addition lines of Japanese bunching onion (*Allium fistulosum* L.) with extra chromosomes from shallot (*A. cepa* L. Aggregatum group). Genes & Genetic Systems **72**(4): 181–6.

Singh R J, Kollipara K P and Hymowitz T. 1998. Monosomic Alien Addition Lines Derived from *Glycine max* (L.) Merr. and G. tomentella Hayata: Production, Characterization, and Breeding Behavior. *Crop Sci* **38**: 1483–9.