Isolation and identification of monosomic alien addition lines (MAALs) of the wild rice *Oryza brachyantha* A.Chev.et.Roehr on cultivated rice for developing rice genotypes resistant to YSB

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***Abstract*— *Oryza brachyantha* (FF), the African wild rice, belonging to secondary gene pool of rice which is resistant to Yellow stem borer (YSB) was used to develop Monosomic Alien Addition Lines (MAALs) from the wild species with the objective to transfer gene(s) of resistance to YSB to cultivated rice. Out of 19, 399 BC_1F_1 spikelets artificially pollinated with the recurrent parent, 29 BC_2F_1 hybrids were produced through embryo rescue with germination and crossability efficiencies of 49.1% and 0.55% respectively. Embryos collected between 10 (36.3%) and 12 (44.4%) days after pollination (DAP) were grown successfully on ¼ MS basal medium with an efficiency of 70.0%. The hybrids were morphologically characterised and categorized into 16 plant types (PT) which resembled 8 primary trisomics of rice. Cytologically characterized hybrids exhibiting 2n+1 (2n=25) chromosome arrangement and bearing resemblance to primary trisomics of rice were thus designated as MAALs representing MAAL- 4 (Sterile), MAAL- 5 (Twisted leaf), MAAL- 7 (Narrow leaf), MAAL- 8 (Rolled leaf), MAAL- 9 (Stout), MAAL- 10 (Erect), MAAL- 11(Pseudonormal) and MAAL- 12 (Tall).**

***Index Terms*— MAALs, *O. sativa*, *O. brachyantha*, wild rice.**

I. INTRODUCTION

The productivity of cultivated rice is affected by several biotic and abiotic stresses. The genetic variability for these biotic stresses is limited in cultivated rice germplasm or the cultivars become susceptible due to changes in insect biotypes and different races of pathogens. It is therefore imperative to broaden the gene pool of rice by introgression of alien gene(s) from wild relatives of rice which are known to be resistant to major biotic and abiotic stresses [9, 17, 23] and can serve as a rich source of variability for rice improvement.

The Yellow stem borer (YSB), *Scirpophaga incertulas* (Walker), a major pest of cultivated rice, causes damage to the rice crop in almost all agro climatic ecosystems resulting in yield loss of about 10%-60% in unprotected field conditions [19]. Gene(s) for resistance to YSB have not been found in the primary gene pool of rice. Tough some high yielding rice varieties have been reported to be moderately resistant to YSB, no rice variety truly resistant to YSB has been developed from cultivated rice. It is therefore essential to incorporate alien genes for resistance to YSB from wild species belonging to the secondary gene pool of rice which are reservoirs of such traits. Wild germplasm has been screened against YSB and *O. brachyantha*, *O. officianalis*, *O. redleyi* and *Porteresia coarctata* were found to resistant/tolerant to YSB [5, 10]. Attempts have been made to transfer YSB resistance gene(s) from *O. redleyi* into cultivated rice [6] but much success has not been achieved.

Oryza brachyantha (FF), the wild species widely distributed in Africa belongs to the secondary gene pool of rice and has been found to be highly resistant to Leaf folder, Bacterial Blight (BB), Blast, Yellow stem borer (YSB) and Whorl maggot [5]. Thus it serves as an important reservoir for a wide range of resistant gene(s) which can be transferred to cultivated rice. Genes for BB resistance from *O. brachyantha* have been transferred to cultivated rice by [5] but transfer of YSB resistance from this wild species has not been attempted. While wild species belonging to AA genome can be easily crossed with *O. sativa*, the more distantly related wild species like *O. brachyantha* and others are difficult to cross due high genomic incompatibility rendering the F_1 hybrids completely sterile. Introgression of alien genes from these distantly related wild species is possible through the development of Monosomic Alien Addition Lines (MAALs) employing embryo rescue. MAALs have an extra chromosome (2n=25) ideally from the wild species in addition to a complete chromosome complement of the cultivated species. MAALs representing 6–12 extra chromosomes have been reported in *O. officinalis* (CC), *O. minuta* (BBCC), *O. latifolia* (CCDD), *O. australiensis* (EE), *O. brachyantha* (FF), *O. granulata* (GG), and *O. ridleyi* (HHJJ) [3,4,6,15,25] which have been characterized based on morphological and cytological characterization, Flourescence- *in situ*- Hybridization (FISH) and molecular markers.

In this investigation we have attempted to develop a complete set of 12 MAALs from *O. brachyantha* which will be further used as introgression lines or pre-breeding lines to develop rice genotypes truly resistant to YSB.

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II. MATERIALS AND METHODS

Development of backcross hybrids

BC_1F_1 (*O. sativa* cv Savitri /*O. brachyantha*//*O. sativa* cv Savitri) interspecific backcross hybrids were again backcrossed with the recurrent parent, *O. sativa* cv Savitri following the scheme for the production of monosomic alien addition lines as suggested by [2]. The spikelets of BC_1F_1 hybrids were treated with solutions, Solution A (NAA 25mg/L+ Sucrose 5g/L), and Solution B (GA3 50mg/L+KN 5mg/L+NAA 5mg/L), before and after pollination to overcome pre- and post-fertilization barriers respectively. Solution A was sprayed on to the emasculated spikelets before pollination and Solution B sprayed after pollination twice a day till 5 days [8, 15]. Artificial pollination was done by hand to maximize chances of obtaining more number of hybrids.

Caryopses with immature hybrid embryos were collected between 7-16 days after pollination (DAP) before embryo abortion and the percentage of embryo survival was evaluated by culturing them on artificial growth medium to establish an appropriate age of hybrid embryos for rescue before abortion (Table 1). The caryopses were then aseptically excised, treated adopting a protocol suggested by [24] and inoculated on 3 different artificial growth media to assess their regeneration efficiency and establish an optimum growth medium for embryo rescue (Table 2). Culture tubes were grown in dark at 25±2 ^oC until germination and germinated embryos were then grown in light-dark cycle of 16 hours.

Plantlets at three-leaf stage after 2-4 weeks of growth with well developed roots were acclimatized following four different methods (i) Direct transfer method, (ii) Modified [21] method, (ii) Modified soil: sand method [22], (iv) Peat moss fortified with Hoagland's solution and the responses were recorded to establish an optimum acclimatization protocol for the interspecific hybrids. The surviving plantlets were then transferred to the net house for further growth and analysis.

Isolation of Monosomic Alien Addition Lines (MAALs)

The BC_2F_1 (*O. sativa* cv. Savitri/*O. brachyantha*//*O. sativa* cv. Savitri///*O. sativa* cv. Savitri) hybrids, were characterized, at vegetative, mature and flowering stages following a set of qualitative and quantitative morphological characters (Table 5) based on the Hutchinson's Polygraph method and categorized different plant types (PT) like PT-1, PT-2 and so on (Table 4) based on their morphological features. More than one plant showing similarities to a particular trisomic were grouped into the respective PTs. Immature panicles from BC_2F_1 hybrids at suitable stage were collected and cytologically characterized to identify their chromosome arrangements and identify the MAALs.

Identification of Monosomic Alien Addition Lines (MAALs)

BC_2F_1 hybrids having a chromosome arrangement of 2n+1(2n=25) chromosomes and bearing resemblance to primary trisomics of rice were designated as Monosomic Alien Addition Lines (MAALs).

III. RESULTS AND DISCUSSION

Development of backcross hybrids

A total of 19,399 BC_1F_1 spikelets were artificially pollinated and treated with hormonal solutions before and after pollination to overcome strong pre- and post-fertilization barriers between the two parental species [18]. It was observed that the use of the hormonal solutions greatly improved viable embryo formation. Out of 5248 spikelets with expected fertilized embryos that could be harvested 916 were viable embryos and rest of the caryopses were without or with aborted embryos or filled with watery endosperm. Of the 916 embryo inoculated only 450 embryos germinated in culture thus showing an efficiency of 49.1% (Table 1). Crossability between the wild species and the cultivated species as evidenced from 29 BC_2F_1 hybrids obtained was found to be 0.55% (Table 1).

Embryo survival efficiency was evaluated with embryos collected between 7 to 16 days after pollination (DAP) and it was found that embryos collected between 10 to 12 DAP showed maximum rates of survival i.e, 36.3% and 44.4% (Table 2). Similar results have been reported in crosses

Table1. Percentage crossability and germination of BC_1F_1 population and recurrent parent

***O.sativa* cv. Savitri / *O.brachyantha* // *O.sativa* cv Savitri**						
Total no. of spikelets pollinated	**Total no. of embryos cultured**	**Total no. of embryos germinated**	**Germination (%)**	**Total no. of plantlets in culture**	**Total no. of plants in Pots**	**Crossability (%)**
5248	916	450	49.1	131	29	0.55

Table 2. Percentage Embryo survival at different days after pollination (DAP)

Days after pollination (DAP)	**BC_2F_1 (*O.sativa* cv. Savitri / *O.brachyantha* // *O.sativa* cv. Savitri) population**			
	No. of spikelets pollinated	**No. of spikelets fertilized**	**No. of embryos inoculated**	**Embryos rescued (%)**
7	32	15	2	13.3
10	27	11	4	36.3
12	36	9	4	44.4
14	23	8	1	12.5
16	32	8	0	0

between *O. sativa* and *O. brachyantha* [1, 18, 20]. It has been also pointed out that embryos collected between 8 and 12 DAP were ideal for embryo rescue irrespective of the wide cross combination [12]. Of the three different growth media used for testing embryo germination and plant growth (Table 3), ¼ MS basal medium showed the highest percentage of germination of 70%. Several earlier reports [7, 8, 11, 14] have used ¼ MS basal medium in different cross combinations of *O.sativa* with different wild species.

Direct transfer of plantlets with healthy 4-5 roots into sterilized soil in small earthen pots resulted in 80% survival supplemented with Hoagland's solution instead of tap water was found to be the ideal method for acclimatization.

Isolation of Monosomic Alien Addition Lines (MAALs)

Out of 29 BC_2F_1 hybrids transferred to net house, 21 plants survived and were studied for their qualitative and quantitative morphological characters and were categorized into the following 13 plant types (PT) based the observed morphological characters. Two or more plants exhibiting similar morphological characters were grouped into one PT. Plants exhibiting mixed characters were grouped separately.

Plant type (PT)- 1: Normal looking, medium height, dark green leaves, flag leaf medium broad and long, ligule is twice the length of *O. sativa* parent, morphologically indistinguishable from disomic except for reduced growth and vigour. BC_2F_1- 1 and 30 were grouped under PT-1.

Plant type (PT)- 2: Short height, slow growing, leaves medium dark green rolled and curved inwards, short ligule, short and dense panicle, short spikelet with beak shaped apiculus. BC_2F_1- 4, 7, 28 and 37 were grouped under PT-2.

Plant type (PT)- 3: Short height, low tiller number, dark green narrow leaves slightly rolled, short ligule, panicles incompletely exerted, slender spikelets with short awns. BC_2F_1- 5 was grouped under PT-3.

Plant type (PT)- 4: Medium tall, high tiller number, thick (stout) tillers, semi spreading appearance, leaves long, calcareous, dark green, folded giving a boat appearance, panicles long, dense, completely exerted, spikelets long, bold without awn. BC_2F_1- 8 and 36 were grouped under PT-4.

Plant type (PT)- 5: Almost as tall as O.sativa parent, low tiller number, erectly growing, leaves dark green, thick, narrow, slightly folded, panicle small, exerted, short spikelets. BC_2F_1- 10 and 32 were grouped under PT-5.

Plant type (PT)- 6: Short height, bushy appearance, high tiller number, leaves narrow, light green, twisted, long flag leaf, short ligule, auricle present, panicles short, incompletely exerted, spikelets slender without awn. BC_2F_1- 13, 33, 34 and 37 were grouped under PT-6.

Plant type (PT)- 7: Short height, medium tiller number, leaves slightly shorter, dark green, short panicles incompletely exerted, spikelets short without awn. BC_2F_1- 27 was included under PT-7.

Plant type (PT)- 8: Tallest among all plants, high tiller number, robust tillers, slightly spreading appearance, leave long, broad, light green colour, long ligules, panicles long with bold spikelets, spikelets have very short awn. BC_2F_1- 31 was included under PT-8.

Plants showing mixed characters are as follows:

Plant type (PT)- 9: Short height, low tiller number, leaves long, dark green, medium broad, long ligules, hairy auricles, panicles short, incompletely exerted, slender spikelets with short awn. BC_2F_1- 29 was included under PT-9.

Plant type (PT)- 10: Short height, low tiller number, appears erect, leaves long, dark green, slightly folded, ligule and hairy auricle, panicles short, incompletely exerted, slender spikelets. BC_2F_1- 35 was included under PT-10.

Plant type (PT)- 11: Very short height, very low tiller number, very slow growth, slight erect appearance, thick, short, dark green leaves, long ligule and hairy auricle, panicles short, incompletely exerted, short spikelets without awn. BC_2F_1- 39 was included under PT-11.

Plant type (PT)- 12: Very short height, low tiller number, very

Table 3. Regeneration efficiency of hybrid embryos growing in different growth media

***O.sativa* cv. Savitri / *O.brachyantha* // *O.sativa* cv Savitri**				
Embryo excised at (DAP)	**Medium**	**No. of embryos inoculated**	**No. of embryos regenerated**	**Regeneration efficiency (%)**
12	MS medium Basal (¼ conc) + Sucrose (3%) + Agar (0.7%)	10	7	70.0
12	MS medium (¼ conc) + NAA (0.5mg/l) + KN (2.0 mg/l) + Sucrose (3%) + Agar (0.7%)	10	4	40.0
12	MS medium (¼ conc) + IAA (0.5mg/l) + KN (1.0 mg/l) + Sucrose (3%) + Agar (0.7%)	10	6	60.0

slow growth, slight erect appearance, thick, medium broad, dark green leaves, folded, ligule, panicle short, incompletely exerted, short spikelets without awn. BC_2F_1- 40 was included under PT-12.

Plant type (PT)- 13: Very short height, low tiller number, very slow growth, slight semi-spreading appearance, thick, broad, dark green leaves, ligule, small auricle, panicle short, incompletely exerted, short spikelets, some spikelets with small awn. BC_2F_1- 41 was included under PT-13.

Each plant type was then morphologically compared with primary trisomics of rice. Summary of resemblances of PTs to primary trisomics of rice (Triplo) and their grouping are presented in Table 4.

PTs 9, 10, 11, 12 and 13 exhibited mixed characters. Cytological analysis of the plants revealed 15 plants out of the 16 plants resembling primary trisomics of rice exhibited a typical trisomic chromosome arrangement of 2n+1 (Table 4). BC_2F_1-7 showing resemblance to Triplo-8 was found to have 2n+1+1 chromosome arrangement.

Identification of Monosomic Alien Addition Lines (MAALs)

Fifteen BC_2F_1 hybrids with 2n+1 chromosome arrangement and bearing resemblance to primary trisomics of rice were designated as Monosomic Alien Addition Lines (MAALs). Eight MAALs were identified which are as follows- MAAL-4 (Sterile) represented by PT-7 (BC_2F_1- 27), MAAL- 5 (Twisted leaf) represented by PT-6 (BC_2F_1s- 13,33,34 and 37), MAAL- 7 (Narrow leaf) represented by PT-3 (BC_2F_1- 5), MAAL- 8 (Rolled leaf) represented by PT-2 (BC_2F_1s- 4 and 28), MAAL- 9 (Stout) represented by PT-4 (BC_2F_1s- 8 and 36), MAAL- 10 (Short grain) represented by PT-5 (BC_2F_1s-10 and 32), MAAL- 11 (Pseudo-normal) represented by PT-1 (BC_2F_1s- 1 and 30) and MAAL- 12 (Tall) represented by PT-8 (BC_2F_1- 31). The detailed comparative morphological data of the 8 MAALs and the parents are presented in Table 5. PTs 9, 10, 11, 12 and 13 exhibiting mixed characters were designated as chromosome variants. Thus 15 plants represented 8 MAALs with addition of extra chromosome from *O. brachyantha*. The morphological photographs of different MAALs are presented in Fig 1 (a-h). Similarly, MAALs have been isolated from different wild species through identification by morphological and cytological analysis [5, 7, 8, 11, 15, 16, 25].

IV. CONCLUSION

Oryza brachyantha is an African wild species of rice representing FF genome and important source of resistance to BB, Blast, and YSB which can be transferred to cultivated rice for crop improvement. While BB resistance from *O. brachyantha* has already been transferred to cultivated rice, transfer of YSB resistance is important and has not been successful. The low germination and crossability percentages suggest a strong hybridization barrier between the two species *O.brachyantha* (FF) and *O. sativa* (AA) and emphasize the importance of embryo rescue technique hybridizing distantly related species of rice. Apart from embryo rescue, the effectiveness of morphological characterization in identifying and establishing MAALs from wild species to cultivated species is also demonstrated. In the present study 8 MAALs from *O. brachyantha* could be identified. Work is in progress to identify the remaining 4 MAALs to produce a complete set of 12 MAALs which can be used as pre-breeding lines to transfer gene(s) for resistance to YSB from *O. brachyantha* ultimately develop rice genotypes with inbuilt resistance to YSB.

Table 4. Morphological and cytological analysis and grouping of BC2F1 hybrids

Morphological Grouping			**Cytological Grouping**	
Plant types	**Primary trisomic**	**BC_2F_1 hybrids**	**Chromosome arrangement**	**No. of plants**
PT-1	Triplo 11 (Pseudonormal)	BC_2F_1- 1, BC_2F_1-30	2n+1	15
PT-2	Triplo 8 (Rolled leaf)	BC_2F_1- 4, BC_2F_1-7, BC_2F_1- 28	2n+1+1	5
PT-3	Triplo 7 (Narrow leaf)	BC_2F_1- 5	2n +1+1+1	1
PT-4	Triplo 9 (Stout)	BC_2F_1- 8, BC_2F_1-36		
PT-5	Triplo 10 (Short grain)	BC_2F_1- 10, BC_2F_1-32		
PT-6	Triplo 5 (Twisted leaf)	BC_2F_1-13, BC_2F_1-33, BC_2F_1-34, BC_2F_1-37		
PT-7	Triplo 4 (Sterile)	BC_2F_1- 27		
PT-8	Triplo 12 (Tall)	BC_2F_1-31		

Table 5. Comparative morphological characters of parents, F_1 hybrid, BC_1F_1 hybrid and MAALs

Characters	**P1**	**P2**	**F_1 hybrid**	**BC_1F_1 hybrid**	**MAALs**							
					MAAL 4	**MAAL5**	**MAAL7**	**MAAL8**	**MAAL9**	**MAAL10**	**MAAL11**	**MAAL12**
Stem	Nrh	Nrh	Nrh	Nrh	Nrh	Nrh	Nrh	Nrh	Nrh	Nrh	Nrh	Nrh
Plant height (cm)	97.2±1.50	105.8±0.76	98.6±3.61	90.0±1.75	72.0	100.0	61.0	20.0	80.0	127.0	72.0	138.0
EBT	8.8±0.31	80.6±1.87	35.8±2.04	16.3±4.8	9.0	19.0	18.0	15.0	12.0	14.0	13.0	13.0
Leaf colour	DG	G	G	G	DG	LG	DG	DG	DG	G	DG	G
Blade pubescence	P	MP	MP	MP	NP	MP	MP	MP	P	P	MP	P
Flag leaf L (cm)	26.8±0.97	22.6±1.16	30.7±1.14	38.3±2.02	20.0	23.0	20.0	19.0	45.0	30.0	22.0	24.0
Flag leaf B (cm)	1.7±0.04	0.8±0.03	0.9±0.03	1.0±0.24	0.5	1.4	0.5	0.5	1.8	1.0	0.5	1.0
LSB colour	W	W	W	W	W	W	W	W	W	W	W	W
Ligule colour	W	-	W	W	W	W	W	W	LG	W	W	W
Ligule shape	S	-	S	S	S	S	S	S	S	S	S	S
Ligule length (cm)	1.7	-	1.5	1.0	0.8	1.2	0.2	0.4	0.6	0.3	1.0	0.9
Auricle colour	W	-	-	-	A	W	A	W	A	A	W	W
Junctura colour	W	W	W	W	LG	LG	LG	LG	LG	LG	LG	LG
Panicle type	C	L	L	L	C	L	C	C	C	L	C	C
Panicle exsertion	E	E	E	E	IE	IE	IE	E	E	E	E	E
Panicle length (cm)	23.3±0.88	17.1±0.72	17.4±0.24	24.2±0.81	11.0	24.0	11.0	11.0	15.0	28.0	17.0	20.0
Spikelet L (cm)	0.6±0.0	0.8±0.0	0.7±0.0	0.8±0.0	1.0	0.7	0.5	0.6	1.0	0.8	0.9	0.9
Spikelet B (cm)	0.3±0.0	0.2±0.0	0.2±0.0	0.2±0.0	0.5	0.2	0.2	0.4	0.8	0.3	0.3	0.4
Apiculus colour	W	W	W	W	W	W	W	W	W	W	W	W
Awn length (cm)	A	16.8±0.42	9.3±0.2	6.2±0.3	0.0	0.0	3.0	0.0	0.0	0.0	0.0	0.2
Stigma colour	W	P	P	P	P	P	P	P	P	P	P	P
Stigma shape	B	B	B	B	B	B	B	B	B	B	B	B

P1- *O.sativa* cv Savitri; P2- *O.brachyantha*; Nrh- non- rhizomatous; W- white; G- green ;LG- light green ; DG- dark green ; GW- greenish white; P- pubescent ;MP- medium pubescent ; NP- non-pubescent ; LSB- leaf sheath base; S- split; NS- non-split; A- absent ; C- compact ; L- lax ; IE- incomplete exsertion; E- exserted; P- purple ; B-bifid

Morphological data of P1, P2, F_1 and BC_1F_1 hybrids have been reproduced from [20]

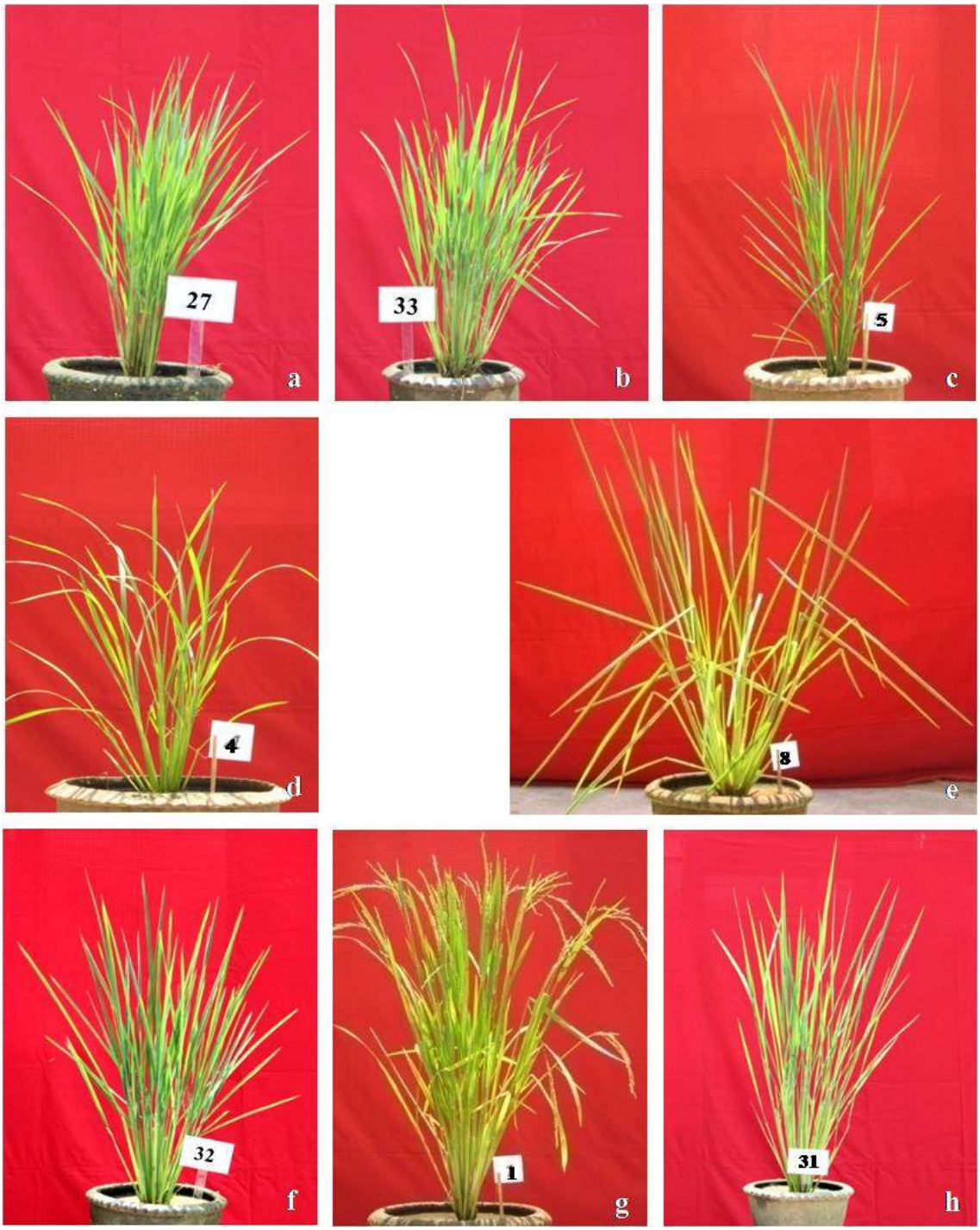

Fig1. (a) BC2F1- 27 representing MAAL- 4, (b) BC2F1- 33 representing MAAL- 5, (c) BC2F1- 5 representing MAAL- 7, (d) BC2F1- 4 representing MAAL- 8, (e) BC2F1- 8 representing MAAL- 9, (f) BC2F1- 32 representing MAAL- 10, (g) BC2F1- 1 representing MAAL- 11, (h) BC2F1- 31 representing MAAL- 12

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He has also been involved in various training programmes as an instructor and has presented papers and IEC materials in various conferences and symposia across the country.

Notable publications:

Aveek Narain, P. Sen. 2012. Isolation and identification of monosomic alien addition lines (MAALs) of the wild rice *Oryza brachyantha* A.Chev.et.Roehr on cultivated rice for developing rice genotypes resistant to YSB. International Journal of Science, Engineering and Technology Research 1(6). Accepted

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He has been awarded the Jawaharlal Nehru Award by ICAR (1979) for outstanding contribution in the field of Genetics and Plant Breeding for developing primary trisomics in rice from *Indica* rice. He has recently been awarded Emeritus Scientist position by ICAR (2008) for his contribution in wide hybridization in rice. He has also guided many students for their Ph. D and M. Sc. programmes.

Notable publications:

Aveek Narain**, P. Sen**. 2012. Isolation and identification of monosomic alien addition lines (MAALs) of the wild rice *Oryza brachyantha* A.Chev.et.Roehr on cultivated rice for developing rice genotypes resistant to YSB. International Journal of Science, Engineering and Technology Research 1(6). Accepted

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