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# Development and morphological characterization of monosomic alien addition lines (MAALs) from *Oryza brachyantha* A.Chev.et.Roehr to transfer Yellow Stem Borer (YSB) resistance gene(s) on cultivated rice *O. sativa* L.

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ABSTRACT

*Oryza brachyantha* (FF), the African wild rice, which is resistant to Yellow stem borer (YSB), was used to develop Monosomic Alien Addition Lines (MAALs) on cultivated rice. A total of 19, 399 $BC_1F_1$ spikelets were artificially pollinated with the recurrent parent, out of which 29 $BC_2F_1$ hybrids were produced through embryo rescue with the crossability efficiency of 0.14%. Embryos collected at 10- 12 days after pollination (DAP) were grown successfully on ¼ MS basal medium with embryo germination of 35.8%. The plantlets with 4-5 healthy roots were acclimatized by direct transfer method and grown. The hybrids were morphologically characterised into 16 different plant types and each plant type was cytologically analyzed. Hybrids resembling with the morphology of primary trisomics of rice and exhibiting 2n+1 (2n=25) chromosome arrangement were designated as MAALs. A total of 8 MAALs were thus identified representing MAAL- 4 (Sterile), MAAL- 5 (Twisted leaf), MAAL- 7 (Narrow leaf), MAAL- 8 (Rolled leaf), MAAL- 9 (Stout), MAAL- 10 (Erect), MAAL- 11 (Pseudo-normal) and MAAL- 12 (Tall).



## 1. Introduction

The productivity of cultivated rice is affected by several biotic and abiotic stresses. The genetic variability in cultivated rice germplasm in terms of resistance/tolerance to abiotic and abiotic stresses is either limited or the cultivars are becoming susceptible to various biotic and abiotic stresses due to the changed climatic conditions, cropping practises, insect biotypes and/or disease races. 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 (Heinrichs *et al*., 1985; Swaminathan, 1986; Sitch, 1990) 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 and in all stages of growth causing an annual yield loss of 5-10% damaging crops upto 60% in unprotected field conditions (Pathak and Khan, 1994). Though some high yielding rice varieties have been reported to be moderately resistant to YSB (Pathak and Khan, 1994; Maqbool *et al*., 1998), 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 rice germplasm has been screened against YSB and *O. brachyantha*, *O. officianalis*, *O. redleyi* and *Porteresia coarctata* were found to resistant/tolerant to YSB (Padhi and Sen, 2002).

*Oryza brachyantha* (FF), the wild species widely distributed in Africa belongs to the secondary gene pool of rice. 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 to high genomic incompatibility rendering the $F_1$ hybrids completely sterile. Introgression of alien genes from these distantly related wild species is possible through the

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development of Monosomic Alien Addition Lines (MAALs) employing embryo rescue. The MAALs thus produced characteristically 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) (Shin and Katayama, 1979; Jena and Khush, 1989; Brar *et al*., 1991, Brar and Khush, 2002, 2006) which have been characterized based on morphological and cytological characterization, Flourescence- *in situ*-Hybridization (FISH) and molecular markers.

Attempts have been made to develop of MAALs from *O. brachyantha* and introgress BB resistant genes from this species to cultivated rice (Brar *et al*., 1996). In the absence of any rice cultivar truly resistant to YSB, in this study we have attempted to develop a complete set of 12 MAALs from *O. brachyantha* on *O. sativa* cv Savitri with the ultimate goal to introgress gene(s) for resistance for YSB, into cultivated rice.

## 2. Materials and Methods

Back crossing of $BC_1F_1$ (*O. sativa* cv Savitri */O. brachyantha//O. sativa* cv Savitri) interspecific backcross hybrids was carried out with the recurrent parent, *O.sativa* cv Savitri following the scheme for the production of monosomic alien addition lines (2*n*=25) suggested by Brar and Khush (1997). The spikelets of $BC_1F_1$ hybrids were treated with solution A (NAA 25mg/L+ Sucrose 5g/L), sprayed on to the emasculated spikelets before pollination and solution B ($GA_3$ 50mg/L+KN 5mg/L+NAA 5mg/L), sprayed after pollination twice a day till 5 days (Jena and Khush, 1989; Multani *et al*., 1994) to overcome pre- and post-fertilization barriers respectively.

Spikelets with expected fertilized embryos were collected between 7-16 days after pollination (DAP) before embryo abortion. Then they were aseptically excised, treated adopting a protocol suggested by Ko *et al*. (1983) and cultured on ¼ MS medium with 3 different hormonal combinations to confirm an optimum growth medium for embryo rescue and establish an appropriate age of hybrid embryos for rescue. The embryos in culture tubes were incubated 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 4-5 healthy roots were removed from culture tubes and acclimatized following four different methods (i) Direct transfer method, (ii) Modified Iyer and Govilla (1964) method, (iii) Modified soil: sand method (Niroula *et al*., 2004), and (iv) Peat moss fortified with Hoagland's solution and the responses were recorded to establish an optimum acclimatization protocol for the interspecific hybrids. Methods described by Iyer and Govila (1964) and Niroula *et al.* (2004) were adopted and modified by including the use Hoagland's solution. The surviving plantlets were then transferred to the net house for further growth till maturity.

The surviving $BC_2F_1$ (*O. sativa* cv. Savitri / *O. brachyantha // O. sativa* cv. Savitri /// *O. sativa* cv. Savitri) progenies, were characterised, at vegetative, mature and flowering stages following a set of qualitative and quantitative morphological characters and categorized into different plant types (PT) based on their morphological features. Each of the plant type was morphologically compared to primary trisomics of rice (Khush *et al*., 1984; Misra *et al*., 1985, Sen and Misra, 1988). More than one plant showing similarities to a particular plant type were grouped accordingly. These hybrids were cytologically characterized to identify their chromosome arrangements. Hybrids resembling with the morphology of primary trisomics of rice and exhibiting 2n+1 (2n=25) chromosome arrangement were designated as MAALs.

## 3. Results and Discussion

### *3.1 Development of backcross hybrids*

A total of 19,399 $BC_1F_1$ spikelets were artificially pollinated treating the spikelets with hormonal solutions before and after pollination to overcome strong pre- and post- fertilization barriers between the parent species *O. sativa* and *O. brachyantha* which have also been reported earlier by Sitch and co-workers (1989, 1990). It was observed that treatment with hormonal solutions before and after pollination greatly improved viable embryo formation as they helped in pollen germination, pollen tube development, overcome post- fertilization barriers and embryo abortion. Such observations have also been reported by Jena and Khush (1986, 1989), Multani *et al.* (1994, 2003) and Niroula *et al.*, (2004, 2005). As a result, 5,248 expected fertilized embryos could be harvested, out of which 1256 were viable embryos and rest of the spkikelets were with aborted embryos or filled with watery endosperm. The overall crossability was found to be 0.14% (Table 1). Of the 1256 embryos inoculated in growth media, only 450 embryos germinated in culture thus showing an efficiency of 35.8% (Table 1).

Percentage of embryo survival 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 (Table 2) i.e, 36.3% and 44.4%, thus were the ideal period to harvest

embryos for rescue. Similar observations have been reported in crosses between *O. sativa* and *O. brachyantha* by Sitch *et al*. (1989), Panda and Sen (2006) and Behura *et al.* (2011). Kumari *et al*. (2005) have also pointed out that embryos collected between 8 and 12 DAP were ideal for embryo rescue irrespective of the wide cross combination. 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 (70%). Several earlier reports by Jena and Khush (1984, 1989, 1990), Yasui and Itawa (1991), Multani *et al*. (1994, 2003) have used ¼ MS basal medium in different cross combinations of *O.sativa* with different wild species. These results suggest that irrespective of any wide cross combination, embryo survival and plant regeneration depend on a combination of cross combination, time of embryo excised and growth medium used.

Out of 4 methods used to acclimatize plantlets before transferring them to net house/green house, the direct transfer method of planting plantlets with 4-5 healthy roots in earthen pots containing sterilized soil supplemented with Hoagland's solution, resulted in 70% survival. The modified method of Iyer and Govila (1964) using Hoagland's solution resulted in 55% survival of the hybrid plantlets whereas the modified sand and soil (1:1) method resulted in 45% plantlet survival. Peat moss method resulted in complete loss of plantlets with no survival. There are limited reports describing hardening methods of embryo rescue regenerated plants. Iyer and Govila (1964) have reported 50% survival rate of plantlets. Jena and Khush (1984) have reported hardening of embryo rescued plants through culture in nutrient solution for 10 days but have not provided details of the nutrient solution used. Though Niroula *et al*. (2004) have reported 100% survival in 3 wild species in their experiments using soil: sand method, in our experiments the method resulted in only 45% survival.

Table 1
Percentage of crossability and germination of $BC_1F_1$ population

| Total SP | Total EFS | Total EC | Total EG | Germination(%) | Total PIC | Total PIP | Crossability(%) |
|---|---|---|---|---|---|---|---|
| 19399 | 5248 | 1256 | 450 | 35.8 | 131 | 29 | 0.14 |

SP- spikelets pollinated; EFS- Expected fertilized spikelets; EC- embryos cultured; EG- embryos germinated; PIC- plantlets in culture; PIP- plants in pots

Table 2
Percentage of 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 |

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 | ¼ MS medium basal + Sucrose (3%) + Agar (0.7%) | 10 | 7 | 70.0 |
| 12 | ¼ MS medium basal + NAA (0.5mg/l) + KN (2.0 mg/l) + Sucrose (3%) + Agar (0.7%) | 10 | 4 | 40.0 |
| 12 | ¼ MS medium basal + IAA (0.5mg/l) + KN (1.0 mg/l) + Sucrose (3%) + Agar (0.7%) | 10 | 6 | 60.0 |

*3.2 Isolation and establishment of Monosomic Alien Addition Lines (MAALs)*

Twenty four surviving $BC_2F_1$ hybrids were studied for their qualitative and quantitative morphological characters and were categorized into 16 plant types (PT) based the observed morphological characters. Two or more plants exhibiting similar morphological characters were grouped into one plant type. Plants exhibiting mixed characters were grouped separately. The detailed characters of each plant type are as follows-

*Plant type (PT)- 1*: Normal looking, medium height, semi-erect, non-rhizomatous, intermediate tillers; leaves medium in length, intermediate in width, green in colour, intermediate pubescence; leaf sheath base green in colour, junctura light green in colour; flag leaf medium in length, intermediate in width, ligule long, split, whitish in colour, auricles present, white in colour; panicles short, completely exserted, lax type, non- shattering; spikelets short, bold with white apiculus and without awn; The plant is morphologically indistinguishable from disomics. $BC_2F_1$- 1 and 30 were grouped under PT-1. Spikelet fertility was found to be 85.7±0.8.

*Plant type (PT)- 2*: Short height, open, very poor growth, non- rhizomatous, low tiller number; leaves narrow and thin, dark green in colour, intermediate pubescence; leaf sheath base green coloured, junctura light green coloured; ligules short, acute, whitish in colour, auricles absent; plant is susceptible to *Cercospora janseana* infection. $BC_2F_1$- 3 was grouped under PT-2.

*Plant type (PT)- 3*: Short height, open, non- rhizomatous, slow growing, intermediate tiller number; leaves long, narrow, incurved, dark green in colour, intermediate pubescence; leaf sheath base green in colour, junctura light green in colour; flag leaves long, narrow; ligule short, split and whitish in colour, auricle present, white in colour; panicles short, completely exserted, compact, non-shattering and dense; spikelets short, bold with white apiculus, without awn. $BC_2F_1$- 4 and 28 were grouped under PT-3. Spikelet fertility was found to be 74.0±0.4.

*Plant type (PT)- 4*: Medium height, semi-erect, non-rhizomatous, low tiller number; Leaves intermediate in length, narrow, slightly rolled, dark green in colour, intermediate pubescence; flag leaves intermediate in length, narrow; leaf sheath base green in colour, junctura is light green in colour; ligule short, split, whitish in colour, auricles absent; panicles very short, incompletely exerted, compact, non- shattering; spikelets slender, short awns. $BC_2F_1$- 5 was grouped under PT-4. Spikelet fertility was found to be 45.2±0.0.

*Plant type (PT)- 5*: Short height, erect, with poor growth, non- rhizomatous, very low tiller number; leaves short, narrow, green in colour, intermediate pubescence, at right angles to the culm; flag leaves short, narrow; leaf sheath base green in colour, junctura is light green in colour; Ligules short, split, whitish in colour, auricles absent; panicles very short, very poor exertion, narrow spikelets without awn. $BC_2F_1$- 6 was included under PT-2.

*Plant type (PT)- 6*: Medium tall, semi-spreading, non-rhizomatous, thick (stout) tillers, high tiller number; leaves long, broad, green in colour, boat shaped appearance; flag leaves long and broad; leaf sheath base green in colour, junctura light green in colour; ligules medium, split, whitish, auricles absent; panicles medium, dense, completely exerted, compact, non-shattering; spikelets short, bold, without awn. $BC_2F_1$- 8 and 36 were grouped under PT-6. Spikelet fertility was found to be 71.2±0.6.

*Plant type (PT)- 7*: Medium tall, erect, non-rhizomatous, slow growing, intermediate tiller number; Leaves intermediate in length, narrow, thick, slightly folded inwards, dark green in colour, intermediate pubescence; flag leaves intermediate, narrow; leaf sheath base green in colour, junctura is light green in colour; ligules short, acute, whitish, auricles white coloured, hairy; panicles very short, completely exserted, lax type, non- shattering; spikelets short slender, without awn. $BC_2F_1$- 10 and 32 were grouped under PT-7. Spikelet fertility was found to be 82.0±0.4.

*Plant type (PT)- 8*: Very short height, semi-spreading, non-rhizomatous, very poor growth, very low tiller number; leaves very short, narrow, thin, rolled, green in colour, intermediate pubescence, at right angles with the culm; leaf sheath base green in colour, junctura light green in colour; ligules are short, acute, whitish in colour, auricles;.$BC_2F_1$- 12 was included under PT-8.

*Plant type (PT)- 9*: Medium height, open, appears bushy, non- rhizomatous, medium high tiller number; leaves long, intermediate width, twisted, light green in colour, intermediate pubescence; flag leaves long, intermediate width; leaf sheath base colour green, junctura light green in colour; ligules short, acute, whitish in colour, auricles present, white in colour; panicles very short, incompletely exserted, lax type, low shattering, spikelets slender without awn. $BC_2F_1$- 13, 33, 34 and 37 were grouped under PT-9. Spikelet fertility was found to be 62.4±0.9.

*Plant type (PT)- 10*: Medium height, erect, non- rhizomatous, low tiller number; leaves intermediate in length, narrow, thick, glabrous, dark green in colour, intermediate flag leaves; leaf sheath base green in colour, junctura is light green in colour; ligules medium in length, split, whitish, auricles

absent; panicles very short, incompletely exserted, compact, non- shattering; spikelets short without awn. $BC_2F_1$- 27 was included under PT-10. Spikelet fertility was found to be 0.0.

*Plant type (PT)- 11*: Tallest among all plants, slightly open, non-rhizomatous, robust tillers, intermediate tiller number; leaves long, broad, green in colour, pubescent; flag leaves long, broad; leaf sheath base green in colour, junctura light green in colour; ligules medium in length, with fringe of hairs, whitish in colour, auricles absent; panicles medium in length, completely exserted, compact, non- shattering; spikelets long, bold, with tip awn. $BC_2F_1$- 31 was included under PT-11. Spikelet fertility was found to be 94.7±0.0.

*Plant type (PT)- 12*: Medium height, semi-erect, non-rhizomatous, low tiller number; leaves long, intermediate width, green in colour, intermediate pubescence; flag leaves long, intermediate; leaf sheath base colour green, junctura light green; ligules short, split, whitish in colour, hairy auricles present; panicles moderately short, incompletely exerted, compact and non-shattering; spikelets slender with tip awn. $BC_2F_1$- 29 was included under PT-12.

*Plant type (PT)- 13*: Medium height, appears erect, non- rhizomatous, intermediate tiller number; leaves long, narrow, slightly folded, dark green in colour, intermediate pubescence; leaf sheath base green in colour, junctura is light green in colour; ligule short, split, and whitish, hairy auricle present; panicles very short, incompletely exerted, compact, moderately shattering; spikelets slender, without awn. $BC_2F_1$- 35 was included under PT-13.

*Plant type (PT)- 14*: Medium height, appears semi-erect, very slow growth, non- rhizomatous, low tiller number; leaves are short, intermediate width, thick, dark green in colour, pubescent; leaf sheath base colour green, junctura light green in colour; ligules medium in length, split, and whitish, hairy auricles are present; panicles very short, incompletely exerted, lax type, moderately shattering; spikelets short, without awn. $BC_2F_1$- 39 was included under PT-14.

*Plant type (PT)- 15*: Very short height, semi- erect appearance, non- rhizomatous, very slow growth, low tiller number; leaves short, narrow, thick, folded, dark green in colour, pubescent; leaf sheath base green in colour, junctura light green in colour; ligule medium, split, whitish, auricles absent. $BC_2F_1$- 40 was included under PT-15.

*Plant type (PT)- 16*: Medium height, appears semi-spreading, non- rhizomatous, very slow growth, low tiller number; leaves intermediate in length, broad, broad, dark green in colour, leaves, intermediate pubescence; leaf sheath base colour green in colour, junctura light green in colour; ligule short, split, whitish, small auricle present; panicles very short, partly exerted, lax type, non- shattering; spikelets short, some spikelets have tip awn. $BC_2F_1$- 41 was included under PT-16.

Plant types were morphologically compared with the primary trisomics of rice. One or more than one plant showing similarity to a particular trisomic was grouped into the respective group (Table 4). PT-1 ($BC_2F_1$s- 1 and 30) resembled Triplo- 11, PT-3 ($BC_2F_1$s- 4 and 28) resembled Triplo-8, PT-4 ($BC_2F_1$- 5) resembled Triplo-7, PT-6 ($BC_2F_1$s- 8 and 36) resembled Triplo-9, PT-7 ($BC_2F_1$s- 10 and 32) resembled

Table 4
Morphological grouping of $BC_2F_1$ hybrids

| Plant types | $BC_2F_1$ hybrids | Morphological resemblance with Rice primary trisomic |
|---|---|---|
| PT-1 | $BC_2F_1$- 1 | Triplo 11 (Pseudonormal) |
| | $BC_2F_1$-30 | |
| PT-2 | $BC_2F_1$-3 | Did not survive |
| PT-3 | $BC_2F_1$- 4 | Triplo 8 (Rolled leaf) |
| | $BC_2F_1$- 7 | |
| | $BC_2F_1$- 28 | |
| PT-4 | $BC_2F_1$- 5 | Triplo 7 (Narrow leaf) |
| PT-5 | $BC_2F_1$-6 | Did not survive |
| PT-6 | $BC_2F_1$- 8, | Triplo 9 (Stout) |
| | $BC_2F_1$-36 | |
| PT-7 | $BC_2F_1$- 10 | Triplo 10 (Short grain) |
| | $BC_2F_1$-32 | |
| PT-8 | $BC_2F_1$-12 | Did not survive |
| PT-9 | $BC_2F_1$-13 | Triplo 5 (Twisted leaf) |
| | $BC_2F_1$-33 | |
| | $BC_2F_1$-34 | |
| | $BC_2F_1$-37 | |
| PT-10 | $BC_2F_1$- 27 | Triplo 4 (Sterile) |
| PT-11 | $BC_2F_1$-31 | Triplo 12 (Tall) |
| PT-12 | $BC_2F_1$-29 | Triplo 12+ Triplo 10 |
| PT-13 | $BC_2F_1$- 35 | Triplo 4+ Triplo 10 |
| PT-14 | $BC_2F_1$-39 | Triplo 4+ Triplo 9 |
| PT-15 | $BC_2F_1$-40 | Triplo 4+ Triplo 9 (Did not survive) |
| PT-16 | $BC_2F_1$-41 | Triplo 5+ Triplo 7+ Triplo 9 |
| Total | 24 | |

Triplo- 10, PT-9 ($BC_2F_1$s- 13, 33, 34 and 37) resembled Triplo-5, PT-10 ($BC_2F_1$- 27) resembled Triplo-4 and PT-11 ($BC_2F_1$-31) resembled Triplo- 12. Plant types 2, 5, 8 and 15 could not be compared to any primary trisomic because of non-survival of the plants and plant types 11, 13, 14, and 16 exhibited mixed characters of 2-3 primary trisomics. Cytological analysis of the hybrids was carried out and plants exhibiting a typical trisomic chromosome arrangement of 2n+1 (2n=25 chromosomes) were identified. As per the cytological observation, out of 24 plants, 16 plants ($BC_2F_1$ hybrids) with the chromosome arrangement of 2n+1 and displaying morphological resemblance to primary trisomics of rice were designated as MAALs. Thus 16 plants represented 8 MAALs with addition of an extra chromosome from the wild rice *O. brachyantha*. The 8 MAALs identified are as follows- MAAL- 4 (Sterile), MAAL- 5 (Twisted leaf), MAAL- 7 (Narrow leaf), MAAL- 8 (Rolled leaf), MAAL- 9 (Stout), MAAL- 10 (Short grain), MAAL- 11 (Pseudo-normal) and MAAL- 12 (Tall). The detailed comparative morphological data of the 8 MAALs are presented in Table 5 and photographs of different MAALs are presented in Fig 1 (a-h). Shin and Katayama (1979), Jena and Khush (1985, 1989, 1990), Brar *et al.* (1996) Multani *et al*. (1994, 2003), and Yasui and Itawa (1991) have similarly isolated MAALs from different wild species and characterized their respective MAALs by comparing the observed morphological characters with that of primary trisomics of rice.

Table 5
Comparative morphological characters of MAALs

| Characters | MAALs | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | MAAL4 | MAAL5 | MAAL7 | MAAL8 | MAAL9 | MAAL10 | MAAL11 | MAAL12 |
| Stem | Nrh | Nrh | Nrh | Nrh | Nrh | Nrh | Nrh | Nrh |
| Plant height (cm) | 72.0 | 100.0 | 61.0 | 20.0 | 80.0 | 127.0 | 72.0 | 138.0 |
| EBT | 9.0 | 19.0 | 18.0 | 15.0 | 12.0 | 14.0 | 13.0 | 13.0 |
| Leaf colour | DG | LG | DG | DG | DG | G | DG | G |
| Blade pubescence | NP | MP | MP | MP | P | P | MP | P |
| Flag leaf L (cm) | 20.0 | 23.0 | 20.0 | 19.0 | 45.0 | 30.0 | 22.0 | 24.0 |
| Flag leaf B (cm) | 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 |
| Ligule colour | W | W | W | W | LG | W | W | W |
| Ligule shape | S | S | S | S | S | S | S | S |
| Ligule length (cm) | 0.8 | 1.2 | 0.2 | 0.4 | 0.6 | 0.3 | 1.0 | 0.9 |
| Auricle colour | A | W | A | W | A | A | W | W |
| Junctura colour | LG | LG | LG | LG | LG | LG | LG | LG |
| Panicle type | C | L | C | C | C | L | C | C |
| Panicle exsertion | IE | IE | IE | E | E | E | E | E |
| Panicle length (cm) | 11.0 | 24.0 | 11.0 | 11.0 | 15.0 | 28.0 | 17.0 | 20.0 |
| Spikelet L (cm) | 1.0 | 0.7 | 0.5 | 0.6 | 1.0 | 0.8 | 0.9 | 0.9 |
| Spikelet B (cm) | 0.5 | 0.2 | 0.2 | 0.4 | 0.8 | 0.3 | 0.3 | 0.4 |
| Spikelet fertility (%) | 0.0 | 62.4±0.9 | 45.2±0.0 | 74.0±0.4 | 71.2±0.6 | 82.0±0.4 | 85.7±0.8 | 94.7±0.0 |
| Apiculus colour | W | W | W | W | W | W | W | W |
| Awn length (cm) | 0.0 | 0.0 | 3.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.2 |
| Stigma colour | P | P | P | P | P | P | P | P |
| Stigma shape | B | B | B | B | B | B | B | B |

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.

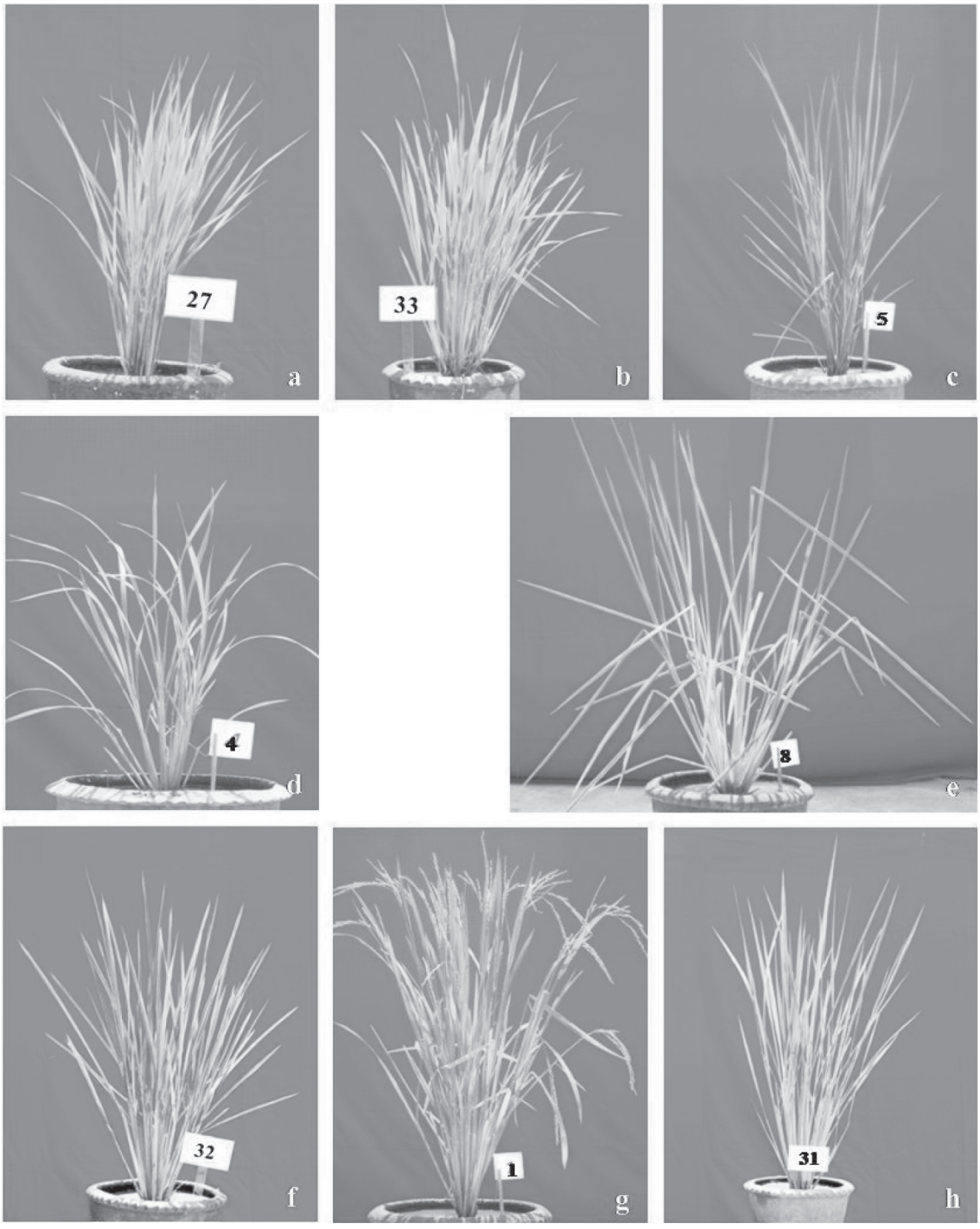


Fig. 1. (a) $BC_2F_1$- 27 representing MAAL- 4, (b) $BC_2F_1$- 13 representing MAAL- 5, (c) $BC_2F_1$- 5 representing MAAL- 7, (d) $BC_2F_1$- 4 representing MAAL- 8, (e) $BC_2F_1$- 8 representing MAAL- 9, (f) $BC_2F_1$1- 32 representing MAAL- 10, (g) $BC_2F_1$- 1 representing MAAL- 11, (h) $BC_2F_1$- 31 representing MAAL- 12

## 4. 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 imminent since it causes wide spread damage to the rice crop in all agro-climatic conditions. During wide hybridization between the two species *O.brachyantha* (FF) and *O. sativa* (AA), low germination and crossability percentages were observed which suggest strong hybridization barriers between them and emphasize the importance of hormonal treatment and embryo rescue technique when working with rice species so genetically apart. 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. Further use of cytological investigations and advanced techniques like Fluorescent-*in situ*- hybridization (FISH) and its variant Genomic-*in situ*-hybridization (GISH) and molecular markers will help to confirm the introgression of alien genes resistance to YSB into cultivated rice more effectively. These MAALs can be used to identify gene(s) for resistance to YSB and develop introgressed lines from *O. brachyantha*, which can be used as pre-breeding lines to develop a rice variety with inbuilt resistance to YSB. As a result the production and productivity of rice will be enhanced.

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