Summary & Conclusions

India has the largest 45 million ha of harvested rice area under rice in the world and covered by 28% rainfed lowland, 46% irrigated, 12% rainfed upland, and 14% flood-prone ecosystems in which Eastern India alone accounts for 26 m ha (57%) of rice area. However, nearly 70% of the rice area in these region falls under the unfavorable and high-risk ecosystem like rainfed lowlands that include deep-water conditions. With the majority of the rice area is under unfavorable ecosystem, Eastern India could contribute only 50% to the total rice production to the national pool. The traditional cultivars grown in these areas are highly vulnerable to periodic cycles of both abiotic and biotic stresses and as a result, yields in these lands are low (1.8 t ha⁻¹). Since the onset of green revolution in the country, there has not only been a constant increase in the number of insect pests but also a concomitant shift in their diversity, spread and intensity in rice. Among the major pests, stem borers have shown geographical variation in its species composition with occurrence of four species viz., yellow stem borer, white stem borer, dark headed borer and pink stem borer, across the country and yield losses due to Yellow stem borer accounts to 671,000 tons in Eastern India alone. Genetic base control of insect pest's damage is most economic and important but strong sources of resistance to stem borers are not available in the gene pool even after screening more than 30,000 rice varieties. For this reason, despite many years of sustained efforts by conventional breeders, much progress could not be made in the development of suitable cultivars resistant to Yellow stem borer. Similar is the case with rice leaf folder for which also strong sources of resistance are not available. The high costs involved in the recurrent use of the agrochemicals for the control of these insects, associated environmental hazards, do not permit the economically poor framers to adopt such practices in these ecosystems. In this context, genetic engineering techniques holds great promise as development of plants with improved natural defense capabilities through transgenic approach is a realizable goal.

Keeping these issues in view, experiments were undertaken on several indica cultivars of different ecosystems (rain fed lowland, irrigated, rain fed upland, and flood-prone) of eastern India with a view to improve their response to somatic cell culture and somatic embryogenesis for exploitation in further transgenic research. Similarly, for the development of a reproducible
transformation protocol for indica rices, studies were undertaken to study the factors that promote Agrobacterium infection through activation of vir genes of Agrobacterium and also to increase plant cell's susceptibility against different Agrobacterium strains. The studies also included the development of transgenic indica rice cultivars through incorporation of resistance to Yellow Stem Borer (YSB) and Leaf folder (LF). The experimental findings are summarized below.

In somatic cell culture, media supplemented with 2.0 mg l⁻¹ 2, 4-D+0.5 mg l⁻¹ Kn +maltose (in place of sucrose) for induction and regeneration media supplemented with 0.5 mg l⁻¹ NAA + 0.5 mg l⁻¹ Kn + 1.5 mg l⁻¹ BAP + maltose were found to be best for both high frequency of callus induction and recovery of green plants at a high frequency through somatic embryogenesis. In addition, the additive effect of anti-necrotic compounds like silver nitrate, adenine sulphate or partial desiccation of calli prior to transferring to regeneration was also studied and both the treatments seem to have a positive influence on regeneration frequency as well as green shoot induction. Of the treatments tested, partial desiccation of callus gave better results. The plants developed through somatic embryogenesis were morphologically uniform and same as the parental genotypes (data not shown).

A series of experiments demonstrated that AS (25 -100 µM) in the preinduction medium and MS medium + 2, 4-D (2.0 mg l⁻¹)+ maltose for co-cultivation were found to be optimal in Agrobacterium mediated approach for getting a high frequency of transient transformation in different rice genotypes infected with different Agrobacterium strains. Additional supplements did not show any positive effect on the transient transformation efficacy of different indica rice cultivars mediated by different Agrobacterium strains. Similar kind of high transient transformation frequencies were obtained in mature seed derived calli of different indica rice cultivars by the infection of Agrobacterium strains pre induced on AB minimal medium containing 0.5% of glucose/galactose/mannose followed by co-cultivation on MS medium + 2, 4-D (2.0 mg l⁻¹)+ sugar (3% w/v) and further supplemented with either 0.5% glucose / galactose or 0.2% mannose. The results indicate that the transient GUS expression levels and the Zones obtained in treatments with effective sugars (glucose/galactose/mannose) were not significantly different from those obtained with addition of acetosyringone of the two Agro strains tested, EHA105 infected calli had shown a higher percentage of tissue samples staining blue than the ones infected with LBA4404/pSB1. The
number of blue spots and intensity of staining of each callus was significantly different among the strains containing single and multiple copies of virG. Early detection of GUS was seen with strains containing multiple (LBA4404/pSB1) copies of virG (data not shown) than with the strains containing a single copy (EHA105) of virG. The results demonstrated that simple sugars can effectively substitute acetosyringone in Agrobacterium mediated transformation of indica rices, and the result was confirmed through recovery of putatively transformed transgenic plants from indica rice cultivar Pusa Basmati1 by using simple sugar combinations for producing transgenics. Moreover, based on the morphology, the white compact and pale yellow colour embryogenic calli derived from MS medium supplemented with casein hydrolysate were best suited for transformation of recalcitrant indica genotypes like Swarna.

By using the modified protocol, seventy seven plants (from thirteen independently transformed calli) and eleven plants (from four independently transformed calli) were recovered from Pusa Basmati1 and Swarna respectively. The stable integration of the transgene in all the putatively transformed plants was confirmed by GUS histochemical analysis. In addition, the stable integration of selectable marker gene hygromycin and GUS reporter gene was confirmed by polymerase chain reaction and the integration pattern, copy number of pin2 gene (0.63 kb in size) in both cultivars (Pusa Basmati1 and Swarna) was confirmed by Southern blot analysis. All the transgenics showed the same banding pattern except two plants of Pusa Basmati 1. The independent status and rearrangement of transgene in putative transgenics were determined by differential banding pattern, when Southern blots for pin2 were reprobed with 1.1 kb coding sequence of hygromycin. In Pusa Basmati1 transgenics, 1- 4 hph specific hybridization bands of different molecular sizes were observed. Few plants of Pusa Basmati1 were showing a single band integrated in two different loci. In case of Swarna, two different banding patterns were observed. The transgenic plants of Swarna were morphologically similar to non-transformed control plants and fully fertile (>90%). In case of Pusa Basmati, transgenic plants showed > 80% seed set, except two plants that were sterile. The segregation pattern of the transgenes is Mendelian as evidenced by the studies onT1 and T2 generations and the transgenics are stable. The bioassay results with Yellow Stem Borer showed minimum 40-100% larval mortality in T2 homozygous and hemizygous lines of Pusa Basmati 1 and Swarna. Some of the transgenic lines of both Pusa Basmati 1 and Swarna showed enhanced levels of resistance to Rice Leaf Folder also. This study proves the utility
of protease inhibitor genes to confer enhanced levels of resistance to serious insect pests like Yellow Stem Borer and Rice Leaf Folder in indica rices.

All these homozygous transgenic lines could be field-tested with the biosafety requirements in place. As the future thrust in transgenic research is in the area of development of marker free transgenics (antibiotic resistance gene), alternative systems like mannose as the selectable marker gene can be explored as this study convincingly proved the efficacy of mannose. Use of mannose as a selectable marker system can circumvent the problems facing transgenics with the antibiotic markers. Future efforts could be on use of matrix attachment regions (MARs) and MAT vectors to prevent the possibility of gene silencing /inactivation resulting from methylation, position effect etc., and development of transgenics which are free from sequences of vector backbone and persistence of the Agrobacterium in the plant tissues after the transformation event. The other important consideration for the future research include the use of tissue specific promoters, use of a combination of genes like Bt and protease inhibitor genes for effective control of major insect pests like stem borer and leaf folder.
Bibliography


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