ABSTRACT

Successful application of available gene transfer techniques to rice requires the development of effective and reproducible plant regeneration protocols. The work presented in this thesis is focused on the establishment of reproducible somatic cell and protoplast culture systems in indica-javanica breeding lines [cvs. IR 65597-134-2 (IR 65597) and IR 65598-112-2 (IR 65598)], and the possible application of the somatic cell culture system developed to *Agrobacterium tumefaciens*-mediated gene transfer studies in these indica-javanica lines.

During the present study, embryogenic calli were initiated from mature seed scutellum (MSS), leaf base (LB) and stem base (SB) explants of the breeding lines cultured on LS 2.5 medium. Plant regeneration from tissue-derived calli was achieved on MSKN medium using a two-step plant regeneration procedure. This procedure involved the subculture of embryogenic calli on the regeneration medium containing high [1% (w/v)] agarose for 2 weeks in the dark. The plant regeneration frequency from MSS-derived calli was further improved by substituting sucrose [3 % (w/v)] in the regeneration medium with 3 % (w/v) maltose. Flow cytometric analysis revealed a prevalence of diploids amongst MSS-calli-derived plants. More than 50 % of MSS-calli-derived plants of both breeding lines transferred to glasshouse conditions flowered, but very few set seed compared to seed-derived controls. Tissue culture-derived plants of both breeding lines exhibited significant variations in several agronomic characters. The majority of these variations showed a shift towards the negative direction of the mean value of the agronomic character. Friable embryogenic cell suspensions, suitable for isolating viable and regenerable protoplasts, were established by transferring MSS-derived embryogenic callus into AA2 liquid medium. The cell suspensions were maintained by weekly subculture and protoplasts were isolated by overnight incubation of suspension cells in enzyme solution.

The protoplast yield was dependent on cell suspension age. Protoplast culture, using a membrane filter-nurse culture method, was critical to induce protoplast division and subsequent colony formation in both rice breeding lines. *Lolium multiflorum* and Black Mexican Sweet maize (BMS) suspensions served as nurse cells. The age of rice cell suspensions (more than 3-4 months) used as a source of protoplasts and high protoplast plating density (1-2 x 10^6 ml^{-1} for cv. IR 65597 and 1 x 10^6 ml^{-1} for cv. IR 65598) were critical in maximizing protoplast plating efficiency. Plant regeneration from protoplast-derived colonies was achieved in both rice breeding lines on MSKN medium using a two-step plant regeneration procedure. Amongst the factors which significantly affected the plant regeneration frequency, were the choice of cell line and feeder (nurse) cells (cv. IR 65597) and the choice of feeder cells and carbohydrate source in the regeneration medium (cv. IR 65598). Albino plant regeneration was observed in all the cell lines tested for protoplast isolation; albino plant regeneration frequency was 100 % in protoplasts derived from the cell line IR 65597-(2).

Flow cytometric analysis revealed an increased number of tetraploids among protoplast-derived plants compared to MSS-callus-derived plants. Protoplast-derived plants of cv. IR 65598 showed a higher mortality under *ex vitro* conditions compared to those of cv. IR 65597. This was attributed mainly to the poor root growth in protoplast-derived plants of cv. IR 65598.

During the present study, an assessment was made of the possible application of *Agrobacterium*-mediated gene transfer to indica-javanica breeding lines. T-DNA transfer into MSS-derived calli was observed as indicated by transient *gus* gene expression in tissues 7 d post inoculation. The presence of acetosyringone (100 μM) in the co-cultivation medium was found to be crucial for T-DNA transfer. The efficiency of T-DNA transfer was enhanced by a prolonged co-cultivation period and a reduced pre-incubation time of the explants. None of the inoculated tissues survived

transfer to selection medium containing 50 mg l^{-1} hygromycin, whereas inoculated tissues transferred to antibiotic free medium proliferated callus which, in turn, regenerated non-transformed rice plants. The inability to proliferate transformed calli on antibiotic medium was attributed mainly to a low level of foreign gene transfer, as indicated by restricted transient GUS activity.

The results presented in this thesis demonstrate, for the first time, that the indica-javanica breeding lines (cvs. IR 65597 and IR 65598) are amenable to reproducible tissue and protoplast culture and plant regeneration. Despite an inability to regenerate transformed plants within the time-frame of the current study, investigations demonstrated that *Agrobacterium*-mediated gene transfer to these breeding lines is possible in terms of transient *gus* gene expression. The results obtained in this study will provide the basis for future studies on improvements to *in vitro* culture systems and *Agrobacterium*-mediated transformation of these rice breeding lines.