M. Phil 73

Development of *Arabidopsis STH2* gene over expressing transgenic rice (*Oryza sativa L.*) to improve its productivity.

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Abstract

Light is a critical energy source for photosynthetic organisms. Plants continuously monitor the intensity, quality and duration of light to optimize their growth and development. *Arabidopsis* STH2 protein can activate light dependent transcription and positively regulate light mediated development of plants by interacting with HY5 and COP1, which are the two key regulators of light signalling pathway. It has been shown that the productivity of crop plants might be enhanced by over-expressing central regulators of light signalling pathway. In order to increase the productivity of Bg250 and Bg360 *indica* rice varieties by transferring *Arabidopsis STH2* gene via *Agrobacterium* – mediated transformation method, we successfully established simple and efficient *in vitro* plant regeneration protocol along with *Agrobacterium* mediated transformation protocol for agronomically important selected rice varieties. Furthermore, we report here the over-expression of *Arabidopsis STH2* gene in *indica* rice variety Bg250.

Experiments were carried out to induce embryogenic calli and plant regeneration from three different *indica* rice cultivars: Bg250, Bg360 as candidate and Bg94-1 as control. Using mature embryos as ex-plant, all three the cultivars could develop scutelum derived calli and regenerate plants. N_6B_5 medium was used for callus induction and shoot regeneration. Large variability in callus growth and plant regeneration potential were revealed among the cultivars tested. The maximum callusing frequency of 90% was observed in Bg250 variety after 21 days followed by 4 days of incubation on callus induction medium under dark. The control variety Bg94-1 showed 78.33% of callus induction frequency and the other candidate variety Bg360 showed 41.25%. Highly significant regeneration difference was observed in partially desiccated calli of Bg360 in comparison to non-desiccated calli. It took 5-6 weeks for callus to regenerate into a complete plant. Plantlets regenerated from calli were successfully established in the soil. Callus induction and plantlet regeneration severely depend on the genotype and the culture conditions.

Agrobacterium mediated rice transformation protocol was standardized using pCAMBIA 1303 binary vector which contains hygromycin marker and GUS reporter gene. The maximum transformation efficiency of 20% was obtained for Bg250 variety using 500 mg/L cefotaxime as a bacteriostatic agent to inhibit growth of *Agrobacterium*. 100 mM acetosyringone in co-cultivation medium and co-cultivation for 3 days were the optimum conditions for maximum transformation. The expression of GUS gene revealed that the calli were successfully transformed.

Arabidopsis STH2 gene over-expressing heterozygous Bg250 were developed by co-cultivating rice calli with *A.tumefaciens* strain harbouring pPZP200-*STH2* plant binary vector. The expression of the transgene was driven by CaMV 35s promoter. Compared with non-transgenic plants, mature transgenic plants showed increased plant height, leaf length and area, bushy appearance due to the production of increased number of tillers per plant and also increased number of panicles and grains per panicle under greenhouse conditions. These results demonstrated the potential of manipulating plant light signal transduction pathways to enhanced grain productivity.