RESEARCH PAPER

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# Transformation of elite rice cultivars ASD16 and IR64 with *cry2Ac* gene for resistance to rice lepidopteran pests

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With the view to imparting resistance to major lepidopteran pests of rice, attempts were made to transform elite *indica* genotype ASD16 and IR64 with *cry2Ac* gene. *Agrobacterium* and particle bombardment-mediated transformation were carried out using the vector pS2AcP2 harbouring *cry2Ac* gene, the selectable marker gene, *hph* and the scorable marker gene, *gusA*. In *Agrobacterium*-mediated transformation experiment with mature seed derived calli, two lines in ASD16 were regenerated with a regeneration frequency of 1.1 per cent. In particle bombardment-mediated transformation experiments with immature embryos, 15 lines in IR64 were regenerated with a regeneration frequency of six per cent. Presence of transgenes in these lines could not be demonstrated through PCR.

Key words : Rice, *Agrobacterium tumifaciens*, *Bacillus thuringiensis*, Stemborer, Leaffolder, *cry2Ac* gene, Biolistic gun

## INTRODUCTION

Rice is one of the most important cereal crops, providing staple food for nearly one-half of the global population (FAO, 2004). Globally rice is cultivated in 154 m.ha with an annual production of around 645 MT and average productivity of 4.12 tonnes per hectare (USDA, 2007). At least 114 countries grow rice and more than 50 have an annual production of 100,000 tonnes or more.

Savary *et al.* (2000) reported that 24-41% of rice yield was lost every year because of rice stemborer and other insect pests, diseases and weeds. The estimated biotic stress causes annual rice yield loss upto 40% (Oerke and Dehne, 2004).

The most destructive insect pests of the rice crop are yellow stemborer (YSB; *Scirpophaga incertulas* Walk.) and rice leaffolder (RLF; *Cnaphalocrocus medinalis*). Globally, YSB alone causes yield losses of 10 MT and accounts for 50% of all insecticides used in rice field (Huesing and English, 2004). For a long time, the control of these pests has depended chiefly on the use of large amounts of poisonous chemical insecticides, mostly as sprays, which cause considerable environmental pollution and represent a health hazard to farmers as well as significantly increasing the costs of rice production (Tang *et al.,* 2006).

The different insecticidal gene used for the control of insect pests includes protease inhibitors, lectins, amylase inhibitors and ä-endotoxins (*Bt* gene) produced by the soil bacterium, *Bacillus thuringiensis.* Among them, *Bt* gene offers a great scope for controlling insect pests (Shelton *et al.,* 2000).

Keeping the above points in mind the present investigation has been envisaged to evolve transgenic indica rice cultivars expressing Bt toxin to provide protection against lepidopteran pests with the following objectives:

- Confirmation of *cry2Ac* gene construct through molecular analysis
- *Agrobacterium* and particle bombardment-mediated transformation of elite *indica* rice cultivars, IR64 and ASD16 using $pS_2AcP_2$ harbouring *cry2Ac* gene

## MATERIALS AND METHODS

***Gene construct :***

The binary vector, $pS_2AcP_2$ (based on pCAMBIA1301; Fig.1) containing *cry2Ac* gene (source: Dr. V. Udayasuryan, Department of Plant Molecular Biology and Biotechnology, CPMB) driven by *CaMV*35S promoter, *hph* and *gusA* gene. *Agrobacterium*- strain LBA4404 ($pS_2AcP_2$) was used for transformation experiments.

***Back-transformation of E. coli DH5á :***

*Agrobacterium total DNA isolation:*

Total DNA was isolated from *Agrobacterium* strain LBA4404 harbouring $pS_2AcP_2$ by following a modified protocol of Chen and Kuo (1993).

*Preparation of DH5α competent cells :*

Single colony of DH5α was inoculated in 3 ml of LB (10 g/l tryptone, 5 g/l yeast extract, 10 mg/l NaCl, pH 7.2) broth and allowed to grow overnight. One millilitre