*Karnataka J. Agric. Sci., 20(4), (845-847) 2007*

# *Agrobacterium*-mediated Genetic Transformation of BPT-5204, a Commercially Grown Indica Rice Variety*

BPT-5204 (GEB24/TN1//Mashuri), though released during 1986, has spread to almost all the states in India (Anonymous, 2004). It is known for its excellent cooking quality; but is susceptible to stem borer and brown plant hopper. Among various approaches used for improvement, genetic transformation with appropriate gene(s) seems to be promising. In rice, considerable difference exists for response to callus induction, plant regeneration and transformation between indica and japonica types (Hiei *et al*., 1997). Tissue culture and genetic transformation in japonica rices has been well studied compared to indica types (Lin and Qifa, 2005; Yukoh and Toshihiko, 2006; Yookongkaew *et al.,* 2007). With this background, development of a protocol for *Agrobacterium*-mediated genetic transformation of three different explants viz., mature embryo, scutellum and scutellum-derived callus was attempted with *cry1Ac* gene in BPT-5204, an indica rice.

*Agrobacterium* strain LBA4404 harbouring the binary vector pRD400 (Fig. 1) containing *cry1Ac* codon optimized gene in fusion with CaMV35S promoter and *nos* terminator was provided by Dr. Altosaar, Canada. This vector carries nptII gene as the selectable marker (Cheng *et al*., 1998).

Fresh seeds of BPT-5204 were obtained from ARS, Mugad, Dharwad. Three different explants viz., mature embryo, scutellum and scutellum-derived callus were used for co-cultivation. Mature embryos were obtained for co-cultivation by first de-husking the seeds, and washing them with 70% ethanol for 30 sec followed by thorough rinsing with sterile distilled water. The seeds were soaked overnight over moistened sterile filter paper in Petri dishes and then mature embryos were excised under sterile condition. Scutellum explants were collected by excising from 4-5 days old in vitro grown seedlings. However, the scutellum-derived calli were obtained by culturing the scutellum on callus induction/maintenance (CIM) medium of MS (Murashige and Skoog, 1962) supplemented with 2, 4-D (1 $mgl^{-1}$) and benzylaminopuirne (BAP, 0.25 $mgl^{-1}$). One month old scutellum-derived callus was taken for co-cultivation.

Freshly subcultured (for 5 d) embryogenic calli (2-4 mm in size) were soaked in bacterial suspension for 10 min. Calli were then placed on CIM medium with acetosyringone (200 µM) and co-cultivated for 3 days in the dark at 25°C. Calli were then washed thoroughly but gently in sterile water supplemented with 500 $mgl^{-1}$ cefotaxime, blot dried on filter paper, placed on CIM medium supplemented with 50 $mgl^{-1}$ kanamycin and 300 $mgl^{-1}$ cefotaxime, and cultured for 3-4 weeks in the dark at 27°C. Resistant callus clusters were transferred onto fresh medium (CIM containing 50 $mgl^{-1}$ kanamycin) and cultured for 2-3 weeks (dark, 27°C). Resistant calli were then transferred to regeneration medium in which 2, 4-D was replaced with BAP (3 $mgl^{-1}$) and NAA (1 $mgl^{-1}$), and kept in the light. Regenerated shoots were transferred to basal MS medium for rooting.

The procedure followed for scutellum and mature embryo co-cultivation and transformation was essentially same as that used for scutellum-derived callus except for the starting plant material. These explants after the co-cultivation were placed on CIM and MS medium for callus induction and direct germination, respectively. Putative transformants (survivors on kanamycin plates) were tested for the marker gene (*nptII*) using PCR with specific primers (npt_F: 5' GAGGCTA TTCGGCTATGACTG 3' and npt_R: 5' ATCGGGAGC GGCGATACCGTA 3') to check for the real transformants. Transformation efficiency was calculated as number of PCR positive plants regenerated from the initial explants used for co-cultivation. Regenerants (Fig. 2) from three different explants such as mature embryo, scutellum and scutellum-derived callus, which survived on kanamycin selection, were compared. Considerable difference was noticed for the frequency of putative transformants (calculated as the number of plants, which survived kanamycin selection from a co-cultivated explant) among the three different explants used for co-cultivation. Out of 320 mature embryos co-cultivated with *Agrobacterium*, 220

Table 1. Transformation efficiency with different explants of BPT-5204

| Explant | No. of explants co-cultivated | Plants regenerated on selection media | Putative transformants (plants survived on selection media) | PCR +ve plants |
|---|---|---|---|---|
| Embryo | 320 | 220 | 55 | 1 |
| Scutellum | 120 | 10 | 6 | 1 |
| Callus | 120 | 64 | 19 | 2 |

* Part of the M. Sc. (Agri.) thesis submitted by the senior author to the University of Agricultural Sciences, Dharwad-580 005, India.

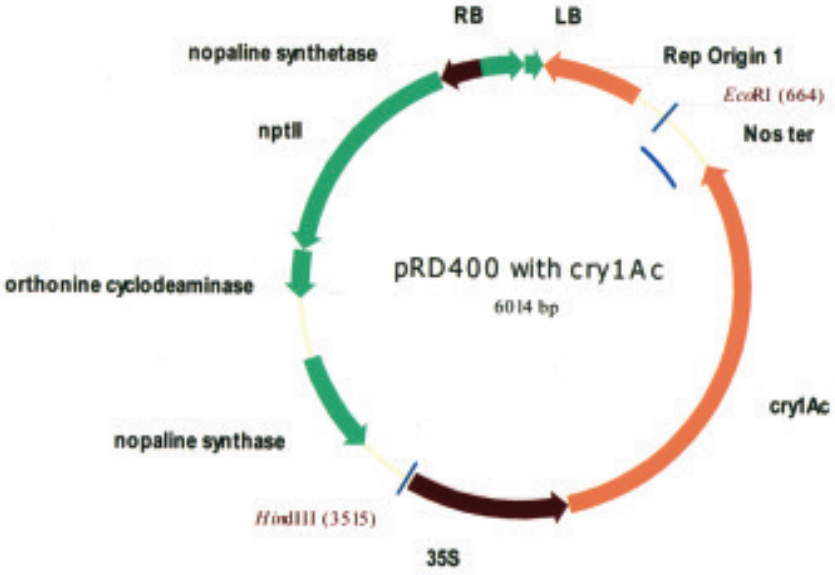


Figure 1. pRD400 vector with codon optimized *cry1Ac*

Figure 2. Putative transformants

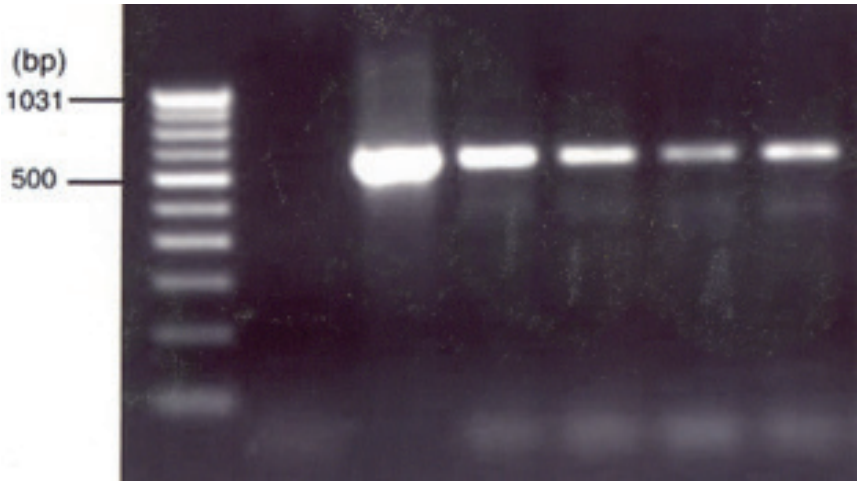


Figure 3. PCR detection of transformants
(Lane1: 100bp ladder, lane2: negative control, lane3: positive control, and lane4-7: transgenics)

could produce plants initially, but only 55 (0.17) plants survived upon selection with kanamycin. Out of 120 scutellum co-cultivated, only 40 could produce callus, which subsequently produced 10 plants and only 6 (0.05) could tolerate kanamycin. Finally, 120 co-cultivated calli produced 64 plants of which 19 (0.16) survived the kanamycin selection (Table 1).

Transformation efficiency indicates the number of transformants that are PCR positive for the transgene (*cry1Ac*) or marker gene (*nptII*). In general, scutellum-derived callus recorded the highest transformation efficiency (1.67%) followed by scutellum and mature embryo (Table 1).

A number of studies have shown that not all plants regenerated upon kanamycin selection are true transformants (Toriyama *et al.*, 1988; Ayres and Park, 1994). In this study, repeated PCR check was done to find out how many of the putative transformants were real by using *nptII* specific primers against the template DNA obtained from all the putative transformants. Out of 80 such plants, only 4 were found to carry *nptII* (Figure 3). Two transformants originated from scutellum-derived callus, and one each from mature embryo and scutellum explant. It is well known that a given transgenic event need not result in plants with desired level of expression of the transgene (Butaye *et al*., 2005). Thus in any crop plant transformation programme, it is necessary to obtain large number of independent transgenic events to pick up the best among them. Hence using more efficient selectable marker genes such as *hph* (for hygromycin) and *bar* (for basta) may prove useful as reported in several studies (Cao *et al.*, 1992; Ayres and Park, 1994; Aldemita and Hodges, 1996).

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*(Received: November, 2006)*

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