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Research Article

An efficient high throughput plant regeneration and transformation protocol for production of transgenics tolerant to salt in finger millet

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Abstract : Finger millet [*Eleusine coracana* (L.) Gaertn.] is the primary food source for millions of people in tropical dry land regions of the world. Development of efficient and genotype-independent tissue regeneration system is an essential prerequisite for successful production of transgenic plants. In this direction we attempted *in vitro* plant regeneration and transformation study in finger millet crop using *PDH45* as a candidate gene to develop transgenics for salt tolerance using *Agrobacterium* mediated gene transfer method (*in vitro* method). Here we used actively dividing embryogenic seed calli as explantt. The seed calli was co cultivated with *Agrobacterium* carrying binary vector pCAMBIA contains PDH45 gene, *nptII, hptII* , and *GUS* reporter gene driven by CaMV 35S promoter. The co cultivated callus was regenerated in half strength MS media with 0.5 mg.L^{-1}BA, 3.0 mg.L^{-1} 2, 4-D, and hygromycin antibiotic supplemented with acetosyringone (100 mg.ml^{-1}), a potent inducer of virulence genes. Successful transformation at callus stage was initially confirmed by *GUS* histochemical assay. By PCR amplification genomic DNA of putative transformed calli showed positive for *hptII* primers. The results by RT-PCR showed that the level of transcripts overexpression in transformed calli was relatively higher than nontransformed control calli. The regenerated transgenic plants were confirmed by PCR amplifying the genomic DNA.

Key Words : Agrobacterium, Callus, Finger millet, *PDH45*, Regeneration

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INTRODUCTION

Among eight minor millets, finger millet [*Eleusine coracana* (L.) Gaertn.], also known as African millet, has outstanding attributes as a subsistence food crop. It is grown globally in more than 4 million ha. and is the primary food source for millions of people in tropical dryland regions. Finger millet constitutes about 81 per cent of the minor millets produced in India. Finger millets also have nutritional qualities superior than that of rice and is at par with that of wheat (Sastri, 1989).

The first experiments to culture plant cells under *in vitro* conditions were conducted more than one hundred years ago (Haberlandt, 1902). It took decades until their detection, isolation and subsequently the observation made by Skoog and Miller (1957) on the auxin/cytokinin ratio controlling root and/or shoot formation from tobacco (*Nicotiana tabacum*) pith tissue cultures *in vitro*, being a milestone for the development of plant tissue and cell culture. However, more than twenty years after that breakthrough and promising results with dicots, success with monocots, especially with the cereals was rare (King *et al.*, 1978). Plant regeneration has been reported to

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