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Title of the Thesis : **“Studies on Micropropagation and Genetic Transformation of Banana (*Musa sp.*).”**

Due to the various polyploidy levels, sterility of most edible cultivars, limited genetic variability and long generation time of the *Musa* spp. are not amenable to breeding. The genetic engineering with breeding programs could proved a powerful approach to overcome these limitations as specific genes can be introduced for improvement of the crop for various traits. An efficient regeneration system is a prerequisite for achieving this goal. It is doubted that the use of suitable gene constructs may lead to the production of incorporate other characteristics, such as, fungal, pest, salt and draught tolerance thus extending the geographic spread of banana and plantain production, and contributing significantly to food security and poverty alleviation in developing countries. With the background in kind, this study was taken up to establish a reproducible regeneration system of *Musa spp*. Pidi monthan, which could be utilized for genetic transformation. Following is the summary of the considerable achievements of this study:

Regeneration through organogenesis

Regeneration through multiple shoot clumps (MSC) was achieved in Indian *Musa* variety Pidi Monthan (ABB). The explants used for the study of regeneration through multiple shoot clumps were primarily shoot tips cultured in modified MS medium supplemented with different working concentrations of BAP and IAA. The particular Pidi monthan cultivar produced the about 16 shoots per explants cultured on modified MS medium supplemented with BAP (100 µM) and IAA (1µM). BAP and IAA at the concentration of 10 ☐M and 1 ☐M respectively were employed to achieve shoot elongation on modified MS medium. Rooting was achieved on MS medium supplemented with 1µM IAA. Regenerated plants developed via multiple shoot clumps were successfully hardened and transferred to the glass house.

Regeneration through somatic embryogenesis

Plantlet regeneration through somatic embryogenesis was tried in *Musa* variety Pidi Monthan (ABB). The MS medium supplemented with BAP (100 µM) and IAA (1 µM) gave highly proliferating compact clumps of meristematic buds called „scalps" using the sh explants. Most of the shoot-tip explants developed scalps within 12-16 weeks of the culture. Embryogenic calli like mass was formed from scalps on MS medium supplemented with 2, 4-D (5 µM) and zeatin (1 µM). Maturation of embryogenic calli was not achieved due to blackening of the culture, which is responsible to block the nutrition of the culture.

Genetic transformation of Musa variety Pidi Monthan with Chitinase gene

The *Chitinnase gene* (accession no. AJ308438) isolated from the insect ***Xenorhabdus nematophillus*** and has an important role in plant defence against fungal infection.

An efficient, simple and rapid *Agrobacterium* mediated transformation system has been developed for the production of transgenic *Musa* variety Pidi monthan (ABB). An optical density of bacteria was found to be 0.8, co-infection time of 45 min., co-cultivation media type B2LAS, supplementation of acetosyringone at 100µM concentration in-cultivation medium, co-cultivation time and effect of vacuum infiltration (400 mmHg) were observed for a high transformation frequency. Transient GUS expression was observed in developing scalp of the putative transgenic plants. Agrobacterium mediated transformation of Pidi Monthan was achieved with Chitinase gene, which is also known as 1, 4-β-poly-N-acetylglucosaminidase. Its plays a potential role in alleviation of biotic stresses.

Putative transgenic *Musa* plants growing on selection medium containing 10 mgl-1 hygromycin were subjected to PCR analysis using Chitinase gene specific primers. GUS expression through histochemical confirmed the successful expression of gus in putative transgenic plantlets. The gus gene already present in binary vector as a reporter gene in pCAMBIA, where chitinase gene at Multiple cloning site. The PCR positive plants had been confirmed with southern hybridization for gene integration by Southern analyses, where the presence of band (3kb) confirms the presence of gene and the expression of gene was confirmed by RT-PCR. Bioassay of the transgenic line (fungal resistance) is in progress.