EXPERIMENT- 9

AIM: *Agrobacterium tumefaciens*-mediated plant transformation and transient reporter gene expression assay.

PRINCIPLE: *Agrobacterium* is the only known organism capable of genetically transforming a plant cell. The foreign DNA to be transferred to plant cell, is cloned between the borders in T-DNA of Ti plasmid and mobilized to *Agrobacterium vir* strain and used for plant transformation.

The steps involved are:

1. Infection of plant tissues with overnight grown *Agrobacterium* culture
2. Cocultivation
3. Post-cocultivation wash and Transient expression assay
4. Culture in selective medium
5. Selection of putative transformed plants
6. Molecular analysis of putative transformed plants

MATERIALS: *In vitro* grown Tobacco plants, Cork borer, *A. tumefaciens* culture, X-gluc (5-bromo-4-chloro-3-indoxyl-beta-D-glucuronic acid, cyclohexyl ammonium salt), Na-EDTA, Potassium ferocyanide, Potassium ferricyanide, Triton X 100, $Na_2HPO_4.2H_2O$, KH_2PO_4

PROCEDURE:

1. Raise the desired *Agrobacterium* strain in 20 ml of LB medium with appropriate antibiotics, agitated overnight at 200 rpm at 28^0 C
2. Concentrate the cells at 5000 rpm, resuspend the cells.
3. Prepare the explants. Infect for 10-30 min.
4. Cocultivate the explants in tissue culture growth conditions for 2-4 days.
5. Wash the explants with sterile dd water for eliminating *Agrobacteria.*
6. Put few explants in GUS substrate, incubate at 37^0 C. Observe transient GUS expression.