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Agrobacterium-mediated genetic transformation of tobacco cells and annexin gene cloning using pTZ57R/T vector

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Abiotic stresses adversely affect growth and productivity and trigger a series of morphological, physiological, biochemical and molecular changes in plants. Drought, temperature extremes, and saline soils are the most common biotic stresses that plants encounter. When a plant is subjected to abiotic stress, a number of genes are turned on, resulting in increased levels of several metabolites and proteins, some of which may be responsible for conferring a certain degree of protection to these stresses. Annexin is a gene which controls the expression of the drought tolerant plants. By attempting the annexin gene cloning that attempts the regulatory transcription factors. Annexin was traditionally thought of as calcium dependent phospholipids-binding proteins, but recent work suggests a more complex set of functions. Annexin gene was successfully amplified from the 17 blackgram genotypes. Annexin gene from LBG756 and PBG107 were cloned into pTZ57R/T vector. The clones were confirmed by colony PCR, Restriction digestion by using BamH1 and Kpn1 to confirm the 930bp annexin gene. Plant transformation is the process by which DNA is introduced into plant cells or tissues. The DNA can come from virtually any source. Gene transfer methodology has become part of an essential technology to manipulate plants for both scientific and commercial purposes. Transgenic plants, the products of this methodology, are useful for dissecting the mechanism(s) of plant gene regulation. This technology is also useful for identifying and evaluating agriculturally useful traits (genes) as well as for their introduction into commercially valuable crops. One of the most efficient methods for gene transfer employs *Agrobacterium tumefaciens* and takes advantage of the naturally evolved crown gall-inducing mechanisms of DNA transfer present in this common soil pathogen. This information has been applied to develop methods that result in the formation of gall-free, genetically transformed plants. This thesis describes a detailed protocol for *Agrobacterium*-mediated transformation of tobacco cells and their subsequent selection and regeneration into transgenic plants. Protocol for the *Agrobacterium* mediated transformation of tobacco standardized using pCAMBIA 1303 vector. Putative pCAMBIA 1303 vector transgenic were confirmed using PCR and RT-PCR analysis using hpt primers. These standardized protocols can be used for transformation of 1303 vector into crop plants.

Biography

Jithender D has done research on annexin gene cloning from Central Research Institute of Dry Land Agriculture, ICAR, Hyderabad, India. He completed his MTech in Biotechnology and Biochemical engineering from Osmania University. He has the authership for annexine gene sequencing from NCBI with accession number gu137310. He is well experienced in downstream processing of HIB Vaccine from Biological E. Limited.

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