

Abstract

Peanut belongs to the genus *Arachis*, a member of the family Leguminosae. Peanut, a protein rich oilseed legume native to South America and cultivated as a major crop in India. It is attacked by a variety of diseases. The Tikka (leaf spot) caused by *Cercospora arachidicola* inflicts a heavy loss in peanut yield. The seed is attacked by *Aspergillus flavus*, a fungus which liberates aflatoxin and makes the seed harmful for consumption. Genetic improvement of this crop will thus be beneficial to increase the yield and cultivate resistant varieties. The conventional methods of crop improvement include introduction of new cultivars via hybridisation. This process is slow due to the fact that peanut is a self pollinated crop. The new in vitro approaches for crop improvement involving genetic transformation, somaclonal variation and other methods offer several opportunities. To fully utilise these approaches however, efficient and effective protocols to facilitate whole plant regeneration are a prerequisite. Reports on regeneration of peanut plants via tissue culture are however very few, suggesting the need for development of a reproducible technique. With this objective, the present studies were undertaken with a view to developing an efficient in Vitro system. The thesis has been divided into seven chapters. The first two Chapters consist of the Introduction and Materials and Methods respectively. Chapters 3-7 have been further divided into two parts each consisting of an Introduction, Results and Discussion.

Chapter I Introduction This chapter deals with general information on the legumes, a family of plants to which the peanut belongs. The status of transformation, somaclonal variation and regeneration in the legumes has also been included with special reference to peanut.

Chapter II Materials and Methods The brand and source of the chemicals, glassware and plasticware used have been included in this chapter. The chapter also describes the procedures followed for cleaning of glassware, composition of the media, and the analytical methods used.

Chapter III Plant Regeneration via Somatic Embryogenesis from embryo explants Somatic embryogenesis was induced from the immature embryo explants using the auxin 2,4-D, the optimal concentration being 13.75 μM . This chapter deals with optimisation of protocols for induction, maturation and conversion of the somatic embryos. Field data of the plants raised from somatic embryos has also been included. The plants derived were found to be uniform for the morphological characters screened. The protein and lipid contents of the seed was comparable to the controls.

Chapter IV Embryogenesis from embryonal leaf explants Plant regeneration via somatic embryogenesis was obtained from embryonal leaf explants on 2,4-D containing medium. 90.50 μM of 2,4-D was found optimum for induction of callus free somatic embryogenesis. Maturation occurred on lowering the 2,4-D concentration to 13.75 μM and in the presence of 6% sucrose. Germination of these embryos took place on MS media containing 5% activated charcoal. The plants obtained grew to maturity and were fertile. The process of somatic embryogenesis was repetitive and secondary embryogenesis occurred with subculture to fresh media provided with 13.75 μM 2,4-D.

Chapter V Plant Regeneration via Organogenesis from embryonal leaf explants MS medium supplemented with 5.36 μM NAA as auxin and 4.40 μM BAP as cytokinin induced direct caulogenesis in the embryonal leaf explants. On this media 85% of the explants induced shoot buds. Multiplication and elongation of the shoot buds occurred in MS medium provided with 13.20 μM and 2.20 μM BAP respectively. Rooting of the shoots obtained was induced on hormone free half strength MS medium. The plants obtained were fertile.

Chapter VI In vitro mutagenesis in peanut This chapter deals with studies carried out to estimate the effect of UV radiation on whole seeds and the regeneration of shoots from embryonal leaf explants. In one experiment the surface sterilised seeds were irradiated for various time intervals . The plants obtained from these seeds showed distinct variations such as variegation and curling of the leaves on exposure to sunlight. In the second experiment the embryonal leaflets excised from the seed were irradiated. The regeneration potential of the irradiated leaf explants decreased with increase in exposure time. Multiplication of the regenerated buds was also very slow. The plants obtained showed dwarfism and lack of lateral branches. These plants flowered earlier as compared to the controls and viable seed was obtained.

Chapter VII Effect of lysine and threonine stress on the regeneration of embryonal leaflets. This chapter describes experiments carried out to test the effect of lysine and threonine stress on regeneration of leaf explants. The regeneration protocol described in Chapter V was used for all the experiments. Lysine and threonine were supplemented in the induction and multiplication media. Lysine and threonine severely inhibited regeneration when used beyond all concentrations greater than 1.8 mM. The embryonal leaf explants showed greater tendency towards dedifferentiation in the presence of the stress. These effects were more pronounced if the leaf explants were irradiated with UV light prior to exposing them to the lysine and threonine stress.