ABSTRACT

Name : Anju Arora  
Id. No. : 20642  
Semester and year of admission : 1st semester, 1999-2000  
Degree : Ph.D  
Major : Genetics and Plant Breeding  
Minor : Molecular Biology and Genetic Engineering  
Thesis title : "In vitro regeneration and Agrobacterium mediated transformation in chickpea (Cicer arietinum L.)"

Advisor : Dr. H.S. Chawla

In vitro regeneration and Agrobacterium transformation studies were conducted in chickpea (Cicer arietinum L.) for the development of transgenics. Four explants viz. immature embryo, immature cotyledon, mature embryo axis and mature cotyledon explants from two genotypes Pusa 256 and PG 186 were used for regeneration. Multiple shoots were induced directly from all the four explants on different shoot induction media containing 1 to 3 mg/l BAP with or without 0.05 mg/l NAA auxin and rooted on 1.5 mg/l IBA or NAA. Immature embryo explants were most effective with shoot induction frequency of 70.6% and 7.0 shoots/explants followed by embryo axis explants with 67.9% shoot induction frequency and 5.6 shoots/explant.

Shoot regeneration via callus induction was also studied. Calluses were induced on media containing 0.5 to 2 mg/l NAA with or without BAP which were regenerated on media with varying concentrations and combinations of BAP and NAA. Callus induced on 2 mg/l NAA alone showed better shoot regeneration on 2mg/l BAP containing regeneration media.

Somatic embryogenesis approach was also studied in four explants. Somatic embryos were induced on MS Media containing 0.5 to 2 mg/l 2,4-D with or without 1 mg/l BAP. A higher embryogenic callus induction frequency of 90% and 88% was observed from immature and mature embryonic or cotyledon explants, respectively, when calluses were induced on 2 mg/l 2,4-D for embryonic explants and on 2 mg/l 2,4-D with 1 mg/l BAP for cotyledon explants. Embryogenic calluses induced on MS media containing 0.5 mg/l 2,4-D showed embryo maturation and regeneration with a frequency of 55-75% on regeneration media with either 1 mg/l BAP alone or in combination with 0.05 mg/l NAA.

Agrobacterium mediated GUS expression was seen using two vectors pBI121 and pCAMBIA2301 containing GUS gene with 35S promoter. The explants of immature embryo and mature embryo axis were treated with SCFs for different time interval GUS expression with 11-12% frequency was observed using pBI121 and pCAMBIA2301 after wounding with SCFs for 2 min or 5 min.