Abstract

Efficient micropropagation protocols were developed for black pepper *(Piper nigrum* L.), a popular spice and long pepper (*Piper longum* L.), an important medicinal plant. Plant regeneration was obtained through shoot organogenesis and somatic embryogenesis in both the species. SH medium supplemented with 6.0 and 8.0 mg/l BAP was optimal for direct shoot regeneration in black pepper using juvenile internodes and apical buds respectively. E1 medium supplemented with 3.0 and 4.0 mg/l BAP was found suitable for long pepper using nodal explants. Callus induced on E1 medium supplemented with 4.0 mg/l NAA and 2.0 mg/l BAP from juvenile apical buds of black pepper regenerated shoots on SH medium containing 1.0 - 3.0 mg/l BAP. In long pepper callus induction and regeneration was obtained as a single-step process on E1 medium containing optimal concentration of 3.0 mg/l BAP from internode explants. *In vitro* grown shoots were rooted on hormone-free or auxin supplemented basal medium in black pepper and on induction medium itself or hormone-free basal medium in long pepper. Plantlets regenerated were acclimatized under green house conditions with 90% survival. Direct somatic embryogenesis and plant regeneration were obtained for the first time in black pepper from the germinating seeds on SH medium containing 1.5 %, 2.0% and 3.0 % sucrose under 24 h dark period. Growth regulators were ineffective in inducing somatic embryogenesis from seeds of black pepper. Callus mediated somatic embryogenesis was induced in long pepper from petiole explants cultured on E1 medium containing 1.0 mg/l BAP under 16 h photoperiod. Maturation and germination of somatic embryos occurred on the induction medium itself in both the cases. Secondary cyclic embryogenesis was observed in black pepper at high frequency. The secondary embryos were formed from the suspensor region of primary embryos. Addition of ABA (1.0 - 3.0 mg/l) increased the proliferation of secondary embryos in black pepper. Germination and conversion of somatic embryos were achieved in high frequency in solid as well as liquid medium. The nature and orientation of explant, genotype, amount of

available nitrogen, sucrose concentration and media supplements markedly influenced *in vitro* propagation of black pepper and long pepper. Among these genotype found to be most critical. Cultivar Karimunda of black pepper and Viswam of long pepper found to be highly morphogenic. Embryogenic suspension culture was successfully established in black pepper and plant regeneration was obtained by plating as well as directly in the liquid medium. Ontogeny of shoot buds and somatic embryos in black pepper and long pepper were traced. Shoot buds originated *de novo* as well as by axillary bud proliferation in both the species. Somatic embryos have a single cell origin in black pepper and multicellular origin in long pepper. A marked similarity in the developmental pattern of zygotic embryos and somatic embryos was observed in black pepper right from the globular stage. The differences observed were the larger size of somatic embryos during early stages and smaller size of cotyledons during later stages. Somatic embryos of black pepper were successfully encapsulated, stored at various conditions and germinated into plantlets. The results described here provide a simplified protocol for micropropagation of black pepper and long pepper which can be effectively used for large scale multiplication of elite genotypes. Furthermore, the protocol can be employed for production of genetically engineered plants for resistance to diseases and germplasm conservation.