

Study of *Ephedra Spp.* Micropropagation

Abstract

Ephedra botanically belongs to chelamidospem class and Ephedraceae. *Ephedra* species are ever green and almost leafless shrubs. The stems are slender, erect or reclining. Flowers are dioecious, bone in round. Fruit (cone) is globular, usually red, occasionally yellow. *Ephedra* is the source of two valuable alkaloids; 1- ephedrine and d-pseudoephedrine. Dried stems of *Ephedra* species are used to alleviate symptoms caused by common cold, influenz, asthma, bronchitis, and hay fever. The *in vitro* micropropagation *Ephedra procera* and *Ephedra strobilacea* were investigated.

A number of experiments were conducted to identify suitable procedure for *in vitro* shoot multiplication of *Ephedra procera*. In first experiment different concentrations of kinetin were studied. Axillary buds explants of *Ephedra procera* were cultured on MS medium supplemented with 0.1 mg^l⁻¹ kinetin and 0.01 mg^l⁻¹ IBA was optimum. Two different nutrient media (MS , DCR) and different explants (axillary and axial buds) were studied. The MS media was significantly better than DCR. Axillary buds explant were more better than axillary buds explants. Combination of 0.05 mg^l⁻¹ BA and IBA was most suitable for shoot fresh weight, main shoot length and number of axillary shoots of *Ephedra procera* axillary buds. Utilization of 1.0 mg^l⁻¹ kinetin and 1.0 mg^l⁻¹ NAA was the best for callus formation. Suspension cultures of *Ephedra procera* were established in MS medium supplemented with 1.5 mg^l⁻¹ kinetin and 1.0 mg^l⁻¹ NAA. A number of experiments were carried out to find a suitable method for micropropagation of *Ephedra strobilacea*. Murashige and Skoog (MS) medium was used as a basal medium in this species. The most suitable concentration of calcium hypochlorit for seeds surface disinfection was 5.0% for 40 minutes. Utilization of 0.15 mg^l⁻¹ BA and 0.05 mg^l⁻¹ IBA was the best for shoot multiplication of *Ephedra strobilacea*, using seeds as explant. The effects of different concentrations of NAA and 2,4-D in combination with BA for callus formation was studied. High frequency of callus formation occurred with 1.0 mg^l⁻¹ 2,4-D combined with 0.01 mg^l⁻¹ BA, friable callus was produced. The effects of different concentrations of NAA and 2,4-D in combination with 1.0 mg^l⁻¹ kinetin on callus formation was studied. Kinetin at 1.0 mg^l⁻¹ in combination with 1.0 mg^l⁻¹ 2,4-D was the best for compact callus formation. The most suitable medium for root formation proved to be half strength Murashige and Skoog (1/2MS) medium supplemented with 2% sucrose, 0.01 mg^l⁻¹ BA and 2mg^l⁻¹ NAA. The *in vitro* raised plantlets were acclimatized and transferred to soil with 50% success.

Keywords: Micropropagation; *Ephedra procera*; *Ephedra strobilacea*; Nutrient medium; Explant; *In vitro* shoot multiplication; Callus formation; Suspension cultures; Root