Internat. J. Plant Sci. Vol.2 No.2 July 2007 : 228-230

New methods for sterilization of explants and hardening of cucurbitaceous plants

MAHIPAL S. SHEKHAWAT
Department of Botany, Arignar Anna Govt. Arts College, KARAIKAL (PONDICHERRY) INDIA

(Accepted :June, 2007)

SUMMARY
Studies were conducted to develop special methods of sterilization of selected Cucurbitaceous female plants. Nodal explants were harvested for surface sterilization and initiation of cultures. The cut ends of nodal explants were sealed in liquid wax and then surface sterilized. 100 % survival rate of explants was observed in tissue culture by this method. On the other hand, when the plants were hardened, they should get physical support and they also require habitat soil for the rapid development of root system which is the prerequisite for survival of these plants. In this method 97 % plants survived in the field conditions.

Key words : Cucurbitaceous plants, Liquid wax, Habitat soil.

The Cucurbitaceae are essentially a tropical family. In India it is represented by 37 genera with many species, several of which are cultivated throughout India. This family provides large numbers of fruits which are eaten raw or cooked. Gourd (*Luffa cylendrica*), Bottle gourd (*Lagenaria siceraria*), Spine gourd (*Momordica dioica*), Cucumber (*Cucumis sativus*), Red pumpkin (*Cucurbita maxima*), Melon (*Cucumis melo*) etc. are some common species known in cultivation. Plants are trailing or decumbent, annual or perennial herbs, dioecious and usually climbing by means of tendrils.

Tissue culture of cucurbitaceous plants have been studied by several workers (Moreno et al., 1985; Dirks and Buggenum, 1989; Reynolds, 1994). Burza and Malepszy (1995) achieved plant regeneration from leaf explants. Burza et al. (1996) studied the effect of simple and recurrent *in vitro* regeneration on a cucumber inbred-line under field cultivation. Malepszy et al. (1996) characterized the cucumber somaclonal variant with paternal inheritance. Plant regeneration from protoplast through direct somatic embryogenesis was achieved by Burza and Malepszy (1995) in *Cucumis sativus.* Filipecki et al (1997) reported on the isolation and characterization of cDNA and genomic clones of MADS-box genes which are expressed in cucumber fruit and somatic embryos. Biosynthesis of defense-related proteins and ribosome inactivating proteins from hairy root cultures of *Trichosanthes kirilowii var. japonicum* and *Luffa cylindrica* have been reported by Savary and Flores (1995) and Sanito di Toppi et al. (1996) respectively. Transformation and genetic engineering of some cucurbitaceous plants have been also achieved (Reynolds, 1994; Dabauza et al., 1997).

The root and stem of the plants of this family contain large vessels in the xylem and hollow central pith in their anatomy. It seems that the cucurbitaceous shoots and their cut ends uptake / absorb the $HgCl_2$ very rapidly through vascular system, which is highly toxic. Secondly the hardening of micropropagated plantlets of this family member has been the most difficult part. Experiments were conducted to overcome these problems to improve the survival rate.

MATERIALS AND METHODS

Sterilization of explants:

The plants establish in the Botanical Garden were used as sources of explants. Fresh shoot sprouts of female plants were harvested with 1-2 nodes used as explants. These were surface sterilized by the following procedures. The cut ends were sealed with wax by giving quick dip in the molten (liquid) wax. The explants with wax sealed cut ends were surface sterilized with 90% ethanol and followed by $HgCl_2$ solution. The wax sealed ends of the surface sterilized explants were cut and removed and then transferred to the culture initiation media.

Hardening of in vitro regenerated plants:

The *in vitro* regenerated plantlets were transferred for hardening after10-15 days of root initiation. Rooted plantlets were taken out from the culture vessels, washed thoroughly with sterile water in order to remove adhered nutrient agar and potted in bottles containing soilrite moistened with 1/4th Murashige and Skoog's (MS) salts. These plantlets were kept capped under 60-80% relative