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Mutagenic studies on nodal explants of *Capparis zeylanica* L.

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ABSTRACT

Micropropagation involves multiplication of genetically identical individuals by sexual reproduction within a short span of time with tremendous potential for the production of high quality plant based medicines. The present study established reliable and reproducible protocol for rapid multiple shoot induction from node explants of *Capparis zeylanica* using different concentration and combination of cytokinins. Murashige and Skoog (1962) medium supplemented with 0.5 to 2.0 mg/l BAP was found to be optimum to induce shoots directly from the node explants. Since very scarce information is available about micro propagation of this important medicinal plant, an attempt was made to develop a reproducible protocol for multiple shoot induction form nodal explants of one the culture. Significant increase in the number of shoots per explants was found ion M.S. medium supplemented with 2.0 mg/l BAP and 14 mg/l adenine sulphate. All the tested combinations have effect on increasing the number of shoots. Nodal explants derived shoot cultures were sub cultured to M.S. medium fortified with same concentration of hormone for shoot elongation. The percentage of explants exhibiting shoot induction was found to be between 50-60 i. most of the concentrations of benzyl amino purine. Several workers in past have micro propagated. Some of the important Asclepiadaceae members such as *Ceroegia bulbosa* (Britto *et al,* 2003), *Holostemma ada – kodien* (Martin, 2002-2003).

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Key words : Mutagenic studies, Nodal explant, Multiple shoots *Capparis zeylanica* L.

INTRODUCTION

The present study was undertaken to explore the immune modulatory activity of ethonolic and water extracts of *Capparis zeylanica* Linn (family capparidaceae). Immuno modulatory activity was also assessed by serological haematological tests. The study comprised the acute toxicity and preliminary phyto chemical screening of the ethano land water extracts. Pre-treatment of water extract (300 mg/kg, oral) of *Capparis zeylanica* evoked a significant increase in neutrophyil adhesion to nylon fibers. The augmnetaton of humoral immune response to sheep red blood cells by athanolic and water extracts (150-300 mg/kg) is evidenced by increase in antibody titres in mice. Oral administration of ethanolic and water extracts of *Capparis zeylanica* leaves, at doses of 150 and 300 mg/kg in mice, dose dependently potentiated the delayed type hyper sensitivity reaction induced by sheep red blood cells. A dose related increase in both primary and secondary antibody titre was observed. Large climbing shrubs with hooked spines, stems woody, rough broon tomentose. Leaves ovate or elliptic – oblong, 3.5-6.5X2.3-4 cm, rusty tomentose when young glabrous at maturity, cuneate, entire, apex mucronate. Flowers 3-5 cm across white, turning pink. Berries 2.5-6 X 2-4.5 cm; ripe red, globese. *Cappparis zeylanica* extracts prevented myelo suppression in mice treated with cyclophosphamide drug.

MATERIALS AND METHODS

The nodal raised from control seeds could produce only callus on MS with different supplements which has regenerated into a single shoot. Well filled undamaged and uniform sized seeds were handpicked from the seed lot and equilibrated to the moisture content of 12 percent. For each dose of physical mutagen and random sample of 100 seeds were treated in *Capparis zeylanica.* The recommended agronomic practices and plant protection measures were fallowed uniformly for all treatment nodal explants. In the present studies, the inductions of multiple shoots were reported from stem explants after gamma rays treatment. The seed were removed, and the cotyledons were surface sterilized with 2 ml teepol, in 98 ml sterilized distilled water for 2 minutes, and then rinsed twice with distilled water; later stem were sterilized in 0.1% mercuric chloride for 5-7 minutes, followed by rinsing four times in sterile distilled water, were closed with cotton plugs and were sterilized by autoclaving. Different explants were excised aseptically and were inoculated on the MS based medium supplemented by kinetin or BAP at concentrations ranging from 0.5 to 5 mg/l. cultures were incubated under 10 h fluorescent light at 25 ± 2^0c temperature. Later, the seeds were kept for

germination on the filter paper bridges. After 8 days of germination the resulted seedlings were irradiated.

RESULTS AND DISCUSSION

The maximum number of shoots obtained at dose of 4 and 5 kR and they ranged from 12-16 from nodal cultures. Mutagen (gamma rays) treated nodal explants were tested for their effectiveness. The lower doses induced callus and greening of callus in stem cultures (Plate I, Fig 1). The doses 4 kR induced high frequency of multiple shoots (Plate I, fig 3). The higher doses 10, 15 and 20 kR induced excessive callus growth and browning of callus. The higher dose of 20 kR reduced the number of shoots and only greening of callus (Plate I, Fig 2). And doses beyond that totally suppressed the formation of shoots from *Capparis zeylanica* nodal explants (Table 1). With the increase in the level of BAP (2.0 to 4.0 mg/l). The maximum numbers of shoots on the explants were observed at 4.0 mg/l BAP or 5.0 mg/l kinetin, but higher levels of BAP or kinetin, the formation of callus had taken place and the number of shoots per explants was reduced. The exact cause for the multiple shoot induction by gamma rays is not known, but it may by possibly due to the stimulation of endogenous hormones by the physical mutagen (gamma rays) which in turn induces multiple shoots. The nodal treated with 1, 2, 3, 4, 5, 10, 15 and20 kR irradiation produced a number of multiple shoots. 4 or 5 kR is most potent in inducing most number of multiple shoots. The isolated *in vitro* raised shoots of 1-2 cm long, rooted profusely on MS medium with BAP (2mg/l) + NAA (1 mg/l) within 30 days resulting in the formation of complete plantlets.

Table 1 : Multiple shoot induction from nodal explants of *Capparis zeylanica*. L

% of growth regulators (mg/l)	Nodal explants	
	Mean number of soots per shoot tip	% of callus production
MS + 1.0 BAP + 1.0 NAA	10.3 ± 2.2	44
MS + 2.0 BAP + 1.0 NAA	9.3 ± 2.4	42
MS + 3.0 BAP + 1.0 NAA	6.2 ± 1.6	30
MS + 4.0 BAP + 1.0 NAA	1.4 ± 0.2	35
MS + 5.0 BAP + 1.0 NAA	6.6 ± 1.3	25
MS + 2.0 BAP + 1.0 L-Glutamicacid	12.4 ± 1.0	20
MS + 3.0 BAP + 1.0 L-Glutamicacid	15.6 ± 0.5	18
MS + 4.0 BAP + 1.0 L-Glutamicacid	14.4 ± 0.4	15
MS + 10 % CM + 0.5 BAP	11.3 ± 2.2	14
MS + 15 % CM + 0.5 BAP	11.6 ± 2.3	10
MS + 20 % CM + 0.5 BAP	10.4 ± 1.6	6

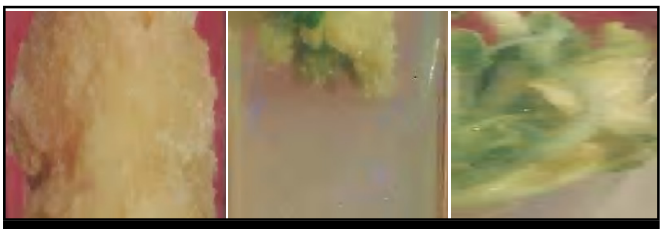

Plate 1 : Induction from nodal explants of *Capparis zeylanica*. L.

Conclusion:

The efficiency of mutagenic agent not only depends on the biological system but also of physical damage, chromosomal aberration and sterility induced in addition to mutation. The nodal explants transferred to a fresh medium containing the some concentration of growth regulators, again resulted in the formation of multiple shoots. In the present study the effectiveness of different doses by gamma rays was assessed by their mutagen dose and the efficiency was assessed on four different biological parameters.

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