STUDIES ON *IN VITRO* SOMATIC EMBRYOGENESIS & ISOLATION OF PROTOPLASTS OF *CAPSICUM ANNUUM* L. VAR. ACCUMINATUM FINGERH (CHILLI)

By

JEYARANI NANDAKUMAR

Thesis

Submitted in partial fulfilment of the requirements

for the degree of

MASTER OF PHILOSOPHY

in the

POSTGRADUATE INSTITUTE OF AGRICULTURE

of the

UNIVERSITY OF PERADENIYA

SRI LANKA

April, 1998

ABSTRACT

Chilli [*Capsicum annuum* L. var. accuminatum Fingerh] is one of the important spice crops of Sri Lanka. Diseases such as Chilli leaf curl complex, Chilli narrow leaf disorder, Anthrocnose & Collar rot are the major constraints in the production. In order to reduce loss of yield, disease resistant Chilli plants could be produced by traditional breeding methods. However new biotechnological techniques such as somatic hybridization followed by plantlet development through *in vitro* organogenesis allow the elite, wild disease resistant characters to be hybridized in cultivated Chilli plants (variety MI_2) i.e. quick genetic improvements are possible in breeding programmes. This *in vitro* culture study of Chilli (Bird chilli variety or Kochchi. 'S.') further helps to conserve valuable wild species of Chilli. Somaclonal variations induced in *in vitro* culture techniques like somatic embryogenesis could enable to select disease-resistance.

The objective of this study was to develop good *in vitro* culture techniques such as protoplast culture, somatic embryogenesis and organogenesis that could be utilised for somatic hybridization and somaclonal variation studies.

The experiments were done in five stages viz,

1. *In vitro* seedling establishment, callus induction, proliferation & maintenance
2. The establishment of embryogenic cell suspension culture
3. Somatic embryogenesis from established embryogenic cell suspension culture & histological studies
4. Organogenesis from callus.
5. Evaluation of a suitable protocol for the protoplast culture.

Successful callus induction was obtained on leaves and cotyledons of two week old seedlings in MS medium containing 1 mgl^{-1} 2,4 - D after incubation in the dark for two to three weeks. A combination of kinetin (0.2 mgl^{-1}) and 2,4 - D (1 mgl^{-1}) promoted a higher callus proliferation. Cell suspension cultures were established using 2 g of four weeks old leaf and cotyledon calli in 20 ml of liquid MS medium in 100 ml. Erlenmeyer flasks. Weekly sub culturing was performed. MS medium with 2,4 - D (1 mgl^{-1}) stimulated embryogenesis on cotyledon callus after 12 weeks in culture. Embryogenic calli formed are pale yellow to brown, compact, organized and nodular in appearance. It comprised small, richly cytoplasmic cells without large vacuoles. Both initiation of embryogenic cells and the subsequent development of these cells into embryoids occurred in the same MS (2,4 - D 1 mgl^{-1}) medium. In a period of five to seven days, 12 weeks old embryogenic cells in the 20 ml suspension produced 12 - 14 proembryoids and after 7 - 14 days they developed into heart stage and then to mature embryoids. Plantlet development did not occur so far with the tested MS media, containing activated charcoal, zeatin, IBA and GA3.

Satisfactory shoot regeneration was achieved in cotyledon callus of Chilli variety MI_2. Shoot regeneration was obtained in MS modified medium supplemented with casein hydrolysate (400 mgl^{-1}), BAP (2 mgl^{-1}) & IAA (1 mgl^{-1}); followed by shoot development on the same shoot induction medium. An average of 3.5 shoots of 2.2 cm height were obtained 4 weeks after shoot bud initiation from 2 g of callus. Root regeneration did not occur with the tested MS media.

In order to isolate the protoplasts from leaves of chilli varieties MI_2 and Bird chilli different incubation periods were tested. Three hours of incubation using 2% cellulase & 0.4% of macerozyme was the best for the tested varieties of Chilli. The yield was $5x10^8$ protoplasts/ml leaf tissue in both varieties. Of the tested NT, MS,

B5 & Potato protoplast culture media modifications, it was found that mixed nurse method using NT medium supplemented with 2,4 - D, NAA & BAP (each 1 mgl^{-1}) & 1.2% Sea Plaque agarose gave the positive results (some small colony formation). But one of the major constraint was bacterial contamination (70% contamination) of plates that was difficult to avoid.