SOMATIC EMBRYOGENESIS IN MUNG BEANS (Vigna radiata L.)

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Abstract: Somatic embryos were obtained from hypocotyl region of Vigna radiata seeds. MS medium supplemented with various growth regulators was used for callus induction. The callus is developed from hypocotyl region. Medium composition is MS+ 5 mg/L NAA+ 2mg/L 2, 4 D. After callus development this callus transferred into liquid MS medium with Proline, 2, 4 D. After application of liquid medium embryos are developed & this embryo transfer into another MS liquid medium with BAP and L- Proline and the developmental stages of embryo under observed the Research microscope.

IndexTerms- hypocotyl, MS medium, Vigna radiata, growth regulator.

I. INTRODUCTION

The present experiment was carried out on important legume plant Vigna radiata L. It is the rich source of protein, vitamins, and minerals. Mung bean is considered as an important pulse crop and it is mostly cultivated all over India mainly in a semi-arid area during a rainy season (Vajravel Sindhuja et al. 2005). Somatic embryogenesis is a process where a cell or cells from somatic tissue is used for formation embryo. In somatic embryogenesis, the somatic cells are used for the formation of an embryo. Somatic embryogenesis is uncommon in leguminous crops, especially in Vigna radiata. The limited work has been done on in vitro regeneration from a callus in Vigna radiata. Therefore; the aim of this study is to develop callus culture and induction of somatic embryogenesis from hypocotyl region of Vigna radiata. In-vitro propagation is carried out by using artificial climatic condition. MS medium supplemented with various growth regulators was used for callus induction. The callus is developed from the hypocotyl region. The present investigation gives information about embryo development through somatic embryogenesis using cell suspension cultures.

II. RESEARCH METHODOLOGY

Seed preparation:

- Seeds were soaked in sterile distilled water for 12 hr.
- The socked seeds were washed under running tap water for 3 times
- The seed coats were then removed and hypocotyls are use as explant.

Media preparation:

- a) Callus Induction Media:
- Murashige and Skoog's medium (MS, 1962) supplemented with various growth regulators was used for callus induction. Medium composition is MS+ 5 mg/L NAA+ 2mg/L 2, 4 D
- b) Embryo Induction Media:
- The liquid medium supplemented with MS+ 50mg/L proline+ 2mg/L 2, 4 D and MS+ 25mg/L proline+ 2mg/L 2, 4 D.

pH of the medium was maintained to 5.8-6.0 with 0.1N HCl or NaOH. Embryo cultures are maintained in a growth chamber at 24 °C, 70% humidity.

Procedure:

The hypocotyl region is used as explant for inoculation suggested by K. Khatoon and N. Ara 1995 and Shamsudeen Varisai Mohamed et al. 2005. These were placed in flasks and covered with cloth and washed for 30 minutes under running tap water to remove all the adhering dust particles and microbes from the surface. Then it washed with liquid detergent (1% v/v) for another 10-15 minutes. Further, it sterilized in the laminar airflow chamber with 0.1% mercuric chloride (HgCl2) solution for 4-5 minutes depending upon the explants. The explants were then washed (4 - 5 washings) with sterilized distilled water to remove the traces of HgCl2. The explants cut into small pieces of size 0.5 to 1.0 cm and inoculated on the callus induction medium supplemented with MS+ 5 mg/L NAA + 2mg/L 2, 4 D. The cultures are incubated at temperature 25±2oC with 16/8 dark/light period. The callus initiation was observed after 15 days of inoculation. After 25 days of inoculation, callus is subjected to embryo induction media supplemented with MS+ 50 mg/L proline+ 2mg/L 2, 4 D and MS + 25 mg/L proline + 2mg/L 2, 4 D. It allowed developing on the above medium for 3 weeks for embryo induction. The induced embryos are subjected to microscopic analysis under research microscope.

III. RESULTS AND DISCUSSION

Mung bean is considered as an important pulse crop and it is mostly cultivated all over India (Shamsudeen Varisai Mohamed et al. 2005). But Somatic embryogenesis is uncommon in leguminous crops, especially in Vigna radiata. The limited work has been done on in vitro regeneration from a callus in Vigna radiata. So the current study targeted the development callus and induction of somatic embryogenesis from hypocotyl region of Vigna radiata. Hypocotyl region was used as explants. It shows maximum callus induction. This observation is in agreement with the findings of S. Girija et al. (2000). Callus induction was observed after 15 days on Medium composed MS+ 5 mg/L NAA+ 2mg/L 2, 4 D. After 5 weeks embryo was obtained on liquid medium composed MS+ 50mg/L proline+ 2mg/L 2, 4 D and MS+ 25 mg/L proline+ 2mg/L 2, 4 D. and those embryos observed under research microscope.

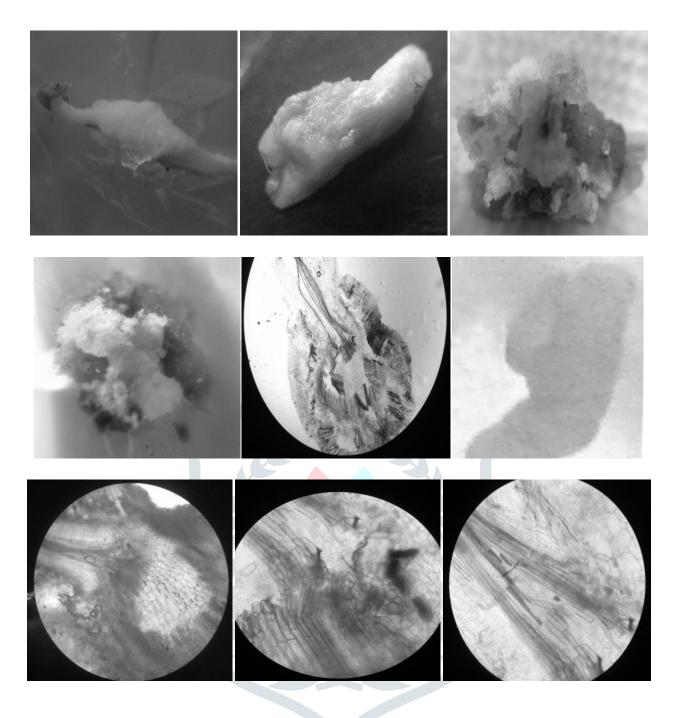


Fig: - Somatic embryogenesis in Mung beans — Images showing callus development from hypocotyl region and microscopic analysis of embryos shows various stages of embryo development

IV. ACKNOWLEDGMENT

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