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In Vitro Culturing of *Capsicum annuum* and *Euphorbia hirta*

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Abstract

The present study deals with the *in vitro* culturing was mostly used *Capsicum annuum* and medicinal plant *Euphorbia hirta*. In this study growth factors was used with the varying combinations along with the basal MS Media. Such combination gives us an idea about the requirement of that plant and hence by use of such combinations these plants can be easily grow in *in vitro* conditions.

Keywords: Explants; Chilli; Propagation; Phytochemicals

Abbreviations: BAP: 6-Benzyle Amino Purine; NAA: a-Naphthaleneacetic Acid; IBA: Indole-Butyric Acid; IAA: Indole-3-Acetic Acid; TDZ: Thidiazuron; MS: Murashige and Skoog's (1962) medium; FEC: Friable Embryogenic Callus; GDT: Gresshoff and Doy Tyrosine Medium.

Introduction

Capsicum annuum is an economically important plant and two main used types are pepper spice and vegetable are prevalent throughout the world [1] capsicum or pepper is a genus of flowering plants in the nightshade family, solanaceae. Its species are native to the Americas, where they have been cultivated for thousands of year by the people of the tropical Americans and now cultivated worldwide [2-8]. Chilli pepper is of great importance in Native American medicine and capsaicin is used in modern family mainly in tropical medication, as a circulatory stimulant and analgesic [9]. Plant regeneration via organogenesis and somatic embryogenesis from diverse explants using different concentration and combinations of auxins and cytokinins has been described in paper [10-15]. The plant *Euphorbia hirta* or Duddhi belongs to family *Euphorbiaceae*. *Euphorbia hirta* is addressed as the Asthma Plant, pill-bearing spurge, snake weed and just euphorbia. In Siddha and Tamil it is called as Amman, where as in Ayurveda it is called as Dudhi, Dugdhikaa, aagaarjuni and Vikshirini. In Unani it is known as Khurd [16]. A number of chemical compounds have been reported to be contained in the extract of its stems, roots and leaves. Some of these chemical compounds are Gallic acid, Phytosterin, Jambulol, Palmitic acid, Linoleic acid and a number of phytochemicals like fatty acids, flavones, essential oil, phenols and sterols etc. [17-18]. In spite of above mentioned reports reproducible methods for routine propagation are not available and existing methods are not satisfactory. [19-20].

Material and Methods

Plant material

Capsicum annuum and Euphorbia hirta: The seed of the *Capsicum annuum* was purchased from the local market of the Landran, Mohali. And the fresh plant of *Euphorbia hirta* was collected from local region of Chandigarh and Roopnagar.

Sterilization of material: Seeds of chilli pepper were first washed with distilled water and then sterilized with warm 5% KNO_3 solution overnight and followed with 0.01% $HgCl_2$ for 3 min and 70% alcohol for 1 min washing with sterile aqua dust were conducted 3 times. Sterilization of explant of *Euphorbia hirta* was done with the standard method with minor modifications [21]. The surface sterilized seeds were inoculated in petri dish containing sterile filter paper soaked in sterile distilled water and incubated in dark for 10-12 days at 25 ± 2°C. after germination the seeds were transferred to the flasks containing MS media (Murashige and Skoog, 1962) basal medium [22-26]. Shoot tip leaf explants were derived from four-five week old *in vitro* germinated seedling shoot apices were trimmed from four week old seedlings and inoculated on a shoot bud induction medium consisting of MS medium supplemented with different concentration of cytokine [27-28]. In case of *Euphorbia hirta* the explants were firstly cut with the sterilized scalpel, and then these were washed 2-3 times with the distilled water. Then washing was done with the help of the fungicide the mainly used fungicide was bengard. After this tween-20 was used and then autoclaved water. After all these 70% alcohol was used followed by again with distilled water [29,30].

Growth factors: The growth factors were in lyophilized form. So before uses these are first dissolve in 1N NaOH and final volume was made up to 200 ml.

Results

Capsicum annuum

The growth of the capsicum from seeds to complete plant with proper roots and shoots indicates that the growth method was perfect with proper concentration of the growth hormones (Figure 1).

Euphorbia hirta

The explants of the *Euphorbia hirta* gives best growth in the flasks containing IBA 2 ml and BAP 1 ml. the other combination of the growth hormones were also used but satisfactory results were not obtained (Figure 2). The other combinations used were as follow:

Combination 1 IBP – 2 ml
Zeatin – 1 ml
Combination 2 IBA – 2 ml
TDZ – 1 ml
Combination 3 IBA – 1 ml
BAP – 1 ml
Combination 4 2,4-D – 2 ml

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Figure 1:
a. Initialization.
b. Callus formation from seed.
c. Shooting and rooting initialization from seed callus.
d. Shooting and leaflets formation.
e. Separation of each growing explant into new medium.

Figure 2:
a. Initialization of the shoot and root buds from the explants.
b. Shooting.
c. Separate growth the growing explant.

BAP – 1 ml

These combinations were used with normal MS media.

Conclusion

The *Capsicum annuum* shows the good growth from normal seeds to proper plant by the use of the firstly filter paper and then this growing seeds in the normal MS media under the suitable temp control of 25. In other case the explants of *Euphorbia hirta* gives the best result in the flasks containing IBA 2 ml and BAP 1 ml. The other combinations not give the satisfactory results. Further trials needed to clarify the certain issues regarding best growth media and contamination prevention issues.

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