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Callus induction from the explant of Atidesma menasu – a folklore medicinal plant

Dhanya P[a], Rama Bhat P[b]

[a]Flat no-004, Akshara Residency, Mallika extension, Kadri Kambla, Mangaluru, Dakshina Kannada, 575005 - India
[b]Post graduate department of Biotechnoogy, Alva's College, Moodbidri, Dakshina Kannada District -574227, India

Abstract

Antidesma menasu is a folklore medicinal plant belongs to the family Euphorbiaceae. This particular study reveals an in vitro culture of the research plant by using Murashige and Skoog(MS) medium with different growth regulators at its different concentration levels. Explant selected for this particular study was leaf. The proliferation of the callus was seen in different concentrations of 2,4-D and 2,4-D in combination with Kinetin.

Keywords: Antidesma menasu; Murashige and Skoog;

1. Introduction

All living cells of a plant are capable of differentiating into whole plant. This inherent property of the cells is called 'cellular totipotency' has led to the concept of tissue culture studies. Plant tissue culture was originally developed as a research tool in order to study the biochemistry and physiology of plants. Plant tissue culture has advanced the knowledge of fundamental botany, especially in the field of agriculture, horticulture, plant breeding, forestry, somatic cell hybridization, phytopathology and industrial production of

plant secondary metabolites etc. This technique has turned into a standard procedure for modern biotechnology and has become one of the cornerstones of present day agriculture [1].

Tissue culture techniques are becoming increasingly popular as alternative means of plant vegetative propagation. The significant advantage offered by the aseptic methods of clonal propagation of the conventional methods is that in a relatively short span of time and space a large number of plants can be produced starting from a single individual [2]. It is useful for multiplying and conserving the species, which are difficult to regenerate, by conservation methods and save them from extinction. Improved cell and tissue culture technologies would help in producing the active compounds in vitro with better productivities without cutting down the natural resources. It can be employed in conservation of the flora in relatively shorter time. It is useful for multiplying and conserving the species, which are difficult to regenerate, by conservation methods and save them from extinction. Improved cell and tissue culture techniques would help in producing the active compounds in vitro with better productivities without destruction of the natural resources.

India has great wealth of traditional knowledge and wisdom, and the value of medicinal plants related trade in India is estimated at 50 crores per annum. As the demand for the plant derived pharmaceutical compounds is increasing, possibilities for mass production need to be explored. Plant tissue culture techniques offer 22 rare opportunity to tailor the chemical profile of a phytochemical product by manipulation of the Availability of the plant is subjected to seasonal variation, leading to uncertainity in stable supply throughout the year. Plant production under controlled conditions of in vitro system can eliminate these problems. Therefore establishing a suitable micropropagation protocol for the high yielding lines will have the potential of providing a better source for continuous supply of plants in the field of drug research as well as manufacturing of drugs[3].

Euphorbiaceae comprises nearly 322 genera and 8910 species, many of which have their own economic value. The members of Euphorbiaceae are valuable source of different kinds of useful products like dyes, edible tubers, oil crops, furniture, agricultural implements, ornamental plants, pharmacological products, rubber, timber and aesthetic items [4]. Jatropha curcas L. a multipurpose drought resistant, perennial plant belonging to Euphorbiaceae family is gaining lot of importance for the production of biodiesel [5]

Phyllanthus fraternus Webster is an important medicinal plant of family Euphorbiaceae. This particular plant is having high demand in domestic and international market for making herbal formations. Pharmaceutical industries need huge volume of raw materials of this valuable species. In vitro micropropagation techniques offer a viable tool for rapid mass multiplication and germplasm conservation of important medicinal plants for meeting the pharmaceutical needs [6].

Antidesma menasu is found commonly throughout South Canara district of Karnataka in India during rainy season. It is a folk remedy for the management of low backache, arthritis, muscle pain, neuralgias by folklore practitioners of Udupi. These symptoms are mainly associated with inflammation and there is a rising scope for traditional medicines [9]. The crude aqueous and ethanolic leaf extracts of Antidesma menasu were tested against four bacterial strains and two fungal strains. Among this, the ethanol extract has got significant antibacterial activity on Staphylococcus aureus [10].

2. Materials and methods

A systemic fungicide, M-45(2g/liter) was sprayed two days prior to the inoculation of explants to avoid systemic infection. After two days of spray, leaves were collected, washed with running tap water for one hour. Leaves were kept in 1% Bavastin for 45 minutes and rinsed 5 times with distilled water. Leaves were treated with 1:5 Sodium hypochlorite for 5 minutes and rinsed with sterile distilled water, inside the Laminar Air Flow cabinet. Again leaves were treated with 0.1% Mercuric chloride and rinsed 3 times with sterile distilled water, dissected and inoculated into the desired medium.

3. Culture medium

The culture medium utilized for this study was Murashige and Skoog medium. Stock solutions were prepared for micronutrients, macronutrients, iron source, vitamins separately [7]. All the stock solutions were sterilized and maintained in dark colored bottles inside the refrigerator. Even the stock solutions for different growth regulators were prepared and stored inside the refrigerator. MS medium was prepared by mixing proper quantities of each stock solution and growth regulators. pH of the medium was adjusted to 5.6 to 5.8 by using 0.1N HCL and 1N NaOH. At the end Agar is added melted using microwave oven, poured into bottles and autoclaved.

4. **Results and Discussion**:

Regeneration of callus from leaf explants were successful in MS medium supplemented with different concentrations of 2,4-D. 2,4-D at its lowest concentration that is at 0.5mg/L did not induce any callus. At 1mg/L, the explants took 16 days for callus induction. At 2mg/L it took 20 days for induction of callus and 16 days at the concentration of 3mg/L. At 4mg/L the explants took least number of days for callus regeneration that i2s 11 days. Explants took 17 days for callus induction in MS medium supplemented with 2,4-D in combination with Kinetin (1mg/L and 1mg/L). Leaf explants were responded well for MS medium supplemented with different concentrations of BAP that is 2mg/L and 4mg/L and IAA (0.5mg/L) in combination with Adenine sulphate-40mg/L. The percentage contamination was found to be 17.857%.

As the seeds of the research plant exhibits seed dormancy even under normal environmental conditions, the above method could be applicable in producing large number of plants from the leaf explant in a short period of time. Since the research plant has got anti-inflammatory property, secondary metabolites could be isolated from the callus and further studied.

Table I. Explant of Antidsma menasu showing callus induction in MS medium supplemented with different growth regulators.

Explant	Media utilized	Concentration(mg/L)	Number of days took by the explants for callus initiation
Leaf	2,4-D	0.5	-
		1	16
		2	20
		3	16
		4	11
Leaf	2,4-D+Kinetin	1+1	17
Leaf	BAP+IAA+Adenine sulphate	2+0.5+40	28
		4+0.5+40	32

Fig.1. Callus growth in 2,4-D at 2mg/L

Fig.2. Callus growth in 2,4-D at 3mg/L

Fig.3. Callus growth in 2,4-D at 4 mg/L

Fig.4. Callus growth in 2,4-D+Kinetin at 1mg/L

Fig.5.BAP+IAA+Adenine sulphate(2mg/L+0.5mg/L+40mg/L)

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