Callus induction from the explant of *Antidesma menasu* Miq. – a folklore medicinal plant

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Abstract

Antidesma menasu is a folklore medicinal plant belongs to the family Euphorbiaceae. This particular study reveals an *in vitro* culture using Murashige and Skoog(MS) medium with auxins and cytokinins as plant growth regulators at different concentrations in individual and combinations. Leaf explants were used for the callus culture. The proliferation of the callus was seen in cultures with different concentrations of 2, 4-D and 2,4-D in combination with kinetin.

Key words*: Antidesma menasu, Murashige and Skoog medium, callus*

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 conventional 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 opportunities 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 the family 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 this 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 [6]. 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 [7,8].

Antidesma menasu is found commonly throughout Dakshina Kannada district of Karnataka 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]. In one of the recent study, the aqueous and ethanolic leaf extracts of *Antidesma menasu* were tested against four bacterial strains and two fungal strains, of which, the ethanolic extract has showed significant antibacterial activity against *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, the 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. Then treated with 1:5 Sodium hypochlorite solutions for 5 minutes and rinsed with sterile distilled water, inside the laminar air flow cabinet. Again the leaves were treated with 0.1% mercuric chloride and rinsed 3 times with sterile distilled water, trimmed and inoculated onto the medium. The inoculated cultures were incubated at 20+2°C with 80% RH, 2000 Lux light intensity and 16 hour light photo period.

Culture medium

The culture medium utilized for this study was Murashige and Skoog medium (1962). Stock solutions were prepared for micronutrients, macronutrients, iron source, vitamins separately. 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.

3. Results and Discussion

Induction of callus from leaf explants of *Antidesma menasu* with different concentrations of auxins and in combinations with cytokinin showed different results in terms of induction as well as their mass, appearance and type (Table 1). The number of days of incubation varied from 16 to 32 days. 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 is 11 days. Explants took 17 days for callus induction in MS medium supplemented with 2, 4-D in combination with kinetin at equal concentrations (1mg/L each). 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 (Fig. 1-5). Jayaraman et al [11] used leaf explants of *Aquilaria malaccensis* and were incubated in MS basal medium using auxins and cytokinins for 30 days in the dark. Callus induced at 1.1 µM NAA which had the highest biomass dry weight (17.3 mg); however, the callus was of a compact type. The auxin was then combined with either 6-benzylaminopurine (BAP) or kinetin at 0.55, 1.1, 2.2 or 3.3 µM to induce growth of friable callus. The 1.1µM NAA + 2.2µM BAP combination produced friable callus with the highest biomass (93.3mg). They also found that sucrose at 15g/L and pH at 5.7 yielded highest biomasses. The calluses in the present study are also of friable type and color varied from cream to green. The percentage contamination was found to be 17.857%. There are some earlier reports that supports the present experimental results [4,12,13].

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 explants 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 1: Callus induction in leaf explants of *Antidsma menasu* in MS medium supplemented with different plant 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

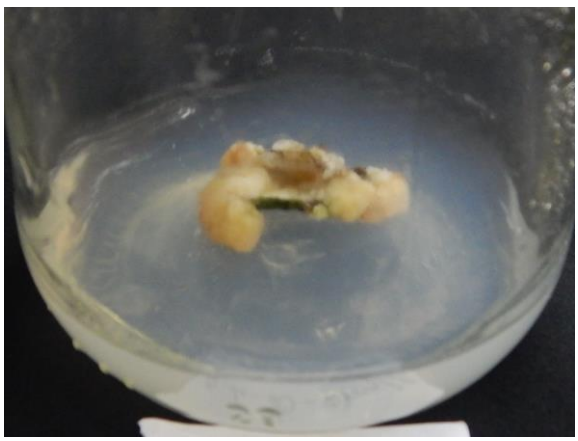

Fig. 1: Callus growth in 2,4-D at 2mg/L

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

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

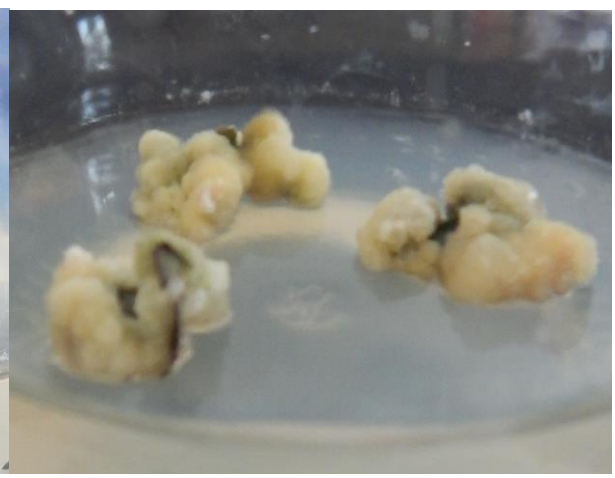

Fig. 4: Callus growth in 2,4-D+Kinetin at 1mg/L

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

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