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In vitro callus induction of Melothria purpusilla, a traditional medicinal plant in Manipur

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ABSTRACT
Melothria purpusilla, a member of Cucurbitaceae, is an endemic species found in North-Eastern part of India. The plant is used traditionally by the people of Manipur in the treatment of jaundice and its roots in fever and diarrhoea. Tissue culture of medicinal plants was performed as a measure for the conservation of endangered medicinal plants, *Melothria perpusilla*. Morphogenetic changes were observed in *Melothria perpusilla* explants in the MS medium supplemented with different concentrations of PGRs. Different colours of callus formation were observed in MS supplemented with BAP, kinetin and IBA. The best callus induction was observed with MS media supplemented with combination of 1BAP mg/l + 1 IBA mg/l and combination of 1Kinetin mg/l + 1 IBA mg/l.

Key words: *Melothria perpusilla,* callus formation, PGRs, BAP, kinetin and IBA.

I. INTRODUCTION

Medicinal plants are considered as the important source of medicine in the treatment of ailment by the majority of world's population. According to World Health Organisation, most populations still rely on traditional medicine for their physiological and physical health requirement [1]. These are not only used for primary health care not just in rural areas in developing countries, but also in developed countries as well where modern medicines are predominantly used. While traditional medicines are derived from medicinal plants, mineral and organic matter, the herbal drugs are prepared from the medicinal plants only. It is estimated that about 80,000 species of plants [2] are utilized in some form or other by different systems of Indian medicine and these plants have been studied on the basis of clearly defined biological parameters like taste, metabolic property, quality, biological effects and potency.

Medicinal plants are very important due to the presence of secondary metabolites like alkaloids, glycosides, coumarins, flavanoids, steroids, etc. and find use in a number of pharmaceutical compounds. However, sustained supply of the source material often become difficult due to the factors like environmental changes, cultural practices, diverse geographical distribution, labour cost, selection of the superior plant stock and over exploitation by pharmaceutical industry. Various technologies have been adopted for enhancing bioactive molecules [3], conservation of the natural resources and the capability to utilize them in sustained manner. Understanding of the biological and ecological background of the species in their normal habitat is also essential to understand their conservation biologically as well as to predict their behaviour under artificial cultivation. Biotechnological tools are important for the multiplication and the genetic enhancement of the medicinal plants by adopting techniques such as *in vitro* regeneration and genetic transformation. The principle advantages of this technology are the high volume production of pharmaceuticals, neutraceuticals and other beneficial substances.

Present study is the culture of plant tissues or organs for the conservation of threatened species of plants under sterile condition which lead to the cell multiplication or regeneration of organs or whole plants as it has many advantages over conventional methods of vegetative propagation to overcome several limitations. Micropropagation greatly increased the rate of multiplication and also permits the production of pathogen free material. Micropropagation of various plant species, including medicinal plants has been reported [1,3,4] . Plant regeneration from shoot and meristem has yielded encouraging results in medicinal plants like *Catharanthus roseus, Cinchona ledgeriana, Digitalis* sp, *Rehmannia glutinosa, Rauvolfia serpentina, Iroplexis canariensis* [5,6,7] , *Atropa belladonna [*8], *Picrorhiza kurroa* [9], *Nothapodytes foetida* [10], *Zingiber spectabile* [11], *Cherodendrum colebrookianum* [12], *Clerodendron serratum* [13,14,15], *Phlogacanthus thyrsiflorus* [16] , *Celosia argentea*[17]. No work has been reported on *In vitro* regeneration of *Melothria purpusilla*.

The present study is the tissue culture of medicinal plant *Melothria purpusilla. Melothria purpusilla,* a member of Cucurbitaceae, is an endemic species found in North-Eastern part of India.

The plant is used traditionally by the people of Manipur in the treatment of jaundice and its roots are used in fever and diarrhoea.

II. MATERIALS AND METHOD

Young shoots were collected from healthy *Melothria purpusilla* plants grown at the Botanical Garden, Department of Botany, Modern college Porompat, Imphal, Manipur. The shoots were cleaned thoroughly by repeated washing with running tap water and it was followed by few drops of labolene. Surface disinfestations were done with mercuric chloride (0.1%$HgCl_2$) for 5 min and 70% ethanol for 30 sec. It was followed by 2-4 rinses with sterile distilled water under laminar air flow.

The shoot tips and the nodal parts were excised measuring 5-8mm sized shoots from the shoot tips. The excised shoots were used as explants for the present study. The outer sheaths of the shoots were removed and all the sides were wounded with a surgical sterile blade. The explants were inoculated on various media.

Callus induction

Murashige and Skoog medium (MS medium) was used with 3% (w/v) sucrose, 0.8% (w/v) agar and supplemented with various plant growth regulators (PGRs). MS basal medium were supplemented with Indole-3-butyric acid (IBA), 6-Benzylaminopurine (BAP) and kinetin singly or in combination and were tested. The pH of the medium was adjusted to 5.8 with 1N NaOH or 0.5N HCl. The MS media were autoclaved at 121^{o}C and 15 lbs psi pressure for 20 min. The MS media were supplemented with various hormone concentrations and dispensed into separate tubes which was sealed with non absorbent cotton wool plugs.

The explants were cultured on MS media supplemented with BAP and Kinetin (0.5 – 1.5mg/l) alone, combination of BAP and Kinetin and in combination with IBA (1mg/l) .The cultures were incubated at 25±2^{o}C under 16h photoperiod provided for callus induction. The effect on callus induction was studied with different concentrations of Plant growth regulators (PGRs).

III. RESULTS AND DISCUSSION

The data on the effect of MS basal media supplemented with different concentrations of PGRs on *Melothria purpusilla* shoot tip explants are given in table 1. During the culture periods,in the controls (MS media without PGR),neither shoot nor root formation were observed throughout the investigation. After 1-3 weeks, MS media supplemented with PGRs responded with varied colour of friable calli formation by the explants based on the hormone and the concentration. The comparative study of different hormones with different concentrations for the induction of callus was also shown by graphical representation given in Fig.2.

Among the different treatments of growth regulators, combinations of 1BAP mg/l + 1 IBA mg/l and 1 Kinetin mg/l + 1 IBA mg/l was shown the better response than any other treatments. The MS media supplemented with 1BAPmg/l + 1 IBA mg/l and 1Kinetin mg/l + 1 IBA mg/l was shown proliferation and turned into a small friable callus in a short period of time whereas MS media supplemented with kinetin, BAP singly and in combination of 0.5 mg/l kinetin and 0.5 mg/l BAP shows slower response compared to combination of 1BAP mg/l + 1 IBA mg/l and combination of 1Kinetin mg/l + 1 IBA mg/l. The friable microcallus initiated from the cut surface of the explants. The best callus induction was observed with MS media supplemented with combination of 1BAP mg/l + 1 IBA mg/l and combination of 1Kinetin mg/l + 1 IBA mg/l. It was observed that the callus remain healthy in different concentrations of BAP and Kinetin in combination with IBA. The study of graph(Fig.2) shows the effect of different concentrations of hormone on induction of callus.

Table1. Morphogenetic changes showed by the *Melothria purpusilla* plants in the MS supplemented with different concentrations of different Plant Growth Regulators (PGR)

Plant Growth Regulators (PGRs)	Concentration(mg/L)	Rate of callus induction (%) within 1-3 weeks	Colour of callus
Kinetin	0.5	66.66%	Light green
	1	50%	Green
	1.5	0%	No induction of callus
BAP	0.5	66.66%	Light green
	1	41.66%	Green
	1.5	33.33%	Light green
BAP + Kinetin	0.5 +0.5	70.58%	Whitish callus with green spots
BAP + IBA	1 + 1	87.5%	Green callus
Kinetin + IBA	1 + 1	87.5%	Green callus

Fig 1. Callus induction from explants of *Melothria purpusilla* cultured on MS media supplemented with different concentrations of different plant growth regulators: (A) light green callus, (B) whitish callus with green spots and C) Green callus

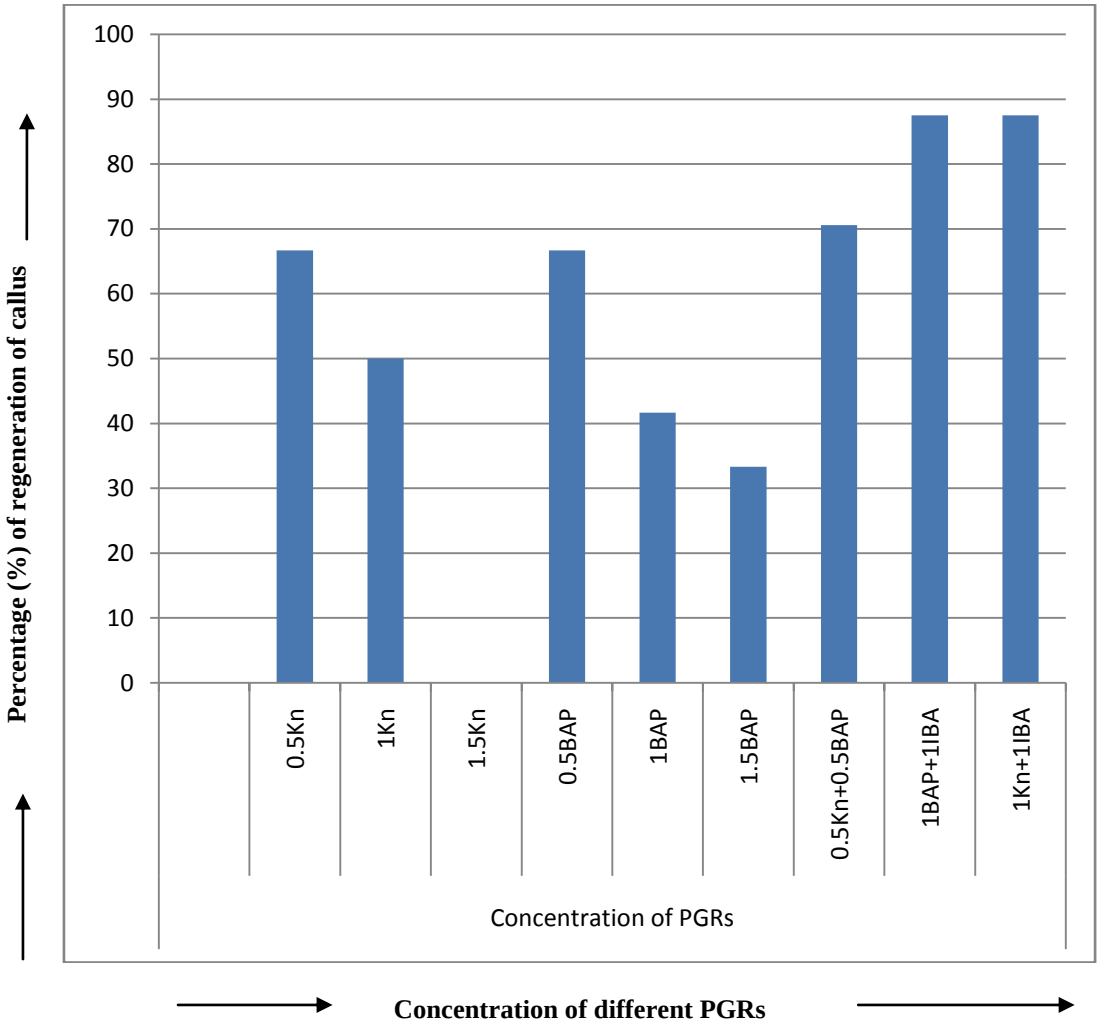

Fig2. Effects of different Plant Growth Regulators (PGRs) on callus induction in *Melothria Purpusilla*

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