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IN VITRO STUDIES IN KYDIA CALICINA ROXB

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ABSTRACT

Liver plays a very important role in the metabolism of foreign compounds entering the body. The exposure to the foreign compounds may be through consumption of alien/contaminated foods from exposure to chemical substances in the occupation environment or through synthetic drugs consumed for various pathological conditions; these compounds have many toxic effects on the human liver. The liver gets injured also by viruses, chemicals, alcohol and autoimmune diseases. Liver diseases remained one of the serious health problems, so medicinal plants and herbs have been used for treating such problems as in the Indian traditional systems of medicine, especially Ayurveda. Recently, a scientific basis was proved to justify the various medicinal uses of herbs like *kydia calicina*.

Kydia calicina is an important medicinal plant used in treatment of skin diseases, curing arthritis and lumbago and relieving body pains. In the present investigation, efforts have been made to develop a suitable experimental protocol for regeneration and micropropagatrion of this important plant *in vitro*. Explants from mature plant of this species were collected and cultured on MS medium supplemented with various concentrations (0.5, 1.0 2.0 and 3.0 mg l-1) of cytokinins (BAP and Kn) and auxins (IAA, NAA and 2, 4-D) alone and in various combinations under controlled conditions. Successful regeneration ~~method~~ was achieved by this method.

Key Words: *Kydia calicina, regenerate, in vitro.*

INTRODUCTION

Kydia calycina Roxb (Malvaceae) synonyms *Kydia fraterna* Roxb, *Kydia roxburghiana* Wight are distributed in tropical Himalayas from the Indus eastwards to Myanmar (Burma) and in peninsular India from Northern Maharastra and Madhya Pradesh southwards, chiefly in mixed, moist and deciduous forests. The leaves of *K. calycina* were 7.5 to 15 cm long and wide, usually 3 to 7 lobed, apex angled or rounded, base cordate, palmately 7-nerved and hoary-tomentose beneath; petioles 2.5 to 5 cm (Parrot, 2001). Among the Santalis, A paste of the grounded leaves of *K. calycina* is applied to relieve body pains, arthritis and lumbago and a poultice of the leaves is traditionally used to treat skin diseases (Parrot, 2001; Ramarao and Henry, 1996). In the present investigation, we have evaluated the hepatoprotective effect of methanolic extract of the leaves of *K. calycina* on carbon tetrachloride induced hepatotoxicity in albino rats.

MATERIAL AND METHODS

Preparation of Explants:

Explants of *Kydia calicina* were collected from Botanical garden, Department of Botany Dr. Babasaheb Ambedkar Marathwada University, Aurangabad. Apical shoot, Axillary bud, node and Meristimatic tissue of *Kydia* were collected from two months old plants. All these explants were used from donor plants during present study. The explants were washed carefully in running tap water for 10 minutes and followed by distilled water for 5 minutes. Explants were surface sterilized for 5 minutes with 0.3% mercuric chloride followed by three subsequent rinses with sterilized double distilled water in a laminar flow. All these explants were dissected into small pieces and treaded so that maximum part can be exposed to media.

Culture Media

MS medium (Murashige and Skoog, 1962), variously supplemented with BA, KIN was used for multiple shooting from apical shoots, Axillary buds and nodal explants of *Kydia calicina*. For rooting, half strength MS medium, Supplemented with various concentrations of auxins IAA, IBA, and NAA, were examined.

Culture Conditions

MS medium containing 3% sucrose was gelled with 3 gm/L solidified agent Clerigel, and the pH was adjusted to 5.8, after addition of the growth regulators. The media were steam sterilized in an autoclave under 15 psi and 121° C. after inoculation, culture tubes and culture vessels were transferred to culture room under a 16 h photoperiod supplied by cool white fluorescent cool tube lights and 25 ± 2 ^{0}C temperature. At least ten cultures were raised for each treatment. Data was measured after 25days for five replicates for shoot multiplication and shoot length Mean (µ) values with the standard error (S.E.).

RESULTS AND DISCUSSION

Apical shoot, Axillary bud and nodal explants of *Kydia*, grown on hormone free MS medium showed no effect on multiple shoots formation. MS media with different concentrations of BAP 1.0, 1.2, 1.4, 1.6, 1.8, 2.0 mg/l and combinations of IBA, NAA gave maximum average percentage of multiplication.

Effect of BAP on shoots multiplication

Table 1:- Effect of BAP and IAA for multiplication of different explants

Explant	Conc. of growth regulator (mg/L)		Shoot length (Mean± SE)	% of shoot formation
	BAP	IAA		
apical shoot tip	1.0	0.2	1.88± 0.073	30
	1.2	0.2	2.64± 0.129	32
	1.4	0.2	2.52± 0.122	37
	1.6	0.2	2.90± 0.149	51
	1.8	0.2	2.16± 0.160	49
	2.0	0.2	2.24± 0.172	47
axillary bud	1.0	0.2	1.70± 0.130	35
	1.2	0.2	2.04± 0.143	37
	1.4	0.2	2.14± 0.140	44
	1.6	0.2	2.62± 0.139	52
	1.8	0.2	1.76± 0.214	50
	2.0	0.2	1.82± 0.149	49

*After 25 days, mean ± SE of 5 replicates

The present investigation of an apical shoot tip, Axillary bud and nodal explant was essential for the development and formation of multiple shoots in *Kydia*. Out of the two Cytokinins tested, BAP was found to be more effective than KIN for shoot multiplication. MS media containing 3% sucrose, 3 mg/L Clerigel and different concentration of BAP 1.0, 1.2, 1.4, 1.6, 1.8, 2.0 mg/l, alone with IAA 0.2, 0.4, 0.6, 0.8, 1.0 mg/L, concentration of BAP with combination NAA 0.2, 0.4, 0.6, 0.8, 1.0 mg/L produced average percentage of multiple shooting of *Kydia*. (Fig.1) Maximum average percentage of multiple shoot was recorded BAP 1.6 mg/L, with combination IAA 0.2 mg/L (Fig.2).

Fig.1 Multiple shoots formation along with callus

Fig.2 Multiple shoot formation

Apical shoot tip and Axillary bud explants were inoculated on MS medium supplement with 3% sucrose, 2.5% Clerigel and various combinations of growth hormones as shown in table 1 Maximum average shoot length and multiple shoot formation percentage of *Kydia* was recorded in 1.6 mg/L BAP combination with 0.2 mg/L IBA. Repeated sub-culturing was said to be one of the methods of maintaining juvenility (Johnson, 1999). In the present work highest number of shoot percentage was recorded in third sub culturing. Somatic embryos were developed into plantlets and subsequently grown to maturity. Similar experimental results, indicating that nodal explants have high competence for somatic embryogenesis, were also reported earlier by Devendra Srinivas and Sandeep Reddy (2011) in *Eclipta alba*. Various combinations of IAA were added to the MS medium to achieve rooting. *In vitro* rhizogenesis was achieved by adding 0.5 mg/lit IAA. Plants were hardened and introduced in soil for *in vivo* trails. *In vitro* regenerated plants had shown 65 % viability *in vivo*.

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