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RESEARCH ARTICLE

INDUCTION OF CALLUS IN YELLOW VARIETY OF LANTANA CAMARA L

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ARTICLE INFO ABSTRACT Article History: Leaf and Stem, excised from young and healthy part of yellow colored variety of Lantana camara

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In vitro culture, 2, 4-D, KN, callus, friable, *Yellow variety of Lantana camara, multicolored variety*

Leaf and Stem, excised from young and healthy part of yellow colored variety of *Lantana camara* were taken as explants and cultured on Murashige and Skoog medium containing sugar (3.0%) and Agar (0.8%) and maintain pH at 5.8. Callus induced at combination of concentrations of 2, 4-D and KN. Leaf parts showed more response than stem. Leaf and stem callus was friable, watery and posses light brown color. This variety showed slight variation in characteristics of callus developed with that of multicolored variety.

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INTRODUCTION

Lantana camara L. (family Verbenaceae) native to tropical and sub-tropical areas of America is an important medicinal plant. It is terrestrial noxious weed (Sharma, 1988) and popular ornament garden plant (Ghisalberti, 2000). Plant tissue cultures have been investigated for industrial production of many useful secondary metabolites (Chowdhury *et al.*, 2007). Secondary metabolites are economically important as drugs, flavor and fragrances, dye, pigments, pesticides, and food additives (Hussain *et al.*, 2012). In folk medicine it is used for treatment of cancers, chicken pox, measles, asthma, ulcers, swellings, eczema, tumors, high blood pressure, bilious fevers, catarrhal infections, tetanus, rheumatism and malaria (Begum *et al.* 1995, Day *et al.*, 2003). The current study reveals that Callus of *Lantana camara* developed successfully in the presence of 2, 4-D and kinetin.

MATERIAL AND METHODS

Leaf and stem from the healthy plant *Lantana camara* from university of Rajasthan, Jaipur, taken as explants for experiments. This plant can be seen in wild, along footpath, farms, deserted field, forests plantation, grasslands, along with riverbank (Kohli *et al.*, 2008) and invasion along streams in a heterogenous landscape (Ramaswami, 2014). Axillary shoots and axillary leaves were used as explants.



Yellow flower variety of Lantana camara L.

Development of callus of Lantana

Explants of yellow colored Lantana (leaf and stem segments) were taken for *in vitro* studies. Leaves and nodal segment washed under the running tap water for 10 min to wash out all dust and surface was sterilized with 0.1% (w/v) mercuric

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chloride solution for10 minutes and thoroughly washed 3 times with sterile Distilled water. The explants (4 in each flask) were transferred on MS (Murashige and Skoog, 1962) medium containing 0.8% (w/v) agar, 3% (w/v) sucrose supplemented with different concentrations of 2,4-D, and a mixture of NAA and BAP as described in Table 1. The pH was adjusted to 5.8 before sterilization. Inoculation process was done on laminar air flow cabinet to protect culture from contamination. The medium was autoclaved at 20 lbs pressure for 20 min before inoculation.

Twenty replicates were prepared for each concentration. The culture was maintained at a temperature $25\pm2^{\circ}$ C under continuous fluorescent light. Callus cultures thus obtained were collected and kept in a hot air oven at 80°C, dried, weighed and powdered in pestle and mortar. Callus was sub cultured after 25 days on the original callus inducing medium. Callus induction frequency (Cip) was calculated using the following formula:

(Cip) = Number of explants forming callus/Total number of used explants X 100.

RESULTS

Callus Initiation

Best callus was initiated in combination of hormone i.e. 2, 4- D +KN at concentration 0.3 mg/l+.2 mg/l in comparison to individual hormones (Table 1 and 2). Callus was initiated within 15 days in auxins IAA and 2, 4- D at 0.2-0.3 mg/l but growth was slow (Table 1) whereas no callus was developed at lower concentration and only shoots developed at 0.2mg/l concentration of KN (Table 2). The best growth was obtained at 2, 4- D +KN of concentration 0.3+0.2 mg/l in combination. Callus showed high regeneration potential and fast growing (Table 3).

Table 1 Effect of different concentrations of auxins and 2	2-
4D on callus induction	

Hormones (mg/l)	Response	Remarks				
	Auxins-IAA					
Control	-	No callusing				
.05	_	no response				
0.1	C+	little response 2-4 flask				
0.2	C++	yellowish green, becomes pale				
0.3	C+++	yellow, friable, slow growing, poor response				
0.5	C –	no response response				
2, 4-D						
.05	-	no response				
0.1	_	no response				
0.2	C++	pale greenish, pale yellow colour, friable callus				
0.3	C+++	slow response				
0.5	C+	little response flask				

Medium: MS+sucrose (3.0%) +Auxins viz. 2, 4-D, IAA

Explant: Mature Nodal Explant and leaf

Incubation: At 30±2°C in 16 hours photoperiod 2500-3000 lux up to four weeks.

Effect of 2, 4-D + KN on callus induction frequency

Callus induction frequency of 2, 4-D + KN at concentration 0.2+0.3 mg/l for as shown Table 4. The highest callus induction frequency is found 90% in stem explants and 75%

induction in leave explants. Callus induction better in stem explants with better response and energetically growing cells.

Table 2 Effect of different C	Cytokinin on callu	s induction.
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Cytokinin (mg/l)	Response	Remarks
• • • •	Kinetin	1
Control		no callusing
0.1		only shoots
0.1	_	developed
0.2		only shoots
0.2	-	developed
0.3	-	swelling in explants
0.5	_	

Control without hormones, (-) No response, IAA- Indole-3 acetic acid. C-Callus +: Slight regenerative, ++: Moderate, +++: High ++++

 Table 3 Effect of 2, 4-D in combination with cytokinins on callus induction in L. camara.

2,4-D mg/l + KN mg/l	Response	Remark
0.0 0.0	C–	
0.1 .05 0.25	C –	
$\begin{array}{c} 0.2 & .1 \\ 0 & .5 \end{array}$	C +	very less response
0.3 .1 0.1	C++	light yellow colour
0.3 .15 0.15	C++++	Friable, fast growing
$\begin{array}{ccc} 0.3 & 0.2 \\ 0.4 & 0.2 & 0.3 \end{array}$	C++++ C +	High regenerative potential. very less response

Medium: MS+sucrose (3.0%) +Auxins viz. 2, 4-D, IAA Explant: Mature Nodal Explant and leaf

Incubation: At $30\pm 2^{\circ}$ C in 16 hours photoperiod 2500-3000 lux up to four weeks.

Table 4 Characteristics of Callus

		Characteristics of Callus		
S.No. Explant	2-4 D +KN	Colour	Callus Nature induction frequency Amount of Callus	

				70	
					High amount of
1	Stem	Pale yellow 0.3 +0.2 to dark brown	Friable callus		callus, better
				90%	response,
					energetically fast
					growing callus
2	Leaf	0.3 +0.2 Greenish to light brown	Friable 7 callus		Better response but
				75%	slow growing in
					comparison to stem
C-Callus, +: Slight regenerative, ++: Moderate, +++: High ++++					
*15	*15 flasks showed positive result out of 20 replicates of each concentration of				

*15 flasks showed positive result out of 20 replicates of each concentration of leaves explants

*18 flasks showed positive result out of 20 replicates of each concentration of nodal segment

(-) - Negative response of hormones

(+) - Moderate response of hormones

KN- Kinetin 2, 4-D- Dichlorophenoxy acetic acid

DISCUSSION

It is very important to regenerate plants via callus formation for the creation of genetic variability in industrially important medicinal plants. With this obvious reasons it is suggested that in future, in tissue culture programme of *Lantana* some more auxin and cytokinin additives viz; 2-4, D and kinetin may be used for successful plantlet regeneration from calli. Here a direct in vitro regeneration protocol was developed. However, further study is needed with different explants for standardization of this protocol of regeneration from calli. The protocol developed here could be used for future improvement of *Lantana* through in vitro culture and genetic transformation.



Dark brown callus of nodal (top) Light brown callus of leaf segment (bottom)

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