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RESEARCH ARTICLE

CALLUS INDUCTION FROM A WILD MEDICINAL PLANT TEPHROSIA HOOKERIANA WIGHT AND ARN

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ABSTRACT

The present investigation was conducted to develop a protocol for rapid callus induction. Tephrosia hookeriana Wight & Arn was collected from Ayyalur, Dindigul District. The explants were cultured on Murashige and Skoog's (MS) medium. The callus culture from leaf, node and internode explants in MS medium supplemented with different concentration and combination of 2, 4-D, NAA, IAA. The highest Percentage (92.35%) of callus from leaf explants was obtained by supplementation of (BAP-0.25mg/L-1 + 2, 4-D-2.00mg/L-1) and the lowest percentage of callus (8.00%) was obtained with the addition of (IAA-1.00mg/L-1) in MS medium. The callus was responded in 30 days from the leaf, node and internode explants. Different colors such as brown, green, greenish white, yellowish green, greenish yellow and brownish white callus were formed. This callus induction protocol was standardized from different explants for successful production of plant lets. There are no earlier reports on in vitro characterization of T.hookeriana.

INTRODUCTION

Leguminosae is one of the most important family of the plant kingdom with the novel characteristics of nitrogen fixation and there by enriching the fertility of soil. One of the most important problems in the woody legumes is the establishment of propagules of high quality regeneration (Mamun *et al*., 2004). *Tephrosia hookeriana* Wight & Arn (Syn:*T. noctiflora, T. amoena*), local name is Kallu kollingi a woody and multipurpose medicinal plant collected from Ayyalur, Dindigul district, Tamil Nadu. It is a perennial shrub up to 1.5m height, originated from Africa, introduced in to South India, West Bengal and Jammu, which is closely allied to *T.purpurea*. Callus induction is a prime phase in vitro tissue culture studies, callus is an undifferentiated mass of dividing plant cells. This callus can solve the problem of unavailable plant materials for in vitro studies. Different physiological and morphogenic responses can also be observed through callus culture like somaclonal variations, somatic embryogenesis, and organogenesis. It also paves the way for isolating economically valuable phytochemicals, which can avoid the collection of plant materials from natural sources (Flick *et al*., 1983; Ogita *et al*., 2009; Berkov *et al*., 2009). Micropropagation has proved as a potential technology for mass scale production of medicinal plant species (Wawrosch *et al*., 2010; Martin *et al*., 2003; Faisal *et al*., 2003). There are no earlier reports on *in-vitro* propagation of *T.hookeriana*, therefore the present study has been focused in *in-vitro* callus induction from various explants.

MATERIALS AND METHODS

Collection of explants and surface sterilization

The leaf, node and internode of *Tephrosia hookeriana* were collected from the place Ayyalur, Dindigul district, Tamil Nadu. The explants were treated under running tap water for 30 minutes to remove all soil particles and treated with 5%Teepol for 5 minutes followed by repeated running tap water for 20 minutes, and then rinsing with distilled water. Further sterilization was done under aseptic conditions in a Laminar Airflow chamber. Explants were surface sterilized with 0.1% mercuric chloride for 1 minute. Finally the explants were washed thoroughly with sterilized distilled water for several times to remove traces. The leaf, node and internode were cut into convenient size (0.5 to 1.0cm) for inoculation.

Culture

For callus induction, MS (Murashige and Skoog., 1962) medium was used with different concentration and combinations of auxins and cytokinins. Throughout the experiments full strength MS medium with 30g/L^{-1} (W/V) sucrose and 8.4 g agar was used. The pH of all media (supplemented with respective growth regulators) was adjusted to 5.6 with 1N NaOH or 1N HCL prior to autoclaving at 121^{o}C for 20min. Once the callus developed, they were further cultured for regeneration in the medium having different concentrations and combinations of plant growth hormones.

Environmental conditions

In callus induction experiment, cultures were kept at 25^{o}C in complete light/dark conditions with 16/8 h light/dark photoperiods with 140 µmol m^{-2} s^{-1} light from cool white fluorescent Lamps.

RESULTS

Effect of 2, 4-D

Within four weeks of culture period 64% callogenic response was achieved when the medium was fortified with 2,4-D (3.00mg/L^{-1}) for both types of explants leaf, node and internodes (Table:1) the highest response was achieved in leaf

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64.66% followed by node 39.33% and internode 42.33%. At the lower concentration of 2, 4-D the callus induction was 28% in leaf, 8.66% node and 9.66% in internode.

Effect of NAA

Higher callus was formed in MS medium fortified with NAA (3.00mg/L^{-1}) 60% for leaf explants. Whereas node and internodal explants produced moderate level of callus. All the treatments with NAA results in the formation of embryonic callus in the medium composition as described in the Table: 1.

Effect of IAA

For callus induction IAA was added in MS medium with different concentration. The different explants were used in the callus cultures, the highest percentage of callus (27%) was observed when 3.00mg/L^{-1} of IAA added to the medium. On the other hand, the lowest percentage of callus induction was 8% on the MS medium supplemented with IAA (1.00mg/L^{1}, - Figure: 1).

Effect of auxins and cytokinins on callus induction

In order to explore the possibility of callus induction, explants cultured on MS medium fortified with 2, 4-D, NAA, IAA either alone or in combination with BAP, KN, AdS. The best response was observed (92%) in leaf explants on MS medium with 2,4-D(2.mg/L^{-1})+ BAP(0.25mg/L^{-1} (Table: 2).The minimum percentage of callus (20%) was observed when MS medium fortified with 2,4-D (1.00mg/L^{-1}) + AdS (0.5mg/L^{-1}) at nodal segment (Figure: 2).

Table 1 Percentage of the callus induction from *T. hookeriana* in different explants leaf, node and internode at various levels of 2, 4-D, NAA and IAA after 4 weeks culture.

Plant Growth Regulators mg/L^{-1}			Effect of explants		
2,4-D	NAA	IAA	Leaf	Node	Internode
0.5			28.66±0.33	8.66±0.33	9.66±0.33
1.0			45.00±0.57	19.66±0.33	24.00±0.57
2.0			54.00±0.57	31.00±0.57	35.33±0.33
3.0			64.66±0.33	39.33±0.33	42.33±0.33
	0.5		19.33±0.33	25.32±0.12	17.00±0.00
	1.0		33.33±0.33	19.33±0.33	23.00±0.00
	2.0		51.33±0.33	22.67±0.33	36.66±0.33
	3.0		60.00±0.00	30.00±0.00	44.33±0.17
		0.5	20.00±0.02	13.00±0.11	16.00±0.21
		1.0	13.00±0.00	8.00±0.00	11.67±0.33
		2.0	18.66±0.33	10.00±0.00	15.00±0.00
		3.0	27.00±0.00	16.00±0.00	23.00±0.00

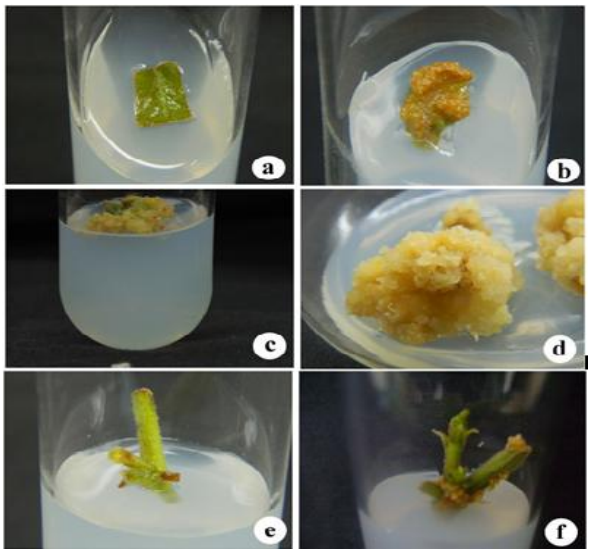
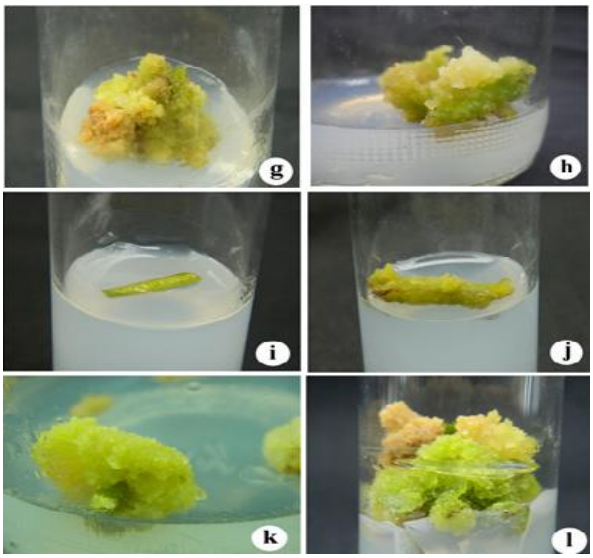


Figure 1 Percentage of the callus induction from *T. hookeriana* in different explants leaf, node and internode at various levels of 2, 4-D, NAA and IAA after 4 weeks culture.

a) Leaf explants, b) Initiation of callus, c) Callus induction from leaf explants, d) Callus proliferation from leaf, e) Node explants, f) Callus Initiation from nodal explants,



g) Callus induction from nodal explants, h) Callus proliferation from node, i) internodal explants,
j) Initiation of callus, k) Callus induction from internodal explants, l) Callus proliferation from internodal explants.

Table 2 Effect of different combination of 2, 4-D, BAP, KN and AdS response to callus multiplication on MS medium after 4 weeks

Plant growth regulators mg/L^{-1}				Frequency of callus induction (%)		
2,4-D	BAP	Kin	Ads	Leaf	Node	Internode
1.0	0.25			70.00±0.35	43.67±0.33	55.36±0.37
	0.5			58.00±0.57	33.00±0.57	41.35±0. 76
2.0	0.25			92.35±0.76	67.32±0.33	86.33±0.77
	0.5			81.66±0.23	50.35±0.89	70.34±0.33
1.0		0.25		56.33±0.89	36.34±0.33	32.33±0. 71
		0.5		45.34±0. 46	29.23±0.77	27.61±0.24
2.0		0.25		73.36±0.33	50.33±0. 89	58.32±0.91
		0.5		58.66±0.23	41.37±0. 76	41.66±0.23
1.0			0.25	65.33±0.89	33.38±0.33	49.33±0.57
			0.5	49.35±0.33	20.34±0. 18	33.35±0.33
2.0			0.25	82.66±0.77	58.32±0.57	69.00±0.00
			0.5	71.67±0.23	40.30±0. 21	55.34±0.32

The values represent the means (Mean ± SE). Mean values within column followed by the significantly different by Duncan's multiple range test (P>0.05).

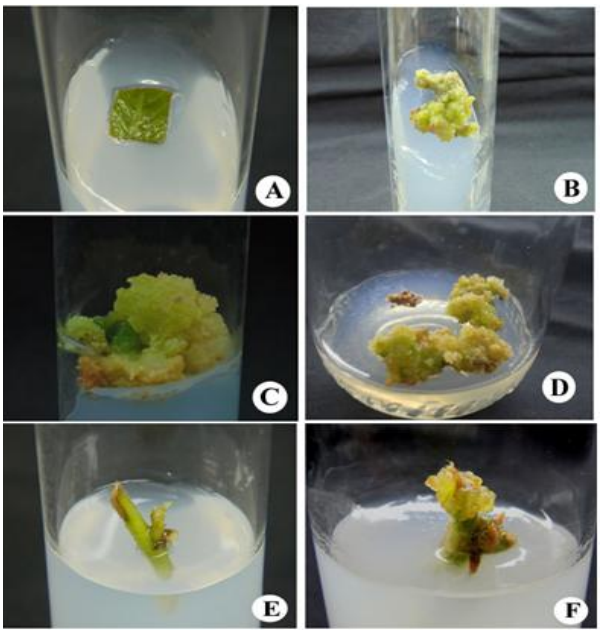
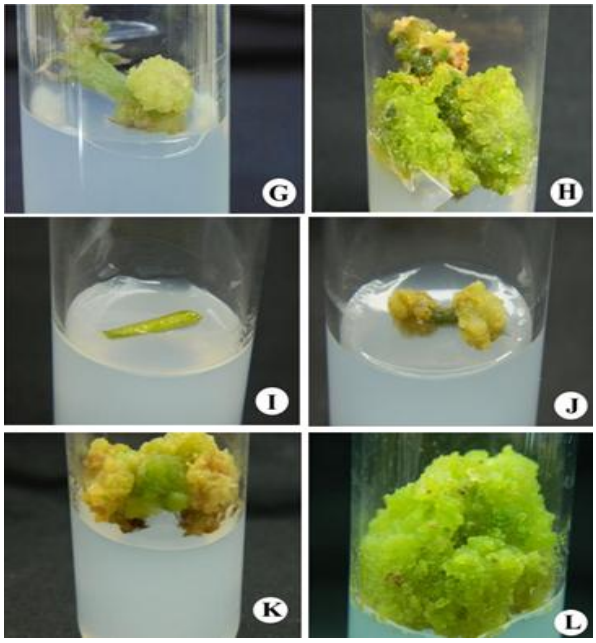


Figure 2 Effect of different combination of 2, 4-D, BAP, KN and AdS on response of callus multiplication in MS medium after 4 weeks

A) Leaf explants, B) Initiation of callus, C) Callus induction from leaf explants, D) Callus proliferation from leaf, E) Node explants, F) Callus Initiation from nodal explants



G) Callus induction from nodal explants, H) Callus proliferation from node, I) internodal explants, J) Initiation of callus, K) Callus induction from internodal explants, L) Callus proliferation from internodal explants.

DISCUSSION

The callus culture forms an important preliminary stage to the regeneration of whole plants. The growth regulators requirement for callus initiation has been modified with the nutritional status of the medium. In the present investigation, among various growth regulators tested individually or in combination. The MS medium fortified with 2, 4-D and BAP was found to be superior for callus production from all explants of *T.hookeriana.* The role of 2, 4-D in callus induction was reported by Saravanan *et al*. (2007) in *Pedalium murex*. Munshi *et al*. (2007) described callus induction in *Brassica oleraceae* using MS fortified with 1 mg L-1 2,4-D and 0.5 mg L-1 NAA. Similar results have been recorded by Jawahar *et al.,* (2003) in *Solanum nigrum* from leaf explants on MS medium supplemented with IAA, BAP and GA3. The best frequency of green compact callus and multiple shoots were obtained on MS medium containing 2.0 mg/l BAP and 0.01 mg/l GA3. Similar observations were noticed by Sivakumar *et al,* (2004) in *Gloriosa superba* which revealed that the highest frequency of callus was obtained when the MS medium was supplemented with 4.52 µM 2, 4-D and 2.32 µM Kin in all types of explants. Mohan *et al*., (2004), reported that when stem explants of *Solanum nigrum* were cultured on MS medium supplemented with 2.0 mg/l IAA or NAA and 1.0 mg/l BAP and 0.01 mg/l GA3 gave highest percentage (73.0) of green compact calli. From the perusal of data it was obvious that in *T. hookeriana* the 2, 4-D and BAP plays a vital role in the callus initiation compare to other plant growth regulators.

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