Study of Impact of Different Concentrations and Combinations of Cytokinins and Auxins on Callus Induction in Abutilon indicum (L.) an Important Medicinal Herbs.

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ABSTRACT:

In the recent past, plant-based medicines are getting popularity even in developed countries, as it has no side effects. Most of the herbal medicinal plants are growing in wild habitat, from where they are being brutally harvested. Loss of habitat due to urbanization and industrialization and unplanted harvesting have exerted pressure on such populations, due to which several species of medicinal plants have become extinct or are on the verge of extinction. Plant tissue culture techniques are the best strategies through such plants can be conserved. In the present work tissue culture study to induce calli on two different explants such as leaf and internodal segments were performed. It was noted that BAP alone at different supplemented in B5 could induce calli on both the explants. But B5 + 2.0 mg/l BAP could induce calli in 95.38% of leaf explants, whereas at these concentrations, it could induce calli in 93.38% in the internodal explants. Time taken for callusing in leaf explants was 14 days whereas in the internodal explants it was 15 days. B5 + 4.0 mg/l BAP could in callogenesis in 96.62% of the explants, where the time taken for callusing was 14 days. Kinetin alone also induced callusing in both the explants but maximum callusing was in B5 + 2.5 mg/l KN that was 88.51%. Here time taken was 15 days. In internodal explants, the maximum percentage of response was 88.45, and time has taken 16 days. Both the explants were also inoculated in B5+ 2.0 mg/l BAP + five different concentrations of NAA. Here higher percentage of response in leaf explant was 98.78 in B5 + 2.0 mg/l BAP + 1.5 mg/l NAA. Time taken for callusing was 17 days. In a similar condition, the percentage of response for nodal explants was 97.32 days and the time taken for callusing 13 days. MS + KN 2.5 mg/l + 1.5 mg/l NAA could induce calli in 92.62% of explants which was the highest. In a similar concentration, the internodal segment could induce calli in 91.66 percent of the explant, and the time taken was 14 days. The colour of the calli ranged from white to white brown or white-yellow, white the texture ranged from compact to lose to friable. Above raised calli were subcultured and maintained in the laboratory.

KEY WORDS:

Herbal medicine, Tissue culture, Explant, Friable, Compact, White brown, MS Medium, Subculture.

INTRODUCTION:

Medicinal plants are being used in the tradition medicines as well as modern medicine. In spite of its demand, even today most of them are growing in wild populations and only few percent are being cultivated. Due to brutal harvesting, and destruction of habitat we are losing several rare species from its natural habitat. *Abutilon indicum*, commonly known as '**Kanghi'**. It is a small shrub and belongs to family *Malvaceae*. Plant bears hairs on stem and leaves. Flowers are yellow on maturity it becomes woody. Plants are being used to cure different diseases by local people and Vaidaya. All parts of the plants are used such as roots, stem leaves and flower buds. They are being used to cure tuberculosis, bleeding disorders, it cures burning sensation. Leaves are locally applied to boils and ulcers. It is also used as laxative, expectorant and analgesic. Roots are prescribed in fever. Chest affection and urethrites. Decoctions of leaves are sued to cure piles.

Tissue culture techniques are being applied in medicinal plants also. With the help of this, rapid propagation, conservation and enhanced production of desired secondary metabolites have become possible. We get different references for callus induction and micropropagation, production of desired secondary metabolites from different medicinal plants. Some of them are being mentioned here such as; Roja and Heble (1991) in *Withania somnifera* L., Tandan and Rathore (1992) in *Coptis teeta*; Cheng and Yang (1995) in *Taxus marei*; Thakur *et al*; (1998) in Melia *azadirachta*; Tiwari *et al*; (1998) in *Bacopa monnieri* L., Zafar and Sagar (1999) in *Eclipta alba*. Shahzad *et al*; (1999) in *Solanum nigrum*. Komala Valli and Rao (2000) in *Gymnema sylvestre*. Chen and Chang (2002) in *Oncidum*; Rani *et al*; (2003) in *Withania somnifera* L; Pandey *et al*; (2004) in *Aconitum balfourii*; Parekh *et al*; (2006); Husain and Anis (2006) in *Eclipta alba*; Khalekuzzaman *et al*; (2008); Ling *et al*; (2009) in *Mirabilis jalapa*; Baskaran and Jayabalan (2009) in *Psoralea corylifolia*; Chituri *et al*; (2010) for the production of secondary metabolite, Witheferin A. Shukla *et al*; (2010) in *Withania somnifera*; Joshi and Padhya (2010) in *Withania somnifera*; Meena *et al*; (2010) in *Citrullus colocynthis*; Arumugam and Gopikrishna (2011) in *Withania somnifera* L.; Sharma *et al*; (2013); Sen *et al*; (2014) in *Achyranthes aspera*. Considering all these ideas in mid tissue culture study in *Abutilon indicum* was performed to induce callus on leaf and internodal explants *in vitro*. Although this plant has several medicinal importance but they are most neglected plant as they grow in wild habitat.

MATERIALS AND METHODS:

Plant material *Abutilon indicum* was located on the road side at Muzaffarpur. It was identified based on the previous knowledge and confirmed with the help of text book o taxonomy.

Healthy and young branches were collected and brought in the laboratory within half an hour. Both the lower and

apical portions were excised with the help of sharp knife. Leaves were taken from the stem. Similarly, internodal segments were prepared. They were washed under running tap water for 30 minutes. Then they were washed with detergent Tween-20 for 5 minutes. It was rinsed with distilled water again. These explants were surface sterilized with 0.1% with aqueous solution of mercuric chloride ($HgCl_2$) for 2 min. Finally, all the explants were rinsed with sterilized distilled water for three times. All these works were done under aseptic conditions of laminar airflow cabinet. Explants were stored in pre-sterilized and moistened muslin cloth for inoculation.

Culture Medium:

MS (Murashige and Skoog, 1962) medium was supplemented with 3% sucrose, different concentrations of BAP, (1.0 mg/l – 4.0 mg/l), KN (1.0-4.0 mg/l) alone and with NAA (0.25 mg/l – 2.0 mg/l) separately. The medium was gelled with 0.8% Agar solution. Before this the pH of the medium was adjusted to 5.8 with the help of N HCl. Semi jelled medium was dispensed in the culture flaks of 125 cc volume at the rate of 20 ml each. The mouth was plugged with non adsorbent cotton plug, covered with muslin cloth. Before autoclaving the plugs were wrapped with aluminum foil to prevent wetting during autoclaving. Autoclaving was done at 15 lb pressure for 20 min. Culture flasks were taken out and allowed to cool at room temperature. They were stored at low temperature in freeze and were used for inoculation after three days. Flasks showing contamination were discarded after autoclaving. Inoculation was done in the aseptic cabinet of laminar airflow cabinet. All precautions were taken during inoculation to avoid contamination. Culture flasks containing explants were inoculated in culture room where, temperature 26 ± 1^0C, moisture 66% and light intensity 3000 lux were maintained artificially. Observation for periods of callus induction, number of explants responding for callusing, callus colour and texture etc. was made on an alternate day. Culture showing contamination were discarded after autoclaving. All experiments were done in triplicate and mean of the data was tabulated in table-1 and table-2 used for discussion. In first cultures browning of culture was observed. To get rid from this problem 50 mg/l ascorbic acid and 50 mg/l citric acid were added during medium preparation.

RESULTS AND DISCUSSION:

In the present work leaf and internodal explants were inoculated in MS + various concentrations of BAP and KN alone and with different concentrations of NAA. From the table-1, it was noted that the leaf explants induced calli in all the concentrations of BAP, but MS + 2.0 mg/l BAP alone was most suitable as here the percentage of response for callusing was 95.35% time taken for callus induction was 14 days and calli had white green colour and friable texture, followed by MS + 2.5 mg/l BAP, where the percentage of response was 92.68, time taken for callusing 15 days and calli had white green colour and friable texture. Lowest percentage of response 81.28 days maximum time taken for callusing 18 days and white brown and compact calli were produced in MS + 4.0 mg/l BAP alone. Highest percentage of response for callus induction in leaf explants was observed in MS + 2.5 m/gl KN, where the response was 88.51%, time taken 15 days and the calli had

white green colour and loose texture. This was followed by 87.84% response time taken for callus induction 16 days and the calli had white brown colour and loose texture. Here minimum percentage of response 78.64, maximum time taken 18 days for callus induction was noted in MS + 4.0 mg/l KN the calli had white brown colour and loose texture.

In case of internodal explants highest percentage of response 94.38, minimum time 14 days was observed in MS + 2.0 mg/l BAP alone. This was followed in MS + 1.5 mg/l BAP where the percent response was 92.56%, time taken for callus induction 15 days with white colour and friable texture calli. Here minimum percentage of response for callusing 87.25, maximum period 18 days was found when the explants were inoculated in MS + 1.0 mg/l BAP. Explants inoculated in MS + 2.5 mg/l KN gave better response as here percentage of response was 88.45 and time taken 16 days with white loose calli. This was followed in MS + 2.0 mg/l KN, where percentage of response was 87.68, time taken 17 days, with white loose calli. Minimum percentage of response 78.64 times taken 18 days was noted in MS + 4.0 mg/l KN.

Experiments were also performed to observe the synergistic effects of cytokinin and auxins on callus induction in leaf and internodal explants of *Abutilon indicum*. Maximum percentage of response 98.78, minimum periods for callogenesis 14 days were noted when the leaf explants were inoculated in MS+ 2.0 mg/l BAP + 1.5 mg/l NAA. Here white friable calli were produced. This was followed in MS + 2.0 mg/l BAP + 1.0 mg/l NAA, where the percentage of response was 98.24, time taken 14 days and colour white and texture friable. Minimum percentage of response 96.16, maximum time taken for callusing 16 days was noted in MS + 2.0 mg/l BAP + 0.25 mg/l of NAA. In case of MS + 2.5 mg/l KN+1.5 mg/l NAA, the percentage of response was 92.62 and time taken 15 days. Here the calli were white friable. MS + 2.5 mg/l KN + 2.0 mg/l NAA gave the lowest percentage of response 87.55, time taken 18 days and the calli were white compact.

For internodal explants, highest percentage of response for callusing 97.32, time taken for callusing 13 day were noted in MS + 2.0 mg/l BAP + 2.0 mg/l NAA. This was followed in MS + 2.0 mg/l BAP + 1.5 mg/l NAA and the time taken for callusing 14 days. Further, in MS + 2.0 mg/l BAP + 0.25 mg/l NAA the minimum percentage of response 93.87, maximum time taken for callusing 15 days was noted. The calli were white, loose to friable here. Maximum percentage of response for internodal explants in MS + 2.5 mg/l KN + 1.5 mg/l NAA was 91.66, time taken was 14 days and the calli were white friable. This was followed in MS + 2.5 mg/l KN + 1.0 mg/l NAA, where percentage of response was 90.22, time taken 15 days and calli were white and friable. Minimum response 80.68, maximum period 17 days were noted in MS + 2.5 mg/l KN + 0.25 mg/l NAA.

DISCUSSION:

Callus induction in different medicinal plants has been reported by different workers. Baskar and Jayabalan (2009) in *Psoralea corylifolia* L.. Ling *et al*; (2009) in *Mirabilis jalapa*; Adhikari and Pant (2013); Sen *et al*; (2014) in *Achyranthes aspera* L., Bhoyar *et al*; (2015) in *Withania somnifera*, Ritika & Mamta (2018) in *Capparis spinosa*.

All have reported that synergistic impact of cytokinin and auxin has better response for callusing I different medicinal plants. In this way findings of the present works corroborate with the above findings.

CONCLUSION:

With respect to evaluate the callogenic potential of different explants of *Abutilon indicum*, here leaf and internodal the leaf explants are more promising with respect to callus induction than that of the internodal one. Similarly, in comparison to single plant growth regulators say cytokinin or auxin the synergistic action of cytokinins and auxins are more suitable. Therefore, such protocol may be exploited at commercial scale to obtain large biomass of calli. These calli may be used for extraction or cell suspension culture, for the production of desired secondary metabolites. Further, the rate of synthesis of secondary metabolites may be enhanced with suitable elicitors.

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TABLE-1

Showing impact of different concentrations of cytokinins on callus induction in leaf and internodal explants of *Abutilon indicum*.

Plant Growth Regulators (mg/l)		Explant	% of response for callusing	Time taken	Callus Nature
BAP	KN				
1.0	--	Leaf Explant	88.62	17.0	WBL
1.5	--		89.85	17.0	WBL
2.0	--		95.38	14.0	WGF
2.5	--		92.68	15.0	WGF
3.0	--		87.46	17.0	WC
4.0	--		81.28	18.0	WBC
--	1.0		85.28	17.0	WYC
--	1.5		87.46	16.0	WBL
--	2.0		87.84	16.0	WBL
--	2.5		88.51	15.0	WGL
--	3.0		81.32	18.0	WYL
--	4.0		78.54	18.0	WBL
1.0	--	Internodal Segment	87.25	18.0	WC
1.5	--		92.56	15.0	WF
2.0	--		94.38	14.0	WF
2.5	--		92.22	15.0	WF
3.0	--		90.62	16.0	WF
4.0	--		82.34	18.0	WC
--	1.0		85.28	17.0	WC
--	1.5		87.68	17.0	WL
--	2.0		88.45	16.0	WL
--	2.5		87.52	16.0	WL
--	3.0		78.64	18.0	WBL
--	4.0				

TABLE-2

Showing synergistic impact of cytokinin and different concentrations of auxin on callus induction in leaf and internodal explants.

Plant Growth Regulator			Explant	% Response	Days	Callus Characteristics
BAP	KN	NAA				
2.0	--	0.25	Leaf	96.16	16	WL
2.0	--	0.5		97.54	16	WGL
2.0	--	1.0		98.24	14	WF
2.0	--	1.5		98.78	14	WF
2.0	--	2.0		96.38	15	WL
--	2.5	0.25		88.34	17	WL
--	2.5	0.5		88.78	17	WYL
--	2.5	1.0		90.44	16	WF
--	2.5	1.5		92.62	15	WF
--	2.5	2.0		87.55	16	WC
2.0	--	0.25	Internodal	93.87	15	WL
2.0	--	0.5		94.52	14	WF
2.0	--	1.0		95.74	14	WF
2.0	--	1.5		96.87	14	WF
2.0	--	2.0		97.32	13	WF
--	2.5	0.25		88.68	17	WBL
--	2.5	0.5		89.74	16	WYL
--	2.5	1.0		90.22	15	WF
--	2.5	1.5		91.66	14	WF
--	2.5	2.0		88.72	16	WC
W = White, L = Loose, B = Brown, Y = Yellow, C = Compact, F = Friable, G= Green						

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