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In-vitro callus induction and multiplication of inter-nodal explants in plants *Dicoma tomentosa* and *Alhagi maurorum*

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ABSTRACT

Dicoma tomentosa and *Alhagi maurorum* are the two medicinal plants with fast in-vitro growth. Both the plants have high economic values. Both the plants were investigated on nodal segments and on leaves. The plants were cultured in five different conditions of medium ranging from MS1- MS5. The hormones were used in these mediums in different concentrations. BAP, NAA, Kinetin, and 2,4 D were use. The MS medium in combination with BAP (2.0 and 2.0mg/ml) with NAA 0.1 mg/ml with kinetin 0.25 mg/ml with 2-4 D were taken, where BAP 1 mg/ml with 2 mg/ml of NAA, BAP 2 mg/ml with 0.5 mg/ml of NAA showed better results with callus growth and root-shoot initiation. The best rooting medium found was MS medium supplemented with IAA and IBA 0.5mg/ each. The culture medium was used in different concentrations for estimation of primary metabolites. Maximum protein and lipid percentage were noticed in leaves of both the plants. It can be concluded that both the studied plants have high medicinal importance and can be used as raw material for industry.

Keywords: - *Dicoma tomentosa; Alhagi maurorum; Plant hormones; MS media.*

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INTRODUCTION:

Dicoma belongs to *Compositeae* and have great medicinal values (1). The group has great structural and physiological importance, with higher economic valuation. It can also be said that Compositeae is a group of innumerable species having successful metabolic values (2). Large numbers of plants of this genus have ornamental values and are cultivated in homes and gardens. Few members of Compositeae have medicinal importance. Member of genus Dicoma have 35 species distributed in tropical region of South Africa and one species is found in Asia. In this genus mostly herb and shrub are included only a few trees are found. *Dicoma tomentosa* is a tree of this genera, it is used for its medicinal properties most often by people of Western Africa (3). The plant is used as tooth cleaner (4) and also as febrifuge in Belgium (5). Africans use it as wound healing agent against putrescent wound. It has been also reported as antiplasmodiac (6-8). The another plant being studied in this study was *Alhagi maurorum* of family Fabaceae. It is commonly pronounced as Caspian manna, camelthrom and Persian manna. It is a thorny, branches, perennial shrub with deep roots. The plant have potent medicinal properties such, it is analgesic, antipyretic (9), diuretic, antirheumatic (10), expectorant, antiasthmatic, antiulcer and laxative (11).

Plant tissue culture has been proved to be an effective, reliable and rapid mean for germplasm conservation. Optimal culture has been used to establish dry cell mass and for production of secondary metabolites (12). Callus appears as undifferentiated mass that appears on explants in few weeks of transfer of growth medium with required hormones (13). It is a process considered as reverse process to cell differentiation (14). In present study we examined internodal explants in different hormonal concentrations in MS medium (15). In recent time destruction of this herb is often seen due to use of chemicals and pesticides, because of rare and poor seed germination. These conditions emphasized us to study the callus culture under lab conditions to protect medicinally important plants.

MATERIAL AND METHODS:

Plant material and sterilization

The plants *D. tomentosa* and *Alhagi maurorum* was stored in (Udaipurwati, Jhunjhunu and Mehlan, Jaipur) Rajasthan and was authenticated in Department of Botany, the University

of Rajasthan. The crude extract was obtained by extraction. Healthy segments from nodal and intermodal parts were excised and thoroughly washed with fresh water, followed by 5 drops of tween 20 in 100 ml for the time duration of 10 minutes in constant agitation. Further the explants were kept emerged in 0.2% $HgCl_2$ (w/v) for 2 - 3 minutes. The internodal and nodal explants were rewashed wit double distill water. The explants were kept vertically on the culture media.

Media preparation and incubation:

For shoot proliferation and callus induction MS media 4.44g (14) add in 1000ml Distilled water was taken in 0.8% agar and 3 % of sucrose, supplemented with varying concentration of α-napthalene acetic acid, 6-benzyl amino purine (BAP) and 2,4-D (2,4-dichlorophenoxyacetic acid) either alone or in different combinations in culture tubes or culture bottles for induction of callus culture and shoot proliferation. IAA and IBA were used in different concentration in MS media for rooting. pH of the media was maintained to 5.8 by using NaOH and HCL 0.1 N, before mixing the agar to the media and was autoclaved for 15 minutes at 121°C. Maintain of the culture was done for 12 hours photoperiod at 25 °C, the intensity of light was supplied was 6000 lux by the use of cool- white fluorescence lamp. After the duration of 4 weeks of inoculation the culture was routinely transferred in the bottle with fresh media.

In-vitro culture condition and Shoot regeneration

The nodal and internodal segments with the calli explants were now inoculated on MS media and were supplemented with varying hormonal concentration and combinations. The concentration taken were 0.2-3mg/L of NAA with 0.5-2mg/L of 2,4-D and 0.5-4mg/L of BAP. Effects of these combinations on regeneration and callus formation as growth regulators were recorded after a certain time interval in regulated manner. MS media with 1 mg/ml of BAP with 0.5 mg/L of NAA for nodal explants showed higher percentage of regeneration of shoot. For callus explants on internodal segment higher percentage of regeneration was seen in 2 mg/ml of BAP and 0.5 mg/ml of NAA. Formation of brown colored compact calli and degree for better response was also found and recorded in 2 mg/L NAA. After the duration of 4 weeks from culturing the percentage of regeneration of shoot was evaluated, length of shoot and mean number was also evaluated.

Root generation

After 4 weeks regenerants were taken from culture and were separated in small pieces, they were further placed in 1-3mg/L IBA or IAA containing MS medium for root generation. The culture tubes having 0.75% of agar with 25ml of medium were used for proliferation of new emerging roots. Root appeared after 25-30 days of inoculation. Subculture was prepared, number of roots grown per explants and root response percentage was recorded after 4 weeks from preparation of subculture.

Hardening and acclimatization

The shoots with developed roots grown upto 5cm were taken after 4 weeks of culture gently in the culture tubes and washed with distill water for removing the adhered agar medium. They were then transferred to tray- beds of vermiculite and sterilized soil in 1:1 ratio. The plantlets were kept for hardening in maintained culture chamber with 85% humidity and temperature of 25 ° C for 35 days. The regenerants were now kept in natural environment for exposure.

Quantification of primary metabolites:

The residues which were obtained after filtration were used for estimation of starch by Anthrone method (15). Intensity of color change was observed at 649nm and amount of starch quantified starch was measured from standard curve. For analysis of soluble sugar the method used was of Dubois., 1956 and absorbance was measured at 420 nm (16). Estimation of reducing sugar was done by DNS method of Miller 1959 (17). The absorbance was measured at 540nm. Glucose was estimated by the help of soluble sugar. Lowry 1951 was used for protein determination (18). Free amino acids were measured at 570nm by the method of Sadasivam and Manickam 1992 (19) and standard curve was made for leucine.

Statistical analysis:

For monitoring the growth parameters percentage response of different parameters were analyzed for shoot regeneration. Height of shoot, number of multiple shoot and regenerants from callus explants were monitored. The data of all the experiments were taken in replicates of 6, SD mean was measured as per new Duncan's multiple range test (20).

RESULTS:

Showing mg/g dry weight and percentage wise distribution in leaf and stem are both the plant. Where maximum percentage of lipid was seen in leaves of both the plants and increased percentage of protein was also seen in both the plants. Leaves of *Alhagi maurorum* and *Dicoma tomentosa* showed better percentage of proteins.

From MS1 to MS 5 all the medium showed better growth of callus. But MS 5 was found most appropriate medium for callus formation from nodal and internodal segments. Good calli growth results were seen in MS1 medium. Medium growth was seen in MS2, MS3 and MS4 medium (table 1 and 2, figure 2 and 3). MS 5 was found to be the medium for adventitious growth. After the duration of one month good shoot and bud primordials were observed (fig 4).

Better results were obtained in the medium with combination of Auxin and cytokinin. But comparatively better results were obtained in medium with combination of IAA+IBA.

On illustrating from table 3 values of TSS, Starch, protein, phenols and lipids highest percentage of lipid was obtained in both the plants. Better protein percentage was found in leaves of both the plants.

Figure: - 1. Showing nodal and internodal growth of *Dicoma tomentosa*.

1. BAP + NAA concentration of a) *Dicoma tomentosa*

2. BAP + NAA + KINETIN concentration of a) *Dicoma tomentosa*

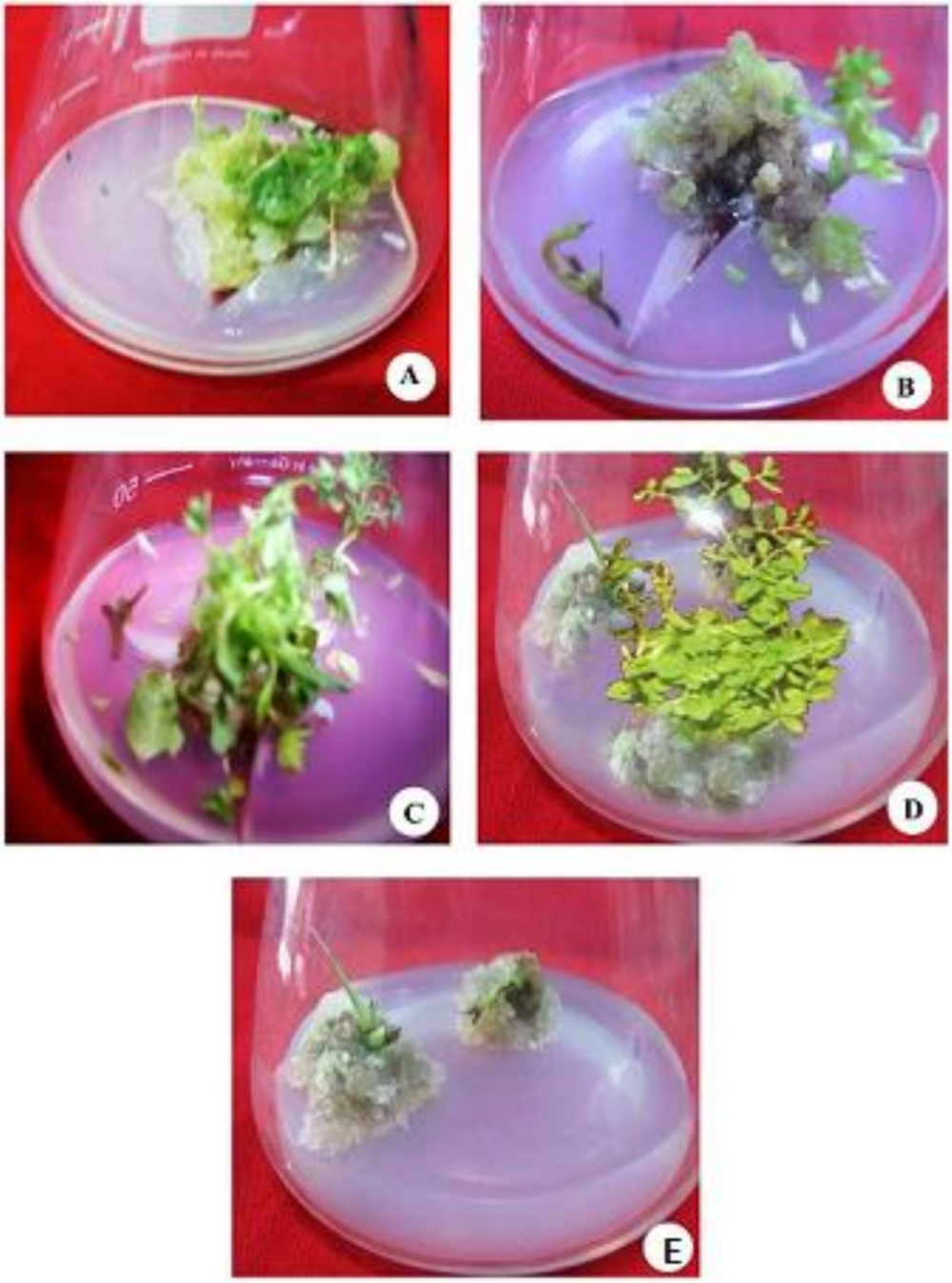
3. BAP + 2-4D concentration of a) *Dicoma tomentosa*

4. BAP + IBA concentration of a) *Dicoma tomentosa*

5. IAA + 2-4D concentration of a) *Dicoma tomentosa*

Showing nodal and internodal growth of *Dicoma tomentosa* with different hormonal concentration in primary stage

Figure: - 2. Showing callus growth of *Dicoma tomentosa* in different concentration of MS medium after the duration of 5 weeks.

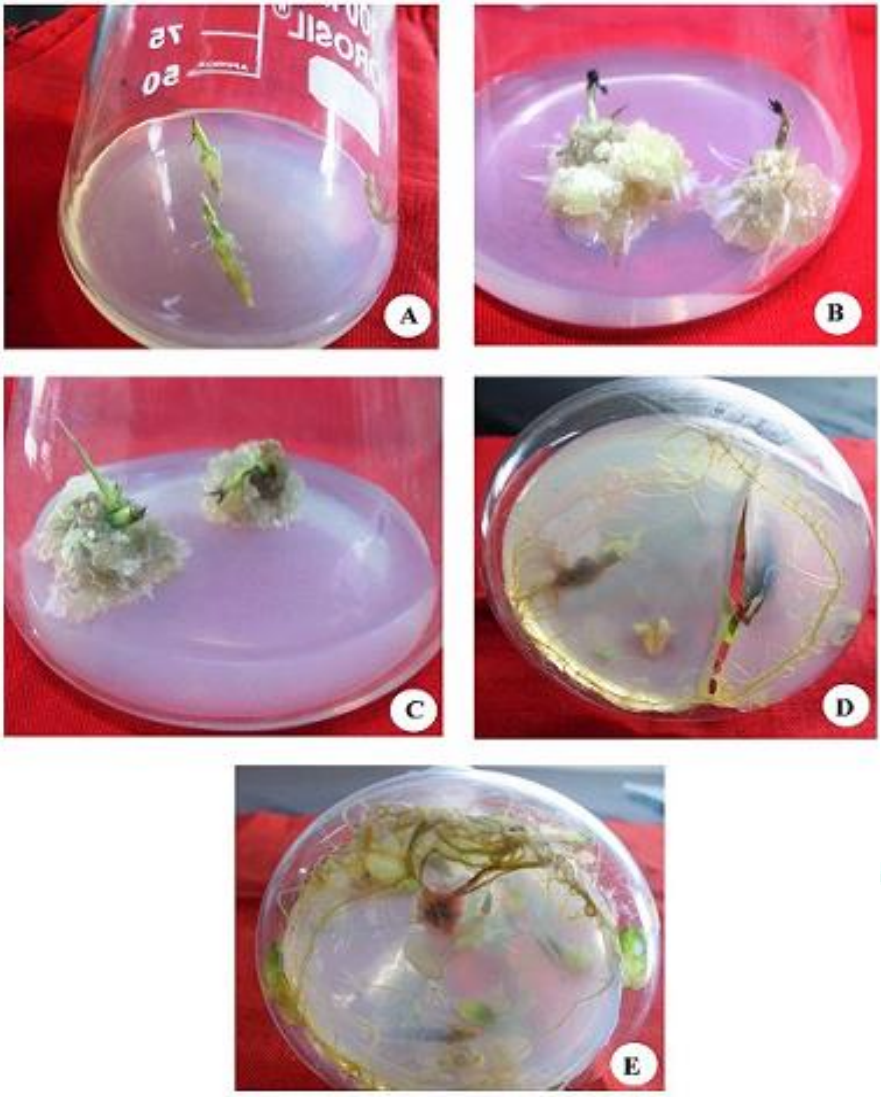


Showing callus growth after the duration of 5 weeks in different concentration of MS mediums A) MS medium 1 B) MS medium 2 C) MS medium 3 D) MS medium 4 and E) MS medium 5

Figure: - 3. Showing callus growth of *Alhagi maurorum* in different concentration of MS medium after the duration of 5 weeks.

Showing callus growth after the duration of 5 weeks in different concentration of MS mediums A) MS medium 1 B) MS medium 2 C) MS medium 3 D) MS medium 4 and E) MS medium 5.

Figure: - 4. Showing growth of root regeneration of both the plants in-vitro after the duration of one month.



Showing A) root regeneration at initial stage, B) regenerated shoot of *Dicoma tomentosa* C) regenerated shoot of *Alhagi maurorum* D) regenerated growth of *Dicoma tomentosa* E) regenerated growth of *Alhagi maurorum*.

Table 1: - Showing effect of growth regulators enzymes for initiation of callus culture from nodal and internodal segments and percentage wise calli formation in plant *Alhagi maurorum*

Medium	BAP mg/ml	NAA mg/ml	Kin mg/ml	IBP mg/ml	Nodal	Internodal	Bud from calli in %
MS1	1.01	-	-	-	71.57±0.08	80.04±0.03	92
MS2	2.01	-	-	-	80.13±0.01	89.01±0.02	91
MS3	1.1	0.2	-	0.1	82.18±0.05	89.05±0.01	91
MS4	1.05	0.19	0.21	0.16	79.04±0.06	86.02±0.06	89
MS5	.5	2.15	0.21	0.22	85.06±0.08	98.08±0.08	97

Table 2: - Showing effect of growth regulators enzymes for initiation of callus culture from nodal and internodal segments and percentage wise calli formation in plant *Dicoma tomentosa*

Medium	BAP mg/ml	NAA mg/ml	Kin mg/ml	IBP mg/ml	Nodal	Internodal	Bud from calli in %
MS1	1.04	-	-	-	69.01±0.03	76.02±0.02	89
MS2	2.03	-	-	-	76.12±0.03	86.3±0.04	87
MS3	1.01	0.19	-	0.09	79.14±0.05	85.04±0.02	89
MS4	1.02	0.15	0.24	0.21	75.18±0.06	86.03±0.04	90
MS5	0.3	2.05	0.24	0.25	90.01±0.03	97.05±0.06	94

Table 3: - Estimation of primary metabolites in *Alhagi maurorum* and *Dicoma tomentosa in* **(mg/g dry weight** and **%)**

S. No.	Parameters	*Alhagi M*		*Dicoma T*	
		Leaf	**Stem**	**Leaf**	**stem**
1	TSS	1.42	2.12	1.86	0.413
2	Starch	1.12	1.23	1.32	1.23
3	Proteins	39.8	32.8	36.6	33.36
4	Phenols	2.1	2.32	1.98	2.0
5	Lipids	5.4%	1.1%	6.0%	2%

DISCUSSION:

Callus formation, growth differentiation rooting and shoot multiplication:

After the duration of 3 to 4 weeks calli and bud initiation was observed from the leaves which were surface sterilized and from the nodal segments. The calli obtained from leaves were non-fragile and blackish. Green and fragile calli were observed and recorded from the nodal segments of both the plants. From tested medium MS 5 showed better results, it was combination of auxin and cytokinin (table 1 &2 fig 1&2) (21). In MS 5 medium best callus initiation and bud break was seen from leaves and nodal segments. The new buds were sub cultured on same medium and in MS1 medium compact green callus and bud appeared from the nodal segment (fig2 and 3).

The choice of hormones for tissue culture was oriented as per morphogenetic response and on the status of used metabolites by (22). Different callogeneic capacity was observed on subjection of different genotype (23). MS 3 and MS 1were found to be the best medium because of evaluation of increased adventitious bark number. In several plant species higher nodal explants with shoot multiplication was found (24). Increased number of adventitious bud and shoot were observed when the calli was shifted from MS 4 and MS 5 to the next medium different hormonal concentration. Similar callus growth was seen in a different species of Alhagi (25). The shoot was restudied in MS medium with 0.5 mg/ml IAA or in combination of IAA and IBA 0.5 mg/ml both. The MS medium with combination of the two potent growth promoting hormones showed better growth for both the studied plants. It can be said that low mineral concentration and in presence of appropriate callus medium root and shoot regeneration was positively induced (26). On phytochemical estimation of leaves and stem of both the studied plants high lipid and protein percentage was found. Higher primary metabolites were found effective for better callus growth.

CONCLUSION:

The reserve food plays a major role in economic importance of plants in the form of protein, lipid and carbohydrate. In present study high amount of primary metabolites was found positively effective in callus growth and it positively trigger in-vitro root and shoot regeneration. It can be said that developing primary metabolites in culture medium can be an eco-friendly approach. In present study MS5 medium was found effective for root and shoot regeneration with combination of IAA and IBA. Primary metabolites influenced expression of significant callus growth.

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