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EFFECT OF PLANT GROWTH REGULATORS ON CALLUS INDUCTION IN GLOBALLY ENDANGERED ETHNOMEDICINAL TREE SPECIES *ADANSONIA DIGITATA* L.

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ABSTRACT

The present study reports on the effect of various plant growth regulators (PGRs) on callus induction from different types of explants of *Adansonia digitata* L. a globally endangered ethnomedicinal tree. The explants viz., hypocotyl, cotyledonary leaf, leaf, root were cultured on MS medium fortified with different concentrations (0.5–6.0 mg/L) of PGRs (2,4-D, IBA, BAP, KIN) alone and also in combination of NAA+BAP. The highest percentage of responding cultures from cotyledonary leaf explants (97.6% at 4.0 mg/L 2,4 D) followed by root explants (94.7% at 4.0 mg/L 2,4 D), leaf explants (93.7% at 4.0 mg/L 2,4 D) and hypocotyl explants (94.7% at 4.0 mg/L 2,4 D) were found. More amount of callus formation was also observed from all the explants at 4.0 mg/L 2,4 D. 2, 4 D was found to be the potent PGR for callus induction and proliferation. Whereas we have observed the shoot buds formation directly from the hypocotyl explants at 0.5/2.0 mg/L BAP. Faster callus proliferation and very high yield of callus mass were produced within 4 weeks on MS medium augmented with 2,4 D followed by IBA, BAP, KIN and NAA+BAP. Callusing ability was found to be more in cotyledonary leaf cultures followed by root, leaf and hypocotyl explants. In the present investigation, the callus development from different explants was subcultured thrice on the fresh medium supplemented with the same concentrations

and cytokinins, but we didn't find any morphogenic event for organogenesis. Since it is medicinally important, the protocol developed for the induction of callus can be used for isolation of pharmaceutically important bioactive molecules in *A. digitata*.

Keywords: Cotyledonary leaf, Hypocotyl, Leaf, Root explants, Plant growth regulators, Callus, *A. digitata*

INTRODUCTION

Adansonia digitata L. is known as baobab tree belongs to family Malvaceae. Baobab is one of the eight species of the genus *Adansonia*, but it has until recently been considered the only native species on African mainland. The tree may reach upto 25 m in height and live for hundreds of years. It is very important in the economy of rural and urban people for food, fodder, medicine, shelter and numerous other products **[1, 2]**. Adansonia has high water holding capacity and its hollow trunk keeps the water potable for many years and serves as a reservoir during drought. The tree extracts are also used as food, fuel, cosmetics, medicines and in the tropical treatment of muscle wounds, dandruff and other skin conditions **[3, 4]**.

Even today, small rural communities acknowledge the importance of baobab as a source of food, in the formulation of medicines and livelihood **[5]**. In fact, baobab tree may be considered a sustainable tree as all vegetative parts are used viz., (1) pulp is used as food or medicine, due to the presence of bioactive compounds, such as phenolic compounds **[5, 6]**, ascorbic acid, minerals or vitamins **[7]**; (2) fibers are used for making ropes; (3) bark is used for producing decorative objects or as firewood; (4) seeds are used as feedstock, in the production of flour and oil extraction for cosmetic applications **[6]**. Baobab seeds are numerous and large, surrounded by a whitish naturally dry pulp, and remain viable over 5 years **[8]**. Literature survey shows that the high potential of baobab seeds as a functional food ingredient, due to the content of phytosterols, proteins, fibers, minerals, vitamins (A, C, E, and D3), playing a key role in cell regeneration, delaying aging **[9]**.

In many areas, particularly in the tropical countries, traditional medicinal plants remain one of the main sources in prevention and treatment of varying ailments through self-medication **[10]**. The results of phytochemical screening of ethanol and chloroform extracts of *A. digitata* using percolation and Sohxlet extraction method revealed the presence of flavonoids and steroids in the extracts **[11]**.

Callus formation is a desirable prerequisite for plant regeneration because callus offers the greatest opportunity for *in vitro* selection and production of genetic variations **[12-16]**. Generally, high concentration of auxins and less amount of cytokinins in the medium promote abundant cell proliferation with the formation of callus **[17]**. The present study was undertaken to know the effect of PGRs on induction of callus for isolation of medicinally important bioactive compounds in *A. digitata*.

MATERIALS AND METHODS

Germplasm collection

Fruits of *A. digitata* were collected from Dodla Kousalyamma Government Degree College for Women (DKW), Nellore, Andhra Pradesh. The seeds were separated and soaked for 2-3 hrs to remove the white pulp and coir surrounding them. Viable seeds were air-dried to avoid fungal contamination, sealed in Zip-lock bags and stored at 4°C in a refrigerator. Seeds were sown for germination in medicinal Arbor and the germplasm was maintained at the Department of Biotechnology, Kakatiya University, Warangal- 506 009 (TG), India.

In vitro seed germination

The viable seeds were washed thoroughly under running tap water followed by distilled water. Then surface sterilized with 0.1% $HgCl_2$ for 7-8 min. They were rinsed thoroughly with sterile distilled water 3-5 times to remove the traces of $HgCl_2$. These sterilized seeds were subjected to pre-treatment techniques before inoculation to break seed dormancy. The pre-treatment of seeds, with the utmost care, the seeds were treated with conc. H_2SO_4 for 18 hr. After acid treatment, the seeds were washed thoroughly with running tap water to remove the remnants of acid and soaked overnight in sterile distilled water. Later, these sterilized seeds were dried on sterile tissue paper and transferred onto MS medium with 3% sucrose (paper-boat method) for *in vitro* seed germination **[18]**.

Culture media and culture conditions

The *in vitro* derived seedling explants viz., hypocotyl, cotyledonary leaves, leaves and roots were inoculated on MSO/MS medium fortified with different concentrations (0.5-6.0 mg/L) of PGRs (2, 4-D/IBA/BAP/KIN) alone and also NAA in combination with BAP. Medium was adjusted to pH 5.7±1.0 either with 0.1 N HCl or 0.1 N NaOH before addition of 0.8% (w/v) Difco-bacto agar and autoclaved at 121°C (15 lbs) for 15-20 min. Medium was dispensed into different culture tubes (15 mL medium) and each tube was inoculated with one explant. These cultures were incubated at 25±1 °C for 16 h photoperiod under white fluorescents with light intensity of 40-60 μmol m^{-2} s^{-1}. The calli obtained were sub-cultured for every 3 weeks

interval on fresh MS medium supplemented with the same concentration of PGRs for further proliferation.

Data Analysis

All the experiments were conducted with a minimum of 25 replicates and each experiment was repeated at least thrice. The cultures were periodically examined and the data were statistically analyzed using IBM-SPSS 20 software. The collected data were subjected to one-way Analysis of Variance (ANOVA). Duncan multiple range tests (P≤0.05) is used for the significance of difference among the means.

RESULTS

Hypocotyl culture

The hypocotyl explants of *A. digitata* were cultured on MS medium supplemented with different concentrations (0.5-6.0 mg/L) of PGRs (2,4-D/ IBA/ BAP) alone and also in-combination with NAA+BAP (Table 1; Fig. 1a-c). The initiation of callus was observed from the 4th week of incubation in all the concentrations of PGRs used but varied in percentage of response and morphology of callus. Green compact callus was induced on MS medium fortified with all the concentrations of 2,4-D and NAA+BAP tested. The highest percentage of response (88.7±0.88) was observed at 2.0 mg/L NAA+1.0 mg/L BAP (Table1). White compact callus was induced at 4.0-6.0 mg/L BAP. Light green friable callus was developed in all the concentrations of IBA used. Shoot buds have been formed directly at 0.5 mg/L BAP whereas shoot buds with callus was found at 2.0 mg/L BAP. High amount of callogenesis was recorded at 4.0 mg/L 2,4- D/ IBA, 6.0 mg/L BAP and 3.0 mg/L NAA+ 1.0 mg/L BAP (Table 1). 0.5 mg/L BAP induced the development of shoots (45.0±1.15 shoots/explant) (Fig. 1c). While, initiation of shoots with callus was observed on MS medium supplemented with 2.0 mg/L BAP.

Cotyledonary leaf culture

The results on callus induction from cotyledonary leaf explants of *A. digitata* using MS medium augmented with different concentrations (0.5-6.0 mg/L) of PGRs (2,4-D/ IBA/ BAP/ KIN) alone and also in combination with NAA+BAP are shown in Table 2. Callus was induced from all the concentrations of PGRs used after 3-4 weeks of culture, but the frequency of response and the morphology differed according to PGRs and their concentrations used. MS medium without any PGRs (MSO) did not yield callus. Compact callus was induced in all the concentrations of 2,4-D/ BAP/ KIN alone and also in the combination of NAA with BAP tested. The highest percentage (97.6± 0.33) of response was found on MS medium fortified

with 4.0 mg/L 2,4-D with more amount of callus; followed by 2.0 mg/L 2,4-D (96.3± 0.88%) (Fig. 1d-f). Whereas, green friable callus was induced in all the concentrations of IBA tested. The green friable calli developed on IBA were sub-cultured on 4.0 mg/L IBA for 3 cycles (Fig. 1f). Later, the calli developed at 4.0 mg/L IBA after 3rd subculture, shifted on to 2.0 mg/L BAP and also 6.0 mg/L IBA+ 2.0 mg/L BAP. Even after 3 subcultures, we didn't find any shoot buds formation except the meristemoids development.

Leaf culture

The results on callus induction from leaf explants of *A. digitata* are presented in Table 3. The leaf explants were cultured on MS medium augmented with various concentrations (0.5-6.0 mg/L) of 2,4-D/ IBA/ NAA/ BAP/ KIN alone and also in combination of NAA+BAP. Callus was formed in all the concentrations of PGRs and their combinations used after 4-5 weeks of culture (Fig. 1g-i). Maximum percentage (93.7 ± 0.67) of callus induction response was found at 4.0 mg/L 2, 4-D followed by 4.0 mg/L IBA (Table 3). The texture and morphology of callus were found to be varied depending upon the type of PGRs and their concentrations tested (Fig. 1g, h). Green compact callus was developed in all the concentrations of IBA and 2.0/ 3.0 mg/L NAA+BAP (0.5-1.0 mg/L). Whereas, white-compact callus was induced at 0.5- 6.0 mg/L BAP/KIN (Fig. 1h). It was interesting to find that white-friable callus was induced in all the concentrations of 2,4-D used. The same callus was sub-cultured for 3 cycles and shifted on to MS medium augmented with 2.0 mg/L BAP for shoot buds morphogenesis. But even after 3 subcultures, further morphogenesis was absent.

Root culture

The results on callus induction from root explants of *A. digitata* are presented in Table 4. Callus was induced in all the concentrations of PGRs used and also in combination of NAA+ BAP. White compact callus was induced from root explants inoculated on MS medium supplemented with 0.5- 6.0 mg/L 2,4-D/ BAP (Fig. j-l). MS medium supplemented with 2.0/3.0 mg/L NAA+BAP combination and 0.5-6.0 mg/L KIN alone induced light green-compact textured callus after 4-5 weeks of incubation. The highest percentage (97.0±0.00) of response was observed at 3.0 mg/L NAA+ 0.5 mg/L BAP. The least percentage of response (59.3±0.67) was observed at 0.5 mg/L BAP. White-friable callus was induced at 0.5- 6.0 mg/L IBA (Fig. 1j) which was sub-cultured thrice on fresh medium at 2.0 mg/L IBA for further proliferation. The same calli pieces were subcultured for 2 cycles on MS+ 2.0 mg/L

BAP, but we didn't find any further morphogenic event for organogenesis.

DISCUSSION

In the present investigation, the efficacy of different concentrations of PGRs (2,4-D/IBA/BAP/KIN) alone and also NAA in combination with BAP on callus induction from four different explants viz., hypocotyl, cotyledonary leaf, leaf and root of *A. digitata* was studied. The cut ends of the explants initiated the formation of callus after 2 weeks of inoculation. The texture of the callus obtained was found to be compact, friable, yellow, green and white. The results recorded differed depending on the type of explant and the PGRs used.

Among the four explants used, cotyledonary leaf explants inoculated on MS medium supplemented with 2, 4-D/IBA/BAP/KIN (2.0-4.0 mg/L) showed maximum response with high amount of callus development in comparison to all other explants cultured. This was followed by root, leaf and hypocotyl explants. Almost all the explants responded well for callus induction but only hypocotyl explants induced organogenesis on MS medium augmented with 0.5 mg/L/ 2.0 mg/L BAP. These explants with shoot buds were sub-cultured on MS medium fortified with 0.5 mg/L IBA+ 0.5 mg/L BAP for further proliferation and elongation of shoots.

BAP is reported to be the best over other cytokinins in many studies **[19-23]**. BAP (2.0 mg/L) in combination with NAA (10.0 mg/L) to induce 90% of callus from leaf explants and it was also reported to be the best for nodal, leaf and hypocotyl explants of *A. digitata* **[24]**. In the present investigations, KIN was observed to induce compact callus in all the concentrations (0.5-6.0 mg/L) used but varied according to the explants used. White compact callus was induced from leaf explants whereas, green compact callus was developed from cotyledonary leaf and root explants.

In an exceptional case, it is interesting to note that, beside white callus, the formation of roots from leaf explants was also observed rarely on MS medium fortified with 0.5 mg/L KIN in *A. digitata* (Fig. 1i). This favors the study which reported a high concentration of KIN to inhibit root formation and in low concentration it allows the formation of roots **[25]**. A similar observation was also reported where less concentration of KIN in combination with NAA to yield roots from leaf explants of *Ocimum sanctum* **[26]**.

Exogenous supply of auxins is reported to be essential for an explant to induce callus but often this condition varies with the species and the type of explant used as there is a need to supply cytokinin for the induction of callus in some species **[27]**. The PGRs and their

preferred concentration also act as a catalyst in promoting the induction of callus. Whereas in the present investigation, 2,4-D is observed to produce a maximum percentage of response with a high amount of callus yield of *A. digitata*. A similar observation was also reported where at high concentration of 3.0–4.0 mg/L 2,4-D of *S. torvum* **[28]**.

White compact callus was obtained from cotyledonary leaf explants on MS medium supplemented with 2,4-D. The obtained callus was sub-cultured thrice with a time interval of 3 weeks on the same fresh medium. Change in the color and texture of callus to light green friable was observed but organogenesis was not observed.

Auxins like IBA and NAA used in the present study also favored the induction of green calli. The cotyledonary leaf explants inoculated on MS medium amended with IBA (0.5-6.0 mg/L) alone yielded green friable calli but did not support organogenesis. Light green friable callus was obtained from hypocotyl explants incubated on MS medium with IBA (0.5-6.0 mg/L). White friable and green compact callus was observed from root and leaf explants respectively.

In the present investigation, the callus obtained was sub-cultured thrice on the same fresh medium supplemented with the same concentration of auxins and cytokinins, but we didn't find any morphogenic event for organogenesis. Besides this, browning of callus was also not observed from any explant in our study. Hence, the concentration of NaCl used in MS medium is satisfactory to avoid salt stress.

Thus, the present study concludes that the cotyledonary leaf explants were found to be the best in the percentage of response for callus induction which is followed by root, leaf and hypocotyl explants. In terms of PGRs used, 2, 4-D is found to be the best auxin in yielding high amount of callus and followed by BAP and IBA.

From the foregoing discussion, it is evident that the different explants were cultured on the same concentration of cytokinin and auxin-cytokinin combinations showed varied results in the same species for induction of callus. Though we have tried to develop the callus mediated regeneration protocol from these different explants using various PGRs and their combinations, it was quite difficult to induce the shoot buds organogenesis in *A. digitata* except the formation of meristemoids. The protocol developed for induction of callus is useful for isolation of pharmaceutically important bioactive compounds from different parts of *A. digitata* inview of its medicinal importance.

Table 1: Effect of different PGRs on *in vitro* callus induction from hypocotyl explants of *A. digitata*.

Conc. of PGRs (mg/L)	No. of days for Callus induction (±SE)^{a}	Response (%) (±SE)^{a}	Morphology of callus	Amount of callus
MSO	-	-	-	-
2,4-D				
0.5	42.0±0.00^{cd}	66.0±0.58^{g}	Green compact	+
2.0	36.3±0.67^{h}	84.3±0.67^{bc}	Green compact	++
4.0	30.3±0.88^{de}	91.7±0.33^{ef}	Green compact	++
6.0	47.7±0.67^{a}	61.7±0.88^{h}	Green compact	++
IBA				
0.5	45.0±0.00^{b}	73.7±0.67^{e}	Light-green friable	++
2.0	38.0±0.58^{g}	86.3±1.33^{b}	Light-green friable	++
4.0	41.0±0.58^{cde}	72.7±0.33^{ef}	Light-green friable	+++
6.0	42.0±0.00^{cd}	48.3±1.67^{j}	Light-green friable	++
BAP				
0.5	27.7±0.67^{j}	45.0±1.15^{k}	Shoots	–
2.0	33.3±0.67^{i}	43.3±0.88^{k}	Shoots*	+
4.0	34.3±0.33^{i}	65.7±0.67^{g}	White compact	++
6.0	44.3±0.67^{b}	59.3±0.67^{i}	White compact	+++
NAA BAP				
2.0 0.5	38.7±0.67^{fg}	70.7±0.67^{f}	Green compact	++
2.0 1.0	38.3±0.33^{g}	88.7±0.88^{a}	Green compact	++
3.0 0.5	41.7±0.88^{cd}	83.7±0.67^{c}	Green compact	++
3.0 1.0	44.0±0.58^{b}	73.3±0.67^{e}	Green compact	+++

(±SE)^{a} - ^{a}Mean±Standard Error; *with callus; + Low; ++ Moderate; +++ High

Table 2 Effect of different PGRs on *in vitro* callus induction from cotyledonary leaf explants of *A. digitata*.

Conc.of PGRs (mg/L)	No. of days for callus induction (±SE)^{a}	Response (%) (±SE)^{a}	Morphology of callus	Amount of callus
2,4-D				
0.5	31.6±0.33^{ij}	67.3±0.33^{f}	White compact	++
2.0	30.6±0.33^{jk}	96.3±0.88^{a}	White-compact	+ ++
4.0	34.0±1.00^{gh}	97.6±0.33^{a}	White compact	++++
6.0	33.6±0.88^{gh}	81.6±1.67^{b}	Greenish-yellow compact	++
IBA				
0.5	32.6±0.33^{hi}	50.3±0.88^{k}	Yellowish green friable	+
2.0	34.0±0.00^{gh}	60.3±1.45^{h}	Light green friable	+++
4.0	29.6±0.33^{k}	75.0±0.58^{c}	Light green friable	+++
6.0	36.6±0.33^{de}	53.0±0.00^{j}	Green friable	+
BAP				
0.5	36.0±0.58^{ef}	63.0±0.00^{g}	White-green compact	++
2.0	34.6±0.88^{fg}	70.6±0.67^{de}	White-green compact	+++
4.0	35.3±0.33^{efg}	81.0±0.58^{b}	Green compact	+++
6.0	39.3±0.33^{bc}	72.6±0.33^{d}	Green compact	++
KIN				
0.5	37.0±0.58^{de}	56.3±0.33^{i}	Green compact	+
2.0	32.6±0.67^{hi}	70.0±0.00^{e}	Green compact	++++
4.0	37.0±0.58^{de}	66.0±0.58^{f}	Green compact	+++
6.0	36.6±0.33^{de}	49.6±0.33^{k}	Green compact	++
NAA BAP				
2.0 0.5	41.6±0.88^{a}	60.3±0.33^{h}	White compact	++
2.0 1.0	36.0±0.58^{ef}	71.0±0.58^{de}	White compact	+++
3.0 0.5	38.0±0.00^{cd}	67.6±0.33^{f}	Light green compact	++
3.0 1.0	40.6±0.67^{ab}	59.0±0.58^{h}	Light green compact	++

(±SE)^{a} - ^{a}Mean±Standard Error; *with callus; + Low; ++ Moderate; +++ High

Table 3: Effect of different PGRs on *in vitro* callus induction from leaf explants of *A. digitata*.

Conc. of PGRs (mg/L)	No. of days for callus induction (±SE)^{a}	Response (%) (±SE)^{a}	Morphology of callus	Amount of callus
2,4-D				
0.5	33.7±0.88^{def}	72.3±0.33^{f}	White friable	++
2.0	31.3±0.67^{gh}	84.0±0.00^{c}	White friable	+++
4.0	32.0±0.00^{fg}	93.7±0.67^{a}	White friable	+++
6.0	36.3±0.33^{bc}	71.7±0.33^{f}	White friable	+++
IBA				
0.5	40.7±0.67^{a}	64.7±0.88^{h}	Green compact	++
2.0	36.7±0.33^{b}	72.3±0.33^{f}	Green compact	+++
4.0	34.7±0.33^{d}	93.0±0.58^{a}	Green compact	+++
6.0	37.0±0.00^{b}	84.7±0.33^{c}	Green compact	+++
BAP				
0.5	39.3±0.67^{a}	69.0±0.58^{g}	White compact	+
2.0	34.3±0.33^{de}	88.3±1.20^{b}	White compact	++
4.0	35.0±0.00^{cd}	75.0±0.00^{e}	White compact	+++
6.0	33.7±0.88^{def}	59.3±0.67^{ij}	White compact	++
KIN				
0.5	32.7±0.67^{efg}	58.0±0.00^{j}	White compact	+
2.0	30.3±0.88^{hi}	70.0±1.15^{g}	White compact	++
4.0	37.0±0.00^{b}	60.0±0.00^{i}	White compact	++
6.0	37.0±0.58^{b}	58.7±0.67^{ij}	White compact	+
NAA BAP				
2.0 0.5	34.3±0.33^{de}	60.3±0.33^{i}	Green compact	++
2.0 1.0	29.3±0.67^{ij}	80.7±0.33^{d}	Green compact	+++
3.0 0.5	28.7±0.67^{j}	65.7±0.33^{h}	Green compact	+++
3.0 1.0	32.7±0.33^{efg}	50.0±0.00^{k}	Green compact	++

(±SE)^{a} - ^{a}Mean±Standard Error; + Low; ++ Moderate; +++ High

Table 4 Effect of different PGRs on *in vitro* callus induction from root explants of *A. digitata*.

Conc. of PGRs (mg/L)	No. of days for callus induction (±SE)^{a}	Response (%) (±SE)^{a}	Morphology of callus	Amount of callus
2,4-D				
0.5	29.7±0.33^{ij}	70.7±0.67^{ghi}	White compact	++
2.0	29.3±0.33^{ij}	87.3±0.88^{d}	White compact	+++
4.0	27.0±0.00^{k}	94.7±0.88^{b}	White compact	+++
6.0	32.7±0.33^{ef}	65.3±0.88^{j}	White compact	++
IBA				
0.5	34.0±0.00^{d}	67.7±0.67^{i}	White friable	++
2.0	31.3±0.33^{gh}	90.0±0.00^{c}	White friable	+++
4.0	33.0±0.58^{def}	80.7±0.33^{e}	White friable	+++
6.0	37.0±0.58^{b}	64.3±0.33^{j}	White friable	++
BAP				
0.5	32.3±0.33^{fg}	59.3±0.67^{k}	White compact	++
2.0	28.7±0.67^{j}	80.7±0.67^{e}	White compact	+++
4.0	30.3±0.33^{hi}	93.7±0.67^{b}	White compact	+++
6.0	32.3±0.33^{fg}	77.7±1.45^{f}	White compact	++
KIN				
0.5	35.3±0.33^{c}	68.7±0.88^{hi}	Light green compact	+
2.0	32.0±0.00^{fg}	70.0±0.00^{ghi}	Light green compact	+++
4.0	33.7±0.88^{de}	91.0±0.58^{c}	Light green compact	+++
6.0	37.3±0.33^{b}	82.7±1.45^{e}	Light green compact	++
NAA BAP				
2.0 0.5	40.0±0.00^{a}	76.3±0.88^{f}	Light green compact	++
2.0 1.0	37.3±0.33^{b}	90.0±1.20^{b}	Light green compact	+++
3.0 0.5	32.7±0.33^{ef}	87.0±0.00^{a}	Light green compact	+++
3.0 1.0	33.0±0.00^{def}	72.3±0.67^{ghi}	Light green compact	+

(±SE)a - aMean±Standard Error; + Low; ++ Moderate; +++ High

Figure 1a-l: ***In vitro*** **callus induction (Callogenesis) from different explants of** ***A. digitata***
a-c) Hypocotyl explants: a) Induction of white compact callus on MS+6.0 mg/L BAP, b) Green compact callus on MS+4.0 mg/L 2,4-D, c) Initiation of shoot buds on MS+0.5 mg/L BAP; d-f) Cotyledonary leaf explants: d) White-green compact callus on MS+ 2.0 mg/L BAP, e) White callus induced on MS+0.5 mg/L 2,4-D, f) MS+ 4.0 mg/L IBA induced light green friable callus; g-i) Leaf explants: g) Green compact callus developed on MS+2.0 mg/L NAA+1.0 mg/L BAP, h) White compact callus formed on MS+2.0 mg/L KIN, i) Exceptional case of Induction of root from leaf explant on MS+ 0.5 mg/L KIN; j-l) Root explants: j) Induction of white friable callus on MS+2.0 mg/L IBA, k) Light-green callus formed on MS+ 4.0 mg/L KIN, l) White compact callus developed on MS+ 4.0 mg/L 2,4-D.

CONCLUSIONS

The present protocol developed for inducing callus and proliferation from different explants can be used for mass multiplication of the species and also for isolation of pharmaceutically important compounds. Thus, the success of callus induction protocol developed in the present investigations can be used to isolate bioactive molecules from *A. digitata* which are in therapeutic uses. All these pharmaceutically important compounds can be isolated through cell suspension culture techniques.

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AUTHOR'S CONTRIBUTION

SN and RM, were carried out experiments, evaluated the data and wrote the manuscript. The research was planned by RN, who also oversaw, edited, and reviewed the paper. The manuscript has been viewed and approved final version by all authors and is now ready for publishing.

CONFLICT OF INTEREST

The authors declare there is no conflict of interest concerning the publication of the manuscript.

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