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Callus Induction and Plant Regeneration of Hyoscyamus Niger (In Vitro)

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Abstract

Black henbane, also known as Hyoscyamus niger, is a herb that is a member of the Solanaceae family and is thought to contain a natural reservoir of secondary metabolites. We made an effort to demonstrate a method for callus induction and plantlet regeneration. In both light and dark environments, callus induction from (leaf, stem, and root) was studied using (MS) medium with various doses of BA or NAA (0, 0.5, 1, 1.5, and 2 mg/l). Our findings were as follows, in brief: in light condition, the Leaf explant produced considerably significant more callus induction than other explants, with high overall mean fresh and dry weights (77.2%, 745.3, and 46.1 mg, respectively). Regarding the interfering effects of the BA and NAA concentrations, the combination of (1.5 + 1 mg/l) BA+ NAA was chosen as the maintenance medium because it considerably produced the highest value of mass weight of callus originating from leaves in light circumstances (1119, 78.7 mg), respectively. If a stem Since treatment BA and NAA (0.5 + 1.5 mg/l) resulted in the highest significant fresh and dry weight values (692.2, 41.2 mg), they were selected as the maintenance medium under light conditions. The Leaf explant clearly outperformed the other explants in terms of callus induction, with a high significant total mean of fresh and dry weight visible in dark (68.8 percent , 608 and 38.9 mg, respectively). The combination of (1.5 + 1.5 mg/l) BA+ NAA significantly generated the maximum fresh and dry weight of callus coming from leaves (1242.5, 77.8 mg), and the medium chosen as a maintenance medium. stem The combination of BA and NAA at (0.5, 1 mg/l) was chosen as a maintenance medium because it produced a significant maximum value of fresh and dry weight (408.8, 18.8 mg, respectively) in dark conditions. Organogenesis was acquired by growing a callus from leaf explants in maintenance medium with a 2 mg/l BA concentration, yielding a high success rate and a substantial number of shoots (90 % , 18 shoots, respectively). With 2 mg/l of IBA, the optimal rooting rate 60 % was achieved at a long (3 cm) root length and root number (4.6 roots) .

Keywords

Hyoscyamus Niger. Black henbane, in vitro, callus induction, organogenesis, rooting.

Native to the temperate regions of Europe, Western and Northern Asia, and Northern Africa, H. niger is a biennial herb belonging to the Solanaceae family (1). It can reach heights of 0.3-0.7 metres and has grayish-green leaves, white or pale yellow blooms with purplish veins, and dark grey seeds, all of which contribute to its well-known traditional use as a hallucinogen (2). Medicinal uses for H. niger include the treatment of stress and memory-related conditions like Alzheimer's disease (4). Also, it shows great promise for use in cancer therapy (5). Plant growth regulators and the kind of explant play important roles in the process of inducing callus in vitro because different species and types of explants require different combinations and

concentrations of exogenous application of plant growth regulators (6). Experts agree that H. niger leaf explants produced the best results for the induction callus, and that using these explants allowed for the generation of the greatest amount of callus biomass at both KN and NAA (7). Callus development was most successful with leaf explants at NAA (12 uM) + Kn (1.5 uM), although stem (8) growth was not detected, as reported by Shah et al. Root explants cultured on MS media supplemented with (1.0 mg/l) BA and (0.5 mg/l) NAA produced the most callus after two months. (9). Maximum callus induction (percent 100) from H. niger leaf explants was observed in MS medium containing (2 mg/L NAA, 2 + 0.5 mg/L) NAA with BA , (2 + 1 mg/L) NAA with BA, and (2 + 2 mg/L)NAA with BA (10). Datura leaf explants developed the most shoots from callus when cultured in MS medium with (1mg/l) 2,4-D and Kin. Growing explant of Datura metel in MS-medium enriched with (1 mg/l) of NAA and BA produced the best callus development results (11). Marvin et al. showed that MS media with IBA (0.5 mg / l) added to shoot medium can be used for H. niger root cultivation (12). The average number of roots, root length, and responsiveness of H. niger (13) were all enhanced when the media was supplemented with growth regulators (0.5 mg/l IBA and 1 mg/l BA) from the modified MS formulation. Some laboratory experiments demonstrated that adding (0.5 mg/l) IBA to MS media aided in the root development of young plants (14). The current study aims to determine the optimal PGR concentration and combination for organogenesis and rooting of newly emerging plants, as well as for the best callus production from different H. niger explants under different conditions.

Materials and methods

Seeds germination

Seeds of H. niger were soaked with (GA_3) in (250 mg /l) for 48 h at room temperature (15).

preparation Culture media

For inducing calluses, regenerating them, and finally rooted them, we used MS medium, which we made by dissolving sucrose and various concentrations of growth regulators like BA and NAA (0, 0.5, 1, 1.5, and 2 mg/l). Agar was added at a concentration of 0.7%, and the mixture's pH was adjusted to fall in the range of 5.7 to 5.8. After that, was placed in an autoclave and subjected to steam at a temperature of 121 degrees Celsius for 15 minutes.

Seed sterilization and plantation

Soaking seeds in GA3 were sterilised under aseptic circumstances by immersing them for 30 seconds in 95 percent ethyl alcohol, then 10 minutes in 2 percent sodium hypochlorite with a few drops of tween-20 (16). They were then washed three to five times in distilled water (under Laminar airflow). sterile seedlings were culture In an incubator, were fed MS media (17) and subjected to a (16-hour light/8-hour dark) cycle at 3000 lux. H. niger explants were obtained from in vitro-grown seedlings (18).

Callus induction

Different concentrations of BA and NAA were used to graft different types of explants (leaf, stem, and root) onto MS media sources. Each treatment was incubated at 25 $\pm$ 2 C^o degrees for 5 weeks, with either of two different light circumstances dark and incubators condition.

Measurement of percentage, fresh and dry weights of initiated callus

After five weeks in incubation, the callusing rate (in percent) was recorded. Dry mass was calculated by first weighing a starting callus fresh, then drying it at (45 °C for 24 hours in a laboratory oven) (19).

Regeneration media

MS was exposed to regeneration media containing varying doses of BA only (0, 0.5, 1, 1.5, and 2 mg/l) and BA+NAA (0, 0.5, 1, 1.5, and 2 mg/l) for comparison. For four weeks, the finest possible callus was grown in an incubator on regeneration media at 25 $\pm$ 2 C^o .

Rooting media

Lab-grown shoots were separated from their shoot proliferation cluster and planted in a container containing MS culture media. adding plant hormones (IAA, NAA, IBA) to the MS medium at (1, 2, 3, 4 mg/l). After four weeks in optimal conditions, the rooting rate, root number, and root length were evaluated.

Statistical analysis

ANOVA was performed on the gathered data, and the differences in treatment means were evaluated at the 5 percent level using LSD

and Duncan tests (20). A completely randomised design (CRD) was utilised for the experiments (21).

Results and discussion

Selection of explants and culture condition in percentage of callus induction

Callus development rates in light and dark environments for the three explant types are shown in Table (1). Callus tissue development was inhibited in the control group regardless of environments. Leaf explants formed a callus at a rate of 77.2% on average in the light environment, while stem explants formed a callus at a rate of only 54.8% on average. Leaves explants under dark conditions induced a callus at a rate of 68.8 percent, significant higher than the rate of 47.2 percent for stem explants. Possibly an internal shortage in hormones that limit stimulation and production of callus tissue explains the lack of callus induction in transplanted explants on a growth regulator-free media (control treatment) (22). Based on the data, we determined that the plant's roots did not respond to either light or darkness by developing calluses, thus we did not include them in the subsequent studies. Callus formation requires variable amounts of time to get going depending on the explant and lighting a period. It took around two weeks for the leaves about two weeks , but it took about four to five weeks for the stems. The callus tissue was compact in texture and white-green in colour in both light and darkness period .

Table 1. The percentage (%) of callus induction from different explant H. niger in light and dark conditions.

Media code	PGRs (mg/l)		Callus induction (%)					
	BA	NAA	light			Dark		
			leaf	Stem	Root	leaf	Stem	Root
M1	0	0	0	0	0	0	0	0
M2	0	0.5	30	50	0	40	60	0
M3	0	1	70	40	0	60	60	0
M4	0	1.5	40	40	0	60	40	0
M5	0	2	40	20	0	100	20	0
M6	0.5	0	80	70	0	80	70	0
M7	0.5	0.5	70	80	0	100	60	0
M8	0.5	1	80	80	0	80	60	0
M9	0.5	1.5	80	80	0	60	60	0
M10	0.5	2	60	50	0	100	40	0
M11	1	0	100	80	0	80	90	0
M12	1	0.5	100	30	0	100	40	0
M13	1	1	100	60	0	40	30	0
M14	1	1.5	100	40	0	40	20	0
M15	1	2	100	30	0	40	20	0
M16	1.5	0	60	80	0	60	70	0
M17	1.5	0.5	100	100	0	100	80	0
M18	1.5	1	100	80	0	80	80	0
M19	1.5	1.5	90	50	0	80	50	0
M20	1.5	2	90	50	0	80	40	0
M21	2	0	80	80	0	100	70	0
M22	2	0.5	90	80	0	60	40	0
M23	2	1	90	40	0	60	30	0
M24	2	1.5	90	40	0	60	30	0
M25	2	2	90	20	0	60	20	0
Total Mean			77.2 a	54.8 b	0 c	68.8 a	47.2 b	0 c
			Significant			Significant		
LSD			LSD = 12.2			LSD= 11.4		

H. niger explants grown in light or dark settings exhibited different rates of callus induction and showed differences in callus shape and pigmentation. Different explants may have responded differently to the plant hormones used in the research due to inherent genetic variances (23). Variations in explant type, totipotency of cells from different explants, quantity of dividing cells, and hormone content within cells may all contribute to the observed variation in the success rate of inducing callus from several explants (24). Multiple investigations have shown that H. niger leaf explants are superior than stem

explants for creating callus. This is because of the cells' rapid responsiveness to PGRs and their tolerance of tissue culture conditions, as well as their genetic and tissue nature and adequate hormone content in connection to the PGRs' type and concentration (25). In contrast, under both light and dark conditions, no callus was induced from root explants, probably because the cells were differentiating and not dividing or may be needs longer time for division and growth (26). This may be because of the micronutrient and hormone levels in the medium, as well as the type of tissue, its degree of totipotency, and the composition of the medium (27).

Influence light and dark conditions on fresh and dry weight of callus

As can be seen in Table 2, the explant used had a major impact on the average fresh weight of the induced callus. The total mean fresh weight of callus was greatest significant (745.3 mg) in the leaf explant under light conditions, whereas it was least in the stem explant (311.0 mg). In a similar vein, the leaf explant substantially outperformed the other explants in dark settings, with a total mean fresh weight of 608 mg. In contrast, the stem explants had the lowest significant total mean, at 247.8 mg.

Table 2. The fresh weights of callus initiated from H. niger explants in light and dark conditions.

Media code	PGRs (mg/l)		Callus induction fresh W.			
	BA	NAA	Light		Dark	
			leaf	Stem	leaf	Stem
M1	0	0	0	0	0	0
M2	0	0.5	522.6	250.4	435.2	262.2
M3	0	1	720.4	257.0	409.6	166.6
M4	0	1.5	361.4	209.8	215.2	148.8
M5	0	2	373.6	172.8	232.6	132.2
M6	0.5	0	631.7	321.0	665.4	210.8
M7	0.5	0.5	873.4	500.4	881.0	402.8
M8	0.5	1	885.0	595.4	769.8	408.8
M9	0.5	1.5	830.2	692.2	683.8	379.2
M10	0.5	2	834.3	537.8	209.2	289.6
M11	1	0	796.0	331.6	650.0	201.8
M12	1	0.5	929.9	363.6	810.4	326.6
M13	1	1	949.3	463.4	712.8	378.4
M14	1	1.5	831.4	264.2	645.5	285.4
M15	1	2	747.4	220.0	500.6	249.8
M16	1.5	0	729.3	353.2	540.5	211.4
M17	1.5	0.5	939.3	371.8	1043.2	301.4
M18	1.5	1	1119	335.2	1011.4	280.2
M19	1.5	1.5	873.3	272.8	1242.5	218.2
M20	1.5	2	736.0	245.4	651.0	192.8
M21	2	0	716.3	311.6	563.2	132.0
M22	2	0.5	842.6	236.4	644.8	291.8
M23	2	1	879.2	160.8	594.0	285.4
M24	2	1.5	788.3	158.0	600.4	224.8
M25	2	2	723.1	150.2	489.0	214.2
Total Mean			745.3 a	311 b	608 a	247.8 b
			LSD = 93.2		LSD = 98.7	

The callus had the same dry weight as its wet weight. In contrast to the stem explants, which averaged 23.8 mg in dry weight under light, the mean dry weight of the leaf explants was 46.1 mg (16.9 mg). The mean dry weight of the leaf explants was formed significant 38.9 mg in the dark, but the mean dry weight of the stem explants was only 15.7 mg (12.4 mg) Table 3. the results Possible explanations for this finding include differences in the hormone content, metabolism, and maturation levels of the H. niger explants used (28) and the composition of the tissue itself (29). Each of these determinants reflects a unique physiological response that shifts in intensity according to the amount of exogenous PGRs that is

supplemented (30). The higher proportion of callus induction in the leaves compared to the percentage of callus induction in the stem (Table 1) may explain why the fresh and dry weight of the leaves increases in both light and dark circumstances.

Table 3. The Dry weights of callus initiated from H. niger explants in light and dark conditions.

Media code	PGRs (mg/l)		Callus induction Dry W.			
	BA	NAA	Light		Dark	
			leaf	Stem	leaf	Stem
M1	0	0	0	0	0	0
M2	0	0.5	33.6	13.2	26.6	13.2
M3	0	1	45.2	13.2	22.6	9.8
M4	0	1.5	20.6	12.6	17.0	10.2
M5	0	2	22.8	11.2	17.4	8.8
M6	0.5	0	40.7	13.2	37.4	10.8
M7	0.5	0.5	54.5	33.2	56.2	19.4
M8	0.5	1	54.6	36.6	45.4	18.8
M9	0.5	1.5	54.8	41.2	41.8	15.8
M10	0.5	2	56.8	32.2	15.2	14.6
M11	1	0	43.2	14.4	38.6	10.6
M12	1	0.5	54.8	16.2	54.2	15.2
M13	1	1	59.6	28.8	42.4	16.6
M14	1	1.5	53.2	15.6	40.0	13.8
M15	1	2	46.4	13.8	37.0	12.2
M16	1.5	0	42.6	14.6	37.2	11.2
M17	1.5	0.5	58.5	15.2	70.6	12.8
M18	1.5	1	78.7	14.6	72.6	14.2
M19	1.5	1.5	57.6	13.1	77.8	13.0
M20	1.5	2	42.2	12.4	39.2	11.4
M21	2	0	39.4	13.4	37.3	9.2
M22	2	0.5	52.2	11.8	42.4	13.0
M23	2	1	54.2	11.6	40.2	12.8
M24	2	1.5	46.8	11.4	40.6	11.8
M25	2	2	41.6	11.2	24.0	10.8
Total Mean			46.1 a	16.9 b	38.9 a	12.4 b
			LSD = 6.1		LSD = 6.2	

Interfering effect of BA and NAA concentrations in the fresh and dry weight of callus arising from leaves in light and dark conditions.

As can be shown in Table 4, the concentrations of both BA and NAA had a major impact on the fresh and dry weight of the callus generated from the leaf under light conditions. Compared to treatments without auxin (0.0 mg/l NAA), those with 1 mg/l NAA had significantly larger total mean fresh and dry weight (910.6 and 58.5 mg,) respectively (574.7 and 33.2 mg) respectively. Mean fresh and dry weights were highest significant in the 1.5 mg/l BA treatment (879.4 and 55.9 mg, respectively), and lowest significant in the cytokinin-free control group (0.0 mg/l BA) (395.6 and 24.4 mg, respectively). The combination (1.5 +1 mg/l) BA+ NAA respectively significantly produced the greatest value of mass weight (1119 ,78.7 mg) respectively which chosen as maintenance medium in this condition. While a fresh and dried weight of 361.4 and 20.6 mg, respectively, under light conditions , the combination of 0 + 1.5 mg/l BA + NAA was deemed insufficient as a growth medium.

Table 4. Effect of BA and NAA concentrations in fresh and dry weight (mg) of callus tissue arising from leaves in light conditions

Fresh weight (mg)						
PGR (mg/l)	NAA concentration					
BA concentration	0	0.5	1	1.5	2	Mean
0	0.0	522.6	720.4	361.4	373.6	395.6
0.5	631.7	873.4	885.0	830.2	834.3	810.9
1	796.0	929.9	949.3	831.4	747.4	850.8
1.5	729.3	939.3	1119.0*	873.3	736.0	879.4
2	716.3	842.6	879.2	788.3	723.1	789.9
Mean	574.7	821.6	910.6	736.9	682.9	---
LSD: BA: 71.94, NAA: 71.94 , BA x NAA: 129.63						
Dry weight (mg)						
PGR (mg/l)	NAA concentration					
BA concentration	0	0.5	1	1.5	2	Mean
0	0	33.6	45.2	20.6	22.8	24.4
0.5	40.7	54.5	54.6	54.8	56.8	52.3
1	43.2	54.8	59.6	53.2	46.4	51.4
1.5	42.6	58.5	78.7 *	57.6	42.2	55.9
2	39.4	52.2	54.2	46.8	41.6	46.8
Mean	33.2	50.72	58.5	46.6	42.0	---
LSD: BA: 6.07 , NAA: 6.07 , BA x NAA: 11.45						

Total fresh weight (762.9) and dry weight (50.0 mg) were substantially higher when treatments with (0.5 mg/l) NAA were used in dark conditions significantly when the NAA concentration was increased above 0.5, mg / l both the wet and dry weights dropped dramatically: to (416.5) to (26.6) mg at a concentration of 2 mg/l respecting (as shown in Tables 5). The overall mean fresh and dry weights (897.7 and 59.5 mg) were substantially higher significantly when BA concentrations were (1.5 mg/l). The mean fresh and dry weights were considerably lower significantly in BA-free treatments (258.5 and 16.7 mg, respectively). The best wet and dry mass values significantly were achieved by combining BA and NAA at a concentration of (1.5 + 1.5 mg/l), yielding 1242.5 and 77.8 mg, respectively. Because of its unique properties including its high fresh and dry weight, quick induction time, and tolerance of maintenance conditions this mixture was chosen as the maintenance medium (shown in Figure 1-A). On the other hand, the lowest significant value was found at (0.5 + 2) mg/l BA + NAA (209.2, 15.2 mg) (as shown in Table 5).

Table 5. Effect of BA and NAA concentrations in fresh and dry weight (mg) of callus tissue arising from leaves in dark conditions

Fresh weight (mg)						
PGR (mg/l)	NAA concentration					
BA concentration	0	0.5	1	1.5	2	Mean
0	0.0	435.2	409.6	215.2	232.6	258.5
0.5	665.4	881.0	769.8	683.8	209.2	641.8
1	650.0	810.4	712.8	645.5	500.6	663.9
1.5	540.5	1043.2	1011.4	1242.5	651.0	897.7
2	563.2	644.8	594.0	600.4	489.0	578.3
Mean	483.8	762.9	699.5	677.5	416.5	---
LSD: BA: 102.53, NAA: 10.53 , BA x NAA: 182.33						
Dry weight (mg)						
PGR (mg/l)	NAA concentration					
BA concentration	0	0.5	1	1.5	2	Mean
0	0.0	26.6	22.6	17.0	17.4	16.7
0.5	37.4	56.2	45.4	41.8	15.2	39.2
1	38.6	54.2	42.4	40.0	37.0	42.4
1.5	37.2	70.6	72.6	77.8	39.2	59.5
2	37.2	42.4	40.2	40.6	24.0	36.9
Mean	30.1	50.0	44.6	43.4	26.6	
LSD: BA: 6.44 * , NAA: 6.44 * , BA x NAA : 10.98						

The fresh and dry weight of callus grown from leaf tissue showed that the fresh weight increased as the BA concentration rose, with the optimal concentration being reached. This could be because cytokinins produce a dramatic increase in cell division, especially in meristematic cells, which causes various plant tissues size (31). One possible explanation for the correlation between auxin concentration and greater callus induction and fresh weight is that auxins boost enzyme efficiency, which in turn stimulates more cell division and callus formation (32). When auxin is added to culture media at quantities above the optimum, it may interfere with the activity of enzymes responsible for forming cellular walls. This affects the mechanical properties, which influences cell division and callus development, which influences the fresh and dried weight of the callus (33). What permits for healthy callus development in the culture media is the delicate equilibrium between the two growth hormones Auxin and cytokinin. Callus growth is slowed if one is prioritised over the other, which has consequences for both wet and dry weights (34).

Interfering effect of BA and NAA concentrations in the fresh and dry weight of callus arising from stems in light and dark conditions.

The results of PGRs under dark condition levels are shown in Table (6). The total mean fresh and dry weights were 362.4 and 21.0 mg, respectively, for the treatments containing (1 mg/l) NAA, while they were only 263.5 and 11.1 mg, respectively, for the treatments without NAA. Both the wet and dry weights of the 1.5 and 2 mg/l NAA treatments showed no discernible difference from the free NAA treatment. The total mean fresh and dry weight was considerably higher significantly in the (0.5 mg/l) BA treatment (529.4 and 31.3 mg, respectively), whereas it was significantly lower in the free BA treatments (178.0 and 10.0 mg, respectively). Fresh and dry weight were significantly reduced when BA concentrations were increased over 0.5 mg/l. Since the growing callus tissue was healthy and did not necessitate rapid cultures, the

treatment (0.5 + 1.5 mg/l) BA and NAA was selected as the maintenance medium for stem. It resulted in significantly larger fresh and dry weight (692.2, 41.2 mg), respectively. BA and NAA both had the lowest significant value at 2 mg/l for both the wet and dry weights (150.2 and 11.2 mg), respectively.

Table 6. Effect of BA and NAA concentrations in fresh and dry weight (mg) of callus tissue arising from stems in light conditions

Fresh weight (mg)						
PGRs(mg/l)	NAA concentration					
BA concentration	0	0.5	1	1.5	2	Mean
0	0.0	250.4	257.0	209.8	172.8	178.0
0.5	321.0	500.4	595.4	692.2	537.8	529.4
1	331.6	363.6	463.4	264.2	220.0	328.6
1.5	353.2	371.8	335.2	272.8	245.4	315.7
2	311.6	236.4	160.8	158.0	150.2	203.4
Mean	263.5	344.5	362.4	319.4	265.2	---
LSD: BA: 68.24, NAA: 68.24 , BA x NAA: 109.73						
Dry weight (mg)						
PGRs(mg/l)	NAA concentration					
BA concentration	0	0.5	1	1.5	2	Mean
0	0	13.2	13.2	12.6	11.2	10.0
0.5	13.2	33.2	36.6	41.2	32.2	31.3
1	14.4	16.2	28.8	15.6	13.8	17.8
1.5	14.6	15.2	14.6	13.1	12.4	14.0
2	13.4	11.8	11.6	11.4	11.2	11.9
Mean	11.1	17.92	21.0	18.8	16.2	---
LSD: BA: 5.31 , NAA: 5.31 , BA x NAA: 9.02						

Table (7) shows that the fresh and dry weights of callus grown from stem explants were significantly affected by the concentrations of BA and NAA used. In terms of both wet and dry weights, the treatment with 0.5 mg/l of NAA yielded the best results (mean values of 317.0 and 14.7 mg, respectively). In contrast, the total mean of fresh and dry mass was lowest significantly in the auxin-free regimens (151.2 and 8.2 mg, respectively). Compared to other treatments, the mean fresh weight (338.2 mg) and dry weight (15.9 mg) of those given BA at a concentration of 0.5 mg/l were significantly higher. The treatment devoid of BA, in contrast, resulted in the lowest significantly mean fresh and dry weights (142.0 and 8.4 mg, respectively). Maintainance medium was determined to be a mixture of 0.5 mg/l BA and 1 mg/l NAA due to its ability to promote the maximum significantly fresh and dry weights (408.8 and 18.8 mg, respectively). The fresh and dry weight values were significantly lower in the treatment with 0 and 2 mg/l of BA and NAA (132 and 8.8 mg, respectively).

Table 7. Effect of BA and NAA concentrations in fresh and dry weight (mg) of callus tissue arising from stems in dark conditions

Fresh weight (mg)						
PGRs(mg/l) BA	NAA Fresh Dark					
concentration	0	0.5	1	1.5	2	Mean
0	0	262.2	166.6	148.8	132	142
0.5	210.8	402.8	408.8	379.2	289.6	338.2
1	201.8	326.6	378.4	285.4	249.8	288.4
1.5	211.4	301.4	280.2	218.2	192.8	240.8
2	132.1	291.8	285.4	224.8	214.2	229.6
Mean	151.2	317.0	303.9	251.3	215.7	---
LSD: BA: 48.51 , NAA: 48.51 , BA x NAA: 76.25						
Dry weight (mg)						
PGRs (mg/l)	NAA concentrations					
BA concentrations	0	0.5	1	1.5	2	Mean
0	0	13.2	9.8	10.2	8.8	8.4
0.5	10.8	19.4	18.8	15.8	14.6	15.9
1	10.6	15.2	16.6	13.8	12.2	13.7
1.5	11.2	12.8	14.2	13	11.4	12.5
2	9.2	13	12.8	11.8	10.8	11.5
Mean	8.2	14.7	14.4	12.9	11.6	---
LSD: BA: 3.83 , NAA: 3.83 , BA x NAA: 5.11						

Whether used alone or in conjunction with Cytokinin, Auxin plays a pivotal role in the effective induction of callus. Both Auxin and Cytokinin play important roles in cell division and metabolism; Auxin initiates cell-wall breakdown and promotes cell division, while Cytokinin controls sugar and protein synthesis and stimulates tubulin activity and cell division (35). An appropriate physiological balance between Auxin and Cytokinin is required for callus to grow in culture conditions. The development of callus might be negatively impacted by an imbalance in hormone levels (36).

Effect of BA or NAA concentration on shoot formation

Callus tissue was created from leaf and stem explants in both light and dark condition maintained on a maintenance medium until to obtain a sufficient quantity of callus. The callus was then re-cultured on regeneration media for multiple. However, during the course of the investigation, the medium supplied with (NAA + BA) and (BA alone) did not cause the differentiation of the induced callus from leaves in light circumstances and stems in both light and dark conditions into vegetative branches. However, when grown on regeneration media supplemented with varied doses of (BA alone), callus formed from dark-grown leaf explants changed behaviour and differentiated into vegetative branches. Treatment with 2mg/l BA considerably increased the percentage of differentiation and the number of shoots per explant (90 percent and 18 shoots, respectively). (Refer to Table 8 and Figure 1B).

Table 8. Effect of (BA) concentrations on developing shoots induced from leaves callus on MS medium.

Parameter	BA concentration (mg/l)				
	0.0	0.5	1	1.5	2
%	20 d	70 c	80 b	90 a	90 a
No. of shoots	1.5 d	6.6 c	15 b	16 a b	18 a

Lower levels of endogenous auxin and cytokinin for this plant segment, depending on cell type (37), may account for the low percentage and quantity of shoots formed in the control treatment. The high proliferation and differentiation rates of meristematic cells may explain why a callus formed from a leaf can be used to create new shoots. Likewise, the potential energy of the cells and their quantity underpin the rates of cell division, callus induction, and differentiation, as do the levels of internal plant hormones that were in balance with the current growth regulator in the regeneration medium (38). Researchers show a positive relationship between BA concentration and number of shoot branches , which regard that BA had in internal structural chemical composition from benzene ring made a prominent cytokine in used to push the plant towards branch formation (39) , also BA had number of double bonds that were owned in a chain of side (40). Kurakawa etal (41) Considering BA's superiority over other cytokines in promoting cell division and growth. These reasons made the importance of BA in regeneration and multiplication branches in plant culture (42).

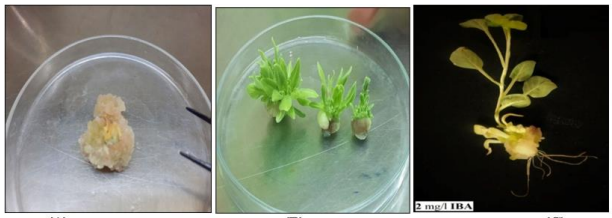

Figure 1. (A) Leave callus tissue in maintenance medium (1.5 + 1.5 mg/l) BA& NAA.
(B) Organogenesis of shoots on regeneration medium (2mg/l) BA.
(C) root induction at (2 mg/l) IBA

Effect of IAA, NAA and IBA concentration on root induction

The inability of the branches to respond to stimulation of rooting at any concentration of (NAA) shows that these concentrations had an adverse effect on the rooting process (Table 9). At the control treatment, where the number of roots and their length both reached (1.6, 1.4), the proportion of rooted branches reached 30%. which showed no significant differences in root number or root length between the (IAA) treatment and control at (1, 3, 4 mg/l). IBA at a dosage of 2 mg/l considerably increased significant rooting percentage, root number, and root length, as shown in Table (9). As seen in Figure 1(C).

Table 9. Effect of IAA , NAA, IBA concentrations on rooting (%) , root number and root length (cm), after 4 weeks of culture in MS medium

PGR Concentration (mg/l)	Rooting (%)	Root number	Root length (cm)
control	30	1.6 d	1.4 c
IAA 1	30	2.2 b c d	1.6 c
IAA 2	40	3.2 b c	1.8 c
IAA 3	50	2.6 b c d	2.3 a b c
IAA 4	40	2.2 b c d	1.9 c
NAA 1	0.0	0.0 e	0.0 d
NAA 2	0.0	0.0 e	0.0 d
NAA 3	0.0	0.0 e	0.0 d
NAA 4	0.0	0.0 e	0.0 d
IBA 1	30	2.0 cd	1.8 c
IBA 2	60	4.8 a	3.0 a
IBA 3	50	3.4 b	2.6 a b
IBA 4	40	3.0 b c	1.9 b c

The data showed that the amount of auxin present in shoot culture had a major impact on the induction of development (root primordial) at the bottom of cultured plant branches. If cells derived from roots divide or not is dependent on the amount of auxin present in the culture medium. Auxin's physiological effects include a proliferation of cells due to the differentiation of mature cells that specialise in the shoot's base into meristematic cells, which then give rise to the development of meristems roots (43). Inactive auxin levels are increased when IAA is present in high concentrations in the growth media, which may explain why root length and number are reduced. This is because root development (root primordia) is inhibited, and as a result, less root growth occurs. Rooting was inhibited by increasing concentrations of NAA, possibly because this compound induces the formation of ethylene, a hormone that stunts root growth and development (44). Shoots can be rooted in vitro using IAA alone or in conjunction with other plant growth regulators (PGRs), most notably IBA (45). Applications of plant growth regulators that are too dilute or use the wrong technique will have no impact or may be detrimental. There is evidence from studies (46), indicating that IBA can promote the mobilisation of photosynthates that stimulate root development by facilitating the breakdown of amyloplast in the cell wall and activating the cambium activity. Optimal levels of the IBA hormone promote root system development and longer roots by accelerating the rate of cambium cell differentiation, hydrolytic activity, and callus formation (47).

Conclusion

Hyoscyamus niger is one of the medicinal biennial herb and it consider a natural pool of secondary metabolites. The production of callus and its physical characteristics, such as its shape and color, varied depending on the growth conditions of the H. niger explants, whether in light or dark conditions. The leaf explants of H. niger were found to be the most effective in generating callus. The combination (1.5 + 1.5 mg/l) BA+ NAA was chosen as a maintenance medium in dark condition. In general , BA is considered most effective cytokinin in shoot regeneration and multiplication. It can be said that IBA a root inducing hormone

Abbreviations: Murashige and Skoog (MS) ; plant growth regulators (PGRs) ; 6-benzyladenine (BA) ; 1-naphthaleneacetic acid (NAA) ; indole acetic acid (IAA) ; Indole-3-butyric acid (IBA) ; gibberellic acid (GA_3)

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