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Original Contribution

IN VITRO HYOSCYAMINE AND SCOPOLAMINE PRODUCTION OF BLACK HENBANE (*HYOSCYAMUS NIGER*) FROM SHOOT TIP CULTURE UNDER VARIOUS PLANT GROWTH REGULATORS AND CULTURE MEDIA

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ABSTRACT

Tropane alkaloids, especially hyoscyamine (HYO) and scopolamine (SCO), are widely used in medicine for their antispasmodic, anticholinergic, and sedative properties. This study was undertaken to investigate the root and shoot tropane alkaloids production from *in vitro* shoot tip (as explant) culture of *Hyoscyamus niger* under different plant growth regulators including auxins (IAA, 2, 4-D, IBA, NAA), cytokines (KIN ، BAP) with different concentrations (0.5, 1 and 2 $mg.l^{-1}$) and two media of full and modified MS (½ NH_4NO_3 and ½ KNO_3 compared to those concentration in full MS medium) treatments. Alkaloids extracted were analyzed by gas chromatography /mass spectra (GC-MS) analysis. The results showed that the highest alkaloid content values in root (HYO: 0.2 %DW; SCO: 0.076 %DW) obtained in full MS medium containing 0.5NAA and 1BAP. Whereas, in the shoot organ the highest HYO (0.394 %DW) and SCO (0.234 %DW) found on modified MS medium supplemented with 1BAP and 0.5IBA. It was obvious that HYO was the prevalent alkaloid in root and shoot tissues. The maximum total alkaloids (HYO + SCO) yield (0.269 $mg.plant^{-1}$) was also obtained on full MS medium supplemented with 0.5NAA and 1BAP.

Key words: *Hyoscyamus niger*; Tropane alkaloids; Hyoscamine, Scopolamine, *in vitro*

INTRODUCTION

Black henbane (*Hyoscyamus niger*) a species in Solanaceae family has long been used as a medicinal plant. A strong-scented annual or biennial herb, which all it's parts contain tropane alkaloids such as HYO and SCO (1). These secondary metabolites are used in medicine as anticholinergic, antispasmodic, hypnotic and sedative (2). Due to fewer side effects on nervous system, SCO is much preferred and has higher commercial demand (3). Because of the complex chemical structures of these alkaloids, industrial synthesis has been found to be prohibitively expensive and therefore they are mainly obtained from plant resources (4) especially Solanaceae plant. Young roots without secondary growth has been found to be the major site of tropane alkaloids production, where the main enzymes of their biosynthetic pathway are localized (5).

In recent years, many experiments have been conducted on the production of these alkaloids by hairy root cultures, but it has not been commercially exploited yet (6, 7). The content of

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secondary metabolites including tropane alkaloids (particularly SCO to HYO ratio) in cultivated plants can be considerably influenced by environmental conditions and other factors such as soil, salt concentration and agrotechnical parameters (8). Nowadays, cell and tissue cultures have been suggested as an alternative method to enhance, and to achieve desire compounds.

The production of significant levels of secondary compounds such as tropane alkaloids depends on the degree of organogenesis of culture (9). Existing reports indicated that for SCO biosynthesis in *H.niger* differentiation is required (10). The content and the number of alkaloids in *Erythrina Americana* were reduced when the number of subcultures *in vitro* increased (11).

Plant hormones play a key factor in a variety of process including growth, development and secondary metabolites biosynthesis (12). Production of xanthones in *Gentianella austrica* shoots cultures were strongly affected by presence of BAP (13). Auxins at lower concentrations in combination with cytokinins have an important role in plant regeneration (14). The highest alkaloid production of *Frangula alnus* was observed in the shoots grown on the MS medium containing NAA and TDZ to concentration of 0.1 $mg.l^{-1}$.

Nutrients like nitrogen in the culture medium serve as a signal for resource allocation; it also has a great effect on rate of cell growth, differentiation and secondary metabolite production (15, 16). Plant tissue culture can provide useful experimental methods for the study of plant secondary metabolism and mass production of secondary compounds, especially from threatened and slow-growing plant species (17, 18). Most plant tissue culture media is still based on the nutrient composition reported by Murashige and Skoog (MS, 1962), which is qualified by high levels of nitrogen in the forms of NH_4NO_3 and KNO3 (19).

It is of a significant importance to have high overall alkaloids yield rather than content for *H.niger* because it is not consumed directly as pharmaceutical and the desired active compounds such as HYO and SCO should be extracted from the plant raw materials. Therefore, it is necessary to have mass production of plant materials. With regarding to the all matters, which mentioned above, we used shoot tip culture for this experiment. Because no subculture are required when larger tips are cultured. In addition once root production is stimulated in cultured shoot-tips, shoot enlargement and growth rapidly follow (20). A review of literature revealed that in most of the studies no investigations have been performed regarding alkaloids yield and content of plant root and shoot via direct regeneration under *in vitro* conditions.

The objective of this study was to prepare the optimal culture conditions by combination of various plant growth regulators (PGRs) and culture media for shoot tip of *H. niger* plants as explant to rapid proliferation and maximum production of HYO and SCO in the both root and shoot organs.

MATERIALS AND METHODS

1. Plant materials: seed preparation and germination

Seeds of *H.niger* were provided by Agricultural and Natural Resources Center, Esfahan, Iran. Black Henbane seeds generally have low germination rate even under normal lab conditions. In order to breaking dormancy and accelerating germination, seeds were treated with 250 mg.l gibberellic acid (GA_3) for 48 h at room temperature (25±0.5˚C). After that seeds were surface-sterilized in 70 % ethanol for 2 min and then in 25 % commercial bleach (containing 6 % sodium hypochlorite) for 10 min and finally rinsed with double distilled sterilized water. Subsequently, seeds were cultured on agar-supported hormone free MS medium. After 4 days, 90% of seeds germinated steadily (with 1mm radicle length). Two weeks after germinating, plantlets have been obtained for explants preparation.

2. Culture media and culture conditions

Shoot apex explants were excised aseptically from shoot (14-d-old *in vitro* grown plantlets) and cut into pieces approximately 0.7± 0.1 cm long. These explants were dissected by discarding the 0. 1 cm of shoot apex and then placed vertically on the medium (120 ml medium) in test containers (500 mL flask). Two types of media culture, Full MS (Murashige and

Skoog, 21) and Modified MS (half-strength MS nitrogen, ½ NH_4NO_3 and ½ KNO_3), consist of 3% sucrose (w/v) and 0.7% agar (w/v) were used and also were supplemented with different concentrations ($mg.L^{-1}$) and combinations of auxins and cytokinins including 0.5 NAA +1 BAP, 1 NAA + 1 KIN, 2 NAA + 0.5 BAP, 0.5 IAA + 1 BAP, 1 IAA + 1 KIN, 0.5 2,4-D + 0.5 KIN, 1 2,4-D + 1 BAP, 0.5 IBA +1 BAP, 2 IBA + 1 KIN.

PGRs were filter-sterilized using a Milipore filter and added to hot autoclaved medium before dispensed into culture containers. The pH of media were adjusted to 5.6- 5.8 with 1 M NaOH or 1 M HCl before autoclaving at 121 ºC for 20 min.

All culture test containers were covered with autoclaved polypropylene sheet and tightened with rubber band. The cultures were kept in growth chamber at 25±2 ċ under a 16-h photoperiod (supplied by two Philips TL 40W fluorescent tubes) and 8-h dark periods with a light intensity of 2500 Lux. After 45 days of culture, all plantlets materials including root and shoot were separated individually and their traits such as the number of rooted shoots, the number of roots per shoot and mean root and shoot length, HYO and SCO content and yield were recorded. Also, some plantlets prepared for acclimatization, which explained below.

3. Acclimatization

Plantlets gently washed in distilled water to remove agar from their roots and were planted in plastic cups (6 cm diameter and 8 cm deep) containing sand, field soil and vermiculite (1:1:1), which sterilized by autoclaving at 105◦C for 60 min over three consecutive days and maintained inside growth chamber with 80% humidity. After 3 weeks, the plants were transferred to greenhouse and kept in shade (approximately 50% shade) for a period of 3 weeks for acclimatization. After that, plants transplanted to pots (25 cm diameter × 30 cm deep) containing field soil and then transferred to out side (under natural conditions) to receiving maximum sunlight up to flowering stage (**Fig. 1**).

4. Alkaloid extraction and analysis

Shoot and root samples were air dried, grinded into fine powder and sieved with laboratory mesh (size 30, mesh opening 545 µm). A subsample of 0.5 grams from each shoot and root was added to appropriate volume of $CHCl_3$: MeOH: NH_4OH 25%, (15:5: 1), sonicated for 20 min and then kept at water bath (40◦C) for one hour. Subsequent sample preparation and alkaloids extraction were based essentially on the method described by Kamada (22). Alkaloids extracted were identified by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) analysis. Gas chromatography analysis was performed using a GC system equipped with a flame ionization detector (FID) and HP-5MS capillary column (30 m × 0.25 mm, film thickness 0.25 µm). Injector and detector temperatures were set at 220 and 290 ◦C, respectively. The column temperature was initially kept at 50 ◦C for 5 min, then gradually increased to 300 ◦C at a rate of 3ºC/min and maintained for 3 min. The flow rate of gas helium was 0.8 mL/min. Then 1 µL of extract was directly injected into the gas chromatograph. Each extraction was replicated three times and the compound percentages are the means of the three replicates. GC-MS analysis was carried out on an Agilent 6890 gas chromatograph (Agilent Technologies, Palo Alto, USA) fitted with a fused silica HP-5MS capillary column (30m×0.25mm×0.25µm). Oven temperature was programmed from 50ºC to 285ºC at 3ºC/min, and helium was used as carrier gas (0.8 mL/min), Mass spectra were obtained in an Agilent 5973 system operating in electron impact mode (EIMS) at 70 eV, coupled to an GC system. The identification of alkaloids was based on the comparison of their GC retention time and mass spectra (MS) data with their standards substances (HYO. HCl and SCO. HBr, Merck). The total tropane alkaloids (HYO + SCO) yield was quantified by both alkaloid content and biomass production; Total alkaloid yield ($mg.plant^{-1}$) = Alkaloid content (% d.w) × Plant dry weight ($mg.plant^{-1}$).

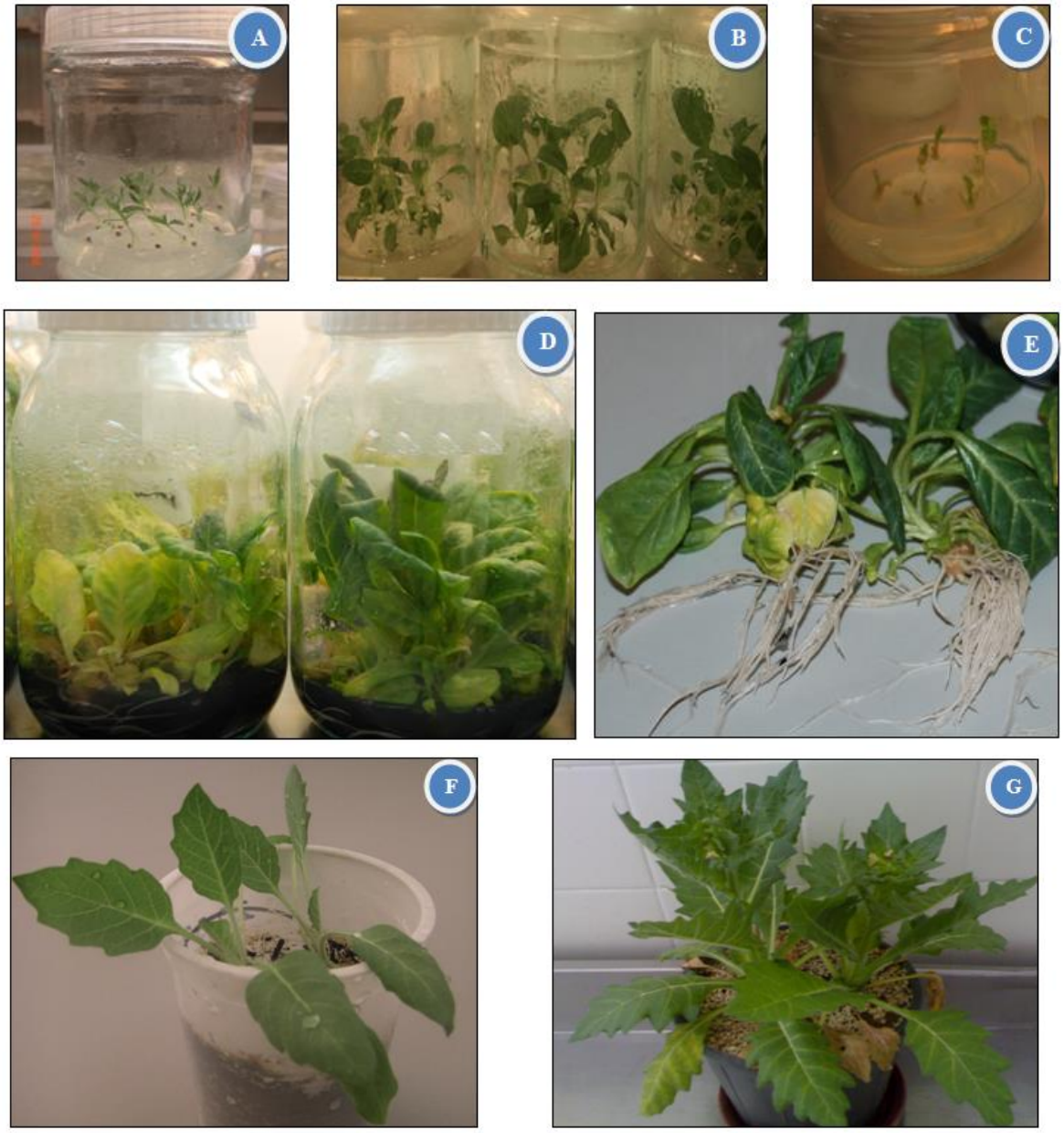

Fig. 1. Shoot organogenesis and plant regeneration in *Hyoscyamus niger*. **A**: Seeds were cultured on agar-supported hormone free MS medium (1 week after germination). **B**: Plantlets for preparing shoot tip explants (2 weeks after germination). **C**: Culture of shoot tip explants. **D**: Development of shoot tip explants on full MS (right) and modified MS (left) medium supplemented with 0.5 mg l^{-1} NAA and 1 mg l^{-1} BAP. **E**: Root induction from shoot tip explant on full MS medium (right) and modified MS (left) medium supplemented with 0.5 mg l^{-1} NAA and 1 mg l^{-1} BAP after 45 days of culture. **F**: *In vitro* generated plantlets for acclimatization. G: Complete plant in plastic pot after acclimatization (acclimated plant at flowering stage).

5. Statistical analysis

The experiments were arranged in factorial experimental based on Completely Randomized Design (CRD) with three replicates per each treatment and fifteen explants per replicate. Statistical analysis of experimental data was performed using SAS program, and probabilities of significance were used to test for significance among treatments and interactions, and LSDs ($P<0.05$) were used to compare means. Values obtained were expressed as mean ± S.D. (standard deviation) from three replications (n=3) of each treatment.

RESULTS AND DISCUSSION

1. Root and shoot morphogenesis

The shoot tip explants showed various morphogenesis responses on two media culture containing different PGRs in the end of culture period **(Table 1)**. All the PGRs combinations in both media culture which tested in this study produced multiple shoots and roots from shoot meristems of *H.niger* cultured *in vitro*. Modified MS medium proved to be superior to full MS medium on root morphology traits in the all PGRs combinations by producing maximum number of roots per proliferated explant, which significantly higher than that on full MS medium compared to the each respective PGRs combinations treatment.

Table 1. *Effect of various PGRs combinations and culture media on morphogenesis of H.niger shoot tip explants (mean ± SD).*

Culture Medium	PGRs combinations ($mg.L^{-1}$)	Root morphology ($explant^{-1}$)			Shoot morphology ($explant^{-1}$)		
		Number	Length (cm)	Dry weight (g)	Number	Length (cm)	Dry weight (g)
Full MS	0.5 NAA +1 BAP	15.5±1.1	29.90±2.36	0.147±0.004	21.17±1.57	10.04±1.03	0.524±0.029
	1 NAA + 1 KIN	12.1±0.95	23.60±2.50	0.126±0.008	17.97±1.98	9.41±0.82	0.460±0.012
	2 NAA + 0.5 BAP	20.3±1.95	36.37±3.78	0.176±0.008	12.57±1.56	7.74±0.44	0.332±0.012
	0.5 IAA + 1 BAP	10.1±1.49	19.63±2.61	0.112±0.010	15.93±2.10	8.82±0.65	0.413±0.020
	1 IAA + 1 KIN	5.4±1.04	11.07±1.21	0.064±0.013	17.50±2.56	9.45±0.70	0.466±0.019
	0.5 2,4-D + 0.5 KIN	9.3±1.50	18.23±2.27	0.106±0.011	14.30±1.91	8.56±0.80	0.363±0.013
	1 2,4-D + 1 BAP	11.1±1.12	21.87±2.86	0.113±0.009	16.50±1.60	9.04±0.75	0.444±0.019
	0.5 IBA +1 BAP	14.0±1.57	28.20±2.62	0.139±0.007	11.50±1.85	7.95±0.74	0.332±0.013
	2 IBA + 1 KIN	17.2±1.35	32.47±3.26	0.164±0.012	10.07±1.76	7.14±0.22	0.324±0.013
Modified MS	0.5 NAA +1 BAP	21.50±2.22	52.17±2.93	0.197±0.010	7.40±1.21	5.27±0.45	0.227±0.011
	1 NAA + 1 KIN	16.40±2.10	34.83±3.02	0.152±0.012	12.97±1.80	7.29±0.18	0.325±0.017
	2 NAA + 0.5 BAP	18.63±2.00	42.87±2.46	0.181±0.006	9.33±0.71	5.50±0.29	0.266±0.016
	0.5 IAA + 1 BAP	13.60±0.88	27.97±2.10	0.143±0.012	10.80±1.67	6.50±0.54	0.286±0.013
	1 IAA + 1 KIN	14.87±1.56	33.13±1.95	0.165±0.010	11.80±1.05	6.98±0.28	0.327±0.009
	0.5 2,4-D + 0.5 KIN	14.07±1.30	31.97±2.72	0.163±0.010	11.27±1.45	6.54±0.34	0.315±0.015
	1 2,4-D + 1 BAP	10.20±1.49	22.57±2.54	0.121±0.006	13.73±2.15	7.07±0.34	0.344±0.015
	0.5 IBA +1 BAP	29.53±2.00	58.93±4.31	0.236±0.011	8.37±1.01	6.23±0.30	0.240±0.014
	2 IBA + 1 KIN	21.70±2.03	45.73±2.21	0.191±0.012	16.87±1.22	8.38±0.27	0.319±0.010

Modified MS media with growth regulator (0.5 $mg.l^{-1}$ IBA and 1 $mg.l^{-1}$ BAP) supplements produced better results in terms of average root number (29.53), length (58.93 cm) and dry weight (0.236 g) response compared to the rest PGRs combinations in the both media **(Table 1).** The second highest root morphology response was also recorded for modified MS media at 0.5 $mg.l^{-1}$ NAA and 1 $mg.l^{-1}$ BAP. In the full MS media, the highest mean root number (20.3),length (36.37 cm) and dry weight (0.176 g) were observed at 2 $mg.l^{-1}$ NAA and 0.5 $mg.l^{-1}$ BAP.

The use of low salt MS medium (like modified MS in our current study) for rooting of the *in vitro* induced shoots is a very common practice

(23). The results indicate that modified MS + 0.5 mg l^{-1} IBA (auxin) and 1 mg l^{-1} BAP (cytokinin) produced maximum number of roots per explant, whereas full MS medium produced only half number of roots per explant at same concentration of auxin and cytokinins. Ahmad *et al.*, (24) reported that the effect of growth regulators can be strongly modified by the medium on which the cultures are grown.

Statistical analysis showed that the significant interaction ($p<0.05$) between culture media and growth regulators supplements for the shoot morphology traits **(Table 1).** The highest and lowest shoot number (21.17 and 7.40), length (10.04 and 5.27 cm) and dry weight (0.524 and 0.227 g) were recorded for full MS and modified MS in growth regulators of 0.5 mg.l^{-1} NAA and 1 mg.l^{-1} BAP, respectively. Generally, shoot morphogenesis was better in the full MS culture media, which had two-fold NH_4NO_3 and KNO_3 compared to those concentrations in modified MS medium.

Nitrogen may function as a signal molecule of plant growth by increased gene expression for enzyme responsible for the uptake and utilization of nitrate (25). Growth on a poor nitrogen source is not sufficient to cause the induction of nitrate reductase and nitrite reductase enzymes essentially required for the consumption of nitrate (26). Reduced nitrogen forms especially ammonium and nitrate content in modified MS medium may act as key factor on root growth. Adversely, there is growing evidence for the usefulness of these supplemented nitrogenous compounds in the culture medium which can enhance cell division, differentiation, growth and development of multiple shoots *In vitro* (27, 28).

Selection of concentration and combination of plant growth regulators is critical to shoot regeneration. Combination of BAP at 1 mg l^{-1} with 0.5 mg l^{-1} NAA (in full MS medium) or IBA (in modified MS medium) proved to be more efficacious by raising the number of roots and shoots per explant as compared to other PGRs with less or more concentration. The combined use of PGRs with BAP has also proved better for shoot multiplication in several species (29-31). All these studies indicated that different species need different PGRs combinations and concentrations. In present study, BAP at concentration of 1 mg l^{-1} has shown the synergistic effect on root and shoot growth with NAA and IBA when used at 0.5 mg l^{-1} as compared to its other combinations.

2. Alkaloids accumulation

There was a significant interaction in alkaloids content of root and shoot in *Hyoscyamus niger* plantlets under two media culture with different PGRs combination treatments **(Table 2).**

In roots of the *in vitro* propagated *Hyoscyamus niger* plantlets, maximum HYO (0.2 % DW) and SCO (0.076 % DW) content were recorded in full MS medium with 0.5 mg l^{-1} NAA and 1 mg l^{-1} BAP supplements. In shoots, however, both HYO and SCO content significantly increased under 0.5 mg l^{-1} IBA and 1mg l^{-1} BAP in the modified MS medium compared to the rest of PGRs combinations.

Maximum HYO and SCO content in plantlets root and shoot tissues, which described above were much more than those of acclimated plants (Root: HYO, 0.012 % DW and SCO, 0.007 % DW; Shoot: HYO, 0.198 % DW and SCO, 0.024 % DW). Similar results reported by Kang *et al.* (32) found that tropane alkaloid contents were higher *in vitro* propagated plants than in native growing plants and acclimated plants. Also our observations are in agreement with Pudersell *et al.* (33) who reported the production of tropane alkaloids of the root cultures in MS and Knop-M media is much higher if compared with the roots from the field cultures. These results show that tropane alkaloid contents differ according to development stage and plant tissue. Variations in alkaloids accumulation between two media culture could be attributed to their compositions. For many plants containing tropane alkaloids, potassium usually decrease the yield of alkaloids. It has been suggested that potassium deficiency increases tropane alkaloids synthesis in *Atropa acuminate* (34). In our study, modified MS medium could have improving effect on alkaloid production due to less potassium amount than full MS medium, which is appeared in shoot alkaloid content. It is generally reported that tropane alkaloids, HYO and SCO are mainly biosynthesized in the roots and then transported to aerial plant parts (35). In current study, we investigated tropane alkaloid contents in both root and shoot tissues **(Table 2).** Many reports have indicated that PGRs could be used to

increase both the variety and quantity of secondary metabolites in tissue culture. The general viewpoint is that 2, 4-D has a negative effect on the synthesis of secondary metabolites (17, 18) However, there are conflicting reports (7). In our study, also PGRs in combination with 2, 4-D produced minimum alkaloids under both media culture.

Table 2. *Effect of various PGRs combinations and culture media on hyoscyamine (HYO) and scopolamine (SCO) content of H.niger organs (mean ± SD).*

Culture Medium	PGRs combinations ($mg.L^{-1}$)	Root alkaloids content (% DW)		Shoot alkaloids content(% DW)	
		HYO	SCO	HYO	SCO
Full MS	0.5 NAA +1 BAP	0.200±0.015	0.076±0.012	0.303±0.010	0.133±0.006
	1 NAA + 1 KIN	0.123±0.010	0.043±0.009	0.272±0.012	0.117±0.008
	2 NAA + 0.5 BAP	0.175±0.008	0.065±0.007	0.386±0.011	0.186±0.007
	0.5 IAA + 1 BAP	0.154±0.014	0.054±0.010	0.221±0.009	0.092±0.013
	1 IAA + 1 KIN	0.112±0.011	0.034±0.008	0.183±0.010	0.075±0.010
	0.5 2,4-D + 0.5 KIN	0.042±0.013	0.021±0.006	0.108±0.012	0.044±0.009
	1 2,4-D + 1 BAP	0.026±0.009	0.010±0.005	0.102±0.017	0.042±0.006
	0.5 IBA +1 BAP	0.153±0.014	0.051±0.009	0.224±0.010	0.116±0.008
	2 IBA + 1 KIN	0.113±0.009	0.033±0.004	0.202±0.013	0.105±0.010
Modified MS	0.5 NAA +1 BAP	0.094±0.010	0.026±0.009	0.213±0.012	0.122±0.007
	1 NAA + 1 KIN	0.102±0.009	0.031±0.006	0.292±0.014	0.124±0.008
	2 NAA + 0.5 BAP	0.147±0.010	0.033±0.006	0.245±0.013	0.136±0.007
	0.5 IAA + 1 BAP	0.037±0.009	0.012±0.005	0.135±0.008	0.064±.0008
	1 IAA + 1 KIN	0.025±0.005	0.007±0.006	0.113±0.010	0.013±.006
	0.5 2,4-D + 0.5 KIN	0.143±0.011	0.038±0.011	0.239±0.012	0.117±0.011
	1 2,4-D + 1 BAP	0.103±0.009	0.027±0.006	0.200±0.013	0.092±0.014
	0.5 IBA +1 BAP	0.173±0.010	0.057±0.008	0.394±0.007	0.234±0.010
	2 IBA + 1 KIN	0.143±0.008	0.046±0.008	0.321±0.014	0.155±0.008

Root and shoot alkaloids (HYO and SCO) yield under all employed treatments are given in **table 3**. The highest value of root HYO (0.041 $mg.plant^{-1}$) and SCO (0.013 $mg.plant^{-1}$) yield were observed in modified MS medium under 0.5 $mg.l^{-1}$ IBA and 1 $mg.l^{-1}$ BAP treatment. In shoots of the *in vitro* propagated *H.niger* plantlets, maximum HYO (0.159 $mg.plant^{-1}$), SCO (0.070 $mg.plant^{-1}$) and total alkaloids (0.269 $mg.plant^{-1}$) yield were recorded in full MS medium with 0.5 $mg.l^{-1}$ NAA and 1 $mg.l^{-1}$ BAP supplement **(Table 3).**

Oksman-Caldentey *et al.* (4) reported that the highest yield of SCO was found in the root cultures of *H. niger* grown in the MS medium and this is over 60-fold higher than in field culture roots and more than 6-fold higher than in the root cultures grown in the Knop-M medium. Differences are obviously caused by the differences in the content of macro-elements in

MS and Knop-M media. Knop-M does not contain ammonia. Also suggested that NH_4^+/NO_3^- ratio had significant effect on the growth and tropane alkaloids production in the hairy root cultures of *Hyoscyamus muticus* L.

Table 3. *Effect of various PGRs combinations and culture media on hyoscyamine (HYO) and scopolamine (SCO) yield of H.niger organs (mean ± SD).*

Culture Media	PGRs combinations ($mg.L^{-1}$)	Root alkaloids yield ($mg.plant^{-1}$)		Shoot alkaloids yield ($mg.plant^{-1}$)		Total alkaloid yield ($mg.plant^{-1}$)
		HYO	SCO	HYO	SCO	
Full MS	0.5 NAA +1 BAP	0.029±0.003	0.011±0.002	0.159±0.014	0.070±0.007	0.269±0.026
	1 NAA + 1 KIN	0.016±0.002	0.006±0.001	0.125±0.009	0.054±0.005	0.200±0.018
	2 NAA + 0.5 BAP	0.031±0.003	0.011±0.002	0.128±0.008	0.062±0.004	0.232±0.017
	0.5 IAA + 1 BAP	0.017±0.003	0.006±0.002	0.091±0.008	0.038±0.007	0.153±0.020
	1 IAA + 1 KIN	0.007±0.002	0.002±0.001	0.085±0.008	0.035±0.006	0.130±0.017
	0.5 2,4-D + 0.5 KIN	0.005±0.002	0.002±0.001	0.039±0.006	0.016±0.004	0.062±0.012
	1 2,4-D + 1 BAP	0.003±0.001	0.001±0.001	0.046±0.009	0.019±0.003	0.069±0.039
	0.5 IBA +1 BAP	0.021±0.003	0.007±0.001	0.075±0.006	0.039±0.004	0.142±0.014
	2 IBA + 1 KIN	0.019±0.003	0.005±0.001	0.066±0.007	0.034±0.004	0.124±0.015
Modified MS	0.5 NAA +1 BAP	0.019±0.003	0.005±0.002	0.048±0.005	0.028±0.003	0.100±0.013
	1 NAA + 1 KIN	0.016±0.002	0.005±0.001	0.095±0.009	0.040±0.004	0.156±0.017
	2 NAA + 0.5 BAP	0.027±0.003	0.006±0.001	0.065±0.007	0.036±0.004	0.134±0.015
	0.5 IAA + 1 BAP	0.005±0.002	0.002±0.001	0.039±0.004	0.018±0.003	0.064±0.010
	1 IAA + 1 KIN	0.004±0.001	0.001±0.001	0.037±0.004	0.004±0.002	0.047±0.008
	0.5 2,4-D + 0.5 KIN	0.023±0.003	0.006±0.002	0.075±0.007	0.037±0.005	0.142±0.018
	1 2,4-D + 1 BAP	0.012±0.002	0.003±0.001	0.069±0.007	0.032±0.006	0.116±0.016
	0.5 IBA +1 BAP	0.041±0.004	0.013±0.002	0.094±0.006	0.056±0.005	0.205±0.017
	2 IBA + 1 KIN	0.027±0.003	0.009±0.002	0.103±0.008	0.050±0.004	0.189±0.017

CONCLUSION

In conclusion, the modification of the nutrient composition especially content of nitrogen and potassium in the MS medium could affect the morphology and the growth of the shoot tip culture of *H. niger*, also the content of tropane alkaloids (HYO and SCO) in root and shoot organs of regenerated plants increased much more than those in acclimated plants. The concentration of tropane alkaloids in regenerated plants are comparable to those reported previously for levels of these metabolites in other Solanaceae plants. Also, our findings could facilitate and accelerate studies toward efficient propagation and metabolic engineering of *H. niger* for secondary metabolites production particularly SCO.

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Abbreviation:

HYO: Hyoscyamine
SCO: Scopolamine
IBA: Indole-3-Butyric Acid
IAA: Indole Acetic Acid
NAA: Naphthalene Acetic Acid
2, 4-D: 2, 4-Dichlorophenoxyacetic acid
KIN: Kinitin
BAP: Benzylaminopurine
PGR (s): Plant Gowth Regulator (s)

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