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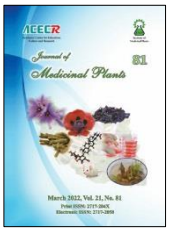

Research Article

Callus induction and withanolides production through cell suspension culture of *Withania coagulans* (Stocks) Dunal

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ABSTRACT

Background: *Withania coagulans* (Stocks) Dunal is a well-known medicinal plant due to its many healing properties. **Objective:** The aim of the present study was to induce friable callus and subsequently establish the plant cell suspension cultures for the production of two important withanolides *i.e.* withaferin A (WFA) and withanolide A (WNA). **Methods:** *In vitro* callus induction was carried out from young leaf and internodal explants cultured on MS medium fortified with various concentrations (0, 1.0, 1.5, 2.0 and 2.5 mg/L) of auxins (2,4-D, NAA, and IAA) solely or in combination with BAP (0., 0.5 and 1.0 mg/L) in a factorial experiment based on a completely randomized design with five replications. The plant cell culture was then established for the production of both withanolides. **Results:** The percentage of callogenesis from the leaf (25.0-96.0 %) was higher than internodal explants (23.2-85.4 %). The high percentage of friable calli was achieved from leaf explants cultured on MS medium fortified with 2.5 mg/L 2,4-D + 0.5 mg/L BAP. Cell suspension culture was established from derived friable callus cultured on MS medium supplemented with 1.5 mg/L IAA + 0.5 mg/L BAP. The highest accumulation of biomass (172 g/L fresh weight and 15 g/L dry weight) and the production of both withanolides were observed in the fourth week of the culture period. The plant cells produced 0.08 and 21 µg/L WFA and WNA at this time, respectively. **Conclusion**: These results can be used for future research on biosynthesis pathways of withanolides as well as their production in bioreactors.

1. Introduction

The Solanaceae family encompasses 84 genera and ca. 3000 species, of which the medicinal genus *Withania* with 33 known species grown in drier regions of the tropics and subtropics, from the Mediterranean area to northern Africa and southwest Asia [1, 2]. *W. somnifera* (L.) Dunal and *W. coagulans* (Stocks) Dunal are two species with the outstanding roles and extensive applications in Unani and

Abbreviations: WTDs, Withanolides; WFA, Withaferin A; WNA, Withanolide A; PGRs, Plant Growth Regulators; IAA, Indole-3-Acetic Acid; 2,4–D, 2,4-Dichlorophenoxyacetic Acid; NAA, 1-Naphthaleneacetic Acid; BAP, 6-Benzylaminopurine; MS Medium, Murashige and Skoog Medium; HPLC, High Performance Liquid Chromatography

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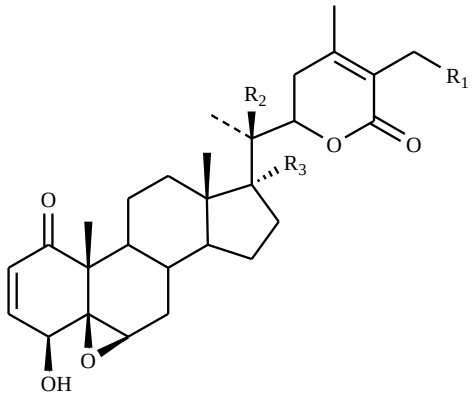
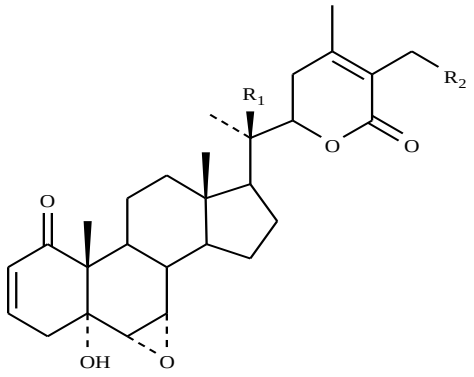
Ayurvedic as traditional local medicine of South East Asia. For example, some properties of *Withania* such as a sexual enhancer, sedative, rejuvenating and life prolonging are listed in Ayurveda [3]. The extensive pharmacological properties of the plant such as anti-inflammatory, anti-tumor, hematopoietic, antioxidant, cardioprotective and immune booster have been reported [4].

Groups of natural compounds C28- steroidal lactones known as withanolides (WTDs) are responsible for the above-mentioned biological activities [4, 5] (Fig. 1). One of the most effective WTDs for cancer treatment derived from *Withania* species is withaferin A (WFA) [6]. In addition, withanolide A (WNA) improves neuronal growth and synaptic reconstruction and seems to be effective in treating Alzheimer's and Parkinson's diseases [7, 8]. *Withania coagulans*, known as *Paneerbad* in Persian, is commonly growing in Iran, Pakistan, Afghanistan and East India. The plant has been previously reported as a source of WFA and WNA [9-11].



Withaferin A R_1=OH, R_2=H, R_3=H
Withanolide D R_1=H, R_2=OH, R_3=H
Dihydrodeoxywithaferin A 2,3-diH, R_1=H, R_2=H, R_3=H
Dihydrowithaferin A 2,3-diH, R_1=H, R_2=OH, R_3=H
Withanolide G 14α-OH, 14=OH, R_1=OH, R_2=H



Withanolide A R_1=OH, R_2=H
Withanone 17α-OH, R_1=H, R_2=H
Withanolide T 14α-OH, 17α-OH, R_1=OH,
Withanolide B R_1=H, R_2=H

Fig. 1. Chemical structure of withanolides [12]

In vitro plant cell cultures are promising approaches for the production of desired specialized metabolites [13]. The rapid proliferation of cells and condense cell cycle in cell suspension culture leads to a higher rate of metabolism than plants grown in the field [14]. On the other hand, *in vitro* plant cell cultures are not affected by ecological, environmental and climatic factors resulted in the growth of the cells faster than the cultivated whole plant in the field. Therefore, this technique represents an effective approach for producing plant-specific metabolites due to the maximum growth rate of cells [9].

The ability to produce larger amounts of specialized metabolites in a shorter time, as well as the possibility to study the biosynthetic pathways are the benefits of large-scale cell culture. On the other hand, homogeneous cell suspension cultures permit uniform access of nutrients, growth regulators, and facilitate various biotechnological approaches such as elicitation, precursor feeding, and biotransformation [15]. The production of WTDs in *W. somnifera* cell suspension cultures affected

by various media cultures, plant growth regulators (PGRs), pH, carbohydrate source, and inoculum density has been previously reported [16, 17]. Our literature survey revealed that several biotechnological investigations on *Withania* species such as cell/organ cultures and genetic manipulation have also been accomplished to increase the WTDs [11, 18-20], but there is no report on the cell suspension culture establishment of *W. coagulans* for the purposes. Thus, this study aimed to induce friable callus and subsequently establish cell suspension cultures to WTDs production as well as optimization of cell culture for next studies on WTDs biosynthesis.

2. Materials and Methods

2.1. Chemicals and reagents

All media components such as salts, sucrose, vitamins, agar and plant growth regulators (PGRs) were provided from Merck (Darmstadt, Germany) and Sigma (Sigma-Aldrich Corporation, USA). Standards of WFA and WNA were purchased from Phytolab GmbH & Co. KG (Germany). Analytical HPLC grade of Methanol was purchased from Merck (Darmstadt, Germany). HPLC grade water was utilized for chromatographic measurement.

2.2. Plant material

The seeds were collected from a wild plant grown at Hizabad village, Zaboli Rural District (27° 06′ N, 61° 37′ E and in altitude of 1280 m), Mehrestan County, Sistan and Baluchestan Province (Fig. 2A-2C). A voucher specimen (MPH-602) has been deposited at the Herbarium of Medicinal Plants and Drugs Research Institute (MPH), Shahid Beheshti University, Tehran, Iran. *In vitro* seedlings (Fig. 2D) were obtained from surface-sterilized seeds as described previously [9].

2.3. Callus induction

Sterile young leaf and internodal segments (1-2 cm) were excised from young seedlings grown in *in vitro* conditions (Fig. 2E) and were placed on MS medium [21] containing 3 % sucrose and strengthened with 0.8 % agar supplemented with different concentrations of auxins such as 2, 4–D, IAA and NAA (1.0, 1.5, 2.0 and 2.5 mg/L) solely or in combination with cytokinin BAP (0.5 and 1.0 mg/L). The pH of the medium was fixed to 5.8 and autoclaved at 121°C and 15 lbs pressure for 20 min. then, the cultures were kept at 25 ± 2°C under 2000 lx illumination (12 h photoperiod). The potential of each explant for callus production (Fig. 2E) after 28 days under different concentrations of PGRs was evaluated as callus induction rate (%) according to below:

Callus induction rate (%) = (Total number of explants produced callus/ Total number of explants cultured) × 100 %. Five replicates were evaluated in each treatment. Proliferated friable calli (Fig. 2F) were used for the establishment of cell suspension culture (Fig. 2G).

2.4. Determination of cell biomass

A 0.5 μm stainless steel sieve was employed for separation the plant cells from the media.

Fresh weight was recorded for each treatment. The cells were dried at 60 ° C and their dry weight was measured until they reached a constant weight.

2.5. Establishment of cell suspension culture

Friable calli (500 mg) were transferred to a flask containing 10 ml MS medium fortified with 1.5 mg/L IAA and 0.5 mg/L BAP in order to establish the cell suspension culture and then incubated in a shaker rotating at 120 rpm, room temperature as well as dark condition for 5 weeks.

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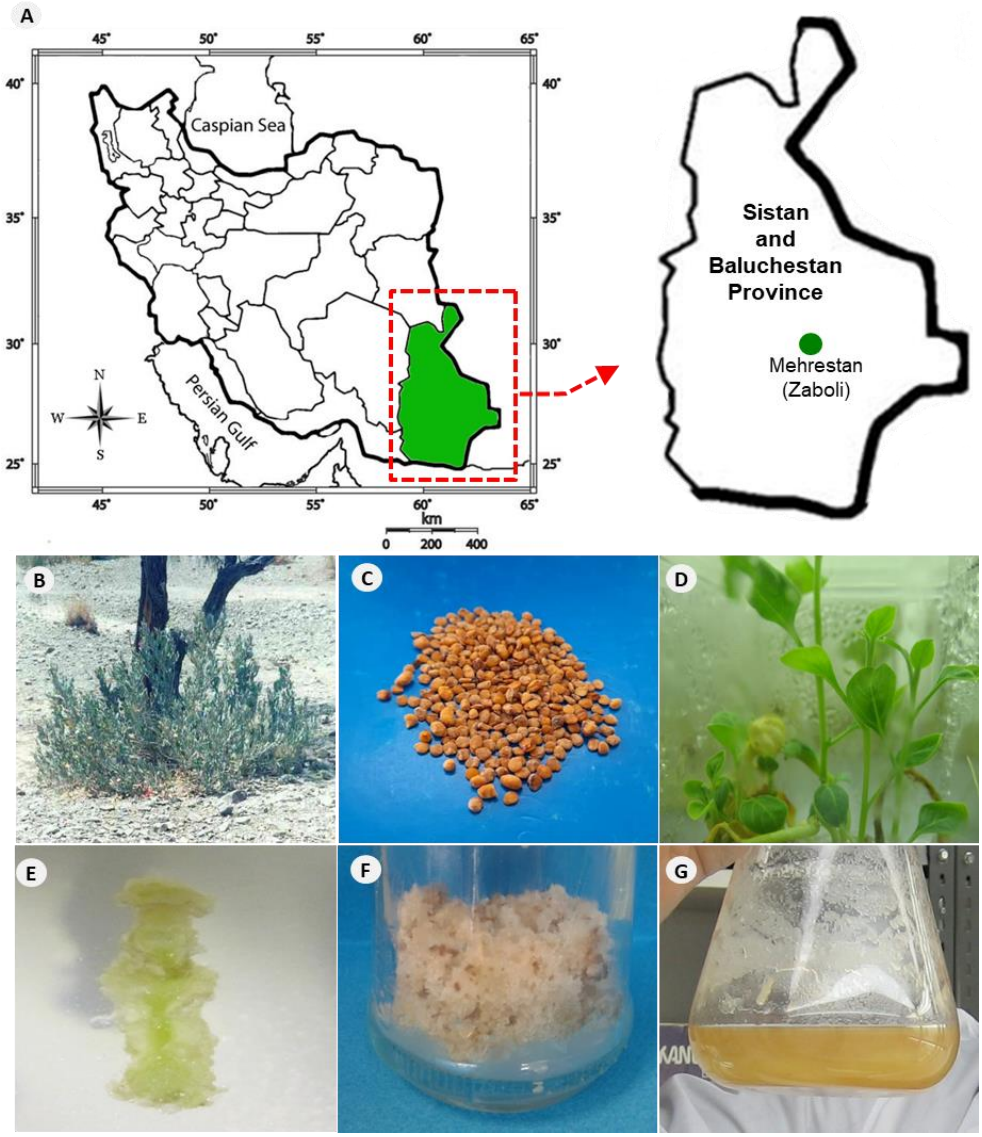

Fig. 2. (A) Collection map and establishment of *Withania coagulans* cell suspension culture (B-G). (B) A wild plant that used as a seed source. (C) The plant seeds that are used for *in vitro* culture. (D) 5-Month-old *in vitro* seedlings that served as explant source. (E) Induced calli from the explants cultured on the media culture. (F) Proliferated friable calli. (G). Cell suspension culture of the plant established in flasks.

2.6. Extraction and Phytochemical analysis

Powdered lyophilized cells (1 g) were soaked in 10 ml methanol and were then sonicated for 30 min. Chromatographic quantification of withanolides was performed according to previous reports [9, 22], wherein a KNAUER High-Performance Liquid Chromatography (HPLC) device with a C18 column (25 cm × 4.6 mm, particle size 5 µm) connected to a K-2800 photodiode-array detector (PDA) was employed for phytochemical analysis using a mixture of methanol and water with 65:35 ratio as mobile phase in isocratic elution program. The flow rate and wavelength were 1 mL/min and 250 nm, respectively. The standard solutions with concentrations of 1-100 µg/mL were used for plotting the calibration curve.

2.7. Statistical analysis

All the experiments were set up in a factorial experiment based on a completely randomized design with five replications. Analysis of variance (ANOVA) and mean comparisons of different treatments were performed using Duncan's Multiple Range test by SAS 9.4 version.

3. Results

3.1. Induction frequency, fresh and dry weight of callus

The experiment outputs showed that the leaf explants resulted in better average callus induction, more friable and fresh callus than internodal segments. The callus induction percentage was ranged from 25.0 ± 2.5 - 96.0 ± 2.5 % for the leaf and 23.2 ± 2.9-85.4 ± 1.8 % for the internodal explants. Therefore, the leaf explants showed qualitatively and quantitatively better results.

Although the three treatments including 2.5 mg/L 2,4-D in combination with 1 mg/L BAP using both explants, and 2.5 mg/L 2,4-D in combination with 0.5 mg/L BAP derived from internode explant produced compact calli, other treatments provided friable calli. The application of 2.5 mg/L 2,4-D in combination with 0.5 mg/L BAP led to the highest callus induction using the plant leaf (96.0 ± 2.5 %) and internodal (85.4 ± 1.8 %) segments (Table 1). The maximum fresh weight (940.4 ± 1.5 mg) and dry weight (108.5 ± 0.9 mg) of callus were recorded in the treatment of 2.5 mg/L 2,4-D in combination with 0.5 mg/L BAP. Using the internodal segment and application of 1 mg/L 2, 4-D resulted in minimum callus induction (56 ± 2.5 %), fresh weight (558.7 ± 1.6 mg) and dry weight (58.2 ± 1.0 mg) of callus (Table 1, Fig. 3).

Among PGRs tested, 2.5 mg/L NAA mixed with 0.5 mg/L BAP was the best combination for callus induction (68.4 ± 1.5 %), maximum fresh weight (693.6 ± 1.1 mg) and dry weight of callus (77.5 ± 1.7 mg) using the leaf explant after four weeks of culturing (Table 1 and Fig. 4). On the other hand, application 2.5 mg/L NAA in combination with 0.5 mg/L BAP was responsible for the highest callus induction (64.8 ± 2.9 %) through internodal segments cultures of *W. coagulans* (Table 1). The lowest callus induction rates were obtained in the treatment of 1 mg/L NAA using both leaf (25.0 ± 2.5 %) and internode (23.2 ± 2.9 %) as explant, respectively.

At most 2, 4-D concentrations alone, the callus induced on both explants was friable, while the nature of the callus was compact at a concentration of 1 mg/L. The nature of the callus was also compact at 2.5 mg/L NAA. At other levels, the application of this hormone solely produced friable callus in both explants (Table 1).

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Table 1. The percentage of induction and nature of callus induced from different explants of *Withania coagulans* treated with plant growth regulators (PGRs) after 4 weeks.

Explants Source	PGRs (mg/L)		Nature of callus	Callus induction (%)
	2,4-D	BAP		
Leaf	1.0	0	Light yellow, friable	62.8 ± 1.9
	1.5	0	Brown loose, friable	72.2 ± 1.0
	2.0	0	Creamish brown, friable	76.2 ± 2.7
	2.5	0	Dark brown, friable	83.3 ± 2.2
	2.5	0.5	Light brown, friable	96.0 ± 2.5
	2.5	1.0	Dark green, compact	87.0 ± 2.7
Internode	1.0	0	Whitish brown, friable	56.0 ± 2.5
	1.5	0	Light brown, friable	61.2 ± 2.3
	2.0	0	Light brown, friable	66.6 ± 2.2
	2.5	0	Dark brown, friable	72.2 ± 2.9
	2.5	0.5	Light green, compact	85.4 ± 1.8
	2.5	1.0	Dark green, compact	74.0 ± 1.9
	NAA	**BAP**		
Leaf	1.0	0	Light yellowish white, friable	25.0 ± 2.5
	1.5	0	Brown loose, friable	44.2 ± 2.4
	2.0	0	Brown loose, friable	54.4 ± 2.1
	2.5	0	Dark brown, compact	47.8 ± 1.9
	2.5	0.5	Light green, friable	68.4 ± 1.5
	2.5	1.0	Dark green, compact	65.2 ± 2.4
Internode	1.0	0	Whitish yellow, friable	23.2 ± 2.9
	1.5	0	Yellow loose, friable	41.3 ± 1.9
	2.0	0	Cream loose, friable	52.5 ± 2.7
	2.5	0	Light brown, friable	45.8 ± 2.4
	2.5	0.5	Brownish green, friable	64.8 ± 2.9
	2.5	1.0	Green, compact	61.0 ± 2.5

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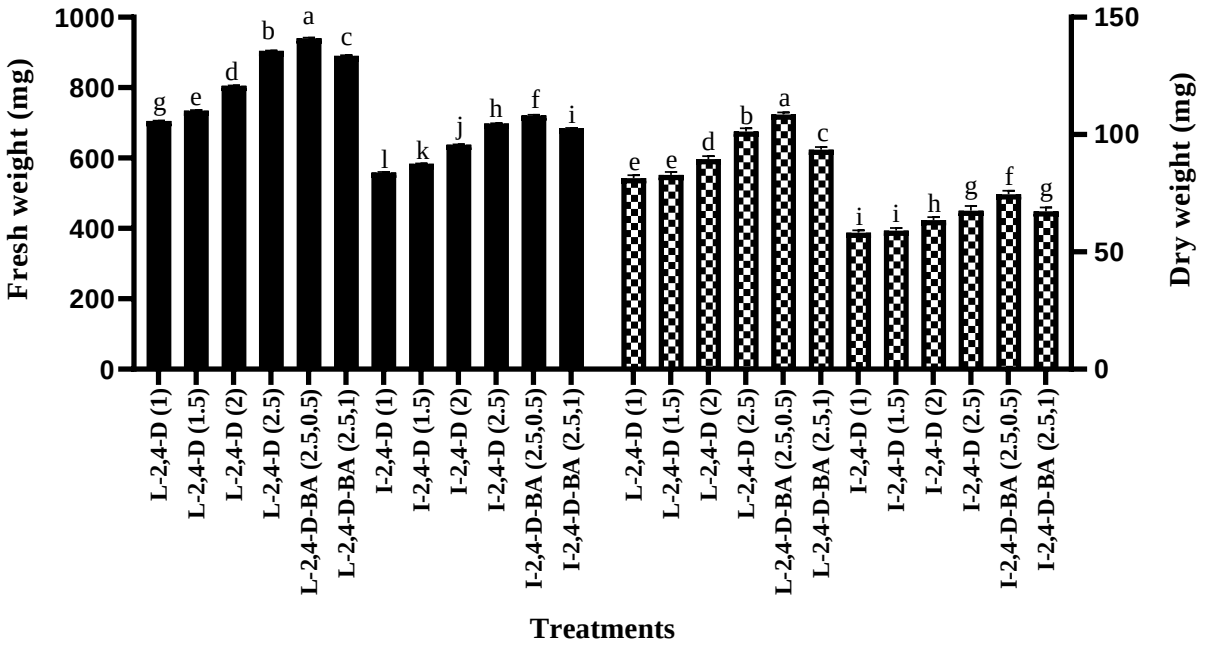

Fig. 3. Effect of different treatment of 2, 4-D concentrations and explant types on fresh and dry weights of *Withania coagulans* callus. L: Leaf explant. I: Internode explant. The numbers in parentheses indicate the concentrations of 2,4-D (mg/L) and BA (mg/L), respectively.

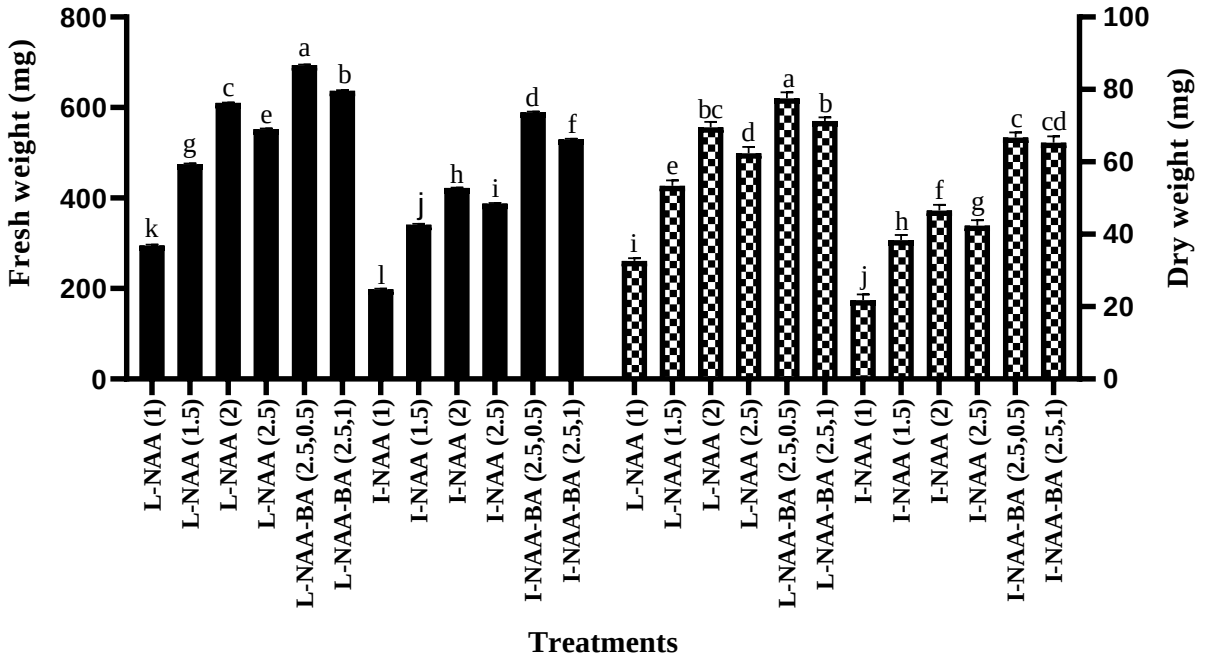

Fig 4. Effect of different treatment of NAA concentrations and explant types on fresh and dry weights of *Withania coagulans* callus. L: Leaf explant. I: Internode explant. The numbers in parentheses indicate the concentrations of NAA (mg/L) and BAP (mg/L), respectively.

The application of 2.5 mg/L IAA in combination with 0.5 mg/L BAP resulted in the highest callus induction (85.2 ± 1.9 %), fresh (841.6 ± 1.6 mg) and dry weights of callus (96.7 ± 0.7 mg), using the leaf explant after four weeks of culturing (Table 2). The application of IAA in combination with both concentrations of BAP resulted in better callus induction, higher fresh and dry weight of callus than the application of IAA solely using leaf explant.

The induced calli derived from leaf explants on culture medium supplemented with various concentrations of IAA solely or in combination with BAP were not friable. Nature of calli induced from internodal explants cultured on culture medium supplemented with 1.0 and 1.5 mg/L IAA were compact and rhizogenic. Only internodal explants cultured on a medium containing 2 and 2.5 IAA alone and a combination of 2.5 mg/L IAA with 0.5 mg/L BAP produced friable calli (Table 2).

Table 2. Callus induction from different explants of *Withania coagulans* after 4 weeks of culturing on MS medium supplemented with different concentrations of IAA (1.0, 1.5, 2.0, 2.5 mg/L) alone and in combination with BA (0.5, 1.0 mg/L).

Explants Source	Growth regulators (mg/L)		Nature of callus	Callus induction (%)	FW (mg)	DW (mg)
	IAA	BA				
Leaf	1.0	0	Brown loose, compact	51.8 ± 1.5^{e}	603.6 ± 1.2^{d}	68.7 ± 1.9^{d}
	1.5	0	Brownish white, compact	63.2 ± 2.5^{cd}	686.7 ± 0.8^{c}	78.6 ± 1.5^{c}
	2.0	0	Light brown, compact	41.8 ± 2.9^{fg}	483.1 ± 1.8^{f}	55.0 ± 1.1^{fg}
	2.5	0	Dark brown, compact	37.8 ± 1.9^{g}	463.6 ± 1.2^{h}	53.4 ± 1.5^{g}
	2.5	0.5	Brownish green, fragile	85.2 ± 1.9^{a}	841.6 ± 1.6^{a}	96.7 ± 0.7^{a}
	2.5	1.0	Light green, fragile	76.0 ± 2.2^{b}	766.7 ± 1.9^{b}	89.1 ± 1.0^{b}
Internode	1.0	0	Light brown, rhizogenous compact	43.0 ± 2.5^{f}	310.6 ± 1.3^{j}	40.2 ± 1.4^{h}
	1.5	0	brownish white, rhizogenous compact	54.6 ± 1.8^{e}	414.5 ± 1.4^{i}	56.5 ± 1.6^{f}
	2.0	0	Creamish brown, friable	36.8 ± 2.8^{gh}	260.7 ± 1.5^{k}	36.3 ± 1.4^{i}
	2.5	0	Light brown, friable	32.4 ± 2.3^{h}	228.2 ± 1.7^{l}	29.6 ± 1.3^{j}
	2.5	0.5	Creamish green, friable	67.2 ± 2.4^{c}	525.3 ± 1.2^{e}	65.1 ± 0.6^{e}
	2.5	1.0	Dark green, compact and gemmiferous	61.0 ± 2.5^{d}	469.4 ± 1.4^{g}	59.4 ± 1.5^{f}

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3.2. Time course of growth and withanolides production

The growth kinetics of *W. coagulans* cells followed a general growth pattern of sigmoid curves. The growth rates of the cell were initially slow but over time, they increased significantly and accumulated great amounts of biomass (about 3.5 fold for both FW and DW) over a period of 28 days. As can be seen in Fig. 5A, Plant cells grew well by the fourth week and the cells achieved a maximum FW (172 g/L) and DW (15 g/L). The cell cultures started to brown after this period and no increase in their biomass was observed. In relation to WFA and WNA production, the cells produced the highest both WTDs studied at the fourth week (Fig. 5B). The plant cells produced 0.08 ± 0.003 and 21 ± 0.4 µg/L WFA and WNA at this time, respectively.

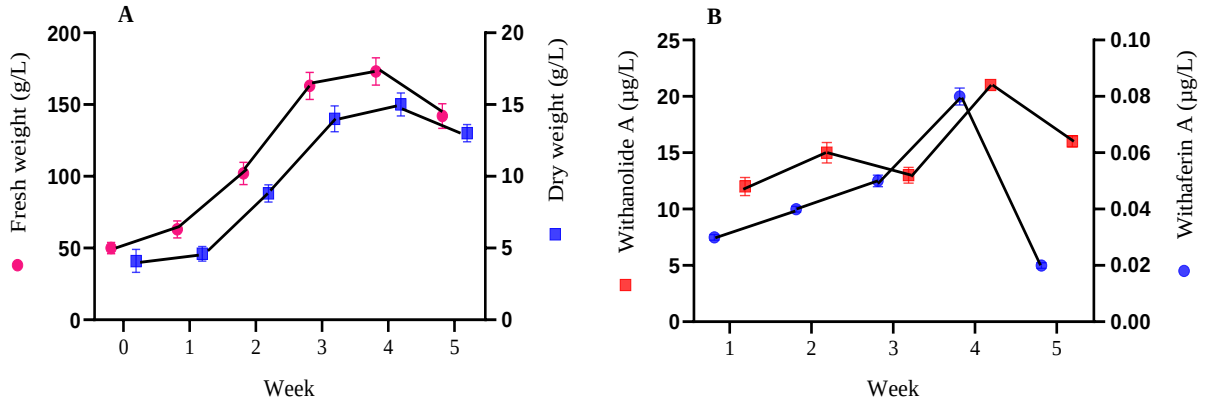

Fig. 5. Time course of growth (A) and withanolides production (B) of cell suspension culture of *Withania coagulans*, during the culture period (five weeks) in MS medium containing 1.5 mg/L IAA and 0.5 mg/L BAP.

4. Discussion

In the present study, the leaf explants showed qualitatively and quantitatively better results than internodal explants. Rani and Grover [23] reported that the maximum callus induction was achieved from cotyledonary leaf segments of *W. somnifera*, which is in agreement with obtained results. 2,4-Dichlorophenoxy acetic acid is the most common callus-inducing auxin on the explants because it is able to change the explant cells to dedifferentiation state [24].

In this study, the application of 2.5 mg/L 2,4-D in combination with 0.5 mg/L BAP led to the highest callus induction using the plant leaf and internodal segments. Similar results were also reported by Rani et al. [25] in *W. somnifera*, wherein the highest callus induction was achieved using MS medium fortified with 2 mg/L 2,4-D + 0.5 mg/L KN. On the other hand, Singh et al. [26] showed that the maximum callus of *W. somnifera* was obtained on MS medium fortified by 1.0 mg/L 2, 4-D and 0.5 mg/L IBA. Nagella and Murthy [27] obtained callus then established the cell suspension cultures of *W. somnifera* from leaf explants. The maximum callus induction (98 %) was also observed on MS medium supplemented with 0.5 mg/L of 2, 4-D and 0.2 mg/L KN by Chakraborty et al. [28] in *W. somnifera*. In the present study, fresh and dry weights of callus obtained from leaf segments were found to be higher than callus derived from internodal segments. The callus formation from an explant consists of three steps including induction, cell division, and differentiation, which their duration can be affected by the physiological status of the explant cells and

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environmental conditions. The proportional and intermediate concentrations of both auxins and cytokinins have been shown to induce callus, while a high ratio of auxin-to-cytokinin or cytokinin-to-auxin induces root and shoot regeneration, respectively [29]. Also, the important role of cytokinins in the enhancement of callus formation in *Arabidopsis thaliana* cultured on cytokinin-containing media may be due to the activation of some transcription factors and the expression of some involved genes as *Arabidopsis* response regulators (ARRs) [30]. Recent advances in biotechnology and plant cell culture have opened a new window into the commercial production of active ingredients from rare plants. In fact, the biotechnological process was applied for the investigation of plant cell suspension cultures, resulted in a promising bio-production platform for valuable metabolites. The culture of plant cells in liquid media is preferred to produce the desired specialized metabolite in the bioreactor [31]. Although the basic techniques for medicinal plant cell suspension cultures are well established, their use for large-scale production is still confined to a few processes. Identification of cell lines with high metabolite production capability, optimization of medium culture, use of elicitors and precursors, use of co-culture systems and metabolic engineering demonstrate new approaches to produce valuable metabolites [32]. The outputs of the present research revealed that cell growth in suspension culture was highest after 28 days. Similar to our result, Nagella and Murthy [33] showed that the highest accumulation of cell biomass of *W. somnifera* at the end of the fourth week of cell culture. We reported the rapid growth of *W. coagulans* suspension culture in flasks, wherein the growth curve of *W. coagulans* cell suspension culture revealed that the highest accumulation of biomass (172 g/L fresh weight and 15 g/L dry weight) was observed in the fourth week of the culture period. The nature of PGRs is one of the factors affecting the production of valuable drugs such as WNA and WFA in *in vitro* condition. In the previous study, a cell suspension culture of *W. somnifera* has been established for WNA production [27]. Also, it has been reported that the use of bioreactors in a *W. somnifera* cell culture increased the cell biomass (1.13 fold) as well as valuable metabolites of WNA (1.7 fold) and WFA (1.5 fold) compared to shake flask [34]. Sivanandhan et al. [35] has been stated that *in vitro* cultivation of *W. somnifera*, produced higher levels of WFA (1.14-fold) and WNA (1.1-fold) metabolites than plants grown in the field. Field acclimatized *in vitro* regenerated *W. coagulans* plants showed more amounts of WFA (1.4) and WNA (1.6) than wild-type plants [19].

5. Conclusion

The present study showed that cell suspension culture of *W. coagulans* is a viable and potent choice for the production of valuable medicinal compounds WFA and WNA. It can also be seen that *W. coagulans* cells followed a general growth pattern of sigmoid curve. The maximum cell biomass was obtained at the fourth week of culture which can be interesting for further elicitation experiments to increase the production of WTDs. Interestingly, the highest production of both WTDs was also observed in the plant cells at the fourth week of culture. This information can be used for future research on biosynthesis pathways of WTDs especially WFA and WNA as well as their production in bioreactors.

Author contributions

MH. M.: contributed to the conception of the study, plant materials collection, *in vitro* culture establishment, formal analysis, wrote and revised

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the manuscript. H. E.: helped in statistical analysis, data interpretation, and drafting the manuscript. Both authors read and approved the final manuscript.

Conflicts of Interest

The authors declare that they have no competing interests.

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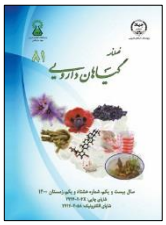

مقاله تحقیقاتی

القای کالوس و تولید ویتانولیدها از طریق کشت سوسپانسیون سلولی گیاه دارویی پنیرباد

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اطلاعات مقاله

گل‌واژگان:
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چکیده

مقدمه: گیاه پنیرباد (*Withania coagulans* (Stocks) Dunal) به دلیل خصوصیات درمانی فراوان، یک گیاه دارویی شناخته شده است. **هدف:** در این مطالعه، القای کالوس نرم و ایجاد کشت سوسپانسیون سلولی گیاه به منظور تولید ویتانولیدهای مهم ویتافرین A و ویتانولید A انجام شد. **روش بررسی:** القای کالوس در گیاه با استفاده از ریزنمونه‌های برگ و میانگره بر روی محیط کشت موراشیگ و اسکوگ (اِم اِس) دارای غلظت‌های مختلف (صفر، ۱، ۱/۵، ۲، و ۲/۵ میلی‌گرم بر لیتر) اکسین‌های توفوردی، نفتالن استیک اسید و ایندول استیک اسید به تنهایی یا در ترکیب با هورمون بنزیل آمینو پورین (صفر، ۰/۵ و ۱ میلی‌گرم بر لیتر) در قالب آزمایش فاکتوریل بر پایه کاملاً تصادفی با پنج تکرار انجام شد. سپس ایجاد کشت سوسپانسیون سلولی به منظور تولید ویتانولیدها انجام شد. **نتایج:** درصد کالوس‌زایی در ریزنمونه برگ (۲۵ تا ۹۶ ٪) بیشتر از ریزنمونه میانگره (۲۳/۲ تا ۸۵/۴ ٪) بود. درصد بالایی از کالوس نرم از ریزنمونه برگ کشت شده بر روی محیط کشت MS همراه با ۰/۵ میلی‌گرم توفوردی و ۰/۵ میلی‌گرم بنزیل آمینو پورین بدست آمد. کشت سوسپانسیون سلولی از کالوس‌های نرم روی محیط کشت MS همراه با ۱/۵ میلی‌گرم بر لیتر ایندول استیک اسید و ۰/۵ میلی‌گرم بر لیتر بنزیل آمینو پورین ایجاد گردید. بیشترین تجمع زیست‌توده (۱۷۲ گرم بر لیتر وزن تر و ۱۵ گرم بر لیتر وزن خشک) و تولید هر دو ویتانولید در هفته چهارم کشت مشاهده شد. سلول‌های گیاه در این زمان، به ترتیب ۰/۰۸ و ۲۱ میکروگرم بر لیتر ویتافرین A و ویتانولید A تولید کردند. **نتیجه‌گیری:** نتایج این مقاله می‌تواند برای تحقیقات بیشتر بر روی مسیرهای بیوسنتز ویتانولیدها و تولید آنها در بیوراکتور مورد استفاده قرار گیرد.

مخففها: WTDs، ویتانولیدها؛ WFA، ویتافرین A؛ WNA، ویتانولید A؛ PGRs، تنظیم‌کننده‌های رشد گیاهی؛ IAA، ایندول-۳- استیک اسید؛ 2,4-D، ۲و۴- دی کلروفنوکسی استیک اسید؛ NAA، نفتالن استیک اسید؛ BAP، ۶- بنزیل آمینوپورین؛ MS Medium، محیط کشت موارشیگ و اسکوگ؛ HPLC، کروماتوگرافی مایع با کارایی بالا

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