emergent
Life Sciences Research

Research Article

Effect of different concentrations of sucrose on Alkaloids and Steroids production *in vitro* from *Withania somnifera* (L) Dunal (Ashwagandha)

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Abstract

Present work has been conducted on *Withania somnifera* commonly known as Ashwagandha to study the influence of different sucrose concentrations on the production of high secondary metabolite compounds. With the addition of different sucrose concentrations 30, 60, 90 g / L to MS medium in *in vitro* conditions, production of secondary metabolite compounds increased. Ten compounds with different production rates were produced. Production rate of compound 17- hydroxy 27 deoxy withaferin, 27-hydroxy withanolide D and Withanone was maximum and significant under 30 g / L, 60 g / L, and 90 g / L sucrose treatment, respectively. However, MS medium containing 120 g / L sucrose led to burning of shoots and turned it in to brown.

Keywords secondary metabolite compounds, sucrose

Received: 20 October 2017
Accepted: 10 December 2017
Online: 11 December 2017

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Emer Life Sci Res (2017) 3(2): 32-36

E-ISSN: 2395-6658
P-ISSN: 2395-664X

DOI: http://dx.doi.org/10.7324/ELSR.2017.323236

Introduction

Withania somnifera (L.) Dunal is a member of the Solanaceae family, popularly known as 'Ashwagandha' [1-2]. It is a green woody shrub of 2-8 m height, found throughout the South East Asia including India, Pakistan, Bangladesh, Sri-Lanka, Nepal and different other parts of Australia, Africa and America [3-6]. It is widely distributed in Indian provinces of Madhya Pradesh, Uttar Pradesh, Punjab, Gujarat and Rajasthan [1]. It is an important medicinal plant that has been used in Ayurvedic and indigenous medicine for over 3,000 years [7]. Additionally, it has multipurpose medicinal applications including antidiabetic, immunomodulatory, hemopoietic, neurological inflammatory disorders and Parkinson's disease, it is also useful as antioxidant, abortifacient, antibiotic, aphrodisiac, de-obstruent, diuretic and sedative [8-9]. Ashwagandha roots are constituent of over 200 formulations in Ayruvedha Siddha and Unani medicines, which are used in the treatment of various physiological disorders. [10-11].

The major biochemical constituents of *W. somnifera* are withanolides (steroidal lactones with ergostane skeleton). Withanolides are highly oxygenated phytochemicals, and the oxidation at various sites of skeleton is responsible for the structural variations in different classes of withanolides [12]. There are different classes of formulations that have been isolated from this plant and characterized including withanosides, glycowithanolides, sitoindosides, alkaloids, saponins, amino acids, phenolic compounds, flavonoids and many other secondary bioactive metabolites of the plant with broad-spectrum therapeutic activity [13]. Its roots are rich in alkaloids (withanine) [14] and the other parts are useful for the treatment of other inflammatory conditions and tuberculosis and exhibit excellent antitumor and anti-bacterial properties [15-16].

The conventional method of propagating this species is through seeds, but it exhibits low germination and seed viability is very poor and vegetative propagation of this plant is not too successful. Tissue culture propagation could offer a valuable alternative and a reliable procedure for propagation [17].

The aim of this investigation was to study the possibility of stimulating the Ashwagandha plant using different concentrations of sucrose to increase the secondary metabolite compounds production.

Methodology

Tissue culture experiment was conducted in the laboratory of the College of Education for Pure Sciences / University of Diyala during 2014-2015. The seeds of the Ashwagandha plant were obtained from the medicinal and aromatic plant cultivation project / General Company for Horticulture and Forestry, Ministry of Agriculture.

Due to the low rate of seed germination, Gibberllin treatment was conducted. The seeds were treated with different concentration of Gibberellin (GA3) to adopt the optimal concentration to achieve highest germination rate. The seeds were washed in running water for 10 minutes followed by distilled water and were soaked with different concentrations of GA3 (250, 500, 750 mg / L) for 12 and 18 hours. Later, seeds were planted in pots containing peat moss. The highest germination rate was found in the seeds treated with Gibberellin at a concentration of 500 mg /L for 18 hours [18].

Finally, for the main experiment, seeds were submerged with GA3 with a concentration of 500 mg / L for 18 hours at room temperature. Further, seeds were washed under running tap water to remove dust and dirt followed by washing with Sodium hypochlorite NaOCl for 15 minutes and rinsed thrice with sterile distilled water to remove the traces of NaOCl. The seeds were inoculated on MS medium [19] containing 0.7% (w/v) agar supplemented with various concentrations of vitamins for 30 days prior to the autoclave. The medium was adjusted to the pH 5.8. The primary shoots formed *in vitro* were separated aseptically and cultured on MS medium supplemented with 1mg/L BAP (6-Benzyl aminopurine) for multiplication of shoots for 30 days, then it was cultured on MS medium with different concentrations of sucrose (30, 60, 90, 120 g / L). The quantitative content of total steroids and alkaloids was estimated for the total vegetative phase of formed shoots on MS medium in *in vitro* by using (High-Performance Liquid Chromatography, HPLC). The experiment was conducted in completely randomized design (CRD) with 10 replications to each compound and the data was analyzed by One-way Analysis of Variance (ANOVA) [20].

Table 1. Effect different concentrations of sucrose on Secondary metabolite compounds production in MS medium in vitro for Ashwagandha

	Secondary metabolite compounds	Sucrose 30 g / L	Sucrose 60 g / L	Sucrose 90 g / L	Sucrose 120 g / L
1	27-hydroxy withanolides	63.33 d	0.0 h	4.25 f	**0.0**
2	17-hydroxy withanolides A	66.26 d	31.23 g	85.76 e	**0.0**
3	17- hydroxy 27 deoxy withaferin	88.87 a	200.60 d	231.38 b	**0.0**
4	Withaferin A	31.35 g	235.10 c	0.0 g	**0.0**
5	Withanolide D	36.88 f	244.01 b	114.37 d	**0.0**
6	27-hydroxy withanolide D	73.48 c	393.04 a	0.0 g	**0.0**
7	Withanolide A	83.38 b	95.25 f	209.36 c	**0.0**
8	Withanone	28.44 g	124.26 e	354.00 a	**0.0**
9	deoxy withaferin A	48.40 e	0.0 h	0.0 g	**0.0**
10	Total Alkaloids	0.02 h	0.13 h	0.12 g	**0.0**
	CD (0.05)	**3.236**	**3.495**	**2.012**	**0.0**

Results and Discussion

Concentrations of sucrose 30, 60, 90 g / L were efficient to promote high secondary metabolite compounds production in *Withania somnifera* as compared to 120 g / L sucrose. It was observed that the shoots planted on the MS medium containing 120 g sucrose were turned to brown, which means the vegetable part was not responsive and the high concentration of sucrose may have led to the toxicity and death of the planted plant part; hence, chemical analysis of this treatment could not be performed (Table 1). Ten compounds with different rates and significant differences were produced from the addition of different concentrations of sucrose to the MS medium. Production rate of compound 17- hydroxy 27 deoxy withaferin, 27-hydroxy withanolide D and Withanone was maximum and significant under 30 g / L, 60 g / L, and 90 g / L sucrose treatment, respectively. Plant *in vitro* cultures offer possibilities for the production of secondary metabolites in bioreactors, manipulation of metabolic pathways and metabolic engineering. Studies showed that the sucrose could influence secondary metabolites production in cell and organ cultures [21-22]. The results of many investigations have concluded that the increase of sucrose within certain limits leads to increased production of alkaloids in tissue cultures of various plants [23-25]. Matsumoto et al [26] reported that increase of sucrose in the tissue culture medium leads to an increase of phytochemicals.

The sucrose is not only an essential carbon and energy resource for plant cells, but it also importantly affects the assembly of metabolites of the phenylpropanoid pathway [27]. The chemistry of *Withania* species has been extensively studied and several groups of chemical constituents such as steroidal lactones, alkaloids, flavonoids, tannin etc. have been identified, extracted, and isolated [28-33]. At present, more than 12 alkaloids, 40 withanolides, and several sitoindosides (a withanolide containing a glucose molecule at carbon 27) have been isolated and reported from aerial parts, roots, and berries of Withania species. The major chemical constituents of these plants, withanolides, are mainly localized in leaves, and their concentration usually ranges from 0.001 to 0.5% dry weight (DW) [34-35]. Sahwar et al. [36] reported that addition of different concentrations of sucrose in the middle of plantations *Catharanthus roseus* has stimulated the production of some alkaloids; however, the high concentration of sugars may have led to the toxicity and death of the plant.

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