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The Effect of Nutrients Electrical Conductivity on Growth Performance and Biochemical Analysis of Hydroponically Grown *Catharanthus roseus* (L.) G. DON.

Research Article

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Abstract

High demand and low synthesis of effective chemotherapeutic agents against cancer harvested from *Catharanthus roseus* has fostered research for its increased production. Cellular receptor of plants responds to electrical conductivity (EC) of nutrient media and subsequently causes morphological and biochemical changes. Optimization of EC in controlled culture media can be used for potential modulation and consequent enhancement of secondary metabolites in plants. In the current study, effect of EC of nutrient media were studied in *Catharanthus roseus* through hydroponic system. The results revealed that plant growth, biomass production, chlorophyll and total soluble protein content gradually increased with increase in EC strength but significantly decreased at 4.0 dS/m. Accumulation of total soluble sugar and proline considerably increased with the increase in EC strength. Based on the growth performance and biochemical criteria studied, the optimal EC for growing *Catharanthus roseus* was found to be 2.0 dS/m which can be further utilized to enhance secondary metabolites production.

Keywords: Electrical conductivity (EC); Hydroponics; *Catharanthus roseus;* Terpenoid Indole Alkaloids

Introduction

Catharanthus roseus (L.) G. Don is a perennial, evergreen herb of the family Apocyanaceae commonly known as periwinkle, Vinca and as "Sadavahar". This is native to Madagascar but widely cultivated and naturalized around south eastern world. The genus consists of two species categorized as 'Rosea' for pink flowered plant and 'Alba' for white flowered plant [1]. *Catharanthus* is one of the most common ornamentals plant but also a widely investigated medicinal plant. It is known to synthesize more than 130different types of terpenoid indole alkaloids (TIAs) with pharmacological activity [2]. Traditionally it was used as a hypoglycemic agent but the present interest in this plant is due to the fact that it is the sole source of chemotherapeutic agents Vinblastin and Vincristine with powerful anti-cancerous activities [3]. Apart from this, some other valuable TIAs from *C. roseus* are ajmalicine, serpentine used for treating hypertension, heart arrhythmias, circulatory disorder, cathrenine for treating diabetics. These plants extensively grown as an annual plant but the content of terpenoid indole alkaloids (TIAs) are very low. Their effectiveness in different medicinal treatment, high market value and low synthesis in nature has fostered research to determine their biosynthesis pathways to develop alternative production methods. However, the synthesis and accumulation of secondary metabolites is highly influenced by many genetic, morphogenic and environmental factor [4]. Environmental factors such as temperature, humidity, light, water, mineral concentration and CO_2 influence the plant growth and have direct impact on biochemical pathway affecting the production of secondary metabolism [3]. For this reason, the application of various abiotic stresses has become a very promising and cost-effective alternative solution for elicitation of the secondary metabolites. However, success can be achieved by establishing a balance between the synthesis of secondary metabolites of interest and stress metabolites needed by

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the plants to tolerate the stress [5]. The quality of the plants growing under natural environment condition is difficult to control as so many factors influence its ecophysiology and the actual biosynthetic pathways are difficult to elucidate. For this, there is a necessary to study ecophysiology of stress response in plants under a controlled environment condition. Plants are among the few organisms that can synthesize all the required metabolites from inorganic ions, water and CO_2 using the energy captured from the sun. Hydroponics is a method of growing plants that takes advantage of this fact by providing all of the nutrients, in their inorganic form, in a liquid solution [6]. In this system plants are grown inside enclosures designed to control air, light, temperature and plant nutrition. Hydroponic systems have been extensively used by scientists for exploring nutrient requirements and also the toxicity of some elements in *Arabidopsis* and other plant species [7]. In plants nutrients plays a great role in affecting the physiology, growth and biosynthesis of primary and secondary metabolites. Inadequate management of nutrient solution or imbalanced ion composition inhibits plant growth due to nutrient toxicity or deficiency [8]. In order to provide required strength of nutrients to plant it is necessary to maintain optimum EC (electrical conductivity) of nutrient solution. Various kinds of nutrient used are basically salt when they are dissolved in water it breaks into cation and anion in the nutrient solution and are capable of conducting electricity, so a higher concentration of nutrient solution means greater electrical ions and greater degree of EC.

The objective of the present study was therefore to document the growth response of *Catharanthus roseus* to various electrical conductivity (EC) of the nutrient solution and to analyse the stress related biochemical changes which can be further utilized to enhance secondary metabolites production.

Materils & Methods

Plant materials and experimental design

A cultivar of *Catharanthus roseus* (L.) G. Don "Alba" was used for the study. The sterilized seeds of *Catharanthus roseus* var. "Alba" obtained from RPRC, Bhubaneswar and were grown in our college garden. The seeds from the field grown plants were grown in rock wool cubes (125 cm^3) in a cultivation room with photoperiod set to 16 h per day provided by cool white fluorescent lamps. Air temperature and relative humidity were set to 26°C and 60-80% respectively. When the seedlings have grown to four leaf stage with fully expanded leaves, they were transplanted to plastic tanks (22 x 16 x 8cm) containing nutrient solution with different EC strength. In this study six EC strength were arranged in a randomized block design with three replicates. The pH of each treatment solution was adjusted to 6.0. A portable conductivity meter was used to measure and set different EC of each nutrient solution tanks. The original nutrient solutions were based on Hoagland's solutions. The nutrient solutions with varying EC were prepared with deionized water and the original Hoagland nutrient solution. The six different EC treatments includes: EC-0 (contained only single distiled water), EC- 0.25, EC-0.5, EC-1.0, EC-2.0 and EC- 4.0 (contained original Hoagland solution diluted with single distiled water to 0.25 dS/m, 0.5 dS/m, 1 dS/m, 2 dS/m and 4 dS/m respectively). After 30 days of culture, the plants from the culture tanks were harvested and analysed for different growth and biochemical parameter.

Measurement of Plant Growth Parameter: The plants along with roots were harvested after 45 days of culture from the different EC treatment. The plant height shoots and root fresh weight (SFW and RFW) were measured. They were dried in an oven at 60^{0}C for 48 hrs and reweighed for shoot and root dry weight (SDW and RDW).

Biochemical Assay and estimation

Total Chlorophyll: Total Chlorophyll of leaves was determined according Arnon's method for all the EC treatment. 100 mg of leaf samples were weighed and extracted with 10 mL 85% acetone. The extract was filtered and analysed using a UV- Vis spectrophotometer on wavelength of 645nm and 663 nm. The concentration of total chlorophyll was calculated using the following equation: Total Chlorophyll: 20.2(A645) + 8.02(A663).

Total soluble proteins: Fresh leaves (500 mg) of *C. roseus* were harvested from each treatment and homogenised in1mL phosphate buffer (0.1M, pH-7.0). The protein concentration was determined following Bradford assay method taking absorbance reading at 595 nm.

Total soluble sugars: Total soluble sugars (TSS) were extracted from leaves for all EC treatment using the method of Irigoyen *et al.,* (1992). A sample of 200mg of leaves were homogenized in 5 mL of 96% ethanol and washed with 5mL of 70% ethanol. The extract was centrifuged at 3500 x g for 10 min and supernatant was stored at 4^{0}C prior to measurement. Each TSS concentration was determined by reacting 0.1 mL of the ethanolic extract with 3mL of freshly prepared anthrone reagent by placing it in a boiling water bath for 10 min. After cooling absorbance was recorded at 625 nm.

Proline: Free Proline content was estimated according to the procedure described by Bates *et al.,* (1973). 50 mg of sample were homogenized in 10 ml of 3% (v/v) aqueous sulphosalicylic acid and then the homogenate was filtered. 2 ml of the filtrate was then mixed in a test tube with 2mL of ninhydrin solution and 2 mL glacial acetic acid and incubated at 100 0 C for 1 hour. The reaction was than terminated by placing the mixture in ice bath. When the reaction mixture cooled, 6 ml of toluene was added and the combination transferred to a separating funnel. After thorough mixing, the chromophore containing toluene was separated and the absorbance was read at 520 nm in a spectrophotometer against a toluene blank.

Statistical Analysis: Statistical analysis was performed using the one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). The values are mean ± SE for three samples in each group. *P* values < 0.05 were considered as significant.

Results

Growth and morphology: In studying the effect of different EC treatment of culture media on *Catharanthus roseus*, it was observed that the effect of different level of EC on all parameters under the study is significant (Figure 1). Plant growth recorded after 45 days of culture showed clear variations in the different treatment of EC. The overall plant growth was more in the treatment with EC 2.0 dS/cm than the plants in other EC treatments. The plant height gradually increases with the increase in EC of the nutrient solution but was inhibited at EC 4.0. Stastical analysis revealed that there was significant effect of

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EC on plant height but the effect was significantly high at 2.0dS/cm (Table 1). The EC level also affected the total fresh weight of shoot and root. Statistical analysis indicated that EC had significant effect on the fresh weight of both shoot and root (SFW and RFW). The plant grown with EC treatment 2.0 dS/m had highest shoot and root fresh weight (Figure 2A) and were superior to EC 4.0 dS/m. The different EC level of nutrients solution affected the shoot and root dry weights (SDW and RDW) and statistically that were significant (Table 1). The SDW and RDW grown in EC2.0 had attended higher dry weight but were statistically same with that of RDW of EC 1.0 dS/m (Figure 2B).

Total Chlorophyll, Total Soluble Protein, Total Soluble Sugar and Proline Content: The total chlorophyll content gradually increased as the EC level is increasing. The statistical analysis showed a significant difference between the different EC treatments (Table 2). The chlorophyll content reached the highest value in EC 2.0 dS/m and then decreased significantly at higher EC i.e., 4.0 dS/cm (Figure 2C). Similarly, the total soluble protein in shoots increases significantly with the increase from EC 0 to EC 2.0 dS/cm and then decreased at EC 4.0 treatment (Figure 2D). The Total Soluble sugar content of leaves increased significantly with increasing EC strength (Table 2). The maximum amount was observed at 4.0 dS/m EC and lowest was related to the control (Figure 2E). Similarly, the Proline content varied significantly in different EC treatment. With the increase in the EC level accumulation of Proline in leaves increased and maximum Proline content was recorded at 4.0 dS/cm (Table 2, Figure 2F).

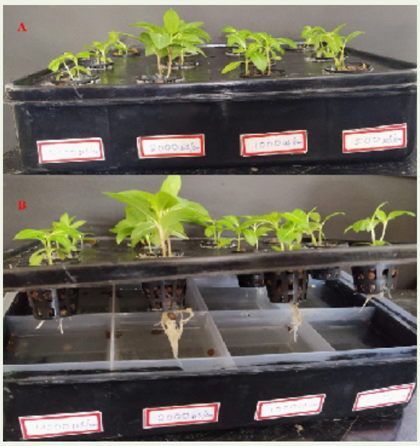

Figure 1: Effect of EC of Hydroponic culture medium on plant Shoot growth (A) and Root growth (B) after 45 days of treatment.

Table 1: Effect of electrical conductivity (EC) of culture media on Morphology of hydroponically grown plants after 45 days.

Treat ments	Plant height (cm)	Shoot FW (g)	Root FW (g)	Shoot DW (g)	Root DW (g)
EC-0.00	2.12 ± 0.06^{e}	3.83 ± 0.09^{e}	0.55 ± 0.07^{e}	0.51 ± 0.01^{d}	0.13 ± 0.03^{bc}
EC-0.25	2.47 ± 0.13^{d}	4.30 ± 0.15^{d}	1.01 ± 0.12^{d}	0.56 ± 0.01^{cd}	0.16 ± 0.03^{b}
EC-0.50	2.78 ± 0.13^{c}	5.53 ± 0.15^{c}	1.46 ± 0.11^{c}	0.61 ± 0.04^{c}	0.18 ± 0.02^{b}
EC-1.00	3.55 ± 0.14^{b}	6.43 ± 0.20^{b}	1.93 ± 0.04^{b}	0.75 ± 0.03^{b}	0.25 ± 0.03^{a}
EC-2.00	4.19 ± 0.11^{a}	8.27 ± 0.21^{a}	2.99 ± 0.08^{a}	0.87 ± 0.02^{a}	0.29 ± 0.02^{a}
EC-4.00	2.64 ± 0.13^{c}	4.43 ± 0.19^{d}	1.26 ± 0.05^{bc}	0.62 ± 0.02^{c}	0.11 ± 0.01^{c}

Data represents the mean ± SE (n=3) and values denoted by different letters are significantly different at p ≤ 0.05.

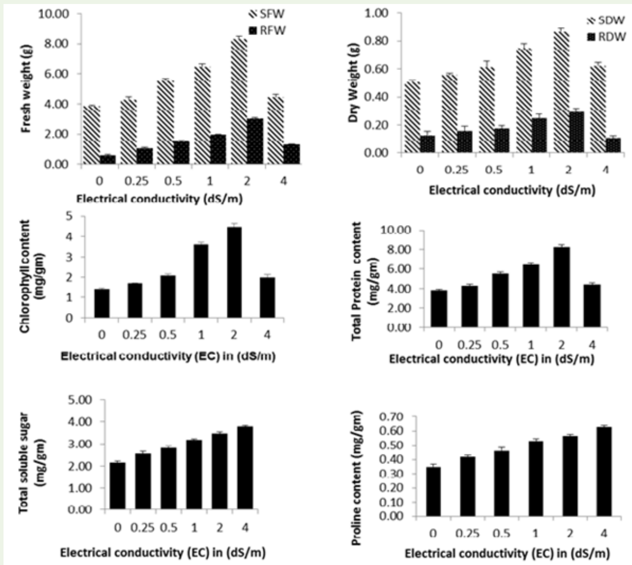

Figure 2: Effect of EC of Hydroponic Culture Media on Plant growth: Fresh weight of Shoot and root (A), Dry weight of Shoot and root (B) Total Chlorophyll Content (C), Total Protein (D), Total soluble Sugar (E), Proline Content (F) after 45 days of treatment. Data represent the mean ± SE, (n=3).

Table 2: Biochemical changes in plants with response to the electrical conductivity (EC) of the Hydroponic culture media after 45 days.

Treatmet	Total Chlorophyll	Total Protein	Total Soluble Sugar	Proline Content
EC-0.00	1.42 ± 0.04^{e}	5.92 ± 0.11^{c}	2.16 ± 0.05^{f}	0.35 ± 0.02^{d}
EC-0.25	1.70 ± 0.03^{d}	6.17 ± 0.04^{bc}	2.55 ± 0.09^{e}	0.42 ± 0.01^{c}
EC-0.50	2.09 ± 0.06^{c}	6.75 ± 0.05^{b}	2.80 ± 0.09^{d}	0.46 ± 0.03^{c}
EC-1.00	3.59 ± 0.12^{b}	7.11 ± 0.03^{a}	3.18 ± 0.04^{c}	0.53 ± 0.02^{b}
EC-2.00	4.47 ± 0.18^{a}	7.62 ± 0.13^{a}	3.46 ± 0.07^{b}	0.57 ± 0.01^{b}
EC-4.00	1.98 ± 0.16^{cd}	6.46 ± 0.03^{b}	3.77 ± 0.05^{a}	0.63 ± 0.01^{a}

Data represents the mean ± SE (n=3) and values denoted by different letters are significantly different at p ≤ 0.05.

Discussion

Increasing EC level causes a significant increase in plant height, fresh weight and dry weight of both root and shoots up to 2.0 dS/m. The increase in EC is due to the increase in the concentration of nutrients and elements which are important for plant growth and development. So with the increase in the concentration of the nutrient there is an increase the growth parameter of *Catharanthus roseus*. The highest EC treatment EC 4.0 dS/m shows a significant decrease in growth parameter which may be due to the toxic effect of high nutrient concentration in solution [9-11]. Albornoz and Lieth (2015) found that increase in EC at root zone reduces the yield of lettuce due combined effect of decrease leaf area and stomatal conductance [4]. Increase in EC above 2.0 dS/m increases salinity of the medium and causes water stress which may have caused the reduction of turgor pressure within cell and restricted cell expansion [12,13]. Osmotic effect, nutritional deficiency on account of ionic imbalance and decrease in many metabolic activities due to high EC reduces water absorption by root that attributes to the reduction in plant growth [14-16]. Similar results were observed by various author

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where increase in concentration of nutrients in medium leads to water deficits and reduction of vegetative characters [5,15]. The uneven supply of nutrient concentrations considerably affects physiological process of the plants. In our present study we found that the total chlorophyll content was relatively low in low EC treatments which may be probably due to low nutrient concentration and deficiency of nutrient such as N, Mg and Fe that are important for chlorophyll biosynthesis. There is a gradual and significant increase in chlorophyll content with the increasing EC treatment with respect to control. But at EC treatment of 4.0 dS/m the chlorophyll content decreases which could be associated with the accumulation of Na+ in leaves and decreased pigmentation concentration [2]. Conversely, it has been reported by many authors that there is an increase of chlorophyll content with increase in EC above 2.0dS/m [11,17-26]. Total protein content determines the nitrogen content of a plant. In this study the total protein increased with increase in EC due to availability of more nitrogen element. There is an increase in protein content of plants in EC treatment up to 2.0 dS/m which is similar to results reported for Pakchoi and Corn [11,21]. At higher EC treatment of 4.0 dS/m the protein content in *Catharanthus* decreases. It may be suggested that nitrogen and carbon containing compound plays an important role during stress as osmotic adjustment (Misra and Gupta, 2006). So, there is a reduction of protein content at higher EC treatment of 4.0 dS/m as the nitrogen and carbon will be used as osmotic adjustment. On the other hand, there is a significant increase in the level of Proline in our present study with the increasing EC strength. For maintaining the ionic balance in vacuoles, cytoplasm accumulates low molecular weight compatible solute which does not interfere with normal biochemical reaction by replacing water in biochemical reaction (Ahmad and john, 2005). Several authors have suggested strong correlation between cellular Proline level and the capacity to survive different environmental stress [7,19,22]. Increased level of proline has also been recorded to correlate with enhanced tolerance to water deficient stress in *Catharanthus roseus* [2]. Proline and it intermediates helps in the regulation of stress responsive gene (Khedr*et al.,* 2003) and enhanced expression of osmoregulatory gene [24]. Moreover, Proline was reported to function as radical scavenger, electronic sink, stabilizer of macromolecules and a cell wall component [18]. There is a significant increase in the total soluble sugar content with increasing EC strength. Similar results were reported by Amalfitano *et al.,* (2017) in 'Friariello' pepper fruit and Ramezani (2011) in *Echium amoenum* [27-29]. Accumulation of soluble sugars in response to the increasing environmental stress has been widely reported despite specific reduction in net CO_2 assimilation level [9,19]. Sugar in addition to the role of regulating osmotic balance acts as metabolic signals in the stress condition.

Conclusion

Effect of different EC treatments on *Catharanthus roseus* culture grown hydroponically were shown better growth in EC strength of 2.0 dS/m as evident from increased morphological and biochemical parameters. Too low EC strength limits plant growth while high EC strength is inhibiting growth because of salinity stress. Considerable increase in accumulation of proline and soluble sugar in higher EC also indicates stress tolerance capacity of the plant, which will be helpful for monitoring the elicitation of secondary metabolites.

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