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Original Research Article

Hydroponically grown *Catharanthus roseus* (L.) G. DON: Impact of electrical conductivity of nutrients on growth rate and biochemical analysis

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ABSTRACT
Research into increasing the production of *Catharanthus roseus* has been prompted by the high demand and low synthesis of efficient chemotherapeutic medicines against cancer. Plants' cellular receptors react to the electrical conductivity (EC) of their nutritional media, which results in morphological and biochemical alterations. Plant secondary metabolites may be modulated and subsequently enhanced by the optimization of EC in controlled culture medium. The current study used a hydroponic system to examine the effects of EC of nutrient media on *Catharanthus roseus*. Plant growth, biomass output, chlorophyll, and total soluble protein content all grew progressively as EC strength increased, but they all sharply declined at 4.0 dS/m, according to the results. Proline and total soluble sugar accumulation significantly increased as EC strength rose. The ideal EC for growing *Catharanthus roseus* was determined to be 2.0 dS/m based on the growth performance and biochemical criteria examined. This EC can then be used to improve the synthesis of secondary metabolites.

1. Introduction

Perennial and evergreen, *Catharanthus roseus* (L.) G. Don belongs to the Apocyanaceae family and is sometimes referred to as "Sadavahar," Vinca, and periwinkle. Although it originated in Madagascar, this plant is widely grown and naturalized throughout the southeast region of the world. The two species in the genus are called "Alba" for white-flowered plants and "Rosea" for pink-flowered plants [1]. In addition to being one of the most popular ornamentals, catharanthus is a therapeutic plant that has been extensively studied. More than 130 distinct terpenoid indole alkaloids (TIAs) having pharmacological action have been synthesized using it [2]. Originally employed as a hypoglycemic medication, this plant is currently of interest since it is the only source of the potent anti-cancer chemotherapeutic drugs vinblastin and vincristine [3]. Other useful TIAs from C. roseus include cathrenine, which is used to treat diabetics, ajmalicine, and serpentine, which is used to treat circulatory disorders, heart arrhythmias, and hypertension. Although these plants are widely grown as annuals, they include very little terpenoid indole alkaloids (TIAs). Their high market value, low synthesis in nature, and usefulness in many medical treatments have encouraged research into their biosynthesis processes in order to create alternate production techniques. However, a variety of genetic, morphogenic, and environmental factors have a significant impact on the production and accumulation of secondary metabolites [4]. Temperature, humidity, light, water, mineral concentration, and CO_2 are examples of environmental elements that affect plant growth and directly alter the metabolic pathway that produces secondary metabolism [3]. Because of this, applying different abiotic stresses has emerged as a very viable and affordable substitute method for eliciting secondary metabolites. Achieving success, however, requires striking a balance between the production of secondary metabolites of interest and those that the plants require in order to withstand the stress [5]. It is challenging to regulate the quality of plants growing in their natural environments because a wide range of factors affect their ecophysiology and it is challenging to identify the precise biosynthetic pathways. Studying the ecophysiology of plants' responses to stress in a controlled setting is required for this. One of the few species that can use the energy they absorb from the sun to create all the necessary metabolites from inorganic ions, water, and CO_2 is a plant. One technique for growing plants that makes use of this feature is hydroponics, which uses a liquid solution to supply all of the nutrients in their inorganic form [6]. Under this approach, plants are cultivated in enclosures that regulate temperature, light, air, and plant nutrition. Scientists have employed hydroponic systems extensively to investigate the nutritional requirements and toxicity of certain elements in Arabidopsis and other plant species [7]. Plant physiology, growth, and the biosynthesis of primary and secondary metabolites are all significantly impacted by nutrition. Due to nutrient toxicity or inadequacy, plant growth is inhibited by improper nutrient solution management or an unbalanced ion composition [8]. The ideal EC (electrical conductivity) of the nutrition solution must be maintained in order to supply the plant with the correct strength of nutrients. Many types of nutrients are essentially salts that, when dissolved in water, separate into cations and anion in the nutrient solution. Because of their electrical conductivity, a higher concentration of nutrient solution indicates a higher degree of EC and more electrical ions.

Therefore, the goal of this work was to record how *Catharanthus roseus* grew in response to different electrical

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conductivities (EC) of the nutrient solution and to examine the biochemical alterations brought on by stress that may be used to improve the synthesis of secondary metabolites.

2. Materials and methods

2.1 Plant materials and experimental design

For the investigation, a cultivar of *Catharanthus roseus* (L.) G. Don "Alba" was employed. We grew the sterilized *Catharanthus roseus* var. "Alba" seeds in our campus garden after obtaining them from CCS-HAU, Hisar. In a growing environment with a photoperiod of 16 hours per day supplied by cool white fluorescent lamps, the seeds from the field-grown plants were cultivated in rock wool cubes (125 cm^3). Relative humidity and air temperature were set at 60–80% and 27°C, respectively. The seedlings were moved to plastic tanks (22 × 16 × 8 cm) with nutritional solutions of varying EC strengths once they reached the four-leaf stage with completely developed leaves. This study used a randomized block design with three replicates to arrange six EC strengths. Every treatment solution's pH was brought down to 6.0. Each nutrient solution tank's EC was measured and adjusted using a portable conductivity meter. Hoagland's solutions served as the model for the initial nutrition solutions. De-ionized water and the original Hoagland nutrient solution were used to create the nutrient solutions with different EC. EC-0 (containing only one distilled water), EC-0.25, EC-0.5, EC-1.0, EC-2.0, and EC-4.0 (containing the original Hoagland solution diluted with one distilled water to 0.25 dS/m, 0.5 dS/m, 1 dS/m, 2 dS/m, and 4 dS/m, respectively) are the six distinct EC treatments. The plants from the culture tanks were taken out and examined for various growth and biochemical parameters after 30 days of cultivation.

Plant Growth Parameter Measurement: After 45 days of culture from the various EC treatments, the plants and roots were collected. Root fresh weight (SFW and RFW) and plant height shoots were measured. After 48 hours of drying at 600C in an oven, they were reweighed to determine the shoot and root dry weights (SDW and RDW).

2.2 Biochemical assay and estimation

Total Chlorophyll: For each EC treatment, the total chlorophyll of the leaves was calculated using Arnon's method. Ten milliliters of 85% acetone were used to extract 100 milligrams of leaf samples. The extract was filtered and examined at wavelengths of 645 and 663 nm using a UV-Vis spectrophotometer. The following formula was used to determine the total chlorophyll concentration: A total of 20.2 (A645) plus 8.02 (A663) chlorophyll.

Total soluble proteins: 500 mg of fresh C. roseus leaves were taken from each treatment and mixed in 1 mL of 0.1M, pH-7.0 phosphate buffer. The Bradford assay method, which takes an absorbance reading at 595 nm, was used to determine the protein content.

Total soluble sugars: Using Irigoyen et al. [12] method, total soluble sugars (TSS) were extracted from leaves after every EC treatment. After homogenizing 200 mg of leaves in 5 mL of 96% ethanol, the sample was rinsed with 5 mL of 70% ethanol. Before being measured, the extract was centrifuged for 10 minutes at 3500 x g, and the supernatant was kept at 40C. By reacting 0.1 mL of the ethanolic extract with 3 mL of freshly made anthrone reagent in a boiling water bath for 10 minutes, each TSS concentration was ascertained. Absorbance was measured at 625 nm after cooling.

Proline: The method outlined by Bates et al. [8] was used to estimate the free proline content. After homogenizing 50 mg of the material in 10 milliliters of 3% (v/v) aqueous sulphosalicylic acid, the homogenate was filtered. After that, 2 milliliters of the filtrate, 2 milliliters of ninhydrin solution, and 2 milliliters of glacial acetic acid were combined in a test tube and incubated for one hour at 100°C. After that, the mixture was placed in an ice bath to stop the process. Six milliliters of toluene were added to the reaction mixture once it had cooled, and the liquid was then moved to a separating funnel. Following extensive mixing, the toluene-containing chromophore was separated, and the absorbance at 520 nm was measured in a spectrophotometer in comparison to a toluene blank.

Statistical Analysis: The one-way analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) were used for statistical analysis. For each group, the values are mean ± SE for three samples. P-values less than 0.05 were deemed significant.

3. Results

Growth and morphology: It was found that the impact of varying EC levels on all research parameters was substantial when examining the effects of various EC treatments of culture media on *Catharanthus roseus* (Figure 1). After 45 days of culture, plant development revealed distinct differences between the various EC treatments. Compared to plants in other EC treatments, the plants in the EC 2.0 dS/cm treatment grew more overall. Although it was inhibited at EC 4.0, the plant height progressively increased as the nutrient solution's EC increased. EC had a considerable impact on plant height, according to statistical analysis, however the effect peaked at 2.0dS/cm (Table 1).

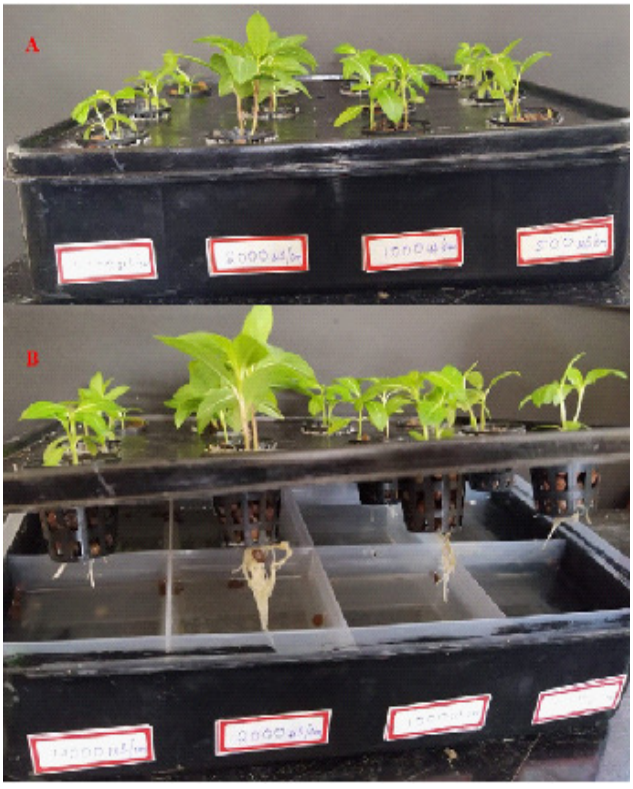

Figure 1: Effect of EC of Hydroponic culture medium on plant Shoot growth (A) and Root growth (B) after 45 days of treatment.

The total fresh weight of the shoot and root was likewise impacted by the EC level. The fresh weight of the shoot and root (SFW and RFW) was significantly impacted by EC, according to statistical analysis. In comparison to EC 4.0 dS/m, the plant cultivated with EC treatment 2.0 dS/m had the maximum fresh weight of shoots and roots (Figure 2A). The shoot and root dry weights (SDW and RDW) were impacted by the varying EC level of the nutrient solution, and this effect was statistically significant (Table 1). Despite having larger dry weights, the SDW and RDW developed in EC2.0 were statistically identical to the RDW of EC 1.0 dS/m (Figure 2B).

Proline content, total soluble protein, total soluble sugar, and total chlorophyll: As the EC level rose, the total chlorophyll content progressively increased as well. A substantial difference between the various EC treatments was revealed by the statistical analysis (Table 2). The amount of chlorophyll content peaked at EC 2.0 dS/m and subsequently sharply declined at higher ECs, or 4.0 dS/cm (Figure 2C). In a similar manner, the amount of total soluble protein in shoots grows dramatically when EC 0 to EC 2.0 dS/cm increases, and subsequently decreases at EC 4.0 treatment (Figure 2D). As the EC strength increased, the leaves' total soluble sugar content rose noticeably (Table 2). The control had the lowest quantity, whereas the highest was recorded at 4.0 dS/m EC (Figure 2E). Likewise, there were notable differences in the proline content among several EC treatments. Proline accumulation in leaves increased as the EC level rose, reaching a maximum value of 4.0 dS/cm (Table 2, Figure 2F).

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

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

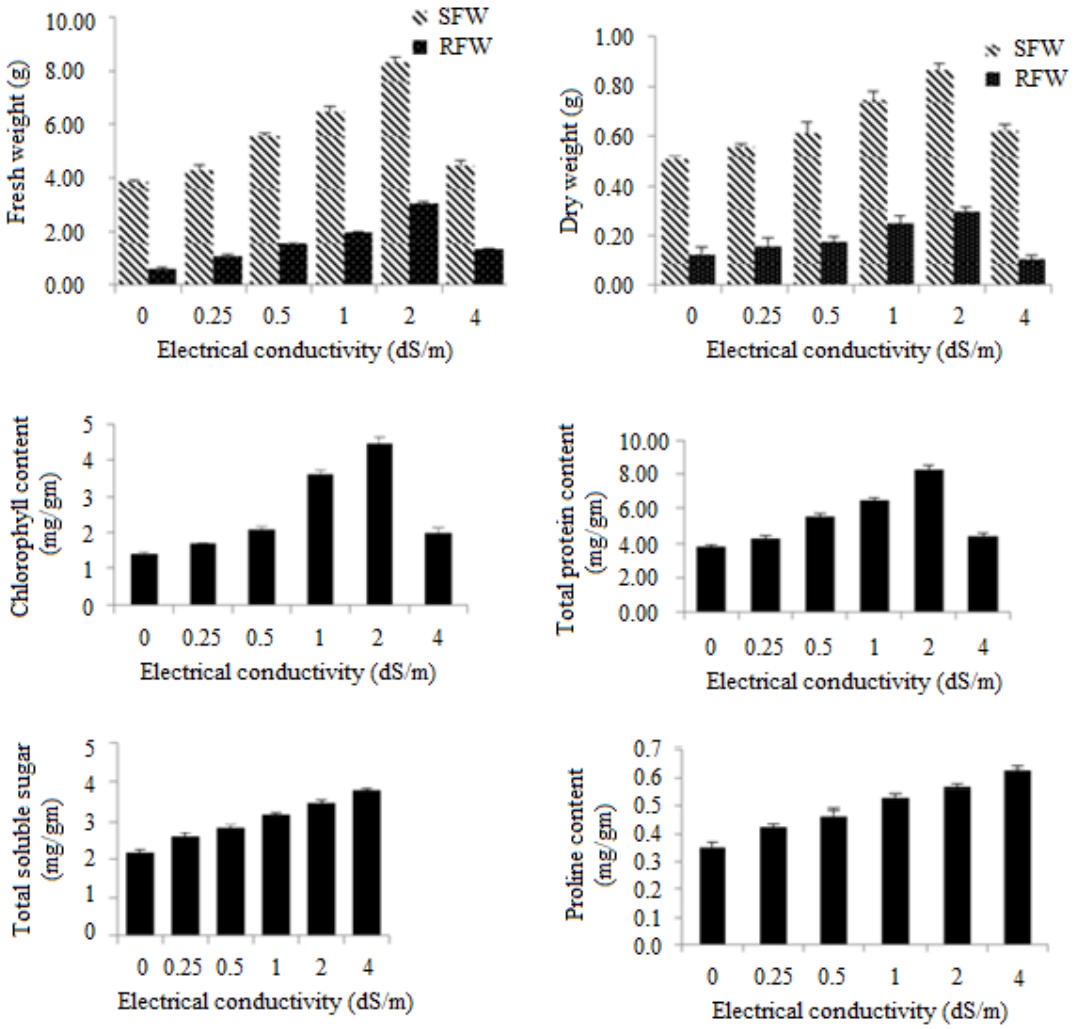

Figure 2: Effect of EC of Hydroponic Culture Media on Plant growth: Fresh weight of Shoot and root (A), Dry weight of Shoot and root (B) Total Chlorophyll Content (C), Total Protein (D), Total soluble Sugar (E), Proline Content (F) after 45 days of treatment. Data represent the mean ± SE, (n = 3).

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

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

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

4. Discussion

Plant height, fresh weight, and dry weight of roots and shoots all significantly increase with increasing EC level—up to 2.0 dS/m. The concentration of nutrients and other elements that are critical to plant growth and development has increased, which is the cause of the rise in EC. Therefore, the growth parameter of *Catharanthus roseus* increases as the nutrient content increases. The growth parameter significantly decreases with the greatest EC treatment, EC 4.0 dS/m, which could be the result of the harmful effect of high nutrient content in solution [9–11]. A rise in EC in the root zone lowers lettuce output because it also decreases leaf area and stomatal conductance [4]. A rise in EC above 2.0 dS/m raises the medium's salinity and induces water stress, which could have led to a decrease in internal turgor pressure and limited cell growth [12, 13]. Water absorption by roots is decreased by the osmotic impact, nutritional deficiencies brought on by ionic imbalance, and a reduction in several metabolic processes as a result of high EC, all of which contribute to a loss in plant development [14–16]. Several authors have reported similar outcomes, namely that a rise in nutrient concentration in the medium causes water shortages and a decrease in vegetative characteristics [5, 15]. The physiological processes of plants are significantly impacted by the unequal distribution of nutrient concentrations. According to our current investigation, the total chlorophyll content was comparatively low in low EC treatments. This could likely be the result of low nutrient concentrations and deficiencies in nutrients like N, Mg, and Fe that are critical for the manufacture of chlorophyll. In comparison to the control, the chlorophyll content gradually and significantly increases as the EC treatment increases. However, the chlorophyll content drops with 4.0 dS/m EC treatment, which may be related to the buildup of Na^+ in leaves and a drop in pigmentation concentration [2]. On the other hand, numerous scientists have noted that as EC rises above 2.0dS/m, the amount of chlorophyll increases [11, 17–26]. A plant's nitrogen content is determined by its total protein content. Because there was more nitrogen available in this study, the total protein rose as the EC climbed. The protein content of plants after EC treatment increases by up to 2.0 dS/m, which is comparable to the findings for corn and pakchoi [11, 21]. Catharanthus's protein concentration drops at higher EC treatments of 4.0 dS/m. It might be argued that compounds combining carbon and nitrogen are crucial for osmotic adjustment under stress. Because the nitrogen and carbon will be required for osmotic correction, the protein concentration decreases with higher EC treatments of 4.0 dS/m. However, in our current investigation, the level of proline significantly increases as the EC strength increases. By substituting water in metabolic reactions, the cytoplasm builds up low molecular weight compatible solutes that do not disrupt regular biochemical reactions and help maintain the ionic equilibrium in vacuoles. Numerous writers have proposed a substantial relationship between the amount of proline in cells and the ability to withstand various environmental stressors [7, 19, 22]. In *Catharanthus roseus*, elevated proline levels have also been linked to improved resistance to water-deficient stress [2]. Proline and its intermediates aid in the control of stress-responsive genes and promote the expression of osmoregulatory genes [24]. Proline has also been shown to have anti-radical, anti-electronic, macromolecule-stabilizing, and cell wall-supporting properties [18]. As the EC strength increases, the amount of total soluble sugar increases significantly. Ramezani [23] in Echium amoenum and Amalfitano et al. [6] in "Friariello" pepper fruit showed similar findings [27–29]. Despite a specific decrease in net CO_2 absorption, there has been widespread reporting of soluble sugar accumulation in response to rising environmental stress [9, 19]. In addition to controlling osmotic equilibrium, sugar serves as a metabolic signal during stressful situations.

5. Conclusions

The effects of various EC treatments on hydroponically produced *Catharanthus roseus* cultures were demonstrated by improved growth at EC strength of 2.0 dS/m, as indicated by elevated morphological and biochemical markers. Plant growth is restricted by too low EC strength, but salt stress caused by high EC strength inhibits growth. A significant rise in proline and soluble sugar accumulation at higher EC also suggests the plant's ability to withstand stress, which will be useful for tracking the elicitation of secondary metabolites.

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