Research Article

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Morpho-physiological and biochemical responses of coconut seedlings in aerated static-solution culture

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Abstract

Coconut (*Cocos nucifera* L.) is a valuable perennial crop with potential applications in food, feed, and nutraceutical industries. Compared to soil-based cultivation, hydroponic systems offer shorter growing cycles and reduced water waste. This study aimed to analyze the growth of coconut seedlings in hydroponic cultivation systems using Hoagland nutrient solution and water over 1 to 7 months post-germination. Morpho-physiological observations revealed significant differences in total and shoot height, chlorophyll index, stomatal resistance, transpiration rate, and chlorophyll fluorescence traits in seedlings grown in Hoagland nutrient solution compared to those grown in water. Additionally, the leaf area index of seedlings in Hoagland nutrient solution showed a marked increase at 7 and 8 months post-germination, suggesting periodicity in coconut growth and warranting further investigation. Differences in root and shoot nutrient uptake efficiencies were also discussed. This study paves the way for designing effective hydroponic systems for examining phenology and nutrient dynamics in coconut seedlings.

Keywords: hydroponics, coconut, nutrient uptake, plant nutrition, growth parameters, leaf area index

Introduction

Coconut (*Cocoa nucifera* L.) is an important commercial plantation crop grown in tropical and subtropical regions. Its production is spread over 85 countries, with Indonesia, the Philippines, and India being the three largest producers of coconut in the world. In India, the highest coconut production is from the state of Kerala, followed by Karnataka and Tamil Nadu. The total productivity is about 9,018 nuts per hectare (CDB, 2022-23). The cultivation aspects of coconut has witnessed robust changes owing to technological advances in crop management practices.

In many horticultural crops, hydroponics is widely favored worldwide due to its efficient use of resources and the production of high-quality food. This method of controlled environment agriculture allows for precise adjustments to create optimal conditions for plant growth, thereby boosting crop yield and enhancing crop quality. Hydroponic production offers several advantages over traditional soil-based agriculture, particularly in terms of space and resource efficiency (Rivera Mendez *et al*., 2013; Hebbar *et al*., 2022).

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Extensive research has been conducted on how the physical and chemical properties of hydroponic nutrient solutions—such as pH, temperature, dissolved oxygen levels, composition, and nutrient concentrations—affect crop growth (Wortman, 2015). Recently, there has been growing interest in understanding how the flow rate of nutrient solutions affects crop growth (Sakamoto and Suzuki, 2020; Baiyin *et al*., 2021). Balanced nutrient elements are crucial for the successful growth and development of seedlings, and the inert growing medium of hydroponics allows for efficient water and nutrient use (Rivera Mendez *et al*., 2013).

In this research, we present the comparative growth parameters of coconut seedlings maintained in a hydroponics setup. One set of seedlings was maintained under nutrient solution, whereas the control seedlings were placed in clean water to study the effect of nutrient solution on the morpho-physiological parameters of coconut seedlings.

Materials and Methods

Experimental site

The experiment was carried out at the polyhouse of the ICAR Central Plantation Crops Research Institute in Kasaragod, Kerala, India. The experimental site is located at 12° 18' N latitude, 75° E longitude, and an altitude of 10.7 meters above mean sea level.The average maximum temperature in summer is about 33.5 ± 2°C.

Plant material

Eight nuts from a selected coconut palm of the same bunch and growth stage were placed inside a container, which was then filled with water until the seed nuts were completely submerged. After two months, 50% of nuts germinated and 50% failed to germinate. The four seedlings of same growth stage were selected for the experiment. Among these seedlings, a set was transplanted to the Hoagland's solution and another set was maintained in water devoid of any external nutrition.

Growth conditions

The temperature inside the polyhouse, where the seedlings are grown, slightly varied from normal atmospheric condition (25°-33° C ±2° C). Normal or plain water and Hoagland's solution were used to study their impact on water use, nutrient use, and biomass gain in coconut seedlings. The details of growth of coconut seedlings in the hydroponic or aerated static-solution culture system are described else where (Hebbar *et al.* 2021).In brief, 100-liter plastic drums were used to grow the coconut seedlings during the study period.Out of the four drums, two were filled with 30 liters of water, and the other two were made up with 30 liters of full-strength Hoagland's solution. The nutrient composition of Hoagland's solution is as follows: macronutrients (in milli Molar) were: KNO_3 (5); $Ca(NO_3)_2.4H_2O$ (4); $MgSO_4.7H_2O$ (1); KH_2PO_4 (2); and micronutrients KCl (0.05); H_3BO_3 (0.025); $MnCl_2$ (0.002); $ZnSO_4$ (0.002); $CuSO_4$ (0.0005); Na_2MoO_3 (0.0001) and Fe-Na-EDTA (0.064).

The germinated seedlings were placed into drums filled with Hoagland's solution and water through a hole drilled in the center of the upper lid. This setup ensured that the nuts and roots were submerged while the shoots and leaves remained above the lid. Each drum contained a single seedling. A 1.5 HP compressor supplied air continuously for 3-4 hours through a mainline and micro tubes connected to each pot, providing adequate aeration. The drums' outer surfaces were painted black to block sunlight and prevent algae growth. Hoagland's solution and water were replaced every ten days, and the pH of the fresh solution was adjusted to 5.8 using alkali [sodium hydroxide (NaOH)] or acid [hydrochloric acid (HCl)]. Leaf, stem, and root samples were collected at the end of the experiment to assess accumulation of dry biomass.

Growth responses

The growth parameters of the seedlings are measured as follow. The total number of leaves was counted from outside towards inner whorls. The leaves in the outer whorls are considered as the older and inner leaves as younger leaves. The height of the plant was measured from the base to the tip of the fully opened leaf. The circumference of the stem, just above the nut attachment or at the base of the stem, was recorded using a measuring tape and referred to as the collar girth (in centimeters). The length and width of each leaf were measured to calculate the total leaf area of the plant using the linear regression equation [$y = 3.9325 + 0.7044x$, where y represents the leaflet area and x is the length multiplied by the width at the broadest part of the leaflet], as described by Mathes *et al.* (1989).The length of the leaf was measured from end point of stem just above to tip of leaf and width was measured by stretching the upper middle leaflets of either side of the petioles. To measure the fresh biomass, the whole plant was removed from the pot and separated into leaf, shoot, root and nut. These separated parts were solar dried for 7 weeks and dry weights were recorded to calculate the total dry biomass production and also for the estimation of relative moisture content of parts; root, shoot and leaf.

Physiological measurements

The first fully opened leaf was considered to measure physiological parameters. Net photosynthetic rate (Pn), stomatal conductance (gs), transpiration, and intercellular CO_2 concentration (Ci) were assessed using a portable photosynthesis system (LI-COR 6400XT, LI-COR, Lincoln, NE, USA). Measurements were taken in triplicate at a constant light intensity of 1000 $\mu mol\ m^{-2}\ s^{-1}$, with CO_2 concentration set at 400 ± 10 $\mu mol\ s^{-1}$. The air flow rate in the measuring chamber was approximately 500 $\mu mol\ s^{-1}$, and the leaf chamber temperature was maintained at 30 ± 2-3℃. The measurements were conducted between 9:00 AM and 11:00 AM on sunny days.

Stomatal resistance of leaf was measured using porometer (porometer AP4, USA). The chlorophyll index was measured using leaf chlorophyll meter (atLEAF handheld chlorophyll meter, USA). The chlorophyll fluorescence was measured using chlorophyll fluorometer (Opti-Sciences, Hudson, MH, USA). Briefly, the dark adaptation clip about 4mm aperture size with a sliding shutter was fixed on the top most leaf surface for 15 min before measuring the chlorophyll fluorescence values.

Biochemical analysis

The first fully opened leaf and root samples were collected and immediately stored in an ice box. Leaf tissue (0.5 g) was ground in 10 mL of 80% ethanol. The mixture was then subjected to roto-spinning (30 minutes) and sonication (30 minutes). The supernatant was collected following centrifugation at 6000 rpm for 15 minutes. The pellet was extracted again with 5 mL of 80% ethanol, and the extraction procedure was repeated. The pooled supernatant was evaporated at 80-85°C in a water bath until only a little residue remained. The residue was then dissolved in 10 mL of distilled water at 80°C. The extract was stored in a deep freezer (-20°C) for biochemical analysis. Total soluble sugar content was measured using the phenol-sulfuric acid method (Thomas *et al.*, 2021), and results are expressed as grams of glucose equivalents per 100 mL based on a standard curve. Total polyphenol content was estimated using the Folin-Ciocalteu method, and the blue color complex formed was quantified with a visible-light spectrophotometer (Shimadzu UV 160A). Total polyphenol content was expressed as gallic acid equivalents (GAE) per gram of fresh weight (Thomas *et al.*, 2021).

Nutrient content estimation

Hydroponic nutrient solution and normal tap water were collected in polyethylene bottles, which were thoroughly rinsed with the respective samples beforehand.

These samples were stored in a refrigerator at 4°C until analysis. The chemical composition of the water was analyzed using standard methods (Beutler, 2014). pH and electrical conductivity (EC) were measured with a Eutech multi-parameter model PC2700. Potassium (K+) was measured with a flame photometer (Elico CL378) (Williams and Twine, 1960). Calcium (Ca^{2+}) and magnesium (Mg^{2+}) levels were determined titrimetrically using an EDTA standard solution (Diehl *et al.*, 1950). Sulfate (SO_4^{2-}) content was analyzed spectrophotometrically using a turbidimetric method (Beutler, 2014). Phosphate (PO_4^{3-}) content was measured using the ascorbic acid spectrometric method (Beutler, 2014), and boron was determined with azomethane H reagent using a UV-Visible Spectrophotometer (Shimadzu UV 160A). Micronutrients such as iron (Fe^{2+}), manganese (Mn^{2+}), zinc (Zn^{2+}), and copper (Cu^{+}) were analyzed using an atomic absorption spectrophotometer (ICE 3300, Thermo Fisher Scientific). The nutrient status of the water and nutrient solution is presented in Table 1.

Table 1. The nutrient profile of the hoagland solution and water used for coconut seedling growth

Parameters	Hoagland solution	Water
Initial pH	5.8 ±0 $_b$	6.27±0.035$_a$
Final pH	7.03±0.459	7.65±0.35
EC (mS/cm)	5.07±0.0071$_a$	0.17±0.06 $_b$
Available PO_4^{3-} (ppm)	16.33±6.48	0.91±0.24
Available K^{+} (ppm)	166.75±20.51$_a$	0±0 $_b$
Exch. Ca^{2+} (ppm)	16975±742.46$_a$	425±106.06 $_b$
Exch. Mg^{2+} (ppm)	2016±118.8 $_a$	57±12.73 $_b$
Available SO_4^{2+} (ppm)	312.83 ±36.27 $_a$	65.155±65.39 $_b$
Fe^{2+} (ppm)	1164.4 ±41.26$_a$	61.0775±39.06 $_b$
Mn^{2+} (ppm)	0.2025±0.081	0.00325±0.0035
Zn^{2+}(ppm)	0.2575±0.209	0.00965±0.013
Cu^{2+} (ppm)	0.10625±0.026	0.037±0
B (ppm)	1.11625$_a$±0.207	0.11125$_b$±0.069

Root and leaf samples were washed with tap water, and then rinsed with double-distilled water. The samples were air-dried and then oven-dried at 70°C for 72 hours. After drying, they were powdered and homogenized. Total nitrogen content was estimated by wet digestion with concentrated sulfuric acid (Bremner, 1996). Phosphorus, potassium, calcium, magnesium, sulfur, iron, zinc, copper, manganese, and boron concentrations were measured after digestion with a nitric-perchloric acid mixture (9:4 ratio). Phosphorus concentration was determined using a UV-visible spectrophotometer (UV-1601, Shimadzu, Tokyo, Japan) following the vanadate-molybdate method (Neenu *et al.*, 2023). Potassium content in the digested extracts was measured using a flame photometer (Elico CL378) (Williams and Twine, 1960). Calcium and magnesium were determined by the versenate titration method (Diehl *et al.*, 1950). Sulfur content was measured by the turbidimetric method using a UV-visible spectrophotometer (Shimadzu UV-1601). Iron, zinc, copper, and manganese concentrations were determined by atomic absorption spectrophotometry (Thermo Scientific iCE 3000 series).

Estimation of biomass gain

The uprooted samples are separated into root, shoot, leaf, and nut and the fresh weight of each plant parts were recorded. Then the samples were sun dried for seven weeks followed by measurement of the sample weight (g) using a laboratory balance.

Statistical analysis

The observations were analyzed in duplicates in a completely randomized design using the WASP 2.0 (ICAR-Central Coastal Agricultural Research Institute).

Results

Changes in morphological traits

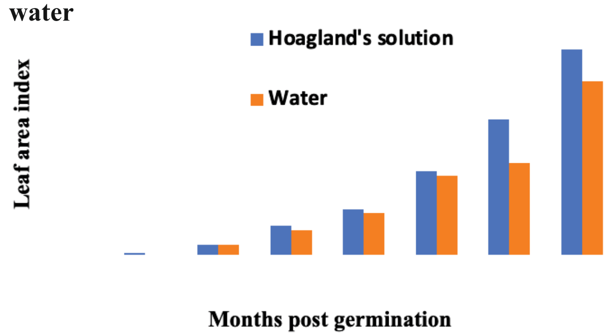
The total height, shoot height and collar girth, total number of leaf and leaf area index (LAI)(cm^2) showed little differences between seedlings grown in water and Hoagland's solution. Among the morphological parameters, total and shoot height, leaf area showed a considerable increase when seedlings were grown in Hoagland's solution. The growth responses in Hoagland's solution and water conditions for collar girth, total number of leaves showed a non-significant difference (Figure.1 and Table. 2). The leaf area index from initial stage of leaf emergence upto final stage showed significant increase in both the growing media. The number of leaves increase and a concomitant increase in LAI was also found (Figure 2).

Figure 1. Hydroponically grown coconut seedlings in a. hoagland's solution, b. water

Table 2. Morpho-physiological responses of coconut seedlings grown in hoagland's solution and water

Morpho-physiological parameters	Coconut seedlings in hoagland solution	Coconut seedlings in water
Collar girth	24.5±4.94	22.4±0
Total height	204.7 ±15.2	175.5±6.36
Shoot height	143±2.83	114.7±57.6
Total leaves	6±1.41	5±0.71
Dry leaves	1±1.41	1±0.71
Fresh leaves	7±0	6±1.41
Leaf area index (LAI) (cm^2)	10,538.8	7431.15
Chlorophyll index (CI) (%)	53.2± 0.306[a]	36.4±1.08[b]
Stomatal resistance (SR) (S cm^{-1})	3.02± 0.16[b]	6.01± 0.403[a]
Fv/Fm (variable fluorescence/ maximum fluorescence)	0.789± 0.006[a]	0.753± 0.004[b]
Photosynthesis (µ mole $m^{-2} s^{-1}$)	7.58 ± 0.487	6.88 ± 0.327
Transpiration (m mole $m^{-2} s^{-1}$)	2.916 ± 0.058[a]	0.5217 ± 0.06[b]
Stomatal conductance (SC) (mole $m^{-2} s^{-1}$)	0.091 ± 0.004	0.089 ± 0.002
Intercellular carbon-dioxide concentration (µ mole mole-1)	248.3 ± 24.98	171.2 ± 40.3
Biomass gain(g)	841±18.4	758±21.9

Figure 2. Leaf area index (LAI-cm^2) of coconut seedlings grown in hoagland's nutrient solution and water



Changes in physiological and photosynthetic traits

Analysis of leaf physiological parameters like stomatal resistance, leaf florescence, chlorophyll index and photosynthetic activity showed a significant difference between the seedlings that are grown in normal water and Hoagland'snutrient solution. The chlorophyll index of seedlings grown in nutrient solution was significantly high (53.25± 0.306) compared to that grown under normal water (36.4[b] ±1.08) suggesting the luxuriant growth. Similarly, chlorophyll fluorescence (Fv/Fm) exhibited a significant variationbetween the seedlings. Analysis of stomatal resistance of seedlings in Hoagland's solution showed significantly low values (3.02s $cm^{-1,}$) compared to the seedlings grown in water (6.01 s cm^{-1}).It indicates that the stomata in the former seedlings are open whereas under normal water conditions stomata are relatively closed.

Analysis of photosynthetic parameters revealed that no significant differences were observed between the seedlings grown in Hoagland's solution and water. However, in accordance with the stomatal resistance data, transpiration rate (m mole m^{-2} s^{-1})was significantly high in seedlings grown under Hoagland's solution (2.916 ± 0.058) than those grown in normal water (0.5217 ± 0.06). Interestingly stomatal conductance values between the seedlings grown under different set up showed insignificant differences. Also careful scrutiny of the monthly leaf area index (LAI) of the seedlings revealed that during VII and VIII months a significant increase in LAI of seedlings grown in Hoagland's solution was observed (Figure 2). The biomass gained by the seedlings showed huge differences between the Hoagland's solution (841g) and water (758g) (Table 2).

Biochemical responses and nutrient contents

The total polyphenolic and total sugar contents in root and shoot samples of coconut seedlings grown in both Hoagland's solution and water varied. There is a significant difference in biochemical parameters between shoot and root of seedlings at 5% level. The total sugar in shoot shows significance at 5% level (CD-36.4) and total polyphenols in both shoot and root of Hoagland's medium is comparatively higher than seedlings in water and it was non-significant.

The total nutrient content of leaves and roots of coconut seedlings grown in the water and Hoagland's solution were estimated. It was found that the total nitrogen content in leaf samples of seedlings grown in Hoagland's solution was higher (14.8%) compared to those grown in water. Similarly, the root nitrogen content also showed a notable difference (32%) between Hoagland and water grown plants. Also, there was no significant difference in the total phosphorus content of leaf samples from plants grown in Hoagland's solution versus water. However, the root samples from Hoagland-grown seedlings showed significantly higher total phosphorus content compared to those from water-grown seedlings. Similar to phosphorus the total potassium in leaves of both the plants have not showed significant difference but the root potassium showed significant difference between the Hoagland and water grown seedlings. Total calcium in leaf and root samples of both conditions were not much differ in concentration. The stored calcium in the seed nut was enough for the seedling growth and hence calcium from the nutrient solution might not require for the growth. Hence there was no significant difference in total calcium content of leaf and roots. In the case of total magnesium, the leaf samples from Hoagland and water grown seedlings showed only narrow differences but total magnesium of the root samples showed significant difference between the two medium. In the case of total sulphur content, significant difference was noticed between the Hoagland and water grown seedlings by both the leaf and root samples. The sulphur content in the seed nut might not sufficient to support the proper growth of the seedling and also the sulphur concentration of water was low enough to provide sulphur to the plants. Among the micronutrients there was no significant difference in concentration in the leaf and root tissues of the coconut seedlings grown under both the conditions (Table 3).

Table 3. Biochemical parameters and nutrient analysis of hydroponically grown seedlings.

Parameters	Coconut seedlings in Hoagland's solution		Coconut seedlings in water	
	Leaf	Root	Leaf	Root
Total sugar content (mg/g)	159.2[a]±11.9	119.95±2.33	109.3[b]±0.7	14.78±0.035
Total polyphenolic content (mg/g)	5.576±0.8156	1.017±0.223	4.821±0.036	0.779±0.068
N (%)	1.6975±0.229	1.56 ±0.229	1.44±0.339	1.06±0.339
PO_4^{3-} (%)	0.12±0.042	0.27[a]±0.042	0.1075±0.017	0.035[b]±0.0176
K^+ (%)	1.1725±0.038	0.435[a]±0.039	1.1075±0.127	0.245[b]±0.127
Ca^{2+} (%)	0.44±0	0.485±0	0.4175±0.031	0.375±0.031
Mg^{2+} (%)	0.4075±0.017	0.45[a]±0.017	0.3425±0.0565	0.135[b]±0.056
SO_4^{2+} (%)	0.41[a]±0.028	0.445[a]±0.028	0.295[b]±0.063	0.05[b]±0.063
Fe^{2+} (ppm)	100.125[a]±0.883	172.93[a]±0.884	92.3[b]±1.873	121.05[b]±1.873
Mn^{2+}(ppm)	54.175±10.42	59.31[a]±10.43	49.425±1.378	48.55[b]±1.788
Zn^+(ppm)	13.7[b]±0.353	46.85[b]±0.353	14.95[a]±0.494	20.1[a]±0.494
Cu^+(ppm)	11.5±0.141	10.05±0.141	11.85±0.494	9.3±0.494
B (ppm)	27.875±1.52	41.55±1.52	36.525±2.86	29.45±3.5

Discussion

Soil-less culture techniques have been incorporated into horticultural practices, optimizing the utilization of water and nutrients. Innovative approaches have been adopted to cultivate medicinal plants, perennial grasses, ornamental seedlings and cuttings, fruit trees in hydroponics set up (Honary *et al*., 2011; Rubio-Asensio *et al*., 2020). However, despite these advances, there is a lack of initiative on a analyzing the effect of nutrient versus normal water in hydroponics, particularly for perennials. Previously we have demonstrated the utility of a hydroponics set up in studying the salinity tolerance in coconut and used it to investigate the inherent water use efficiency (WUE) of coconut seedlings (Hebbar *et al*., 2021; Hebbar *et al*.,2022).

In this research, coconut seedlings were evaluated under Hoagland's nutrient solution and normal water to study the efficiency of nutrient uptake and its effect on the morpho-physiological parameters. The results reveal that some of the parameters such as total and shoot height, and leaf area showed a considerable increase when seedlings were grown in Hoagland's solution. However, the growth responses in Hoagland's solution and water conditions for collar girth, total number of leaves showed a non-significant difference. Similar results of improved morphological growth and physiological parameters of crops in soilless culture compared to crops grown in soil are reported (Sharma *et al*., 2022).

The lettuce plants grown in a hydroponic system showed higher plant height, collar girth and number of leaves. Suggesting that the plants cultivated in hydroponic system demonstrated better growth parameter, resulting in higher yield (Camen *et al.,* 2022). Also, the sudden spurt in the increase in biomass due to leaf area index of Hoagland solution grown coconut seedlings warrants further investigation.

Growing seedlings in regular water compared to Hoagland's solution is essential for studying nutrient efficiency in plant development. This approach helps clarify how varying nutrient levels influence growth rate, root development, and overall plant health, thereby informing agricultural practices and strategies to maximize crop productivity and sustainability.The differences in major nutrient contents between the leaves and roots of hydroponically grown coconut seedlings and those grown in water were consistent with the findings of Li and Cheng (2014), Asher and Ozanne (1967), Randle (2000), and Kane *et al.* (2006). The root samples from Hoagland-grown seedlings showed significantly higher total phosphorus content compared to those from water-grown seedlings. This might help the plant to absorb more nutrients and produce more total biomass. In the initial growth period the requirement of micronutrients was low and hence the difference is also negligible. It is well established that the pH of the nutrient solution plays an important role in the process of nutrient absorption (Wortman 2015). The increase in transpiration rate of coconut seedlings grown in Hoagland nutritent solution compared to water grown plants could be attributed to the presence of nutrients such as nitrogen as demonstrated elsewhere (Guidi *et al.*, 1998).

Conclusion

There is a notable difference in elemental composition and total biomass between coconut seedlings grown in Hoagland's solution and those grown in normal water. Seedlings cultivated in Hoagland's solution exhibited greater biomass compared to those grown in normal water. While there were minor differences in the morphological, physiological, and biochemical parameters between the two groups, these differences were not statistically significant.This implies that basic plant growth characteristics and functions were relatively unaffected by the type of water used. The leaf area index (LAI) increased from the germination stage up to the removal stage. This indicates a consistent development of leaf area over time, contributing to the plant's ability to capture light and perform photosynthesis. Despite the higher total biomass in plants grown in Hoagland's solution, seedlings grown in normal water did not exhibit adverse effects on growth and photosynthesis. This suggests that normal water was sufficient to support the essential growth processes and photosynthetic activity of the seedlings. The study indicates that while Hoagland's solution enhances total biomass, normal water is adequate to maintain healthy growth and photosynthesis in seedlings. This could imply that for basic growth, normal water suffices, but for maximizing biomass, nutrient-enhanced water is beneficial.

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