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The Role of Carbon Nanotubes in Improving Drought Tolerance via Upregulation of the Physiological Processes of Peanut Plants Grown in Sandy Soils

Bakry A. Bakry [1], Mervat Sh. Sadak [2], Nagla M. Al Ashkar [2], Omar M. Ibrahim [3], Mohammad K. Okla [4] and Amira M. El-Tahan [3,*]

1 Field Crops Research Department, Agricultural and Biological Research Institute, National Research Centre, 33 El-Bohouth Street, Dokki 12622, Giza, Egypt
2 Botany Department, Agricultural and Biological Research Institute, National Research Centre, 33 El-Bohouth Street, Dokki 12622, Giza, Egypt; naglatarek7@yahoo.es
3 Plant Production Department, Arid Lands Cultivation Research Institute (ALCRI), City of Scientific Research and Technological Applications (SRTA-City), New Borg El-Arab City 21934, Alexandria, Egypt; oibrahim@srtacity.sci.eg
4 Botany and Microbiology Department, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia
* Correspondence: aeltahan@srtacity.sci.eg

Abstract: Drought stress is an important challenge to global food security and agricultural output, and dramatic and rapid climate change has made the problem worse, causing unexpected impacts on the growth, development, and yield of different plants. Understanding the biochemical, ecological, and physiological reactions to these pressures is essential for improved management. Carbon materials' impacts on plants subjected to different stresses are still poorly studied. Thus, this study was carried out investigate the feasibility of applying carbon nanotubes (CNTs) (0, 20, and 40 mg/L) as a foliar treatment for mitigating the effect of water stress (100%, 75%, and 50% irrigation water, IW) on peanut plants growing in sandy soil through assessments of growth and productivity and some physiological and biochemical measurements. Exposure of peanuts to decreased irrigation water led to significant decreases in growth, yield, photosynthetic pigments, indole acetic acid (IAA), and some nutritional components in peanut seeds, but increased levels of osmolytes such as total soluble carbohydrates (TSS) and proline, in addition to free amino acids and phenolics. However, foliar spraying with CNTs could ameliorate the impacts of decreased irrigation water on growth and production via enhancing the studied physiological parameters, such as photosynthetic pigments, IAA, osmolytes, and phenolics. Furthermore, the application of carbon nanotubes improved the nutrient contents, as expressed by the oil yield, protein yield, total carbohydrates, antioxidant activities (DPPH), B-carotene, lycopene, and flavonoids in peanut seeds, either under normal or water stress conditions. The higher level of CNTs (40 mg/L) was more effective than the lower one (20 mg/L) at increasing the above-mentioned parameters. In conclusion, foliar treatment with carbon nanotubes has the ability to enhance peanut drought tolerance and increase its growth and productivity under sandy soil conditions.

Keywords: carbon nanotubes; drought; flavonoids; growth; peanut; osmolytes; yield; DPPH

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1. Introduction

Peanut (*Arachis hypogaea* L.), also called groundnuts, are not only one of the most important summer oil seed and protein crops, but also the king of oil seed crops and legume edible seeds [1]. Peanuts are known as the 13th food crop, 4th oil seed crop, and the 3rd source of vegetable protein. In Egypt, the average area cultivated with peanuts over the last five years was about 62,000 ha. Ref. [2] identified the development scale of peanuts; the growth of peanut plants was divided into two phases (vegetative and reproductive), which were further subdivided into distinct stages. The main phases were the vegetative (V) and reproductive (R) stages of plant development. Letters or numbers were added to denote individual steps within these phases. Peanut's economic benefits are mostly due to its short soil lifespan, which allows for higher economic returns in newly reclaimed lands when compared to other crops. Peanuts are as popular as they are nutritious, containing 40–50% oil, 25–30% protein, 20% carbs, and 5% ash, as well as various minerals such as magnesium and calcium, depending on the variety and agricultural treatment [3]. Moreover, peanut leaves are used as animal feed. Thus, because of its aptitude for growing in newly reclaimed sandy soil, both the government and specialists have paid close attention to this crop. In addition to oil production, peanuts are used to make peanut butter, confectionary, roasted peanuts, snack meals, meat product extenders, soups, and desserts [4].

Drought is one of the world's most serious problems, resulting in significant crop yield losses. Plant growth, productivity, and resistance to environmental challenges are currently the focus of agriculture and new plant-based technologies [5]. Among these stressors, drought, salinity, and alkalinity are the most major global challenges, resulting in significant crop yield losses [6]. Drought is a global issue that impacts a wide range of regions around the world. Water is a critical constraint that affects major metabolic, physio-biochemical, growth, proteomic, and transcriptomic processes that control plant growth, tolerance, and yield [6]. Accordingly, plant researchers are investigating these critical physio-biochemical concerns related to plant water stress tolerance. Plants confront a number of problems as a result of water shortages brought on by a lack of water and hot temperatures. The plant's reaction to the decreased effect of water stress varies by organ; they achieve active osmotic equilibrium by amassing osmolytes and inorganic ions [7]. Osmolytes build up and translocate, interfering with important metabolic functions in order to promote growth and development. Water stress builds up reactive oxygen species (ROS) [8]. Increased levels of reactive oxygen species (ROS) disrupt cell processes by damaging proteins and lipids, resulting in cell death [9].

Nanotechnology is a technique for designing and incorporating nanoparticles into devices. Nanoparticles (NPs) with at least one dimension smaller than 100 nm can be made using this technology. Nanomaterials have recently become widely employed in a wide range of scientific sectors, including pharmaceuticals, biology, and agriculture [10,11]. Furthermore, nanoparticles hold a lot of promise for the external treatment of plants in terms of nutrition and defense [12]. Organic and inorganic nanoparticles are the two types of nanoparticles. Carbon nanotubes, for example, are organic molecules. Carbon nanotubes (CNTs) are a novel kind of carbon that resemble a two-dimensional graphene sheet when folded up. Carbon nanotubes (CNTs) also have the shape of molecular-scale graphitic carbon tubes. CNTs are widely used in a variety of products due to their unique nanostructures and outstanding properties such as a large specific surface area, an enhanced aspect ratio, a high electrical conductivity, and significant thermal stability [13]. Single-walled and multi-walled nanotubes are the two most common types of nanotubes. Exploiting the properties of CNTs will surely open up new possibilities for the development of a variety of nanodevices with specific conductivity, optical, and thermal properties for use in agriculture. Plant scientists employ carbon nanotubes to improve plant production systems, detoxify pollutants (pesticides and fertilizers), improve disease tolerance, and act as plant bio-regulators [14]. CNT-based delivery systems can focus fertilizers or agrochemicals to specific hosts, reducing the amount of chemicals discharged into the environment as well as the damage to other plant tissues [14]. In comparison to non-crystalline, relatively bigger carbon materials, the crystalline tubular structure of the outer layers may facilitate further absorption and interaction with the biological system [15]. Furthermore, carbon nanotubes have been demonstrated to absorb and eliminate organic and inorganic pollutants from water [16]. Therefore, using CNTs in agriculture has shown very promising results [17]. According to Srivastava and Rao [18], using CNTs improved germination percentages and growth of wheat, maize, peanut and garlic plants. Also, Rahimi et al. [19], stated that multi-walled carbon nanotubes enhanced the growth, development and yield of *Alnus sub-*

cordata plant under drought stress conditions. Furthermore, Ref. [20] found that MWCNT treatment enhanced the growth, photosynthetic pigments and physiological processes of barley plant under salinity stress.

Few research groups have looked at the impact of foliar spraying carbon nanotubes onto stressed peanut plants as far as we know. As a result, the goal of this study is to see if foliar spraying carbon nanotubes can reduce the impact of water stress on peanut plants grown in sandy soil. The impact of carbon nanotubes on peanut plant development, yield, and several physiological and biochemical properties, as well as seed nutritional values, was investigated.

2. Materials and Methods

Carbon Nanotubes: The multi-walled carbon nanotubes used in this present investigation were supplied from Sigma-Aldrich Co. (St. Louis, MO, USA). The characteristics of MW CNTs were as follows: purity, 98 wt%; outside diameter, 5–15 nm; inside diameter, 3–5 nm; length, ~50 µm; ash < 1.5 wt%; surface area > 110 m^2g^{-1}; true density 2.1 g cm^{-3}. A uniform mixture of MWCNTs was prepared after suspending them in distilled water and then sonicating at 40 KHz (100 W) for 30 min. Homogeneous suspensions of MWCNTs at different concentrations of 20 and 40 mg/L were prepared according to Sadak et al. [21].

Experimental procedure: Two field experiments were conducted during two successive summer seasons in 2021 and 2022 at the experimental station of the National Research Center (NRC) (latitude 30°30′1.4″ N, longitude 30°19′10.9″ E, and 21 m + MSL (mean sea level)) at Al Nubaryia district, El-Behaira Governorate, Egypt (Figure 1). Prior to the initiation of each experiment, soil samples at 30 cm depth were collected to identify some physical and chemical properties of the site of experimentation. Soil samples were analyzed according to the standard published procedures of [22]. The soil texture was sandy, and the soil had the following characteristics: sand 94.7%; pH 8.6; organic matter 0.8%; $CaCO_3$ 2.4%; EC 0.13 mmhos/cm^3; available N 18.0 ppm; available P 18.0 ppm; available K 104 ppm; and available Zn 0.05 ppm.

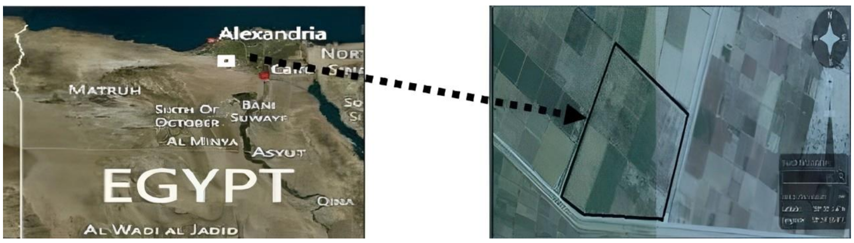

Figure 1. Location of the experimental farm in El Nubaria Region, Egypt.

The experimental design was a split plot design with three replications, where irrigation water (IW) levels of 100%, 75% and 50% were applied to the main plots. Carbon nanotubes at foliar application at rates of 0, 20 and 40 mg/L were randomly applied via foliar spraying until runoff. Before spraying, Tween-20 (0.1%, v/v) as a surfactant was added to promote the optimal penetration of CNTs into leaf tissues. This was performed in sub plots and carried out twice; the first application was 30 days after the sowing date and the second application was 45 days after the sowing date at the vegetative stage of growth and the early reproductive stage [2]. The plot area was 10.5 m^2, consisting of five rows (3.5 m length and 60 cm between rows).

Peanut (*Arachis hypogaea* L.) variety Gize-6 was procured from the Oil Crops Research Section, Field Crops Research Institute, Agricultural Research Center, Giza, Egypt, and was inoculated just before sowing with specific rhizobium bacteria inoculants. Seeds of peanut were sown in the first week of May in both seasons. A phosphorus fertilizer, calcium superphosphate (15.5% P_2O_5), was added during seed bed preparation at a rate of 60 kg P_2O_5/faddan. Potassium sulphate (48% K_2O) at the rate of 50 kg/fad was applied at sowing. Nitrogen fertilizer was added at a rate of 30 kg N/fad as ammonium sulfate (20.6% N) in two equal portions; the first half was added at sowing and the second 30 days later. Three irrigation levels were applied, each representing a different percentage of crop water irrigation requirements (IWRs) as the following: 100%, 75% and 50%.

2.1. Water Irrigation Requirements

The daily reference evapotranspiration (ETo, mm day^{-1}) was determined using the Class A pan (Epan, mm day^{-1}) and the pan coefficient (Kp) according to Allen et al. [23] as follows:

$$\text{ETo} = \text{Epan} \times \text{Kp}$$

Crop evapotranspiration (ETc) was calculated using the crop coefficient (Kc) according to the following equation:

$$\text{ETc} = \text{ETo} \times \text{Kc}$$

The growing peanut plants were irrigated every two days.

The average amount of irrigation water applied with the sprinkler irrigation system was 2500, 1875 and 1250 m^3 Feddan^{-1} season^{-1} (for 100%, 75% and 50%) for the two seasons [24]. (**One Feddan = 4200 m^2**).

The following equation was used to determine irrigation water amounts:

$$\text{IWR} = (\text{ETo} \times \text{Kc} \times 4.2) \times 1.2$$

where IWR: irrigation water requirement (m^3/fed.), Kc: crop coefficient, ETo: reference evapotranspiration (mlm/day), 4.2: for feddan, and 1.2: leaching requirement.

Growth parameters: A sample was taken 60 days after sowing to measure some morphological parameters such as the shoot length (cm), number of branches and leaves/plant, shoot fresh and dry weight (g), root length (cm), and root fresh weight (g).

At harvest: A sample of five plants was taken in each plot at harvest time (120 days from the sowing date); the data of seed yield characteristics were recorded follows: plant height (cm), number of branches/plants, number of pods/plants, plant fresh weight (g), pod weight/plant (g), seed weight/plant (g). The whole plot was harvested and the pods were air dried to calculate the pod yield (kg/feddan), seed yield (kg/feddan), oil yield (kg/feddan), and protein yield (kg/feddan).

2.2. Chemical Analysis

2.2.1. In Peanut Shoots

Photosynthetic pigments such as chlorophyll a and b and carotenoids of peanut plants were determined according to Li and Chen [25]. Indole acetic acid (IAA) was measured according to Gusmiaty et al. [26]. The total phenol content was determined as described by Siddiqui et al. [27]. Total soluble sugars (TSSs) were extracted and determined according to Mecozzi [28]. Proline and free amino acid contents were determined according to the method described by Vartanain et al. [29]. Proline was assayed according to the method described by Versluses, [30]. The free amino acid content was determined using the method described by Tamayo and Pedrol [31].

2.2.2. In Peanut Seeds

Estimation of total carbohydrates was performed via the method of Albalasmeh et al. [32]. The protein content was determined according to Pedrol and Tamayo, [33]. The oil of peanut seeds was estimated according to the AOAC [34]. The free radical scavenging activity of seed extracts was determined according to Gyamfi et al. [35]. The method used for determination of lycopene and β-carotene was as described by Nagata and Yamashita [36]. The flavonoid content was determined by the method proposed by Chang et al. [37].

2.3. ANN Modeling

In the present work, a four-layer feed-forward multilayer perceptron neural network was used to assess the importance of the inputs to the output (seed yield). The structure of the network consisted of one input layer with fourteen neurons, two hidden layers with twenty-three neurons each, and one output layer with one neuron. A back propagation learning algorithm was used to train the network, and the Rectifier activation function (f(x) = max (x,0)) was adopted in hidden layers, while the linear activation function was adopted in the output layer. The goodness of fit was evaluated based on the determination of the coefficient (R^2) and the root mean square of error (RMSE). A total of 80% of the dataset was used to train the network, while 20% was used for testing its performance. After finishing training and testing the network, the connection weights were used to calculate the relative importance of the inputs. Uniform connection weights were used to avoid gradient vanishing problems in the deep learning networks; the learning rate was 0.01, the momentum was 0.9, the number of epochs was 15, and the L2 regularization method was used to avoid the multicollinearity problem among traits.

2.4. Statistical Analysis

The data were statistically analyzed for variance according to the split plot design. Since the trend was similar in both seasons, a homogeneity test, Bartlet's equation, was applied and the combined analysis of the two seasons was performed according to this method. An HSD test was conducted to compare the means at $p < 0.05$ using SPSS software version 24 (IBM, Armonk, NY, USA) [38,39].

3. Results

To achieve the goal of this study, thirty-three traits were measured to examine the potential roles of carbon nanotubes at foliar application at rates of 0, 20 and 40 mg/L in increasing the tolerance of peanut plants to drought stress.

3.1. Changes in Morphological Criteria

The effect of exogenous treatment with carbon nanotubes on the morphological criteria of peanut plants under different irrigation water amounts is shown in Table 1. The data show that water deficit led to significant decreases $p \leq 0.05$ in the studied morphological criteria (shoot length (cm), number of branches/plants, number of leaves/plants, shoot fresh weight (g), and dry weight (g)) compared with those plants grown under 100% IW. Meanwhile, moderate (75% IW) and severe (50% IW) water deficits gradually and significantly increased the root length and root fresh weight compared with 100% WIRs. Foliar application of carbon nanotubes at 20 and 40 mg/L induced significant enhancements in all morphological criteria compared with the control plants for plants grown either under water stress (75% and 50% IW) or normal conditions (100% IW). Application of a higher concentration of CNTs was more superior over lower concentrations regarding increases in all the growth criteria of peanut plants under different water irrigation requirements (Table 1).

Table 1. Impact of carbon nanotubes (CNTs) (0, 20, and 40 mg/L) on morphological criteria of peanut plants grown under irrigation water in sandy soil (results are the combination of two seasons).

WIR	CNTs (mg/L)	Shoot Length (cm)	Branch no./Plant	Leaf no./Plant	Shoot Fresh Weight (g)	Shoot Dry Weight (g)	Root Length (cm)	Root Fresh Weight (g)
100%	0	22.7 ^{d}	9.56 ^{d}	34.44 ^{cd}	56.42 ^{d}	14.79 ^{e}	10.05 ^{c}	0.78 ^{e}
	20	37.76 ^{b}	12.19 ^{b}	38.24 ^{c}	83.98 ^{b}	32.63 ^{b}	11.13 ^{bc}	1.25 ^{de}
	40	42.2 ^{a}	20.35 ^{a}	76.36 ^{a}	131.33 ^{a}	51.53 ^{a}	11.09 ^{bc}	1.58 ^{d}
75%	0	22.15 ^{de}	7.04 ^{e}	21.49 ^{f}	32.51 ^{e}	13.43 ^{e}	12.08 ^{ab}	1.29 ^{de}
	20	32.43 ^{c}	11.36 ^{bc}	36.53 ^{c}	78.93 ^{b}	30.26 ^{b}	13.33 ^{a}	3.32 ^{c}
	40	35 ^{bc}	10.18 ^{cd}	64.7 ^{b}	64.72 ^{c}	32.72 ^{b}	12.73 ^{a}	3.63 ^{c}
50%	0	18.53 ^{e}	6.21 ^{e}	15.05 ^{g}	24.55 ^{f}	13.07 ^{e}	12.74 ^{a}	3.36 ^{c}
	20	21.77 ^{de}	6.58 ^{e}	24.07 ^{ef}	51.22 ^{d}	19.26 ^{d}	13 ^{a}	6.34 ^{b}
	40	21.37 ^{de}	9.92 ^{d}	28.83 ^{de}	56.33 ^{d}	22.36 ^{c}	13.38 ^{a}	7.15 ^{a}

Multiple comparisons were performed using Tukey's HSD test to control type I errors. Different lowercase letters indicate significant differences.

3.2. Changes in Photosynthetic Pigments

Figure 2 illustrates the impact of carbon nanotubes at 20 and 40 mg/L on peanut photosynthetic pigment contents grown at 100%, 75% and 50% IW. The data clearly show that water deficits (75% and 50% IW) led to significant $p \leq 0.05$ decreases in various photosynthetic pigment components (Chlo a, Chlo b, and carotenoids as well as total pigments), while the ratio of Chlo a and Chlo b increased significantly in contrast to those plants grown under 100% IW. Foliar application of carbon nanotubes with 20 and 40 mg/L induced significant $p \leq 0.05$ increases in the various photosynthetic pigment constituents in addition to the Chlo a/Chlo b ratio of the plants grown either under normal irrigation (100%) or drought-stressed irrigation (75% and 50% IW) conditions in comparison to untreated plants.

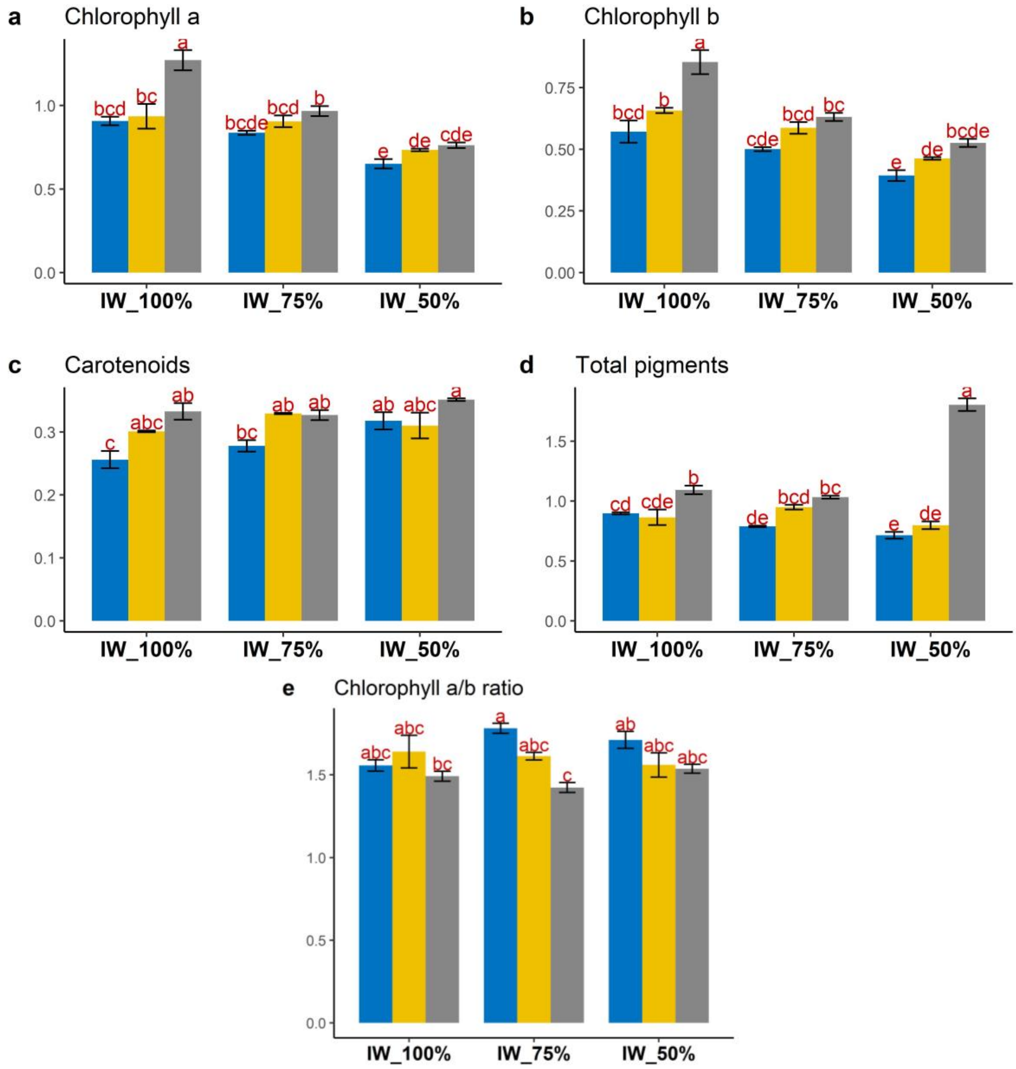

Figure 2. Impact of carbon nanotubes (CNTs) (0.0, 20 and 40 mg/L) on photosynthetic pigment (μg/g fresh weight) contents and the Chlo a/Chlo b ratio in peanut plants grown under irrigation water (IW%) conditions in sandy soil. Different lowercase letters indicate significant differences.

3.3. Changes in Endogenous IAA and Total Phenols

Figure 3 shows the variations in indole acetic acid (IAA) and phenolics in peanuts treated with carbon nanotubes and grown under normal and drought-stressed conditions in sandy soil. The data clearly show that moderate (75% IW) and severe (50% IW) drought stress caused significant $p \leq 0.05$ and gradual decreases in the endogenous IAA content compared with plants irrigated normally (control plants). Meanwhile, the phenolic contents increased significantly and gradually compared with normal irrigated plants. Foliar treatments of peanut plants with carbon nanotubes (20 and 40 mg/L) improved the IAA contents and caused a higher increase in total phenols compared with the controls. The data clearly show the superiority of higher levels of CNTs (40 mg/L) over low levels (20 mg/L) in increasing IAA and phenolic contents under different irrigation levels.

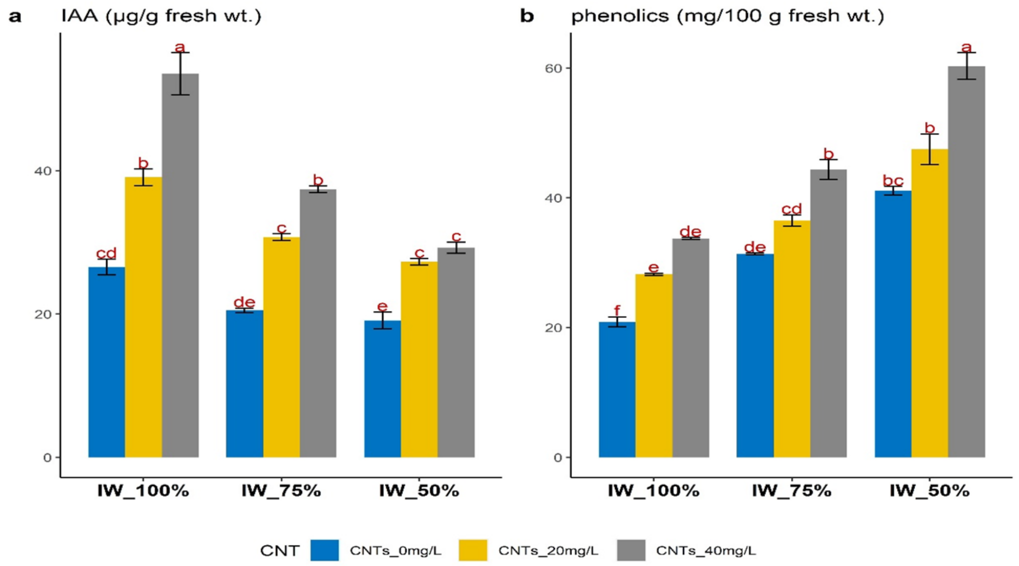

Figure 3. Impact of carbon nanotubes (CNTs) (0.0, 20 and 40 mg/L) on IAA (µg/g fresh wt.) and phenolic (mg/100 g fresh wt.) contents in peanut plants grown under irrigation water in sandy soil. Different lowercase letters indicate significant differences.

3.4. Changes in Osmolytes

The effect of water stress and different levels of carbon nanotubes (CNTs, 20 and 40 mg/L) on the contents of osmolytes such as TSS, proline and FAA in peanut plants is shown in Table 2. Decreasing the IW from 100% to 75% and 50% caused a gradual accumulation of these compatible solutes. Moreover, foliar treatments of different carbon nanotube concentrations caused a greater increase in the studied compatible osmolytes; these increases were significant $p \leq 0.05$ compared with the corresponding controls. Furthermore, higher levels of CNTs caused higher increases than the lower levels in peanut plants under different irrigation levels (Table 2).

Table 2. Impact of carbon nanotubes (CNTs) (0.0, 20 and 40 mg/L) on TSS, proline and free amino acids (mg/100 g dry wt.) in peanut plants grown under irrigation water in sandy soil.

WIR	CNTs (mg/L)	TSS (mg/100 g Dry wt)	Proline (mg/100 g Dry wt)	FAA (mg/100 g Dry wt)
100%	0	1323.28 ^{b}	35.41 ^{g}	230.51 ^{e}
	20	1464.76 ^{ab}	42.45 ^{fg}	241.05 ^{de}
	40	1517.5 ^{a}	47.99 ^{ef}	251.53 ^{de}
75%	0	1554.11 ^{a}	54.12 ^{de}	268.48 ^{cde}
	20	1492.3 ^{a}	59.26 ^{cd}	279.86 ^{bcd}
	40	1576.56 ^{a}	64.4 ^{bc}	298.61 ^{bc}
50%	0	1453.75 ^{ab}	64.87 ^{bc}	302.06 ^{bc}
	20	1466.15 ^{ab}	69.12 ^{b}	318.86 ^{ab}
	40	1491.66 ^{a}	78.33 ^{a}	357.89 ^{a}

Multiple comparisons were performed using Tukey's HSD test to control type I errors. Different lowercase letters indicate significant differences.

3.5. Yield and Yield Components

Tables 3 and 4 show the impact of various levels of carbon nanotubes (20 and 40 mg/L) on the yield and yield components of peanut plants grown under 100%, 75% and 50% IW.

The data clearly show that decreasing the IW to 75% and 50% led to gradual significant decreases ($p \leq 0.05$) in the yield and yield components (plant height, branches and pods number/plant, plant fresh weight, pod and seed weight/plant (g)) compared with control plants (100% WIR). Meanwhile, foliar application of carbon nanotubes (20 and 40 mg/L) led to a significant increase ($p \leq 0.05$) in all the above-mentioned yield components for the plants grown under normal conditions or a water deficit. Application of CNTs (40 mg/L) led to the maximum increase in seed yields kg/Fadden under different water irrigation requirements, ay 90.23, 62.29 and 50.95 kg/plant compared with 61.98, 43.31 and 20.67 kg/Fadden in plants treated with 20 mg/L CNTs under 100%, 75% and 50% IW, respectively.

Table 3. Impact of carbon nanotubes (CNTs) (0.0, 20 and 40 mg/L) on yield and its components for peanut plants grown in irrigation water in sandy soil. (Data are the combination of two seasons.)

WIR	CNTs (mg/L)	Plant Height (cm)	Branch no./Plant	Pod no./Plant	Plant Fresh wt. (g)	Pod wt./Plant (g)	Seed wt./Plant (g)
100%	0	42.09 ^{d}	7.94 ^{cd}	15.14 ^{e}	105.91 ^{e}	51.45 ^{f}	22.26 ^{e}
	20	70.33 ^{a}	11.41 ^{b}	34.29 ^{d}	310 ^{c}	123.75 ^{c}	61.88 ^{b}
	40	70.02 ^{a}	16.16 ^{a}	42.13 ^{a}	511.54 ^{a}	179.6 ^{a}	91.58 ^{a}
75%	0	35.31 ^{e}	6.87 ^{d}	14.19 ^{e}	77.29 ^{f}	27.23 ^{h}	18.41 ^{e}
	20	51.1 ^{c}	7.49 ^{d}	38.7 ^{bc}	170.32 ^{d}	84.01 ^{e}	42.14 ^{d}
	40	73.61 ^{a}	12.68 ^{b}	41.19 ^{ab}	331.32 ^{b}	134.42 ^{b}	63.51 ^{b}
50%	0	36.31 ^{e}	6.7 ^{d}	13.17 ^{e}	62.26 ^{f}	25.58 ^{h}	13.75 ^{f}
	20	46.25 ^{cd}	7.51 ^{d}	15.34 ^{e}	76.26 ^{f}	37.74 ^{g}	20.67 ^{e}
	40	58.89 ^{b}	9.28 ^{c}	36.83 ^{cd}	303.47 ^{c}	97.53 ^{d}	49.72 ^{c}

Multiple comparisons were performed using Tukey's HSD test to control type I errors. Different lowercase letters indicate significant differences.

Table 4. Impact of carbon nanotubes (CNTs) (0.0, 20 and 40 mg/L) on pod yield, seed yield, oil yield and protein yield (kg/fed) of peanut plants grown under different water irrigation requirements in sandy soil. (Data are the combination of two seasons.)

WIR	CNTs (mg/L)	Pod Yield (kg/Fed)	Seed Yield (kg/Fed)	Oil Yield (kg/Fed)	Protein Yield (kg/Fed)
100%	0	1064.52 ^{d}	477.15 ^{d}	209.09 ^{ef}	86.79 ^{de}
	20	2088.54 ^{a}	1158.5 ^{b}	553.52 ^{b}	231.91 ^{b}
	40	2211.67 ^{a}	2006.35 ^{a}	952.25 ^{a}	396.64 ^{a}
75%	0	881.7 ^{d}	397.52 ^{d}	173.41 ^{fg}	65.27 ^{e}
	20	1441.68 ^{c}	940.08 ^{bc}	435.48 ^{d}	160.5 ^{c}
	40	1782.64 ^{b}	1139.53 ^{b}	496.79 ^{c}	213.84 ^{b}
50%	0	496.41 ^{e}	368.62 ^{d}	152.71 ^{g}	60.61 ^{e}
	20	979.24 ^{d}	566.22 ^{d}	256.49 ^{e}	97.43 ^{d}
	40	1373.24 ^{c}	886.38 ^{c}	433.25 ^{d}	160.71 ^{c}

Multiple comparisons were performed using Tukey's HSD test to control type I errors. Different lowercase letters indicate significant differences.

Furthermore, the data in Table 4 indicate that the pod yield, seed yield, oil yield and protein yield (kg/fed) of peanuts decreased significantly in plants subjected to moderate and severs water stress compared with plants irrigated normally. Meanwhile, the data clearly show that treatment of peanuts with various levels of carbon nanotubes (20 and 40 mg/L) significantly increased the studied yield parameters. Foliar application of 40 mg/L CNTs led to the maximum increases under different WIRs over the control, untreated plants as well as the other 20 mg/L CNT-treated plants.

3.6. Changes in Carbohydrates, Protein and Oil Contents

The data presented in Figure 4 show the influence of different concentrations of carbon nanotubes (20 and 40 mg/L) on the carbohydrates, protein and oil contents of peanut seeds subjected to different levels of WIRs. Exposure of peanut plants to moderate drought (75% WIRs) and severe drought (50%) conditions led to non-significant differences in carbohydrates and oil contents, while a gradual and significant decrease in protein content was observed. On the other hand, treating peanut plants with different concentrations of CNTs (20 and 40 mg/L) caused insignificant and gradual increases in the TCH and protein levels compared with untreated plants under different water irrigation requirements (100%, 75% and 50% WIR).

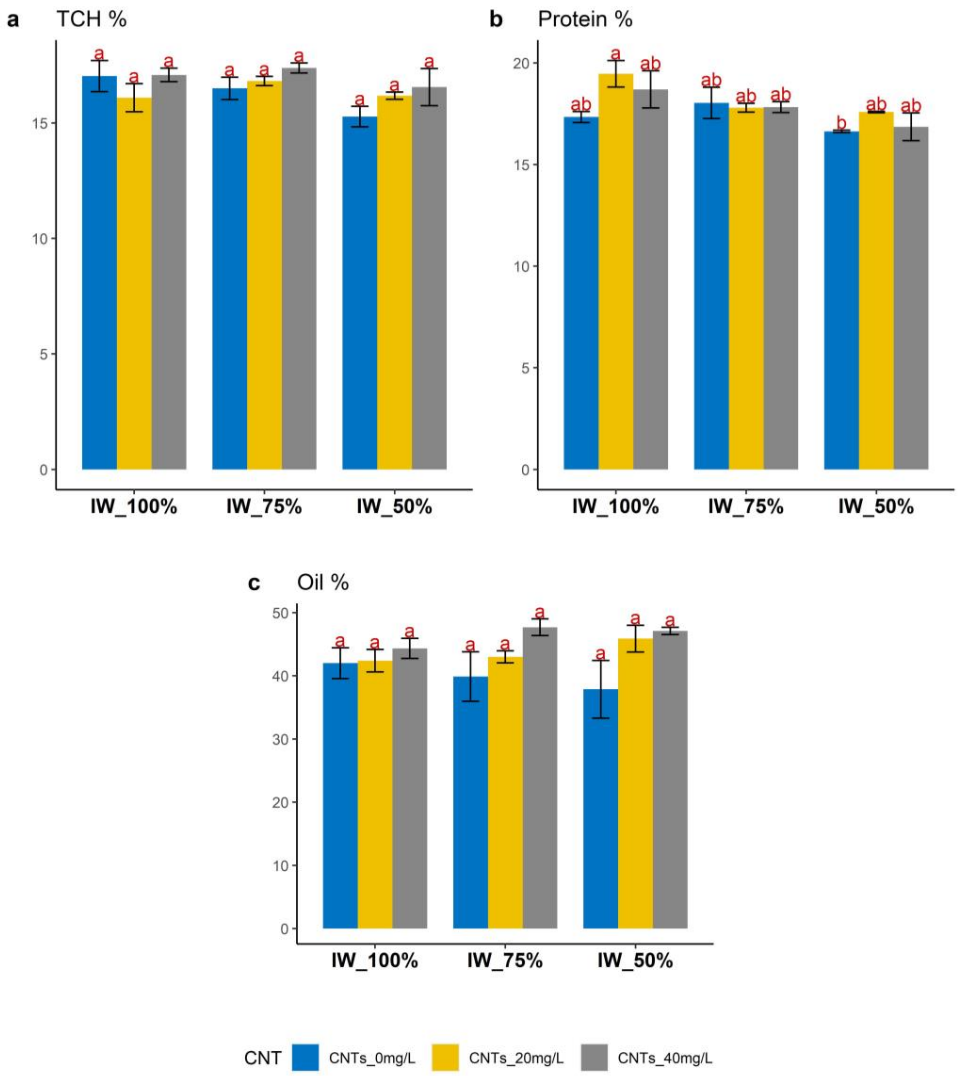

Figure 4. Effect of carbon nanotubes (CNTs) (0.0, 20 and 40 mg/L) on total carbohydrate TCH, protein and oil contents of peanut seeds grown under different water irrigation requirements in sandy soil. Different lowercase letters indicate significant differences.

3.7. Changes in Antioxidant Activities (DPPH%) and Flavonoid Contents

The presented data in Figure 5 show that water deficits (expressed as 75% and 50% IR) caused gradual and significant ($p \leq 0.05$) increases in antioxidant activity, expressed as DPPH%, as well as the flavonoid contents as compared with 100% IR irrigated peanut plants. However, for DPPH% under 75% WIRs, the increase was non-significant. Moreover, different concentrations of CNT foliar treatments caused not only significant increases ($p \leq 0.05$) at 100% WIRs, but also increases under 75% and 50% IR as compared with the corresponding controls (CNTs 0). Furthermore, a concentration of 40 mg/L CNTs was more effective at increasing the DPPH% and flavonoid contents compared with the other concentration of CNTs (20 mg/L).

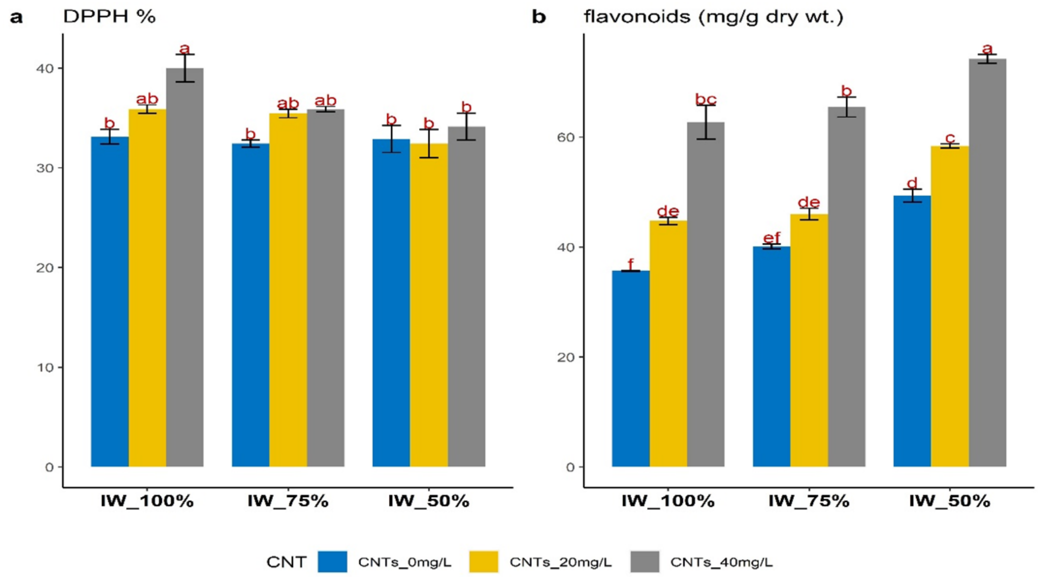

Figure 5. Effect of carbon nanotubes (CNTs) (0.0, 20 and 40 mg/L) on DPPH% and flavonoid contents (mg/g dry wt.) in peanut seeds grown under different water irrigation requirements in sandy soil. Different lowercase letters indicate significant differences.

3.8. Changes in Non-Photosynthetic Pigments

Subjecting peanuts to drought stress by irrigation with 75% and 50% of the WIRs caused significant decreases in the B-carotene and lycopene contents in the yielded seeds (Figure 6) as compared with those plants irrigated with 100% WIRs (control plants), except for 75% WIRs, which decreased the carotene level non-significantly. Meanwhile, foliar treatment of peanut plants with different concentrations of carbon nanotubes (CNTs) (20 and 40 mg/L) caused significant increases in the above-mentioned parameters (B-carotene and lycopene contents) as compared with untreated controls under different WIRs (Figure 5).

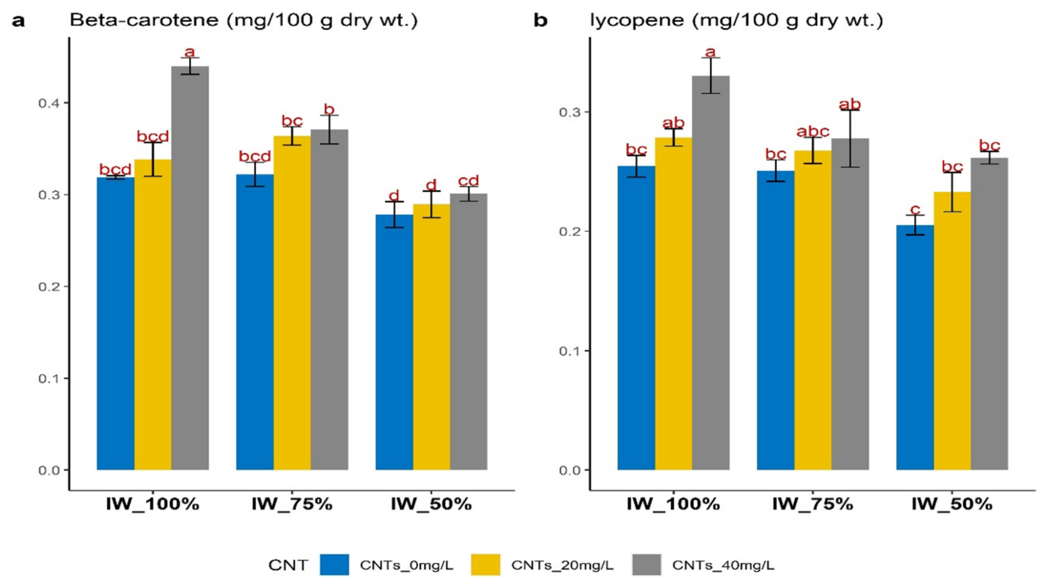

Figure 6. Impact of carbon nanotubes (CNTs) (0.0, 20 and 40 mg/L) on B-carotene and lycopene (mg/100 g dry wt.) contents in peanut seeds in irrigated sandy soil. Different lowercase letters indicate significant differences.

3.9. Artificial Neural Networks (ANNs)

ANNs are a mimic of a biological neural system. They consist of layers, which in turn consist of neurons; each layer is connected to another layer through the neurons by connection weights, but neurons in the same layer are not connected to each other. The strength of the final connection weights after training the network is used to estimate the relative importance of inputs to the output [40]. The network used in the present work is illustrated in Figure 7. It is a four-layer feed-forward multilayer perceptron neural network (MLP) constructed to assess the effect of the studied traits on the seed yield of peanuts expressed as relative importance on a scale (0–1). The regularization method was adopted during training of the network to avoid a high correlation among traits and distinguish the traits that have a direct effect on the seed yield.

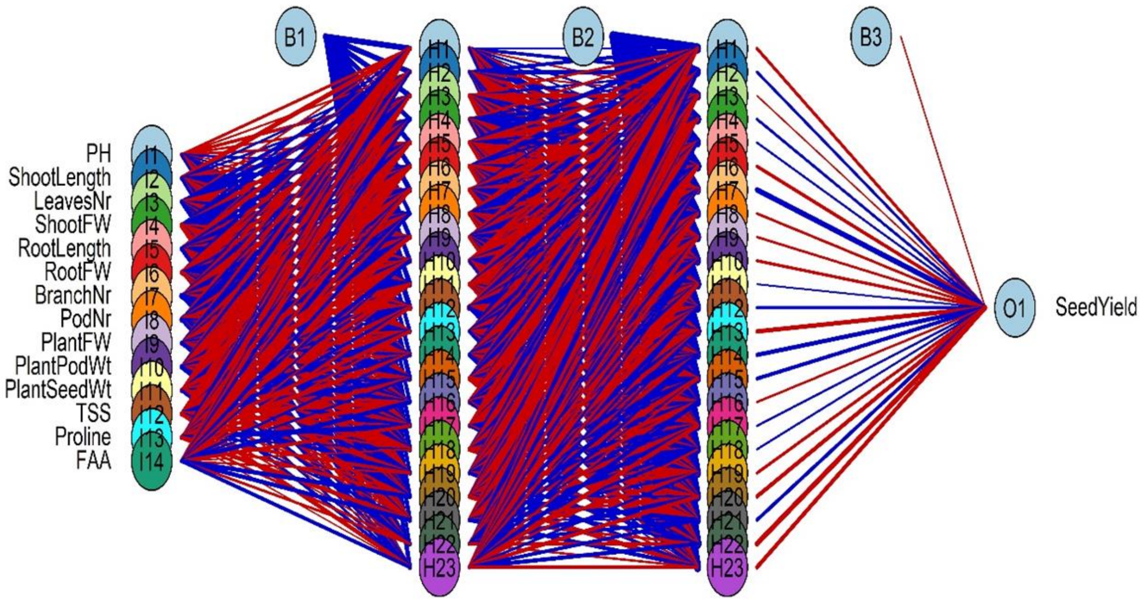

Figure 7. Multilayer perceptron artificial neural network used to estimate the importance of the studied traits to seed yield. The left layer is the input layer, the middle two layers are hidden layers and the right layer is the output layer. Red indicates negative connection weights while blue indicates positive connection weights. I, H, B and O denote input, hidden, bias and output, respectively.

3.10. Relative Importance of Agronomic Traits to Peanut Seed Yield

The final connection weights after training the multilayer perceptron artificial neural network were used to assess the relative importance of inputs to the output (seed yield) after regularization due to the correlation among traits. Figure 8 reveals that the pod number/plant was the most important trait that affected peanut seed yield, followed by the shoot fresh weight, the number of branches/plants, the shoot length and the plant fresh weight. The rest of the studied traits were less important to seed yield, even if they were correlated with the seed yield, because the adopted regularization process was used to overcome multicollinearity among inputs.

3.11. Yield Network Analysis

Every node (circle) in the network plot in Figure 9 represents one trait, and the edges (lines) between nodes represent the correlation. The length of the edge expresses the degrees of separation between each pair of nodes in the network. Positive and negative correlations are expressed in blue and red, respectively. The size of each node reflects the strength of the variable, where the strength is the sum of the weights of edges connected to that node. Figure 9 reveals that all agronomic traits measured at the end of the season, and some that were measured mid-season, were highly correlated and were gathered in one community (nodes with a blue border). These traits had a high strength compared to the other traits. It is also apparent that proline had a negative relationship with agronomic traits (red edges), while TSS had a positive relationship (blue edges). The chemical traits of seeds and leaves were correlated and gathered in another community (nodes with a green border).

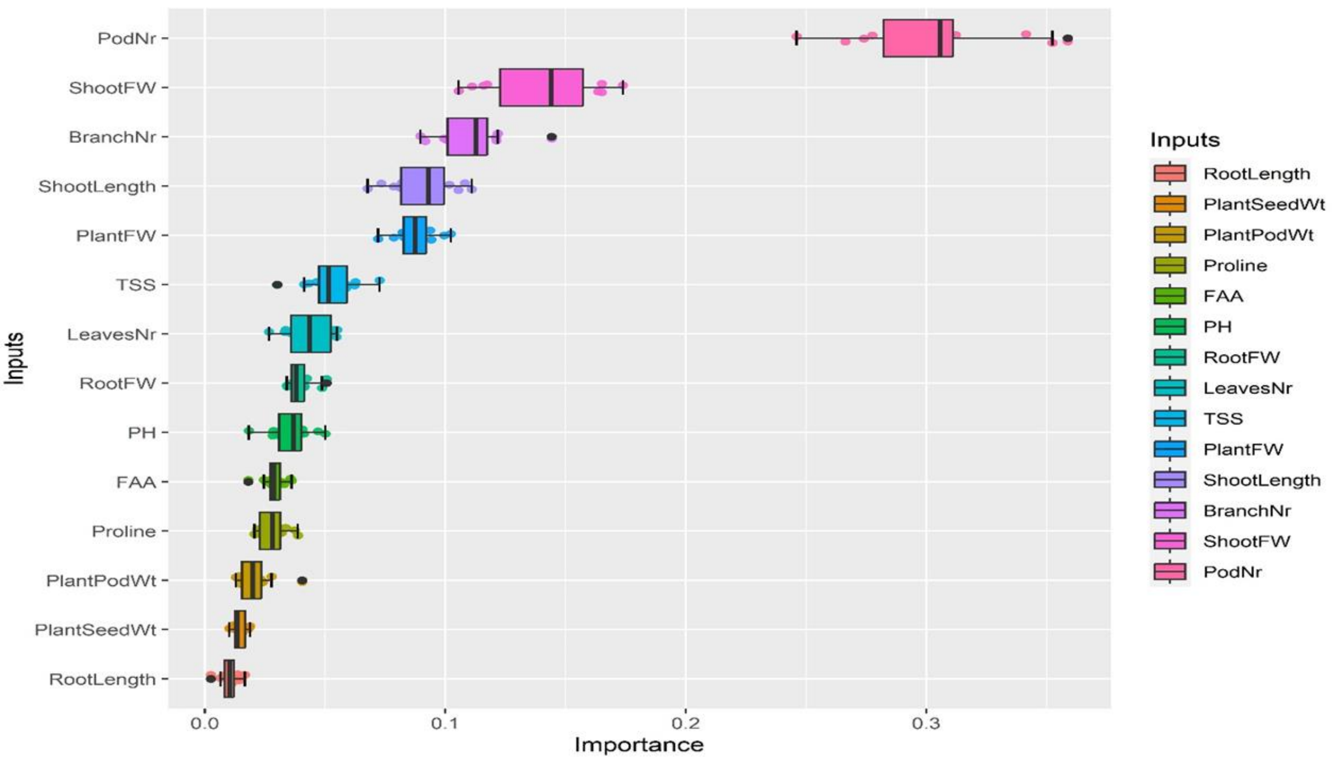

Figure 8. Relative importance of inputs to peanut seed yield expressed on a scale (0–1). (The regularization method was used to avoid the multicollinearity problem among traits.)

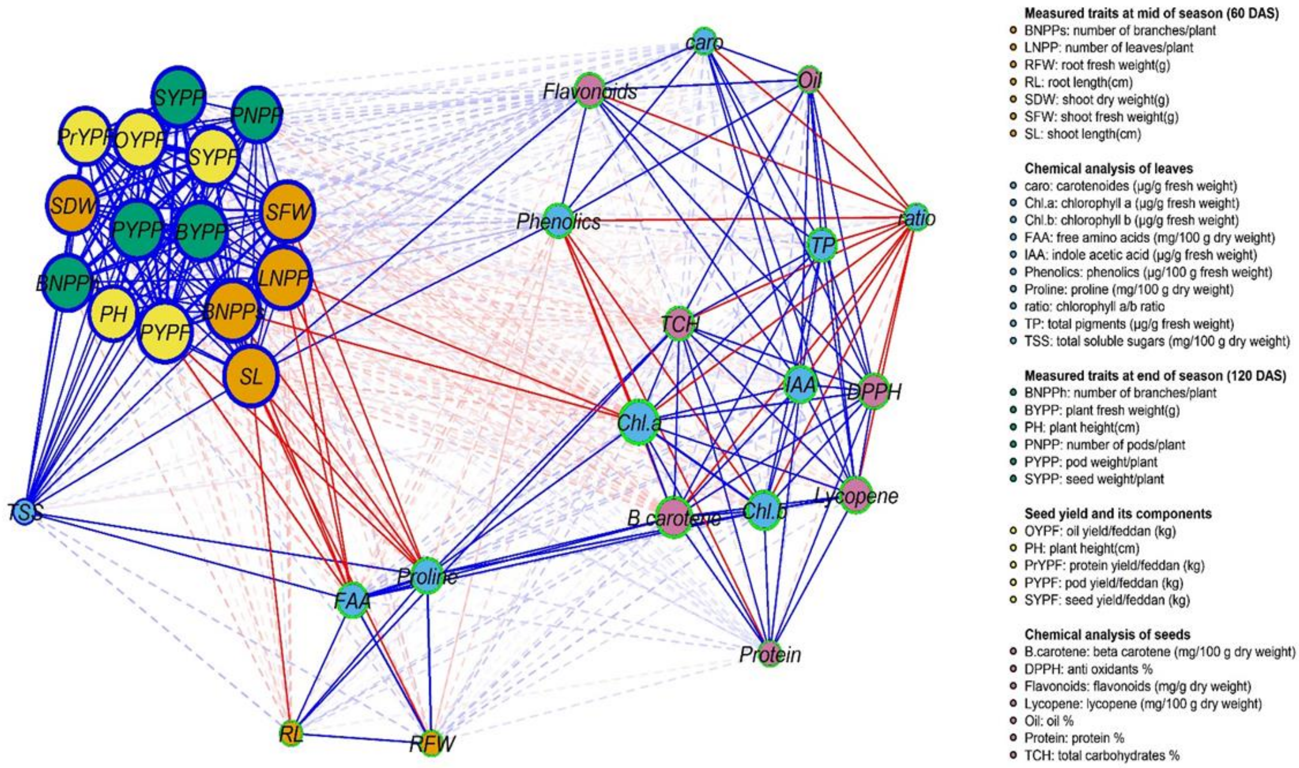

Figure 9. Yield network analysis based on spearman correlation coefficients among 34 traits using the Fruchterman–Reingold algorithm (blue edges represent significant positive relationships, red edges represent significant negative relationships, faded edges represent insignificant relationships and nodes with the same border color are clustered in one community).

4. Discussion

Water stress has a significant impact on crop output, making productive land less profitable. As a result, in the current circumstances, effective management measures are required to improve plants' tolerance to low irrigation water levels. In this context, we investigated the effect of exogenous carbon nanotubes on enhancing peanut drought resistance.

4.1. Changes in Photosynthetic Pigments

Under water stress, chloroplast is the primary source of reactive oxygen species buildup, which leads to the destruction of thylakoid membranes and photosynthetic pigments. Drought stress reduces photosynthetic pigment constituents relative to unstressed plants, as shown in Table 1. These findings are comparable to those of Elewa et al. [41] for quinoa, and [42] for flax. Drought-induced reductions in photosynthetic pigment contents could be due to the instability of the pigment–protein complex and pigment degradation. Furthermore, the decrease in photosynthetic pigments could be linked to the mechanism of defense against free radicals (ROS) by reducing the amount of photosynthetic pigment due to a lack of water. Moreover, Nazarbeygi et al. [43] reported that exposing plants to drought stress increased proline biosynthesis activity, resulting in less glutamate in biosynthesizing chlorophyll molecules (glutamate is a subscriber precursor to chlorophyll and proline biosynthesis). Because chlorophyll b degrades more quickly than chlorophyll a, leaves exposed to moderate and severe water shortage stress had lower chlorophyll concentrations and greater chlorophyll a/b ratios. This could be explained by the fact that chlorophyll b degradation is the first step [44]. Changes in the pigment composition of the photosynthetic apparatus, which contain lower quantities of light-collecting proteins, have been linked to an increase in the chlorophyll a/b ratio. On the other hand, this same effect was induced after treating peanut plants with CNTs under normal and drought-stressed conditions [4,19,45] found that CNT treatments resulted in increased photosynthetic pigment constituents in diverse plant species, as was found in this study. Drought-induced reductions in the chlorophyll content in peanut plants might be mitigated by CNT foliar sprays by increasing the activities of peanut antioxidant enzymes. Furthermore, by increasing the intake of water and nutrients, CNT treatment could boost the formation of chlorophyll.

4.2. Changes in Endogenous IAA and Total Phenols

Drought levels considerably lowered the IAA levels in peanut leaves (Figure 3); with decreasing water irrigation requirements, these declines were more gradual. IAA, as a phytohormone, regulates plants' defensive responses to biotic and abiotic challenges through signaling crosstalk with other hormones such as GA3, BA and ABA [45]. As previously indicated by Elewa et al. [41], drought and stress reduced the IAA levels in various plant species. An increased IAA oxidase activity could cause these reductions [46]. Drought stress causes a decline in many phytohormones, including IAA, which can be attributed to a decrease in the activity of enzymes that participate in phytohormone synthesis or an increase in enzyme activity that contributes to its breakdown. Because CNTs operate as elicitors in IAA biosynthesis, their relieving effect on increases in endogenous IAA contents in peanut plants can be explained by their promotive action on different plant growth regulators [47].

In terms of secondary metabolites, such as phenolic and flavonoid compounds, they increased in peanut plants as a result of drought stress and CNT foliar application (Figure 3). An increased buildup of phenols and flavonoids, as well as an up-regulation of phenylalanine ammonia, has been described in *Dracocephalum moldavica*, which is similar to our findings regarding lyase activity, which a crucial enzyme that controls the synthesis of secondary metabolites according to Naghizadeh et al. [48]. By increasing ROS scavenging, an increased accumulation of important polyphenolic chemicals protects against the oxidative consequences of stress. Earlier, Sowndhararajan and Kang [49] noted increased phenol and flavonoid levels conferred radical scavenging capabilities, which was also observed in the current investigation. To neutralize harmful radicals such as hydroxyl radicals, polyphenolic substances donate hydrogen atoms. As a result, cellular macromolecules are protected. Drought stress has been linked to greater levels of phenols and flavonoids. Carbon nanotubes were found to alter the antioxidant defense system of peanut plants by boosting the phenolic content in this study. Gonzalez-Garcia et al. [50] confirmed that CNTs induce phenolic content increases in tomato plants.

4.3. Changes in Osmolytes

Plants have mechanisms in place to combat the oxidative impacts of stress by removing reactive oxygen species (ROS). CNT application up-regulated the osmo-protectant system by increasing levels of compatible solutes such TSS, proline and free amino acids; these substances protect plants from stress by inducing membrane stabilization and causing enzyme tertiary structure maintenance [51] in peanut plants. Furthermore, these osmolytes had a significant impact on cells' adaptability to various unfavorable environments. They also increased the cytoplasmic osmotic pressure, stabilized proteins and membranes and maintained the relatively high-water content required for cell development and cell functions [52]. Despite a decrease in the CO_2 absorption rate, the plant had increased TSS levels as a result of a reaction to water stress. Furthermore, the increased proline level could be due to a decrease in proline oxidase activity during drought. It is also assumed to be a source of nitrogen and carbon, as well as a regulator of membranes and some macromolecules, a free radical scavenger and an enzyme protector during stress [53]. The results show that varied treatments of carbon nanotubes have a crucial role in regulating plant tolerance by boosting the above-mentioned osmo-protectants. CNTs have the ability to increase proline production, decrease proline degradation, and decrease proline dehydrogenase activity [20]. In stressed *Hyoscyamus niger*, proline levels increased when CNTs were applied [54].

4.4. Changes in Morphological Criteria and Yield

Exogenous application of nanoparticles such as carbon nanotubes (CNTs) ameliorates the effect of drought stress on growth and yield components, as evidenced by increases in several morphological and yield metrics of peanut plants at various CNT concentrations (Table 2). Our findings on the lessened effect of drought stress are consistent with the previous findings of Sadak et al. [55]. In this study, the losses in yield and its components in peanut plants under drought stress were investigated. The lessened effects obtained are in good agreement with Elewa et al.'s [41] finding in quinoa. Water stress is a powerful element that disrupts plant growth, physiological processes, and yield components significantly. It is possible that the reduced effect of water stress on peanut plant growth is related to a decrease in cell enlargement and turgor pressure [56]. Furthermore, decreased water absorption, a low water potential, increased contents of various ions in cells and stomata conductance of leaves could all contribute to this reduced effect. Jabeen et al. [57] found that drought caused oxidative stress, nutritional and hormonal disturbances, protein suppression/deterioration, enzyme deactivation and secondary metabolic disturbances. Furthermore, decreases in growth characteristics resulted in decreases in many yield components of peanut plants. These decreases could be due to a decrease in the chlorophyll content as well as the Calvin cycle enzyme activity [58,59]. According to researchers, lipids in cell membranes, proteins, carbohydrates, and nucleic acids are key cell components that can be harmed by an increase in ROS levels caused by drought stress. Plant growth and tolerance to numerous environmental difficulties, such as drought stress, have recently been improved and enhanced using nanoparticle treatment in agriculture, resulting in a higher plant productivity. These findings are consistent with previous studies using CNTs on tomato plants [60], on Satureja khuzestanica [61], and wheat [62]. CNT treatment of peanut plants at various doses increased growth criteria (Table 2) and yield components (Table 4). Previous research has shown that treating tomato plants with CNTs improves their growth and output by increasing the water intake [63]. Carbon nanomaterials' ability to activate the gene/protein expression required for plant growth and development may be related to their ability to promote growth. As a result, carbon nanomaterials can be employed as a plant growth regulator [15]. Furthermore, CNTs improve plant growth by enhancing the production of endogenous indole acetic acid (Table 3) [64]. Furthermore, enhanced water uptake and transport, seed germination, photosynthesis and IAA, which activate water channel proteins and improve nutrient uptake, may contribute to CNTs' beneficial effects.

4.5. Changes in Carbohydrates, Protein and Oil Contents

Regarding the chemical components of the peanut seed, [56] validated the chemical composition of maize seeds produced under reduced water stress. Carbohydrate modifications are particularly important in terms of variations in carbohydrate contents since they are linked to a number of biochemical functions such as photosynthesis, motility, and respiration. Reduced oil levels in peanut seeds as a result of low irrigation levels could be due to the oxidation of specific polyunsaturated fatty acids [65]. Different treatments with carbon nanotubes resulted in an increase in peanut productivity. These results are in agreement with earlier reports of applying CNTs to tomato plants [60], to Satureja khuzestanica [61], and to wheat [62].

In terms of the nutritious components of the peanut plant seeds, the percentage of carbs, protein and oil in the produced peanut seeds decreased as the water irrigation level was reduced.

In accordance with our findings, Sadak et al. [55], they stated that water stress reduced the glucose content in faba bean and soybean plants. The reductions in growth parameters (Table 2) and photosynthetic pigments are the key reasons for the decreases (Figure 1).

Because of their direct association with physiological activities, including photosynthesis, translocation, and respiration, carbohydrate alterations in the produced seeds are particularly important [55]. Water stress reduced the amount of chlorophyll in leaves, resulting in a drop in photosynthetic activity. As a result, there was less glucose buildup in mature leaves and, as a result, carbohydrate transport from leaves to developing seeds may be reduced [41]. The stimulating effect of CNT treatments on carbohydrate, protein and oil content of produced seeds, on the other hand, could be attributed to increases in growth parameters and photosynthetic pigments. Furthermore, these increases in carbohydrate content could be attributed to enhanced photosynthetic production, which increases carbohydrate synthesis in leaves and consequently boosts carbohydrate translocation from leaves to developing seeds.

Non-photosynthetic pigments like B-carotene and lycopene are the key phytochemicals found in peanut seeds, and they are well known for their ability to act as a powerful antioxidant.

4.6. Changes in Antioxidant Activities (DPPH%), Flavonoid Contents and Non Photosynthetic Pigments

Free radicals, which produce oxidative damage in the body, have been linked to the development of a number of chronic diseases, including cancer, ageing and cardiovascular problems [66]. Lycopene is a precursor of β-carotene which as a fat-soluble carotenoid exhibits a two-fold higher antioxidant activity than β-carotene. Lycopene's potential antioxidant activity is mostly due to its long-chain conjugated double bonds (polyene chains), which have the ability to quench free radicals [67]. Cell signaling and communications are two other significant designated roles of lycopene hormone and immune response regulation, as well as functioning in metabolic pathways [68]. The nutritional values and antioxidant potentials of the peanut seed contents, such as lycopene, β-carotene and flavonoids, and the antioxidant activity, as DPPH percent, in response to CNT foliar application were determined. Lycopene and β-carotene are well known natural antioxidants that, in vitro, are the most effective singlet oxygen quenchers among the typical carotenoids [69]. In accordance with our obtained results, Dorais et al. [70] showed that β-carotene in tomato fruit was significantly decreased under salt stress. As well, Ali and Ismail [71] found that water stress had a detrimental impact on the accumulation of lycopene and β-carotene during tomato ripening. As part of the light-harvesting system, carotenoids are intricately related to photosynthesis, and it is widely understood that stress inhibits photosynthesis [72]. Thus, the decrease in lycopene and β-carotene levels under the current experimental conditions might be linked to a decrease in photosynthetic activities under stress. Stress may block or upregulate the biosynthesis pathway of carotenoids by inhibiting genes encoding enzymes associated with lycopene and β-carotene according to one theory [73]. Recently, Babu et al. [74] found that stress inhibited the expression of the gene encoding lycopene-cyclase, the enzyme that transforms lycopene to beta carotene. Flavonoids, which include flavones and condensed tannins, are secondary metabolites of plants whose antioxidant action is dependent on the availability of free OH groups, particularly 3-OH. In vitro, plant flavonoids exhibit antioxidant activity, and in vivo, they serve as antioxidants. Because this is the first set of data on the antioxidant activity of peanut seeds, detailed phytochemical investigations to determine the active phenolic and flavonoid components should be performed.

Since water stress was accompanied by increased formation of reactive oxygen species, the increased flavonoid content may indicate some type of defense against stress circumstances (i.e., oxidative load) [25,74–78].

Finally, using carbon nanotubes as a cheap economic application could be used as a beneficial method for increasing peanut plant tolerance towards drought stress; thus, peanut plants can be grown in areas which suffer from drought stress.

5. Conclusions

It can be concluded that exogenous application of carbon nanotubes via foliar treatment might be an effective method for improving drought stress tolerance in peanut plants grown in sandy soil. These significant effects mainly arise from the improvement in growth and the seed yield quantity and quality of peanut plants, with the maximum increases if CNTs are applied at a level of 40 ppm. Meanwhile, under conditions of 75% and 50% IW, different yield components are expected to exhibit the maximum increases at 40 ppm. The role of CNTs in enhancing the tolerance of peanut plants under drought stress could be attributed mostly to (1) improvements in photosynthesis, growth and development and (2) improvements in endogenous IAA, phenolic and osmolyte levels [namely TSS, proline and FAA].

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Conflicts of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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