soil systems

MDPI

Article

Effect of Natural Phytohormones on Growth, Nutritional Status, and Yield of Mung Bean (*Vigna radiata* L.) and N Availability in Sandy-Loam Soil of Sub-Tropics

Aasma Parveen [1,†], Muhammad Mahran Aslam [2,3,†], Rashid Iqbal [4,*], Muhammad Ali [3], Muhammad Kamran [5,*], Mona S. Alwahibi [6], Muhammad Akram [7] and Mohamed S. Elshikh [6]

1 Department of Soil Science, Faculty of Agriculture & Environment, The Islamia University of Bahawalpur, Bahawalpur 63100, Pakistan
2 State Key Laboratory of Agrobiotechnology/Beijing Key Laboratory of Crop Genetic Improvement, College of Agronomy and Biotechnology, China Agricultural University, Beijing 100193, China
3 Nuclear Institute of Agriculture (NIA), Tandojam 70060, Pakistan
4 Department of Agronomy, Faculty of Agriculture & Environment, The Islamia University of Bahawalpur, Bahawalpur 63100, Pakistan
5 School of Agriculture, Food and Wine, The University of Adelaide, Adelaide, SA 5005, Australia
6 Department of Botany and Microbiology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia
7 Department of Botany, The Islamia University of Bahawalpur, Bahawalpur 63100, Pakistan
* Correspondence: rashid.iqbal@iub.edu.pk (R.I.); muhammad.kamran@adelaide.edu.au (M.K.)
† These authors contributed equally to this work.

Citation: Parveen, A.; Aslam, M.M.; Iqbal, R.; Ali, M.; Kamran, M.; Alwahibi, M.S.; Akram, M.; Elshikh, M.S. Effect of Natural Phytohormones on Growth, Nutritional Status, and Yield of Mung Bean (*Vigna radiata* L.) and N Availability in Sandy-Loam Soil of Sub-Tropics. *Soil Syst.* **2023**, *7*, 34. https://doi.org/10.3390/soilsystems7020034

Academic Editors: José L. S. Pereira and Vítor João Pereira Domingues Martinho

Received: 26 January 2023
Revised: 28 March 2023
Accepted: 3 April 2023
Published: 10 April 2023

Abstract: Climate changes and poor soil nutrient profiles in sub-tropics are determinant factors to estimate crop productivity. This study aims to evaluate the impact of phytohormones, e.g., indole acetic acid (IAA) and gibberellic acid (GA_3), on mung bean yield, seed nutritional profile, and soil N availability in the sub-tropical region of Pakistan. The mung bean plants were treated with three levels (0, 30, and 60 mg L^{-1}) of IAA and GA_3 individually and/or in combination using a hydraulic sprayer. The amendments were applied in the flowering stage (approximately 25 days after germination) in a randomized complete block design. The results revealed that the 60 mg L^{-1} concentration of IAA and GA_3 led to significant changes in the growth and yield traits compared to non-treated plants. For example, GA_3 positively influenced the biological yield (35.0%), total carbohydrate (7.0%), protein (16.0%), and nitrogen (14.0%) contents in mung bean seeds, compared to the control (CK). Additionally, the combined foliar treatment of IAA and GA_3 ($IAA_2 + GA_2$) displayed a much stronger influence on yield attributes, such as the number of pods by 66.0%, pods' weights by 142.0%, and seed yield by 106.5%, compared with the CK. Mung bean plants showed a significant improvement in leaf photosynthetic pigments under a higher level (60 mg L^{-1}) of sole and combined treatments of IAA and GA_3. Moreover, except abscisic acid, the endogenous concentration of IAA, GA_3, and zeatin was enhanced by 193.0%, 67.0%, and 175.0% after the combined application of IAA and GA_3 ($IAA_2 + GA_2$) compared to the CK treatment. In addition, soil N availability was increased by 72.8% under the IAA_2 treatment and 61.5% under $IAA_2 + GA_2$, respectively, compared with the control plot. It was concluded that the combined treatment of IAA and GA_3 ($IAA_2 + GA_2$) followed by the sole application of GA_3 and IAA at a 60 mg L^{-1} concentration were most effective treatments to improve the morpho-physiology and nutrient profile of mung beans; however, the underlying molecular mechanisms need to be explored further.

Keywords: indole acetic acid; gibberellic acid; *Vigna radiate* L.; photosynthetic pigments; protein; economic yield

1. Introduction

The current cultivation pattern depends on 30 crops which are responsible for providing 95% of the daily caloric requirements of world population [1]. Among them, four crops are a major part of the diet, namely wheat, rice, maize, and potatoes. However, minor crops are still very significant at the national, regional, and local levels. Pulses are rich in protein and contain more than three times higher quality protein than cereals [2]. In addition, pulse crops preserve and improve soil fertility, especially soil N availability, through biological nitrogen fixation, and hence have a significant role in sustainable agriculture. Pulses are popularly known as the "poor man's meat" in most developing countries due to being cheaper and more widely available than animal protein [3]. Mung bean (*Vigna radiata* L.), a short-duration crop (70–90 days), is an important pulse all over the world with admirable economic importance. The grains of mung bean contain 25.67% protein, 1–3% fat, 5.4% carbohydrates, 3.5–4.5% fibers, and 4.5–5.5% ash with very minimal flatulent effects [4], and they are rich in folate and iron [5]. They contain a high amount of protein with a diversity of essential amino acids and are especially rich in lysine [6]. They also contain important forms of fatty acids (linoleic acid and linolenic acid), which are essential in an organism's growth. It is a vital crop to Asian farmers with small land holdings [7].

Mung bean (*Vigna radiata* L.) fixes atmospheric nitrogen and contains high nutritious value for forage and seed purposes. The crop fits well in multi-cropping systems because of its rapid growth and early maturity. Subsequently, the crop is widely grown in marginal and abiotically stressed agro-ecosystems [8], but it experiences considerable yield losses. Worldwide food insecurity affects more than 800 million people. Almost 60% of them live in South Asia and sub-Saharan Africa [9], whereby half of them are livestock keepers and small land holders [10]. Small land holder farmers in dryland areas or areas with erratic rainfall usually lack technologies to diversify their production, making them particularly vulnerable. In Pakistan, the production of pulses has stagnated and has not kept the pace needed to meet consumption demand. The gap has been fulfilled by massive imports [7]. The size of areas suitable for the growth of pulses has been steady since the 1960s, with chickpeas being the dominant pulse grown in Pakistan [11]. However, the area in which mung beans are cultivated increased substantially during 2019–20 by 35%, in which production increased by 65% in Punjab province, 6% in Khyber Pakhtunkhwa province, and 17% in Baluchistan province. About 88% of the area in which mung beans are cultivated is in the Punjab province, which produces 85% of the total production in the country [12]. Still, a lot needs to be done in Pakistan to enhance mung bean production in terms of quality and yield. More recently, the Australian Centre for International Agricultural Research, in collaboration with the Pakistani government, supported research for improving the productivity and marketing of pulses in Pakistan [11]. Therefore, considering this scenario, the total pulse production must urgently be increased to meet the consumption and protein demands [7].

Many artificial and natural soil amendments, e.g., phytohormones [13,14], C-rich organic amendments [15–17], and synthetic minerals nutrients [18–21] have been recognized to govern soil fertility; facilitate progressive methods, from the germination to the harvesting of crop plants; and facilitate improvements in plant production [22]. The natural form of auxin found in plants is indole-3-acetic acid (IAA), which is primarily present in the young leaves and stem apex of a plant. Indole-3-acetic acid (IAA) is one of the main important enzymes, which is non-toxic for plants even at high concentrations [23]. IAA has been recognized in root growth, cell division, cell elongation, adventitious root formation, tissue swelling, embryogenesis induction, callus initiation, as well as the loosening of cell walls even at lower levels of this hormone [24,25]. Gibberellic acid (GA_3) is an important natural plant growth regulator (PGR) which enhances the growth, development, and yield of different crops. GA_3 is a phyto-hormone, and terpenoid compounds containing 19–20 carbon atoms, naturally produced in new leaves and germinated seed embryos, and more than 136 species have been identified [26]. A very small amount of GA_3 enhances stem elongation [27] by increasing cell divisions and cell size [28]; improves plant growth and development [29,30] by inducing metabolic activities [31,32] of many key enzymes such as carbonic anhydrase (CA) and nitrate reductase (NR) [33] and regulating nitrogen utilization [34]; and consequently, increases the dry weight and yield [35,36]. It also induces growth and development by enhancing water uptake in plant tissues [37,38], facilitates the production of photosynthetic pigments and photosynthesis [39], and enables flower formation and fruit set in legumes [36,40].

Keeping in view the importance of mung beans in sub-tropics, this study aimed to improve their morpho-physiology, yield, and nutritional profile when grown in a sandy-loam soil with low-fertility status. In the literature, many researchers have evaluated the individual beneficial effects of indole acetic acid (IAA) and gibberellic acid (GA_3) on many crops under stressful and non-stressful environments [14,35,41]; however, their combinations with various dosage levels still have to be evaluated. Therefore, the individual and/or combined effectiveness of various levels of IAA and GA_3 was assessed in the present study to evaluate mung bean (Var. AEM-96) performance in the sub-tropical region of Pakistan for two consecutive years (2018–19/2019–20). This experiment has huge importance as a reference for the impact of two important phytohormones on mung bean growth, yield, quality, and nutritional contents in the sub-tropical region of Pakistan.

2. Materials and Methods

2.1. Experiment Site and Climate Conditions

The two-year field experiment was carried out in the agronomic research area of The Islamia University of Bahawalpur, Punjab, Pakistan, during the 2019–20 growing seasons. The meteorological data were obtained from Bahawalpur meteorological station and are described in Table 1. The experiment was carried out to determine the properties of the soil at the experimental site. The soil sample test results showed that the soil was sandy loam and had an acidic pH (7.2). The detailed statuses of the soil's physio-chemical characteristics are depicted in Table 2.

Table 1. Meteorological data recorded at the experimental site during the study period of 2019–2020.

Month	T_{max} °C		T_{min} °C		Total Rain Fall (mm)		R.H (%)	
	2019	2020	2019	2020	2019	2020	2019	2020
October	36.1	37.4	21.6	20.5	0	0	58	54
November	31.6	32.20	13.9	11.6	0	0	53	52
December	27.5	26.6	12.2	9.0	0	0	57	53
January	26.4	22.1	11.3	8.3	0	2.5	61	62
February	22.4	24.2	10.2	11.0	0	0	45	50
March	35.1	34.4	18.1	15.5	0	0	48	48
April	38.0	40.2	21.9	18.9	0	0	44	43
May	39.5	46.0	29.0	28.0	0	0	58	56
June	41.0	42.0	28.0	27.0	0	0	47	45
July	45.0	47.0	27.0	25.0	0.7	0	56	53
August	42.0	43.0	28.0	23.0	0	2.6	47	49
September	38.0	41.0	26.0	24.5	0	0	50	52

T_{max} = maximum temperature, T_{min} = minimum temperature, and R.H = relative humidity.

Table 2. The detailed fertility status of the soil at the agronomic research field station used in the current experiment.

Soil Properties	Units	Values
Texture	-	Sandy loam
pH	-	7.2
SOM	%	1.43
Total N	%	0.07
Na	meq/60 g soil	0.07
K	meq/60 g soil	0.12

Table 2. *Cont.*

Soil Properties	Units	Values
Mg	meq/60 g soil	2.1
P	ug/g soil	12.3
S	ug/g soil	16.09
Ca	ug/g soil	3.02
Zn	ug/g soil	1.43
B	ug/g soil	0.38

2.2. Experiment Design and Treatments

The field experiment was conducted under a randomized complete block design (RCBD) with three replications per block. The area was divided into different plots, and each plot size was 10 m^2 containing 6 rows. To fulfill the nutrient profile of the growth media of the experimental site, it was fortified with potassium (K), phosphorus (P), and nitrogen (N) in the form of calcium superphosphate (12%), potassium sulfate (50%), and urea (46%), respectively. The above ratio of fertilizer was maintained, with one fertilizer bag containing 50 kg of DAP (diammonium phosphate) and 10 kg of urea, and it was incorporated in the soil before planting the mung bean genotypes.

The mung bean seeds (Var. AEM-96) were collected and washed three times with distilled water. After being washed, these seeds were spread to dry overnight at room temperature. Later on, the mung bean seeds were sown and thinned after three weeks of germination for the maintenance of the plant-to-plant distance. The application solutions of IAA and GA_3 were prepared according to the treatment specification (0, 30, and 60 mg L^{-1} concentrations), and they were applied on the plant foliage at flowering time after 25 days of germination (DAG) thrice, with an 11-day interval each time. The foliar application with a hand hydraulic sprayer was carried out at dawn and dusk to avoid evaporation losses. Optimal cultural and agronomic practices that affect the yield of the crop were applied efficiently during the whole plant growth cycle. The details of the foliar treatment of IAA and GA_3 are described in Table 3.

Table 3. Treatment layout of the experiment conducted in 2019–2020 in a sandy-loam soil in sub-tropics.

Treatment Number	Treatment Labels	IAA Conc. (mg L^{-1})	GA_3 Conc. (mg L^{-1})
T1	CK	00.0	00.0
T2	IAA_1	30.0	00.0
T3	IAA_2	60.0	00.0
T4	GA_1	00.0	30.0
T5	GA_2	00.0	60.0
T6	$IAA_1 + GA_1$	30.0	30.0
T7	$IAA_1 + GA_2$	30.0	60.0
T8	$IAA_2 + GA_1$	60.0	30.0
T9	$IAA_2 + GA_2$	60.0	60.0

IAA: indole-3-acetic acid; GA_3: gibberellic acid.

2.3. Determination of Growth Traits

The mung bean plant samples which were to be assessed to determine morphological parameters were collected 60 days after sowing (DAS) by randomly selecting plants from each plot for the estimation of growth characteristics. Plant height was recorded with the help of a measuring scale from the ground to the top of the leaf in centimeters (cm). Other growth traits, including leaf fresh and dry weight (wt.), shoot length, number of leaves/plant, and number of branches/plant, were also calculated accordingly.

2.4. Determination of Agronomic Traits

Post-harvesting data and seed-related traits were measured 75 days after sowing (DAS) from the agronomic research area, The Islamia University of Bahawalpur, Pakistan. During harvesting, the mung bean plant samples were collected for the estimation of agronomic and yield-related parameters. The pod weight/plant, the number of pods/plant, and the number of seeds/pod were counted from ten randomly selected plants at harvest. Four rows from each treatment condition were harvested and weighed to compute the biological yield (kg ha^{-1}). However, the seed yield was recorded at harvest, where the mass of seeds collected per square meter (g/m^2) was recorded and converted to kg/ha.

2.5. Estimation of Photosynthetic Traits

The mung bean fresh leaf samples were collected 60 DAS. The determination of chlorophyll pigments was carried out by randomly selecting ten plants/plots. Fresh leaf samples were collected from each treatment and subjected to grinding with 80% acetone. The semi-liquid extract was filtered and centrifuged at 10,000 rpm for 5 min. The supernatant was then subjected to a spectrophotometer (Model Analytikjena Spekol 1500 Germany). For acetone (80% v/v) extraction, the following equations given by Lichtenthaler [42] were used to estimate chlorophyll contents (mg/g FW) in the leaves.

$$\text{Chl. a} = \{(12.25A_{663.2} - 2.79A_{646.8}) \times V\} \div W$$

$$\text{Chl. b} = \{(21.50A_{646.8} - 5.10A_{663.2}) \times V\} \div W$$

$$\text{Carotenoids} = \{(1000A_{470} - 1.82_{\text{Chl.a}} - 85.02_{\text{Chl. b}}) \times V\} \div (198 \times W)$$

where V represents the final volume of chlorophyll extract in 80% acetone, and W is 0.1 g.

2.6. Determination of Total Carbohydrates, Nitrogen, and Protein Contents

The leaves of 3 randomly selected plants 60 DAS were subjected to the estimation of total carbohydrates, nitrogen, and protein contents. The phenol–sulfuric acid method was applied to measure the total carbohydrates from the IAA and GA_3 treatment individually or in combination. Micro Kjeldahl's apparatus was used to estimate the nitrogen contents [43]. The crude protein was determined using a previously reported method [44]. In this method, crude protein was measured by multiplying protein contents with a 5.75 factor.

2.7. Estimation of Endogenous Growth Regulators

The mung bean plant samples were collected randomly from each plot and treatment 75 DAS for the determination of endogenous critical plant regulators. For this particular trait, the plants were screened based on data obtained from morphological and yield attributes. The most promising treatments were then selected for the estimation of endogenous regulators, including IAA, GA_3, ABA, and zeatin. The hormones were extracted using the previously reported method described by Shindy et al. [45]. Briefly, 2 g of fresh leaves was ground in cold 80% (v/v) aqueous methanol with a mortar and pestle for extraction. After extraction, the gas–liquid chromatography (GLC) technique was used to determine the IAA, GA, and ABA contents [45]. Furthermore, the cytokinin contents were estimated using the method previously reported by Müller [46] via HPLC (high-performance liquid chromatography).

2.8. Statistical Analysis

The data were pooled for both the growing years. All the experimental data were recorded and subjected to a one-way analysis of variance (ANOVA) with three replicates to record statistically significant/non-significant differences among different traits of mung bean through a computer program, Statistix Version 8.1 (Analytical Software, 2005).

Moreover, Bonferroni's statistical analysis test was applied to verify the level of significance (5%) among different treatment means.

3. Results

3.1. Influence of Natural Phytohormones on Mung Bean Growth

The statistical data of morphological/vegetative parameters depicted that the application of two phytohormones, i.e., IAA (T_2–T_3) and GA_3 (T_4–T_5), individually or in combination (T_6–T_9), significantly influenced the growth of mung bean plants (Figure 1A–F). A significant increase was observed under IAA_2 + GA_2 treatments in shoot length by 33.8%, leaf fresh weight by 56.0%, leaf dry weight by 57.7%, no. of leaves by 34.8%, and no. of branches per plant by 22.9%, as compared to the control (CK) treatment. The maximum growth performance was observed in the IAA_2 + GA_2 (T_9) treatment compared to the other treatments (T_1–T_8) and the control (T_0).

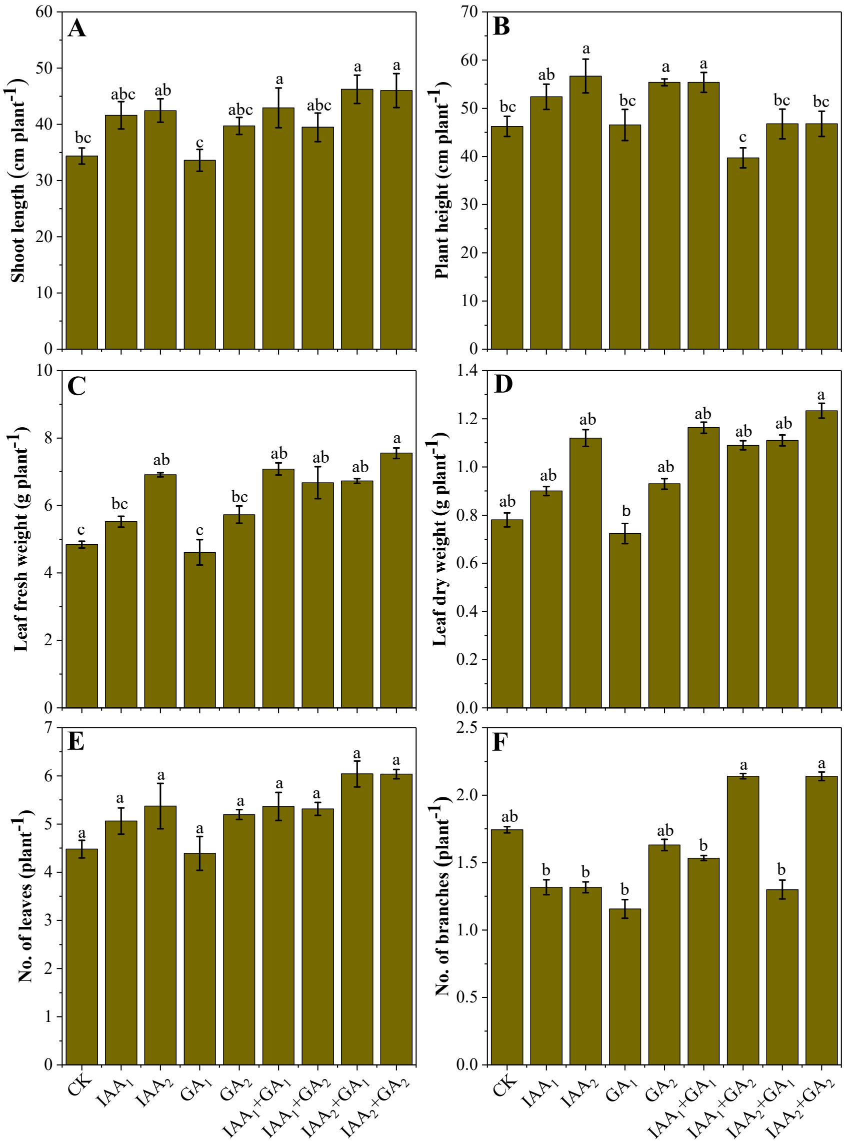

Figure 1. Influence of individual and/or combined foliar application of natural phytohormones on growth and biomass characteristics, e.g., shoot length (**A**), total plant height (**B**), leaf fresh weight (**C**), leaf dry weight (**D**), no. of leaves (**E**), and no. of branches (**F**) of mung bean plants grown in sandy-loam soil in sub-tropics. The bar values are an average of three replicates (n = 3), and the bars not sharing the same lowercase letters indicate significant differences from each other according to Bonferroni's statistical analysis at $p < 0.05$.

3.2. Influence of Natural Phytohormones on Yield and Yield Related Parameters

The foliar application of the singular or combined treatment of IAA and GA_3 hormones positively influenced the mung bean yield and yield-related parameters, as presented in Figure 2A–F. The data analysis showed that the highest yield of each parameter was obtained with the IAA_2 alone and IAA_2 + GA_2 treatment. However, the most effective treatment was found to be IAA_2 + GA_2 (T_9), which led to the highest mung bean yield in terms of seed yield (>230.3%), straw yield (>29.5%), and biological yield (>43.4%), as compared with the CK treatment in which no phytohormone was supplied. In most of the yield attributes, however, there was no statistical difference among the effect of the IAA_2 (T_3) and IAA_2 + GA_2 (T_9) treatment levels. On the other hand, it was noticed that a lower concentration of GA_3 hormone (GA_1 treatment) was less effective in improving mung bean's yield-related traits when compared with the higher dosages of the studied hormones.

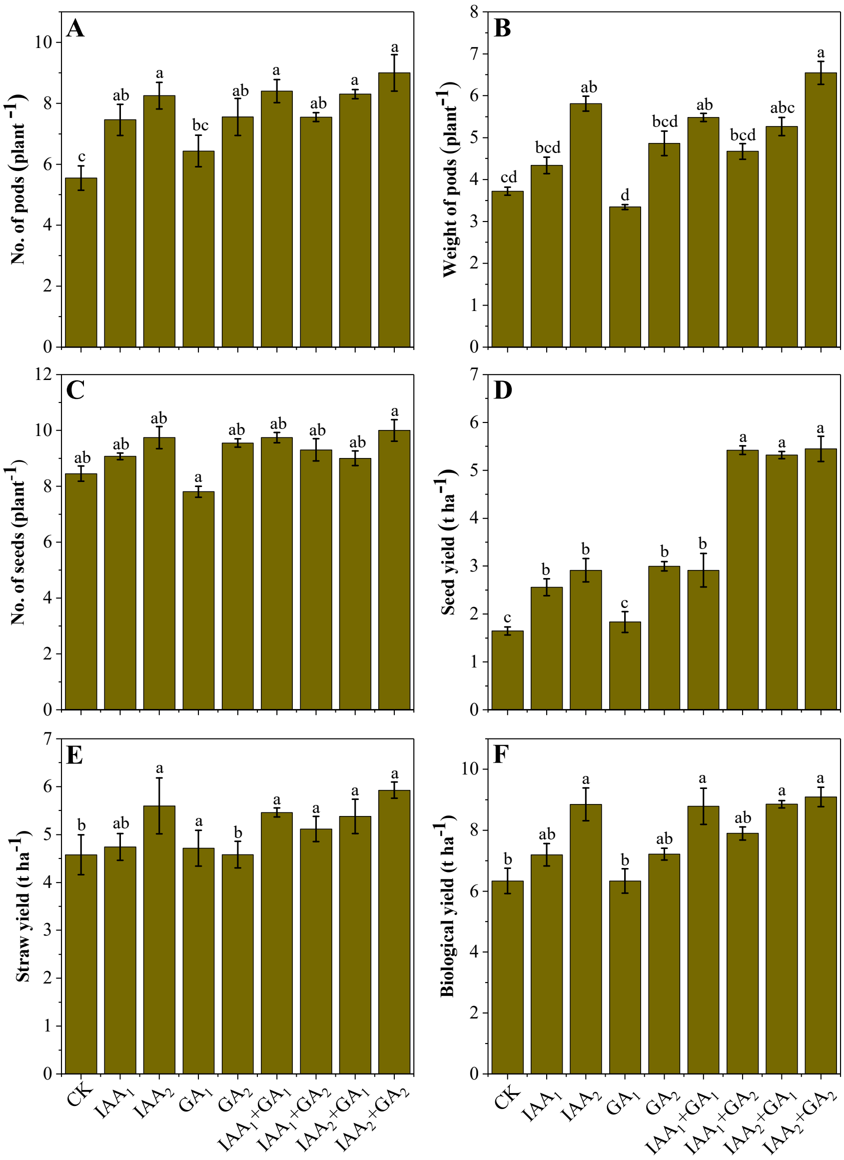

Figure 2. Influence of individual and/or combined foliar application of natural phytohormones on yield-related characteristics, e.g., no. of pods (**A**), weight of pods (**B**), no. of seeds per plant (**C**), seed yield (**D**), straw yield (**E**), and biological yield (**F**) of mung bean plants grown in a sandy-loam soil in sub-tropics. The bar values are an average of three replicates (n = 3), and the bars not sharing the same lowercase letters indicate significant differences from each other according to Bonferroni's statistical analysis at $p < 0.05$ level.

3.3. Influence of Natural Phytohormones on Photosynthetic Pigments

The influence of different concentrations of the spraying of IAA and GA_3, combined or alone, on total photosynthetic pigments, carotenoids, chlorophyll a (Chl a), and chlorophyll b (Chl b) are presented in (Figure 3A–D). Overall, the photosynthetic pigments and carotenoid contents were influenced positively by the foliar application of IAA and GA_3. The analysis of the results showed that IAA and GA_3 treatments caused a significant increase in photosynthetic pigments. Briefly, maximum enhancements of 16.5%, 19.7%, and 17.9% was reported in chlorophyll a, chlorophyll b, and carotenoid contents, respectively, under the combined application of phytohormones (IAA_2 + GA_2), as compared with the plants grown in the control plot. Moreover, quite similar results were observed for the total concentration of pigments in the leaves of mung bean under the application of phytohormones.

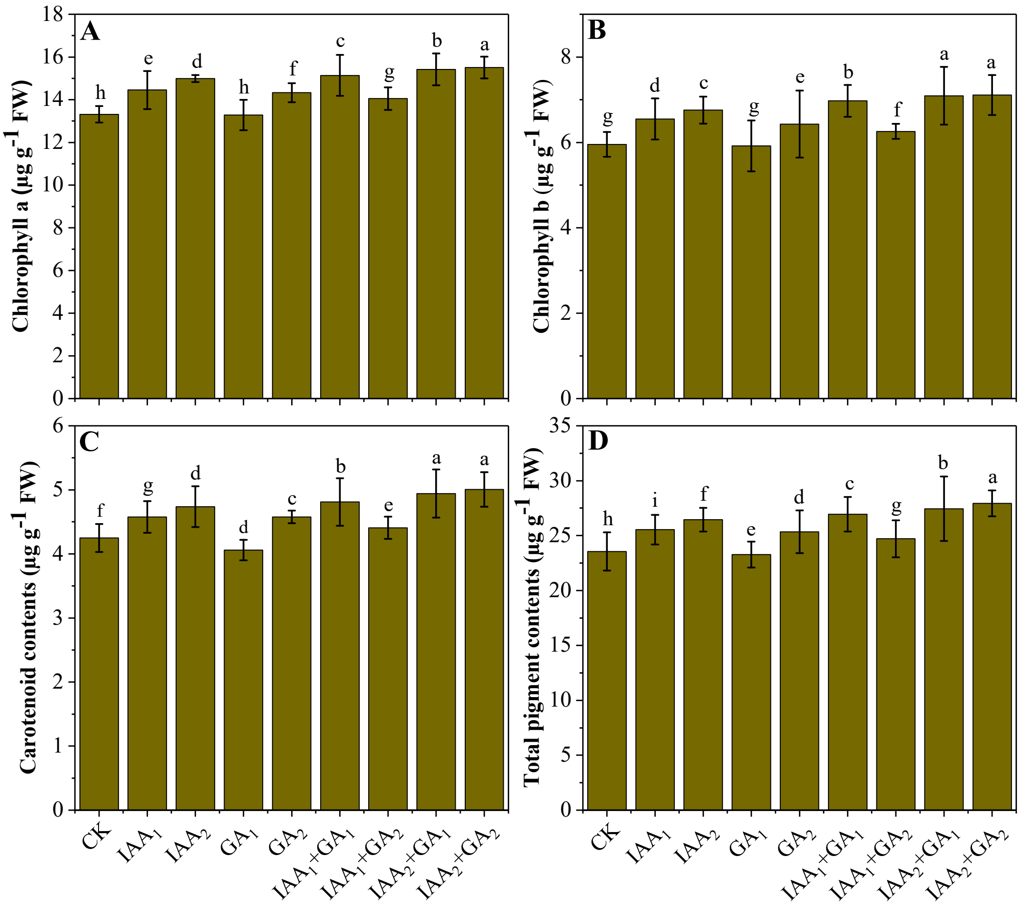

Figure 3. Influence of individual and/or combined foliar application of natural phytohormones on the physiological parameters, e.g., chlorophyll a (**A**), chlorophyll b (**B**), carotenoid contents (**C**), and total pigment contents (**D**) of mung bean plants grown in a sandy-loam soil of sub-tropics. The bar values are an average of three replicates (n = 3), and the bars not sharing the same lowercase letters indicate significant differences from each other according to Bonferroni's statistical analysis at $p < 0.05$ level.

3.4. Influence of Natural Phytohormones on Nutritional Status of Mung Bean Seeds

The findings related to the nutritional status of the seeds of mung bean plants, e.g., total carbohydrates, protein contents, and nitrogen contents, from the current trial are given in Figure 4A–C. The results revealed that the singular or combined foliar application of IAA and GA_3 significantly improved the total carbohydrate, protein, and nitrogen contents in the yielded mung bean seeds as compared with the control (CK) plants. The highest increases in the contents of total carbohydrates (6.6% and 7.5%), protein (17.0% and 17.3%), and nitrogen (13.2% and 14.5%) were observed under the IAA_2 and $IAA_2 + GA_1$ treatments, compared to the other treatments and control plants. On the contrary, it was noticed that the lower concentration of GA_3 hormone (GA_1 treatment) was not effective in improving nutritional traits of mung bean seeds as compared to the other treatments.

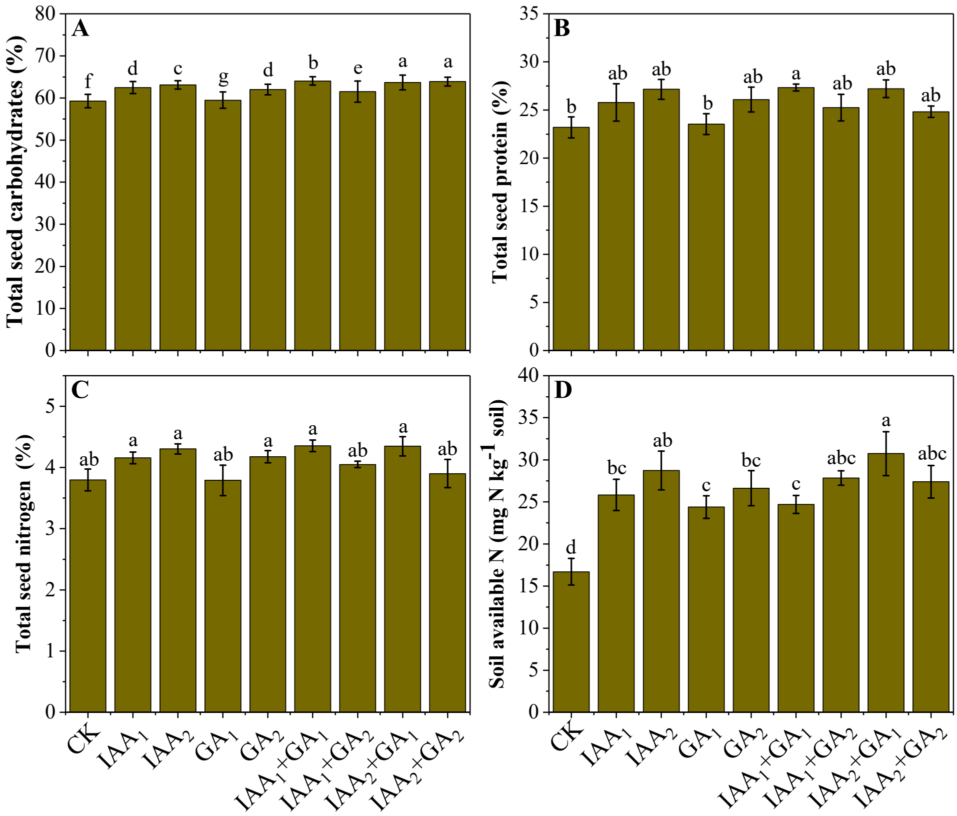

Figure 4. Influence of individual and/or combined foliar application of natural phytohormones on the nutritional status of seeds of mung bean plants, e.g., total carbohydrates (**A**), protein contents (**B**), nitrogen contents (**C**), and soil N availability (**D**) of the studied sandy-loam soil in sub-tropics. The bar values are an average of three replicates ($n = 3$), and the bars not sharing the same lowercase letters indicate significant differences from each other according to Bonferroni's statistical analysis at $p < 0.05$ level.

3.5. Influence of Natural Phytohormones on Soil N Availability

The influence of different concentrations of the spraying of IAA and GA_3, combined or alone, on soil N availability was determined after harvesting in the experiment (75 DAS), and the data are presented in Figure 4D. Overall, the availability of N in the soil was positively influenced with the foliar application of IAA and GA_3. It was observed that the IAA and GA_3 treatments caused a significant increase in N availability in sandy-loam soil. The maximum increase was reported under the IAA_2 (71.8%), $IAA_1 + GA_2$ (66.5%), and $IAA_2 + GA_1$ (83.8%) treatments.

3.6. Influence of Natural Phytohormones on Endogenous Phytohormone Production

The current study determined the changes in the endogenous IAA GA_3, zeatin, and ABA contents after the foliar application of varying concentrations of natural phytohormones on seedlings 60 days after sowing (DAS). The corresponding results are presented in Figure 5A–D. The endogenous production of IAA GA_3, zeatin, and ABA was only evaluated under the higher application doses of IAA and GA_3 based on the results obtained for growth- and yield-related characteristics. It was noticed that the exogenous applications of GA_3 and IAA were correlated with the changes in their endogenous contents of studied phytohormones in mung bean. The combined foliar application of IAA and

GA_3 (IAA_2 + GA_2) markedly increased the IAA, GA_3, and zeatin concentrations, while it declined the ABA contents in the leaves of mung bean seedlings.

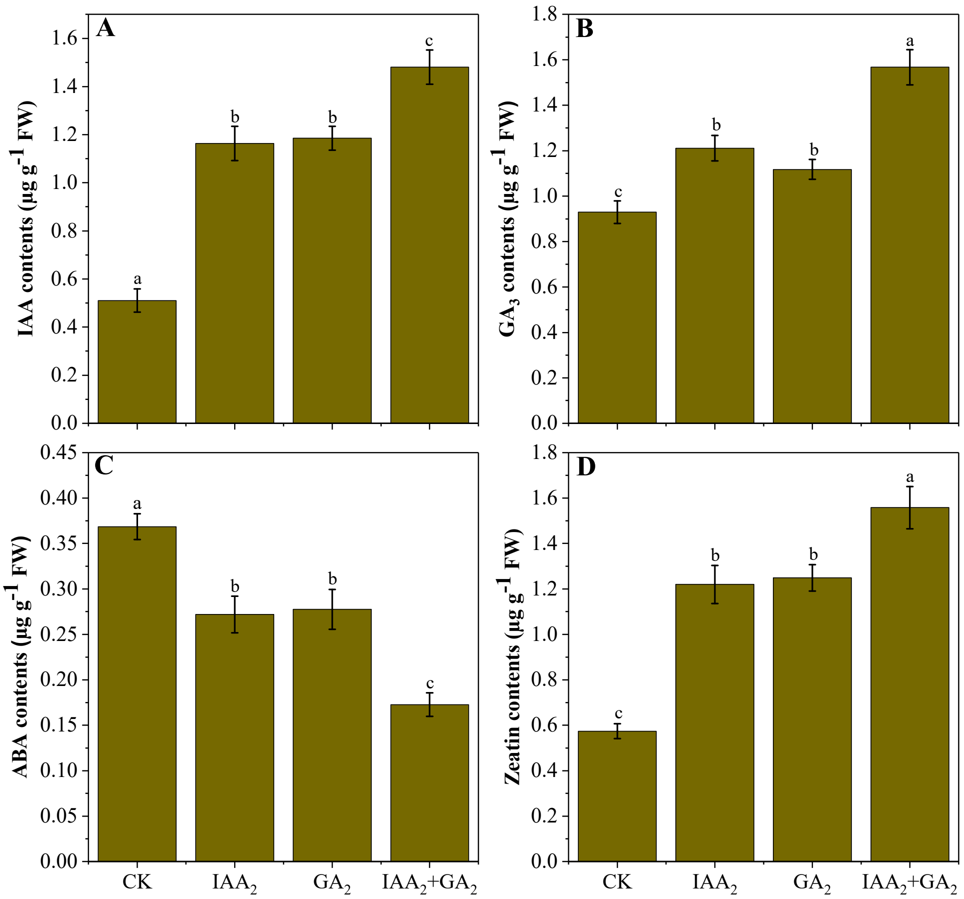

Figure 5. The influence of individual and/or combined foliar application of natural phytohormones on the endogenous concentration of phytohormones, e.g., IAA contents (**A**), GA_3 contents (**B**), ABA contents (**C**), and zeatin contents (**D**) in the leaves of mung bean plants grown in a sandy-loam soil in sub-tropics. The bar values are an average of three replicates (n = 3), and the bars not sharing the same lowercase letters indicate significant differences from each other according to Bonferroni's statistical analysis at $p < 0.05$ level.

3.7. Hierarchical Agglomerative Cluster Analysis

A two-dimensional relationship analysis was carried out to elaborate the distinction between the studied treatments and various physio-biochemical attributes of mung bean plants (Figure 6). The double hierarchical heatmap presented the correlation between the various studied traits of mung bean (row) and different singular and/or combined application levels of natural phytohormones (columns). The column hierarchical dendrogram clearly showed that the effect of IAA_2 and GA_2 and IAA_2 + GA_2 on the studied variables of mung bean seedlings under a subtropical environment was much more superior compared to all the other treatments. On the other hand, the analysis also showed that the effect of these treatments was very useful to boost the growth, yield, and biochemical attributes of mung bean seedlings.

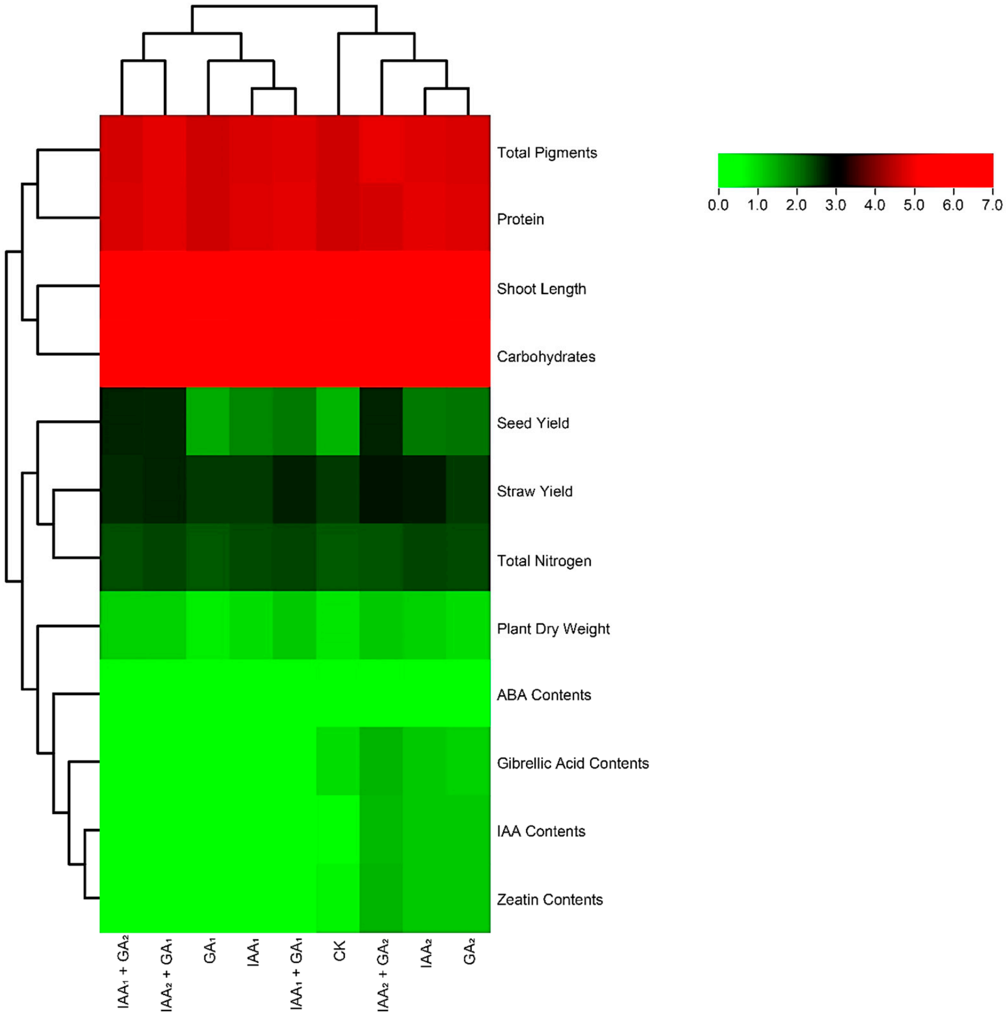

Figure 6. Heatmap analysis based on the correlation matrix of the growth and physio-biochemical variables measured in mung beans (*Vigna radiata* L.) grown in a sandy-loam soil sprayed with two natural phytohormones, viz., IAA and GA_3. The double hierarchical dendrogram reveals the relationship among treatments (column) and among various plant characteristics of mung bean seedlings (row).

4. Discussion

In a natural system, the ratio of various phytohormones is maintained to a required level by finely regulating their synthesis, transport, metabolism, and/or destruction to ensure the coordinated growth of various tissues/organs along a defined pattern of growth and development in the life span of the plant. A limited desired deviation in this set pattern of growth and development may, however, be possible by enhancing the level of any of these regulators by their exogenous application to intact plants or their parts [47]. Therefore, it has been very well established that exogenous hormonal treatment alters plant growth and development by modifying their growth, physiology, and endogenous contents [48,49]. However, it is still ambiguous as to whether the effects of exogenous hormones on growth are direct or whether they are related with changes induced in endogenous hormones [50]. With reference to the comparison of two hormones, IAA foliar application was more efficient in improving the growth attributes of mung bean than GA_3. The present increase in growth is in parallel to the earlier reported studies on various plants, including faba-bean and mung bean [51,52] with the exogenous treatment of plant growth regulators (GA_3 and IAA). Another study also supported the current findings that GA_3, IAA, and the interaction of both phytohormones significantly contributed towards plant height, the number of pods/plant, biological yield, straw yield, seed yield, total carbohydrates, protein and N contents in the seeds [35]. IAA has been recognized to increase growth and photosynthetic pigments' concentration in the leaves of plants, as shown in the current study [53], and stimulate cell division and enhance biochemical traits, i.e., total carbohydrates content and polysaccharides [54]. Furthermore, previously, an important role of GA_3 on the growth traits of the *Simmondsia chinensis* plant was observed under a sub-tropical environment [55]. In the current study, the statistical results revealed that all the treatments, including the singular and/or combined application of phytohormones, had a promotive effect on the growth and yield of mung bean and faba-bean, as reported by [53] and [54], respectively. For example, it was suggested that a combined dose of auxin (1.0 mg L^{-1}) and gibberellin (200 mg L^{-1}) is recommended for the enhancement of seed yield, whereas a 0.5 mg L^{-1} dose of auxin is recommended for the enhancement of vegetative growth. This enhancement of mung bean growth (Figure 1), photosynthetic assimilates (Figure 3), and endogenous phytohormones (Figure 5) after the spraying of plant growth regulators, alone or combined, might lead to the accretion of photo-assimilates in the seed and enhance the transfer rate of these assimilates to boost the yield of mung bean (Figure 2). Additionally, the exogenous foliar application of IAA at a 100 ppm concentration previously resulted in maximum plant height, chlorophyll content, spike length, 1000-grain weight, and grain yield in bread wheat [56]. Moreover, an increase in grain yield could be positively correlated to enhanced grain numbers per spike of two growth hormones (IAA $\times$ 6-BAP) in the booting stage of wheat [57].

Our results were in accordance with the "acid growth theory" of auxin, e.g., the indole-3-acetic acid (IAA) causes acidification (excretion of protons into the apoplast) of cell walls which ultimately increases stem growth by loosening 0f cell walls via cleavage of the bonds [47,58] and also has an impact on the functioning of ionic channels, thereby affecting the direction of the movement of ions and solutes and the turgor of cells [47]. Moreover, auxins also involve genes in inducing certain expressions by altering the type, activity, and level of the proteins [47]. This is possibly the reason for the enhanced rate of photosynthesis in the leaves of mung bean in the current study, and therefore, it may have gave a boost to the growth of the root and the shoot, as expressed in the form of increased fresh and dry mass (Figure 1). On the other hand, GA_3 has been reported to increase the pigment content of Vicia faba [59], and the water use efficiency of wheat [60], and it increased photosynthesis by increasing the carboxylase activity of Rubisco in broad bean and soybean [61] and regulated the transport of ions in plants. Additionally, it might increase water uptake in plant tissues, causing cell expansion and the dilution of sugars in tissues under harsh conditions [13]. Likewise, GA_3 tends to boost the protein content by increasing the nitrate reductase activity in cowpea [62], wheat [60], black cumin [63], and mung bean [41]. The growth-promoting effect of GA_3 may be attributed to the stimulation of the mobility of soil nutrients towards the buds, thereby increasing cell division and/or increasing the differentiation of the vascular tissues. Additionally, the increases in the yield of mung bean plants via the application of different growth hormones might result by breaking the apical dominance of mung bean plants, leading to the increase in flowering, branches, and consequently, the number of fruits. The increase in seed weights might be because of the promotive effect of IAA and/or GA_3 in increasing the assimilates and their translocations from leaves to fruits, where the seed weight increases [64].

Hormonal coordination is an important aspect which regulates leaf growth processes [22]. The current findings related to pigment formation are in line with those obtained for maize [65], wheat [66] and pulses [53,67]. Moreover, the plant growth regulator IAA presumably acts as a coenzyme in the metabolism of higher plants, which directly affects the synthesis or formation of photosynthetic pigments [68]. These increments in photosynthetic pigments may play their role in improving photosynthesis and the retardation of its degradation. It was confirmed that GA_3 is involved in the photosynthates machinery of the *Simmondsia chinensis* plant, and irrespective of the dosage of GA_3 used in the current experiment, it was shown that 300, 200 and 100 ppm of GA_3 differentially modulated the studied traits of mung bean (photosynthetic pigments: carotenoids, chlorophyll a, and chlorophyll b): yield-contributing traits such as pod length, the number of grains pod^{-1}, plant height, the number of pods plant^{-1}, and 100-grain weight; and quality parameters including seed protein and nitrogen [55]. It is important to increase protein rich crops such as mung bean in developing countries, because they can serve both as food and feed. Our findings from the current experiment are also in parallel with several scientific results that state that GA_3 improves the photosynthetic attributes in *V. radiate* [35,41], wheat [69], cotton [70], broccoli leaves [71], *Simmondsia chinensis* [55], and *Visiafaba* [13]. It has also been scientifically proven that the application of GA_3 alters the specific components of plastids that affect the retention of chlorophyll apparatus in maize [72]. Among all photosynthetic species, carotenoids act as a non-enzymatic antioxidant, protecting the "antenna complex" from photo-oxidative damage, enabling wavelengths of light to be available for photosynthesis [73]. Our findings are supported by the previous results from *V. radiata* that GA_3 treatment significantly increased leaf carotenoid contents [35]. It was then recommended that the improvement in carotenoid capacity by GA_3 may be attributed to the ultrastructural morphogenesis of plastids [74].

It has been well established that to prevent oxidative plant injury, plants have evolved adaptive mechanisms, including the upregulation of the antioxidant defense system, which includes ROS-scavenger enzymes, e.g., ascorbate peroxidase (APX), catalase (CAT), and superoxide dismutase (SOD), and non-enzymatic antioxidants such as glutathione, α-tocopherol, ascorbic acid, and phenolic compounds [75–79]. On the other hand, many researchers have explored whether the application of plant growth regulators degrade the activities of various enzymes which are directly related to improving endogenous phytohormone production [13,80]. Therefore, the increased endogenous phytohormone production in our study might have been due to the alleviation of oxidative stress and the improvement in antioxidant capacity, as suggested by various researchers [81,82]. These results are also comparable to those obtained for cowpea [70], wheat [45], and faba-bean [46]. The previous findings suggest that isopentenyl pyrophosphate is a common precursor for the biosynthesis of cytokinin and/or gibberellins and ABA [50]; apparently, the exogenous application of IAA and GA_3 may have caused a shift into cytokinin or gibberellin biosynthesis instead of ABA, which resulted in a decline in ABA content in the current research (Figure 5).

The exogenous application of natural phytohormones such as IAA and GA_3 has been shown to be an emerging trend which can positively regulate growth parameters and total carbohydrate, polysaccharide, proline, free amino acid, and total phenolic contents in faba-beans [54]. Moreover, many studies have shown that foliar and seed-priming-based applications of phytohormones have increased the availability of N in soil by enhancing nitrogenase activity in mung bean crops [83]. Higher nitrogenase activity signifies an increase in the rate of the reduction of nitrogen to ammonia. Therefore, more and more organic forms of nitrogen are made available in plants to be incorporated with keto acids to generate additional quantities of the required amino acids/amides [84]. Simultaneously, the synthesis of additional proteins (Figure 4) could have also sped up the availability of the enzymes (glutamin synthetase and glutamate synthase) involved in the glutamin synthase cycle determining the incorporation of ammonia [85]. It seems quite natural from these observations that the hormones might have elevated the useable form of nitrogen (ammonia) to produce a larger pool of amino acids/amides.

5. Conclusions

The application of natural phytohormones, e.g., IAA and GA_3, affected plant growth, and it enabled the mung bean plants to survive in a sandy-loam soil with nutrient deficiency. Moreover, these phytohormones also enhanced the yield of mung bean in two different growing seasons in 2019–2020. The most effective treatment was 60 mg L^{-1} IAA + 60 mg L^{-1} GA_3 (IAA_2 + GA_2), which improved the growth characteristics, photosynthetic pigments formation, yield attributes, endogenous phytohormone production, and biochemical composition of mung bean seeds. In the current investigation, it was therefore suggested that the mung bean performed better and gave the maximum capability of yield after the singular or combined application of high doses of IAA (60 mg L^{-1}) and GA_3 (60 mg L^{-1}) in a subtropical region of Pakistan. However, the recommended dosages, in future experiments, should be tested in various field environments in different parts of the world to confirm the potential of these two hormones.

Author Contributions: Conceptualization, M.K. and M.M.A.; methodology, A.P. and M.M.A.; software, R.I. and M.A. (Muhammad Ali); validation, M.K., M.S.E. and M.S.A.; formal analysis, A.P., M.K. and M.A. (Muhammad Ali); investigation, A.P.; resources, M.K. and M.S.E.; data curation, R.I.; writing—original draft preparation, A.P., M.M.A. and M.K.; writing—review and editing, M.S.E., M.S.A. and M.A. (Muhammad Akram); visualization, M.A. (Muhammad Akram) and M.S.A.; supervision, M.K. and M.S.E.; project administration, R.I.; funding acquisition, M.S.E. and M.K. All authors have read and agreed to the published version of the manuscript.

Funding: The authors extend their appreciation to the Researchers supporting project number (RSP2023R173), King Saud University, Riyadh, Saudi Arabia.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All the data are available upon request from the corresponding author(s).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Konuma, H. Status and Outlook of Global Food Security and the Role of Underutilized Food Resources: Sago Palm. In *Sago Palm: Multiple Contributions to Food Security and Sustainable Livelihoods*; Ehara, H., Toyoda, Y., Johnson, D., Eds.; Springer: Singapore, 2018; pp. 3–16.
2. Hilger, T.; Lewandowski, I.; Winkler, B.; Ramsperger, B.; Kageyama, P.; Colombo, C. Seeds of Change—Plant Genetic Resources and People's Livelihoods. In *Agroecology*; IntechOpen: London, UK, 2015.
3. Sehrawat, N.; Yadav, M.; Sharma, A.K.; Kumar, V.; Bhat, K.V. Salt Stress and Mungbean [*Vigna radiata* (L.) Wilczek]: Effects, Physiological Perspective and Management Practices for Alleviating Salinity. *Arch. Agron. Soil Sci.* **2019**, *65*, 1287–1301. [CrossRef]
4. Abbas, G.; Ahmed, A.; Amer, M.; Abbas, Z.; Rehman, M.; Hussain, A.; Khan, G.A. Impact of Pre-Emergence Herbicides for the Control of Weeds in Chick Pea (*Cicer arietinum* L.) under Hot Arid Climate. *J. Bioresour. Manag.* **2016**, *3*, 7.
5. Noble, T.J.; Tao, Y.; Mace, E.S.; Williams, B.; Jordan, D.R.; Douglas, C.A.; Mundree, S.G. Characterization of Linkage Disequilibrium and Population Structure in a Mungbean Diversity Panel. *Front. Plant Sci.* **2018**, *8*, 2102. [CrossRef] [PubMed]
6. Mubarak, A.E. Nutritional Composition and Antinutritional Factors of Mung Bean Seeds (*Phaseolus aureus*) as Affected by Some Home Traditional Processes. *Food Chem.* **2005**, *89*, 489–495. [CrossRef]
7. Rani, S.; Schreinemachers, P.; Kuziyev, B. Mungbean as a Catch Crop for Dryland Systems in Pakistan and Uzbekistan: A Situational Analysis. *Cogent Food Agric.* **2018**, *4*, 1499241. [CrossRef]
8. Raina, S.K.; Govindasamy, V.; Kumar, M.; Singh, A.K.; Rane, J.; Minhas, P.S. Genetic Variation in Physiological Responses of Mungbeans (*Vigna radiata* (L.) Wilczek) to Drought. *Acta Physiol. Plant.* **2016**, *38*, 263. [CrossRef]
9. FAO; WFP; IFAD; UNICEF; WHO. *The State of Food Security and Nutrition in the World 2017. Building Resilience for Peace and Food Security*; FAO: Rome, Italy, 2017.
10. Ogunkunle, A. *Management of Nigerian Soil Resources: An Imperative for Sustainable Development*; Ibadan University Press: Ibadun, Nigeria, 2016.
11. Vanzetti, D.; Petersen, E.H.; Rani, S. Economic Review of the Pulses Sector and Pulses-Related Policies in Pakistan. In Proceedings of the Mid-Project Workshop of ACIAR Project ADP/2016/140 "How Can Policy Reform Remove Constraints and Increase Productivity in Pakistan?", Islamabad, Pakistan, 3 April 2017; Volume 3.
12. Ullah, A.; Shah, T.M.; Farooq, M. Pulses Production in Pakistan: Status, Constraints and Opportunities. *Int. J. Plant Prod.* **2020**, *14*, 549–569. [CrossRef]
13. Rady, M.M.; Boriek, S.H.K.; El-Mageed, T.A.A.; El-Yazal, M.A.S.; Ali, E.F.; Hassan, F.A.S.; Abdelkhalik, A. Exogenous Gibberellic Acid or Dilute Bee Honey Boosts Drought Stress Tolerance in Vicia Faba by Rebalancing Osmoprotectants, Antioxidants, Nutrients, and Phytohormones. *Plants* **2021**, *10*, 748. [CrossRef]
14. Sadiq, R.; Maqbool, N.; Hussain, M.; Tehseen, S.; Naseer, M.; Rafique, T.; Zikrea, A.; Naqve, M.; Mahmood, A.; Javaid, A.; et al. Boosting Antioxidant Defense Mechanism of Mungbean with Foliar Application of Gibberellic Acid to Alleviate Cadmium Toxicity. *Plant Physiol. Rep.* **2021**, *26*, 741–748. [CrossRef]
15. Malik, Z.; Jamil, M.; Abassi, G.H.; Nafees, M.; Rafey, M.; Kamran, M. Biochar and Fly Ash Role in Improving Mechanical and Physical Properties of Vertisol. *Sarhad J. Agric.* **2017**, *33*, 151–161. [CrossRef]

16. Kamran, M.; Malik, Z.; Parveen, A.; Huang, L.; Riaz, M.; Bashir, S.; Mustafa, A.; Abbasi, G.H.; Xue, B.; Ali, U. Ameliorative Effects of Biochar on Rapeseed (*Brassica napus* L.) Growth and Heavy Metal Immobilization in Soil Irrigated with Untreated Wastewater. *J. Plant Growth Regul.* **2020**, *39*, 266–281. [CrossRef]
17. Kamran, M.; Huang, L.; Nie, J.; Geng, M.; Lu, Y.; Liao, Y.; Zhou, F.; Xu, Y. Effect of Reduced Mineral Fertilization (NPK) Combined with Green Manure on Aggregate Stability and Soil Organic Carbon Fractions in a Fluvo-Aquic Paddy Soil. *Soil Tillage Res.* **2021**, *211*, 105005. [CrossRef]
18. Chen, G.; Shi, L. A Multi-Element Mineral Conditioner: A Supplement of Essential Cations for Red Soil and Crops Growth. *Clean* **2016**, *44*, 1690–1699. [CrossRef]
19. Chen, G.; Shi, L. Removal of Cd(II) and Pb(II) Ions from Natural Water Using a Low-Cost Synthetic Mineral: Behavior and Mechanisms. *RSC Adv.* **2017**, *7*, 43445–43454. [CrossRef]
20. Chen, G.; Shah, K.J.; Shi, L.; Chiang, P.C.; You, Z. Red Soil Amelioration and Heavy Metal Immobilization by a Multi-Element Mineral Amendment: Performance and Mechanisms. *Environ. Pollut.* **2019**, *254*, 112964. [CrossRef] [PubMed]
21. Huang, G.; Ding, C.; Guo, N.; Ding, M.; Zhang, H.; Kamran, M.; Zhou, Z.; Zhang, T.; Wang, X. Polymer-Coated Manganese Fertilizer and Its Combination with Lime Reduces Cadmium Accumulation in Brown Rice (*Oryza sativa* L.). *J. Hazard. Mater.* **2021**, *415*, 125597. [CrossRef]
22. Iqbal, N.; Khan, N.A.; Ferrante, A.; Trivellini, A.; Francini, A.; Khan, M.I.R. Ethylene Role in Plant Growth, Development and Senescence: Interaction with Other Phytohormones. *Front. Plant Sci.* **2017**, *8*, 475. [CrossRef] [PubMed]
23. Duca, D.R.; Glick, B.R. Indole-3-Acetic Acid Biosynthesis and Its Regulation in Plant-Associated Bacteria. *Appl. Microbiol. Biotechnol.* **2020**, *104*, 8607–8619. [CrossRef]
24. Pérez-Alonso, M.M.; Ortiz-García, P.; Moya-Cuevas, J.; Lehmann, T.; Sánchez-Parra, B.; Björk, R.G.; Karim, S.; Amirjani, M.R.; Aronsson, H.; Wilkinson, M.D.; et al. Endogenous Indole-3-Acetamide Levels Contribute to the Crosstalk between Auxin and Abscisic Acid, and Trigger Plant Stress Responses in Arabidopsis. *J. Exp. Bot.* **2021**, *72*, 459–475. [CrossRef]
25. Taiz, L.; Zeiger, E. Auxin: The Growth Hormone. *Plant Physiol.* **2006**, *4*, 468–507.
26. Hedden, P. The Current Status of Research on Gibberellin Biosynthesis. *Plant Cell Physiol.* **2020**, *61*, 1832–1849. [CrossRef] [PubMed]
27. Keykha, M.; Ganjali, H.R.; Mobasser, H.R. Effect of Salicylic Acid and Gibberellic Acid on Some Characteristics in Mungbean (*Vigna radiata* L.). *Int. J. Biosci. (IJB)* **2014**, *5*, 70–75. [CrossRef]
28. Tan, H.; Man, C.; Xie, Y.; Yan, J.; Chu, J.; Huang, J. A Crucial Role of GA-Regulated Flavonol Biosynthesis in Root Growth of Arabidopsis. *Mol. Plant* **2019**, *12*, 521–537. [CrossRef]
29. Camara, M.C.; Vandenberghe, L.P.S.; Rodrigues, C.; de Oliveira, J.; Faulds, C.; Bertrand, E.; Soccol, C.R. Current Advances in Gibberellic Acid (GA_3) Production, Patented Technologies and Potential Applications. *Planta* **2018**, *248*, 1049–1062. [CrossRef]
30. Rahman, M.; Khan, A.; Hasan, M.; Banu, L.; Howlader, M. Effect of Foliar Application of Gibberellic Acid on Different Growth Contributing Characters of Mungbean. *Progress. Agric.* **2018**, *29*, 233–238. [CrossRef]
31. Saleem, M.H.; Fahad, S.; Adnan, M.; Ali, M.; Rana, M.S.; Kamran, M.; Ali, Q.; Hashem, I.A.; Bhantana, P.; Ali, M.; et al. Foliar Application of Gibberellic Acid Endorsed Phytoextraction of Copper and Alleviates Oxidative Stress in Jute (*Corchorus capsularis* L.) Plant Grown in Highly Copper-Contaminated Soil of China. *Environ. Sci. Pollut. Res.* **2020**, *27*, 37121–37133. [CrossRef]
32. Wang, Y.H.; Zhang, G.; Chen, Y.; Gao, J.; Sun, Y.R.; Sun, M.F.; Chen, J.P. Exogenous Application of Gibberellic Acid and Ascorbic Acid Improved Tolerance of Okra Seedlings to NaCl Stress. *Acta Physiol. Plant* **2019**, *41*, 93. [CrossRef]
33. Ahmad Dar, T.; Uddin, M.; Khan, M.M.A.; Ali, A.; Hashmi, N.; Idrees, M. Cumulative Effect of Gibberellic Acid and Phosphorus on Crop Productivity, Biochemical Activities and Trigonelline Production in *Trigonella foenum-graecum* L. *Cogent Food Agric.* **2015**, *1*, 995950. [CrossRef]
34. Miceli, A.; Moncada, A.; Sabatino, L.; Vetrano, F. Effect of Gibberellic Acid on Growth, Yield, and Quality of Leaf Lettuce and Rocket Grown in a Floating System. *Agronomy* **2019**, *9*, 382. [CrossRef]
35. el Karamany, M.F.; Sadak, M.S.; Bakry, B.A. Improving Quality and Quantity of Mungbean Plant via Foliar Application of Plant Growth Regulators in Sandy Soil Conditions. *Bull. Natl. Res. Cent.* **2019**, *43*, 61. [CrossRef]
36. Sanjida, T.; Alam, M.J.; Rahman, M.M.; Islam, M.S.; Sikdar, M.S.I. Response of Mungbean Growth and Yield to GA_3 Rate and Time of Application. *Asian J. Crop Soil Sci. Plant Nutr.* **2020**, *1*, 28–36. [CrossRef]
37. Ghani, M.A.; Mushtaq, A.; Ziaf, K.; Ali, B.; Jahangir, M.M.; Khan, R.W.; Khan, I.; Azam, M.; Noor, A. Exogenously Applied GA_3 Promotes Plant Growth in Onion by Reducing Oxidative Stress under Saline Conditions. *Tarim Bilim. Derg.* **2021**, *27*, 122–128. [CrossRef]
38. Ahmad, F.; Kamal, A.; Singh, A.; Ashfaque, F.; Alamri, S.; Siddiqui, M.H.; Khan, M.I.R. Seed Priming with Gibberellic Acid Induces High Salinity Tolerance in Pisum Sativum through Antioxidants, Secondary Metabolites and up-Regulation of Antiporter Genes. *Plant Biol.* **2021**, *23*, 113–121. [CrossRef] [PubMed]
39. Karamany, M.F.E.; Sadak, M.S.; Bakry, B.A. Synergistic Effect of Indole Acetic Acid and Gibberellic Acid on Mung Bean Grown under Sandy Soil Conditions. *J. Appl. Sci.* **2019**, *19*, 718–724. [CrossRef]
40. Nandan, R.; Yadav, R.; Singh, S.P.; Singh, A.K.; Singh, A. Effect of Seed Priming with Plant Growth Regulators on Growth, Biochemical Changes and Yield of Mung Bean (*Vigna radiata* L.). *Int. J. Chem. Stud.* **2021**, *9*, 2922–2927. [CrossRef]

41. Islam, M.S.; Hasan, M.K.; Islam, B.; Renu, N.A.; Hakim, M.A.; Islam, M.R.; Chowdhury, M.K.; Ueda, A.; Saneoka, H.; Ali Raza, M.; et al. Responses of Water and Pigments Status, Dry Matter Partitioning, Seed Production, and Traits of Yield and Quality to Foliar Application of GA_3 in Mungbean (*Vigna radiata* L.). *Front. Agron.* **2021**, *2*, 596850. [CrossRef]
42. Lichtenthaler, H.K. Chlorophyll and Carotenoid Determination: Pigments of Photosynthetic Biomembranes. *Method. Ensymol.* **1987**, *8*, 350–382.
43. Miller, L.; Houghton, J.A. The MicroKjeldahl Determination of the Nitrogen Content of Amino Acids and Proteins. *J. Biol. Chem.* **1945**, *169*, 373–383. [CrossRef]
44. Bradford, M.M. A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Anal. Biochem.* **1976**, *72*, 248–254. [CrossRef]
45. Shindy, W.W.; Smith, O.E. Identification of Plant Hormones from Cotton Ovules. *Plant Physiol.* **1975**, *55*, 550–554. [CrossRef]
46. Müller, P.; Hilgenberg, W. Isomers of Zeatin and Zeatin Riboside in Clubroot Tissue: Evidence for Trans-zeatin Biosynthesis by Plasmodiophora Brassicae. *Physiol. Plant* **1986**, *66*, 245–250. [CrossRef]
47. Chapman, E.J.; Estelle, M. Mechanism of Auxin-Regulated Gene Expression in Plants. *Annu. Rev. Genet.* **2009**, *43*, 265–285. [CrossRef] [PubMed]
48. Ahmad, S.; Su, W.; Kamran, M.; Ahmad, I.; Meng, X.; Wu, X.; Javed, T.; Han, Q. Foliar Application of Melatonin Delay Leaf Senescence in Maize by Improving the Antioxidant Defense System and Enhancing Photosynthetic Capacity under Semi-Arid Regions. *Protoplasma* **2020**, *257*, 1079–1092. [CrossRef] [PubMed]
49. Parveen, A.; Ahmar, S.; Kamran, M.; Malik, Z.; Ali, A.; Riaz, M.; Abbasi, G.H.; Khan, M.; Sohail, A.B.; Rizwan, M. Abscisic Acid Signalling Reduced Transpiration Flow, Regulated Na^+ Ion Homeostasis and Antioxidant Enzyme Activities to Induce Salinity Tolerance in Wheat (*Triticum aestivum* L.) Seedlings. *Environ. Technol. Innov.* **2021**, *24*, 101808. [CrossRef]
50. Szalai, G.; Horgosi, S.; Soós, V.; Majláth, I.; Balázs, E.; Janda, T. Salicylic Acid Treatment of Pea Seeds Induces Its de Novo Synthesis. *J. Plant Physiol.* **2011**, *168*, 213–219. [CrossRef] [PubMed]
51. Choudhury, S.; Islam, N.; Ali, M. Growth and Yield of Summer Tomato as Influenced by Plant Growth Regulators. *Int. J. Sustain. Agric.* **2013**, *5*, 25–28. [CrossRef]
52. Hossain, M.E.; Amin, R.; Sani, M.N.H.; Ahamed, K.U.; Hosain, M.T.; Nizam, R. Impact of Exogenous Application of Plant Growth Regulators on Growth and Yield Contributing Attributes of Summer Tomato. *Int. J. Plant Soil Sci.* **2018**, *24*, 1–14. [CrossRef]
53. Naeem, M.; Bhatti, I.; Ahmad, R.H.; Ashraf, M.Y. Effect of Some Growth Hormones (GA_3, IAA and Kinetin) on the Morphology and Early or Delayed Initiation of Bud of Lentil (*Lens culinaris Medik*). *Pak. J. Bot.* **2004**, *36*, 801–809.
54. Sadak, M.S.; Dawood, M.G.; Bakry, B.A.; El-Karamany, M.F. Synergistic Effect of Indole Acetic Acid and Kinetin on Performance, Some Biochemical Constituents and Yield of Faba Bean Plant Grown under Newly Reclaimed Sandy Soil. *World J. Agric. Sci.* **2013**, *9*, 335–344.
55. Atteya, A.K.G.; Genaidy, E.A.E.; Zahran, H.A. Chemical Constituents and Yield of Simmondsia Chinensis Plants as Affected by Foliar Application of Gibberellic Acid and Zinc Sulphate. *Biosci. Res.* **2018**, *15*, 1528–1541.
56. Hanaa, H.; Safaa, A. Foliar Application Foliar Application of IAA at Different Growth Stages and Their Influenced on Growth and Productivity of Bread Wheat (*Triticum aestivum* L.). *J. Phys. Conf. Ser.* **2019**, *1294*, 092029. [CrossRef]
57. Jalali-Honarmand, S.; Rasaei, A.; Saeidi, M.; Ghobadi, M.E.; Khanizadeh, S. The Effects of Foliar Application of Plant Hormones at Booting Stage Non Wheat Yield Components. *Thai J. Agric. Sci.* **2015**, *48*, 35–38.
58. Cleland, R.E. Auxin-Induced Hydrogen Ion Excretion: Correlation with Growth, and Control by External PH and Water Stress. *Planta* **1975**, *127*, 233–242. [CrossRef]
59. Aldesuquy, H.S.; Gaber, A.M. Effect of Growth Regulators on Vicia Faba Plants Irrigated by Sea Water Leaf Area, Pigment Content and Photosynthetic Activity. *Biol. Plant* **1993**, *35*, 519–527. [CrossRef]
60. Aldesuquy, H.S.; Ibrahim, A.H. Interactive Effect of Seawater and Growth Bioregulators on Water Relations, Abscisic Acid Concentration and Yield of Wheat Plants. *J. Agron. Crop Sci.* **2001**, *187*, 185–193. [CrossRef]
61. Yuan, L.; Xu, D.Q. Stimulation Effect of Gibberellic Acid Short-Term Treatment on Leaf Photosynthesis Related to the Increase in Rubisco Content in Broad Bean and Soybean. *Photosynth. Res.* **2001**, *68*, 39–47. [CrossRef] [PubMed]
62. Miri, M.R.; Ghooshchi, F.; Tohidi-Moghadam, H.R.; Larijani, H.R.; Kasraie, P. Ameliorative Effects of Foliar Spray of Glycine Betaine and Gibberellic Acid on Cowpea (*Vigna unguiculata* L. Walp.) Yield Affected by Drought Stress. *Arab. J. Geosci.* **2021**, *14*, 830. [CrossRef]
63. Azadi, F.; Hatami, A.; Salek Mearaji, H. The Effect of Cytokinin Foliar on Morpho-Physiological Traits, Yield and Yield Components of Black Cumin (*Nigella sativa* L.) under Salinity Stress Conditions. *Environ. Stress. Crop Sci.* **2022**, *15*, 975–990.
64. Mostafa, H.A.M.; El-Bassiouny, H.M.S.; Khattab, H.K.I.; Sadak, M.S. Improving the Characteristics of Roselle Seeds as a New Source of Protein and Lipid by Gibberellin and Benzyladenine Application. *J. Appl. Sci. Res.* **2005**, *1*, 161–167.
65. Parida, A.K.; Dagaonkar, V.S.; Phalak, M.S.; Umalkar, G.V.; Aurangabadkar, L.P. Alterations in Photosynthetic Pigments, Protein and Osmotic Components in Cotton Genotypes Subjected to Short-Term Drought Stress Followed by Recovery. *Plant Biotechnol. Rep.* **2007**, *1*, 37–48. [CrossRef]
66. Farhat, F.; Arfan, M.; Wang, X.; Tariq, A.; Kamran, M.; Tabassum, H.N.; Tariq, I.; Mora-Poblete, F.; Iqbal, R.; El-Sabrout, A.M.; et al. The Impact of Bio-Stimulants on Cd-Stressed Wheat (*Triticum aestivum* L.): Insights Into Growth, Chlorophyll Fluorescence, Cd Accumulation, and Osmolyte Regulation. *Front. Plant Sci.* **2022**, *13*, 850567. [CrossRef]

67. Rashid, N.; Khan, S.; Wahid, A.; Ibrar, D.; Hasnain, Z.; Irshad, S.; Bashir, S.; Al-Hashimi, A.; Elshikh, M.S.; Kamran, M. Exogenous Application of Biostimulants and Synthetic Growth Promoters Improved the Productivity and Grain Quality of Quinoa Linked with Enhanced Photosynthetic Pigments and Metabolomics. *Agronomy* **2021**, *11*, 2302. [CrossRef]
68. Mir, A.R.; Siddiqui, H.; Alam, P.; Hayat, S. Foliar Spray of Auxin/IAA Modulates Photosynthesis, Elemental Composition, ROS Localization and Antioxidant Machinery to Promote Growth of Brassica Juncea. *Physiol. Mol. Biol. Plants* **2020**, *26*, 2503–2520. [CrossRef]
69. Pazuki, A.; Sedghi, M.; Aflaki, F. Interaction of Salinity and Phytohormones on Wheat Photosynthetic Traits and Membrane Stability. *Agriculture/Pol'nohospodárstvo* **2013**, *59*, 33–41. [CrossRef]
70. da Costa, W.A.; Ribeiro, V.T.; da Silva, D.C.; Neto, A.; Wanderley Neto, A.d.O.; de Castro Dantas, T.N.; Ferrari, M.; dos Santos, E.S. Low-Energy Nanoemulsified Systems Containing Antioxidant Eutectic Extract from Rhodotorula Mucilaginosa Yeast Cells. *Colloids Surf. A Physicochem. Eng. Asp.* **2022**, *651*, 129715. [CrossRef]
71. Majeed, A.; Kaleem Abbasi, M.; Hameed, S.; Imran, A.; Rahim, N. Isolation and Characterization of Plant Growth-Promoting Rhizobacteria from Wheat Rhizosphere and Their Effect on Plant Growth Promotion. *Front. Microbiol.* **2015**, *6*, 198. [CrossRef] [PubMed]
72. Ashour, N.I.; Neumann, D.; Nieden, U. zur Gibberellic Acid Induced Changes in the Ultrastructure of Chloroplasts and the Content of Chlorophyll in Leaves of Dwarf Maize (*Zea mays* L.). *Biochem. Physiol. Der Pflanz.* **1973**, *164*, 402–413. [CrossRef]
73. Vichnevetskaia, K.D.; Roy, D.N. Oxidative Stress and Antioxidative Defense with an Emphasis on Plants Antioxidants. *Environ. Rev.* **1999**, *7*, 31–51. [CrossRef]
74. Arteca, R.N. *Plant Growth Substances: Principles and Applications*; Springer Science & Business Media: Berlin/Heidelberg, Germany, 2013; ISBN 1475724519.
75. Sabir, F.; Noreen, S.; Malik, Z.; Kamran, M.; Riaz, M.; Dawood, M.; Parveen, A.; Afzal, S.; Ahmad, I.; Ali, M. Silicon Improves Salinity Tolerance in Crop Plants: Insights into Photosynthesis, Defense System, and Production of Phytohormones. In *Silicon and Nano-Silicon in Environmental Stress Management and Crop Quality Improvement*; Elsevier: Amsterdam, The Netherlands, 2022; pp. 91–103.
76. Malik, Z.; Afzal, S.; Dawood, M.; Abbasi, G.H.; Khan, M.I.; Kamran, M.; Zhran, M.; Hayat, M.T.; Aslam, M.N.; Rafay, M. Exogenous Melatonin Mitigates Chromium Toxicity in Maize Seedlings by Modulating Antioxidant System and Suppresses Chromium Uptake and Oxidative Stress. *Environ. Geochem. Health* **2021**, *44*, 1451–1469. [CrossRef]
77. Parveen, A.; Hamzah Saleem, M.; Kamran, M.; Zulqurnain Haider, M.; Chen, J.T.; Malik, Z.; Shoaib Rana, M.; Hassan, A.; Hur, G.; Tariq Javed, M.; et al. Effect of Citric Acid on Growth, Ecophysiology, Chloroplast Ultrastructure, and Phytoremediation Potential of Jute (*Corchorus capsularis* L.) Seedlings Exposed to Copper Stress. *Biomolecules* **2020**, *10*, 592. [CrossRef]
78. Kamran, M.; Danish, M.; Saleem, M.H.; Malik, Z.; Parveen, A.; Abbasi, G.H.; Jamil, M.; Ali, S.; Afzal, S.; Riaz, M.; et al. Application of Abscisic Acid and 6-Benzylaminopurine Modulated Morpho-Physiological and Antioxidative Defense Responses of Tomato (*Solanum lycopersicum* L.) by Minimizing Cobalt Uptake. *Chemosphere* **2021**, *263*, 128169. [CrossRef] [PubMed]
79. Ali, M.; Kamran, M.; Abbasi, G.H.; Saleem, M.H.; Ahmad, S.; Parveen, A.; Malik, Z.; Afzal, S.; Ahmar, S.; Dawar, K.M. Melatonin-Induced Salinity Tolerance by Ameliorating Osmotic and Oxidative Stress in the Seedlings of Two Tomato (*Solanum lycopersicum* L.) Cultivars. *J. Plant Growth Regul.* **2020**, *40*, 2236–2248. [CrossRef]
80. Hashem, M.; Abo-Elyousr, K.A. Management of the Root-Knot Nematode Meloidogyne Incognita on Tomato with Combinations of Different Biocontrol Organisms. *Crop Prot.* **2011**, *30*, 285–292. [CrossRef]
81. Moravcová, Š.; Tůma, J.; Dučaiová, Z.K.; Waligórski, P.; Kula, M.; Saja, D.; Słomka, A.; Bąba, W.; Libik-Konieczny, M. Influence of Salicylic Acid Pretreatment on Seeds Germination and Some Defence Mechanisms of *Zea mays* L. Plants under Copper Stress. *Plant Physiol. Biochem.* **2018**, *122*, 19–30. [CrossRef] [PubMed]
82. Kim, S.K.; Son, T.K.; Park, S.Y.; Lee, I.J.; Lee, B.H.; Kim, H.Y.; Lee, S.C. Influences of Gibberellin and Auxin on Endogenous Plant Hormone and Starch Mobilization during Rice Seed Germination under Salt Stress. *J. Environ. Biol.* **2006**, *27*, 181.
83. Ali, B.; Hayat, S.; Aiman Hasan, S.; Ahmad, A. A Comparative Effect of IAA and 4-Cl-IAA on Growth, Nodulation and Nitrogen Fixation in *Vigna radiata* (L.) Wilczek. *Acta Physiol. Plant* **2008**, *30*, 35–41. [CrossRef]
84. Beevers, L. *Nitrogen Metabolism in Plants*; Springer: Berlin/Heidelberg, Germany, 1981; ISBN 3642679218.
85. Hopkins G, W.; A, H.N.P. *Introduction to Plant Physiology*, 4th ed.; John Wiley & Sons: Hoboken, NJ, USA, 2009.