sustainability

MDPI

Article

Identifying the Role of Biostimulants in Turnip (*Brassica rapa* L.) Production Compared with Chemical Fertilization

Qurat-Ul-Ain Raza [1,†], Muhammad Amjad Bashir [1,*,†], Abdur Rehim [1,*], Yucong Geng [2,*], Hafiz Muhammad Ali Raza [1,3], Sajjad Hussain [4], Ijaz Ahmad [5] and Muhammad Wasif [3]

1 Department of Soil Science, Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan 60800, Pakistan
2 School of History, Geography and Tourism, Chengdu Normal University, Chengdu 610000, China
3 Department of Soil Science, College of Agriculture, University of Layyah, Layyah 31200, Pakistan
4 Department of Horticulture, Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan 60800, Pakistan
5 Department of Agronomy, Engro Fertilizers Limited, Lahore 54000, Pakistan
* Correspondence: amjad.bashir941@gmail.com (M.A.B.); abdur.rehim@bzu.edu.pk (A.R.); tiger86gyc@gmail.com (Y.G.)
† These authors contributed equally to this work.

Abstract: Chemical fertilizers play an integral role in advancing food production to combat the increasing food challenges and ensure food security. Unfortunately, the overuse of these fertilizers has negatively influenced the soil and the environment. Considering this aspect, two pot experiments were performed to evaluate the efficacy of biostimulants in vegetable production systems. The first experiment compared the effects of chemical fertilizers (CF) with glycine (GL), aspartic acid (AA), lysine (LY), and vitamin B complex (VB). The plant's physiological and morphological attributes and yield were studied. The results confirmed that VB has the potential to improve the rate of transpiration (26%), total chlorophyll content (27%), root diameter (213%), and dry matter (289%) compared with CF. In the second experiment, the effects of chemical fertilizers (CF) were compared with Isabion® (I), 25% CF + GL + LY (B1), 25% CF + GL + AA (B2), and 25% CF + AA + LY (B3). Similar attributes were analyzed to identify the influence of the applied treatments on turnip production. The results demonstrated that B2 enhanced the rate of photosynthesis (963%), transpiration (254%), and stomatal conductance (76%). Moreover, B1 improved the plant's fresh weight (6%) and moisture contents (4%) compared to CF. In conclusion, biostimulants (LY, VB, and B1) are capable of improving turnip performance and production compared to CF. Future studies must focus on the efficiency of biostimulants against the long-term application effects on soils, nutrient-use efficiency, and crop production. Furthermore, the mechanism of action needs to be addressed in the future.

Keywords: amino acids; leaf extract; vitamins; lysine; foliar application

Citation: Raza, Q.-U.-A.; Bashir, M.A.; Rehim, A.; Geng, Y.; Raza, H.M.A.; Hussain, S.; Ahmad, I.; Wasif, M. Identifying the Role of Biostimulants in Turnip (*Brassica rapa* L.) Production Compared with Chemical Fertilization. *Sustainability* **2023**, *15*, 11851. https://doi.org/10.3390/su151511851

Academic Editor: Luigi Ledda

Received: 28 June 2023
Revised: 21 July 2023
Accepted: 27 July 2023
Published: 1 August 2023

1. Introduction

Vegetables are a good source of biologically active substances [1], calories, and nutrients; thus, they are highly recommended for humans' daily diet. Plant products are a major part of the human diet and provide dietary fiber, minerals, and phytochemicals that are beneficial for human health. Studies reported that a vegetable-rich diet helps maintain bone strength, blood pressure, heart and mental health, and hormonal production, in addition to providing numerous other health benefits. Moreover, vegetables can also be used to fight against undernutrition, hunger, and poverty and grow the economy even if grown and consumed locally [2]. With the increasing world population, it has been estimated that approximately 9.7 billion people will need to be fed by 2050 [3]. During the last two decades (1997–2017), vegetable production has doubled (from 0.55 to 1.09 billion); therefore, it is essential to provide favorable soil and environmental conditions to fulfill the demands for vegetables in the future [4,5]. The turnip (*Brassica rapa*) is a vital root vegetable and forage crop. It is indigenous to Asia, Europe, Russia, and the Near East and is now widely cultivated throughout the globe as both a vegetable and oil source [6]. In Pakistan, the turnip was cultivated on 9609 hectares of land with 167,065 tons of production in 2020–2021, making it an important vegetable locally [7]. Furthermore, it has also been reported to have various medicinal benefits, including acting as a therapeutic agent against kidney and liver diseases and other ailments. It has also been discovered that turnip has antimicrobial, anti-inflammatory, antitumor, cardio-protective, antidiabetic, analgesic, and nephron-protective properties and aids against metabolic syndrome and obesity [8]. Moreover, it improves growth in response to heavy fertilization [9], which is also a cause of non-point pollution.

Modern agriculture is highly dependent on fertilizers' application for achieving higher crop productivity and is a crucial tool to target food safety challenges. Chemical fertilizers greatly contribute to improving crop productivity and soil nutrient pools. However, the troublesome impacts of high-dose chemical fertilization cannot be ignored in environmental deterioration. The excessive use of chemical fertilizers has adversely affected the soil eco-profile; altered the soil's biological and physicochemical properties; declined soil organic matter; reduced soil fertility; disturbed biodiversity; and polluted the air, water, and soil, respectively [10]. In addition, the excessive use of nitrogenous fertilizers has increased the concentration of nitrates in many vegetables, becoming a significant source of nitrate for humans [11]. The scientific community and agricultural sectors face a massive challenge to enhance crop productivity to feed the growing population worldwide and reduce the hazardous impacts on the environment and human health [12].

Various technologies have been introduced to boost vegetables' growth and minimize the toxic agriculture inputs, i.e., fertilizers and pesticides [13–15]. One of the emerging technologies to proliferate sustainable agriculture production is the use of biostimulants [16]. According to the new European Union regulation (EU; 2019/1009), biostimulants are a product that has the potential to improve the plant's nutritional processes and achieve the following properties to improve nutrient uptake: tolerance against biotic/abiotic stresses, enhanced quality traits, and ability to contribute to nutrients' availability in the rhizosphere [17]. Biostimulants are a mixture of organic and inorganic materials that can improve a crop's growth, productivity, quality, and nutrient uptake. Biostimulants cannot replace fertilizers because they are not nutrients but effectively reduce the requirement for fertilizers and overcome nutrient deficiency [18]. The global market of biostimulants reached USD 2.5 billion in 2019 and is one of the fastest-growing agriculture-associated industries, ultimately reducing the chemical fertilizers market [19].

The beneficial responses of biostimulants regarding improved crop yield have been reported in recent years. Combining zinc–lysine chelates and zinc-solubilizing bacteria significantly improved maize (*Zea mays* L.) yield and grain biofortification [20]. Moreover, growing plants hydroponically improved the pigment content in leaves and positively influenced yellow pepper (*Capsicum annum* L.) yield with biostimulants [21]. Without chemical fertilizers, radish (*Raphanus sativus* L.) root and shoot biomass increased with the integrated use of biostimulants under greenhouse conditions [18]. It is also reported that strawberry (*Fragaria* × *ananassa*) plants can benefit from quality and yield traits with biostimulants under insufficient nutrients [22]. Tomato *Solanum lycopersicum* L. plants also responded positively to exogenously applied amino-acids-based biostimulants, which promoted plant-growth processes [23]. Vitamin B was also reported to improve plants' oxidative stress, ultimately enhancing yield [24] and increasing root growth [18]. Thus, various biostimulants have been reported to improve yield in numerous crops, including the bean (*Phaseolus vulgaris* L.), with an increased number of pods (26%) [25]; Lavandin (*Lavandula* × *intermedia*), with improved fresh weight (47%) and dry weight (38%); white radish, with enhanced fresh weight (478%) [3]; and red radish, with increased root biomass (65%) [18]. However, the impact on the nutritional and functional properties of edible parts might differ depending upon the species, dose, properties of biostimulants, and time of application [8].

The need for this study arises from the necessity to find sustainable solutions for turnip cultivation under stress-less conditions and find an eco-friendly approach to conventional agricultural practices, especially over-fertilization. This study aimed to identify the use of biostimulants for the performance of turnips and improve their performance compared with chemical fertilization. With this aim, two pot experiments were performed with sole and combined application of biostimulants. Our study hypothesized that applying biostimulants would help minimize the use of chemical fertilizers. Prior to the extent of the authors' knowledge, experiments evaluating the effect of exogenously applied biostimulants on turnip growth with reduced fertilization in stress-less environments have not been reported yet. Moreover, various alternatives to chemical fertilization have been reported, but the use of biostimulants and their combinations under normal soil, environmental, and climatic conditions to support turnip growth and its impacts on plant physiology in the absence or limitation of chemical fertilizers endows it with the distinction of being a novel study. Hence, the objectives are to (i) evaluate the influences of biostimulants on turnip growth, photosynthetic activity, and yield, (ii) study the effects of biostimulants in the absence/presence of chemical fertilizers, and (iii) reduce the application of chemical fertilizers to minimize its negative impacts on the environment.

2. Materials and Methods

This study was organized into two pot experiments to evaluate the response of turnips with the application of biostimulants.

2.1. Experimental Details

The first experiment was performed at the experimental station of the Department of Soil Science (30.258° E, 71.515° N), FAS&T, Bahauddin Zakariya University (BZU) Multan, Pakistan. Multan is located in a semi-arid region with hot summers and mild winters. The maximum temperature during the growing season was 36 °C, and the minimum temperature was 7 °C. Multan is the pronounced part of Bari Doab, Punjab plains, and its elevation is 114 to 135 m above sea level, geographically located in the center of Pakistan [26]. This study was conducted during the growing season of 2021 (September–December).

The second study was performed at the experimental area of the College of Agriculture, BZU, Bahadur Sub-campus Layyah (30.97° E, 70.96° N) Pakistan. Layyah City lies in a desert climate with hot days, cold nights, and little annual rainfall. The average annual temperature is 25 °C in Layyah, and during the experimental season, it ranged from 6–36 °C. The trial was conducted from October to January 2021.

2.1.1. Experiment 1

The pots were lined using plastic sheets, and 20 kg of soil was added. The physicochemical properties of the soil used in this experiment are given in Table 1. This study was focused on five treatments with three replicates and was organized in a completely randomized design (CRD). The following treatments were tested:

- Chemical fertilizer (CF; N 62 kg ha^{-1}, P 49 kg ha^{-1}, K 62 kg ha^{-1});
- Glycine (GL; 2.47 g pot^{-1});
- Lysine (LY; 4.79 g pot^{-1});
- Aspartic Acid (AA; 4.37 g pot^{-1});
- Vitamin B complex (VB; 0.46 g pot^{-1}).

The treatments' doses were kept the same according to nitrogen (N) requirements (62 kg ha^{-1}) of turnip. In the case of CF, urea, di-ammonium phosphate (DAP), and sulphate of potash (SOP) were used as a source of N, phosphorus (P), and potassium (K). Chemical fertilizers were not applied to any treatment except CF. Turnip seeds were soaked in water overnight before sowing, and five seeds (*cultivar*; Purple top white globe) per pot were sown on 24 September 2021. Later, two plants per pot were maintained after thinning. Once the seedlings were established, four foliar applications of each treatment were made at ten days intervals on 15 October, 25 October, 5 November, and 15 November 2021. The crop was harvested at maturity on 9 December 2021. Plants were irrigated according to the requirement by maintaining the field capacity level, and weeds were eradicated manually. Plant root and shoot samples were collected at maturity and used to calculate the yield, physiology, and morphological attributes.

Table 1. Physio-chemical properties of soil.

Soil Properties	Experiment 1	Experiment 2
Soil pH	7.7	7.8
Electrical conductivity (dS·m^{-1})	0.5	0.1
Organic matter (%)	0.4	0.7
Saturation (%)	34	28
Texture (USDA System)	Loam	Sandy Loam
Soil total N (mg kg^{-1})	221	450
Soil extractable P (mg kg^{-1})	8.2	7.1
Soil extractable K (mg kg^{-1})	30.1	62.34

2.1.2. Experiment 2

Soil (8 kg) was filled in plastic sheet-lined pots. The soil physicochemical characteristics in this experiment are given in Table 1. Five treatments with three replicates included in the experiment are as follows:

- Recommended chemical fertilizer (62 kg ha^{-1} N, 49 kg ha^{-1} P, K 62 kg ha^{-1} K) (CF);
- Isabion® (contains 100 g L^{-1} amino acids; 1 L ha^{-1}) (I);
- Treatment with 25% CF + Glycine (5 g L^{-1}) + Lysine (1 g L^{-1}) (B1);
- Treatment with 25% CF + Glycine (5 g L^{-1}) + Aspartic acid (2 g L^{-1}) (B2);
- Treatment with 25% CF + Lysine (1 g L^{-1}) + Aspartic acid (2 g L^{-1}) (B3).

Treatment application methods and practices were identical to the first experiment. Five seeds were sown on 21 October 2021 in each pot. Later two healthy plants per pot were later maintained, and the smaller ones were removed. Foliar applications of treatments were carried out on 16 November, 26 November, 6 December, and 16 December 2021. Irrigation was applied according to the crop need, while weed eradication was conducted manually. Turnip roots and shoots were harvested at maturity on 13 January 2022, and all the samples were collected, washed, and used for the required analyses. GL, LY, and AA were purchased from Sigma Aldrich (St. Louis, MO, USA), while VB was purchased from Martin Dow Market Ltd. (Karachi, Pakistan).

2.2. Laboratory Analysis

Before harvesting, two healthy leaves from each replication were selected, and plant photosynthetic parameters, i.e., photosynthetic and transpiration rates, internal CO_2 concentrations, and stomatal conductance were measured in the morning (between 08:30 and 9:30 a.m.) using an infrared gas analyzer (IRGA; Analytical Development Company, Hoddesdon, UK). Photosynthetic pigments, including chlorophyll, were estimated using the colorimetric method. One healthy leaf from each replication was collected, cut into small pieces, dipped in ethanol, and stored in the dark for 24 h for chlorophyll extraction. The spectrophotometer (UV 1600 Shimadzu, Kyoto, Japan) was used to determine chlorophyll a and b contents at 665 and 649 nm wavelengths. Analysis was performed in two replicates to minimize the error [27]. Plant fresh weight was determined immediately after harvesting, while the plants were oven dried at 65 °C until a constant weight was achieved. Plant fresh and dry weights were recorded using a weighing balance. Moreover, turnip root diameter was determined using digital Vernier calipers.

2.3. Statistical Analysis

The one-way analysis of variance (ANOVA) was carried out to analyze the significance of data using Statistix 9. The Least Significance Difference (LSD) at $p \leq 0.05$ was used for pairwise comparison. R studio (2022.12.0) and Microsoft Excel (2016) were the critical software for data processing and graphs preparation.

3. Results

3.1. Experiment 1

The results demonstrated that biostimulants are potentially important in sustainable agriculture (Figure 1). A significant improvement in photosynthetic rate with the use of AA (165%), followed by LY (164%), VB (158%), and GL (128%), compared to CF, was observed (Figure 1A). Transpiration was also increased significantly with VB (26%) and AA (12%) (Figure 1B), whereas stomatal conductance and internal CO_2 were reduced with biostimulants compared to CF (Figure 1C,D). In addition, the photosynthetic pigments (chlorophyll a + b) were significantly improved with the application of VB (26.95%), followed by AA (18%), LY (14%), and GL (5%) compared to CF (Figure 2A). CF significantly enhanced moisture content, followed by GL, LY, AA, and VB (Figure 2B).

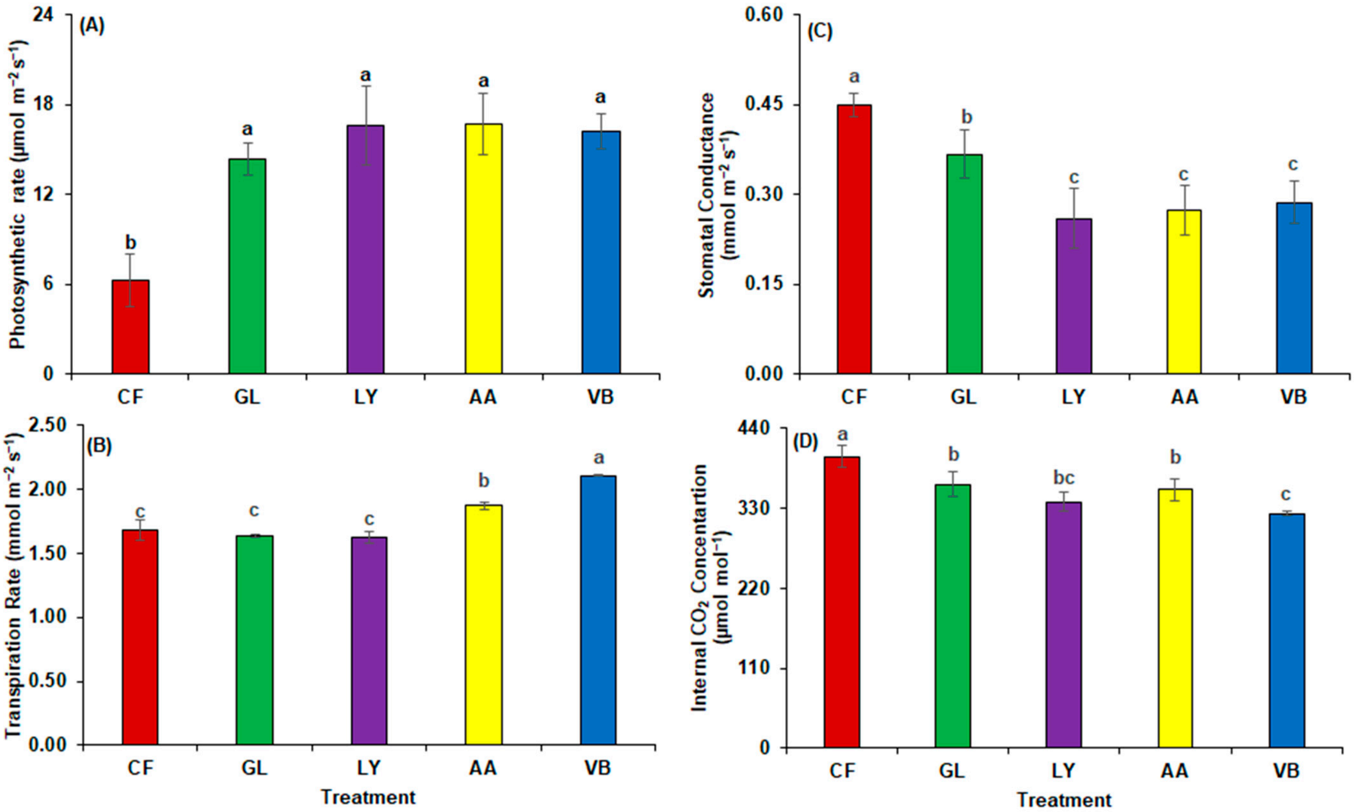

Figure 1. Effect of biostimulants on (**A**) photosynthetic rate, (**B**) transpiration rate, (**C**) stomatal conductance, and (**D**) internal CO_2 concentration, where CF is chemical fertilizer, GL is glycine, LY is lysine, AA is aspartic acid, and VB is vitamin B complex. The data sets are presented as means $\pm$ SD (n = 3). Columns that share the same letter(s) are not statistically significant at $p \leq 0.05$.

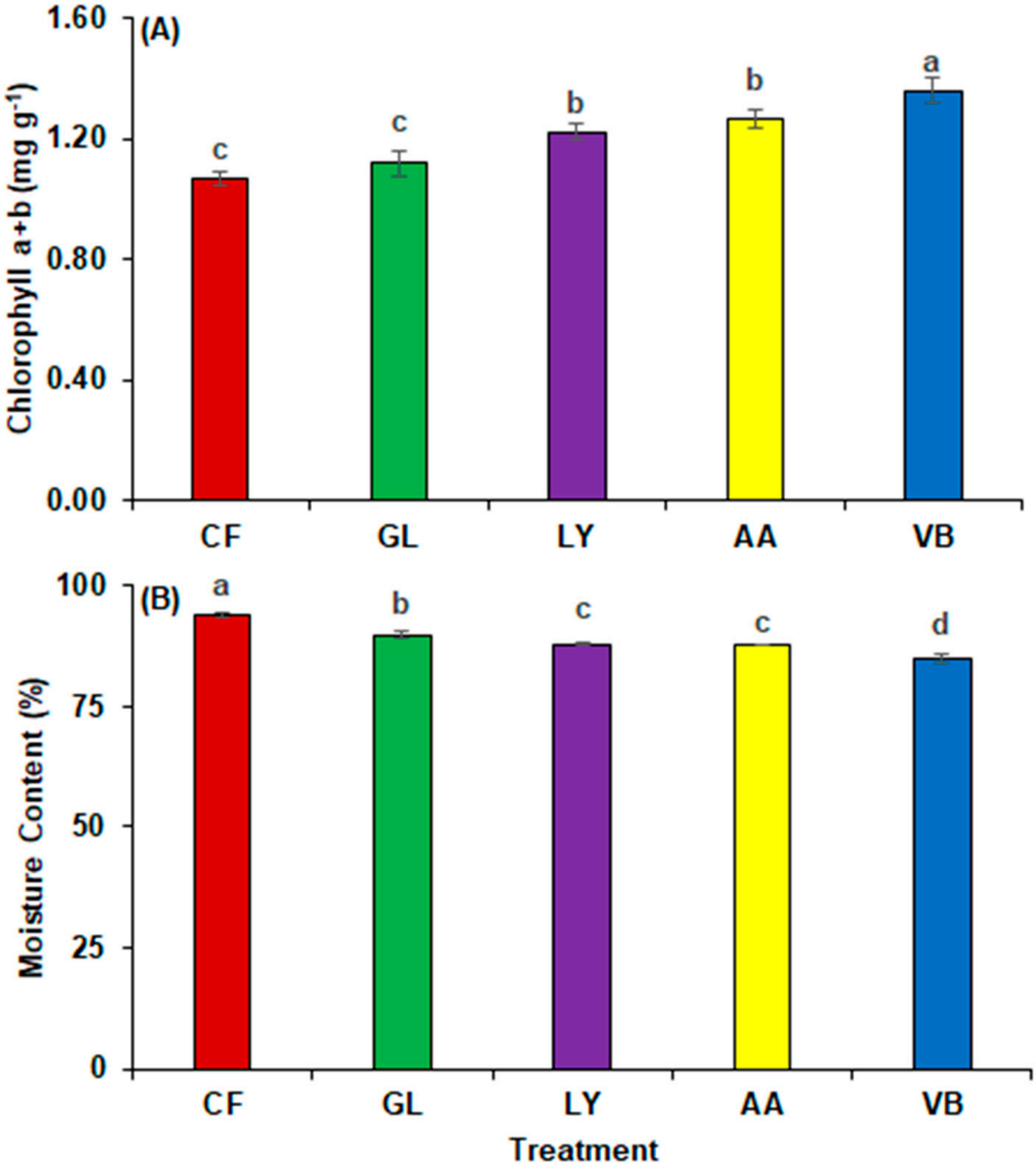

Figure 2. Pigment and moisture content analysis: (**A**) photosynthetic pigments and (**B**) moisture content. CF is chemical fertilizer, GL is glycine, LY is lysine, AA is aspartic acid, and VB is vitamin B complex. The data sets are presented as means± SD (n = 3). Columns that share the same letter(s) are not statistically significant at $p \leq 0.05$.

The plant fresh weight (shoot + root) metric was significantly improved using biostimulants (Figure 3A). LY improved the fresh weight (82%), followed by AA (72%), VB (54%), and GL (51%) in comparison to CF. An increase in plant dry weight was observed with the use of VB (289%), LY (271%), AA (257%), and GL (160%), respectively (Figure 3B). The root diameter metric was increased in VB (213%), AA (185%), GL (164%), and LY (60%) compared with CF (Figure 3C).

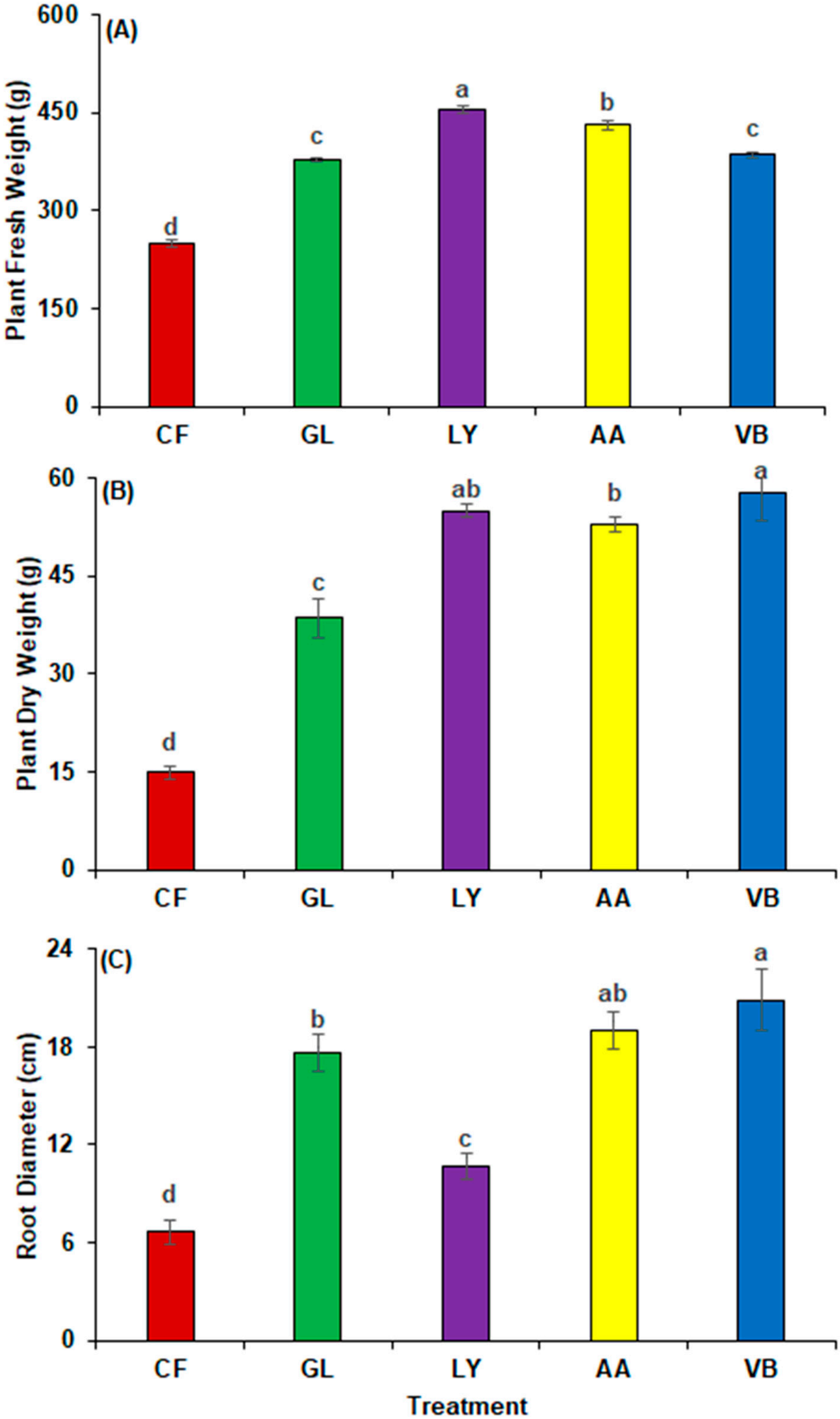

Figure 3. Plant biomass and morphology: (**A**) plant fresh weight, (**B**) plant dry weight, (**C**) root diameter. CF is chemical fertilizer, GL is glycine, LY is lysine, AA is aspartic acid, and VB is vitamin B complex. The data sets are presented as means± SD (n = 3). Columns that share the same letter(s) are not statistically significant at $p \leq 0.05$.

3.2. Experiment 2

The integrated application of biostimulants and chemical fertilizers significantly improved the photosynthetic rates in turnip plants (Figure 4A). Compared to CF, the photosynthetic rate was highest in B2 (963%), followed by B3 (491%), B1 (100%), and I (21%), respectively (Figure 5A). Transpiration was also increased by 254%, 234%, 188%, and 169% in B2, B3, B1, and I, respectively (Figure 4B). Stomatal conductance also experienced significant benefits with the use of B2 (76%), followed by B3 (58%), I (40%), and B1 (29%), respectively (Figure 4C). Internal CO_2 concentration declined as CF (341 µmol mol^{-1}) and I (346 µmol mol^{-1}) showed the highest values (Figure 4D).

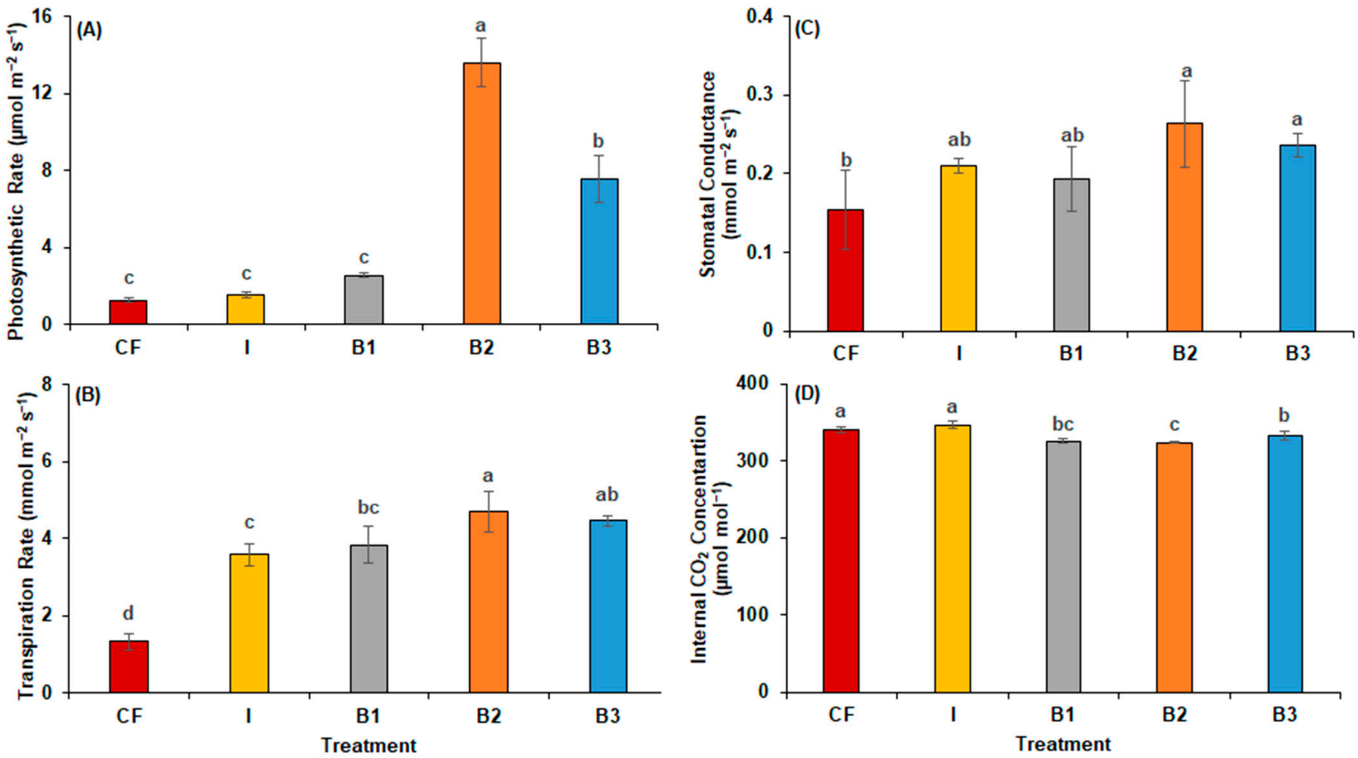

Figure 4. Effect of biostimulants on (**A**) photosynthetic rate, (**B**) transpiration rate, (**C**) stomatal conductance, and (**D**) internal CO_2 concentration, where CF is chemical fertilizer, I is Isabion, B1 is 25% CF + glycine + lysine, B2 is 25% CF + glycine + aspartic acid, and B3 is 25% CF + aspartic acid + lysine. The data sets are presented as means± SD (n = 3). Columns that share the same letter(s) are not statistically significant at $p \leq 0.05$.

Photosynthetic pigments (chlorophyll a + b) were improved with the application of I (98%), B2 (83%), B3 (75%), and B1 (73%), respectively, compared to CF (Figure 5A). Moisture contents were increased with the application of B1, I, and B3 by 4%, 3%, and 2% compared to CF (Figure 5B). Plant fresh weight (shoot + root) was increased in B1 (6%), while the other treatments, I (67.00 g), B2 (50.00 g), and B3 (47.67 g), showed negative results as compared to CF (102.67 g), respectively (Figure 6A). Moreover, dry weight was highest in CF, followed by B1, B2, B3, and I (Figure 6B). In addition, CF and B1 improved the root diameter, while the other treatments, I (2.88 cm), B2 (3.31 cm), and B3 (3.05 cm), showed negative results compared to CF (3.79 cm), respectively (Figure 6C).

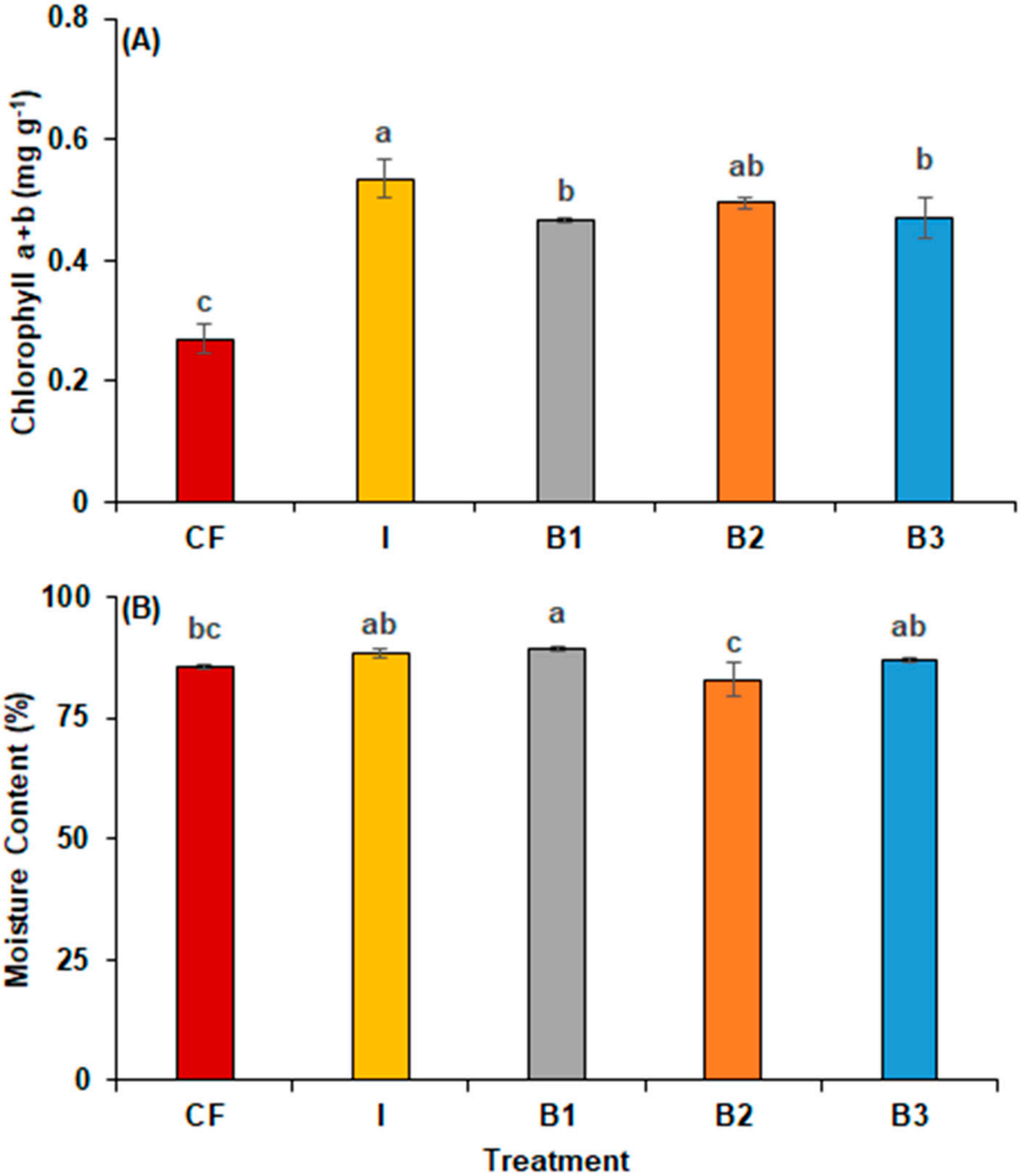

Figure 5. Pigment and moisture content analysis: (**A**) photosynthetic pigments and (**B**) moisture content. CF is chemical fertilizer, I is Isabion, B1 is 25% CF + glycine + lysine, B2 is 25% CF + glycine + aspartic acid, and B3 is 25% CF + aspartic acid + lysine. The data sets are presented as means± SD (n = 3). Columns that share the same letter(s) are not statistically significant at $p \leq 0.05$.

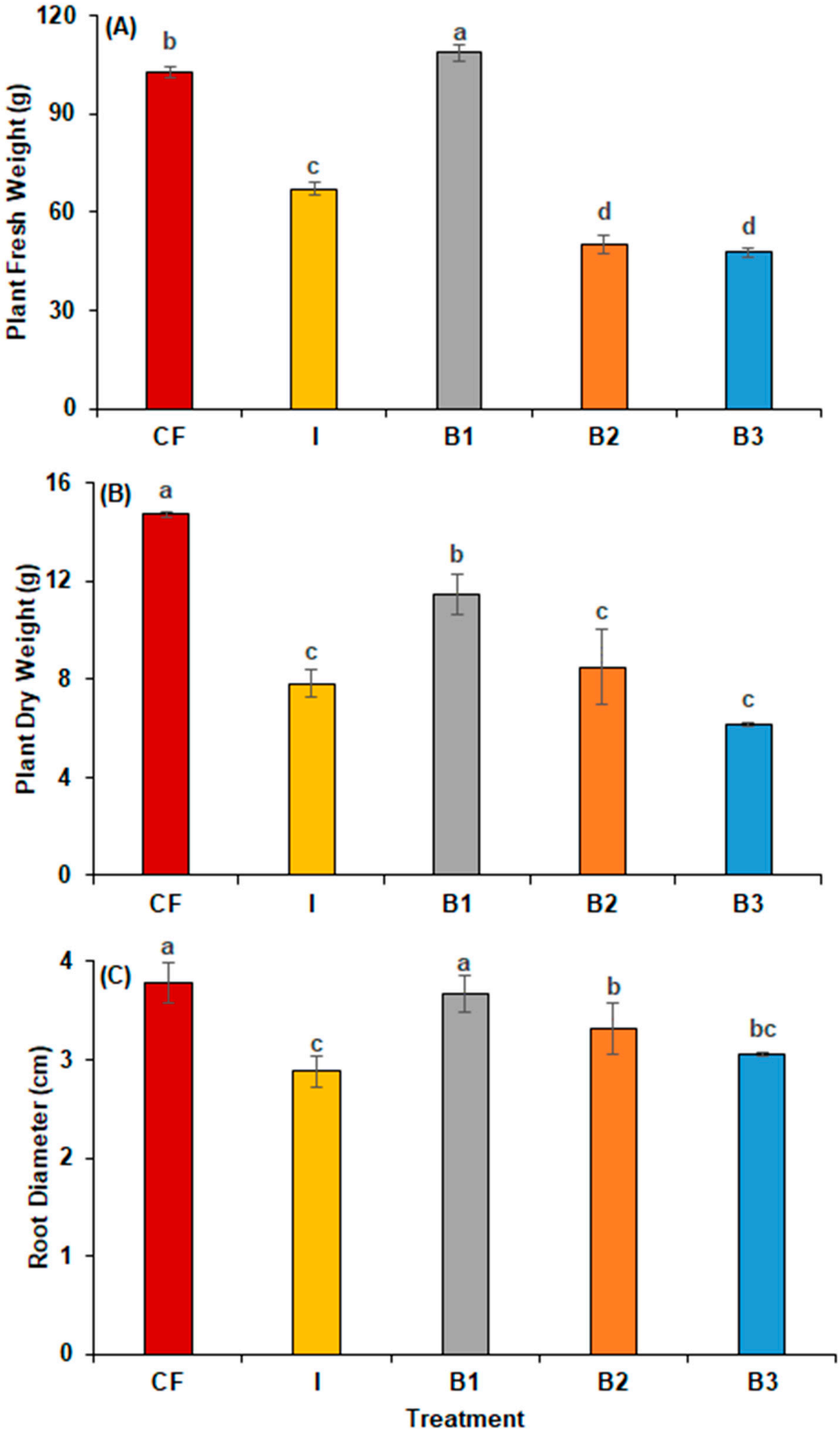

Figure 6. Plant biomass and morphology: (**A**) plant fresh weight, (**B**) plant dry weight, and (**C**) root diameter. CF is chemical fertilizer, I is Isabion, B1 is 25% CF + glycine + lysine, B2 is 25% CF + glycine + aspartic acid, and B3 is 25% CF + aspartic acid + lysine. The data sets are presented as means± SD ($n = 3$). Columns that share the same letter(s) are not statistically significant at $p \leq 0.05$.

3.3. Pearson Correlation

The results demonstrated a moderately positive correlation of photosynthetic rate with stomatal conductance, fresh and dry weight, chlorophyll content, and root diameter. At the same time, it has a weak negative association with internal CO_2 assimilation, transpiration, and moisture content. The transpiration rate strongly correlates negatively with fresh weight, dry weight, and root diameter. It negatively relates to chlorophyll content, internal CO_2 assimilation, and weak correlation with stomatal conductance and moisture content. Stomatal conductance was positively correlated with internal CO_2 assimilation, plant fresh and dry weight, root diameter, and chlorophyll content, while it showed a positive weak relation with moisture content. Internal CO_2 assimilation is positively associated with fresh and dry weight, root diameter, chlorophyll content, and moisture content. A strong positive influence of chlorophyll content on plant biomass and morphology was observed, while moderate positive relation with moisture content was observed. A strong positive association was observed between fresh and dry weight and root diameter, while a slightly positive correlation with moisture content. In addition, moisture content also presented a weak positive correlation with root diameter (Figure 7).

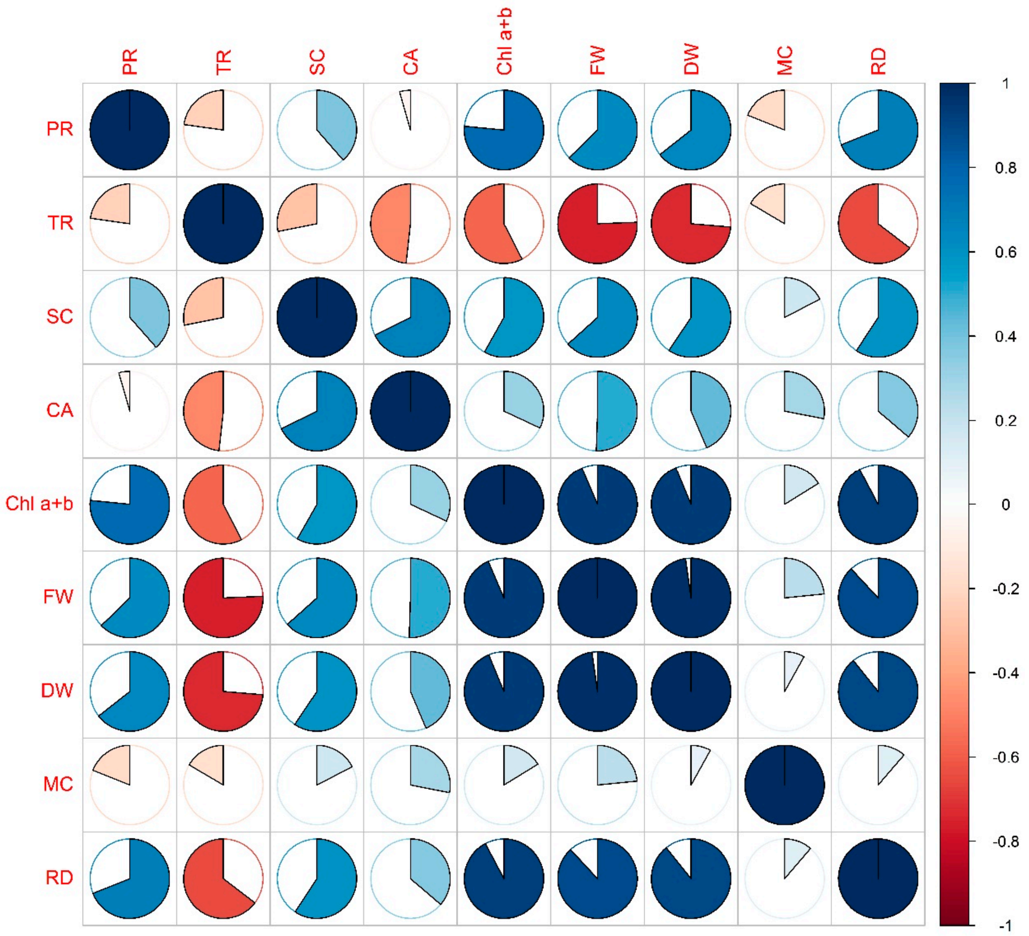

Figure 7. Pearson correlation: PR is photosynthetic rate, TR is transpiration rate, SC is stomatal conductance, CA is internal CO_2 concentration, Chl a + b is chlorophyll content, FW is plant fresh weight, DW is plant dry weight, MC is moisture content, and RD is root diameter.

4. Discussion

Biostimulants can improve crop productivity and decrease the use of chemical fertilizers. Our studies revealed that the foliar application of biostimulants significantly improved crop nutrition, growth, and productivity. In experiment 1, biostimulants improved plants' photosynthesis (Gl, Ly, AA, and VB) and transpiration rates (AA and VB), possibly via the composition of biostimulants and their role in plant mechanisms. Vitamins are significant in cellular metabolism and participate in more than 140 biochemical reactions. It also contributes to the biosynthesis of amino acids, fatty acids, plant hormones, organelle-specific compounds, and neurotransmitters. Furthermore, it is involved in the breakdown of storage compounds, reactive oxygen species scavenging, and chlorophyll synthesis and ultimately benefit photosynthesis [28]. In addition, experiment 2 showed that B1 improved photosynthesis in plants. This shows that the combination of GL and AA helps improve plant physiology. It might be due to amino acids that play a role in primary and secondary plant metabolic processes. It not only impacts plant physiological processes, plant vegetative growth, fruit maturity, seed germination, defense against a/biotic stresses, and reactive oxygen species but also acts as a reverse source of N [29].

Plants are capable of producing amino acids and vitamins, but energy is required for the processes; therefore, the foliar application of such biostimulants aids plants in improving their growth and development [30]. The results revealed that biostimulants improved turnip growth and physiological activities. In both experiments, the turnip growth and yield were significantly enhanced with the foliar feeding of biostimulants. It could be related to improved photosynthetic efficiency, cell enlargement, chlorophyll formation, and participation in the formation of growth hormones, including auxin, gibberellin, and/or cytokine [18]. In experiment 1, LY, AA, and VB improved the chlorophyll contents, whereas all combinations were helpful in enhancing photosynthetic pigments in the second experiment. The substances in biostimulants might be effective against chlorophyll deterioration and benefit the biosynthesis of the thylakoid membrane, thus resulting in improved photosynthesis [12]. In addition, chlorophyll a is directly involved in photosynthesis, whereas chlorophyll b allows plants to absorb light at a broader range of wavelengths, enhances cell metabolism, and involves in the synthesis of plant secondary metabolites [31].

A similar case was observed in both experiments. The combined use of amino acids-based biostimulants significantly improved the plant biomass and root diameter. It might be associated with improved fertilizer assimilation, water and nutrient uptake, and plant photosynthesis [32]. Amino acids also act as chelating agents [30] and contribute to improving nutrient uptake and nutrient use efficiency for macro- and micro-nutrients [33]. Additionally, the plants promptly absorb amino acids, become a stable source of molecule precursors, and contribute to plant metabolic activities [34]. Vitamins also play a significant role in root growth and development, act as an antioxidant against osmotic stress, and involve protein biosynthesis [24].

Amino acids also contribute to improving nutrient uptake and nutrient use efficiency for macro- and micro-nutrients [33]. Various published material reported that the use of amino acids and vitamin-based biostimulants improved radish root and shoot biomass [18], sweet pepper yield [35], physiological and nutritional responses in tomato [29], energy value in pea seeds [36], physiology and leaf properties of the sweet cherry tree [37], and cucumber growth and yield [38]. Moreover, commercial amino acid-based biostimulants have shown improved yield even with the half dose of N fertilizer [33]. It can be associated with stimulating hormone activity, nitrogen and carbon metabolism enzymes, plant biochemical (sugar synthesis, antioxidant enzyme production), and physiological attributes [39].

Lysine plays numerous roles in plant growth and development; it has been reported to induce the jasmonate signaling pathway, energy metabolism, serotonin accumulation, systemic immunity, and multiple roles in plant metabolism [40]. Glycine also helps plants to improve cell membrane stability and protection against peroxidation [41]. It has also been reported that glycine can stabilize the oxygen-evolving activity of the photosystem II complex, maintain cell membrane integrity and its components, and synthesize ATP [42]. Amino acids play a key role in N metabolism by regulating nitrate and ammonium uptake, nitrate reduction, ammonium incorporation, nitrogen remobilization, and protein synthesis and help plant grow even in nutrient-less conditions [41].

The excessive use of chemical fertilizers has adversely affected the soil environment, and this ongoing practice will reduce the soil's ability to serve humanity in the long run with quality food products [43]. Using biostimulants to boost nutrient uptake and plant growth can be helpful in reducing the rates, ultimately minimizing the detrimental impacts of chemical fertilizers on soil and the environment. Our studies reveal that biostimulants can improve crop yield, physiology, and productivity. Moreover, the combined use of biostimulants (B1 with a 75% reduction in fertilizer) can achieve outcomes similar to chemical fertilizers. The impacts of biostimulants on crop biochemistry and quality need to be addressed. Moreover, other application methods, such as root or soil application, could be another aspect to study. The scientific community also needs to testify to the long-term impacts and the response of various crops in field conditions to serve the farming community better.

5. Conclusions

The Green Revolution was a practical approach to food and nutrition security. Moreover, it alleviated extreme poverty and malnourishment. But the excessive use of chemical fertilizers deteriorated the soil and environment. In this scenario, biostimulants can be an alternative method for sustainable production and minimize fertilizer applications to overcome agricultural non-point pollution. The foliar application of biostimulants is a feasible strategy to implement. However, its beneficial impacts depend on multiple aspects, including the dose and time of application and the crop species. Our studies conclude that biostimulants can improve the plant biomass, gaseous exchange attributes, root morphology, and chlorophyll contents significantly in turnip. Moreover, its impacts on other food crops, its doses, and its time of application might differ from our reported results. Our study confirms that the foliar application of biostimulants can improve turnip crop physiology and yield and confirms our hypothesis that biostimulants can reduce the use of chemical fertilizers. The potential benefits of our research state that small-scale farmers and kitchen gardeners can implement biostimulants and grow turnips with less use of fertilization. Furthermore, the use of biostimulants in field conditions and their response on a larger scale needs to be addressed. Its mechanism of action is still a research gap that scientists can identify with the latest analytical techniques and bioinformatics in the future. In addition, biostimulants are economical because they improve the yield, whereas our study lacks sufficient investigation and requires further examination.

Author Contributions: Conceptualization, M.A.B., A.R. and Y.G.; Data curation, M.A.B., Y.G., S.H. and I.A.; Formal analysis, Q.-U.-A.R. and M.W.; Funding acquisition, Y.G.; Investigation, Q.-U.-A.R. and M.W.; Methodology, Q.-U.-A.R., I.A. and H.M.A.R.; Resources, A.R., S.H. and H.M.A.R.; Software, M.A.B.; Supervision, A.R.; Writing—original draft, Q.-U.-A.R.; Writing—review and editing, M.A.B., A.R., S.H., I.A., M.W., H.M.A.R. and Y.G. All authors have read and agreed to the published version of the manuscript.

Funding: The support was received from the Chengdu Normal University youth doctor training program fund.

Data Availability Statement: All the required data have been added to the manuscript in the form of figures and table. Figure data can be taken from the corresponding author upon request.

Acknowledgments: This study was supported by Higher Education Commission (HEC) Pakistan under the indigenous Ph.D. 5000 fellowship program. Qurat-Ul-Ain Raza (520(PH-II) 2AV6-075/HEC/IS/2020) acknowledges HEC for providing financial support to fulfill her degree requirements at BZU.

Conflicts of Interest: The authors declare that they have no competing interest that could have appeared to influence the work reported in this article.

References

1. Bursać Kovačević, D.; Brdar, D.; Fabečić, P.; Barba, F.J.; Lorenzo, J.M.; Putnik, P. Strategies to Achieve a Healthy and Balanced Diet: Fruits and Vegetables as a Natural Source of Bioactive Compounds. In *Agri-Food Industry Strategies for Healthy Diets and Sustainability: New Challenges in Nutrition and Public Health*; Academic Press: Cambridge, MA, USA, 2020; pp. 51–88. ISBN 9780128172261.

2. Buturi, C.V.; Mauro, R.P.; Fogliano, V.; Leonardi, C.; Giuffrida, F. Mineral Biofortification of Vegetables as a Tool to Improve Human Diet. *Foods* **2021**, *10*, 223. [CrossRef]
3. Raza, Q.-U.-A.; Bashir, M.A.; Rehim, A.; Ejaz, R.; Raza, H.M.A.; Shahzad, U.; Ahmed, F.; Geng, Y. Biostimulants Induce Positive Changes in the Radish Morpho-Physiology and Yield. *Front. Plant Sci.* **2022**, *13*, 2475. [CrossRef] [PubMed]
4. Dong, J.; Gruda, N.; Li, X.; Tang, Y.; Zhang, P.; Duan, Z. Sustainable Vegetable Production under Changing Climate: The Impact of Elevated CO2 on Yield of Vegetables and the Interactions with Environments—A Review. *J. Clean. Prod.* **2020**, *253*, 119920. [CrossRef]
5. Magama, P.; Chiyanzu, I.; Mulopo, J. A Systematic Review of Sustainable Fruit and Vegetable Waste Recycling Alternatives and Possibilities for Anaerobic Biorefinery. *Bioresour. Technol. Rep.* **2022**, *18*, 101031. [CrossRef]
6. Sun, R. Economic/Academic Importance of *Brassica rapa*. In *The Brassica rapa Genome*; Springer: Berlin/Heidelberg, Germany, 2015; pp. 1–15. ISBN 9783662479018.
7. Government of Pakistan, Ministry of National Food Security & Research Economic Wing Islamabad. 2021. Available online: http://www.mnfsr.gov.pk/Detail/NzJlNWE2N2MtNzY5Ni00ZWUwLWFkNTItZjk4MmM4MWY1MWE1 (accessed on 12 May 2023).
8. Paul, S.; Geng, C.A.; Yang, T.H.; Yang, Y.P.; Chen, J.J. Phytochemical and Health-Beneficial Progress of Turnip (*Brassica rapa*). *J. Food Sci.* **2019**, *84*, 19–30. [CrossRef]
9. Nawaz, M.Q.; Ahmed, K.; Qadir, G.; Rizwan, M.; Nawaz, M.F.; Sarfraz, M. Growth and Yield of Turnip (*Brassica rapa* L.) in Response to Different Sowing Methods and Nitrogen Levels in Salt-Affected Soils. *Pakistan J. Agric. Res.* **2020**, *33*, 126–134. [CrossRef]
10. Pahalvi, H.N.; Rafiya, L.; Rashid, S.; Nisar, B.; Kamili, A.N. Chemical Fertilizers and Their Impact on Soil Health. In *Microbiota and Biofertilizers*; Springer: Cham, Switzerland, 2021; Volume 2, pp. 1–20.
11. Salehzadeh, H.; Maleki, A.; Rezaee, R.; Shahmoradi, B.; Ponnet, K. The Nitrate Content of Fresh and Cooked Vegetables and Their Health-Related Risks. *PLoS ONE* **2020**, *15*, e0227551. [CrossRef]
12. Rouphael, Y.; Colla, G. Editorial: Biostimulants in Agriculture. *Front. Plant Sci.* **2020**, *11*, 40. [CrossRef] [PubMed]
13. Jamal, A.; Saeed, M.F.; Mihoub, A.; Hopkins, B.G.; Ahmad, I.; Naeem, A. Integrated Use of Phosphorus Fertilizer and Farmyard Manure Improves Wheat Productivity by Improving Soil Quality and P Availability in Calcareous Soil under Subhumid Conditions. *Front. Plant Sci.* **2023**, *14*, 1034421. [CrossRef]
14. Khan, I.; Amanullah; Jamal, A.; Mihoub, A.; Farooq, O.; Farhan Saeed, M.; Roberto, M.; Radicetti, E.; Zia, A.; Azam, M. Partial Substitution of Chemical Fertilizers with Organic Supplements Increased Wheat Productivity and Profitability under Limited and Assured Irrigation Regimes. *Agriculture* **2022**, *12*, 1754. [CrossRef]
15. Geng, Y.; Bashir, M.A.; Zhao, Y.; Luo, J.; Liu, X.; Li, F.; Wang, H.; Raza, Q.U.A.; Rehim, A.; Zhang, X.; et al. Long-Term Fertilizer Reduction in Greenhouse Tomato-Cucumber Rotation System to Assess N Utilization, Leaching, and Cost Efficiency. *Sustainability* **2022**, *14*, 4647. [CrossRef]
16. Hamid, B.; Zaman, M.; Farooq, S.; Fatima, S.; Sayyed, R.Z.; Baba, Z.A.; Sheikh, T.A.; Reddy, M.S.; Enshasy, H.E.; Gafur, A.; et al. Bacterial Plant Biostimulants: A Sustainable Way towards Improving Growth, Productivity, and Health of Crops. *Sustainability* **2021**, *13*, 2856. [CrossRef]
17. Bashir, M.A.; Rehim, A.; Raza, Q.-U.-A.; Muhammad Ali Raza, H.; Zhai, L.; Liu, H.; Wang, H. Biostimulants as Plant Growth Stimulators in Modernized Agriculture and Environmental Sustainability. In *Technology in Agriculture [Working Title]*; IntechOpen: London, UK, 2021; ISBN 978-1-83881-922-4.
18. Rehim, A.; Amjad Bashir, M.; Raza, Q.-U.-A.; Gallagher, K.; Berlyn, G.P. Yield Enhancement of Biostimulants, Vitamin B12, and CoQ10 Compared to Inorganic Fertilizer in Radish. *Agronomy* **2021**, *11*, 697. [CrossRef]
19. Rašovský, M.; Pačuta, V.; Ducsay, L.; Lenická, D. Quantity and Quality Changes in Sugar Beet (*Beta vulgaris* Provar. Altissima Doel) Induced by Different Sources of Biostimulants. *Plants* **2022**, *11*, 2222. [CrossRef]
20. Bashir, S.; Basit, A.; Abbas, R.N.; Naeem, S.; Bashir, S.; Ahmed, N.; Ahmed, M.S.; Ilyas, M.Z.; Aslam, Z.; Alotaibi, S.S.; et al. Combined Application of Zinc-Lysine Chelate and Zinc-Solubilizing Bacteria Improves Yield and Grain Biofortification of Maize (*Zea mays* L.). *PLoS ONE* **2021**, *16*, e0254647. [CrossRef]
21. Paradiković, N.; Vinković, T.; Vinković Vrček, I.; Žuntar, I.; Bojić, M.; Medić-Šarić, M. Effect of Natural Biostimulants on Yield and Nutritional Quality: An Example of Sweet Yellow Pepper (*Capsicum annuum* L.) Plants. *J. Sci. Food Agric.* **2011**, *91*, 2146–2152. [CrossRef] [PubMed]
22. Soppelsa, S.; Kelderer, M.; Casera, C.; Bassi, M.; Robatscher, P.; Matteazzi, A.; Andreotti, C. Foliar Applications of Biostimulants Promote Growth, Yield and Fruit Quality of Strawberry Plants Grown under Nutrient Limitation. *Agronomy* **2019**, *9*, 483. [CrossRef]
23. Alfosea-Simón, M.; Simón-Grao, S.; Zavala-Gonzalez, E.A.; Cámara-Zapata, J.M.; Simón, I.; Martínez-Nicolás, J.J.; Lidón, V.; Rodríguez-Ortega, W.M.; García-Sánchez, F. Application of Biostimulants Containing Amino Acids to Tomatoes Could Favor Sustainable Cultivation: Implications for Tyrosine, Lysine, and Methionine. *Sustainability* **2020**, *12*, 9729. [CrossRef]
24. Asensi-Fabado, M.A.; Munné-Bosch, S. Vitamins in Plants: Occurrence, Biosynthesis and Antioxidant Function. *Trends Plant Sci.* **2010**, *15*, 582–592. [CrossRef]
25. Kocira, A.; Lamorska, J.; Kornas, R.; Nowosad, N.; Tomaszewska, M.; Leszczyńska, D.; Kozłowicz, K.; Tabor, S. Changes in Biochemistry and Yield in Response to Biostimulants Applied in Bean (*Phaseolus vulgaris* L.). *Agronomy* **2020**, *10*, 189. [CrossRef]

26. Abbas, M.; Atangana Njock, P.G.; Wang, Y. Influence of Climate Change and Land-Use Alteration on Water Resources in Multan, Pakistan. *Appl. Sci.* **2022**, *12*, 5210. [CrossRef]
27. Yuan, Z.; Cao, Q.; Zhang, K.; Ata-Ul-Karim, S.T.; Tan, Y.; Zhu, Y.; Cao, W.; Liu, X. Optimal Leaf Positions for SPAD Meter Measurement in Rice. *Front. Plant Sci.* **2016**, *7*, 719. [CrossRef]
28. Parra, M.; Stahl, S.; Hellmann, H. Vitamin B6 and Its Role in Cell Metabolism and Physiology. *Cells* **2018**, *7*, 84. [CrossRef]
29. Alfosea-Simón, M.; Simón-Grao, S.; Zavala-Gonzalez, E.A.; Cámara-Zapata, J.M.; Simón, I.; Martínez-Nicolás, J.J.; Lidón, V.; García-Sánchez, F. Physiological, Nutritional and Metabolomic Responses of Tomato Plants After the Foliar Application of Amino Acids Aspartic Acid, Glutamic Acid and Alanine. *Front. Plant Sci.* **2021**, *11*, 2138. [CrossRef] [PubMed]
30. Popko, M.; Michalak, I.; Wilk, R.; Gramza, M.; Chojnacka, K.; Górecki, H. Effect of the New Plant Growth Biostimulants Based on Amino Acids on Yield and Grain Quality of Winter Wheat. *Molecules* **2018**, *23*, 470. [CrossRef]
31. Toscano, S.; Romano, D.; Patanè, C. Effect of Application of Biostimulants on the Biomass, Nitrate, Pigments, and Antioxidants Content in Radish and Turnip Microgreens. *Agronomy* **2023**, *13*, 145. [CrossRef]
32. Haghighi, M.; Saadat, S.; Abbey, L. Effect of Exogenous Amino Acids Application on Growth and Nutritional Value of Cabbage under Drought Stress. *Sci. Hortic.* **2020**, *272*, 109561. [CrossRef]
33. Halpern, M.; Bar-Tal, A.; Ofek, M.; Minz, D.; Muller, T.; Yermiyahu, U. The Use of Biostimulants for Enhancing Nutrient Uptake. *Adv. Agron.* **2015**, *130*, 141–174. [CrossRef]
34. Rouphael, Y.; Colla, G. Toward a Sustainable Agriculture through Plant Biostimulants: From Experimental Data to Practical Applications. *Agronomy* **2020**, *10*, 1461. [CrossRef]
35. Rodrigues, M.; Baptistella, J.L.C.; Horz, D.C.; Bortolato, L.M.; Mazzafera, P. Organic Plant Biostimulants and Fruit Quality—A Review. *Agronomy* **2020**, *10*, 988. [CrossRef]
36. Biel, W.; Podsiadło, C.; Witkowicz, R.; Kępińska-Pacelik, J.; Stankowski, S. Effect of Irrigation, Nitrogen Fertilization and Amino Acid Biostimulant on Proximate Composition and Energy Value of *Pisum sativum* L. Seeds. *Agriculture* **2023**, *13*, 376. [CrossRef]
37. Serapicos, M.; Afonso, S.; Gonçalves, B.; Silva, A.P. Exogenous Application of Glycine Betaine on Sweet Cherry Tree (*Prunus avium* L.): Effects on Tree Physiology and Leaf Properties. *Plants* **2022**, *11*, 3470. [CrossRef] [PubMed]
38. Zargar Shooshtari, F.; Souri, M.K.; Hasandokht, M.R.; Jari, S.K. Glycine Mitigates Fertilizer Requirements of Agricultural Crops: Case Study with Cucumber as a High Fertilizer Demanding Crop. *Chem. Biol. Technol. Agric.* **2020**, *7*, 19. [CrossRef]
39. Al-Karaki, G.N.; Othman, Y. Effect of Foliar Application of Amino Acids Biostimulants on Growth, Macronutrient, Total Phenols Contents and Antioxidant Activity of Soilless Grown Lettuce Cultivars. *S. Afr. J. Bot.* **2023**, *154*, 225–231. [CrossRef]
40. Yang, Q.; Zhao, D.; Zhang, C.; Sreenivasulu, N.; Sun, S.S.M.; Liu, Q. Lysine Biofortification of Crops to Promote Sustained Human Health in the 21st Century. *J. Exp. Bot.* **2022**, *73*, 1258–1267. [CrossRef]
41. Mohammadipour, N.; Souri, M.K. Beneficial Effects of Glycine on Growth and Leaf Nutrient Concentrations of Coriander (*Coriandrum sativum*) Plants. *J. Plant Nutr.* **2019**, *42*, 1637–1644. [CrossRef]
42. Díaz-Zorita, M.; Fernández-Canigia, M.V.; Grosso, G.A. Applications of Foliar Fertilizers Containing Glycinebetaine Improve Wheat Yields. *J. Agron. Crop Sci.* **2001**, *186*, 209–215. [CrossRef]
43. Kopittke, P.M.; Menzies, N.W.; Wang, P.; McKenna, B.A.; Lombi, E. Soil and the Intensification of Agriculture for Global Food Security. *Environ. Int.* **2019**, *132*, 105078. [CrossRef]