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Phthalanilic Acid with Biostimulatory Functions Affects Photosynthetic and Antioxidant Capacity and Improves Fruit Quality and Yield in Cowpea (*Vigna unguiculata* (L.) Walp.)

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Abstract: The widespread application of biostimulants with a growing trend represents sustainable practices aimed at improving growth and yield and alleviating stresses in green agricultural system. Phthalanilic acid (PPA), with biostimulatory functions, has been increasingly applied to fruit and vegetable production. However, its specific biostimulatory effects on growth and development of cowpea (*Vigna unguiculata*) plants is still unclear. In this study, the regulatory function of foliar spraying PPA at the flowering timing in morphometric (length, width, single pod weight and yield), physiological (relative electrical conductivity), and biochemical (antioxidant enzymes activity, photosynthetic pigment, malondialdehyde, vitamin C, soluble protein, and soluble sugar content) parameters of cowpea plants were investigated. In general, PPA treatments exhibited higher antioxidant enzymes activities (with an increase of 11.89–51.62% in POD), lower relative conductivity (with a decrease of 22.66–62.18%), increased photosynthetic pigment levels and amounts of free proline (with an increase of 24.62–90.52%), and decreased malondialdehyde. Furthermore, the length, width and weight of single pod, podding rate (with an increase of 19.64%), vitamin C, soluble protein (with an increase of 18.75%), and soluble sugar content were increased by 200 mg·L^{-1} PPA. These data, together with an increased yield of 15.89%, suggest that PPA positively regulates the growth and development, improving fruit quality and yield, especially at 200 mg·L^{-1}. This study indicates that PPA has biostimulatory effects in cowpea production and shows application prospect in field cultivation.

Keywords: biostimulants; photosynthetic characteristics; antioxidant activity; fruit

Citation: Ma, T.; Wu, Q.; Liu, N.; Zhang, R.; Ma, Z. Phthalanilic Acid with Biostimulatory Functions Affects Photosynthetic and Antioxidant Capacity and Improves Fruit Quality and Yield in Cowpea (*Vigna unguiculata* (L.) Walp.). *Agriculture* **2021**, *11*, 1082. https://doi.org/10.3390/agriculture11111082

Academic Editor: Cinzia Margherita Bertea

Received: 15 September 2021
Accepted: 26 October 2021
Published: 1 November 2021

1. Introduction

Better fruit quality and higher yield are always the goals for agricultural production. However, abiotic stresses, such as drought, salinity, heat, and chill, severely threaten field cultivation. In recent years, the applications of biostimulants have become an effective strategy for the enhancement of sustainable practices to mitigate stresses and improve growth and yield by regulating plant physiological and biochemical processes in field green production [1–4]. In general, biostimulants, with multiple components, are categorized as silicon, protein hydrolysates, seaweed extracts, and humic substances [5–7]. Specifically, the stimulation on crop growth and development and is ascribed to peptides, algal polymers, and molecular inducing the production of phytohormones [8,9], antioxidants decreasing stresses [10,11], and plant growth regulators [12,13].

For standard biostimulants, the constituents are always complex and triggered effects are the synergy of the mixture; thus, the mechanism of action is unlikely to be completely exposed [1]. Apart from the mixture, some small molecules, showing biostimulatory effects, positively alert physiological and biochemical processes, including seed germination, root nodulation formation of legume crops, light utilization, crop nutrient absorption,

and adverse stresses tolerance [14–18]. Triacontanol promoted growth, photosynthetic capacity [19], and functioned as an antioxidative agent through inhibiting peroxidative damage of cellular proteins and lipids [20]. Brassinolide application improved the drought tolerance in maize through modulation of enzymatic antioxidants and leaf gas exchange [21]. Similarly, abscisic acid (ABA) and brassinolide application promoted antioxidant activity in tall fescue under water stress [22]. In addition, exogenous application of glycinebetaine up-regulated photosynthetic capacity and antioxidase activities in salt-stressed maize plants [23].

Phthalanilic acid (PPA), as a crystalline amido acid, with melting point of 168 °C ± 1 °C, which is soluble in anhydrous methanol, ethanol, acetone, and other organic solvents, was first developed by the Hungarian Neviki Institute of Chemical Industry in 1982 [23,24]. PPA promotes flower and fruit retention, significantly increasing the yield of apples, cherries and plums [25–28], and mitigate the unfavorable effect on crops [29]. PPA was used in fruit trees, corn, alfalfa, rape, sunflower, and some ornamental plants to promote higher yield and obtain more economic benefits [24,30]. Post-PPA applications enhanced stress resistance of capsicum in field trials, along with the promotion of chlorophyll content, and antioxidant enzyme activities, as well as increased yield, were observed in previous research [27]. In spite of positive regulations on capsicum and some plants, whether PPA has positive biostimulatory effects on other vegetables needs further research.

Cowpea (*Vigna unguiculata* L.), an important legume crop, is rich in protein and phenolic compounds, which is not only used as fresh vegetables but also as a dry grain to supplement the cereal-based diet for protein needs [31,32]. Compared to other vegetables, it grows well in the barren soil without needing an extra nitrogen application [33]. For all the above reasons, it is widely cultivated in throughout Southeast Asia, Africa, the southern United States, and Latin America, as well as in Mediterranean countries [34]. In modern cowpea production, substances with regulatory effects were introduced into field cultivation. Spraying exogenous IAA significantly decreased the number of flowers abscised from cowpea plants [35]; GA_3 that was applied to cowpea also increased the yield [36]. However, there is no report about using PPA on cowpea by now.

In view of the above, this study systematically determined photosynthetic, antioxidant, nutrient, and other physiological and biochemical indexes to comprehensively assess the biostimulation effects of PPA on the growth, final yield, and fruit quality of cowpea plants. These results would provide guidance for its rational use in cowpea fields, as well as lay the foundation for the subsequent reveal of the biostimulatory mechanism.

2. Material and Methods

2.1. Plants and Chemicals

Cowpea (*Vigna unguiculata* L.) seeds were purchased from Yangling Agricultural High-Tech Development Co., Ltd., Yangling, China. 20% PPA AS (Aqueous Solution) was provided by Shaanxi Sunger Road Bio-Science Co., Ltd., China. 0.0075% Brassinolide AS was purchased from Chengdu New Sun Crop Science Co., Ltd., China. Other reagents, polyvinylpyrrolidone, riboflavin, bovine serum albumin, etc., were obtained from Sigma-Aldrich, St. Louis, MO, USA.

2.2. Experimental Design

A field trial was conducted from May to July 2016 at the Northwest A&F University experimental base. The soil for field trials was Guanzhong soil with medium soil fertility. The ploughing layer (10–20 cm in depth) contained 10.2 g/kg of organic matter, 1.20 g/kg of total nitrogen, 0.18 g/kg of total phosphorus (P_2O_5), 5.37 mg/kg of effective phosphorus (*p*), and150.0 mg/kg of quick-acting potassium. During the experimental periods, average daily maximum and minimum temperatures were 28 °C and 16 °C, respectively. Cowpea seeds were sowed on 30 April at the rate of two seeds per hole, with the spacing of 35 cm × 65 cm. All treatments were arranged in a randomized complete block design with three replicates (15 m^2 per replication), and ten plants with the similar growth trend

were selected from each plot for assays. The experiment consisted of the following five treatments: the blank control, 0.075 mg·L^{-1} brassinolide (PGR control), 133.3 mg·L^{-1} PPA, 200.0 mg·L^{-1} PPA, and 266.7 mg·L^{-1} PPA. PPA and brassinolide were applied to cowpea seedlings by high volume spray during the seedling stage (3–4 true leaves; spray volume of 450 kg·hm^{-2}) and the early flowering period (spray volume of 900 kg·hm^{-2}). Ten plants with the same growth were selected from each plot, and the middle leaflets of compound leaves at the same position were used for experiments.

2.3. Physiological and Biochemical Measurements

The net photosynthetic rate (Pn), stomatal conductance (Gs), intercellular CO_2 concentration (Ci), and transpiration rate (Tr) were determined by the portable photosynthesis system (LI-6400XT) with standard 6 cm^2 leaf chamber and red-blue LED light source. At the seedlings (5–6 true leaves), full-bloom, and early podding stage, fully-expanded mature leaves (the upper third leaf) of cowpea were measured on sunny days, and results were collected from five randomly selected plants for each plot. In the course of measurement, saturating light intensity was kept at 1500 mmol·m^{-2}·s^{-1}, and three readings (1-min interval for each reading) were recorded averagely to make the measurement stability and precision [37]. Data were reported as the mean value of three replicates (five seedlings per replicate) for each treatment.

The chlorophyll content of the cowpea leaves was determined by previously described procedure [38], and the electrolyte leakage was assayed by the relative conductivity following the method of Ekmekci and Terzioglu [39]. The malonaldehyde (MDA) content was measured by the thiobarbituric acid (TBA) method [40]. Proline content was determined according to the method [41] and expressed as μg/g dry weight. For estimation of antioxidant enzymes, the pretreatment of the sample was based on the methods of Xiong et al. (2016) [42]. Superoxide dismutase (SOD) activity was determined by measuring the inhibition of the photoreduction of nitro blue tetrazolium (NBT) at 560 nm [43]. Peroxidases (POD) was detected by testing the initial rate of guaiacol oxidation at 470 nm [43]. Catalase (CAT) activity was evaluated by assaying the initial rate of H_2O_2 decrease through measuring the absorbance change at 240 nm [43]. On the 3rd, 7th, 11th, 14th, and 21st day after the second PPA application, the upper third leaflets from the ten cowpea plants, which were randomly selected as the fixed point for each plot, were used for assays.

2.4. Assessment of Fruit Quality and Yield

2.4.1. Fruit Quality

The determination of pod length, width, and single pod weight was according to the method of Masuthi et al. (2010) [44]. Ten cowpea pods without difference on appearance and maturity were chosen randomly from each plot and then were broken by a homogenizer, with pulp for testing the content of soluble sugar, soluble protein, and vitamin C. Concentration of soluble sugar was determined by the anthrone colorimetric method, while the content of soluble protein was detected by the coomassie brilliant blue G-250 method [37]. The 2, 6-dichlorindophenol titration method was applied to assay vitamin C content [37].

2.4.2. Podding Rate and Yield

The podding rate was measured by the method [45]. Specifically, thirty flowers were marked for each plot, and then the number of pods was counted after two days. The flower numbers were marked three times during the entire flowering period, with data representing the mean of three statistics for per treatment. Overall yield was determined as the sum of cowpea weight for choosing commodity pods during the harvest period, and it was extrapolated to kg/hm^2.

2.5. Statistical Analysis

All data were represented as the mean ± standard deviation (SE), and all analyses were carried out using SPSS 22 statistical software (IBM Corporation, Somers, NY, USA).

Statistical differences between photosynthetic indexes (within each individual time point), and between antioxidant indexes (within each individual time point), and between nutrient indexes, and between the final yield treated by phthalanilic acid brassinolide, were determined using one-way analysis of variance (ANOVA), followed by Duncan's Multiple Range Test ($p < 0.05$).

3. Results

3.1. Effect of PPA on Photosynthetic Properties of Cowpea

The results of PPA on photosynthetic parameters in cowpea leaves are displayed in Figure 1. Four photosynthetic parameters had no obvious difference at the cowpea seedling period, among all treatments.

Compared with the blank control, at the full-bloom period, Pn increased by 15.31% at 133.3 mg·L^{-1} PPA. At the early podding period, and Pn increased by 21.98–28.84% under three PPA treatments (Figure 1A). PPA treatments brought a significant decrease of 27.08–45.83% and 30.26–36.84% in Gs at the full-bloom and early podding period, respectively (Figure 1B). However, Ci (at the early podding period) was slightly increased by 6.45% and 9.47% at 133.3 and 200 mg·L^{-1} PPA, respectively (Figure 1C). Tr decreased significantly by 10.84–23.27% at the full-bloom and early podding period (except 133.3 mg·L^{-1} PPA) under PPA treatments; however, no significant differences were observed at the seedling stage (Figure 1D). In addition, brassinolide treatment has the similar effect on photosynthetic properties of cowpea with PPA treatments.

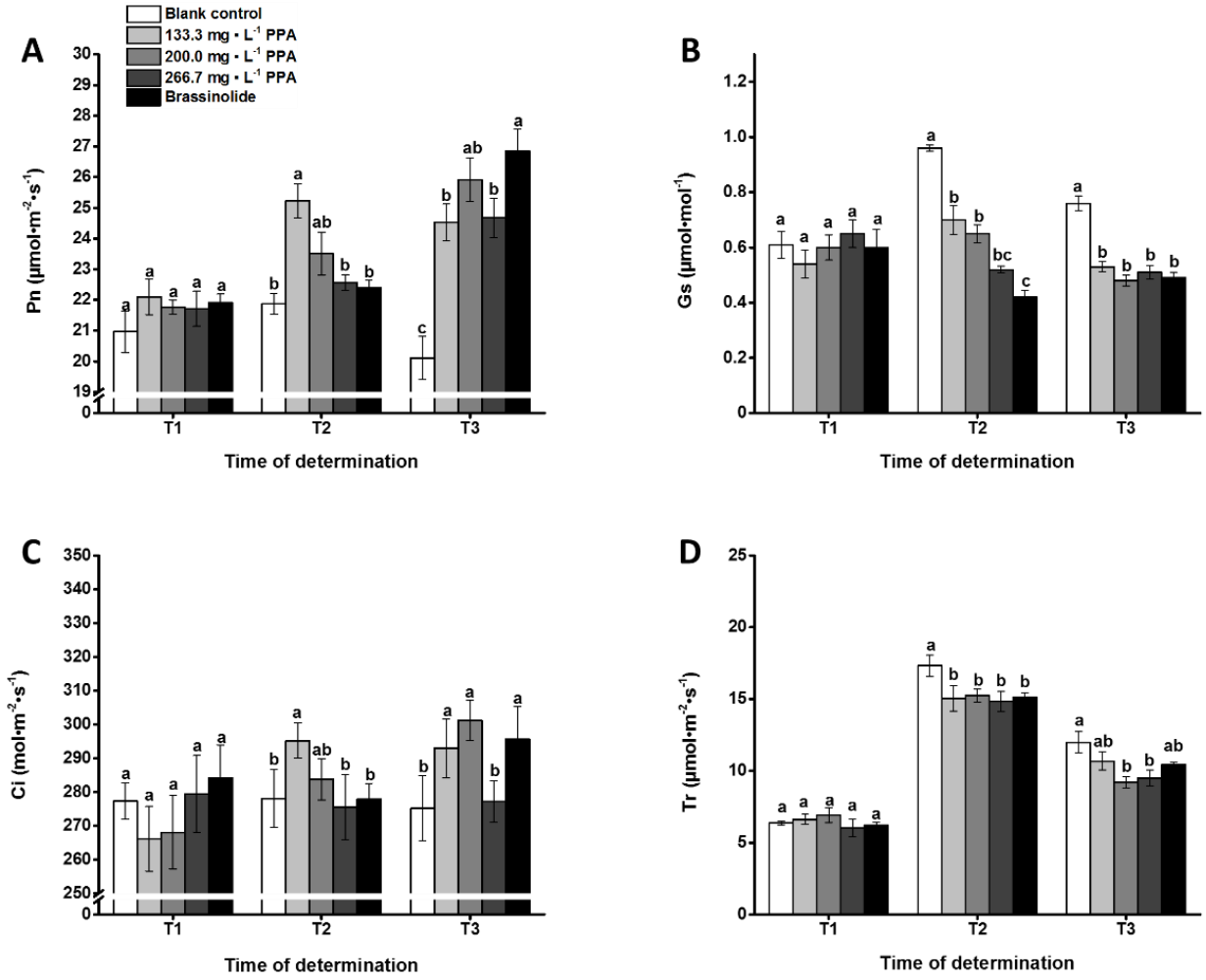


Figure 1. *Cont.*

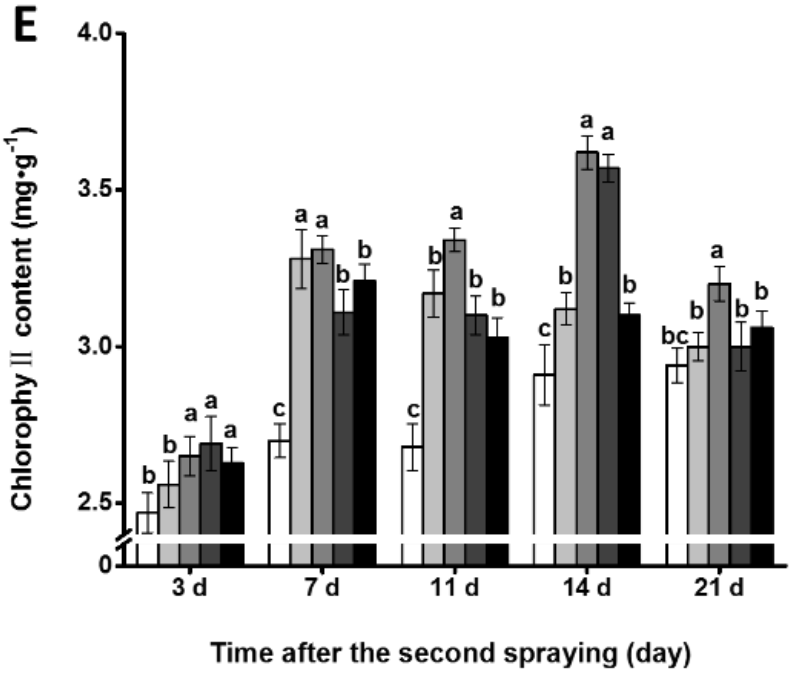


Figure 1. Effect of phthalanilic acid (PPA) on photosynthetic properties of cowpea leaves.T1, T2, and T3 indicate the stage of the seedlings, full-bloom, and early podding period, respectively. Chlorophyll content was detected after the second spraying. (**A**) represents the detected net photosynthetic rate (Pn); (**B**) represents the detected stomatal conductance (Gs); (**C**) represents the detected intercellular CO_2 concentration (Ci); (**D**) represents the detected transpiration rate (Tr); (**E**) represents the detected chlorophyll content. The concentration of brassinolide applied to cowpea plants was 0.075 mg·L^{-1}. Pn, Gs, Ci, and Tr represent photosynthetic rate, stomatal conductance, intercellular CO_2 concentration, and transpiration rate, respectively. The data are represented as the means (±SE) of three replications. Values with different lower-case letters are significantly different ($p < 0.05$) by Duncan's multiple range test at each same time point.

As can be seen from Figure 1E, different concentrations of PPA treatments increased the chlorophyll content of cowpea leaves. Compared with the blank control, PPA applications (except 133.3 mg·L^{-1}) and brassinolide significantly increased the chlorophyll content by about 11% on day 3 after the second spraying. On day 7, all the treatments presented a significant increase of 15.18–22.59%. A statistical increase of 10.06–24.63% was observed on days 11 and 14. However, on day 21, only 200 mg·L^{-1} PPA showed a significant increase of 10.23%.

3.2. Effect of PPA on the Lipid Peroxidation and Electrolyte Leakage of Cowpea

The results (Figure 2) show that electric conductivity significantly decreased by 22.66–62.18% on days 11, and 21 post-PPA applications. However, no statistical differences were observed on days 3 and 7 (except 200 mg·L^{-1} PPA).

MDA content statistically decreased by 14.41%, 18.25%, 13.65%, and 20.20% on days 3 (133 mg·L^{-1} PPA), 11 (200 mg·L^{-1} PPA), 14 (200 mg·L^{-1} PPA), and 21 (brassinolide), respectively. However, the rest treatments were not significantly different from the control.

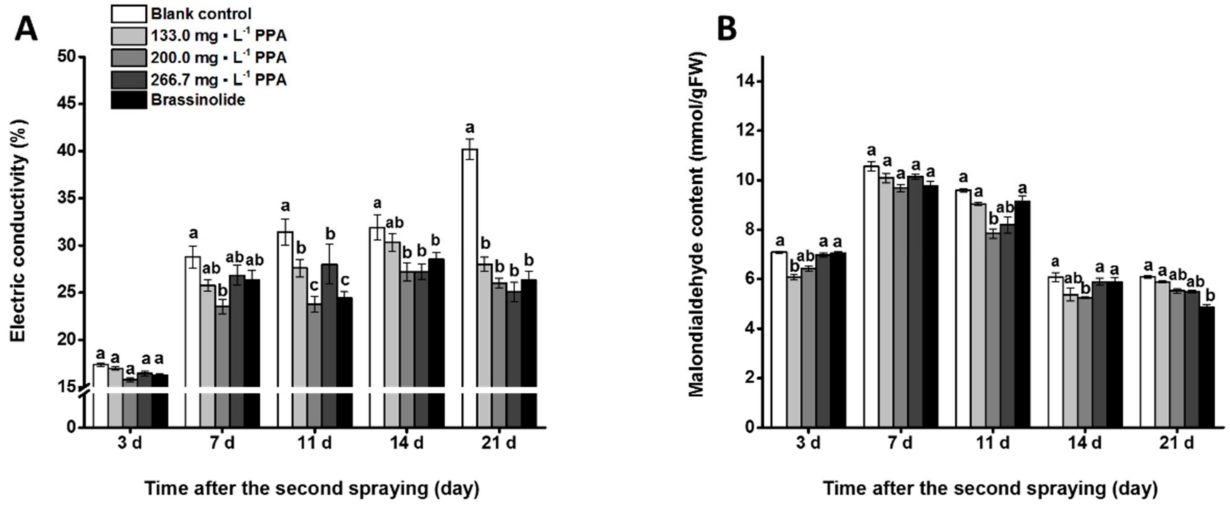
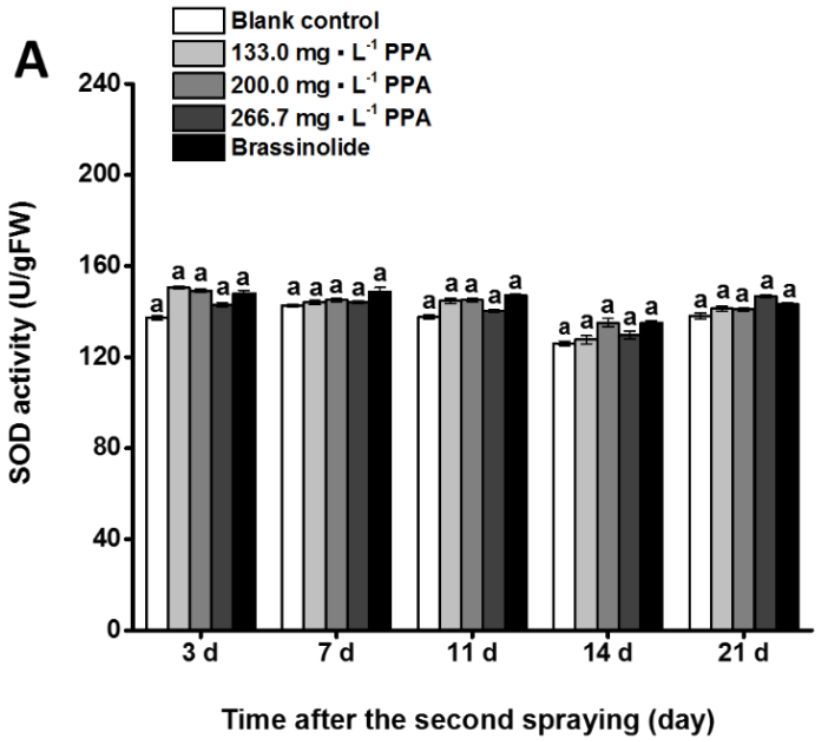


Figure 2. Effect of phthalanilic acid (PPA) on electric conductivity and malondialdehyde (MDA) content of cowpea leaves. The electric conductivity and MDA content were determined after the second spraying. (**A**) represents the detected electric conductivity and (**B**) represents the detected MDA content. The concentration of brassinolides applied to cowpea plants was 0.075 mg·L^{-1}. The data are represented as the means (±SE) of three replications. Values with different lower-case letters are significantly different ($p < 0.05$) by Duncan's multiple range test at each same time point.

3.3. Effect of PPA on Antioxidant Enzyme Activities in Cowpea

The data of SOD, POD, and CAT activities in cowpea leaves are presented in Figure 3. Compared with the blank control, no significant differences were found on SOD from day 3 to day 21 after PPA and brassinolide treatments.



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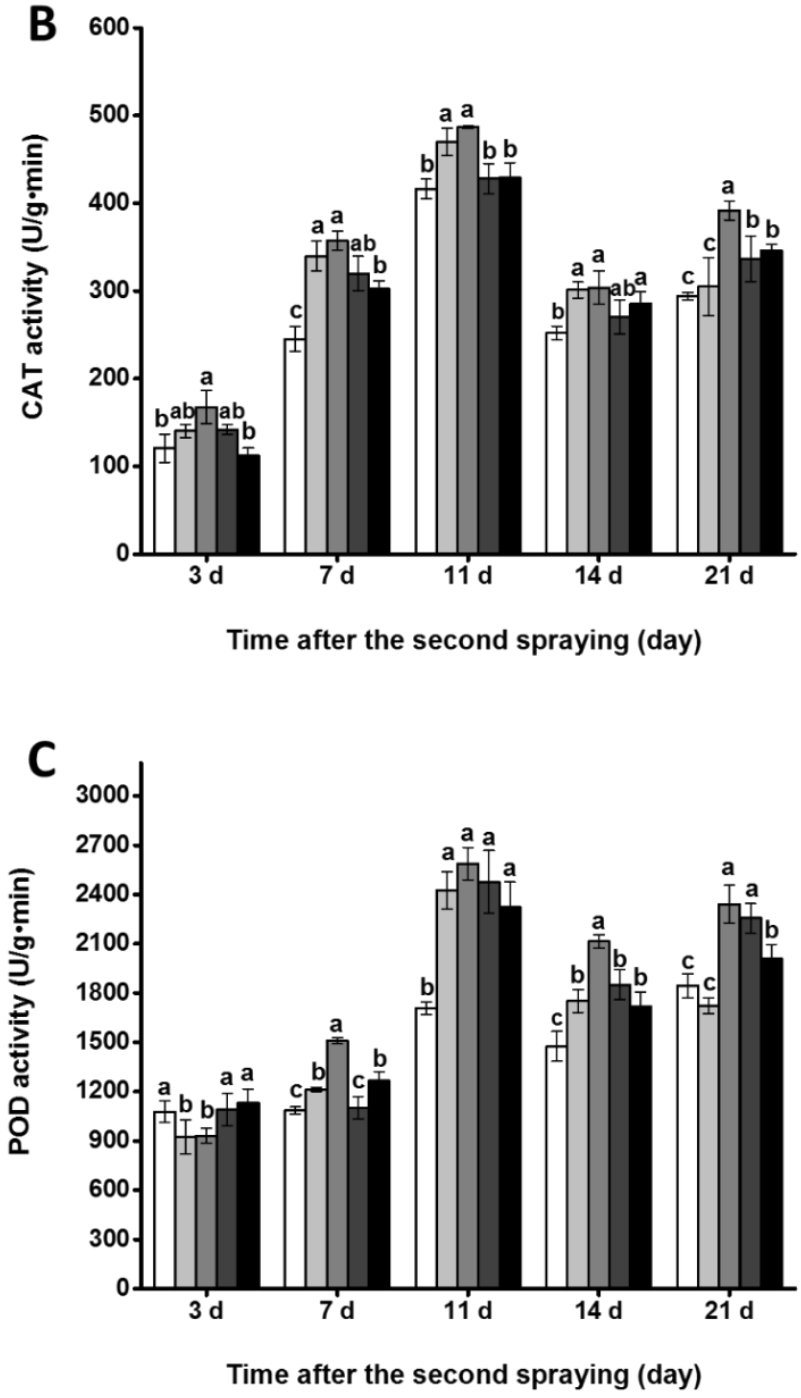

Figure 3. Effect of phthalanilic acid (PPA) on SOD, CAT, and POD activity in cowpea leaves. SOD, CAT, and POD activity were assayed after the second spraying. (**A**) represents the detected SOD activity; (**B**) represents the detected CAT activity; (**C**) represents the detected POD activity. The concentration of brassinolides applied to cowpea plants was 0.075 mg·L^{-1}. The data are represented as the means (±SE) of three replications. Values with different lower-case letters are significantly different ($p < 0.05$) by Duncan's multiple range test at each same time point.

POD activity was significantly induced by an increment of 11.89–39.07%, 36.15–51.62%, 16.47–43.28%, and 10.68–26.95% on days 7 (except 266.7 mg·L^{-1} PPA), 11, 14, and 21 (except 133 mg·L^{-1} PPA), respectively.

CAT activity was significantly increased by PPA and brassinolide treatments on day 7, and the increase rate was 23.34–38.67%. A significant increase of 13.11–20.45%

and 17.66–33.21% was observed on days 14 (except 266.7 mg·L^{-1} PPA) and 21 (except 133 mg·L^{-1} PPA), respectively.

3.4. Effect of PPA on the Free Proline Content in Cowpea

Free proline content in leaves of treated cowpea plants are elucidated in Figure 4. PPA and brassinolide increased the free proline content, and the increase rate was 16.25–80.42%, 36.34– 80.71%, 13.46–24.62%, and 73.84–94.16% and on days 3, 7, 11 (except 266.7 mg·L^{-1} PPA and brassinolide), 14 (except 133 mg·L^{-1} PPA), and 21, respectively. From day 3 to day 21, proline content increased significantly by 200 mg·L^{-1} PPA treatment. The same effect was found in brassinolide treatment, but being lower than 200.0 mg·L^{-1} PPA, except on days 14 and 21.

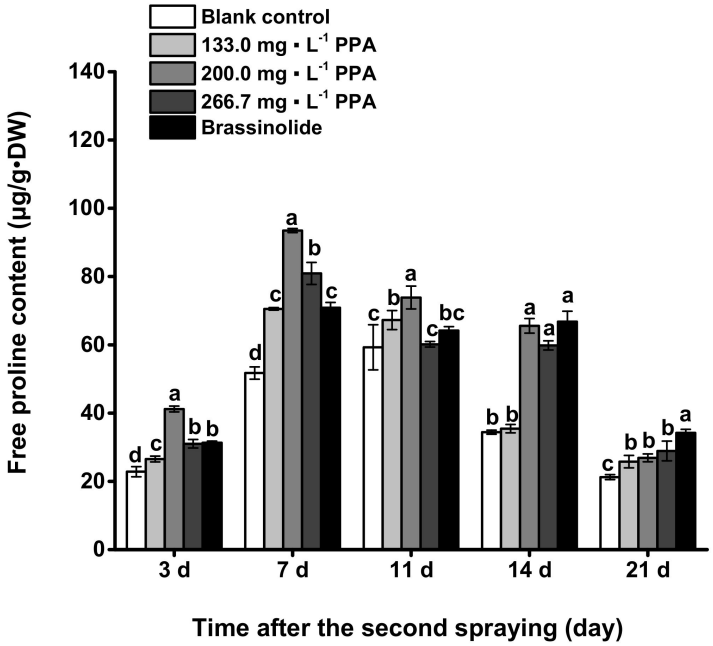

Figure 4. Effect of phthalanilic acid (PPA) on the free proline content of cowpea leaves. Free proline content was detected after the second spraying. The concentration of brassinolides applied to cowpea plants was 0.075 mg·L^{-1}. The data are represented as the means (±SE) of three replications. Values with different lower-case letters are significantly different ($p < 0.05$) by Duncan's multiple range test at each same time point.

3.5. Effect of PPA on the Quality of Cowpea

The data in Table 1 indicate that PPA possessed positive effects on appearance quality of cowpea pods. At 200.0 mg·L^{-1} PPA, the pod length increased by 8.67%, while the single pod weight increased 15.94%. The pod length and single pod weight of the brassinolide treatment were not significantly different from that of the blank controls.

Table 1. Effect of phthalanilic acid (PPA) on appearance quality of cowpea.

Treatment	Concentration (mg·L^{-1})	Length (cm)	Width (cm)	Single Pod Weight (g)
Blank control	0	50.62 ± 3.24 b	0.60 ± 0.03 ab	10.10 ± 0.67 b
Phthalanilic acid	133.3	54.00 ± 1.91 a	0.61 ± 0.05 a	10.89 ± 1.72 ab
	200.0	55.01 ± 3.05 a	0.61 ± 0.02 a	11.71 ± 1.08 a
	266.7	51.96 ± 1.83 ab	0.57 ± 0.02 b	10.30 ± 1.26 b
Brassinolide	0.075	50.95 ± 2.07 b	0.59 ± 0.02 ab	10.55 ± 0.46 ab

Note: The data are represented as the means (± SD) of three replications. Values in the same column with different letters are significantly different ($p < 0.05$) by Duncan's multiple range test.

The nutrient qualities of cowpea were improved (Table 2). Vitamin C content was significantly increased by 200.0 mg·L^{-1} PPA. In addition, PPA (200.0 mg·L^{-1}) and brassinolide displayed an increase of 18.75% and 30.10% on soluble protein content, but other treatments were not significantly different from the control. All the treatments promoted the content of soluble sugar except for 266.67 mg·L^{-1} PPA.

Table 2. Effect of phthalanilic acid (PPA) on the nutrient quality of cowpea.

Treatment	Concentration (mg·L^{-1})	Vc Content (mg/100 g·FW)	Soluble Protein Content (mg/g·FW)	Soluble Sugar Content (mg/g·FW)
Blank control	0	13.04 ± 0.50 b	25.12 ± 0.74 c	50.93 ± 1.11 b
Phthalanilic acid	133.3	13.99 ± 0.93 b	26.09 ± 0.88 c	53.92 ± 2.23 a
	200.0	16.85 ± 0.55 a	29.83 ± 0.68 b	54.00 ± 1.43 a
	266.7	14.31 ± 0.46 b	26.06 ± 0.53 c	51.87 ± 3.73 ab
Brassinolide	0.075	13.35 ± 0.95 b	32.68 ± 1.19 a	54.21 ± 2.30 a

Note: The data are represented as the means (± SD) of three replications. Values in the same column with different letters are significantly different ($p < 0.05$) by Duncan's multiple range test.

3.6. Effect of PPA on the Podding Rate and Yield

PPA (200.0 mg·L^{-1}) performed a statistical increment of 19.64% on the podding rate cowpea (Table 3); however, no significant difference was observed at 133 and 266.7 mg·L^{-1} PPA. Similarly, 200.0 mg·L^{-1} PPA significantly improved the yield, with the increase of 15.89%, while brassinolide treatment possessed no significant difference from the blank control on these two indexes (Table 3).

Table 3. Effect of phthalanilic acid (PPA) on podding rate and yield of cowpea.

Treatment	Concentration (mg·L^{-1})	Podding Rate (%)	Yield (kg/hm^2)	Yield Growth Rate (%)
Blank control	0	57.58 ± 2.92 b	$2.14 \times 10^4 \pm 1.24 \times 10^3$ b	—
Phthalanilic acid	133.3	61.11 ± 3.93 b	$2.37 \times 10^4 \pm 1.60 \times 10^3$ ab	10.75
	200.0	68.89 ± 3.85 a	$2.48 \times 10^4 \pm 1.34 \times 10^3$ a	15.89
	266.7	63.33 ± 4.77 ab	$2.29 \times 10^4 \pm 1.49 \times 10^3$ ab	7.01
Brassinolides	0.075	61.11 ± 5.09 b	$2.28 \times 10^4 \pm 1.03 \times 10^3$ ab	6.54

Note: The data are represented as the means (± SD) of three replications. Values in the same column with different letters are significantly different (P < 0.05) by Duncan's multiple range test.

4. Discussion

During the long-term evolution, plants have formed the adaptability to environment stresses by utilizing antioxidant and non-antioxidant systems to alleviate injury from overproduction of reactive oxygen species (ROS) [46–49]. The antioxidant enzyme system primarily consists of SOD, POD, and CAT [50]. Non-antioxidant enzyme systems contain flavonoids, total phenolics, free proline, etc., which act as an osmotic regulator of plant cytoplasm [51], or an effective ROS chelator [52,53]. Under their combined action, peroxidation products are quickly and effectively diminished, so as to make sure that plants normally grow under the adverse environment [54]. 5-aminolevulinic acid (ALA) defensed water-deficit stress by activating antioxidative and non-antioxidative defense systems in *Brassica napus* L. [55]. Additionally, the promotion in growth and metabolic activities induced by brassinolide treatment maintained the water potential and antioxidant enzyme activity of tissues reducing lipid peroxidation under drought conditions [21]. ABA and brassinolide significantly reduced the relative conductivity and malondialdehyde content of tall fescue leaves and increased the antioxidant enzyme activity and proline content of the leaves [22]. In addition, exogenous glycinebetaine application alleviated the adverse effects of salinity stress on maize plants by enhancing the photosynthesis capacity and the activity of some antioxidant enzymes [23].

In this research, the antioxidant and non-antioxidant enzyme systems in cowpea plants treated by PPA were investigated. The activity of the antioxidant enzyme system was

enhanced in varying degrees, especially for POD. PPA treatments significantly increased POD activity, with an increase of 26.95–51.64%. Similar results of elevated activity of antioxidant enzyme system were observed by PPA treatments in capsicum [27,56]. The increase in the activity of antioxidant enzymes enhances the ability of plants to scavenge active oxygen free radicals, which is beneficial to resist the surge of active oxygen in plants under stress conditions, keeping free radicals at a low level, thereby reducing damage to cells [57]. The promoted antioxidant enzyme system by PPA in cowpea would resist ROS stress and would further mitigate the oxidative damage.

When plants suffer from adversity stress, the destruction of cell membranes of leads to increased or even loss of membrane permeability, so that the electrolyte in the cell is exuded, which is manifested in the increase of relative electrical conductivity [58]. Post-PPA treatments, the relative electrical conductivity was deceased, which would suggest that cells function well. In the meanwhile, changes in the content of osmotic adjustment substances (such as proline) are a strategy for plants to resist stresses [59]. Free proline content in cowpea was increased by 24.62–90.52% post-PPA applications, showing the potential of fighting against stresses. Additionally, MDA is one of the end products of membrane lipid peroxidation, and the MDA content is one of the representative indexes to assess the adversity to plant cells [60–63]. The decreased MDA content and relative electrical conductivity indicated the low degree of lipid peroxidation of the cell membrane, which would facilitate to prevent cytoplasm leakage and maintain the integrity and stability of the biofilm. The enhanced antioxidant enzyme system, together with improved non-antioxidant enzyme system by PPA, would potentially help cowpea plants to enhance the adaptability to environmental stresses.

The quality of cowpea mainly depends on the appearance and nutrition, including pod length, pod width, single pod weight, the content of soluble sugar, vitamin C and phenols, and other important indicators [64,65]. In particular, the soluble protein is the most important nutrient substance of cowpea [65]. Significantly, both the appearance and internal quality of pods were improved post-PPA application. PPA markedly increased the pod length and single pod weight. Moreover, increased contents in vitamin C, soluble sugar, and soluble protein were also observed, suggesting that PPA, at 200.0 $mg{\cdot}L^{-1}$, improved the nutrition content in cowpea. However, neither 133.3 nor 266.7 $mg{\cdot}L^{-1}$ treatment displayed significant impact on internal quality. Interestingly, the similar effects of improving fruit quality also were found in capsicum post-PPA application [56]. As above, PPA improved the appearance and internal quality of cowpea by increasing the contents of VC, soluble protein, and soluble sugar content in pods.

Effective improvement of the crop yield is the ultimate aim of using biostimulants. PPA application at 200.0 $mg{\cdot}L^{-1}$ performed a significant yield-increasing effect on cowpea, with the increment of 15.89%. Similarly, yield-increasing effects were also observed in sweet cherry, sour cherry, apple, and capsicum after PPA applications [25,26,32]. Photosynthesis is the basis of plant organic matter synthesis and energy storage, and transformation and enhanced photosynthesis is conducive to the accumulation of assimilation products in plants [64]. In this research, PPA applications increased the chlorophyll content and Pn, implying that PPA applications enhance photosynthesis and promote the transformation and assimilation of dry biomass in cowpea leaves. In addition, the foliar application of salicylic acid increased the integral biomass and Pn of corn plants, resulting in increased yield [66]. Moreover, PPA application induced cowpea leave partial stomata closure, resulting in Gs and Tr reduction, and Ci increasing. These physiological responses together affected the photosynthesis ability of cowpea leaves, which is similar with that of ABA and IAA treatments [66]. In addition, small molecules with biostimulatory effects improved resistance of crops to biological or non-biological stress to maintain normal growth and development, thus laying the foundation for the increase of production [15,67–69]. Analogously, antioxidant and non-antioxidant defenses were also elevated after PPA applications, which would improve the tolerance to stresses and would potentially contribute to increased yield.

Taken together, the yield-increasing effect of PPA application on cowpea, on the one hand, might be related to the increased stress resistance and the promoted growth; on the other hand, might be related to the increase of the chlorophyll content and improved photosynthesis, resulting in increased accumulation of assimilation products in cowpea. The yield-increasing effect of PPA may be a comprehensive manifestation of multiple physiological effects. It should be pointed out that the yield-increasing mechanism is very complicated and requires further research.

5. Conclusions

The foliar spraying of PPA presents positive biostimulatory effects on cowpea plants of improving the photosynthetic properties and antioxidant and non-antioxidant defenses, leading to the improved fruit quality and the increased yield. This study indicates that PPA application with biostimulatory regulations is of important significance in modern cowpea production, and it is advisable to apply twice at 200.0 $mg \cdot L^{-1}$ during the flowering period. Finally, it should be stated that the use of PPA is a feasible and sustainable practice to promote the yield and nutraceutical quality for horticultural crops.

Author Contributions: Conceptualization, T.M.; Data curation, Q.W.; Formal analysis, R.Z.; Investigation, Q.W. and R.Z.; Methodology, N.L.; Project administration, Z.M.; Resources, Z.M.; Writing-review & editing, T.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was financially supported by Horizontal Cooperation Project (No. 20151001) and the cooperative partner was Shaanxi Sunger Road Bio-Science Co., Ltd., China.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Conflicts of Interest: This research was jointly completed by Provincial Center for Bio-Pesticide Engineering and Shaanxi Sunger Road Bio-Science Co., Ltd. (SSRB), and SSRB provided partial funding support and developed part field trials. According to the contract, both parties shall publish articles jointly, and the co-author listed in the article meets the requirements of the contract.

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