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Application of *Polygonum minus* Extract in Enhancing Drought Tolerance in Maize by Regulating Osmotic and Antioxidant System

Mingzhao Han¹, Susilawati Kasim^{1,*}, Zhongming Yang², Xi Deng², Md Kamal Uddin¹,
Noor Baity Saidi³ and Effyanti Mohd Shuib¹

¹Department of Land Management, Faculty of Agriculture, Universiti Putra Malaysia, Serdang, 43400, Malaysia

²Natural Medicines and Products Research Laboratory, Institute of Bioscience, Universiti Putra Malaysia, Serdang, 43400, Malaysia

³Department of Cell and Molecular Biology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, Serdang, 43400, Malaysia

*Corresponding Author: Susilawati Kasim. Email: susilawati@upm.edu.my

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ABSTRACT

Drought stress is a major factor affecting plant growth and crop yield production. Plant extracts as natural biostimulants hold great potential to strengthen plants to overcome drought impacts. To explore the effect of *Polygonum minus* extract (PME) in enhancing drought tolerance in plants, a study was set up in a glasshouse environment using 10 different treatment combinations. PME foliar application were designed in CRD and effects were closely observed related to the growth, physiology, and antioxidant system changes in maize (*Zea mays* L.) under well-watered and drought conditions. The seaweed extract (SWE) was used as a comparison. Plants subjected to drought stress exhibited a significant reduction in fresh weight, dry weight, relative water content (RWC), and soluble sugar, but they stimulated the phenolic, flavonoid, proline, glutathione (GSH), malondialdehyde (MDA) and antioxidant enzyme (catalase, CAT; peroxidase, POD; superoxide dismutase, SOD) activities. Foliar application of PME improved fresh and dry weight (FW: 33.1%~41.4%; DW: 48.0%~43.1%), chlorophyll content (Chl b: 87.9%~100.76%), soluble sugar (23.6%~49.3%), and soluble protein (48.6%~56.9%) as well as antioxidant enzyme activities (CAT and POD) compared to CK under drought conditions. while decreasing the level of MDA. Notably, the mitigating effect of PME application with high concentration was more effective than those of SWE. Our study reveals that PME could alleviate drought stress by regulating osmoprotectant content and antioxidant defense system and can be used as an economical and environmentally friendly biostimulants for promoting maize growth under drought stress.

KEYWORDS

Drought; biostimulants; photosynthesis; osmoprotectants; antioxidants; maize

1 Introduction

Maize (*Zea mays* L.), as an economically important food and forage crop, is widely grown all over the world under a various of climates and plays a prominent position in agricultural production [1]. However, the growth of maize is highly susceptible to various abiotic stresses. Studies have shown that maize yield is most sensitive to water deficit at the tasseling stage, because water deficit at the tasseling stage leads to a decline in



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pollen vigor, which also leads to loss in yield [2,3]. Besides, drought stress is the most crucial abiotic stress and about one-third of the world's arable land has been restricted due to unpredictable and seasonal drought which severely threatens agricultural sustainability [4]. Water deficit causes the accumulation of Reactive Oxygen Species (ROS) in plants, and overproduces ROS affects a series of damage such as physiological disorders, stomatal conductance, and membrane lipid peroxidation [5–7].

Plants have developed intrinsic mechanisms to regulate photosynthesis and maintain water conditions by regulating physiological and biochemical functions in the face of abiotic stress conditions [8–12]. However, these endogenous physiological regulations are usually not sufficient to help plants effectively to resist abiotic stress. With these problem in mind, biostimulants, including amino acids, humid substances, plant extracts, protein hydrolysates and beneficial fungi, have been proposed as potential natural agents to promote nutrient absorption and plant development and enhance tolerance of abiotic and biotic stresses [13,14]. At the same time, biostimulants also can reduce the dependence on chemical fertilizer and pesticides [15].

Plant extract, as a kind of biostimulants, contain a variety of phytochemical compounds and nutrients, which improve physiological and biochemical processes of plant [16,17]. In recent years, seaweed extracts (SWE) have been widely used in vegetable and crop research, and study has indicated that application of SWE may increase phytochemical such as phenolic and flavonoids as well as antioxidant capacity [18,19]. Elansary et al. [20] reported that application of SWE can increase plant height and dry weight of *Spiraea nipponica* and *Pittosporum eugenioides* by regulating stomatal conductance and photosynthetic rate. Mutale-Joan et al. [21] found that microalgae liquid extracts improve root and shoot length and dry weight, meanwhile, the maximum uptake of nitrogen, phosphorus, and potassium were increased by 185.17%, 119.36%, and 78.04%, respectively, compared with non-treated plants. Besides, the use of natural extracts from various plant components such as licorice, cypress leaf, and pollen grains also has been reported to enhance plant tolerance to drought stress [16,17,22].

Polygonum minus (*P. minus*), commonly known as 'kesum' in Malaysia, is widely used in cooking and for its medicinal properties, particularly in treating stomach-related illnesses [23]. Previous pharmacological studies have revealed *P. minus* have a high antioxidant content, making it as a subject of considerable interest [24]. However, studies using *P. minus* extract (PME) as a biostimulants to enhance stress tolerance in maize have not been conducted. Thus, we hypothesize that PME is associated with antioxidant activities and may have beneficial effect in promoting plant tolerance to abiotic stresses and this will stabilize the plant growth. To the best of our knowledge, this study needs to be investigated by treating maize with foliar spraying different concentrations of PME. The effects on morphology, physiology, and antioxidant activity of maize under drought condition were identified, and SWE was used as a comparison. This study aim to utilize PME as a drought mitigator for maize planted using sustainable agriculture approach.

2 Materials and Methods

2.1 Preparation and Analysis of SWE and PME

The *Polygonum minus* was purchased from a local supplier. The materials were extracted as details in Taha et al. [22] method with modification. Each 10 g of materials were mixed with 80% methanol using an optimum ratio of 1/8 (w/v) sample weight to solvent volume. Thereafter, the mixtures were continuously shaken at 150 rpm for 24 h using an orbital shaker. The extraction samples were filtered with Whatman No.1 filter paper, and the alcohol and excess water were evaporated under vacuum at 30°C using a rotary evaporator. The *Polygonum minus* extract (PME) obtained was made as an extract at a concentration of 1.0 g/L by dissolving in 10 L distilled water. The extracts were kept in refrigerator at 4°C until use. The seaweed extract (SWE) was purchased from Maize Star Resources Sdn. Bhd., Malaysia, and concentration was configured according to the instructions: *1 g seaweed extract was dissolved in 1 L of distill water*. The chemical constituents of SWE and PME (on a dry weight basis) are shown in Table 1.

Table 1: Chemical constituents of SWE and PME (on a dry weight basis)

Component	Unit	Seaweed ext.	<i>P. minus</i> ext.
Total carbon (C)	%	37.79	49.94
Total nitrogen (N)	%	0.21	7.10
Total phenolic	mg GAE g ⁻¹	5.90	15.93
Total flavoniod	mg Lutin g ⁻¹	15.04	36.38
Free proline	mg g ⁻¹	7.67	7.14
Soluble sugar	mg g ⁻¹	9.67	14.79
Protein	mg g ⁻¹	38.98	53.89
Glutathione (GSH)	mg g ⁻¹	95.26	81.34
Phosphorus (P)	g kg ⁻¹	0.18	2.26
Potassium (K)	g kg ⁻¹	13.98	38.54
Calcium (Ca)	g kg ⁻¹	0.29	0.25
Magnesium (Mg)	g kg ⁻¹	0.35	0.26
Iron (Fe)	mg kg ⁻¹	158.17	67.13
Copper (Cu)	mg kg ⁻¹	6.67	7.17
Zinc (Zn)	mg kg ⁻¹	21.07	20.72
Manganese (Mn)	mg kg ⁻¹	9.50	3.14
Boron (B)	mg kg ⁻¹	118.24	4.37

2.2 Plant Material and Growing Conditions

This experiment was carried out from March to June 2023 in a glasshouse at Faculty of Agriculture (2° 98' N, 101° 73' E), University Putra Malaysia (UPM), Selangor, Malaysia. Maize seeds (*Zea mays* L., Hybrid F1 316, Malaysia) were brought from local supplier. Prior to germination step, growth medium using mixed soil was prepared using sand and mineral soil (Oxisols, pass 2 mm sieve) at 1 to 4 ratio. Later, four seeds were sown in plastic pots (24 cm in diameter, 28 cm in depth) filled with mixed soil (14 kg). The basic properties for mixed soil are: total carbon 1.55 g kg⁻¹, total nitrogen 2.10 g kg⁻¹, total phosphorus 3.87 g kg⁻¹, available phosphorus 0.53 g kg⁻¹, total potassium 3.40 g kg⁻¹, CEC 4.51 cmol kg⁻¹. All pots were placed in a completely randomized design (CRD) and the equal amount of water was added to the pot until imposition of drought stress. After 2 weeks, save only one of the best quality seedlings in each pot. A total of 15 g compound fertilizer (N:P:K, 15:15:15) was applied to each pot: 5 g at sowing, after 28 and 56 days, respectively. The average day and night net glasshouse temperature was 38.80°C ± 2.60°C and 27.52°C ± 1.39°C, whereas average day and night net relative humidity was 40.14% ± 4.45% and 82.00% ± 4.42%, respectively.

2.3 PME Treatments and Experimental Design

The plants grew under normal water condition until tasseling (56 days) before they were divided into well-watered (75% soil field capacity) and drought (35% soil field capacity) conditions. Total 10 treatments were examined as follows: well-watered, well-watered with SWE, well-watered with PME1, well-watered with PME3, well-watered with PME5, drought, drought with SWE, drought with PME1, drought with PME3, and drought with PME5 (1, 3 and 5 indicate different concentrations). The concentration of 1 and 5 g L⁻¹ based on Taha et al. [22] and Pourghasemian et al. [16] recommendation,

and the middle value (3 g L^{-1}) was established as a comparison value. Each treatment was performed 4 repetitions. SWE and PME application were applied by using foliar spraying once a week and at each application, it is important to ensure that all leaves were sprayed evenly. The moisture of each pot was monitored everyday by soil moisture meter and maintained to desired soil water conditions by adding appropriate volumes of water. The top leaves of maize were collected after the last spray (84 days) and stored at -80°C until analysis of plant physiology, antioxidant as well as enzyme activity.

2.4 Morphological Parameters and RWC

The fresh plant weight is measured immediately, and the dry weight is measured after the sample is placed in the oven to constant weight. Plant height was measured by ruler.

Leaf relative water content (RWC) was assessed using the methods described by Wang et al. [25]. Briefly, the leaves were measured to fresh weight (FW) and immersed in distilled water for 24 h to record the turgid weight (TW). After that, the leaves were oven-dried to record the dry weight (DW). The RWC was expressed as follows:

$$\text{RWC (\%)} = [(\text{FW} - \text{DW})/(\text{TW} - \text{DW})] \times 100\%$$

2.5 Chlorophyll Content

Chlorophyll and carotenoid contents were assessed and then calculated as detailed described in Pourghasemian et al. [16]. Samples of 0.3 g fresh leaves were extracted in 20 mL acetone (80%) and left in the dark. After 24 h of the incubation, absorbance (A) was recorded at wavelengths of 646.8, 663.2 and 470 nm. Chl a, Chl b and carotenoids were calculated using the following equation:

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

$$\text{Chl b} = (12.25 \times A_{663.2}) - (2.79 \times A_{646.8})$$

$$\text{Carotenoids} = [(1,000 \times A_{470}) - (1.8 \text{ Chl a} - 85.02 \text{ Chl b})]/198$$

2.6 Leaf Total Phenolic and Flavonoid Content

The leaf extracts were obtained using methanol method [22] with modification. Briefly, 0.3 g dry leaf samples were cut into small pieces and extracted in 10 mL 80% methanol solution for 24 h at room temperature. After centrifuged at $10,000 \times g$ at 4°C , the supernatant was used to determine the total phenolic and flavonoid content using the Folin-Ciocalteu colorimetric method and the method described by Miliauskas et al. [26], using gallic acid and rutin as the reference, respectively.

2.7 Osmoprotectants (Proline and Soluble Sugar)

Soluble sugar content was measured by the anthrone colorimetric method [27]. Briefly, 0.3 g fresh maize samples were homogenized with 10 mL ethanol (80%) and then centrifuged at $10,000 \times g$ at 4°C for 10 min. The extract was mixed with 5 mL of anthrone reagent and then heated at 100°C in water bath for 10 min, and the absorbance was read at 630 nm after cooling to room temperature. Proline quantification was determined following the method of Bates et al. [28] and the absorbance was read at 520 nm.

2.8 Glutathione and Lipid Peroxidation

Glutathione (GSH) contents were quantified using the method described by Griffith [29]. Briefly, 0.3 g fresh leaves were extracted with 10 mL 5 % trichloroacetic acid (TCA) and centrifuged at $4,000 \times g$ for 15 min. The 2 mL of extract was mixed with 0.4 mL of 5, 5'-dithiobis (2-nitrobenzoate) (DTNB), the

absorbance was read at 412 nm. Lipid peroxidation was indicated by Malondialdehyde (MDA) following de Vos et al. [30] method. The same supernatant (same as GSH) was used to determine MDA. The 2 mL of extract was mixed with 2 mL of 0.6% 2-thiobarbituric acid (TBA), and the mixture was heated at 95°C for 15 min and cooled immediately in an ice bath. The absorbance of supernatant was read at 532 nm after centrifugation at $4,000 \times g$ for 10 min.

2.9 Antioxidant Enzyme Activities and Protein

Fresh maize samples were homogenized with potassium buffer at pH 7.8 to assess the antioxidant enzyme activities and protein. The mixture was then centrifuged at $12,000 \times g$ for 10 min at 4°C. The supernatant was used to measure antioxidant enzyme activities using the method described by Zafari et al. [31]. The activity of superoxide dismutase (SOD) was quantified by measuring the inhibition of nitro blue tetrazolium (NBT) reduction under light. The peroxidase (POD) activity was determined by assessing the oxidation of guaiacol after the addition of H_2O_2 . The catalase (CAT) activity was determined by assay kits purchased by Nanjing Jiancheng Bioengineering Institute, China. The protein content was measured according to Bradford [32] using bovine serum albumin as the standard.

2.10 Statistical Analysis

Data were analyzed using SPSS 25.0 (IBM, New York, NY, USA). Significant differences between treatments were calculated by one-way analysis of variance (ANOVA) with LSD test at $p < 0.05$. The regression relationships between all parameters and treatments (well-watered, drought, PME and SWE application) were analyzed by unitary linear regression. Figures and heat map were created by Origin 2018 software (OriginLab, Northampton, MA, USA).

3 Results

3.1 Growth Parameters

Drought stress caused a significant impact on fresh weight (FW), dry weight (DW) and number of leaves (Fig. 1). FW and DW were affected by foliar spraying under both well-watered and drought conditions. The result shows that SWE and PME treatments significantly increased FW and DW under drought condition, and in all PME treatments evaluation parameters, except height, all were significantly higher than CK. Generally, the SWE, and PME5 recorded the highest value, exhibiting a 41.4% and 53.1% higher than CK, respectively ($p < 0.05$). Additionally, it is worth noting that PME3 and PME5 showed significantly increased in FW by 8.6% and 9.3% and DW by 15.1% and 16.4%, respectively, compared to CK under well-watered condition. However, there were no significant differences observed in plant height among all foliar spraying treatments.

3.2 Photosynthetic Pigments and RWC

Chlorophyll a, chlorophyll b and carotenoids were significantly affected by foliar spraying and the drought stress condition (Fig. 2). Under well-watered condition, the PME5 treatment exhibited the highest content of chlorophyll a, chlorophyll b and carotenoids with significant increases of 91.2%, 113.7% and 100.0% compared to the control, respectively ($p < 0.05$). Under drought conditions, SWE and different concentrations of PME treatments able to increase the contents of chlorophyll a, b and carotenoids in different extents compared to the control. Among them, chlorophyll b and carotenoids showed the most significant differences. As for chlorophyll a, only SWE and PME1 showed significant improvements of 30.4% and 16.8% compared to CK, respectively. Furthermore, the results in Fig. 2 indicated that foliar spraying has no obvious influence on relative water content (RWC) under well-watered condition. However, under drought stress, both SWE and PME3 treatments were significantly higher than CK by 21.9% and 26.1%, respectively ($p < 0.05$).

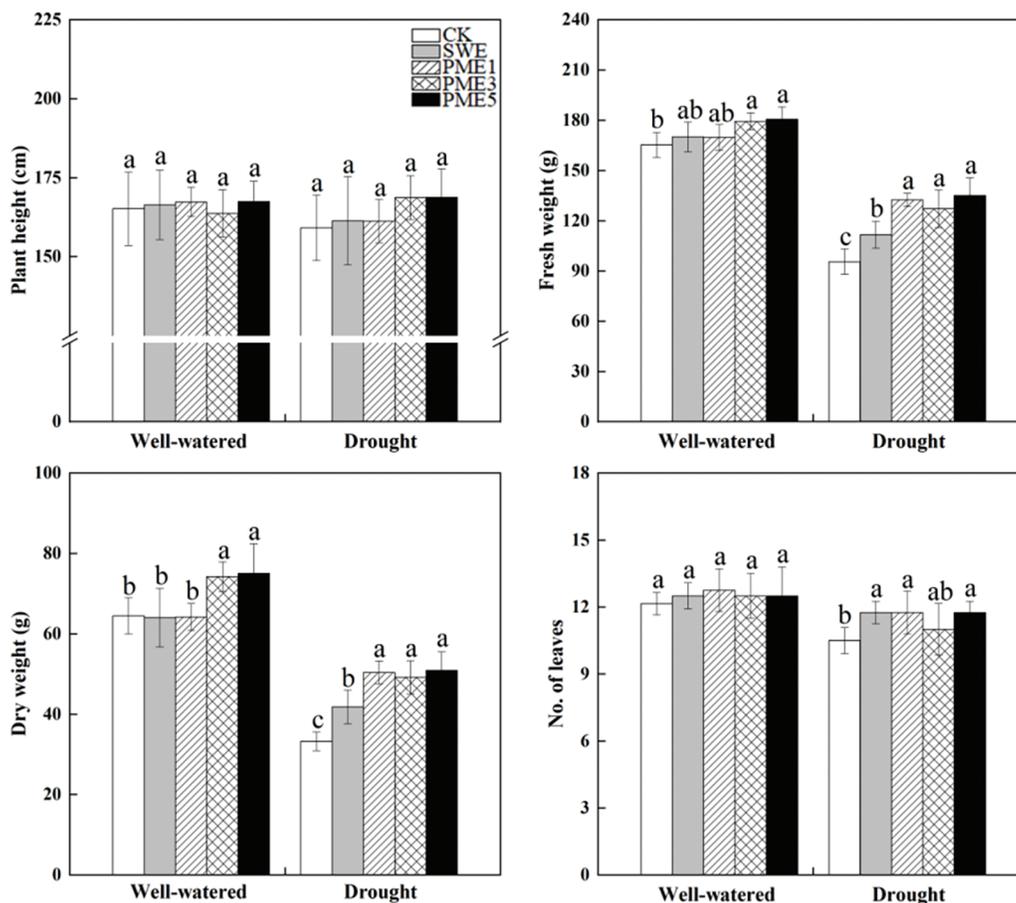


Figure 1: The analysis of variance of the effects of water conditions (well-watered and drought) and foliar application of SWE and PME treatments (1, 3 and 5 as concentration) on growth of maize. Different lowercase letters indicate significant influences between the same water conditions with different treatments ($n = 4$, $p < 0.05$)

3.3 Total Phenolic and Flavonoid Content

Drought stress caused an increase of total phenolic and flavonoid concentration in maize, however, both SWE and PME treatments showed no significant differences compared to CK (Fig. 3). Among them, the PME5 treatment exhibited the highest concentration of total phenolic and flavonoid with increase of 6.7% and 4.8% compared to CK, respectively.

3.4 Proline and Soluble Sugar

Proline content increased by 50.6% under drought conditions, whereas soluble sugar content decreased by 31.5% as shown in Fig. 4. All PME treatments had no significant effect on proline, but SWE treatment significantly decreased 25.9% compared to the CK under drought condition ($p < 0.05$). Regarding soluble sugar content, both SWE and PME treatments led to an increase of the value compared to CK. The highest concentration was observed in SWE treatment under well-watered condition, which was significantly higher by 11.3%. Under drought conditions, the soluble content of SWE, PME1, PME3 and PME5 was significantly increased by 46.4%, 23.6%, 32.9% and 49.3%, respectively ($p < 0.05$).

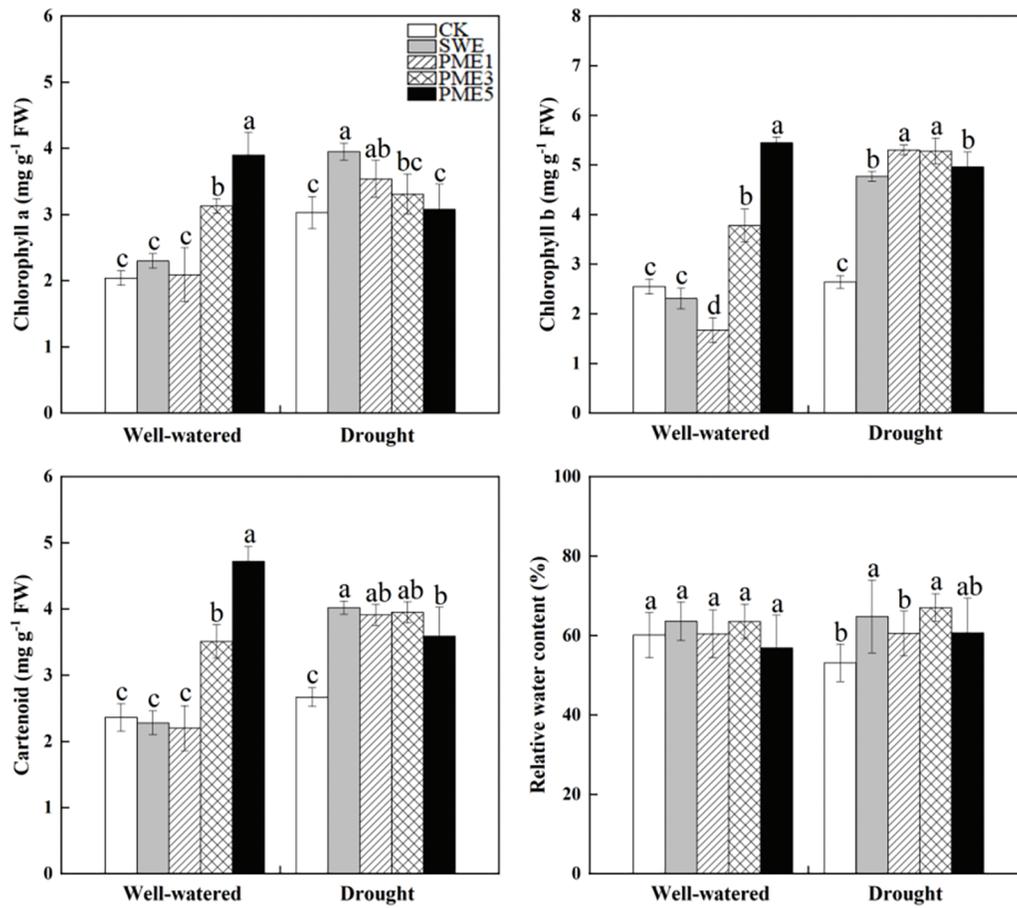


Figure 2: The analysis of variance of the effects of water conditions (well-watered and drought) and foliar application of SWE and PME treatments (1, 3 and 5 as concentration) on chlorophyll and relative water content of maize. Different lowercase letters indicate significant influences between the same water conditions with different treatments ($n = 4$, $p < 0.05$)

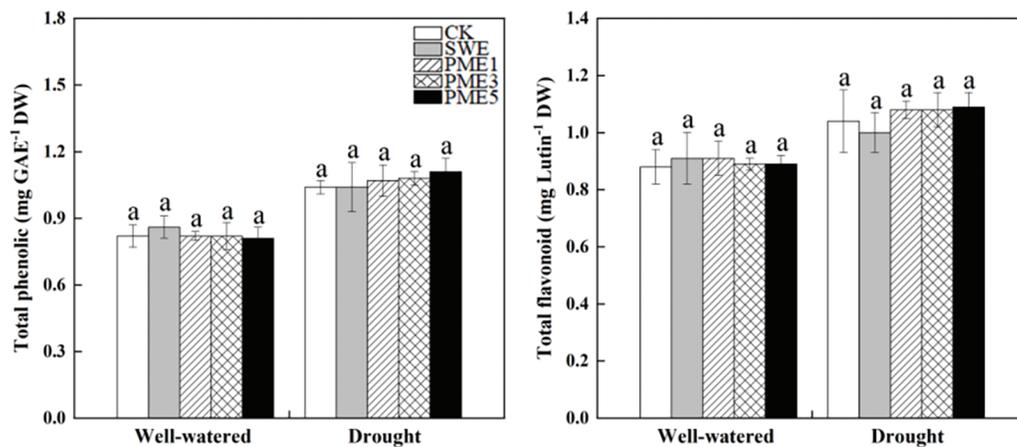


Figure 3: The analysis of variance of the effects of water conditions (well-watered and drought) and foliar application of SWE and PME treatments (1, 3 and 5 as concentration) on total phenolic and flavonoid of maize. Different lowercase letters indicate significant influences between the same water condition with different treatments ($n = 4$, $p < 0.05$)

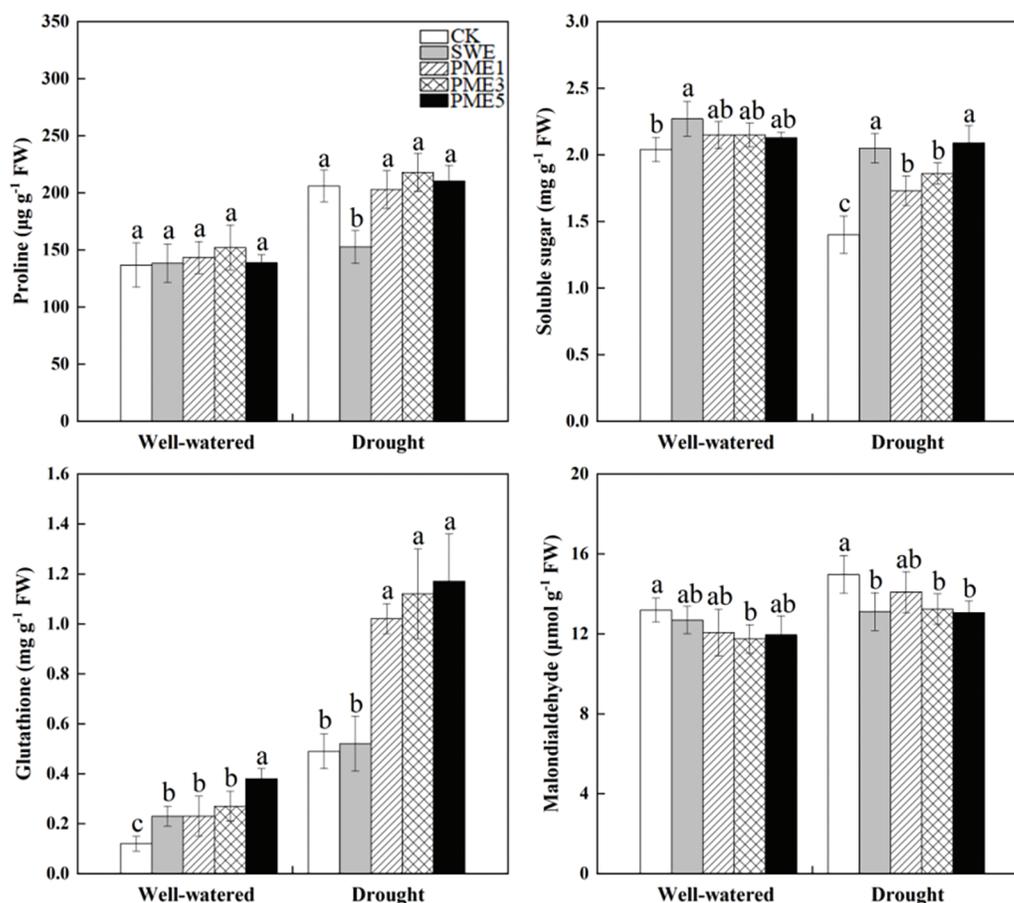


Figure 4: The analysis of variance of the effects of water conditions (well-watered and drought) and foliar application of SWE and PME treatments (1, 3 and 5 as concentration) on proline, soluble sugar, GSH and MDA of maize. Different lowercase letters indicate significant influences between the same water conditions with different treatments ($n = 4$, $p < 0.05$)

3.5 Glutathione and Lipid Peroxidation

Glutathione (GSH) and Malondialdehyde (MDA) concentration were significantly affected by both drought stress and foliar spraying, as depicted in Fig. 4. Under drought stress condition, GSH and MDA accumulation increased by 308.3% and 13.5%, respectively, compared to the well-watered condition. Both SWE and PME treatments led to a significant increase in GSH content compared to the control under well-watered and drought conditions ($p < 0.05$). Notably, under drought condition, PME treatments have the largest improvement compared to CK, where the enhancements ranging from 108.2% to 138.8%. On the contrary, MDA levels displayed a decreasing trend in foliar spraying (Fig. 4). Among the treatments, PME3 treatment had significant difference under well-watered condition, while both SWE, PME3 as well as PME5 treatments had significant differences under drought stress condition ($p < 0.05$).

3.6 Antioxidant Enzyme Activities and Protein

The data showed that antioxidant enzymes activities (CAT, SOD and POD) were affected by both drought stress and foliar spraying of SWE and PME (Fig. 5). All the antioxidant enzyme activities were increased when they received changes amount of water from well-watered and drought stress condition. Under drought condition, both SWE and PME treatments significantly increased CAT activity compared

to CK, while there was no significant difference under well-watered condition. The SOD activity in all foliar spraying treatments, except PME5, was lower than CK. Specifically, under drought stress, SWE, PME1 and PME3 treatments showed a significant reduction in SOD activity by 15.9%, 19.4% and 21.4%, respectively ($p < 0.05$). Furthermore, foliar spraying of both SWE and PME increased POD levels compared to CK treatment, and PME5 significantly increased 22.6% and 15.3%, respectively, under well-watered and drought stress conditions ($p < 0.05$).

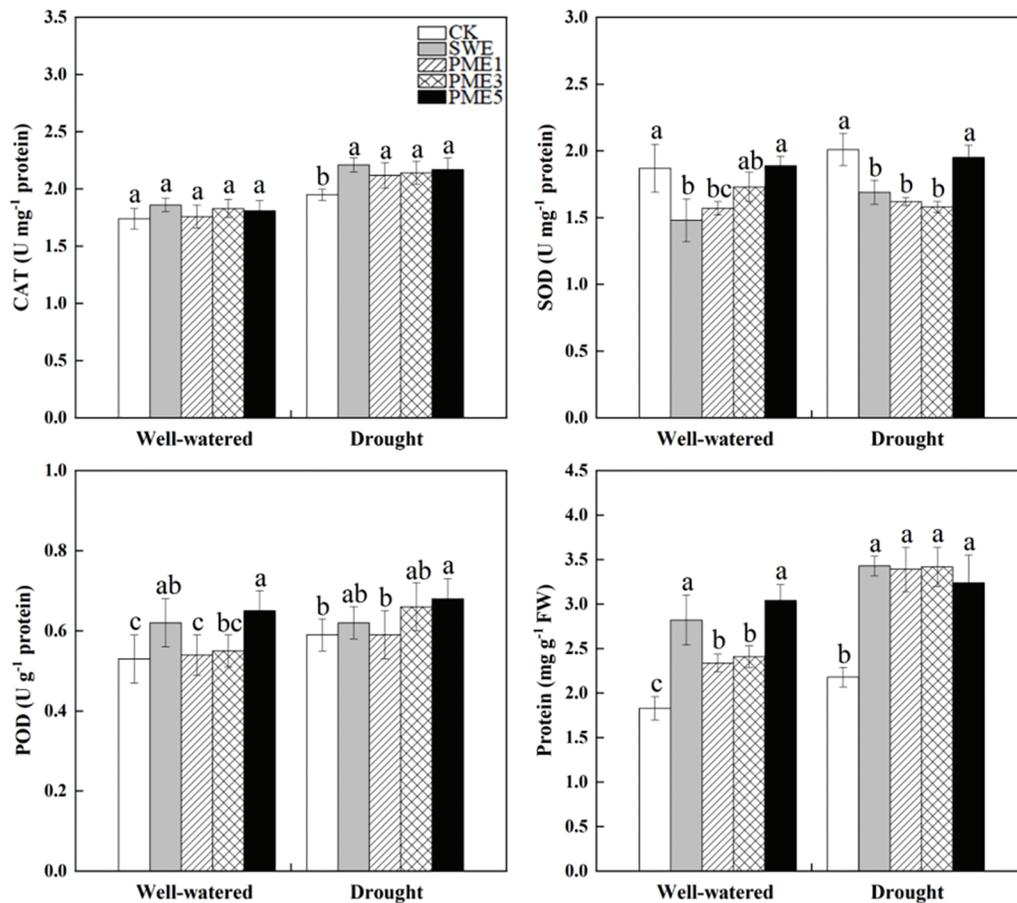


Figure 5: The analysis of variance of the effects of water conditions (well-watered and drought) and foliar application of SWE and PME treatments (1, 3 and 5 as concentration) on antioxidant enzyme activity and protein of maize. Different lowercase letters indicate significant influences between the same water condition with different treatments ($n = 4$, $p < 0.05$)

Protein was significantly affected by foliar spraying of SWE and PME and all treatments were higher in drought stress than in well-watered condition (Fig. 5). Under drought condition, PME treatments have the largest improvement compared to CK, with enhancements ranging from 108.2% to 138.8%. Under the drought condition, the protein content was improved obviously after treated with foliar spraying. Among all treatments, SWE, PME1, PME3 and PME5 were significantly higher than CK by 57.3%, 55.5%, 56.9% and 48.6%, respectively ($p < 0.05$).

3.7 Correlation Analysis of the Physiological Responses after Applying Biostimulants under Drought

In order to investigate potential correlations among various physiological and biochemical characteristics influenced by SWE and PME under drought-induced stress, a heat map be constructed (Fig. 6). The heat map visually represents the physiological and antioxidant-related responses of maize crop under two sets of conditions: well-watered conditions (with CK, SWE and PME treatments) and drought stress conditions (with CK, SWE and PME treatments). It can be seen from the heat map that plant height, FW, DW, number of leaves, RWC and sugar exhibited negative correlations with drought stress. Additionally, under drought stress condition, foliar spraying of PME resulted in significantly positive correlations with FW, DW, Chl b, carotenoid, RWC, sugar, GSH, protein and CAT activity, meanwhile the MDA and SOD activity exhibited a significant negative correlation. The heat map also shown that different physiological responses were increased with SWE treatment.

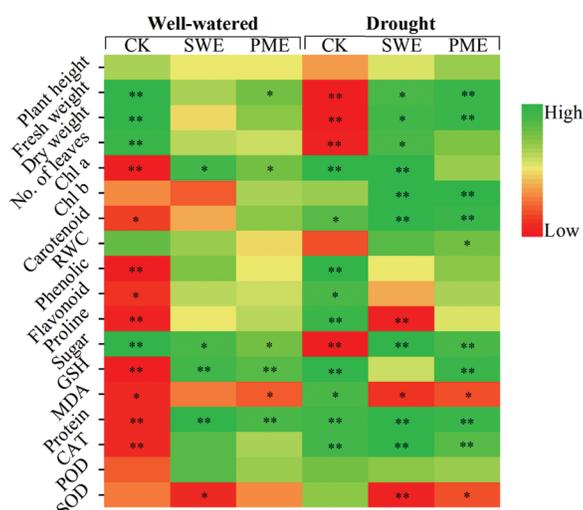


Figure 6: Overall relationship analysis of plant growth and physiological parameters in exogenous biostimulants application under drought stress; * $p < 0.05$; ** $p < 0.01$

4 Discussions

Drought stress has a detrimental impact on plant growth and development, influencing various physiological and biochemical characteristics [33,16]. The choice of maize as the subject of this study because of it is sensitive to water availability, making it a pivotal model for both physiological and agronomic studies. The adverse effects of drought stress on morphological aspects such as plant height and biomass weight and grain yield parameters have been reported in maize [34]. This study also indicated that drought stress reduced morphological parameters (plant height, FW, DW and number of leaves) compared with well-watered plants (Fig. 1). This is because water deficit causes stomatal closure and reduction of CO₂ intake which result in decrease of photosynthesis [25]. Besides, under well-watered and drought stress condition, foliar spraying of PME promoted almost all the morphological parameters compared to the control. This result is similar to that of the Rady et al. [35] and Anjum et al. [34] studies. According to the fact, *Polygonum minus* extract (PME) is rich in osmoprotectants (i.e., proline and soluble sugar), phytochemical compounds (i.e., phenolic and flavonoid), mineral nutrients (i.e., N, P, K, Ca, Mg, Fe, Cu and so on) and glutathione (Table 1). The presence of these key components makes PME potentially beneficial to plant growth. Furthermore, these components might be attributed to the improvement of chlorophyll levels (Fig. 2), osmoprotectant and antioxidant content (Fig. 4). Typically, RWC exhibit a decline when leaves face drought stress due to its strong correlation with water uptake

through the roots and water transpiration [36]. This study showed that application of PME leads to an increase in RWC under drought condition, which might be explained by reduced stomatal closure and increased efficiency of water uptake.

Drought stress primarily leads to the changes in plant water status that subsequently affects the transpiration rate and the stomatal conductance in leaves, limits CO₂ acquisition, and finally inhibits photosynthesis [37]. Interestingly, without the foliar application, drought still increased chlorophyll content (Fig. 2), which due to the concentrating effect caused by the limitation in plant growth [38]. Previous study has reported that foliar and drench application of SWE can improve stomatal conductance, transpiration and photosynthesis rate through reducing stomatal closure and increasing gas exchange values [20]. Our findings showed that the application of PME not only increased chlorophyll content under drought conditions, but also significantly increased chlorophyll content at higher concentrations (PME3 and PME5) under well-water condition (Fig. 2), confirming the ability of SWE to diffuse CO₂ and increase plant photosynthesis rate. This was possibly due to application of PME results in the accumulation of high levels of compatible solutes, decreased ROS levels and improved activities of chlorophyll synthesising enzymes to reduce chlorophyll degradation [39]. Additionally, the enrichment of growth stimulants such as amino acids, phytohormones and many mineral nutrient elements within PME contributes to the maintenance of the photosynthetic machinery through the regulation of the antioxidant defense system [40]. Phenolic and flavonoid compounds are major plant secondary metabolites that have strong antioxidant activities and important function in stress resistance [20]. Proline content has been associated with the accumulation of free radicals generated by abiotic stress factors [41]. In this study, total phenolic, flavonoid and proline content in maize leaves was observed to be higher under drought stress compared to well-watered condition, whereas the improvement effect of SWE and PME was not significant (Fig. 3). This result is not in accordance with Elansary et al. [20], who reported that phenolic and flavonoid content of *Spiraea* and *Pittosporum* were significantly increased with application of SWE. Therefore, the response of phytochemical compound and proline accumulation to exogenous biostimulants is not constant, and the extent of its accumulation may depends on the degree of drought and plant species or genetic factors. In addition, this study showed that the application of PME remarkably increased soluble sugar and protein in maize plants compared to the control under drought stress (Figs. 4 and 5). Similar results have been reported by Wang et al. [25], who revealed the sugar possessing key function in maintaining cell osmotic potential when plant be subjected to drought stress. Consequently, this result further indicated the adjustment ability of PME to osmolyte accumulation in response to stress. GSH as an antioxidant molecule and plays a critical function in maintaining the cellular redox balance and scavenging ROS in plant cells [42]. Our results also showed that foliar spraying of PWE can significantly increase GSH content compared to the control in both well-watered and drought stress conditions (Fig. 4), thus confirming the positive effect on antioxidant defense system. Although maize plants exhibited an increase in MDA levels under drought stress, application of PME significantly reduced MDA concentration compared to the control. MDA is used to characterize the degree of free radical damage to cell membrane caused by oxidative stress. This finding comprehensively elucidated that PME at a certain concentration, have a significant effect in protecting lipid peroxidation under drought stress.

Oxidative damage is closely related to the antioxidant defense mechanism, and plants with abiotic stress tolerance exhibited higher antioxidant enzyme activity [43]. Our observation showed that maize leaves exposed to drought stress displayed higher activity of the antioxidant enzymes (CAT, POD and SOD) compared to well-watered condition, and foliar spraying of PME further increased CAT and POD activities (Fig. 5), suggesting PME has the potential to mitigate oxidative damage by stimulating the activities of antioxidant enzymes. CAT plays a key role in the plant cell and convert H₂O₂ to water and oxygen, which leads to protecting photosynthesis against drought stress [44]. Similarly, application of exogenous

pollen grains extract, which contain amino acids, sugars, and nutrient elements, can increase the activities of CAT and SOD in *Ocimum basilicum* under drought stress [22]. In addition, maize crop was able to maintain POD activity for detoxifying ROS in response to water deficit [34]. Summarily, under both well-watered and drought stress conditions, maize was performed well with PME and SWE. In addition, PME is easy to prepare, extract and it is inexpensive. Therefore, it can be used as a natural bioactive mitigator to help plants to enhance their tolerance to drought stress.

5 Conclusions

Foliar application of PME exhibits notable biostimulatory effects and significantly improves maize growth performance by increasing chlorophyll and osmoprotectant content and stimulating antioxidant under drought stress. In addition, PME application significantly reduced oxidative damage caused by drought stress via improving accumulation of low molecule antioxidants and increasing antioxidant enzyme activities. Our findings clearly indicate that PME is an effective bioactive stimulant to alleviate negative effects of drought stress and has potential to improve crop yield. Nevertheless, the yield-related parameters and molecular mechanisms by which PME regulates drought resistance are not known and will be further validated in future field trials.

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Author Contributions: The authors confirm contribution to the paper as follows: Study conception and design: Mingzhao Han, Susilawati Kasim; data collection: Effyanti Mohd Shuib; analysis and interpretation of results: Zhongming Yang, Xi Deng and Md Kamal Uddin; draft manuscript preparation: Mingzhao Han and Noor Baity Saidi. All authors reviewed the results and approved the final version of the manuscript.

Availability of Data and Materials: The data that support this study will be shared upon reasonable request to the corresponding author.

Ethics Approval: Not applicable.

Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

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