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Morpho-physiological and Production Responses of Peanut (*Arachis hypogaea* L.) to Biostimulant Protein Hydrolysate Under Drought Stress

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ABSTRACT

Abiotic stress has been demonstrated to exert a deleterious effect on plant growth and development, culminating in diminished crop yields. This phenomenon has also been observed in peanuts. Among the various types of abiotic stress, drought is considered the most critical, as limited water availability has a negative impact on plant growth and development, especially in arid and semi-arid regions. This study aims to assess the impact of applying a protein hydrolysate biostimulant on the morphological, physiological, and production responses of groundnuts (*Arachis hypogaea* L.) variety 'Takar-2' under drought conditions. The observed parameters included plant height, root length, number of nodules, percentage of active nodules, chlorophyll a, b, total chlorophyll content, relative water content, and number of pods. The findings indicated that the application of protein hydrolysate led to substantial enhancements in the morphological adaptation of the plants, as evidenced by an increase in plant height, root length, and nodule number. Additionally, the application improved physiological parameters, including chlorophyll a and b, total chlorophyll, and relative water content. Furthermore, utilizing biostimulants positively affected the number of pods under drought conditions. Consequently, protein hydrolysate has the potential to function as a biostimulant, thereby enhancing the resilience and yield of groundnuts in environments with limited water availability.

Keywords: Drought stress, Peanut, Protein hydrolysate, Biostimulant.

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INTRODUCTION

Drought is a primary abiotic factor that impedes crop productivity in various agricultural regions, particularly tropical and subtropical areas. Drought stress has disrupted various physiological processes in plants, including water and nutrient uptake, photosynthesis rates, and vegetative and generative growth [1]. Peanuts (*Arachis hypogaea* L.), a prominent legume crop, exhibit heightened sensitivity to water deficiency, particularly during pivotal growth phases such as flowering and pod filling [2]. Consequently, developing agronomic strategies is imperative to enhance peanut tolerance to water stress conditions.

A growing body of research focuses on biostimulants, including protein hydrolysates [3]. Protein hydrolysates result from the enzymatic or chemical hydrolysis of animal or plant protein sources. They are rich in free amino acids, small peptides, and other bioactive compounds [4]. Many studies have demonstrated that applying protein hydrolysates can enhance water use efficiency, augment antioxidant enzyme activity, and improve plant morphological structure under stress conditions [5].

In the context of peanut cultivation, the application of protein hydrolysate is expected to strengthen plant adaptation to drought stress, both in terms of morphology (e.g., plant height, root length, number of leaves, and number of nodules) and physiology (e.g., chlorophyll content and relative water content) and yield (number of pods) [5]. However, the specific effects of protein hydrolysate on peanut responses to drought stress remain to be fully elucidated and require further research. The simulation of drought stress in plants can be achieved using polyethylene glycol (PEG) 6000 [6].

Considering the aforementioned background, this study aims to evaluate the impact of protein hydrolysate application on the morphology, physiology, and yield of peanuts cultivated under drought stress conditions. The findings of this study are anticipated to furnish a scientific foundation for the advancement of peanut cultivation techniques, rendering them more resilient to the impacts of climate change and water scarcity.

MATERIALS AND METHODS

The experiment was in September 2024 at the Greenhouse and Center for Excellence in Science & Technology for Industrial Plant Biotechnology (PUI-PT BioTIn), University of Jember, East Java, Indonesia (-8.164630 N and 113.716932 E).

Biostimulats Preparation

Peanuts were de-shelled and dried in a hot-air oven at 70 ◦C for 16 h. Dry peanuts were ground, defatted using hexane (1:5) for 6 h, and dried in a hot-air oven at 60 ◦C for 16 h. The defatted peanut powder was mixed with distilled water (1:5, w/v), and the pH of the slurry was adjusted to 10 using 1 M NaOH. The extract was processed using a magnetic stirrer at 50 ◦C for 1.5 h. The mixture was centrifuged at 6000 rpm for 10 min, and the supernatant was collected. The pH of the solution was adjusted to 4.5 using 1 M HCl and centrifuged again. The protein precipitates were collected, dissolved in phosphate buffer pH 8.5, and stirred using a magnetic stirrer overnight. The mixed solution was added with alcalase enzyme at a ratio of 1:10 (w/v) of the total protein and incubated in a water bath at 95◦C for 6 hours. It was then cooled to room temperature to inactivate the enzyme. The solution was then dried using freeze-drying.

Plant Material and Growth Conditions

The peanut seeds of the Takar-2 variety were planted in regosol soil. Peanut seeds were planted in 35x35 cm polybags containing regosol soil as the growing medium. Peanut plants aged 1-34 days were cared for with regular watering and fertilization. At 35 days, 500 ml/polybag of 10% PEG-6000 was applied twice a week for two weeks, along with 2 mg/200ml/polybag of protein hydrolysate. Drought stress was induced first, followed by treatment with protein hydrolysate. PEG-6000 and protein hydrolysate were applied directly to the growing medium. Leaf, root, and nodule samples were collected 50 days after treatment, and morphological and physiological variables were measured. The remaining plants were maintained until 110 days old, then harvested, and the peanut pods were collected.

Morpho-physiological Parameters

Morphological parameters were measured, including plant height, root length, number of root nodules, and number of pods per plant. The height of the plants was ascertained by measuring from the soil surface to the base of the leaf tips. The distance from the root tip to the base of the shoot was measured to determine the root length. The quantity of root nodules was ascertained by enumerating the root nodules affixed to the roots. The number of peanut pods per plant was subsequently enumerated. The measurements were taken after the plants had passed the stress phase, and the number of pods was measured at harvest time (110 days). Leaf samples were collected at 50 days after planting for analysis.

Chlorophyll Content

Chlorophyll contents were determined using 80% methanol to extract 0.3 g of leaf samples from each treatment. The extracts were centrifuged at 10.000 rpm for 10 minutes. Total chlorophyll was calculated based on absorbance (Abs) recorded at 665 nm, 652 nm, and 470 nm, using the equations provided by

$$\text{Chlorophyll a (mg/g)} = \frac{[(16{,}29\ x\ Abs665)-(8{,}54\ x\ Abs652)]}{1000}\ x\ volume/weight$$

$$\text{Chlorophyll b (mg/g)} = \frac{[(30{,}66\ x\ Abs652)-(13{,}58\ x\ Abs665)]}{1000}\ x\ volume/weight$$

$$\text{Total chlorophyll (mg/g)} = \frac{[(22{,}12\ x\ Abs665)+(2{,}71\ x\ Abs652)]}{1000}\ x\ volume/weight$$

Relative Water Content (RWC)

The relative water content was determined using the method of Terzi and Kadioglu [20]. Leaf samples (0.5 g) were saturated in 100 ml of distilled water for 24 hours at 4°C in the dark, and their saturated weights were recorded. The samples were oven dried at 65°C for 48 h, and their dry weights were recorded. RWC was calculated as follows:

$$\text{RWC (\%)} = \frac{\text{FW}-\text{DW}}{\text{SW}-\text{DW}} \text{x } 100\%$$

FW = fresh weight
SW = saturated weight
DW = dry weight

Statistical Analysis

The study used a non-factorial completely randomized design consisting of eight treatments with three replicates. The analysis used was ANOVA, followed by the DMRT test.

RESULT AND DISCUSSION

Response of Morphology in Peanut Plant After Treatment with Biostimulant Protein Hydrolysate and PEG

Applying PEG-6000 and biostimulant protein hydrolysate (PHs) resulted in significant differences in plant growth morphology between treatments. Peanut plants treated with PEG+PHs exhibited optimal height growth, with an average value of 64 cm. Conversely, the lowest recorded height gain was observed in the treatment PEG, with an average value of 55.66 cm. The root length parameter of peanut plants with optimal root length was found in the control treatment, with an average value of 38.18 cm, and the lowest was found in the control treatment PEG with an average of 26.72 cm. Consequently, applying biostimulants to peanut plants treated with PEG effectively suppresses drought-related symptoms.

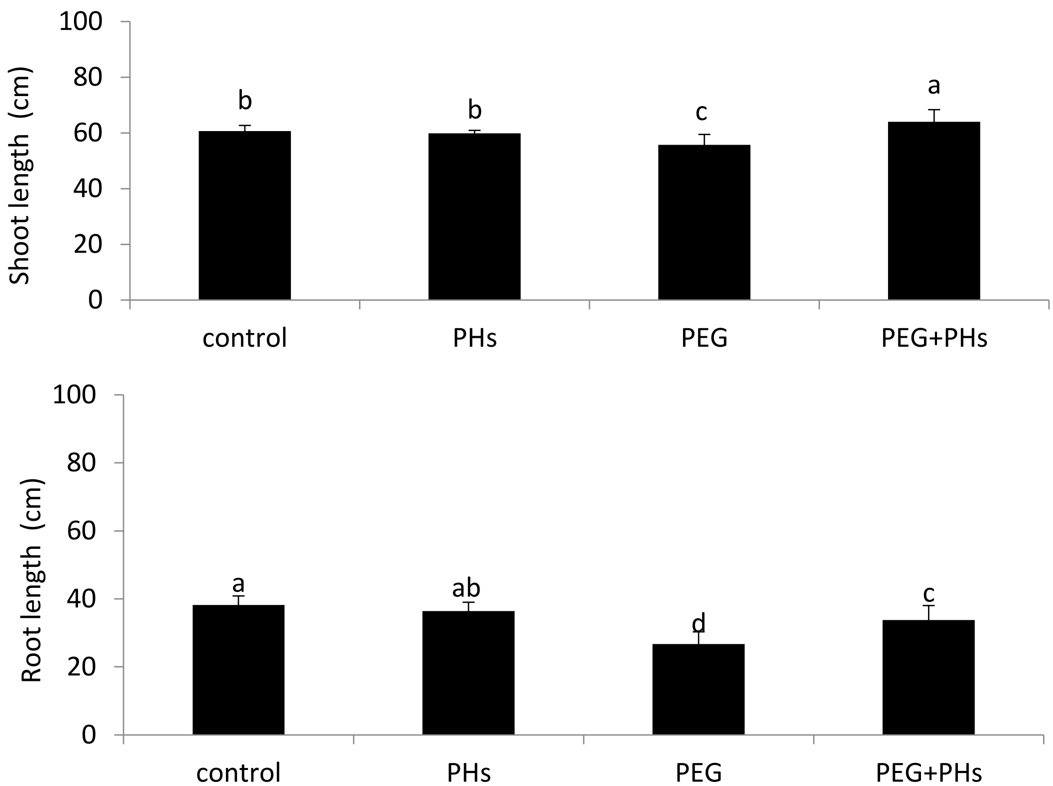

Fig. 1 Plant height and root length for the morphological appearance of peanut plants after treatment with protein hydrolysate (PHs). Different letters at the same level indicate a significant difference ($p < 0.05$)

PEG has been demonstrated to elicit a drought stress response in plants due to its capacity to bind water in the medium, thereby reducing water potential through osmotic stress mechanisms [7]. This results in a deficiency in water availability for plants. Drought has been demonstrated to have a deleterious effect on plant cells, reducing leaf water potential and cell turgor. This, in turn, has been shown to inhibit cell division and elongation. This phenomenon has the potential to influence the final height of the plants. Root growth constitutes a critical survival mechanism in conditions of stress. Acknowledging the significance of root development to optimize water efficiency and nutrient utilization is imperative. Consequently, peanut plant productivity can be enhanced, and the adverse effects of stress conditions can be mitigated [8].

Protein hydrolysate has been demonstrated to enhance nutrient availability, mitigate the adverse effects of chemical fertilizer use, and reduce the deleterious impacts of suboptimal growing conditions on plants [5]. In certain studies, the utilization of protein hydrolysate derived from leguminous plants has been observed to enhance the strength of the root system, thereby augmenting the efficiency of water and nutrient absorption [9]. Furthermore, a study by Parrado [10] demonstrated that applying protein hydrolysate led to an augmentation in plant height and the number of flowers in tomato plants.

Response of Nodulation in Peanut Plant After Treatment with Biostimulant Protein Hydrolysate and PEG

The following study will examine the effects of applying protein hydrolysate biostimulant and PEG-6000 on root nodules of peanut plants (Table 1). The highest number of nodules on peanut plants was observed in the treatment of PHs, with an average of 98 nodules, while the lowest number of nodules, with an average of 40 nodules, was observed in the treatment of PEG. The number of nodules in the treatment PEG differed significantly from each treatment, while the treatment of plants PEG, given the biostimulant, did not differ significantly from the control plants. The number of nodules on the roots of peanut plants was used to determine the percentage of active nodules. The highest percentage of active nodules was found in the PHs treatment, with an average value of 85%. The lowest percentage of active nodules was found in the PEG treatment, with an average value of 43%.

In Table 1, plants that received the control application PEG exhibited the lowest number of root nodules and percentage of active nodules compared to peanuts subjected to alternative treatments. This phenomenon can be attributed to the impact of drought on bacterial activity, which is known to decrease moisture levels in the soil. In moist soil conditions, which are conducive to bacterial growth, the reduction in moisture can hinder the formation of nodules, as observed in the study by [3]. Root nodule symbiosis forms root nodules through interaction between plants and rhizobium bacteria, which are free-living nitrogen-fixing bacteria [11]. Infected and active nitrogen-fixing root nodules exhibit a pink hue due to leghemoglobin.

Table 1: Effect after treatment with protein hydrolysate and PEG on nodulation of peanut plants.

Treatment	Number of nodules plant $^{-1}$	Active nodules plant $^{-1}$ (%)
Control	95 ± 2.52^{b}	71%
PHs	98 ± 2.00^{a}	76%
PEG	40 ± 1.53^{c}	43%
PEG+PHs	94 ± 2.52^{b}	66%

Protein hydrolysate (PHs). Different letters at the same level indicate a significant difference ($p < 0.05$).

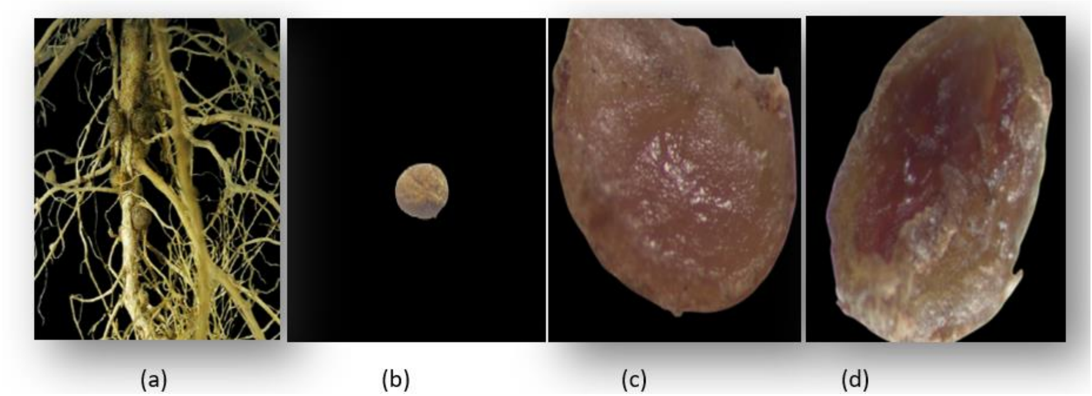

Fig. 2. (a) peanut plant root showing nodules attached to the roots, (b) appearance of intact root nodules, (c) Nodules from a peanut cut open to show the reddish-pink color that indicates an active, healthy nodule, and (d) dark brown or browning indicates inactive nodules.

In contrast, inactive nodules undergo leghemoglobin degradation, resulting in a brown appearance [12]. Biostimulants, in the form of protein hydrolysates, have been shown to enhance soil microbial activity under drought conditions. This effect is attributed to soil microbes, such as bacteria, using biostimulants as a food or nutrient source. Increased bacterial activity has positively impacted the interaction between bacteria and plants [5].

Response Chlorophyll a, b and Total Chlorophyll Content in Peanut Plant After Treatment with Biostimulant Protein Hydrolysate and PEG.

Table 2: Effect after treatment with protein hydrolysate and PEG on the chlorophyll of peanut plants.

Treatment	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Chlorophyll content (mg/g)
control	35.61 ± 0.18 ab	68.23 ± 0.32 b	101 ± 0.49 a
PHs	35.77 ± 0.21 a	68.65 ± 0.39 a	101 ± 0.56 a
PEG	26.93 ± 0.05 d	51.78 ± 0.06 d	76 ± 0.11 c
PEG+PHs	31.00 ± 0.05 c	59.50 ± 0.10 c	87 ± 0.15 b

Protein hydrolysate (PHs). Different letters at the same level indicate a significant difference ($p < 0.05$).

The study's results demonstrated that the levels of chlorophyll a, b, and total chlorophyll in peanut plants after administering protein hydrolysate and PEG varied significantly. In the treatment PEG, the chlorophyll a, b, and total chlorophyll content were lower than in the control treatment, with average values of 26.93, 51.78, and 76 mg/g, respectively. The values of chlorophyll a, b, and total chlorophyll in the PEG treatment were also lower than those in the PEG treatment given the protein hydrolysate biostimulant.

Chlorophyll is a green pigment in plants crucial for photosynthesis [13]. It has been demonstrated that the levels of chlorophyll a, b, and total chlorophyll will undergo a decline when plants are experiencing a state of drought stress. In response to drought conditions, plants have evolved mechanisms to conserve water, including stomata closure and reduced leaf area. This phenomenon can lead to a reduction in the uptake of carbon dioxide and the intensity of light reaching the chloroplasts, consequently decreasing the rate of photosynthesis and inducing chlorophyll degradation. Changes in cell membrane structure under stress cause a decrease in the amount of chlorophyll [14]. The utilization of protein hydrolysate biostimulants has been demonstrated to safeguard plants from declining chlorophyll content within the plant canopy under drought conditions. The application of biostimulants has been demonstrated to stimulate several physiological processes that enhance nutrient use efficiency and stimulate plant development. These processes include chlorophyll synthesis in plants [15]. Protein hydrolysate has been demonstrated to assist plants in counteracting chlorophyll content decline by increasing antioxidant enzymes and enzymes involved in chlorophyll biosynthesis [16]. In the study by Colla et al. [5], using protein hydrolysate combined with microorganisms increased chlorophyll content.

Response of Relative Water Content in Peanut Plant After Treatment with Biostimulant Protein Hydrolysate and PEG

The relative water content parameter is a reliable indicator of stress conditions in peanut plants. The graph above indicates that the control treatment exhibited the highest relative water content, 73.01%. Conversely, the lowest relative water content was observed in the PEG treatment, reaching 44.84%. The water content in plants is influenced by the environmental conditions they are exposed to. The water content in plants is a critical factor in photosynthesis, which requires water and carbon dioxide to produce energy in the form of carbohydrates and oxygen. The relative water content (RWC) measures the degree of hydration in cells and tissues, essential for plant physiological metabolism. It also describes how plants regulate and maintain optimal hydration levels under stress. In drought stress conditions, it becomes challenging for plants to sustain adequate levels of water content. The utilization of biostimulants has been demonstrated to facilitate the maintenance of elevated root water content (RWC) in plants. This augmentation in RWC is achieved through the enhancement of water uptake, the reduction of water loss through transpiration, and the enhancement of the plant's ability to adapt osmotically [17].

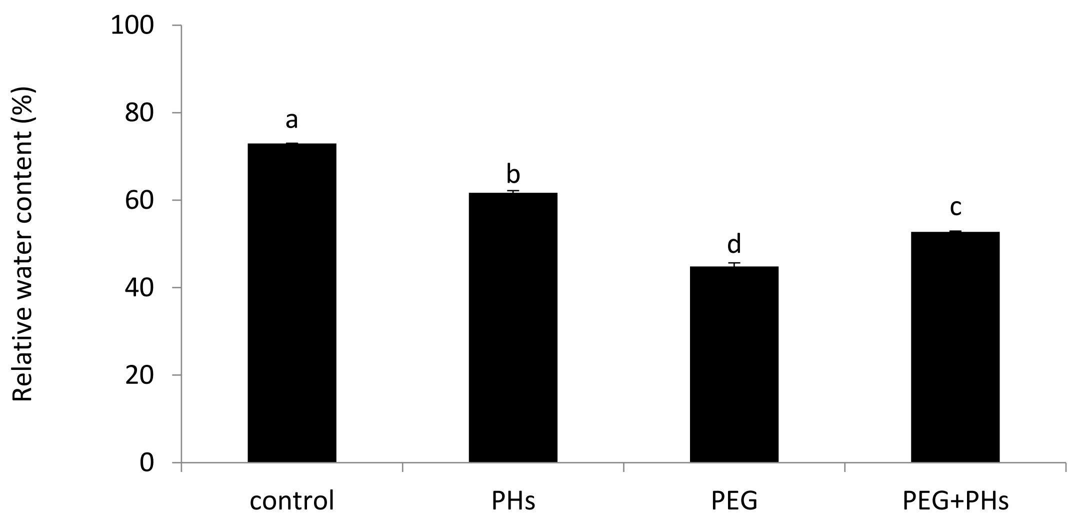

Fig. 3 Relative water content of peanut plants after treatment with protein hydrolysate (PHs). Different letters at the same level indicate a significant difference (p < 0.05)

Response of the Number Of Pods Content in Peanut Plant After Treatment with Biostimulant Protein Hydrolysate and PEG

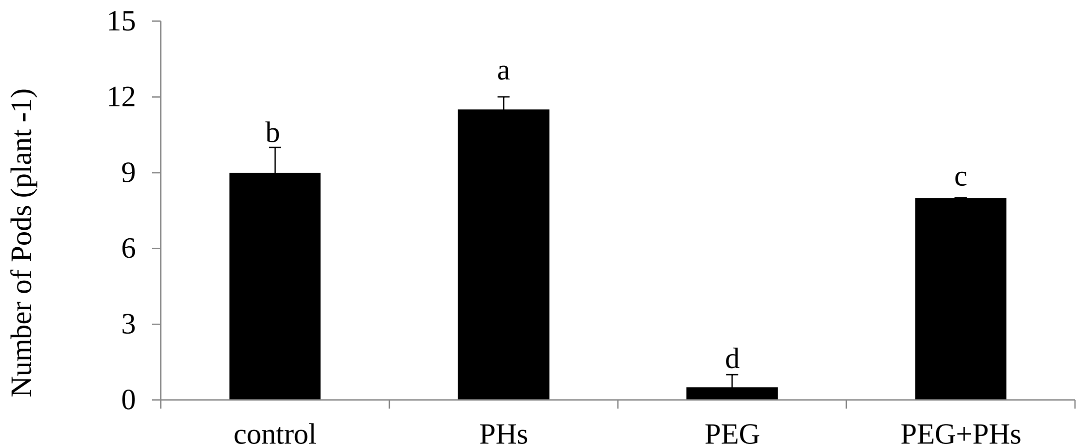

Fig. 4 Number of pods of peanut plants after treatment with protein hydrolysate (PHs) and PEG. Protein hydrolysate (PHs). Different letters at the same level indicate a significant difference ($p < 0.05$)

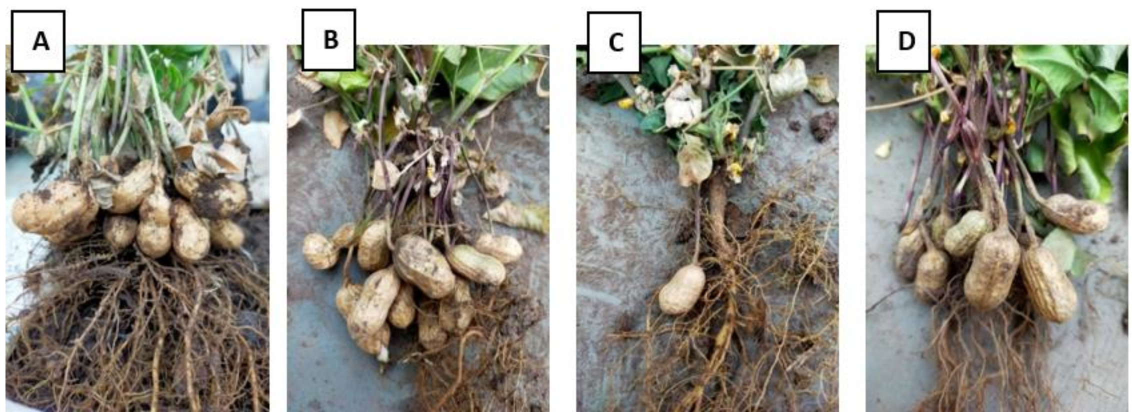

Fig. 5 Peanut pod yield at 110 days. Control (A), PHs (B), PEG (C) and PEG+PHs (D)

Peanut plants experiencing drought stress have been shown to affect the pod formation process, resulting in reduced yields. The highest number of pods was found in the PH treatment with an average value of 11.50 pods plant^{-1}, while the lowest was in the treatment PEG with an average value of 0.50 plant^{-1}. Using protein hydrolysates from legume plants can strengthen the root system, increasing the efficiency with which plants absorb water and nutrients [9]. Pod formation in legume plants is influenced by photosynthesis. Photosynthesis occurs in plant leaves with the help of sunlight. The basic ingredients of photosynthesis are water and carbon dioxide, which produce complex compounds such as carbohydrates, fats, proteins, and oxygen. These compounds are stored in stems, fruits, seeds, and pods. In legume plants, the results of photosynthesis are stored in pods. To optimize photosynthesis, plants must have access to organic material and sufficient water. Drought during the generative phase can inhibit pod formation. Pod formation in plants requires water. If environmental conditions provide sufficient water, photosynthate will accumulate properly in the formation of plant pods [16].

CONCLUSION

The application of protein hydrolysate biostimulants under drought stress affects the growth of peanut plants. The response of peanut plants varies across several parameters, including morphological characteristics, nodule formation, chlorophyll a, chlorophyll b, total chlorophyll, relative water content (RWC), and pod yield per plant. Further research is needed to confirm these findings through antioxidant enzyme activity and gene analysis.

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