
Physiological responses of chili pepper to foliar-applied seaweed biostimulant and humic acid under vegetative-stage drought stress

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Abstract Results showed that physiological responses of chili pepper to foliar application of seaweed extract (SE) from *Sargassum polycystum* and humic acid (HA) during the vegetative growth continued to be positive which is continued to develop under drought stress condition. Higher concentration (6%) of SE is required during the early stage of chili pepper growth and development, but lower SE concentration (2%) was more effective as plants continue to age. On the other hand, the effects of humic acid were much pronounced at the latter stage of chili pepper growth. The higher concentration of humic acid showed better physiological responses (chlorophyll a, chlorophyll b, and total chlorophyll, proline and nutrient uptakes) of chili peppers. Humic acid concentration of 1.50% was generally shown better at lower concentration. The significant interaction between seaweed extract and humic acid concentrations highlighted their complementary roles in regulating photosynthetic pigments and osmoprotective responses. Overall, these findings suggested that targeted biostimulant application enhanced physiological resilience and drought tolerance in chili pepper plants, offering a viable strategy for maintaining productivity in water-stressed environments.

Keywords: Chili pepper, Drought stress, Humic acid, Plant biostimulant, Seaweed extracts

Introduction

Global climate change has brought about significant influences on vegetable production practices by shifting planting season patterns, altering plant physiological responses, influencing the dynamics of pathogen, and increasing the geographical scope of drought-affected territories, especially within coastal zones (Hirpo and Gebeyehu, 2019). It appears that drought stress is considered as one of the most severe abiotic constraints on crop production within the tropical agroecosystems, where limitations on water availability often

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correspond with elevated requirements for evapotranspiration. Since the physiological processes of chili pepper (*Capsicum annuum* L.) is highly sensitive to moisture scarcity, with direct consequences for growth and yield determination (Ntanasi *et al.*, 2025), water deficits during vegetative growth might disrupt essential physiological processes that govern subsequent reproductive growth. During the vegetative stage, drought stress suppresses leaf expansion, chlorophyll content, and nutrient acquisition, thereby reducing biomass accumulation and yield potential. The vegetative phase is critical for accumulating energy investments that nourishes further crop productivity (Tjokrosumarto and Soedjarwo, 2017; Acharya *et al.*, 2022). It is, therefore, very critical to develop sustainable practices to increase drought tolerance during early growth of chili pepper grown under climate-driven water stress conditions.

Recently, the use of natural-based biostimulants has been increasingly practiced as an effective environmental-friendly input in boosting plant resilience to abiotic stresses. According to du Jardin (2015), such efficacy has primarily resulted from the adaptations of physiological and biochemical processes associated with stress tolerance, nutrient acquisition, and growth regulation. Seaweed extracts and humic substances are widely used among the naturally available biostimulants. Seaweed-based biostimulants derived from brown algae, *Sargassum* spp., contain bioactive compounds, including auxins, cytokinins, gibberellins, betaines, alginates, laminarin, fucoidan, amino acids, vitamins, and essential minerals (Shukla *et al.*, 2019). These active compounds increased plant resilience to drought stress conditions by strengthening antioxidant defence systems and promoting osmoprotectant accumulation (Khan *et al.*, 2009). It was reported that seaweed extracts improve photosynthetic efficiency, root system development, chlorophyll stability, and water-use efficiency under drought stress conditions (Rouphael and Colla, 2020). Foliar application of seaweed extracts is very effective in stimulating physiological responses due to the direct uptake of bioactive compounds through the leaf cuticle and stomata. Recently, Fahrurrozi *et al.* (2025) reported that *Sargassum polycystum* had high levels of photosynthetic pigments, water, ash content, proteins, lipids, carbohydrates, total phenolics, and 17 amino acids. Moreover, higher concentrations of *Sargassum*-based seaweed extract significantly enhanced growth performance of red chili plants under drought stress.

Humic acid, an important portion in soil fraction, is another source of natural biostimulant that might be combined with seaweed extracts to improve plant stress tolerance. According to Ampong *et al.* (2022) this substance is a complex organic compound resulted from organic matter humification and influences plant growth, hormone production, nutrient absorption and assimilation, protein synthesis, and yield. It was reported that the elevation of

crop yield and improved abiotic stress mitigation due to foliar or soil application of humic acid was attributed to the increasing production of secondary metabolites in plants (Chandrakant and Verma, 2023). Research conducted by Abu-Ria *et al.* (2025) concluded that under drought stress, humic acid increased activity of antioxidant enzymes, accumulation of osmolytes, and yield traits and quality. Moreover, humic acid also promotes root elongation and branching, thereby increasing the plant's ability to harvest moisture and nutrients at deeper soil layer (Calvo *et al.*, 2014).

The use of seaweed biostimulants together with humic acid might benefits chili peppers grown under drought stress condition due to their mutually beneficial modes of action. Seaweed extracts will primarily regulate physiological and biochemical processes, while humic acid promotes plant-soil interactions and root function. According to Rouphael *et al.* (2018), foliar application of these two components works together to boost photosynthesis, nutrient uptake, and metabolic pathways that respond to drought stress. However, scientific studies assessing the combined effects of these two biostimulants on chili pepper, particularly during the vegetative stage under drought stress, remain limited. Most research have focused on either fruit yield or post-flowering responses, but less information on how early physiological responses determine later productivity.

Extenuating drought stress during the vegetative phase of red chili is very important, since during vegetative growth, flowering, and fruit development represent the most water-sensitive stages in chili production (Wardani and Purwanta, 2008; Ricardez-Miranda *et al.*, 2021). Indeed, vegetative growth is very critical to plant growth since it creates the foundation for structural and physiological for canopy formation and carbohydrate assimilation, as well as the balance between roots and shoots. Preserving growth stability during this phase is crucial to supporting uninterrupted growth and successful reproductive development. The combined application of seaweed-based biostimulants and humic acid is expected to promote physiological resilience to drought stress during vegetative stages. Although much evidence claimed the individual benefits of seaweed-based biostimulants and humic acid, limited information is available regarding their interactive effects when applied foliarly under drought stress, particularly in chili pepper. Clarifying how these amendments affect chili pepper physiological responses is important for creating effective and sustainable drought mitigation strategies in chili pepper production. This is very relevant for smallholder farming systems in regions that are increasingly affected by climate-induced water scarcity. This research aimed to determine the application of seaweed-based biostimulants and humic acid on the physiological responses of

chili pepper, including pigment concentration, proline accumulation, and nutrient uptake, under vegetative-stage drought stress.

Materials and methods

Seaweed extracts and humic acid preparations

Seaweed samples of *Sargassum polycystum* were gathered from Kampung Melayu District, Bengkulu City, Indonesia (3°56'23.6" S, 102°16'43.2" E). The collected samples were washed to remove adhering debris, air-dried, packed in opaque plastic bags, and brought in a cooled container to the laboratory. The seaweed was subsequently air-dried for five days at ambient temperature (26–30 °C) under indirect light, followed by brief oven-drying at 60 °C for 5 min to ensure uniform moisture reduction. The dried material was then crushed and finely ground using a laboratory grinder, and the resulting powder was sieved through a 270-mesh screen to obtain a homogeneous particle size. The seaweed powder was stored in airtight containers until application to chili pepper plants. Commercial humic acid (AH-90; 90% active humic acid) was procured from the local market and used as the humic substance source in this study.

Growing red chilies in a greenhouse

Greenhouse experiment was conducted from August to December 2025 at Experimental Farm of Agriculture Faculty, University of Bengkulu, Indonesia (3°76'83.2" S and 102°26'89.28" E), at elevation of approximately 10 m above sea level. Red chilies (*cv. Tangguh F-1*) were planted using 4-week-old seedlings in 8-kg polybags in the mixture growing medium of soil, rice husks, and vermicompost in a 2:1:1 ratio. The cultivation practices and maintenances of red chili plants followed cultivation standards adapted to research conditions (Herison *et al.*, 2020).

Experimental design and treatments

The study used a three factors factorial experiment in completely randomized design. The first factor was drought stress (DS); (1) DS0=without drought stress and (2) DS1=7 weeks of drought stress. The second factor was seaweed extract (SE) concentration; (1) SE1=2%, (2) SE2=4%, (3) SE3=6%, and the third factor was humic acid (HA) concentration; (1) H1=0%, (2) H2=0.75% and (3) H3=1.50%. Each treatment combination was repeated three times, and each treatment unit contained four plants.

Treatment application

For drought stress treatment, plants were not watered until 75% of the shoots showed wilting symptoms and then watered again to field capacity the following day (Airlangga *et al.*, 2023). Humic acid was mixed evenly with the exogenous seaweed-based osmoprotectant treatment concentrations according to each concentration. The seaweed-based osmoprotectant and humic acid were applied to chili plants aged 20, 45, and 65 days after transplanting (DAT). Applications were conducted in the morning, between 7:00 and 8:30 a.m., evenly wetting all parts of the plant.

Pigment analysis

Pigment contents (chlorophyll a, chlorophyll b, and total chlorophyll) were determined at 45 and 65 days after transplanting. Sample of chili pepper leaves (0.5 g of fresh samples) were extracted using 80% acetone, the absorbances of this extract were then measured at 480 nm (A480), 645 nm (A645), and 663 nm (A663) were measured using a UV/Visible Spectrophotometer. Han *et al.* (2023) formulated the calculation for concentrations (moly/L) of chlorophyll a, chlorophyll b, and total chlorophyll were chlorophyll a (Ca) = $(12.72 \cdot A_{663} - 2.59 \cdot A_{645})$, and chlorophyll b (Cb) = $(22.9 \cdot A_{645} - 4.67 \cdot A_{663})$. For total chlorophyll in plant tissues was calculated by summing Ca + Cb.

Proline analysis

The physiological response of red chili plants observed was the concentration of proline in the leaves, which was observed at 20 (before treatment application), 45, and 65 days after planting. Proline is a very capable chemical indicator for detecting how pepper plants respond to drought stress (Masoumi *et al.*, 2024). Proline determination was conducted followed method proposed by (Prameswari *et al.*, 2025), started with homogenizing fresh leaf tissues (0.2–0.5 g) in 10 mL 3% sulfosalicylic acid and centrifuged (3,000 rpm, 10 min). The supernatant (2 mL) was combined with 2 mL acid ninhydrin reagent and 2 mL glacial acetic acid, then incubated at 100 °C for one hour After cooling, 4 mL of toluene was added and vortexed, and the colourful top phase was recovered. At 520 nm, absorbance was measured in comparison to a toluene blank. A standard curve was created using L-proline, and proline content was expressed as $\mu\text{mol g}^{-1}$ fresh weight based on sample weight and extract volume.

Nutrient uptakes

Determination of nutrient N, P, K and Ca uptakes of chili peppers was conducted at 60 days after transplanting by taking the leaf samples from fully developed leaves, typically at the third leaf from upper most expanded leaves. Samples were cleaned and dried in oven at 60-70°C for further nutrient content analysis. Nutrient content (N, P, K, Ca) determination was carried out with reference to the method according to Kalra (1997) at the Agronomy Laboratory of Bengkulu University. Nutrient uptakes (g plant^{-1}) were then calculated followed Ardakani *et al.* (2011), as $= \text{PNC}/100 \text{ SDW}$, where PNC is plant nutrient (%) and SDW is shoot dry weight per plant (g).

Statistical analysis

Prior to analysis of variance using the Statistical Analysis System (SAS) at $P \leq 0.05$, the data underwent a homogeneity test. Mean treatment comparisons for significant variables were determined by using Least Significant Different test at $P \leq 0.05$.

Results

Result indicated that the three treatment factors, drought stress (DS), seaweed extracts (SE) and humic acid (HA) generally had a highly significant effect on plant physiological variables at both 45 and 65 days after transplanting (DAT). During the initial phase of 45 DAT, the content of chlorophyll a, chlorophyll b, and total chlorophyll had a very significant effect on all single factors and their interactions, except for the three-way interactions ($\text{DS} \times \text{SE} \times \text{HA}$) on total chlorophyll, which had no significant effect (Table 1). It indicated that the combination of treatments is played a greater role in each pair of factors than the interaction of the three treatment combinations.

Results also showed that proline accumulation at 45 DAT had highly significant effects for all treatment factors and their interactions, indicating a physiological response of the plant to treatment conditions, especially under stress. Increased proline content is considered an adaptive mechanism in response to suboptimal environmental conditions. At 65 DAT, the treatment effects showed that all parameters of chlorophyll a, chlorophyll b, total chlorophyll, and proline content had significantly affected on all single treatment factors and their interactions. It indicated that the effect of treatment became cumulative and complex as the plants continued to grow. Nutrient content was significantly affected by the single factors DS and SE, as well as by their

interactions, whereas no significant effect was observed for the single factor HA. Furthermore, P and K uptake were significantly influenced by all single factors and their interactions. It indicated that applying all three treatments enhanced the uptakes of these two elements. Meanwhile, Ca uptake was only significantly affected by the single factors DS and SE.

Table 1. Summary of ANOVA F-values showing the effects of treatments on physiological traits and nutrient uptakes of chili pepper plants at 45 and 65 days after transplanting (DAT)

Variable	DS	SE	HA	DS x SE	DS x HA	SE x HA	DS x SE x HA
<i>45 days after transplanting (DAT)</i>							
Chlorophyll a	1015.58*	248.76**	7.58**	93.17**	18.69**	126.93**	15.26**
Chlorophyll b	315.05**	64.28**	4.37**	55.57**	39.06**	55.68**	13.19**
Total Chlorophyll	2028.41*	466.64**	11.73**	214.91**	83.86**	250.97**	2.15ns
Proline	3.78**	15.56**	2.22**	12.73**	11.17**	2.61**	8.72**
<i>65 days after transplanting (DAT)</i>							
Chlorophyll a	62.21**	471.06**	265.98**	449.67**	286.26**	76.27**	130.59**
Chlorophyll b	639.49**	482.79**	1098.56**	1239.25**	1362.12**	273.39**	265.40**
Total Chlorophyll	3881.49*	10869.07*	10491.85**	15082.26**	12055.48**	2241.12**	3020.35**
Proline	24.49**	283.82**	74.53**	29.68**	262.21**	73.74**	190.81**
<i>Nutrient uptakes</i>							
N	143.20**	17.93**	1.84ns	19.77**	12.08**	7.56**	3.91**
P	1.55**	143.69**	47.81**	107.50**	3.79**	27.27**	42.38**
K	3.17**	143.41**	86.79**	124.04**	4.38**	30.96**	44.80**
Ca	8.77**	4.45*	2.59ns	1.22ns	0.39ns	0.95ns	1.42ns

Noted: DS = drought stress, SE = *Sargassum* sp. extract, HA = humic acid, ** = highly significant difference, * = significant difference at $P \leq 0.05$, ns = not significant effect.

Chili pepper grown under drought stress conditions were significantly differed for all physiological variables at both 45 and 65 DAT, except for proline content at 45 DAT (Table 2). Meanwhile, drought stress was significantly affected N and Ca uptake but had not significantly affected on P and K uptake. Results also indicated that chlorophyll a, chlorophyll b, and total chlorophyll contents showed significant differences between non-drought and drought-

stressed conditions at 45 DAT. Chlorophyll a content decreased from 16.93 mg g⁻¹ (non-drought stress) to 10.68 mg g⁻¹ under drought stress conditions. Likewise, chlorophyll b content decreased from 7.46 mg g⁻¹ (non-drought stress) to 4.44 mg g⁻¹ (under drought stress), and total chlorophyll content also decreased significantly from 24.38 mg g⁻¹ (non-drought stress) to 15.12 mg g⁻¹ (under drought stress). In contrast, proline content at 45 DAT did not significant different between treatments; 0.29 µmol g⁻¹ (non-drought stress) and 0.23 µmol g⁻¹ (under drought stress).

Table 2. The effect of drought stress conditions on physiological variables and nutrient uptakes of chili pepper at 45 and 65 days after transplanting (DAT)

Treatments (Stress)	Variables							
	Chlo a (mg/g)	Chlo b (mg/g)	Total Chlo (mg/g)	Proline (µ mol/g)	Chlo a (mg/g)	Chlo b (mg/g)	Total Chlo (mg/g)	Proline (µ mol/g)
<i>45 days after transplanting (DAT)</i>				<i>65 days after transplanting (DAT)</i>				
Non-drought	16.93a	7.46a	24.38a	0.29a	12.67a	4.70a	17.31a	0.45a
Drought	10.68b	4.44b	15.12b	0.23a	12.16b	4.07b	16.23b	0.40b
<i>Nutrient uptakes</i>								
	N (%)		P (%)		K (%)		Ca (%)	
Non-drought	2.49b		0.28a		0.96a		0.26b	
Drought	3.06a		0.29a		1.00a		0.36a	

Notes: The means sharing same letters within a same column are statistically non-significant (Least Significant Different Tests at P≤0.05).

Similar observations were also found in the variables of chlorophyll a, chlorophyll b, total chlorophyll, and proline content at 65 DAT. Chlorophyll a content was significantly differed between conditions without drought stress and under drought stress, namely 12.67 mg g⁻¹ and 12.160 mg g⁻¹, respectively. In addition to chlorophyll a content, chlorophyll b and total chlorophyll content at 65 DAT was significantly differed, namely 4.70 mg g⁻¹ (non-drought stress) and 4.07 mg g⁻¹ (under drought stress) for chlorophyll b, and 17.31 mg g⁻¹ (non-drought stress) and 16.23 mg g⁻¹ (under drought stress) for total chlorophyll. Meanwhile, the proline content was significantly was significantly differed between conditions without drought stress and under drought stress, namely 0.45 µmol g⁻¹ and 0.40 µmol g⁻¹, respectively. In addition, nitrogen (N) and calcium (Ca) content showed significant differences between treatments without drought stress and drought stress, namely 2.49% (non-drought stress) and 3.06% (under drought stress) for N uptake, and 0.26% (non-drought stress) and 0.36% (under drought stress) for Ca uptake, respectively. However, in contrast, phosphate (P) and potassium (K) uptake showed no significant differences between non-

drought stress and drought stress conditions, namely 0.28% and 0.29% for P uptake, and 0.96% and 1.00% for K uptake, respectively.

Results revealed that the concentration of seaweed extract (SE) treatment showed significant differences in all physiological variables and nutrient uptake in chili plants at both 45 and 65 DAT (Table 3). Chlorophyll a and total chlorophyll content at 45 DAT showed significant differences between SE concentration treatments in chili plants, where the highest chlorophyll a content was at a concentration of 6% Sargassum sp. extract of 16.84 mg g⁻¹, then followed by 4% SE (12.80 mg g⁻¹) and 2% SE (11.77 mg g⁻¹). Meanwhile, the highest total chlorophyll content was 24.15 mg g⁻¹ (SE 6%), followed by 18.09 mg g⁻¹ (SE 4%) and 17.01 mg g⁻¹ (SE 2%). In contrast, proline content at 45 DAT showed a significant difference between SE 6% (0.38 µmol g⁻¹) and SE 4% (0.19 µmol g⁻¹), but not significantly different between SE 2% (0.21 µmol g⁻¹) and SE 4% (0.19 µmol g⁻¹). At 65 DAT, chlorophyll b content showed no significant difference between SE 2% (4.63 mg g⁻¹) and SE 4% (4.68 mg g⁻¹) but was significantly different from SE 6% (3.84 mg g⁻¹). The highest proline content at 65 DAT was SE 2% (0.55 µmol g⁻¹), followed by SE 4% (0.43 µmol g⁻¹) and SE 6% (0.29 µmol g⁻¹).

Table 3. The effect of seaweed extract concentration on physiological variables and nutrient uptakes of chili pepper at 45 and 65 days after transplanting (DAT)

Seaweed extract concentration	Variables							
	Chlo a (mg/g)	Chlo b (mg/g)	Total Chlo (mg/g)	Proline (µ mol/g)	Chlo a (mg/g)	Chlo b (mg/g)	Total Chlo (mg/g)	Proline (µ mol/g)
<i>45 days after transplanting (DAT)</i>				<i>65 days after transplanting (DAT)</i>				
2%	11.77c	5.24b	17.01c	0.21b	13.58a	4.63a	18.21a	0.55a
4%	12.80b	5.29b	18.09b	0.19b	12.51b	4.68a	17.19b	0.43b
6%	16.84a	7.31a	24.15a	0.38a	11.15c	3.84b	14.99c	0.29c
<i>Nutrient uptakes</i>								
	N (%)		P (%)		K (%)		Ca (%)	
2%	2.58b		0.21c		0.76c		0.25b	
4%	2.92a		0.28b		0.90b		0.33ab	
6%	2.83a		0.39a		1.27a		0.36a	

Notes: The means sharing same letters within a same column are statistically non-significant (Least Significant Different Tests at P≤0.05).

N uptake was not significantly differed between SE 4% and SE 6% (2.92% and 2.83%, respectively), but both were significantly different from SE 2% (2.58%). Meanwhile, P and K uptake showed significant differences between the concentrations of seaweed extract, where P and K uptake for SE 2% (0.21% and 0.76%), SE 4% (0.28% and 0.90%), and SE 6% (0.39% and 1.27%), respectively (Table 3).

The effects of humic acid concentrations had a significantly different impact on some physiological variables (chlorophyll a, chlorophyll b, and total chlorophyll) in chili plants except for proline content at 45 DAT, as well as almost all nutrient uptake variables, except for N uptake (Table 4). The total chlorophyll content at 45 DAT had no significant difference between the humic acid (HA) concentrations of 0% and 1.5%, namely 19.91 mg g⁻¹ and 20.26 mg g⁻¹, respectively. However, both were significantly different from HA 0.75% at 19.08 mg g⁻¹. Meanwhile, the proline content at 45 DAT showed no significant difference among HA concentrations, with the highest proline content observed in HA 0% (0.30 µmol g⁻¹), HA 0.75% (0.25 µmol g⁻¹), and HA 1.50% (0.23 µmol g⁻¹). At 65 DAT, chlorophyll a and chlorophyll b contents showed significant differences between various HA concentrations. Chlorophyll b contents showed significant differences between HA 0% (3.61 mg g⁻¹), HA 0.75% (4.52 mg g⁻¹), and HA 1.50% (5.03 mg g⁻¹), respectively. While proline contents showed no significant differences between HA 0.75% (0.45 µmol g⁻¹) and HA 1.50% (0.47 µmol g⁻¹), both were significantly different from HA 0% (0.35 µmol g⁻¹).

Table 4. The effect of humic acid concentration on physiological variables and nutrient uptakes of chili pepper at 45 and 65 days after transplanting (DAT)

Humic acid Concentration	Variables							
	Chlo a (mg/g)	Chlo b (mg/g)	Total Chlo (mg/g)	Proline (µ mol/g)	Chlo a (mg/g)	Chlo b (mg/g)	Total Chlo (mg/g)	Proline (µ mol/g)
<i>45 days after transplanting (DAT)</i>				<i>65 days after transplanting (DAT)</i>				
0%	14.17a	5.74b	19.91a	0.30a	11.39c	3.61c	15.00c	0.35b
0.75%	13.97a	5.80b	19.08b	0.25a	12.74b	4.52b	17.26b	0.45a
1.50%	13.27b	6.30a	20.26a	0.23a	13.13a	5.03a	18.14a	0.47a
<i>Nutrient uptakes</i>								
	N (%)		P (%)		K (%)		Ca (%)	
0%	2.79a		0.24c		0.81c		0.27b	
0.75%	2.71a		0.29b		0.93b		0.36a	
1.50%	2.82a		0.34a		1.20a		0.31ab	

Notes: The means sharing same letters within a same column are statistically non-significant (Least Significant Different Tests at P≤0.05).

In addition, P uptake showed significant differences among the three treatments of HA concentration, with the highest P uptake noticed with HA 1.50% at 0.34%, followed by HA 0.75% at 0.29%, and HA 0% at 0.24%. Along with P uptake, K uptake also showed significant differences among the three HA concentrations. The highest K uptake content was found in HA 1.50% (1.20%), followed by HA 0.75% (0.93%) and HA 0% (0.81%). However, N uptake had no significant difference among the three HA concentrations, with values of 2.71% (HA 0.75%), 2.79% (HA 0%), and 2.82% (HA 1.50%), from the lowest

to the highest absorption value. Ca absorption showed no significant difference between the concentration of HA 0.75% and HA 1.50%, namely 0.36% and 0.31%, respectively, while HA 0.75% was significantly different from HA 0%, where the Ca absorption content value of HA 0% was 0.27% (Table 4).

The interaction between SE and HA concentrations at 65 DAT resulted in significant variations in chlorophyll *a*, chlorophyll *b*, total chlorophyll, and proline contents (Table 5). The combination of 2% SE with 0.75% HA produced a significantly higher chlorophyll *a* content compared with the combinations of 2% SE with 1.50% and 0% HA, yielding values of 14.40, 14.28, and 12.30 mg g⁻¹, respectively.

Table 5. Interaction of seaweed extract concentration and humic acid concentration on chlorophyll *a*, chlorophyll *b*, total chlorophyll, and proline content at 65 days after transplanting (DAT)

Seaweed extracts concentration	Humic Acid (%)		
	0	0.75	1.50
<i>Chlorophyll a (mg/g)</i>			
2%	12.02 b/A	14.40 a/A	14.28 a/A
4%	12.56 ab/A	12.26 b/AB	12.70 a/B
6%	9.53 c/B	11.54 b/B	12.09 a/B
<i>Chlorophyll b (mg/g)</i>			
2%	6.10 a/B	4.85 b/AB	4.73 ab/C
4%	6.58 a/AB	4.22 c/C	5.08 b/B
6%	6.96 b/A	5.87 c/A	9.08 a/A
<i>Total Chlorophyll (mg/g)</i>			
2%	18.17 a/C	16.16 b/B	16.64 ab/C
4%	21.96 a/B	13.60 c/C	18.67 b/B
6%	25.60 a/A	21.37 b/A	25.43 ab/A
<i>Proline (μ mol/g)</i>			
2%	0.230 a/B	0.197 b/B	0.183 b/B
4%	0.170 b/C	0.162 b/C	0.215 a/A
6%	0.490 a/A	0.360 b/A	0.128 c/C

Notes: The means sharing the same lower and upper cases letters are not significant difference among the humic acid and seaweed extract concentration, respectively (Least Significant Different Tests at $P \leq 0.05$).

For chlorophyll *b*, a significant difference was observed between the treatment combining 6% SE with 1.50% HA and that combining 6% SE with 0.75% HA, with corresponding values of 9.08 and 5.87 mg g⁻¹. Moreover, total chlorophyll content differed significantly between the combinations of 2% seaweed extract with 0% humic acid and 2% SE with 0.75% HA, recording 18.17 and 16.16 mg g⁻¹, respectively. A similar interactive effect was evident for proline accumulation, where the combination of 4% SE and 1.50% HA resulted in a significantly higher proline content than the combination of 4% SE and 0.75% HA, with values of 0.215 and 0.162 μmol g⁻¹, respectively.

Discussion

The results showed that drought stress (DS), seaweed extract concentration (SE), and humic acid concentration (HA) significantly affected all physiological variables and nutrient uptake in chili plants. Drought stress significantly reduced chlorophyll a, chlorophyll b, and total chlorophyll content at 45 and 65 DAT, indicating there were biochemical, metabolisms and photosynthesis disruptions in plant grown under water-deficient conditions through reduced leaf pigmentation and impaired chloroplast function. Such reductions are commonly associated with drought-induced inhibition of chlorophyll biosynthesis (Zandalinas *et al.*, 2020). Decreased chlorophyll levels in plants can cause tissue damage, inhibit biochemical pathways, and disrupt plant physiology under limited water-availability condition (Anjum *et al.*, 2020). In addition, the insignificant effects of drought stress on proline level at 45 DAT, but conversely at 65 DAT, indicated there was a gradual adjustment osmotic pressure in chili peppers. It appeared that non-drought stress chili pepper had higher proline contents than drought-stressed plants (Table 2). According to Ashraf and Foolad (2007), proline generally plays a vital role as an osmoprotectant, stabilizing membranes and proteins and maintaining tissue balance under drought-stress conditions. The significant increase in proline content at 65 DAT indicated that an activation mechanism is involved in adjusting osmotic pressure under drought stress. This is suspected because proline content has an important role in ROS absorption, osmoprotection, and plant membrane stability (Szabados and Savaure, 2010). In addition, the increase in nutrient uptake of N and Ca under drought stress indicates a shift in nutrient uptake induced by stress and the involvement of Ca nutrients in sending stress signals and stabilizing membrane structures, which are adaptive responses of plants under abiotic stress, especially drought (Thor, 2019).

The effects of SE generally increased all physiological variables and nutrient uptakes. The use of higher concentrations of SE increased chlorophyll contents and proline contents during early plant growth. Such increases might have been resulted from the bioactive compounds presence in the SE that effectively boosted the growth performance of red-chili peppers grown under drought-stress conditions (Fahrurrozi *et al.*, 2025). Previously, Calvo *et al.* (2014) and Ali *et al.* (2021) suggested that the bioactive compounds, such as cytokinins, auxins, amino acids, betaines, and polysaccharides, present in the SE were able to support chlorophyll biosynthesis and delay aging or damage to plants. Surprisingly, at 65 DAT, the highest chlorophyll content was observed in the 2% SE treatment, and the lowest in the 6% SE treatment. The effectiveness of the 2% concentration indicated that moderate concentration may optimize physiological responses under drought-stress conditions, while excessive

concentrations may disrupt metabolic balance. In contrast, under non-drought conditions, higher SE concentrations (6%) significantly enhanced chlorophyll *a*, chlorophyll *b*, total chlorophyll, and proline contents. The higher concentration of SE might have brought about biochemical and physiological imbalances in plants, thereby inhibiting their metabolic processes. Indeed, Sharma *et al.* (2014) concluded that optimal concentrations of seaweed extract can increase growth, but higher concentrations might inhibit plant growth, physiology, and biochemistry.

The application of humic acid (HA) during the vegetative of red chili growth tended to reduce chlorophyll content (chlorophyll *a*, chlorophyll *b*, and total chlorophyll) and proline. Such response is presumably related to the initial adaptation process of plants to the effects of HA in rhizosphere conditions and the dynamics of nutrient availability. At this stage, the ability of HA the ability to chelate essential nutrients such as Fe brings about a temporary delay in its to plants, so that chlorophyll synthesis does not optimally take place (Chen *et al.*, 2004). Similar delay might also happen to other nutrients responsible in chlorophyll formations. In addition, energy allocations in young plants are more focused on developing the root system in response to HA stimulation, which results in a temporary decrease in photosynthetic activity (Canellas and Olivares, 2014). As chili peppers grow, the promoting effects of HA start to dominate through increased cation exchange capacity, soil microbial activity, and nutrient absorption efficiency the other hand, increase proline levels in the later phase chili pepper indicated the role of humic acid in increasing plant adaptation to stress through osmoregulation mechanisms (Calvo *et al.*, 2014).

The significant interaction between SE and HA during the vegetative stage of chili pepper was interesting due to their mutually beneficial modes of action. This demonstrates the complementary roles of these biostimulants in regulating photosynthetic pigments and osmolyte accumulation. It appeared that the choice of application could be 4% SE with 0.75% HA, or 6% SE with 0% HA. Specific treatment combinations enhanced chlorophyll *a* or chlorophyll *b*, while others preferentially increased proline content, suggesting that application of this combination at particular pairs was able to promote crop photosynthesis as well as to improve drought tolerance mechanisms in red chili peppers. Liang *et al.*, (2013) concluded that proline accumulation under stress conditions functions as an osmoprotectant, stabilizing proteins and membranes while scavenging reactive oxygen species.

In conclusion, foliar application of SE and HA is very promising biostimulant to increase physiological resistance of red chili peppers to drought stress condition. Higher concentration of SE during the initial stage of vegetative growth is crucially important to stimulate initial crop responses. Meanwhile, HA

play more roles in increase nutrient uptakes and crop adaptation during the subsequent growth. Combined applications of SE and HA. It is suggested that for optimal red chili responses, the application of both SE and HA must be adjusted accordingly to the phases of growth and development. Further research might be addressed to specific dosages and time of applications as well as the molecular studies of red chili responses.

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Conflicts of interest

The authors declare no conflict of interest.

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