International Journal of Basic and Applied Science 13 (2) (2024) 61-73

Published by: IOCSCIENCE

International Journal of Basic and Applied Science

Journal homepage: www.ijobas.pelnus.ac.id

Growth and biochemical responses of red chili (*Capsicum annuum* L) under drought conditions with 6-Benzylaminopurine application

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Article Info

Article history:

Received Jul 08, 2024
Revised Jul 17, 2024
Accepted Sep 23, 2024

Keywords:

6-Benzylaminopurine (BAP);
Biochemical response;
Drought stress;
Red chili.

ABSTRACT

The purpose of this research is to assess how red chili plants (Capsicum annuum L.) develop and react biochemically to drought stress, as well as how cytokinin treatment affects these plants. The study employed a factorial Randomized Block Design (RAK) with three replications and two components, namely the degree of drought with three stages, comprising: K1 has an 80% soil water content, K2 has a 60% soil water content, K3 has a 40% soil water content, and S0, S1, S2, and S3 have 0 ppm, 10 ppm, 20 ppm, and 30 ppm of 6-benzylaminopurine concentration, respectively. Plant height, leaf count, root length, flowering age, total and aqueous chlorophyll content, activity of antioxidant enzymes (e.g., superoxide dismutase and peroxide dismutase), and hydrogen peroxide as a signal for plant molecules against dehydration stress are among the parameters assessed. The findings demonstrated that red chili plants under drought stress experienced slower growth, as seen by a reduction in height and leaf count as well as earlier flowering. However, by raising plant height, leaf count, and chlorophyll levels (a, b, and total), cytokinin treatment was able to lessen the deleterious impacts of drought. When treated with 10 ppm 6-Benzylaminopurine, the enzyme activity of superoxide dismutase, peroxide dismutase, and hydrogen peroxide increased, but at other dosages, it tended to decrease, suggesting a slight but noticeable increase in plant defense mechanisms against oxidative stress. Therefore, giving red chili plants 10 parts per million of cytokinin may be a useful tactic for enhancing their resistance to drought stress.

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1. INTRODUCTION

One of the most popular vegetables in the country, red chilies have a high degree of flexibility and economic worth. Because of its importance to the national economy, chili is a critical agricultural product that has drawn the attention of both the government and corporate players. In Indonesia, the area planted to red chili during the previous five years (2018–2022) fluctuates from year to year. In 2018, the area planted with red chili reached 137,596 hectares, the following year, namely in 2019, the area planted with red chili reached 133,434 Ha, a decrease of 0.9%. In 2020, the area of red chili planting reached 133,729 hectares, until finally in 2022 the latest data says that the area of red chili planting

reached 140,137 [1]. Environmental variables including an unpredictable climate, which can result in an extended dry season, low-quality seeds, ineffective cultivation methods, and the use of non-disease- and pest-resistant chili cultivars can all cause fluctuations in the amount of land that is planted [2]–[4]. The lack of fertilizers or growth regulators in chili plants will disrupt the growth of chili plants [5].

A lack of fertilizer or growth regulators for chili plants, as well as an unpredictable climate like droughts, are some of the factors causing Indonesia to shrink its red chili planting area [6]. Drought inhibits photosynthesis and respiration, which has been linked to a drop in the amount of chlorophyll, the stability of cell membranes, the amount of water in leaves, and the activity of antioxidant enzymes [7]. Drought stress disrupts the electron transfer chain and increases the generation of reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2) and superoxide radicals (O_2-), which can cause autocatalytic peroxidation of membrane lipids, which can change the functionalities of the membrane and cause it to lose its semipermeability [8].

Drought is one of the significant abiotic stresses, occurring when water potential and turgor are reduced to the point where they impair the normal metabolic functions and reproductive capacity of plants [9]. Drought can cause nutrient deficiencies, even in fertilized fields, as soil physiochemical properties can lead to reduced nutrient mobility and absorbance [10]. In moderate or even increased drought the content of the hormone cytokinin ensures the maintenance of growth and photosynthesis and accelerates the formation of canopy leaves, which reduces evaporation from the soil surface and, ultimately, promotes a satisfactory harvest [11].

A phytohormone called benzolaminopurine (BAP) stimulates cytokinesis, or cell division, in plant roots and shoots. Cytokinin is a plant growth substance (phytohormone) that encourages cell division, or cytokinesis in plant roots and shoots [12], [13]. Quinine was the term used when 6-Benzylaminopurine (BAP) was initially extracted from yeast cells. 6-Benzylaminopurine (BAP) affects internode length and leaf growth, mediates auxin transport throughout the plant, and helps postpone plant tissue senescence [14].

6-Benzylaminopurine (BAP) can increase resistance to adverse environmental factors, which is achieved mainly through changes in endogenous 6-Benzylaminopurine (BAP) concentrations or exogenous 6-Benzylaminopurine (BAP) applications [15]. Application of exogenous 6-Benzylaminopurine (BAP) can also improve drought resistance in Cucumis sativus [16]. For the reasons above, the researcher intends to conduct research with the title Growth and biochemical response in red chilies (Capsicum annuum L) under drought stress conditions by administering 6-Benzylaminopurine (BAP), and by carrying out this research it is hoped that farmers will be able to continue cultivating chili plants. even in the dry season using the growth agent 6-Benzylaminopurine (BAP) according to the recommended dose after this research.

2. RESEARCH METHOD

2.1 Place and time of research

From October 2023 to February 2024, this study was carried out at the University of North Sumatra's Greenhouse, Faculty of Agriculture, Medan.

2.2 Materials and tools

The resources used in this study were the seeds of the Curly Red Chili Chili Plant Variety TM 999 as plant material, 6-Benzylaminopurine (BAP), top soil, polybag, N, P and KCl fertilizer, distilled water, liquid nitrogen, coomasie brilliant blue, 95% ethanol, phosphoric acid, pvp 1%, NaCl, KH_2PO_4, K_2HPO_4, EDTA, L-methionine, nitro blue tetrazolium, riboflavin, aquabidest, $CaCl_2$, phenol, aminoantipyrine, methyl ester sulphonate, NaOH, trichloroacetic acid, KI, proline, ninhydrin acid, acid sulfosalicylate, glacial acetic acid, toluene, bovine serum albumin, aluminum foil. The tools used in this research were hand sprayer, hoe, analytical balance, sample pack, beacker glass, meter, envelope, oven, filter paper, nitrogen tube, mercury thermometer, room thermometer, test tube, mortal, pH meter, Eppendorf micropipette , Eppendorf tube, yellow tip Eppendorf, stirrer, centrifuge, vortex, spectrophotometer and stationery.

2.3 Research methods

This study employs a factorial Randomized Group Design (RAK) research methodology with three replications and two factors: the three levels of drought (K1: 80% Field Capacity, K2: 60% Field Capacity, and so on) K3: 40% Field Capacity and 6-Benzylaminopurine (BAP) concentration (BAP) with 4 levels including: So: 0 ppm, S1: 10 ppm, S2: 20 ppm, S3: 30 ppm. From these 2 factors, 12 treatment combinations were obtained, so that from 12 treatment combinations repeated 3 times, 36 treatment combinations were obtained. The number of plants per treatment was 2. The total number of plants was 72 plants.

2.4 Research Implementation

2.4.1 Preparation of planting media

Top soil was taken from Sei Mencirim Village, Deli Serdang Regency. Soil extraction is done by hoeing to a depth of approximately 20-30 cm which is the tillage layer. The soil to be used is air-dried and smoothed, then sieved to obtain a more homogeneous soil structure. The sifted soil was weighed and put into pots, each filled with 10 kg of air-dry soil.

2.4.2 Determination of Soil Water Content and Field Capacity

Gravimetric determination of water content was carried out by drying approximately 10 grams of soil samples in an oven at a temperature of 105°C for 24 hours. Then put it in a desiccator, then weigh the oven-dry soil to obtain the average weight of the oven-dry soil. Calculation of soil water content is calculated using the formula:

$$\text{KAKU (\%)} = \frac{BTKU - BTKO}{BTKO} X100\% \quad (1)$$

Noted:
KAKU (%) : Air Dry Moisture Content (%)
BTKL : Air Dry Soil Weight (g)
BTKO : Oven Dry Soil Weight (g)

After obtaining the results of the initial water content (%KA), the field capacity water content (KAKL) is then calculated using the Alhricks method. The stages of KAKL work are as follows: A 500 ml beaker is filled with quartz sand to a height of 1.2 cm, so that the soil does not fall when tapped, then gauze is placed on top of the quartz sand. The glass pipe is placed perpendicular to the sand surface. The beaker was filled with air-dried soil samples to 3.5 cm from the top edge of the beaker. The beaker is tapped 50 times. The top layer of soil is wetted with water by spraying it with a sprayer, but the water is controlled so that it does not wet the sand. The beaker was closed and kept for 24 hours. After 24 hours, Samples of soil were collected at a depth of approximately 2.5 cm from the surface and the water content was determined based on the oven dry weight of the soil at 105°C. Water content/time interval measurements were carried out (every day until the water content remained relatively constant), and an equation was made between water content and time. The field capacity water content value resulting from laboratory experiments (%KAKL) is used as the basis for providing water in pot experiments in plastic houses with the formula:

$$\text{KAKL (\%)} = \frac{BTKU - BTKO}{BTKO} X100\% \quad (2)$$

Noted:
KAKL : field capacity water content
BTKL : field capacity soil weight
BTKO : oven dry soil weight

So, the water content that must be added uses the formula according to [17]:

$$\text{Added water content} = (\%KAKL) - (\%KAKU) \quad (3)$$

Information:
KAKL : field capacity water content (%)

KAKU : Air Dry Moisture Content (%)

2.4.3 Seed Nursery

The seeds used in this research are Curly Red Chili seeds of the TM 999 Variety. The seeds are sown in a nursery that has been prepared in the form of a bed measuring 1 cm wide and the length depends on needs. The red chili seeds are spread evenly on the bed and covered lightly with fine soil, then covered again with banana leaves or plywood [18].

2.4.4 Planting

Plants that are really good for transplanting are selected from the nursery process, namely plants that are healthy and free from disease. Red chili planting is done in the afternoon where one polybag contains one red chili plant

2.4.5 Providing Treatment

The treatment given consisted of two factors, namely 1) 6-Benzylaminopurine (BAP) concentration and 2) drought stress based on field capacity (KL). The treatment process is as follows: Spraying is carried out when the plants are 4 WAP - 12 WAP. The plants to be sprayed are previously given a barrier so that they do not touch other plants. The application of 6-Benzylaminopurine (BAP) is sprayed with a hand sprayer on all the leaves of each plant until they are wet, with a concentration according to the application interval of 1 week. Spraying is done in the morning at around 8:00 a.m. Watering is carried out every day with a watering quantity of 100% of field capacity (KL). Then after the plant is 3 WAP - 12 WAP the watering volume is changed based on the treatment of 80%, 60% and 40% KL. Drought stress treatment looks at the condition of the soil, if it is still damp then wait until the soil dries out then weigh it first before treatment. The amount of water for watering is adjusted to the level of water loss in each watering treatment so that the condition of each polybag is within field capacity.

2.4.6 Maintenance

Caring for red chili plants consists of fertilizing, weeding and managing pests and diseases.

2.5 Observation Parameters

2.5.1 Plant height

Plant height observations are carried out every 4 WAP - 12 WAP. Plant height is measured from the ground surface or base of the stem to the base of the top leaf that has fully developed.

2.5.2 Number of Leaves

The number of leaves is counted once a week at the age of 4 WAP - 12 WAP. For each plant, all leaves that have fully opened are counted, if there are leaf buds that have not fully opened, they are not counted.

2.5.3 Chlorophyll Levels (a,b and Total)

When the plants were 12 weeks old, leaf samples were taken from mature leaves at the base of the stem near the V branch and young leaves at the end of the upper branches. The method used in calculating the amount of chlorophyll a, b and total is the method of Wintermans & De Mots (1965) [19]. Chlorophyll levels are calculated using the formula:

Chlorophyll a = (13.7 x A665) - (5.76 x A649) (5)

Chlorophyll b = (25.8 x A649) - (7.60 x A665) (6)

Total chlorophyll = (6.10 x A665) + (20.0 x A649) (7)

A665 = absorbance of chlorophyll extract at 665 nm

A649 = absorbance of chlorophyll extract at 649 nm

2.5.4 Hydrogen Peroxide (H_2O_2)

H_2O_2 analysis was carried out using the method of [20]. Hydrogen peroxide activity is expressed in µmol/g units. Observations were made at 12 WAP.

2.5.5 Superoxide Dismutase (SOD) Enzyme Activity

SOD enzyme analysis was carried out at 12 WAP. SOD analysis was observed based on the method carried out by Beauchamp and Fridovich (1971). SOD activity is expressed in units/mg protein. Next, it is calculated using the formula:

$$\text{SOD activity}=\frac{Tangen\ control-Tangen\ sample}{\frac{0,5\ x\ Tangen\ control}{mg\ Protein}} \quad (8)$$

Noted:
Tangent control = blank absorbance
Tangent sample = sample absorbance

2.5.6 Peroxide Dismutase (POD) Enzyme Activity

Peroxide dismutase enzyme analysis was carried out at the Faculty of Agriculture, University of North Sumatra, Tissue Culture Laboratory POD analysis was observed based on the method carried out by Standard Operating Procedures (1994). POD enzyme analysis was carried out at 12 WAP. POD activity is expressed in units/mg protein. Next, it is calculated using the formula:

$$\text{POD activity}=\frac{Af-Ai}{mg\ protein} \quad (9)$$

Information:
Af = final peroxidase reading
Ai =Initial peroxidase reading

2.6 Data analysis

Software called the Statistical Analysis System (SAS) 9.4 was used to evaluate the observation data. Statistical Analysis System (SAS) 9.4 software is used to conduct a follow-up test using Duncan's multiple range test if the treatment's impact on variance is statistically different.

3. RESULTS AND DISCUSSIONS

3.1 Results

3.1.1 Plant Height

Plant height was significantly impacted by the combination of 6-Benzylaminopurine (BAP) treatment and drought stress. Meanwhile, drought stress treatment and administration of 6-Benzylaminopurine (BAP) had no significant effect on plant height. The height of red chili plants treated with drought stress and 6-Benzylaminopurine (BAP) can be seen in Figure 1.

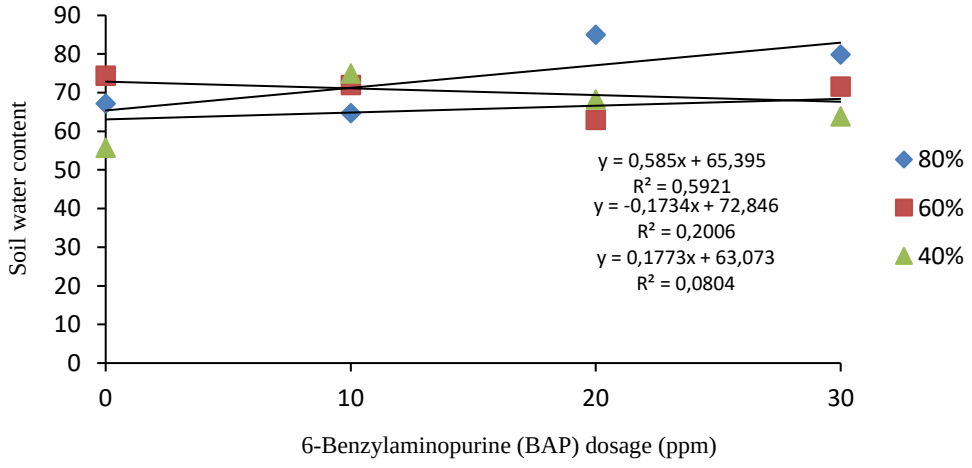

Figure 1. Red chili plant height against drought stress treatment with 6-Benzylaminopurine (BAP) at 12 WAP

Based on Figure 1 above, it is known that in the interaction between treatments, in the treatment with a soil water content of 80%, the highest field capacity of red chili plants was obtained in the 20 ppm treatment.6-Benzylaminopurine (BAP) was not significantly different compared to the 30 ppm 6-Benzylaminopurine (BAP) treatment but was significantly different from other treatments. In the 60% soil water content treatment, the highest field capacity of plant height was obtained in the 0 ppm 6-

Benzylaminopurine (BAP) treatment and was not significantly different from the other treatments. In the soil water content treatment of 40% field capacity, the shortest plant height was obtained in the 0 ppm 6-Benzylaminopurine (BAP) treatment (without BAP treatment) and was significantly different from the 10 ppm 6-Benzylaminopurine (BAP) treatment and not significantly different from the other treatments.

3.1.2 Number of Leaves

The treatment of drought stress, the administration of 6-Benzylaminopurine (BAP), and their combination do not appear to have a substantial impact on the number of leaves (Table 2). Table 2 shows how many red chili leaves were subjected to 6-Benzylaminopurine (BAP) and drought stress.

Table 2. Number of red chili leaves against drought stress treatment with 6-Benzylaminopurine (BAP) at 12 WAP

Soil water content (% KL)	6-Benzylaminopurine (BAP)				Average
	0 ppm	10 ppm	20 ppm	30 ppm	
		.....sheet			
80	87.67	82.17	125.17	101.33	99.08
60	101.00	92.17	80.83	91.83	91.46
40	67.33	99.50	88.50	87.00	85.58
Average	85.33	91.28	98.17	93.39	

3.1.3 Chlorophyll content (a, b and total)

Chlorophyll a, b, and total content were significantly impacted by the drought stress treatment. On the other hand, the administration of 6-Benzylaminopurine (BAP) and their combination had no discernible impact on the levels of chlorophyll a, b, or total content. Table 3 shows the chlorophyll content of red chilies treated for drought stress and given 6-Benzylaminopurine (BAP).

Table 3. Chlorophyll content of red chilies against drought stress treatment with 6-Benzylaminopurine (BAP)

Parameter	Soil water content (% KL)	6-Benzylaminopurine (BAP)				Average
		0 ppm	10 ppm	20 ppm	30 ppm	
				.....mg/g		
Chlorophyll a	80	28.60	27.30	27.86	27.70	27.86b
	60	26,26	27.14	27.12	27.02	26.89ab
	40	23.25	25.25	26.82	27.34	25.66a
	Average	26.04	26.56	27,27	27.35	
Chlorophyll b	80	59.49	66.74	64.34	62.59	63.29b
	60	60.86	61.83	65.93	61.81	62.61b
	40	53.63	55.77	56.17	57.31	55.72a
	Average	57.99	61.45	62.15	60.57	
Total Chlorophyll	80	83.74	92.99	91.16	90.34	89.56b
	60	82.23	89.41	86.38	88.84	86.72b
	40	71.45	75.31	78.36	81.92	76.76a
	Average	79.14	85.90	85.30	87.03	

Note: numbers followed by different letters in different treatment groups are significantly different according to Duncan's Multiple Range Test at the 5% level.

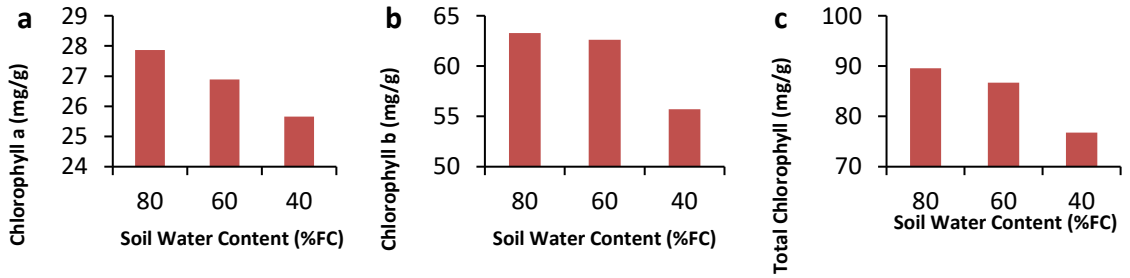

Figure 2. a. Chlorophyll a (mg/g), b. Chlorophyll b (mg/g), c. Total Chlorophyll (mg/g)

According to table 3 dan Figure 2 above, the treatment of soil water content at 40% field capacity produced the lowest chlorophyll a content during the drought stress treatment. This treatment did not differ significantly from the treatment of soil water content at 60% field capacity, but it did differ significantly from the treatment of soil water content at 80% capacity roomy. The soil water content treatment with a 40% field capacity yielded the lowest chlorophyll b content, which differed significantly from the other treatments. The soil water content treatment with a 40% field capacity produced the lowest total chlorophyll content in the total chlorophyll parameter, and this treatment differed considerably from the other treatments.

3.1.4 Hydrogen Peroxide (H_2O_2)

Hydrogen peroxide was significantly impacted by the treatment of drought stress. Meanwhile, hydrogen peroxide was not significantly affected by the administration of 6-Benzylaminopurine (BAP) or the combination of the two. Table 4 shows the hydrogen peroxide content of 6-Benzylaminopurine (BAP) and red chilies during drought stress.

Table 4. Hydrogen peroxide in red chilies against drought stress treatment by administering 6-Benzylaminopurine (BAP)

Soil water content (% KL)	6-Benzylaminopurine (BAP)				Average
	0 ppm	10 ppm	20 ppm	30 ppm	
	.....µmol/g.....				
80	0.138	0.115	0.141	0.142	0.134a
60	0.161	0.159	0.143	0.145	0.152a
40	0.156	0.185	0.159	0.151	0.163b
Average	0.152	0.153	0.148	0.146	

Note: numbers followed by different letters in different treatment groups are significantly different according to Duncan's Multiple Range Test at the 5% level.

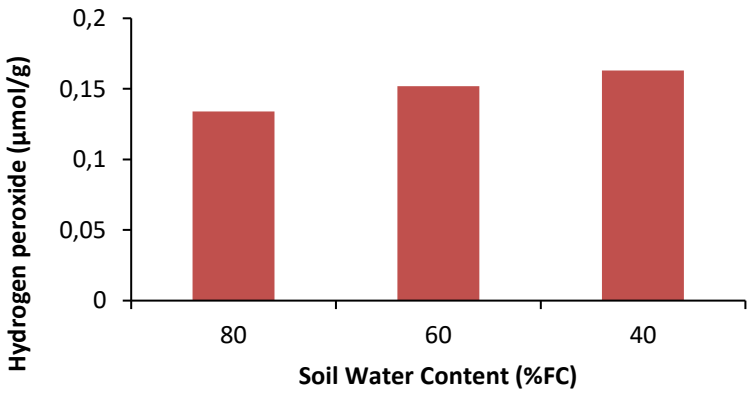

Figure 3. Hydrogen peroxide in red chilies against drought stress treatment by administering 6-Benzylaminopurine (BAP)

Table 4 above indicates that the treatment of soil water content of 40% field capacity produced the highest level of hydrogen peroxide (H_2O_2) activity, which differed significantly from other treatments.

3.1.5 Superoxide Dismutase (SOD) Enzyme Activity

Drought stress treatment had a significant effect on the activity of the superoxide dismutase enzyme. Meanwhile, administration of 6-Benzylaminopurine (BAP) and the interaction between the two had no significant effect on the superoxide dismutase enzyme. The superoxide dismutase enzyme in red chilies treated with drought stress and 6-Benzylaminopurine (BAP) can be seen in Table 5.

Table 5. Superoxide dismutase enzyme activity in red chilies against drought stress treatment by administering 6-Benzylaminopurine (BAP)

Soil water content (% KL)	6-Benzylaminopurine (BAP)				Average
	0 ppm	10 ppm	20 ppm	30 ppm	
		.....units/mg protein			
80	5,11	4.97	4.71	5.58	5.09a
60	6,11	6.21	5.54	5.93	5.95a
40	6.80	7.95	6.31	6.05	6.78b
Average	6.01	6.38	5.52	5.85	

Note: numbers followed by different letters in different treatment groups are significantly different according to Duncan's Multiple Range Test at the 5% level.

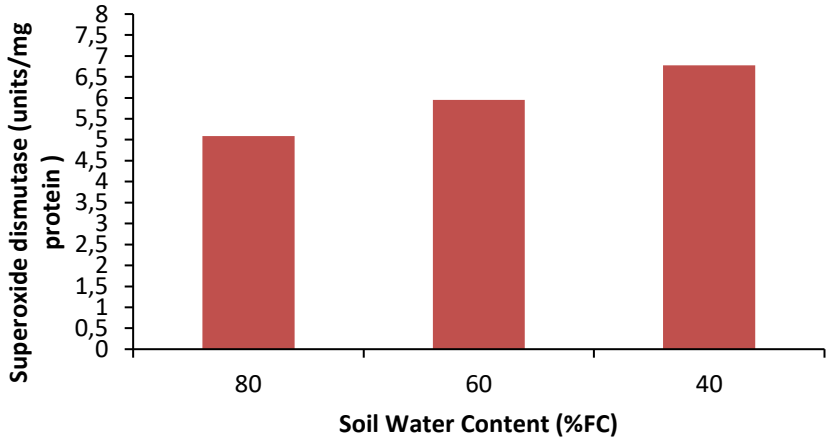

Figure 4. Superoxide dismutase enzyme activity in red chilies against drought stress treatment by administering 6-Benzylaminopurine (BAP)

Drought stress can increasesuperoxide dismutase enzyme activityon red chilies. The more the soil water content decreases, the moresuperoxide dismutase enzyme activitywill increase further.

3.1.6 Peroxidase Dismutase (POD) Enzyme Activity

Drought stress treatment, administration of 6-Benzylaminopurine (BAP) and the interaction between the two had no significant effect on the activity of the peroxidase dismutase enzyme. The activity of the red chili peroxidase dismutase enzyme with drought stress treatment and administration of 6-Benzylaminopurine (BAP) can be seen in Table 6.

Table 6. Activity of red chili peroxidase dismutase enzyme against drought stress treatment by administering 6-Benzylaminopurine (BAP)

Soil water content (% KL)	6-Benzylaminopurine (BAP)				Average
	0 ppm	10 ppm	20 ppm	30 ppm	
		.....units/mg protein.....			
80	0.28	0.35	0.32	0.32	0.32
60	0.36	0.27	0.36	0.35	0.33
40	0.36	0.38	0.41	0.41	0.39
Average	0.33	0.34	0.36	0.36	

3.2 Discussion

3.2.1 Effect of drought stress on growth and biochemical responses of red chili plants

A decrease in soil moisture content reduces plant height and the number of leaves in chili plants with soil moisture content of 80% field capacity to 40% field capacity, the more stressed the plant height and the number of leaves will be less due to disruption of physiological and biochemical processes such as plant turgor pressure which affects the opening and closing of stomata in plants. According to [21], [22] when water is not available in sufficient quantities, turgor pressure decreases which can inhibit cell division and elongation which is essential for stem growth and causes leaves to wilt and become susceptible to damage. Furthermore Bhattacharya & Bhattacharya (2021) [23] said that decreased turgor pressure will inhibit cell division and expansion, which are important for plant growth including

plant height and leaf number. As a result, plant height growth of new leaf formation slows down or stops altogether, while existing leaves can be damaged or fall off because they do not get enough structural support. If turgor decreases, the stomata will close and CO_2 entering the active side of photosynthesis will also decrease [24] without effective photosynthesis, carbohydrate production decreases, resulting in a lack of energy and nutrients needed for plant growth. Closing of stomata serves to reduce water loss through transpiration [21].

Chlorophyll a, b, and total content decrease during drought stress. The findings demonstrated that plant adaptation mechanisms to stress circumstances, such as modifications in enzyme activity, were responsible for the lower chlorophyll a content in plants compared to chlorophyll b content. Liu et al., (2022) [25] report that enhanced activity of the enzyme Chlorophyll an Oxygenase (CAO) can result in higher conversion of chlorophyll a to chlorophyll b under drought stress conditions. This might be an adaptive strategy to raise the proportion of chlorophyll b in comparison to chlorophyll a. Changes in the chlorophyll a/b ratio can result from an increase in CAO enzyme activity during drought stress [26]. Due to a shortage of water, drought stress can cause a reduction in plant chlorophyll, which can interfere with photosynthesis. The green pigment chlorophyll is needed for photosynthesis, which transforms solar energy into chemical energy that plants can use. Drought disrupts photosynthesis in plants because it leaves them lacking in water, which is necessary to move nutrients and keep their cells intact. Plants' chlorophyll content may consequently drop. This is consistent with the claim made by Nio et al., (2019) [27] that chlorophyll content serves as a marker of a plant's ability to withstand drought stress because photosynthesis, which is dependent on water availability, is directly linked to chlorophyll biosynthesis. Drought stress has been demonstrated in several studies to dramatically lower the levels of photosynthetic pigments, including total chlorophyll, chlorophyll a, and chlorophyll b [28], [29]. Reduced gene expression of enzymes involved in the chlorophyll production pathway is the first step in the reduction in chlorophyll biosynthesis. Lowering the amount of chlorophyll will stop toxic tetrapirole, which produces singlet oxygen, from building up. According to Dalal & Tripathy (2012) [30] lowering the chlorophyll concentration will minimize light absorption, which will lower electron transport and lower the generation of ROS (Reactive Oxygen Species).

Stress from drought can make hydrogen peroxide more active. Plants under drought stress may produce more hydrogen peroxide (H_2O_2) in reaction to a water shortage. Reactive oxygen species, or H_2O_2, are produced as byproducts of a number of cellular metabolic processes, particularly when oxidative stress is present. Drought causes oxidative stress in plants, which in turn increases the generation of free radicals like H_2O_2. Impa et al. (2012) [31] claim that the negative effects of drought stress cause modifications in plant cell metabolism, which releases reactive oxygen species (ROS) like singlet oxygen, hydrogen peroxide, and superoxide radicals. However, because H_2O_2 is a very persistent ROS and has significant diffusivity [32], [33] claim that H_2O_2 is frequently used as an important signal molecule for plant cells to respond to various stimuli. Perhaps a crucial warning signal, increased H_2O_2 synthesis boosts the antioxidant defense system or causes cell death as oxidative stress escalates.

In red chili plants, drought stress raises the levels of the antioxidant enzyme superoxide dismutase (SOD). Plants under drought stress respond adaptively to environmental stressors like water scarcity by increasing the antioxidant enzyme SOD in their tissues. The SOD enzyme is in charge of neutralizing free radicals, which have the potential to harm plant cells and interfere with physiological functions. Plants in drought may produce more free radicals in their cells as a result of oxidative stress. Important components including DNA, proteins, and lipids can be harmed by these free radicals, as well as the architecture of cells. Plants react by producing more of the enzyme SOD to neutralize superoxide (free radicals) and shield their cells from oxidative stress-related harm. Similar to most other environmental stresses, drought stress upsets the equilibrium between oxygen radical generation and detoxification, which leads to indirect oxidative stress in plants [34]. Plants' defense systems and stress tolerance are triggered in such circumstances, leading to increased levels of oxygen radical detoxification [35]. Accordingly, the activation and/or increase in the expression profile of associated

genes results in a rise in the antioxidant content in plant cells [36], [37]. An antioxidant enzyme called SOD is involved in the process of dismutating oxygen free radicals. This enzyme controls how different organelles convert superoxide radicals (O2–) into hydrogen peroxide (H2O2) [38]. According to recent research, radial superoxide (SOD) is the most abundant radical in a number of organelles, including chlorophyll cells. This suggests that SOD serves as plants' first line of defense against oxidative stress because it has a high number of stress regulatory elements that enable it to react quickly to environmental changes [39].

3.2.2 Effect of giving cytokinins on the growth and biochemical response of red chili plants

The provision of 6-Benzylaminopurine (BAP) has no significant effect on the height and number of leaves of chili plants due to the influence of an unfavorable growth environment such as drought stress. If plants experience a lack of water or nutrients, the positive effects of BAP can be hindered because the plant does not have sufficient resources to support additional growth (Mall et al., 2021). Under drought conditions, although BAP can stimulate cell division, the lack of water will limit cell expansion and new tissue formation, so the effects of BAP become less noticeable [40].

6-Benzylaminopurine (BAP) had no significant effect on chlorophyll a, b and total content. This is because cytokinin can slow down the process of chlorophyll decomposition. Cytokinin is one type of plant hormone that plays an important role in the regulation of plant growth and development. One of the effects of cytokinins on plants is to slow down the breakdown of chlorophyll, which usually occurs during aging or stressful conditions. Normal conditions for chlorophyll decomposition normally allow it to proceed. Cytokinin, which slows aging, can also prevent chlorophyll decomposition. This is consistent with the assertion made by Ismandari (2021) [41]that the primary role of leaves is to supply nutrients for plant growth via photosynthesis. Cytokinins have multiple effects on the structural and functional components of photosynthesis. Furthermore, cytokinins are efficient in degrading chlorophyll and can postpone the aging of leaves.

6-The activity of free radicals, specifically H2O2, and antioxidant enzymes like SOD and POD were not significantly impacted by benzolaminopurine (BAP). This is because plants go through a variety of physiological reactions to make up for the water shortage when they are stressed by drought. As a plant growth hormone, cytokinin usually has no effect on how plants react to drought stress, including higher SOD, POD, and H2O2 levels. Cytokinins are more frequently linked to abscisic acid (ABA) signaling hormones. Cytokinins are the primary phytohormones that control plant growth and development as well as mediate plant tolerance to drought stress, according to [42]. Cytokinin signaling has developed as a crucial intercellular communication network during water shortage, interacting with other phytohormones including ABA and its regulatory pathways to mediate plant stress responses.

3.2.3 Interaction between drought stress and cytokinin administration on the growth and biochemical response of red chili plants

At 12 weeks of age, the combination of 6-Benzylaminopurine (BAP) treatment with drought stress had a noteworthy impact on plant height. When 6-Benzylaminopurine (BAP) 20 ppm is applied at 40% field capacity, plant height can be increased in comparison to the control. Conversely, plant height decreased with an increase in 6-Benzylaminopurine (BAP) content. Cell division can be impacted by cytokinin, particularly in growing regions like shoot tips. Plants can generate more new cells when there are enough cytokinins present, which contributes to the growth of the plants' height. Drought stress inhibits the growth and development of plants. Drought stress reduces the average plant height [43], [44]. Protoplasmic dehydration causes a considerable reduction in plant height by inhibiting cell division, cell growth, and loss of turgidity [45]. Accordingly, applying benzylaminopurine to plants may result in them becoming taller [46]. This is due to the fact that cytokinins promote cell elongation and expansion. In order for plants to grow tall, stem elongation is brought about by the division, elongation, and expansion of cells in the stem internodes and apical meristem [47], [48]. Excessive cytokinin concentrations, however, can harm or have no effect on plants. This is due to the fact that using ZPT in excess of what is considered appropriate will prevent growth [49].

4. CONCLUSION

Red chili plants under drought stress exhibit stunted growth, which is shown by a reduction in plant height and leaf count as well as an earlier onset of blooming. However, by boosting plant height, leaf count, and levels of chlorophyll (a, b, and total), cytokinin administration was able to lessen the deleterious impacts of drought. Superoxide dismutase (SOD), peroxide dismutase (POD), and hydrogen peroxide (H2O2) enzyme activities increased in response to 6-benzylaminopurine (BAP) 10 ppm treatment, but at other levels tended to decrease, suggesting an increase in plant defense mechanisms against oxidative stress. Giving red chili plants 10 parts per million of cytokinin can therefore be a useful tactic for enhancing their resistance to drought stress. This work advances our knowledge of the mechanisms by which plants adapt to adverse environmental circumstances and offers empirical support for the beneficial effects of growth hormone treatments on drought tolerance in plants. This study suggests that one useful tactic in agriculture to increase plant drought resistance is the administration of cytokines. This is especially important in light of climate change and the growing frequency of droughts, which may endanger agricultural output. In regions where there is a risk of water scarcity, farmers can manage their crops more effectively by using cytokinins. The effects of mixing cytokinins with other hormones or growth regulators should also be investigated in future studies to see whether there are any synergies that can improve plant tolerance to drought.

Acknowledgment

The author would like to thank the Rector of the University of North Sumatra, Prof. Dr. Muryanto Amin S.SOS, M.Si., Dean of the Faculty of Agriculture, Prof. Dr. Ir. Tavi Supriana, MS, Supervisor Prof. Luthfi A. M. SP, MSc, PhD as Chairman of the Supervisory Commission and Dr. Nini Rahmawati SP, M.Si as Member of the Supervisory Commission who have assisted the author in the preparation of this thesis. In particular, the author would like to thank both parents and the entire family who have encouraged and motivated the author to complete this thesis.

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