International Journal of Agriculture and Plant Science
www.agriculturejournal.in
Online ISSN: 2664-7664, Print ISSN: 2664-7656
Received: 21-10-2024, Accepted: 20-11-2024, Published: 05-12-2024
Volume 6, Issue 4, 2024, Page No. 25-30

Thiamine foliar application effects on growth and yield of tomato (*Solanum lycopersicum* L.) under drought stress

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Abstract
Drought stress decreases plant growth and affects the loss of plant production. Thiamine is a primary vitamin that has a role in preventing plant damage under drought stress. The addition of thiamine is expected to help reduce the loss in plant yield. The aim of this research was to study the growth and yield responses of drought-stressed tomato toward application of exogenous thiamine. The study was carried out in Kajoran district of Magelang regency and the Laboratory of Plant Physiology and Breeding, Department of Agriculture, Universitas Diponegoro, Semarang. The design used was factorial in the base of Randomized Complete Design (RCD) with three replications. The first factors were the drought stress (irrigation intervals); one-day interval (K1), three-day interval (K2), and five-day interval (K3) and the second factors were exogenous Thiamine treatment; 0 mg L^{-1} (T0), 50 mg L^{-1} (T1), and 100 mg L^{-1} (T2). Parameters observed were plant height, stem diameter, leaves number $plant^{-1}$, fruits number $plant^{-1,}$ and fruit weight $plant^{-1}$. There was interaction between drought stress and thiamine application on plant height (56 DAS). Separately, drought stress and thiamine application affected the plant height (42 DAS), leaves number, stem diameter, fruits number and weight.

Keywords: Tomato, drought stress, thiamine, growth, yield

Introduction

Global climate change is significantly affecting the agricultural environment (Teshome *et al*., 2020). [33] Horticultural crops are agricultural commodities that are sensitive to environmental stress (Saparso *et al*., 2024). [27] Droughts, floods, and high temperatures are stressful conditions that are intensively faced by horticultural crops. Among the environmental stresses, drought is the main environmental stress that is always faced by horticultural crops (Danish *et al*., 2020), [11] including tomatoes. Drought stress commonly occurs in the tropics (Seleiman *et al*., 2021), [29] due to the intensity of rain, and the distribution of rainy days are disproportionate (Danish *et al*., 2020). [12] Drought stress has a major effect on yield loss in horticultural crops (Teshome *et al*., 2020), [33] including tomatoes. Drought conditions will result in an imbalance of biological responses that can disrupt physiological processes and functional activities in plants (Teshome *et al*., 2020). [33] Drought stress may inhibit water uptake from the soil, decreasing the photosynthesis rate, increasing the respiration rate, and stomata closure. The first response of tomato to water shortage is by closing the stomata. In addition, water limitation also causes the increasing abscisic acid levels which results in the regulation of guard cells. It has an impact on the narrowing of stomata. Closure and narrowing of stomata can limit the CO2 uptake through stomata leaves, thus inhibiting the photosynthesis process. Inhibition of carbon dioxide fixation to the plant mesophyll cell will result in the disruption of sugar or photosynthate mobilization in plant parts (Agurla *et al*., 2018). [2] The advanced responses of plants that caused by drought are plant growth abnormalities; such as leaf yellowing, leaf curling, stunting, leaf senescence, and permanent wilting (Patharkar *et al*., 2019); (Bhusal *et al*., 2020). [26, 8]

Drought stress has a close correlation with oxidative stress. Physiological responses performed in drought-affected plants include an increase in antioxidant levels and activity, one indicator of which is total phenolic content, due to the increasing of Reactive Oxygen Species (ROS). Antioxidants are required to maintain plant physiology in the face of drought stress. This mechanism helps plants to maintain growth rate and biomass accumulation under stress (Jabeen *et al*., 2020). [19] Not only an increase in phenolic content but drought stress results in an increase in biochemical molecules that are responsible for maintaining the plant under stress conditions. An increase in enzymatic antioxidant levels will further be responsible for ROS scavenging. The antioxidant enzymes that contributed to this process are superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) were significantly increased (Jabeen *et al*., 2020). [19] Furthermore, the mechanism of ROS scavenging also largely involves the work of a group of antioxidant components, which are vitamins. Vitamins are produced by plants as a result of secondary metabolites. Vitamins produced by plants are generally used by humans as useful nutrients. However, in stressful conditions, plants need their own vitamins to maintain physiological responses, growth and production (Hamada *et al*., 2009). [18]

Thiamine (vitamin B1) is an important part of the B vitamin group and plays an important role in mitigating drought stress. Thiamine also has many roles in plant cells, Thiamine diphosphate (TDP), is a coenzyme for many primary metabolic enzymes. Several enzymes with Thiamine as a coenzyme involved in both photosynthesis (transketolase, TK) and respiration (pyruvate dehydrogenase, PDH; a-ketoglutarate dehydrogenase, a-KGDH) in the Calvin and TCA cycles of chloroplasts and mitochondria, respectively (Araújo *et al*., 2012); (Joshi *et al*., 2019). [7, 21] As an antioxidant compound, Thiamine has a big role in the scavenging process of ROS (Jabeen *et al*., 2020). [19] Exogenously applied thiamine is reported to have a positive effect on improving the growth, yield, and photosynthesis parameters of drought plants (Hamada *et al*., 2009); (Jabeen *et al*., 2020). [18, 19] Foliar application of thiamine (B1) reduced K+ leakage. Moreover, as foliar treatment, Thiamine maintains the photosynthetic pigments, pigment biosynthesis, leaf and root osmoprotectors, membrane integrity, and increases in enzymatic antioxidants (SOD, POD, and CAT), ascorbic acid content, and total phenols under drought stress conditions. Thiamine application increases the proline and glycine betaine content and reduces malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) even at 50% field capacity (Hamada *et al*., 2009); (Jabeen *et al*., 2020). [18, 19] By those modulations, exogenous thiamine treatment plays an important role in mitigating various reactive oxygen species (ROS) under drought conditions and induces plant tolerance.

According to FAOSTAT (2015), [16] among horticultural commodities, tomato (Solanum lycopersicum) is the most cultivated crop with stable and high economic value, after potato. Tomato contains a high nutritional value and antioxidant content that is useful for the human daily diet (Viskelis *et al*., 2005). [35] The demand for tomato production is increasing every year, however, the production is limited by the abiotic stress factor, mainly by drought stress. The proper strategies for mitigating the effect of drought stress in tomatoes are still being investigated. According to previous research, It is hypothesized that the exogenous application of thiamine can mitigate drought stress effectively in tomato. Thiamine may improve the growth and yield of tomatoes under drought-stress conditions. The application of exogenous thiamine for drought-stressed tomato may become a promising strategy that can be further used by farmers.

Materials And methods

The experiment was conducted at Agroloka Green House, located at Krajan sub-district (7°30'04.9"S latitude, 110°06'37.3"E longitude, altitude approximately 575 m above mean sea level), Kajoran District, Magelang Regency, Central Java Province, Indonesia and Laboratory of Plant Physiology and Breeding, Agriculture Department, Faculty of Animal and Agriculture Sciences, Universitas Diponegoro. The average annual rainfall is 2.589 mm/year. The mean daily maximum and minimum temperatures during the growing season of tomatoes fluctuated between 28.5 to 36.5 ºC and 14.5 to 24.5 ºC, respectively. Similarly, mean daily relative humidity ranged between 60 to 90%.

Research material and plant establishment

The materials used in the experiments were tomato seeds Var. Servo F1 (PT. East-West Seed Indonesia), plastic pots, growing media, consist of soil:cow manure:rice husk (1:1:1), NPK 15:15:15 fertilizer with the dose of 1 t. ha^{-1} (150 kg N ha^{-1}, 150 kg P_2O_5 ha^{-1}, and 150 kg K_2O ha^{-1}), and Thiamine Hydrochloride (Himedia PCT0215). The 2-week-old tomato seedlings were transplanted to the plastic pots of 30 cm in height with top and bottom diameters of 30 and 28 cm, respectively.). After transplanting, all plants were daily irrigated for two weeks until the proper establishment of seedlings. Then, a simulated drought-stress condition was implemented by interval level of irrigation. NPK fertilizer was applied two times (21 and 35 DAS). Thiamine was applied according to the treatment concentration. Disease and pest were controlled using mechanical and chemical technique.

Experimental design and treatment

The experimental design used was factorial in the base of Randomized Complete Design (RCD) with three replications. The first factor was the drought stress (irrigation intervals), and the second factor was exogenous Thiamine treatment. Three irrigation interval levels were applied, namely one-day interval (K1), three-day interval (K2), and five-day interval (K3). Water supply volume was based on the 100% field capacity (FC). The method to determine soil moisture content was based on (Chakma *et al*., 2021) [9] with slight modification, three aluminum trays each with 1 kg of air-dried soil were placed in an oven at 80 ◦C until the weight of the soil became constant. Previously, the weight of the empty aluminum tray was measured, and then further subtracted from the weight of the tray filled with dried soil to determine the dry weight of the soil. Then, the dried soil was placed in a plastic pot (of known weight) and water was used until saturation. The wet weight of moist soil is determined after 24 h when the soil moisture is still available but no drip under the pots. Soil moisture content was calculated using the following equation:

$$\text{Soil moisture content (\%)} = \frac{(\text{soil wet weight} - \text{soil dry weight})}{(\text{soil dry weight})} \times 100 \quad \text{……………………………} (1)$$

The amount of soil moisture content at this condition was considered as 100 % FC. While, three levels of Thiamine concentration were used; 0 mg. L^{-1}, 50 mg. L^{-1}, and 100 mg. L^{-1}. A total volume of 500 ml of Thiamine solution (according to the concentration of each treatment) was applied to tomato. Thiamine was applied as a foliar application for five times (28, 35, 42, 49, 56 DAS). The experiment units were 27, consisting of 10 plants for each unit, therefore the total plant populations were 270 plants.

Observed parameters and statistical analysis

Observed parameters were plant growth component (plant height, number of leaves, and stem diameter), and yield component (number of fruits per plant and fruit weight per plant). The growth parameters were observed at 42 and 56 DAS, while yield component were observed at 77 DAS. The data were statistically analyzed by two-way analysis of variance (ANOVA) using SPSS 25.0 (IBM SPSS Statistic) software program. Differences between means were evaluated using the Duncan Multiple Range Test (DMRT) at $P < 0.05$ (Steel *et al*., 1990). [32]

Results And discussion

Exogenous thiamine improved the growth of drought-stressed tomato

Irrigation interval level is an artificial drought stress which showed moderate (3-day interval) and severe (5-day interval) drought stress conditions. Separately, at 42 DAS drought stress in the form of irrigation intervals and thiamine application showed a significant effect on plant height, while, the interaction between those two factors did not showed a significant effect. At 42 DAS, the drought stress levels significantly reduced the plant height of tomato (Table 1). Irrigation of 5-day intervals has the lowest plant height compared to 3-day and 1-day intervals, respectively. The application of exogenous thiamine could improve the growth of tomato. By giving 100 mg. L^{-1} thiamine, tomato generated the best growth as indicated by plant height. Positive effects of thiamine are also shown on the plant height of tomatoes at 56 DAS (Table 1). The interaction between drought stress and the application of exogenous thiamine significantly affected the plant height (Figure 1). Under 1-day interval of irrigation, each concentration of thiamine gives different result on plant height; 100 mg. L^{-1} thiamine generate the highest plant height. However, under 3-days and 5-days interval of irrigation, there is no significant different between each concentration of thiamine. It means that thiamine is not significantly affected the growth of tomato under drought stress, compared to normal conditions. Increasing in plant height can be seen in normal condition (1-day interval) with thiamine addition, among thiamine concentration, 100 mg. L^{-1} showed the best plant height.

Table 1: Plant height of tomato as affected by drought stress and exogenous thiamine application

	Thiamine concentration (mg. L^{-1})			
Irrigation interval	**0**	**50**	**100**	**Mean**
	Plant height at 42 DAS			
	------cm------			
1-day interval	82.27	84.90	86.90	84.69^{a}
3-days interval	77.17	78.83	80.60	78.87^{b}
5-days interval	68.33	70.23	71.50	70.02^{c}
Mean	75.92^{b}	77.99^{ab}	79.67^{a}	
Plant height at 56 DAS				
	------cm------			
1-day interval	105.60^{c}	112.87^{b}	126.50^{a}	114.99^{a}
3-days interval	88.40^{d}	90.67^{d}	89.93^{d}	89.67^{b}
5-days interval	78.40^{e}	81.63^{e}	79.53^{e}	79.86^{c}
Mean	90.80^{b}	95.06^{ab}	98.66^{a}	

- The values with a different superscript letter in a column are significantly different ($p<0.05$)
- The values with a different superscript letter in a row are significantly different ($p<0.05$)
- Interaction between thiamine and irrigation interval level is significantly different ($p<0.05$) on 56 DAS

In general, drought stress disrupts multiple plant activities, including leaf gas exchange, carbon assimilation rate, cell turgor and oxidative defense system. All these changes lead to reduced yields (Chowdhury *et al.*, 2016); (Shafiq *et al.*, 2019). [10, 31] Plant susceptibility or tolerance to water stress is a complex situation, linked to the activity of key enzymes, nutrient uptake patterns and defense mechanisms, resulting in reduced yields (Anjum *et al.*, 2017); (Todaka *et al.*, 2017). [6, 34]

Thiamine, a vitamin B1, plays an important role as a coenzyme in several metabolic processes, including the pentose phosphate pathway, tricarboxylic acid cycle, branched-chain amino acid formation, and glycolysis in cellular organisms (Goyer, 2010). [17] High concentrations of thiamine in plant seeds, roots and leaves have been recognized to help promote plant growth and productivity in stressful conditions. Involved in negative impact changes (Goyer, 2010). [17] Application of thiamine reduces the negative effects of water stress on some plants. The research found that foliar application of thiamine may enhanced the plant growth because thiamine may suppress the activity of reactive oxygen species (ROS) (Sayed and Gadallah, 2002). [28]

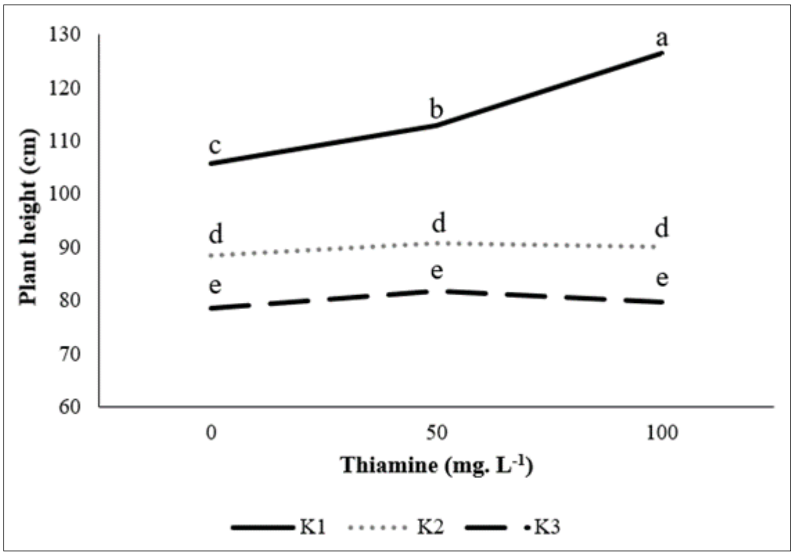

Fig 1: Plant height of tomato under thiamine application and drought stress at 56 DAS

At 42 DAS, drought stress decreases the leaves number. The 1-day interval of irrigation had a significantly higher number of leaves compared with 3-day and 5-day intervals of irrigation. There was no significant difference in leaves numbers between the 3-day and 5-day intervals of irrigation condition. Separately, exogenous application of thiamine showed a significant effect on leaves number. Concentration of thiamine up to 100 mg. L^{-1} significantly increases the number of leaves. However, at 56 DAS, only the thiamine application affected the leaves number (Table 2). Drought stress significantly inhibits the plant growth and reduced yields. Plant growth inhibition effects are shown on the shoot and roots, due to water deficit at every stage of plant growth could impair physio-biochemical processes, such as; modulation of stomata, cell growth (division and elongation), photosynthetic rate, water relations, hormonal regulation and nutrients' uptake and utilization (Duan *et al*., 2017). [14] Stressors such as drought and salinity severely inhibit plant growth and productivity by inducing downregulation of the activity of cyclin-dependent kinase enzymes, resulting in less dividing cells and less cell division and expansion. Area reeds that will reduce the number of leaves and leaves (Zhu, 2002). [36]

Table 2: Leaves number of tomato as affected by drought stress and exogenous thiamine application

	Thiamine concentration (mg. L^{-1})			
Irrigation interval	**0**	**50**	**100**	**Mean**
	Number of leaves at 42 DAS			
	------leaf $plant^{-1}$------			
1-day interval	18.67	22.67	24.33	21.89^{a}
3-days interval	17.33	19.00	19.00	18.44^{b}
5-days interval	16.00	16.33	17.67	16.67^{b}
Mean	17.33^{b}	19.33^{ab}	20.33^{a}	
Number of Leaves at 56 DAS				
	------leaf $plant^{-1}$------			
1-day interval	31.00	33.67	33.33	32.67^{a}
3-days interval	25.00	25.33	25.67	25.33^{b}
5-days interval	24.33	23.67	25.00	24.33^{b}
Mean	26.78	27.56	28.00	

The values with a different superscript letter in a column is significantly different ($p<0.05$)
The values with a different superscript letter in a row is significantly different ($p<0.05$)

Thiamine significantly improved plant growth under different water conditions. Among various plant growth bioregulators, thiamine can be considered a cofactor as it plays an important role in mitigating plant abiotic stress factors (Jabeen *et al*., 2020). [19] Application of thiamine was found to be beneficial in improving drought stress tolerance in beet (Jabeen *et al*., 2020) [19] plants. Interestingly, under stress-free conditions, a previous study found that exogenous application of thiamine, especially in the amount of 500 mg. L^{-1}, improved growth, yield, and biochemical (nitrogen, phosphorus, carotenoid, and chlorophyll b) of coriander and fenugreek plants.

Drought stress significantly decreased the stem diameter of tomato. There was no significant difference between 3-day and 5-day intervals of irrigation on stem diameter. Exogenous application of thiamine did not affect the stem diameter (Table 3). Water stress conditions could limit biomass production, due to the inhibition of photosynthesis and imbalance of water and nutrients in plant cells. This could further impact the growth, including the stem (Duan *et al*., 2017). [14] However, thiamine concentrations up to 100 mg. L^{-1} have not improved the biomass accumulation on the stem. It may be due to Thiamine working primarily to reduce the ROS and osmotic adjustment in the cell.

Table 3: Stem diameter of tomato as affected by drought stress and exogenous thiamine application

	Thiamine concentration (mg. L^{-1})			
Irrigation interval	**0**	**50**	**100**	**Mean**
	Stem diameter at 56 DAS			
	------cm------			
1-day interval	1.01	1.09	1.06	1.05^{a}
3-days interval	0.88	0.88	0.91	0.89^{b}
5-days interval	0.84	0.82	0.89	0.85^{b}
Mean	0.91	0.93	0.95	

The values with different superscript letter in a column is significantly different ($p<0.05$)

Drought stress increases in overproduction of H_2O_2 and MDA have already been observed in cucumber (Li *et al*., 2011), rape (Akram *et al*., 2018), [3] and others. Thiamine could reduce the amount of ROS. Previous studies emphasize that thiamine application was effective in reducing the accumulation of MDA and H_2O_2 levels in cauliflower cultivars at 50% F.C (Jabeen *et al*., 2020). [19]

Exogenous thiamine maintained the yield of tomato under drought stress

Plant sensitivity level to drought stress is also shown in the yield parameters. Drought stress significantly decreased the fruits number and fruits weight per tomato plant. Moderate and severe drought stress performed a similar trend of fruits production. Meanwhile, application of exogenous thiamine did not show a significant effect on drought-stressed tomato (Table 4).

Table 4: Fruits number and weight per plant in different drought stress and thiamine application

	Thiamine concentration (mg. L^{-1})			
Irrigation interval	**0**	**50**	**100**	**Mean**
	Fruit number per plant			
	------(fruits $plant^{-1}$)------			
1-day interval	33.00	35.00	34.33	34.11[a]
3-days interval	27.33	29.33	29.00	28.56[b]
5-days interval	26.33	26.67	27.33	26.78[b]
Mean	28.89	30.33	30.22	
	0 mg L-1	50 mg L-1	100 mg L-1	Mean
	Fruit weight per plant			
	------(kg $plant^{-1}$)------			
1-day interval	1.55	1.61	1.65	1.60[a]
3-days interval	1.09	1.13	1.07	1.09[b]
5-days interval	0.89	1.05	1.09	1.01[b]
Mean	1.18	1.26	1.27	

The values with different superscript letter in a column is significantly different ($p<0.05$)

Water deficit is the most important abiotic stress affecting many physiological parameters of plants, especially photosynthetic performance. During drought, dehydration of plant cells and tissues leads to reduced plant growth and productivity (Krishna *et al*., 2022). [22] Drought stress during the generative stage is involved in the reduction of the number and weight of fruits per plant. It is due to water limit conditions that disrupt the stability of photosynthesis rate and carbohydrate assimilation which was further stored as fruit biomass. Reduced number and weight of fruits also previously be reported on lime (Mira-García *et al*., 2023) and eggplant (Semida *et al*., 2021). [24, 30] Water stress leads to loss of membrane integrity and tissue water starvation, disruption of PSII photosynthetic capacity, and decreased relative chlorophyll content, resulting in severe impairment of growth-related traits and yield structure. The predominant response to soil water deficit is stomatal closure, controlled via root-to-shoot signaling (mainly ABA), which directly affects CO_2 diffusion into leaf tissue, thereby reducing photosynthesis and plant growth is then reduced (Abdelkhalik *et al*., 2019); (El-Mageed *et al*., 2020). [1, 15] Those impaired physiological process contribute to reduce the number and weight of tomato fruits.

Conclusions

Drought stress (in range of 3-day to 5-day irrigation intervals) significantly decreased the plant growth, affecting the reduction in the plant production. Severe growth inhibition and production were performed by 5-day irrigation interval. Application of thiamine improved the growth parameters. Thiamine increased the plant growth in the normal condition (1-day irrigation interval). Based on the research, thiamine does not enhance the productivity of the plant under drought conditions.

Acknowledgments

The authors wish to convey gratitude to the LPPM and Faculty of Animal and Agricultural Sciences Universitas Diponegoro for the research grant and facilities and Agroloka Kajoran Magelang for the technical support of green house.

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