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Stomata character and chlorophyll content of tomato in response to Zn application under drought condition

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Abstract. This experiment was performed in order to evaluate the effects of Zn application under drought condition on tomato, especially its chlorophyll content and stomata character. This experiment was arranged in factorial using randomized complete block design with three replications. The treatment consisted of the Zn application method, namely: soil and foliar, the Zn dosage, namely: 0, 40 and 60 mg ZnSO₄ kg⁻¹ soil and two cultivars of tomato, namely: 'Tyrana' F₁ and 'Permata' F₁. The stress condition was induced by watering every 12 days of 3 weeks after transplanting until harvesting. The results showed that the soil with a Zn application under drought conditions increased the aperture stomata, chlorophyll b and chlorophyll a/b ratio. The response of stomata character, chlorophyll a and total chlorophyll in both cultivars was similar.

1. Introduction

Water shortages in tomato plants will affect a number of physiological and biochemical processes, including the ability of plants to obtain water and nutrients, which directly or indirectly affect the growth of plants and crops. Several studies of drought stress in tomato plants show that drought stress decreases yield, yield components and dry matter production significantly both insensitive and tolerant cultivars [1,2]. Drought stress on tomato growth fell by about 60% [3].

Various cultivation technologies are done to reduce the impact of drought stress; one of them is by using micronutrient Zink (Zn). Zinc possibly reduces the impact of drought because Zn is involved in membrane integrity and protects cells from damage caused by reactive oxygen species [4-6]. Other literature suggests that Zn involvement in stomata opens possibilities due to Zn as part of the carbonate anhydrase enzyme which is necessary to maintain the HCO_3 content in guard cells [7]. In addition, Zn is one of the micronutrients involved in carbohydrate metabolism. Zn deficiency can lead to a reduction of net photosynthesis by 50-70%, depending on the plant species and the severity of the deficiency. Decrease in the efficiency of photosynthesis can be caused, among others, by the decrease in enzyme activity, chlorophyll conditions, stomata conditions that affect the availability of CO₂ in plants as well as environments such as temperature and water. Decreased efficiency of photosynthesis may be due, among others, to a decrease in enzyme activity of carbonic anhydrase and ribulose enzyme 1.5 bisphosphate carboxylase (RuBPC) [8].

However, there is no research report on the effect of Zn on tomato plants on drought condition. Therefore, this article will examine the application of Zn on tomato under drought conditions, especially its stomata character and chlorophyll content.

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2. Methods

2.1. Place and Time

Tomato was grown in Research Field and observation of stomata and chlorophyll analysis was done in Plant Science Laboratory of Faculty of Agriculture, UGM Yogyakarta, Indonesia. Starting from March to August 2014.

2.2. Research design

The study was designed using 3 factors, namely: Zn dosages of 0, 40 and 60 mg kg⁻¹ soil given in the form of ZnSO₄7 H₂O, cultivars, namely: 'Tyrana' F_1 and 'Permata' F_1 and the third factor is application method, namely: soil and foliar. The study was conducted in factorial based on a randomized complete block design. Drought conditions are made by watering every 12 days. Besides, control plants were planted that watered every 2 days. ZnSO₄ application through soil was done by broadcasting around the plants when the plants were 1 week after planting. Foliar application was done by spraying ZnSO4 solution with concentration of 1%. Fertilization in the form of manure and NPK was given as basal fertilizer.

Stomatal and chlorophyll measurements were taken when the plants were 6 weeks after transplanting. Stomata observation was done by smearing the leaves with nail polish after dry lift the layer by using insulation and then attached to the preparation. The measurement of chlorophyll content was done by measuring the chlorophyll content taken from the leaf which has been perfectly cleared on the 9-week-old plant after planting. The measurement of chlorophyll content was performed as described by Islam [10], one gram of leaf sample was cut into pieces and crushed in mortar and then added by 20 ml of 80% acetone. The solution was put aside for a while, then filtered with Whatman filter paper number 42. The filtrate was fed into cuvette until the boundary line and then its absorbance was measured by spectrophotometer at λ 645 and 663 nm. Calculation of chlorophyll content is determined by the formula:

Chlorophyll a = (12.7 x A663 - 2,69 x A645) x (20 ml / 1000 x 1 g)

Chlorophyll b = $(22.9 \times A645 - 4,68 \times A663) \times (20 \text{ ml} / 1000 \times 1 \text{ g})$

Total chlorophyll content = (20.2 x A645 + 8.02 x A663) x (20 ml / 1000 x 1 g)

The data obtained were analyzed by Anova and continued with Duncan test. The analysis was done using SPSS.

3. Result and discussion

3.1. Stomata character

Stomatal observations include stomatal density and stomatal opening width. Stomatal density and stomatal opening width showed no interaction of 3 factors, however, there is interaction between the method and the dosage as well as the dosage and the cultivar.

Table 1 illustrates the difference in stomatal opening widths due to ZnSO₄ dosage applications depending on the application method. The width of stomatal openings under drought conditions is narrower than the width of stomatal openings in plants with sufficient water. The decrease in stomatal opening width under drought conditions is due to the reduced moisture content in the plant which will affect the turgor of guard cell that controls the opening and closing of the stomata. Stomatal closure is an early response to drought-induced plants [10] The application of ZnSO₄ through the soil under drought conditions widened the opening of stomata, while the application through the leaves did not affect the width of the stomatal opening. As ZnSO₄ increases, Zn in the crop will increase. This is also due to the application of ZnSO₄ which increase the surface area and root length [11] so that it will increase water uptake and increase the cell turgor. Some researchers have reported that Zn application influences root length on barley [12], as well as Malik [13] stated that 200 ppm Zn increase root length of Amaranthus. Increased Zn in plants is thought to be able to reduce damage to stomata, because one

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of the functions of Zn is to maintain the integrity of the membrane [4]. However, too high Zn concentrations will also damage stomata morphology and narrow stomata openings [14]

Table 1. Stomatal aperture (μ m) of tomato plants at watering intervals 2 and 12 days with soil and foliar ZnSO₄ application

Mathad	Waterin				
method	2 (normal)		Mean		
application	- ZnSO ₄	0	40	60	_
Soil	23.82 a-b	9.51 d	12.66 c	12.36 c	14.59
Foliar	23.87 a	10.22 d	6.98 e-f	6.11 e-f	11.79
Mean	23.85	9.89	9.82	9.23	(+)

Description: CV = 7.40%. Values in the same row or column followed by the same letter are not different on the Duncan 5% test. Sign (+): there is an interaction.

Under normal watering (every 2 days) without $ZnSO_4$ application, 'Tyrana' F_1 has a significant greater stomatal opening width than the 'Permata' F1. The application of $ZnSO_4$ under drought conditions increased stomatal opening width in both 'Permata' F_1 and 'Tyrana' F_1 , but the stomatal opening width of the $ZnSO_4$ application showed no significant difference with stomatal opening width without the $ZnSO_4$ application. Both cultivars showed no significant difference in stomatal openings (Table 2).

Table 2. The stomatal :	aperture and	stomatal of	density of	f two	tomato	plants	at the	watering	interval	of 2
and 12 days with the ap	oplication of Z	ZnSO4.								

	Watering i				
Cultivar	2 (normal)		12 (drought)	Mean	
	- ZnSO4	0	40	60	
Aperture stomata (µm)					
Permata F ₁	22.45 b	9.62 с-е	10.22 с-е	10.59 c-d	13.22 a
Tyrana F ₁	25.24 a	10.11 c-e	11.42 c	10.88 c-d	14.41 b
Mean	23.85	9.86	10.82	10.74	(+)
Density stomata (per μ m ²)					
Permata F ₁	31.5 b	24.5 d-e	22.0 d-f	24.8 d-e	25.71
Tyrana F ₁	47.8 a	28.3 b-c	29.0 b-c	23.8 d-e	32.25
Mean	39.67	26.42	25.50	24.33	(+)
Mean	39.67	26.42	25.50	24.33	(+)

Description: CV aperture stomata = 7.40%. CV stomatal density = 18.18%. Values on the same row followed by the same letter are not different on the Duncan 5% test. (+): There is interaction.

The stomata density response of the two cultivars to the application of $ZnSO_4$ under drought conditions is different. In the 'Permata' F_1 , stomata density on application of 40 mg Zn kg⁻¹ soil is less than stomatal density in 60 mg Zn kg⁻¹ and without ZnSO₄ application, although it shows no significant difference in density. Different responses are shown by the 'Tyrana' F_1 , in the application of 40 mg Zn kg⁻¹ soils have a large density, although it is not significantly different from the density in plants without the application of ZnSO₄. The application of 60 mg Zn kg⁻¹ soil on 'Tyrana' F_1 decreased stomatal density significantly compared with no application or with application of 40 mg Zn kg⁻¹ (Table 2). The difference in response may be due to the genetic Zn efficiency from the two cultivars are different.

3.2. Chlorophyll Content

Chlorophyll measurements include chlorophyll a, chlorophyll b, total chlorophyll and chlorophyll ratio. There is interaction between dosage and $ZnSO_4$ application method to chlorophyll b and chlorophyll ratio, while chlorophyll a is influenced by Zn dosage and total chlorophyll method is only influenced by the Zn application method.

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Chlorophyll a content under drought conditions either with $ZnSO_4$ application or not is lower than the chlorophyll content in plants with normal watering (Table 3). The content of chlorophyll a in ZnSO₄ applications with a dosage of 40 and 60 mg kg⁻¹ under drought conditions was not significantly different from that without the application. The content of chlorophyll a on the foliar application was less than chlorophyll a in the soil application of ZnSO₄. Similarly, the content of chlorophyll a in both cultivars was not significantly different.

Table 3. Chlorophyll a and total chlorophyll content of two tomato cultivars at watering intervals 2

and 12 days with ZnSO₄ foliar and soil application Chlorophyll a Chlorophyll total Treatment (mg g⁻¹ fresh weight) (mg g⁻¹ fresh weight) Watering interval (day) + $ZnSO_4$ (mg Zn kg⁻¹ soil) 2 (normal) 0 0.258 a 0.468 k 12 (drought) 0 0.195 b 0.433 k 40 0.193 b 0.438 k

Application method ZnSO ₄			_
Soil	0.232 a	0.489 k	
Foliar	0.186 b	0.3971	
Cultivar			
Permata F ₁	0.209 a	0.449 k	
Tyrana F ₁	0.211 a	0.448 k	
Interaction	(-)	(-)	
CV (%)	19.39	18.28	

0 195 h

60

0455 k

Description: Values on the same column followed by the same letter are not different on the Duncan 5% test. (-): There is no interaction.

Table 4 shows the different responses of chlorophyll content b to the increase in $ZnSO_4$ influenced by the $ZnSO_4$ application method. In soil applications, chlorophyll b content in drought condition plants was more than chlorophyll b in plants with sufficient water. The application of $ZnSO_4$ on drought conditions decreased the chlorophyll b content compared to without $ZnSO_4$ application and the chlorophyll content b in the application with $ZnSO_4$ is not significantly different from the chlorophyll content of b in the plant by watering enough water. In the foliar application through leaves, the chlorophyll b content in drought plants without the application of $ZnSO_4$ is less although is not significantly different, whereas in plants with $ZnSO_4$ applications had more chlorophyll content than chlorophyll in plants with normal watering.

There is also interaction between dosage and method to chlorophyll ratio (Table 4). The chlorophyll ratio under drought conditions is generally lower than in normal watering. The application of ZnSO₄ through the soil increased the chlorophyll ratio compared with no application of ZnSO₄ under drought conditions, even the chlorophyll ratio in a dosage application with 60 mg Zn kg⁻¹ soil equaled the chlorophyll ratio at normal watering without the application of ZnSO₄. Different things are seen in the application of ZnSO₄ through the leaves, which decreases the chlorophyll ratio than without the application of ZnSO₄ in both drought and normal watering conditions. Wang also reported that Zn did not affect chlorophyll b and total chlorophyll contents under drought-stressed leaves, instead it caused an increase of chlorophyll b and total chlorophyll contents in well-watered leaves. Chlorophyll a contents in leaves did not change and chlorophyll a/b ratio was increased by Zn treatment [15].

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Although the method and dosage affect the chlorophyll ratio, the total chlorophyll is only affected by the ZnSO₄ application method. Table 4 shows the total chlorophyll content of the ZnSO₄ application through the leaves was less than in the soil. It is thought that foliar application resulted in Zn toxicity affected biosynthesis and chlorophyll degradation resulting in a decrease in chlorophyll content. Excess Zn in the plant results in a decrease in chlorophyll was also reported by Sagardoy [16] in sugar beet grown hydroponically. Table 4 also shows that both cultivars had the same total chlorophyll content, and the total chlorophyll content in both drought conditions without ZnSO₄ application and with ZnSO₄ application is no different from the total chlorophyll content at normal watering and the total chlorophyll content shows only difference in the ZnSO₄ application method.

Annlightign	Waterin						
Application	2 (normal)	normal) 12 (drought)					
method	- ZnSO ₄	0	40	60	-		
	Chloro	ophyll b content	t (mg g ⁻¹ fresh we	eight)			
Soil	0.193 b-e	0.308 a	0.254 a-b	0.272 b-d	0.507		
Foliar	0.227 a	0.170 b-f	0.237 b-c	0.247 a-b	0.469		
Mean	0.210	0.239	0.246	0.260	(+)		
Chlorophyll a/b ratio							
Soil	1.481 k	0.675 m-o	0.819 m	0.905 k-l	0.985		
Foliar	1.104 k-l	1.106 k-l	0.751 m-n	0.591 n-p	0.942		
Mean	1.292	0.891	0.785	0.749	(+)		

Table 4. Chlorophyll b content and ratio chlorophyll of tomato plant at watering interval 2 and 12 days with soil and foliar $ZnSO_4$ application

Description: CV chlorophyll b content = 20,31 %; CV chlorophyll ratio = 20,46 %. Values in the same row or column followed by the same letter are not different on the Duncan 5% test

4. Conclusion

The stomata opening width under drought conditions increases by increasing $ZnSO_4$ application under drought condition, but still smaller than stomata opening width under no drought stress condition. Chlorophyll a and chlorophyll total content are higher in $ZnSO_4$ soil application than foliar application under drought stress. $ZnSO_4$ application method on drought conditions gives a different pattern on chlorophyll b and the chlorophyll ratio along with increasing of $ZnSO_4$ dosage.

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