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Physiological characters and tomato yield under drought stress

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Abstract. Climate change had changed the rainfall patterns and causing tomato plants to suffer from drought in a certain period. It affects the morphology and physiology characters of the plant, and thus these characters can be used as indicators to predict plant tolerance to drought. The study aimed to determine the relationship between several physiological characters with tomato yield under drought stress. The study was conducted using 7 lowland tomato cultivars, namely 'Zamrud', 'Permata F1', 'Mirah', 'Tombatu F1', 'Tyrana F1', 'Ratna' and 'Tymoti F1'. Drought was applied by 8 days interval of watering. Relative water content, membrane stability index, chlorophyll, and proline content were measured when the plant entered the maximum vegetative of growth stage. The results showed a high correlation ($r > 0.6$) between membrane stability index with fruit weight per plant; while the proline, chlorophyll and relative water contents resulted in low correlation ($r < 0.5$) with fruit weight. The membrane stability index was better in indicating the drought tolerance in tomato than other physiological characters

1. Introduction

Climate change affects the rainfall patterns indicated by the increase of rainfall during wet season, but reduce the rainfall in the dry season. This causes the risk of drought during the dry season. Drought resulting in a decrease of agricultural crops productivity in general. In tomato plants, the effects of drought stress lead to various changes in morphological and physiological characters, i.e: the decreases in the plant height, dry weight, shoot root ratio, yield, relative water content and the chlorophyll content [1]. In addition, the drought stress increases the osmotic adjustment and causes differences in the photosynthetic responses between the tomato cultivars. The water shortages will also affect various physiological processes that will ultimately affecting the yields [2].

Most of the development of new drought-tolerant cultivars is based on a decrease in crop yield. It means the ability to survive in dry conditions by considering a decrease in yield. If the decline is small, the plant is considered to be able to withstand in unfavorable conditions. As reported by Zdravkovic who used a susceptibility stress index based on decreasing yields in drought stress for screening genotype drought-tolerant tomato [3]. In plant production, the yield of a plant is a function of various complex components, and also the final products that influencing each other between different morphological and physiological characters. From these relationships, it would be known which character will affects the yield dominantly [4]. Therefore, in the plant breeding programs, it is



very important to understand the relationship between the characters toward the yield and its component. This relationship could be used as a basis for determining the characters that can affect the yield. Therefore, it will improve the efficiency of breeding programs. One of the statistical tools to study this relationship is the simple correlation analysis. It provides the information about the relationship of plant characters and leads to a model for predicting the outcomes [5].

Several researchers have previously reported an association between the tomato yield and its component. The fruit weight positively related to 50% of flowering day and 50% of fruiting day [6]. Islam [7] stated that the weight was very significant and positively correlated with flowers, fruit, length, diameter, and weight in each plant. Souza et al. [8] revealed that the fruit yield had a direct effect and a significant positive correlation with fruits number. Shashikanth et al. [9] also revealed that the number of flowers per bunch and the number of branches highly correlated with fruit yield. However, most of the discussions about the relationship between the yield and its component are in the normal conditions. Only a few articles discussing the relationship of morphological and physiological characters to tomato yield under the drought stress conditions. Therefore, the purpose of this study is to analyze the relationship of various physiological characters with the weight of tomatoes under drought stress condition.

2. Material and method

The analysis and physiological character measurements were carried out both in the Plant Science Laboratory, Faculty of Agriculture, UGM and at the Laboratory of Plant Physiology and Biotechnology, Faculty of Agriculture, UNS, Indonesia. This study was conducted from October 2012 to January 2013, and was designed using a completely randomized design with one factor namely cultivars. The seven tomato cultivars used were 'Mirah', 'Zamrud', 'Ratna', 'Permata' F1, 'Tombatu' F1, 'Tyrana' F1 and 'Tymoti' F1 which were treated with drought and repeated 6 times. Drought conditions were applied by watering every 8 days interval. Soil moisture content before watering ranged from 16.54 -18.32 % mass (50-55% mass of soil moisture from field capacity). After watering with the volume of 2500 ml, the soil moisture content at all treatments ranged from 37.02 to 37.25% mass. As control, each tomato cultivar was also planted under normal condition with watering interval every 2 days.

Tomato seeds were sown in a nursery with sand planting media. Tomato seeds of approximately 3 weeks old with fully opened-2 leaves, were transferred to polybags, containing 8 kg of soil. The fertilizers consisted of compost (10 ton ha⁻¹) and SP-36 (200 kg ha⁻¹) were given before planting. The urea fertilizer (200 kg ha⁻¹) and KCl (100 kg ha⁻¹) were applied twice, one week and four weeks after planting with half of the doses, respectively. Watering according to treatment, pruning, pest and disease management were done manually.

The physiological characters observed including the relative water content (RWC), the membrane stability index (MSI), the chlorophyll and proline content. The measurement of physiological characters taken from the fully expanded leaves and were carried out when the plant entered the phase of maximum vegetative growth.

The measurement of the relative water content of the leaves was done by weighing the fresh weight of the leaves (fw), then it was immediately soaked in distilled water for 48 hours to get the weight of turgid (tw). After that, the leaves were dried in an oven to determine the dry weight (dw). The relative moisture content is calculated by the formula: $RWC = ((fw-dw)/((tw-dw)))$ [10].

The measurement of membrane stability index was carried out by weighing 0.1 g of leaves or roots which has cut into the similar sizes. Then it was put in a test tube containing 10 ml of distilled water in two sets. Each set was measured for electrical conductivity after incubation at 40 °C for 30 minutes (C1) and incubated at 100 °C for 15 minutes (C2). The measurement of electrical conductivity was carried out with EC meters, while membrane stability index was calculated using the formula $[1 - (C1 / C2)] \times 100$ [11].

Chlorophyll analysis was carried out by the method described by Islam et al. [12]. One g of leaf sample was cut into pieces and crushed in a mortar, then was added 20 ml of acetone 80%. The

solution was left idle for a while, then filtered with Whatman Filter paper. no. 42. It was inserted into the cuvette until the boundary line, then was measured by the spectrophotometer at λ 645 and 663 nm, respectively. The calculation of chlorophyll content was determined by the formula: Chlorophyll a = $(12.7 \times A_{663} - 2.69 \times A_{645}) \times (20 \text{ ml}/1000 \times 1 \text{ g})$; Chlorophyll content b = $(22.9 \times A_{645} - 4.68 \times A_{663}) \times (20 \text{ ml} / 1000 \times 1 \text{ g})$ and total chlorophyll content = $(20.2 \times A_{645} + 8.02 \times A_{663}) \times (20 \text{ ml}/1000 \times 1 \text{ g})$.

The measurement of proline content was done by the Bates method [13]. 0.5 g of leaves were pounded with mortar in a solution of 3 % sulfosylsilic acid as much as 10 ml. Then it was filtered with Whatman filter paper No. 1. Two (2) ml of filtrate was reacted with 2 ml of ninhydrin acid and 2 ml glacial acetic acid in a test tube at a temperature of 100 °C for 1 hour, after which the test tube was put into a beaker containing ice. The mixture was extracted with 4 ml of toluen, then shaken out with a stirrer for 15-20 seconds. The red color containing a proline at the top was sucked with a pipette and the absorbance of the solution was observed using Spectronic 21D at a wavelength of 520 NM.

The data were then analyzed by ANOVA [14], and the relationship between physiological characters with fruit weight were analyzed using the correlation and regression [15].

3. Results and discussion

The physiological characters, relative water, proline, chlorophyll content, and the membrane stability index of seven tomato cultivars under drought stress were presented in Table 1. The membrane stability index measures the percentage of damage and describes the ability of the membrane to survive in the drought stress. Water stress resulting changes in cell membrane permeability [11]. Table 1 shows that 8 days of watering interval resulting, decrease in the average membrane stability index (MSI) by 13.5% and 16.7% in the roots and leaves, respectively, compared to normal watering. The MSI in 'Mirah', 'Tyrana' F1 and 'Tombatu' F1 cultivars, were higher and significantly different from 'Zamrud', 'Permata' F1 and 'Ratna' cultivars. In addition, the reduction percentage of 'Tyrana' F1 and 'Tymoti' F1 cultivars were lower than others, which is showing they had the ability to maintain the high membrane integrity during the drought stress. Drought induces the oxidative stress due to increased production of reactive oxygen species (ROS), especially in photosynthetic organelles. This could directly oxidizes the lipid membranes so its permeability increases and cause ion leakage [11]. The decrease in the membrane stability index due to drought stress also occurred in peanuts, ranged from 4.9 to 8.2% in the severe stresses [16]. Meanwhile, for the wheat, it decreased by 35-41% and 47-58% under drought condition at the anthesis and grain filling phase, respectively [11].

As a result of membrane damage in the drought conditions, the process of absorption of water and nutrients by the roots affects the water content in the plants. The relative water content (RWC) indicates the status of water in the cell and is related to the tolerance to drought stress. Drought stresses decreased RWC with an average decrease ranging from 8-23% compared to watering every 2 days (Table 1). In that condition, the relative water content of some tomatoes ranges from 65-79%. It describes the osmotic adjustment so that the plant can maintain the turgor pressure [17]. The reduction of RWC of 'Tyrana' F1 and 'Tymoti' F1 cultivars were greater than others with the lowest. Meanwhile, the RWC of 'Ratna' cultivar was relatively high indicating it could adapt to the drought conditions.

Osmotic adjustment can also be shown by increasing the production of organic compounds. Proline production in tomato with 8 days of watering interval increased from 17 - 119% compared to normal conditions. The proline content on the seven cultivars under drought conditions was extremely diverse, ranged from 5-16 $\mu\text{g g}^{-1}$ fresh weight (Table 1). The proline content in 'Ratna' cultivar was higher than others, even triple than 'Tombatu' cultivar. This indicates that 'Ratna' cultivar attempted to survive in the drought conditions by increasing proline content. Plants accumulate osmolyte compounds such as proline, amino acids, and organic acids to survive in the drought conditions. However, proline is the most accumulated [17][18]. But not all plants show osmotic adjustment through a high accumulation of proline. In tomatoes, proline production and accumulation are depending on genetic, duration stresses and stress conditions [19].

Table 1. The membrane stability index (MSI), relative water content (RWC), proline and chlorophyll contents, fruit weight per plant under the drought conditions

Cultivar	MSI (%)		RWC* (%)	Proline* ($\mu\text{g g}^{-1}$ fw)	Chlorophyll ^{ns} (mg g^{-1} fw)	Fruit weight* (g)
	Root*	Leaf*				
Zamrud	61.08 a (-18.92)	61.73 ab (-15.56)	73.50 ab (-13.95)	12.03 bc (+107.86)	2.22 a (-70.69)	136.07 c (-27.93)
Permata F1	68.86 b (-5.78)	65.26 bc (-12.02)	75.54 ab (-14.81)	12.55 bc (+54.00)	2.34 a (-45.17)	304.41 b (-3.07)
Mirah	75.12 c (-12.96)	69.47 bc (-15.20)	78.90 b (-8.35)	8.09 ab (85.41)	1.79 a (-12.13)	300.61 b (-74.72)
Tombatu F1	72.64 c (-14.84)	69.12 bc (-15.61)	79.38 b (-8.65)	5.74 a (+ 58.39)	2.21 a (-9.12)	459.35 a (-37.28)
Tyrana F1	69.05 b (-20.00)	73.64 c (-14.17)	65.29 a (-23.28)	8.29 ab (29.53)	1.89 a (-23.61)	556.43 a (-68.14)
Ratna	63.49 a (-10.47)	53.28 a (-25.46)	76.89 ab (-8.66)	16.14 c (+119.02)	2.30 a (-57.91)	71.81 c (-148.10)
Tymoti F1	75.10 c (-11.59)	67.40 bc (-18.95)	66.31 a (-22.08)	8.98 ab (+17.06)	1.92 a (5.37)	445.99 a (-102.11)

Description : *: significant; ns: non significant. The means followed by the same letter in the same column show no significant difference in the Duncan test of 5%. The numbers in parentheses show the percentage of decrease (-) or increase (+) compared to normal conditions (watering every two days)

The total chlorophyll on tomato in the drought conditions ranged from 1.7 to 2.3 mg g^{-1} fw (Table 1). The results of the variance analysis showed that there were no significant differences between the tomato varieties. Drought stress resulting in a decrease of total chlorophyll content, which ranged between 9 - 70%. The biggest decrease was found in 'Zamrud' and 'Ratna' cultivars. It showed that these two cultivars suffered a large decrease in the ability to capture light energy, then it would lead to a lower photosynthesis process than others. The total chlorophyll reduction in drought conditions is thought to be related with the decrease in relative water content [20].

The fruit weight is the product of photosynthesis, and the adsorption of water, nutrition and chlorophyll during photosynthesis were affected by drought stress. Tomato fruit weight in the drought conditions decreased from 3-148% compared to the normal conditions. The smallest weight reduction was found in 'Permata' and the greatest decrease was found in the 'Ratna' Meanwhile, in spite of having a high yield decline, the fruit weight of 'Tyrana' cultivar was higher than others, indicating it was able to withstand under the drought conditions. Therefore, yield decreasing should not always indicating drought resistance.

Further analysis with the correlations and regression are presented in Table 2 and 3, respectively. Table 2 shows the relative water content, the content of proline and chlorophyll negatively correlated with fruit weight. It means that the higher relative water content, proline and chlorophyll contents led to lower fruit weight. It is also shown that the correlation coefficients were less than 0.5 and not significant, indicating low relationship of these physiological characters with fruit weight. Table 3 shows that the relative water content, proline and chlorophyll contents resulting in low R^2 , 13.56, 8.44 and 7.93%, respectively. This indicates that the contribution of these characters to fruit weights is very small, fruit weight is more dictated by factors. Therefore, the physiological characters are not appropriately used as an indicator of tomato tolerance in the drought stress. It is possible that the tomato resistance mechanism is not through the increased proline content because not all the plants have the same mechanism. Although proline content can be used as an indicator of drought stress resistance, but it is very important to consider that not all the plants produce it [18][19].

Table 2. The correlation matrix between physiological characters in drought stress conditions

Character	RWC	Chlorophyll	Proline	MSI root	MSI leaf
Chlorophyll	0.050				
Proline	-0.143	-0.131			
MSI_root	0.088	-0.253	-0.142		
MSI_leaf	-0.353	-0.190	-0.045	0.579**	
Fruit weight	0.353	-0.297	-0.152	0.515*	0.634**

* significant at 5%. RWC: relative water content; MSI: membrane stability index

The fruit weight was determined by MSI, as shown in Table 2 whereas the correlation between fruit weight and MSI was positive and with greater coefficient, it was 0.515 and 0.634 in the roots and leaves, respectively. This was also strengthened by the results shown in Table 3, where R^2 between fruit weight with ISM_D and ISM_A was 44.09 and 41.19%, respectively. It might be because the drought not only influences the physiological characters, but also the morphological and anatomical characters. It will affect the photosynthesis process which eventually will affect the fruit weight, too. Therefore, the membrane stability index (MSI) can be utilized as a selection parameter that affects the fruit weight and, it may be used as an indicator of plant resistance to the drought stress. This character has also been reported for use in wheat associated with stress tolerance index (STI) [21].

Table 3. The regression analysis of physiological characters and fruit weight of tomatoes in drought stress conditions.

Character	Regression	R^2
ISM_D	$y = -680.01 + 15.296x$	44.09
ISM_A	$y = -1179.2 + 21.669x$	41.19
RWC	$y = 964.33 - 8.677x$	13.56
Proline	$y = 395.26 - 6.8516x$	8.44
Chlorophyll	$y = 589.71 - 126.26x$	7.93

Notes: R^2 = Determination value

4. Conclusion

The fruit weight was less determined by relative water content, proline, and chlorophyll contents. Since the membrane stability index has a strong, positive and significant relationship with fruit weight under drought stress, it is possible to be used as a tool to predict the resistance of tomatoes to drought.

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