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Tolerance level of three genotypes of cayenne pepper (*Capsicum frutescens* L.) toward drought stress of vegetative phase based on morphological and physiological responses

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Abstract : The aim of this research was to determine the tolerance level of three genotypes of cayenne pepper (*Capsicum frutesecens* L.) toward drought condition in vegetative phase based on some morphological and physiological responses. Drought stresses were given by watering plants equall to 60% of field capacity (mild-stressed plant) and 30% of field capacity (severe-stressed plant). Control plant was watered equally to 80% of field capacity. The treatment of drought stress was applied in the beginning of vegetative phase for 3 weeks with interval watering two days. The result showed that drought stress caused the reduction of plant height, stem diameter, leaves length and leaves width significantly but the drought stress levels (mild and severe-stressed) were not significantly different. Drought stress also decrease RWC and width of stomatal opening significantly. The physiological responses observed after the plant experienced drought stress showed that chlorophyll content of leaves of control plants was higher than the plants which experience the drought stress but carotenoid content in the leaves of control plant was lower. Based on the Stress Sensitivity Index (SSI) value from morphological and physiological responses shown by three genotypes of cayenne pepper in this research obtained results that genotype I and genotype II were included in medium tolerant category toward mild and severe drought stress meanwhile genotype III was included in sensitive category toward mild and severe drought stress in vegetative phase.
Keywords : *Capsicumfrutescens* L., drought stress, vegetative phase, morphological responses, physiological responses.

Introduction

Pepper (*Capsicum* sp) is one of vegetable plants belongs to Solanaceae family[1]. In Indonesia there are two cayenne pepper cultivars being grown, Cakra putih and Cakra hijau. Cakra putih and Cakra hijau cultivars consist of several genotypes that can be distinguised by the shape, size, color of the fruit and level of spiciness. Every genotype of cayene pepper has a lifespan and different levels of tolerance to diseases and environmental stresses (abiotic stresses)[2]. One of abiotic stress beeing concern by farmers is drought. Drought is becoming a raising threat of the world and most of the countries of the world are facing the problem of drought[3]. Drought conditions affect the growth and production of crops including cayenne pepper plant[4]. Drought stress in plants can cause the flow of water through the xylem disturbed. Interrupting the flow of water causes a decrease in turgor cell[5].

Decrease in turgor affect the process of mitosis, elongation and expansion cell thus becomes stunted plant growth and crop production decreased[6]. In drought stress conditions the supply of water in the root zone decreased[7]. The decline led to the amount of water that can be absorbed by plants is reduced. The condition causes a potential drop of water on the leaves so that the relative water content (RWC) changes[3]. The major effect of decreased water availability in the leaf is diminishing leaf carbon fixation (A) due to stomatal closure[8]. Decreased CO_2 diffusion from the atmosphere to the site of carboxylation is generally considered the main cause for decreased photosynthesis[9].

Plant responses to drought stress can differ significantly, depending on the stage of plant development[3]. Drought stress that occurred in the early vegetative phase and during the transition between vegetative phase to the generative phase can cause a decrease in yield[10]. Every plant has a different response to drought conditions. The response depends on the genotype of the plant[11]. *Capsicumannuum* L. cultivars Shanshu-2001 is more resistant to drought stress conditions than cultivars Nongchengjiao-2. Resistance of cutivars Shanshu-2001 seen from the growth rate, yield and higher levels of antioxidants, and has a better ability to maintain RWC[12].

Plant responses to drought conditions, in addition, affected by genotype also influenced by the intensity of the drought experienced by the plant. In mild stress conditions, most of the plants could grow normally after being given optimal watering. But the plants exposed to moderate and severe stress conditions difficult to re-grow normally. The response is also specific, depending on the species or even depending on the genotype of the plant[13]. Plants which still showing increased growth in drought conditions can be regarded as tolerant genotypes[11]. Tolerance level of plants to drought stress can be determined by calculating Stress Sensitivity Index (SSI). SSI is a comparison of the value of a crop parameter in drought stress conditions and normal conditions[14].

Accordingly, in this study will be carried out observations of morphological and physiological responses of three local genotypes of Indonesian cayenne pepper to various conditions of drought stress in vegetative phase. Morphological and physiological responses were observed used as the basis for determining the level of tolerance of the three genotypes of cayenne pepper.

Materials and Methods

Planting and drought stress treatment:

This research was conducted from August 2015 to June 2016. Research was initiated with the selection of seeds to be used from three local genotypes found in the market at Malang Indonesia. Seeds that qualify for selection sowing on seedling media. Seedlings were transplanted to polybag containing 4,5 kg mixture of soil, compost, rice hulls, and organic fertilizer with a ration 2:1:1:1. Plants were grown in the Dau, Malang Regency, Indonesia. The drought stress treatments began at first stages of the vegetative phase (20 days after transplanting) for 21 days. Three levels of drought stress were used: 1) control which is 80% of field capacity; 2) mild stress, 60% of field capacity and 3) severe stress, 30% of field capacity with interval watering two days.

Measurement of morphological characters:

Morphological characters of stressed and unstressed (control) plants in terms of plant height, stem diameter, leaf length, leaf width and number of leaves were recorded at the first, middle and end of the vegetative phase.

Measurement of physiological responses:

Physiological responses were recorded 2 weeks after the plants exposed to drought stress. Physiological responses observed were RWC and width of stomatal opening. Measurement of RWC according to Ranganayakulu *etal*[15]. Leaf material was collected from control and stressed plants. Leaf discs of 1 cm diameter prepared. Fresh weight of five leaf discs, in three replicates, was recorded. The leaf discs were floated in 10 mL of distilled water for 6 h and allowed to gain turgidity. Then turgid weights were recorded and dried in oven at 80^oC for 24 h and dry weight of the samples was recorded. RWC was quantified and expressed in percent using the following formula:

$$RWC = \frac{FW - DW}{TW - DW} \times 100$$

Preparations for observation of stomatal conducted by printing method according to Astuti[16]. The third of leaf from the apex of an individual plant were collected. At each plant leaf there is 3 sample for stomata observations taken at the tip, middle and leaf base. Each sample was observed in three fields of view and in each field of view randomly selected 5 stomata to measure the width of the opening. Microscopic observations performed with 400x magnification.

Analysis of chlorophyll and carotenoid content:

Analysis of chlorophyll and carotenoid content conducted after plants ecperienced drought stress treatment. This observation was done for knowing the ability of plants to recover after experienced drought stress. Sample for analysis is the third leaf counted from the apex of an individual plant. About 1 g of each sample was finely ground and extracted with 100 mL acetone 80%. Extract was filtered using filter paper. Filtrate obtained was inserted into the cuvet for measuring chlorophyll and carotenoid content using spectrophotometer . The optical density was recorded at 645 nm and 663 nm for chlorophyl content while carotenoid content was recorded at 480 nm, 645 nm and 663 nm[17]. Chlorophyll content was quantified using the following formula by Sayyari & Ghanbari[18].

Chlorophyll a (mg/g F.W) = (12,7 x A663) – (2,69 x A645)
Chlorophyll b (mg/g F.W) = (22,9 x A645) – (4,68 x A663)
Total chlorophyll (mg/g F.W)= (20,2 x A645) + (8,02 x A663)

Carotenoid content was quantified using formula by Gayathri *etal*[19].

Carotenoid (mg/g F.W) = A480 + (0,114 x A 663) – (0,638 x A646)

Statistical analysis:

Univariate analyzed was employed for knowing interaction between the research factors. Normal and homogeneous data were analyzed with ANOVA and followed by Duncan test, while normal and not homogeneous data were analyzed with ANOVA and followed by Games-Howell test. Data that is not normal analyzed withKruskal-Wallis test and if there are significant differences ($P < 0.05$) were tested further by using the Mann-Whitney test.Each of morphological and physiological response data were used to calculate the Stress Sensitivity Index (SSI) using the formula by Fischer and Maurer[20].

$$SSI = (1-Ysi/Ypi) / (1-Ys/Yp)$$

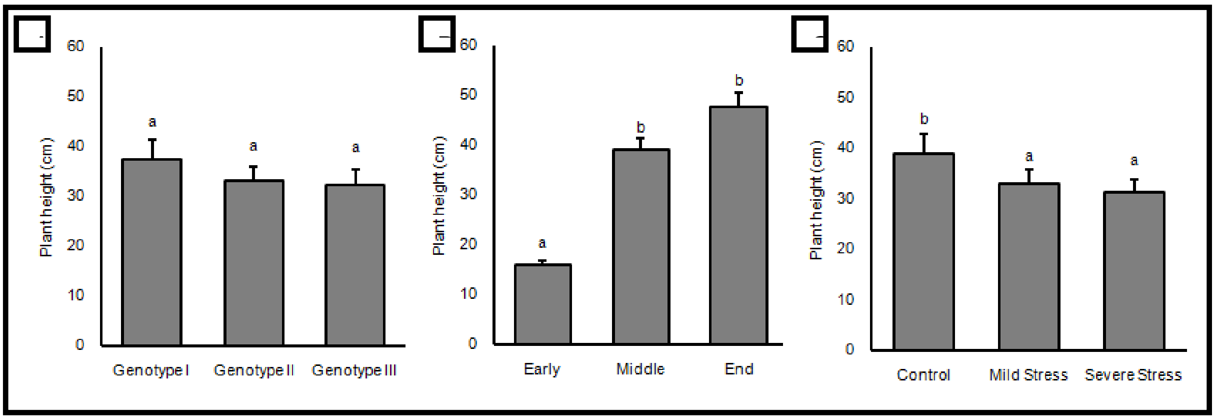
Ypi is grain yield of each genotype in non-stressed conditions; Ysi is grain yield in stressed conditions; Ys average yield of genotypes in stressed conditions and Yp is average yield of genotypes in nonstressed conditions.

SSI value of all the morphological and physiological responses was summed and calculated mean values to determine the criteria for the level of tolerance of each cayenne pepper genotype. The criteria for determining the level of tolerance: if the value of ISC <0.5 were categorized into tolerant genotypes; if 0.5 <ISC <1.0 were categorized into medium tolerant genotypes; and if ISC> 1.0 were categorized into sensitive genotypes[21].

Results

Morphological responses of three genotypes of cayenne pepper:

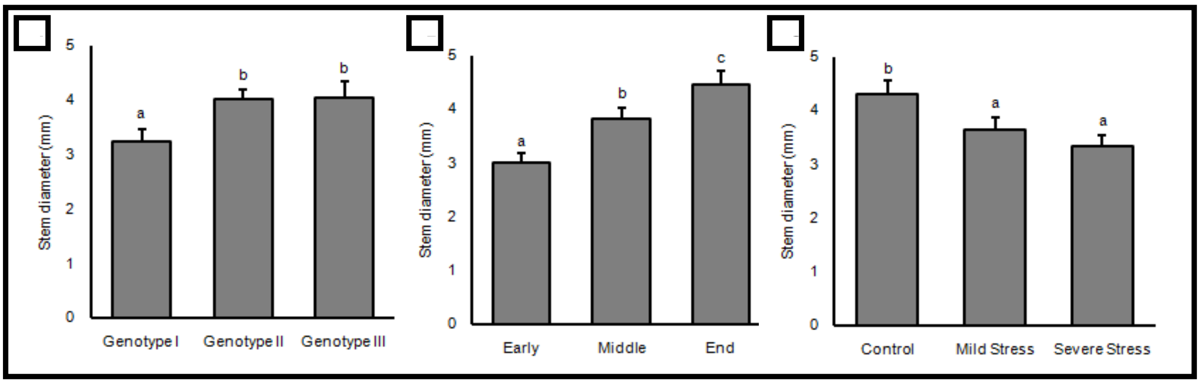
Based on statistical tests, plant height among genotypes did not differ significantly (Figure 1A). In the early stages until middle stage of vegetative phase plant height increased significantly, but at the end of the vegetative phase though still an increase in plant height but not significant (Figure 1B). Treatment of drought stress in plants caused significant decrease in plant height, it is shown in Figure 1C. Control plants taller than plants experience drought stress (mild and severe stress) but the level of drought stress had no significant effect on plant height.



Data are shown by the mean ± SE. The letters above the columns indicate significant differences (P< 0.05).

Figure 1. Plant height of cayenne pepper (*Capsicumfrutescens* L.) based on (a)genotype, (b)stages of plant growth, early (20 days after transplanting/DAT), middle (34 DAT) and the end of the vegetative phase (48 DAT) and (c)level of drought stress

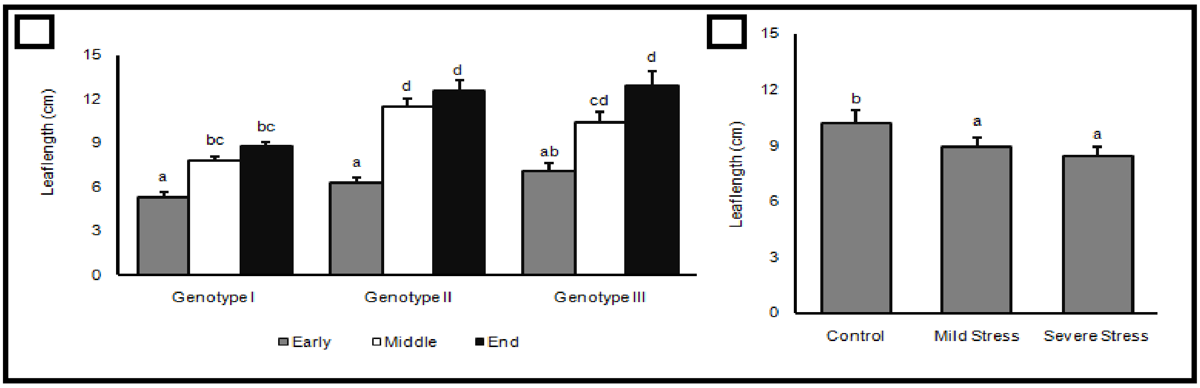
Other morphological responses observed in this research was stem diameter. Stem diameter among genotype I and genotype II and genotype III were significantly different (Figure 2A). Unlike plant height, stem diameter of cayenne pepper in the early stage until the end stage of vegetative phase increase significantly (Figure 2B). Drought stress significantly decreased stem diameter but the level of drought stress (mild and severe stress) had no significant effect on stem diameter (Figure 2C).



Data are shown by the mean ± SE. The letters above the columns indicate significant differences (P< 0.05).

Figure 2. Stem diameter of cayenne pepper (*Capsicumfrutescens* L.) based on (a)genotype, (b)stages of plant growth, early (20 days after transplanting/DAT), middle (34 DAT) and the end of the vegetative phase (48 DAT) and (c)level of drought stress

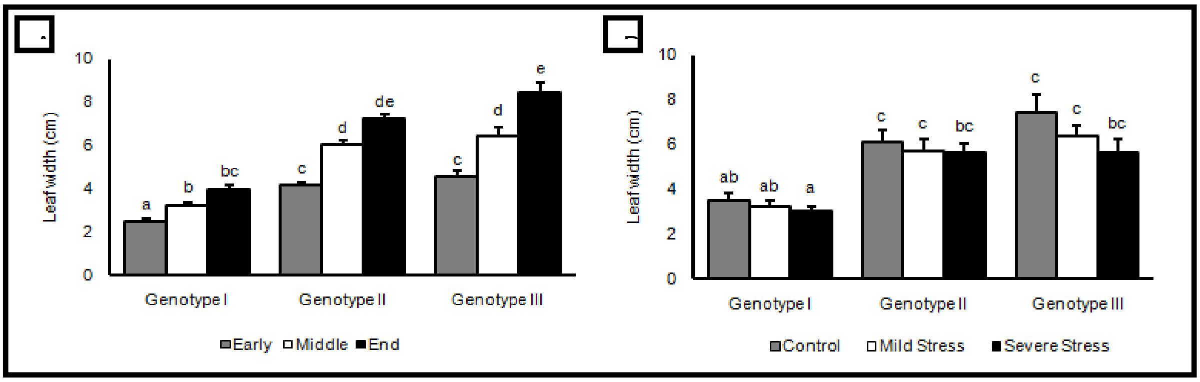
Leaves length of three genotypes of cayenne pepper did not differ significantly in the early stage of vegetative phase but in the middle stage until the end stage of vegetative phase, leaves length among three genotypes were significantly different especially between genotype I and genotype II and genotype III (Figure 3A). Drought stress significantly decrease leaves length of cayenne pepper but as well as plant height and stem diameter, the leaves length of cayenne pepper that suffered mild drought stress did not differ significantly with cayenne pepper that suffered severe drought stress (Figure 3B).



Data are shown by the mean ± SE. The letters above the columns indicate significant differences (P< 0.05).

Figure 3. Leaves length of Cayenne pepper (*Capsicumfrutescens* L.) based on (a) genotype and stages of plant growth, early (20 days after transplanting/DAT), middle (34 DAT) and the end of the vegetative phase (48 DAT) and (b) level of drought stress

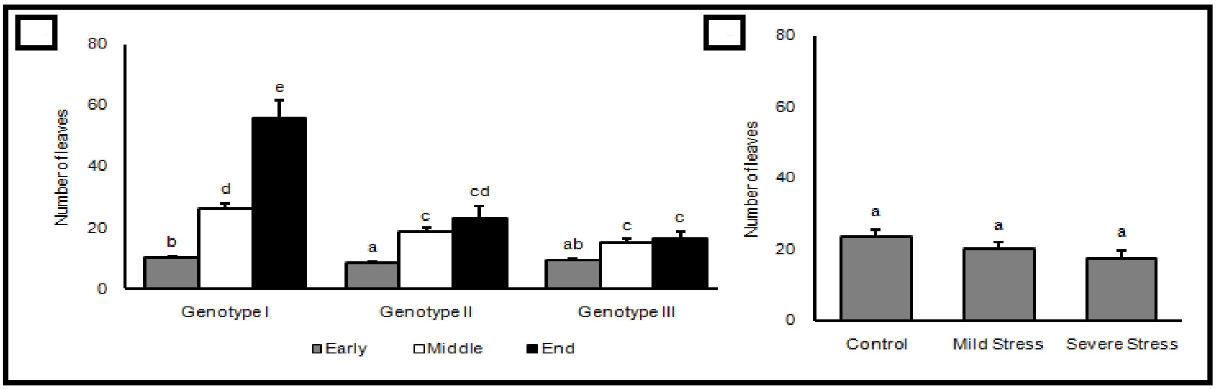
Leaves width of genotype I in the middle stage of vegetative phase increase significantly but in the end stage did not increase significantly while genotype II and genotype III increase significantly from the early stage until the end stage of vegetative phase (Figure 4A). Observation of the effect of drought stress to leaves width indicated that drought stress causes decrease of leaves width along with the severity of drought stress experienced by plants either genotype I, genotipe II or genotype III but the most affected is genotype I. It is shown by the leaves width of genotype I were smaller than genotype II and genotype III (Figure 4B).



Data are shown by the mean ± SE. The letters above the columns indicate significant differences (P < 0.05).

Figure 4. Leaves width of Cayenne pepper (*Capsicumfrutescens* L.) based on (a) genotype and stages of plant growth, early (20 days after transplanting/DAT), middle (34 DAT) and the end of the vegetative phase (48 DAT) and (b) genotype and level of drought stress

In this research, other morphological responses which also observed was the number of leaves. Genotype I is the genotype that showed the most significant increased of the number of leaves of throughout the vegetative phase compared with genotype II and genotype III (Figure 5A). The level of drought stress severity did not significantly affect the number of leaves on the cayenne peper (Figure 5B).

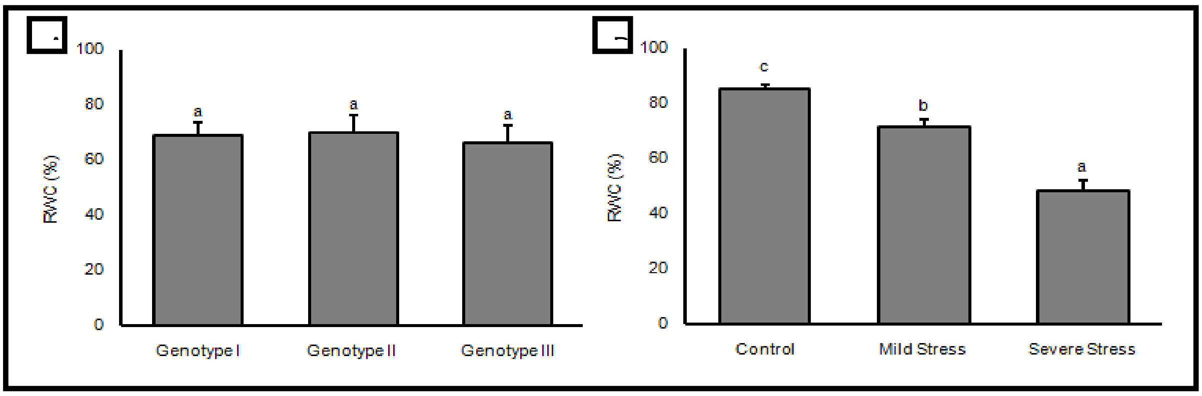


Data are shown by the mean ± SE. The letters above the columns indicate significant differences (P< 0.05).

Figure 5. Number of leaves of Cayenne pepper (*Capsicumfrutescens* L.) based on (a) genotype and stages of plant growth, early (20 days after transplanting/DAT), middle (34 DAT) and the end of the vegetative phase (48 DAT) and (b) level of drought stress

Physiological responses of three genotypes of cayenne pepper:

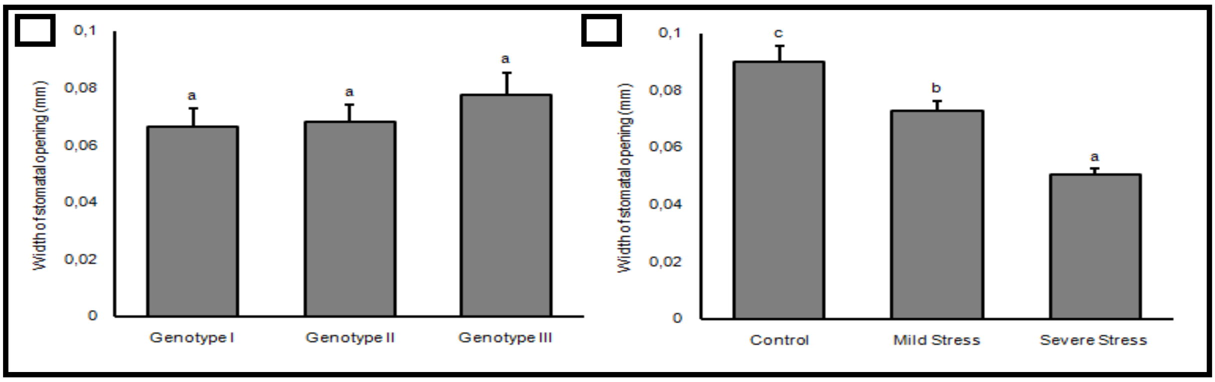
In this research, the physiological responses observed in each genotype which experienced different level of drought stress severity includes RWC, width of stomatal opening, chlorophyll and carotenoid of leaves. RWC is a representation of the water content contained in the plant tissue associated with changes of turgor cells. The water content of the plant tissue can be described with the value of RWC in the tissue[22]. The value of RWC among genotypes did not differ significantly (Figure 6A) but treatment of drought stress in plants causing decrease of RWC significantly (Figure 6B).



Data are shown by the mean ± SE. The letters above the columns indicate significant differences (P < 0.05).

Figure 6. Relative Water Content (RWC) of Cayenne peper (*Capsicumfrutescens* L.) based on (a) genotype and (b) drought stress levels was observed 2 weeks after drought stress treatment

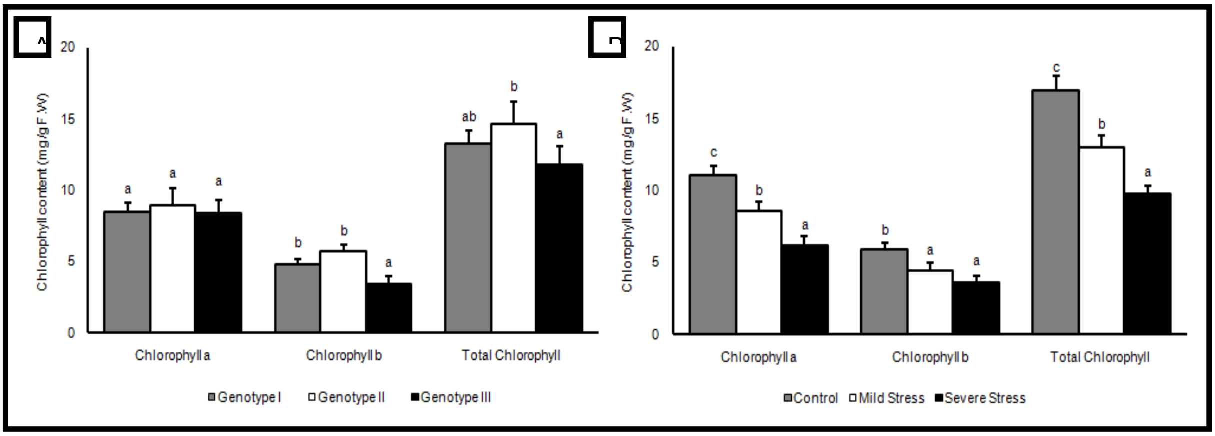
Another physiological responses observed in this research was the width of stomatal opening. Stomatal closure is one way of adaptations made by plants against drought stress. The width of stomatal opening among genotypes did not differ significantly (Figure 7A) but treatment of drought stress in plants causing decrease width of stomatal opening significantly (Figure 7B).



Data are shown by the mean ± SE. The letters above the columns indicate significant differences (P < 0.05).

Figure 7. Width of stomatal opening of Cayenne peper (*Capsicumfrutescens* L.) based on (a) genotype and (b) drought stress levels was observed 2 weeks after drought stress treatment

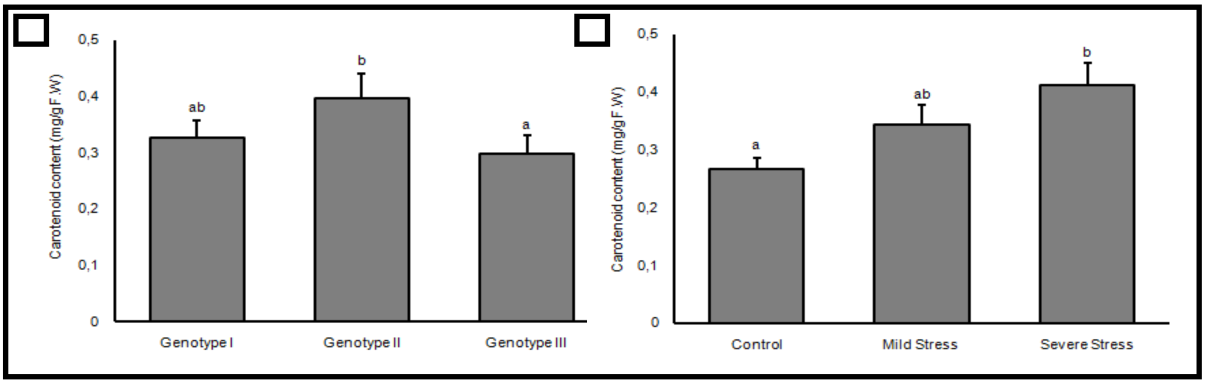
The chlorophyll a, chlorophyll b and total chlorophyll content of leaves , of three genotypes of cayenne peper are relatively similar (Figure 8A). Comparisons between plants that had experienced drought stressed and control plants showed that chlorophyll a, chlorophyll b and chlorophyll total of leaves of the control plants significantly higher than the plant that had experienced mild and severe drought stress. Differences in stress levels also affected chlorophyll content of the plants. Plants that had experienced mild drought stress have chlorophyll content significantly higher than plants that had experienced severe drought stress except for chlorophyll b (Figure 8B).



Data are shown by the mean ± SE. The letters above the columns in each type of chlorophyll indicate significant differences (P < 0.05).

Figure 8. Chlorophyll content of Cayenne peper (*Capsicumfrutescens* L.) based on (a) genotype and (b) drought stress levels experienced by the plant.

Plants that had experienced drought stress undergo changes in metabolic activity[23]. It is evidenced in this research by observing carotenoid content of each genotype that ever and never experienced drought stress. The carotenoid content of genotype II significantly higher than carotenoid content of genotype III (Figure 9A). Carotenoid content of plants that had experienced severe drought stressed significantly higher than control plants (Figure 9B).



Data are shown by the mean ± SE. The letters above the columns indicate significant differences (P < 0.05).

Figure 9. Carotenoid content of Cayenne peper (*Capsicumfrutescens* L.) based on (a) genotype and (b) drought stress levels experienced by the plant

Tolerance level of three genotypes of cayenne pepper:

Drought stress is one of the issues that need to be considered in cultivating cayenne pepper. The use of genotypes of plants that tolerant to drought stress is one of the alternatives to improve plantproduction in water limitation conditions. Based on that, the level of tolerance of the three genotypes cayenne pepper was determinedin this research. Based on the value of SSI, genotype I and genotype II had moderate levels of tolerance while genotype III was sensitive to mild stress. In this research also determined the tolerance level of the three genotypes of Cayenne peper to severe drought stress. The results obtained showed that genotype I and genotype II are genotypes with moderate level of tolerance while genotype III is a genotype that is sensitive to severe drought stress (Table 1).

Table 1. Value of Stress Sensitivity Index three genotypes of Cayenne pepper (*Capsicumfrutescens* L.) toward mild and severe drought stress based on morphological and physiological responses

Plant	Average of SSI value		Category
	Mild stress	Severe Stress	
Genotype I	0,76	0,86	Moderate
Genotype II	0,95	0,99	Moderate
Genotype III	1,08	1,15	Sensitive

Discussion

In this research, morphological responses observed showed increases from the early stage until the end stage of the vegetative phase. In the vegetative phase water used by plant for cell division and cell enlargement, causing increase in plant height and stem diameter. In this phase, the plant also utilizes the majority of primary metabolites resulting from the photosynthesis to support the formation of vegetative organs of plants. Division assimilate in the vegetative phase is directed to the stem which supports plant growth and is directed to the leaves so increase length and width of leaves during the vegetative phase[24]. Drought stress in this research led to a decrease in morphological characters observed in plants that experienced mild and severe drought stress. It is because the water used to maintain cell turgor[5]effect on the process of mitosis, elongation and expansion cell[6]. Drought stress resulted in the reduction or even cessation of cell division activity so there is no increase cell size in plant[24]as well as decreasing the number of cells per tissue of plant[25].

Number of leaves in this research did not influenced by drought stress. Based on theory, drought stress will reduce number of leaves. This finding was inaccordance with the result of research conducted by Adeoye *etal*[26]who observe the effect of different intervals watering to some morphological characters of red pepper one of which was the number of leaves. It was found that the longer interval watering will futher decrease the number of leaves on the plant.

RWC and width of stomatal opening are physiological responses that are accurate to describe mechanisms carried out by plants against drought stress. Decrease of soil water cause the amount of water that can be absorbed by plants is reduced, so the amount of water contained within the tissue of the plants that experiencing drought stress are lower than the plants which obtain optimal watering.. Plants absorb water from the soil and distribute it troughout the plant tissue to maintain cells turgor on the tissue. In the event of a decrease in the amount of water that can be absorbed by the plant, the plant will perform an adaptation mechanisms either by stomatal opening and closing mechanism so the amount of water lost through transpiration is reduced[27].

Plants experience drought stress can re-grow normally after a given water supply is not longer limited (optimal) but the plant experiencing a severe drought stress will be difficult to re-grow normally[13]. It was evidenced in this research based on the data of chlorophyll and carotenoid content of leaves from each plant that have experienced drought stress compared with plants that have never experienced drought stress (control).

The decreased chlorophyll content and increased secondary metabolites (carotenoid) in plant that had experienced drought stress showed that drought condition cause long-term effect on the disruption of the metabolic activity in plants. Decrease in chlorophyll content due to inhibition of the synthesis of chlorophyll a and chlorophyll b due to decrease activity of enzimes involved in the biosynthesis of chlorophyl[28]. Disorder metabolic activity in plants that had experienced drought stress indicated by the increase in carotenoid content compared to the control plants. Carotenoid is one of the photosynthetic pigment that plants need in small amount, but because the plants that had experienced drought stress decreased the chlorophyll content, carotenoid as photosynthetic pigment required in greater numbers to the sustainability of the process of photosynthesis. Dominant pigment in plants is chlorophyll. Carotenoid helps chlorophyll to absorb light energy so that the plants decreased chlorophyll content will be increased carotenoid content because carotenoid as accesory pigment in photosynthesis[17]. Increase of carotenoid in plants associated with the ability of plants to adapt to new environmental conditions[29].

The results obtained in this research indicate that genotype I and genotype II are moderate tolerant genotypes, while genotype III is sensitive genotype to mild and severe drought stress. Based it can be said that genotipe I and genotype II have better adaptability than genotype III. Tolerance of plants to drought stress conditions are the result of a series of morphological and physiological responses are carried out by plants in the face of environmental change.

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