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Physiological responses of some local cowpea from Southwest Maluku (Indonesia) varieties to drought stress

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Abstract. *Karuwal Rl, Suharsono, Tjahjoleksono A, Hanif N. 2017. Physiological responses of some local cowpea from Southwest Maluku (Indonesia) varieties to drought stress. Biodiversitas 18: 1294-1299.* The aim of this study was to analyze the physiological responses of some local cowpea from Southwest Maluku (Indonesia) varieties to drought stress. The physiological responses were analyzed by measuring plant height, number of leaves, relative water content (RWC), and chlorophyll content using Anova and were continued with Duncan test at 5% significance level. Results of this research showed that the varieties and drought stress in the form of watering periods to affect to all variables except plant height. Physiological responses showed that at every ten days watering periods, the crimson varieties gives the highest plant height (33.85 cm), the brown varieties gives the highest number of leaves (30), the dark brown varieties have the highest RWC (88.675%), and the highest content of chlorophyll a (0.5088 mg/L), chlorophyll b (1.595 mg/L), and total chlorophyll (1.5095 mg/L) are found in white varieties. The supply of water at every ten days is the optimum time of drought stress in cowpea. Further research will be needed on the responses of the other variables for screening, selection, then multi-location trials to obtain tolerant local cowpea varieties to drought.

Keywords: Cowpea, drought stress, physiological responses

INTRODUCTION

Cowpea is one of local crop legume that was cultivated in Indonesia, but only by a few of farmers. Morphologically, it has diversity in growing habit, leaf, pod and seeds. Physiologically, it has important traits such as more tolerant to drought, more pest and disease resistant, easier to cultivate, and more ability to produce pods on nutrient poor soil than other legumes (Rukmana and Yuniarsih 2000). Cowpea seeds are used as food materials, livestock, and raw industrial materials. Seeds of cowpea have high protein and low fat just like soybean i.e. between 23,4-25,9% (Purwani and Santosa 1996) and then can be developed as an alternative material to make tempeh (Ratnaningsih et al. 2009).

In Indonesia, a total of 139 accessions of cowpea were listed in germ plasma collection from Java, Sulawesi, and Nusa Tenggara islands (ICABIOGRD 2010). One of areas also having potential of cowpea is Maluku islands namely Southwest Maluku District but it is not listed. In general, the community in this area cultivates cowpea as food materials as carbohydrate complement. Based on exploration results by Polnaya (2008) in Lakor Island, there are ten of local varieties with a local name according to seeds color. According to Esquinas-Alcasar (1993), landrace is a variety growing in a location which can adapt to the adverse condition, with stability of production. Southwest Maluku has harvest area of legumes as much as 149 hectares with the low productivity and human consumption by 50.83 calories (Central of Statistics Southwest Maluku District 2015). From environment factors, small islands in southwest Maluku are an arid area with minimum rainfall and this is limiting factors in the availability of water (Susanto dan Sirappa 2005). According to Pesireron et al. (2013), rainfall distribution in this area consists of eight months of dry season and four months of the rainy season so that water availability is limited and it becomes a limiting factor in the productivity of agricultural crops. Thus, it can also affect the growth and productivity of cowpea due to drought stress as described by Peijic et al. (2013) that drought stress affects cowpea production and it also depends on genotype, intensity and stress periods, stage of plant development. Ndiso et al. (2016) also reported that water stress imposed vegetative growth stage and the impact of water stress on growth is dependent on the cowpea variety.

Drought stress is a condition of reduced water in plants affecting the growth and production (Purwanto and Agustono 2010) depending on period and intensity, and stage of plants development (Bastos et al. 2011). The responses to drought stress at plants growth are the decrease in plant height, stem diameter, number of leaves and also the changing of anatomical structure. Another response is the reduction of water content and chlorophyll content in leaves, and the accumulation of chemical compounds such as the increase of proline content in osmotic adjustment that will inhibit metabolism process (Nio et al. 2011). In the study about breeding for drought tolerance in progenies of cowpea, Abed (2014) reported that the highest amount of chlorophyll content and relative water content is found in the S3 progeny under period of 10 days irrigation in both seasons. While Okon (2013) reported that seedling of brown variety had more adverse effect than white variety on growth performance which was observed based on water regimes (12, 24, and 48 hours) for 30-days in the green house. Also, Kutama et al. (2014) mentioned that in all the varieties after water stress induction, it generally showed a different rate of drought tolerance and it was observed based on some physiological mechanisms.

Various of studies about the response to drought stress on cowpea were conducted by Bastos et al. (2011); Punggulani et al. (2012); Okon (2013); Uzunova dan Zlatev (2013); Abed (2014); Kutama et al. (2014); Ndiso et al. (2016). However, the study about some local cowpea from southwest Maluku varieties was focused on exploration and agronomic characterization while the observation in response to drought stress and to do screening on tolerant varieties had not been done yet. Therefore, character of plants with physiological responses to drought tolerance must be analyzed to obtain tolerant varieties in the local site and to build a data base. The aim of this study was to analyze the physiological responses of some local cowpea from southwest Maluku varieties to drought stress.

MATERIALS AND METHODS

Study area

A field experiment was conducted at a greenhouse in the Department of Biology, Universitas Pattimura, Ambon, Maluku (Moluccas), Indonesia in September-December 2016.

Design

Randomized complete block design (RCBD) was used in this research with two factors and two replicates. The first factor was cowpea varieties consisting of batik, crimson, pink, white, dark brown, russet, black, yellow, and brown which were collected from Sermatang island. The second factor was drought stress which was in the form of watering period of two days (P0), ten days (P1), and 15 days (P2).

Procedures

The soil was obtained from Taeno Rumahtiga village, then it was screened for dirt and litter, and was dried and filtered. A 3 kg of the soil was poured into polyethylene bags with size of 30 x 40 cm and then was watered before the cowpea was planted in it. Two healthy seeds of cowpea were planted in each polyethylene bags with a depth of 3 cm. Supply of water was done in accordance with combination treatments on each variety from the first day of planting and it was 300 mL of water.

Physiological responses

Physiological responses which were measured were plant height in cm, number of leaves/plant, relative water content (RWC) in percent, and chlorophyll content in mg/L. Plant height was measured from the surface of soil to the first branching point using measure tape on tenth week after planting. While, on number of leaves only fully formed leaves were calculated. RWC was measured following the method of Abed (2014) with modification. The second fresh leaf from the shoot of the plant in each pot was detached and was put in a labeled aluminum foil. 0.5 g of fresh leaf was weighed and it was considered as fresh weight (FW), then it was soaked in water for 4 hours, and after being picked up from water, it was weighed again and this time, it was considered as turgid weight (TW). Next, the leaves were dried in an oven at 80° C for 24 hours, then it was weighed again and its weight was considered as dry weight (DW). RWC was calculated using formula:

$$RWC\ (\%) = (FW-DW)/\ (TW-DW) \times 100$$

Chlorophyll content was measured at tenth week after planting following the method from Setiari and Nurchayati (2009) with modification. The second fresh leaf from apex was picked and 0.1 g of it was spread out. This leaf was extracted with 10 mL of acetone 80%. The extract was sifted with Whatman filter paper number 2. Absorbance of filtrate was calibrated on UV-Vis spectrophotometer at a wavelength of 644 nm and 663 nm. Calculation of chlorophyll content was estimated using formula:

$$\text{Chlorophyll a} = 1.07\ (OD\ 663) - 0.094\ (OD\ 644)$$
$$\text{Chlorophyll b} = 1.77\ (OD\ 644) - 0.28\ (OD\ 663)$$
$$\text{Total of chlorophyll} = 0.79\ (OD\ 663) + 1.076\ (OD\ 644)$$

Statistical analysis

The data obtained were analyzed with analysis of variation (ANOVA) and Duncan test at 5% significance level using SPSS 16.0 software.

RESULTS AND DISCUSSION

The effects of drought stress on plant physiology activity are determined by periods and intensity of stress, stage of plant development (Bastos et al. 2011), and also the characteristics of plant (Fathi and Tari 2016). This opinion is also in line with that explained by Peijic et al. (2013) that the production of cowpea exposed by drought is affected by genotype, period and intensity of stress, stage of plant development. Each genotype of plant will give different physiology responses based on its sustainability or controlled by genetic factors. The physiological responses such as plant height, number of leaves, relative water content, and chlorophyll content were measured in this study. The differences in the physiological responses to drought stress among the nine cowpea varieties show that each variety has different ability in drought stress treatment because cowpea is tolerant plant to drought (Bastos et al. 2011). This is the same for the local cowpea varieties from Southwest Maluku, although these varieties are landrace that naturally grows in the same area, their resistance to drought has not been studied yet and therefore, they are still unknown. In general, the highest result of physiological responses was obtained in the period of watering of treatment P1 (every ten days).

Results show that period of watering affects the fluctuation of plant height in each cowpea varieties (Figure 1). The highest plant height is found on crimson variety (33.85 cm) and the lowest is found on brown variety (12.35 cm) on treatment P1. The russet variety gives the highest (33.40 cm) and the lowest is crimson variety (23.75 cm) on treatment P2. Compared to control, on treatment P1 varieties of crimson, pink, white, dark brown are more tolerant than varieties of batik, russet, black, yellow, and brown based on plant height. Results of ANOVA show that varieties, drought stress, and the interaction with them give no effect on plant height. The fluctuation in plant height of cowpea makes them drought tolerant plant varieties (Bastos et al. 2011). Treatment P1 lead to the reduction of water on growing media but the growth of cowpea is optimal on certain varieties, even it has the highest growth number in treatment P2. Besides that, process of growth requires different amounts of water depending on the type of plant (Hendriyani dan Setiari 2009), so there are varieties with optimal plant height in the water with small amount. Treatment P0 on cowpea made the growing media in humid conditions all the time, so it disturbed aeration of soil and it resulted in stunted growth of cowpea. Results of research show that varieties and periods of watering give no affect on plant height of cowpea, because cowpea has character of tolerant to drought. Moreover, the character of cowpea which is resistant to drought will make the plant to reach its maximal height in a longer time at the vegetative stage.

In contrast, the number of cowpea leaves on the treatment showed that each variety had a declining number of leaves in line with the watering period (Figure 2). The highest leaf number was found in the russet variety (30) and the lowest was on brown variety (17) in the P1 treatment. The highest number of leaves was found in yellow variety (22.50) and the lowest was in pink variety (15 strands) in P2 treatment. Compared with controls, all variety in P1 treatment were more tolerant than P2 based on leaf number. ANOVA results show that variety, water intervals, and interactions between them have an effect on the number of leaves. The decrease in the number of leaves, which is in line with the increasing period of water administration in each variety, is significantly due to cell division at the time of leaf formation. According to Yunusa et al. (2014) reduction of leaf number is caused by decrease of leaf initiation and leaf area so that the activity of photosynthesis is also decreased and this is also explained by Kariuki et al. (2016). Gardner et al. (1991) state that in line with the increasing age of the plant there is a reduction in the number of leaves. Once the leaves become mature and aging, the leaves may not be able to meet their own energy needs. On the other hand, the reduction of the number of leaves can be due to the use of more energy in increasing the plant height in which the energy is obtained from its own leaves as a source of energy. At the vegetative growth stage, water is used by plants for cell division and enlargement in plant height increase and leaf propagation.

Similar to the number of leaves, RWC also shows the same trend (Figure 3). In the P1 treatment, dark brown variety gave the highest RWC (88.675%) while the lowest was on batik variety (69.645%). For P2 treatment, dark brown variety gave the highest RWC (89.615%) while the lowest was on batik variety (63.115%). Compared with control, all variety of P1 treatment were more tolerant than P2 treatment. ANOVA results show that variety, watering intervals, and their interactions have effect on RWC.

Water content is relatively associated with water availability in leaf tissues (Pungulani et al., 2012). The height of relative water content in water stress condition indicates the ability of accession to maintain the metabolism process so that it can survive. The results show that the value of relative water content i.e. 77% in cowpeas after being treated with stress for two weeks is high, while in well-watered condition the cowpea has a relative water content value of 88-91%. After four weeks of treatment, the accession of cowpea has water content of above 50% which is significant to the ability of metabolic processes during extreme conditions. Maintenance of high relative water content in some genotypes after four weeks can be used to screen for drought tolerance in cowpea. When compared to this study, the relative water content measured at plant age of six weeks after the drought stress treatment has a range of above 60%. This suggests that cowpea varieties have the ability to survive even under stressful conditions.

The measurement of chlorophyll content consists of chlorophyll a (Fig. 4), chlorophyll b (Fig. 5), and total chlorophyll (Fig. 6). White variety gives the highest levels of chlorophyll a (0.5088 mg/L), chlorophyll b (1.595 mg/L) and total chlorophyll (1.5095 mg/L) while the lowest is on brown variety in P1treatment. Batik variety gives the highest level and the lowest level is black variety in P2 treatment. Compared to control, in P1 treatment, based on chlorophyll content, all variety is more tolerant except for yellow and brown variety. ANOVA results show that the interaction has an effect on chlorophyll a content while the period of watering affects chlorophyll b content and total chlorophyll content.

Chlorophyll as one indicator of drought-stricken plants is synthesized with the aid of light, water, temperature, nutrients, as well as genetic factors and the numbers are different for each species (Hendriyani and Setiari, 2009). The measurement of chlorophyll content can evaluate the process of photosynthesis in tolerant plants. Water as one of the factors influencing chlorophyll synthesis can determine chlorophyll content. This means that plants that absorb water from growing media containing a lot of water lead to high level of chlorophyll. In this study, cowpea variety has decreasing chlorophyll level which is in line

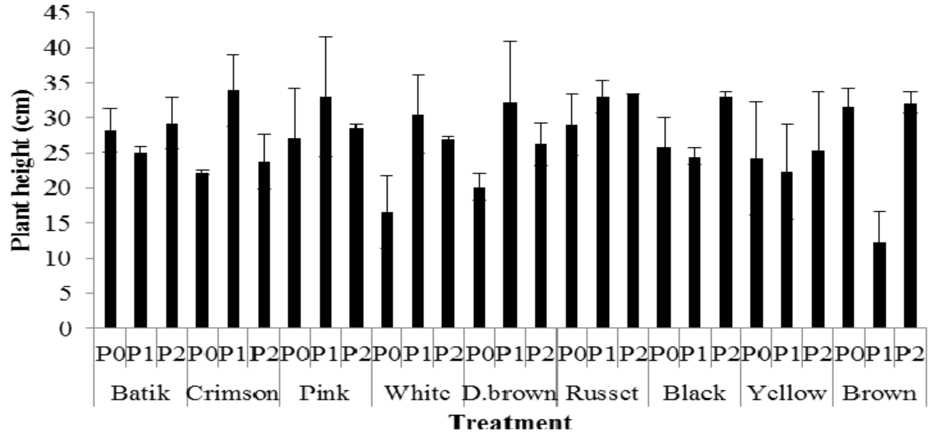

Figure 1. Effect of three periods of watering and nine varieties of cowpea on plant height

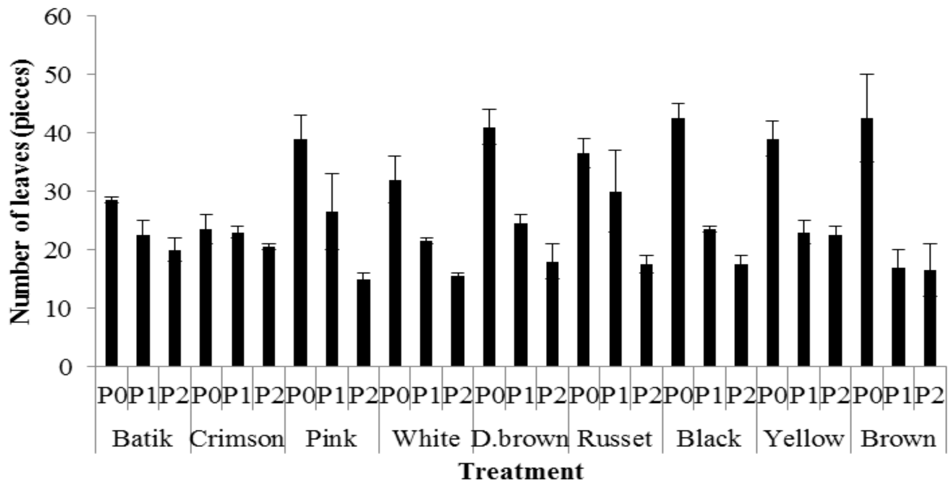

Figure 2. Effect of three periods of watering and nine varieties of cowpea on the number of leaves

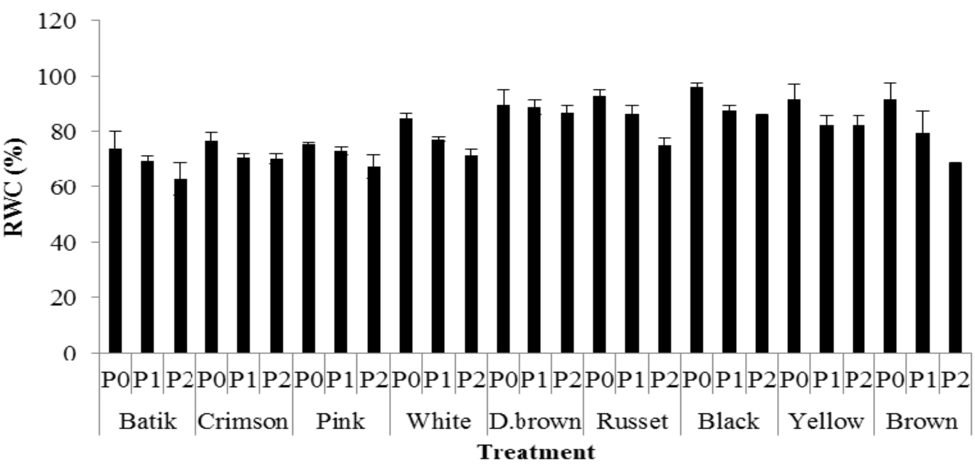

Figure 3. Effect of three periods of watering and nine varieties of caudal on RWC

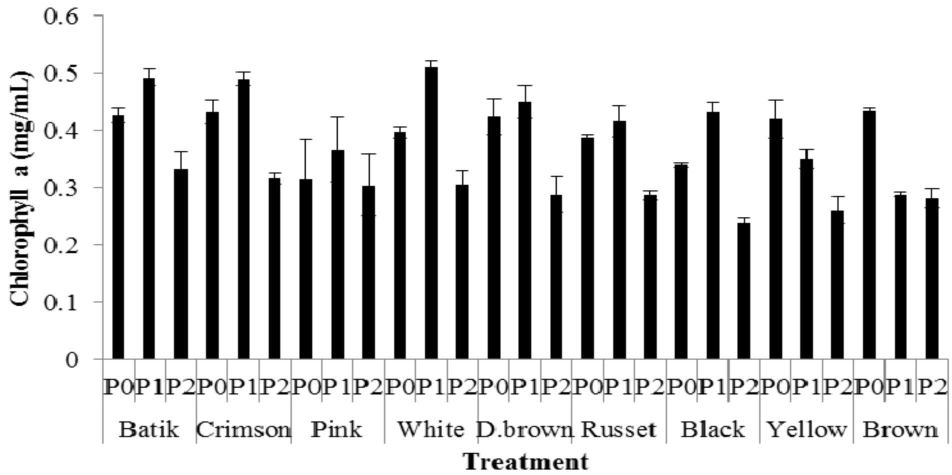

Figure 4. Effect of three periods of watering and nine varieties of cowpea on chlorophyll a

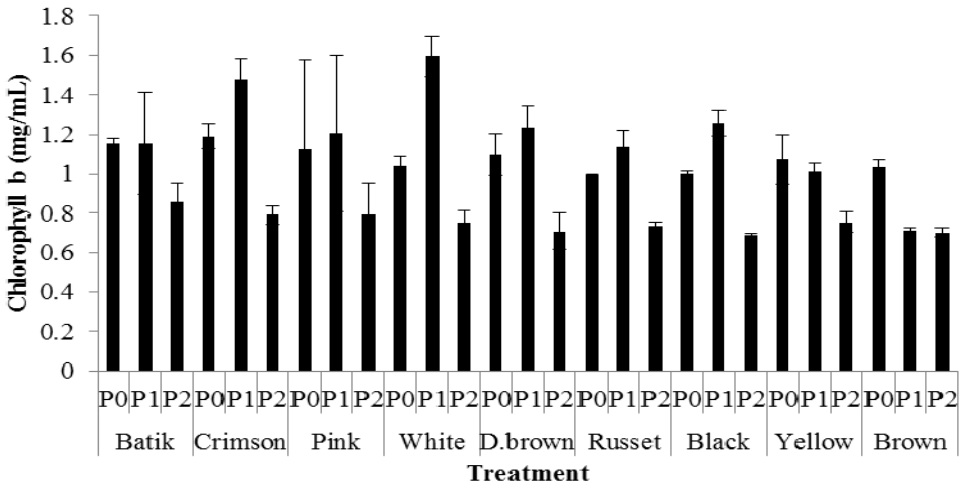

Figure 5. Effect of three periods of watering and nine varieties of cowpea on chlorophyll b

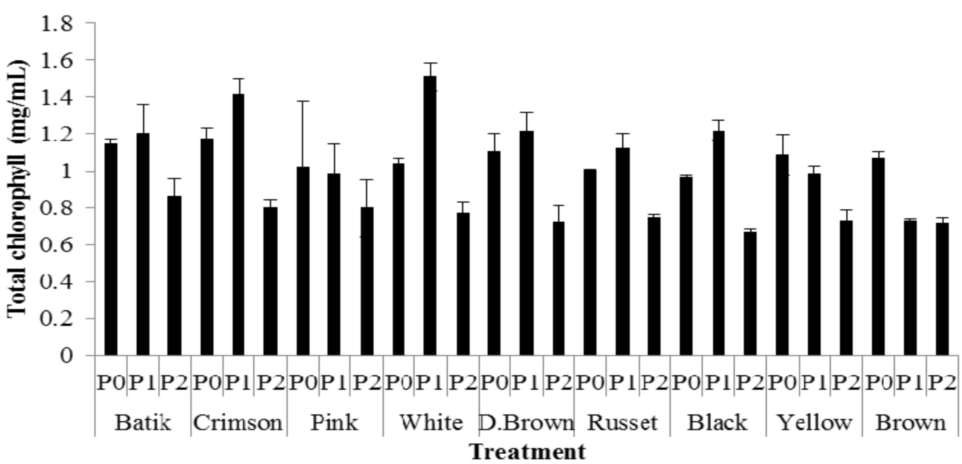

Figure 6. Effect of three periods of watering and nine varieties of cowpea in total chlorophyll

with the period of watering due to the lack of water up taking in planting medium. On the other hand, the synthesis of chlorophyll is determined by the element of nitrogen that can be fixed directly by the cowpea plant having symbiotic relation with the fixing bacteria. In moist or wet conditions of planting media, the fixing bacteria cannot enter the pores of the soil because they are aerobic, on the other hand, they can bind the nitrogen and form the optimal chlorophyll in the lack of water condition.

Based on the result of the research, it can be concluded that the physiological response of nine local varieties of cowpea of Southwest Maluku on drought stress significantly influence leaf number, relative water content, and chlorophyll content and physiological response differences among varieties can be used as indicator of sustainable selection for cowpea variety with drought tolerance. Providing water once every 10 days is the optimum time of drought stress on cowpeas. Through screening and other variable selection and multi-site testing, local cowpeas from Southwest Maluku can be developed to obtain local varieties tolerant to drought.

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