MALAYSIAN JOURNAL OF BIOCHEMISTRY & MOLECULAR BIOLOGY

The Official Publication of The Malaysian Society For Biochemistry & Molecular Biology (MSBMB)
http://mjbmb.org

GROWTH AND PHYSIOLOGICAL RESPONSES OF SWEET POTATO PLANT (*Ipomoea batatas* (L.) Lam.) VAR. CILEMBU TO DROUGHT STRESS

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International E-Conference of Science and Biosphere Reserve 2021

Keywords:

Ipomoea batatas, Sweet potato var. Cilembu, Drought stress, Physiological responses

Abstract

Drought stress is a serious problem in agriculture since it causes a significant decline in agricultural production. This problem is also experienced by cilembu sweet potato farmers in the Sumedang area, West Java, Indonesia, who make cilembu sweet potato as one of the main commodities that support their income. This study aims to understand the growth and physiological response of sweet potato *Ipomoea batatas* (L.) Lam.) var Cilembu (SPC) when grown under drought stress. The plants were grown at various levels of drought stress i.e. 80% field capacity (fc) (as control), 60% fc, 40% fc and 20% fc, over an exposure period of 10 weeks. Parameters measured included plant relative growth rate (RGR), an increase of plant length, number of leaves, leaf area, plant dry weight, relative water content of leaf (RWC), chlorophyll content, proline and glycine betaine contents, mallondialdehyde (MDA) and antioxidant enzyme activity of catalase (CAT) and ascorbate peroxidase (APx). Result showed that SPC plants treated with 20% fc withstand severe drought stress. However, its recorded significantly lowest relative growth rate with 86.7%. Growth parameters such as vine length, number of leaves, leaf area, dry weight, RWC and chlorophyll content also decreased significantly at all levels of drought stress studied. At 20% and 40% fc, the content of proline, glycinebetaine, MDA, antioxidant activity of CAT showed significantly optimum value. On the other hand, the APC activity showed a negative result for a similar treatment. The content of proline, glycinebetaine, MDA, antioxidant activity of CAT were significantly increased at 20% and 40% fc treatments, while the APx activity was not affected. A significant increase of proline, glycinebetaine, MDA and CAT in plant indicated plant adaptation to drought stress. Further study is required to understand the effect of drought to the quality and quantity of tuber production in the field scale.

INTRODUCTION

Sweet potato, *Ipomoea batatas* (L.) Lam, is a perennial crop, a popular staple food of the tropical and subtropical areas with a nutritional benefit. The plant has been used as a staple food in various indigenous populations from central and south America to Papua New Guineans [1]. It is regarded as the sixth most important food crop in the world [1]. Sweet potato is mostly harvested for its tubers, although the leaves are also consumed as vegetables. and it contains phytochemicals, which are beneficial for human health. The Sweet potato is also used for composite ingredient-based foods, starch, and industrial products [2] as its tuber rich in dietary fibre, antioxidants, vitamins, minerals, and contain no saturated fats or cholesterol. [3]

Sweet potato var Cilembu (SPC) is very well known in Indonesia due to its unique honey-sweet taste when the tuberous roots are cooked in an oven. This characteristic is probably due to the fact that the tuberous root of SPC has a sugar content as much as 3.53–6.87%, which is higher than common red sweet potato, which has sugar contain only 2.38%, and SPC was also reported to contain glucose (1.60-

2.67%), fructose (0.6–1.79%), sucrose (0.36–12.6%) and maltose (0.39-1.97%) [4]. Traditional cultivation of sweet potato by local farmers in Indonesia depend very much on rainy season, whereas during dry season farmers usually do not cultivate sweet potato.

Drought stress adversely affects plant growth and it is estimated that more than 40% of crop production is lost to drought [5, 6]. Physiologically, dought causes a decrease in plant water content, water potential and turgor pressure in the cells, leading to a decrease in plant growth Furthermore, drought is also known to cause chlorophyll damage, protein degradation, decreased permeability of membrane and peroxidation as well as stomatal closure, which can lead to a decrease in internal CO_2 concentrations [7]. Plants respond to drought stress by closing their stomata and accumulating compatible solutes to maintain a low water potential and avoid dehydration [8]. Plants also conducted osmotic adjustment and regulation of ion homestasis, controlling the damage repair system, detoxification and removal of reactive oxygen species (ROS). [6]

Plants are also known to have some protective mechanisms to minimize oxidative damage in the form of antioxidant compounds such as ascorbic acid, α-tocopherol and antioxidant enzymes (superoxide dismutase-SOD, catalase-CAT, and ascorbate peroxidase-APx) [9]. Besides that, plants also known to produce multifunctional amino acids i.e. proline and glycinebetaine which act as osmoprotectant during drought stress [10]. The accumulation of proline is one of significant metabolic responses of plants to drought stress. Several studies have reported some mechanism of plant protection against drought stress by increasing its antioxidant enzyme activity such as in sorghum (*Sorghum bicolor* L.) and wheat (*Triticum durum* L.). In tomato (*Solanum lycopersicum*) leaves, increased in level of drought stress stimulated higher levels of its proline content [11].

This paper is a continuous study from our previous research [12] in order to understand how far SPC withstand drought stress and how the plant physiologically adapt to drought stress conditions, and to understand the relationship between growth parameters and physiological parameters such as proline and glycine betaine content, mallondialdehyde (MDA), antioxidant enzyme activity of catalase (CAT) and ascorbate peroxidase (APx). Cilembu variety of sweet potato was chosen since it represents an important agriculture commodity for farmers in West Java area and has some unique and better characteristics than the common sweet potato.

MATERIALS AND METHODS

Shoot Cuttings (15-25 cm) of SPC were obtained from farmers in Cilembu village, Sumedang, West Java. The shoots were planted in 10 kg polybag filled with mixture of sandyclay soil and compost (3:1). [12]

Drought treatment was applied by reducing watering level i.e. 20% field capacity (fc), 40%, and 60% fc, with 80% fc regarded as control [13]. Measurement of field capacity was determined using the method of Coombs *et. al.* [14]. The plants were exposed to drought stress for a period of three months.

Relative growth rate of the plants were measured by comparing plant biomasses before and after treatment using the Radford formula [15]. Plant (vine) length and number of leaves were counted every two weeks. Dry weight of root and shoot of the plants were determined by drying in an oven at 80ºC for 48 hours.

Chlorophyll Measurement

Measurement of chlorophyll content was determined using Arnon method [16]. A total of 0.1 g of fresh leaves were crushed and mixed with 10 mL of 80% acetone. Absorbance was measured using a spectrophotometry (ThemoFisher Scientific) at wavelength of 645 nm and 663 nm. Leaf relative water content (RWC) was determined using Turner formula [17], where fresh leaves (1 cm in diameter) were weighed, soaked in aquades for 24 hours to obtain turgid weight. The leaves were dried in the oven at 80ºC for 24 hours to obtain dry weight.

Proline Content Measurement

Proline content was measured using Bates *et al*. method [18]. Fresh leaves and roots (0.5 g) were ground with liquid nitrogen. Samples were homogenized with 10 mL of 3% (w/v) sulfosalicylic acid followed by filtration. Filtrate (2 ml) was reacted with 2 ml of ninhydrin acid and 2 ml of glacial acetic acid and heated in water (100° C) for 1 hour and the tubes were then placed in ice. The mixture was extracted with 4 ml of toluene, vortexed for 15 to 20 seconds to form two separate layers of liquid. The absorbance was measured using a spectrophotometer at a wavelength of 520 nm.

Glycine betaine (GB) Measurement

Glycine betaine (GB) content was determined using the method of Grieve and Grattan [19]. Leaves and roots (0.5 g) were ground with liquid nitrogen, mixed with 20 ml of distilled water and stirred for 24 hours. The filtrate was then diluted with 1 M sulfuric acid ($H_2 SO_4$) in a ratio of 1: 1. The mixture was transferred to a 0.5 ml microtube and cooled in ice for 1 hour. The mixture was then reacted with a 0.2 ml KI-I_2 solution and homogenized. The mixture was stored at 4° C for 16 hours and centrifuged at 10,000 rpm for 15 minutes to obtain iodide crystals. The crystals were dissolved in 9 ml of 1,2-dichloroethane, and allowed to stand for 2 hours. The absorbance was measured at a wavelength of 365 nm.

Malondialdehyde (MDA)

Malondialdehyde (MDA) content was measured using Heath dan Packer method [20], based on TBARS (Thiobarbituric Acid Reactive Substance Assay). Leaves and roots (0.2 g) were soaked in liquid nitrogen, extracted with 1 ml of 0.5% thichloroacetic acid (TCA) and centrifuged at 9000 rpm for 20 minutes at 4° C. Supernatant (0.5 ml) was then transferred to test tube and 1 ml of 20% TCA containing 0.5% TBA was added. Sample was then heated at 100° C for 30 minutes. The reaction was stopped by placing the test tube in ice, and then centrifuged at 9000 rpm at 4° C for 10 minutes. Absorbance was measured using a UV-VIS spectrophotometer at wavelength of 532 nm.

Catalase (CAT) Activity

Catalase (CAT) activity was measured using the method described by Aebi [21], by controlling the reduction of H_2O_2 at the wavelength of 240 nm using a UV-VIS spectrophotometer. The reaction was carried out in a reaction mixture containing 500 µl of 50 mM phosphate buffer (pH 7), 10 µl of the extracted enzyme, and 500 µl of 30% (v/v) H_2O_2. CAT activity was calculated using an extinction coefficient of 0.036 mM /cm. An enzyme unit (UE) determines the amount of enzyme needed to hydrolyze 1 µmol of H_2O_2 per mg of protein per minute at 25ºC, expressed in EU / mg protein.

Ascorbate Peroxidase (APx) Activity

Ascorbate Peroxidase (APx) enzyme activity was measured using the method described by Nakano and Asada [22], by the oxidation rate of ascorbic acid, dependent on hydrogen peroxide in a mixture containing 50 mM phosphate buffer (pH 7), 0.5 mM ascorbic acid 0.1 mM H_2O_2 and 0.1 mM EDTA (ethylenediaminetetraacetic acid) (pH 7) and enzymes extract. Reaction initiated by adding 100 µL of 0.5 mM H_2O_2 at a wavelength (λ) = 290 nm using a UV-VIS spectrophotometer. APx activity calculated using coefficient extension of 2.8 mM/ cm. One unit of enzyme (EU) determines the amount of enzyme needed to hydrolyze 1 µmol of H_2O_2 per mg of protein per minutes at 25 °C. Enzyme extraction from plant samples was conducted using the method of Mizuno [22]. Frozen leaf sample (0.5 g) were ground in liquid nitrogen, added with 4 ml of 100 mM phosphate buffer (pH 7). The samples were centrifuged at 11,000 rpm for 25 minutes at 4ºC. The Supernatant was used to test the activity of the antioxidant enzyme.

All the data were recorded as mean ± standard error from six replicates. Mean values of each treatment were analyzed using One-way ANOVA to determine the significance of the results between different treatments and then Duncan multiple range tests were performed with $p < 0.05$.

RESULTS AND DISCUSSION

Plant Growth

The results showed that all drought levels reduced plant relative growth rate (RGR)(Figure 1a). The lowest RGR was found in plant grown at 20% fc. Similarly, there was a decrease in plant length at all treatments compared to the control (Figure 1b). The lowest plant length is at the 20% fc,which is lower ± 60% than control. For a comparison, a decreased in plant length by 77% was reported in sweet potato cv. Resisto grown in 30% fc., while in cv. Tainung 57 plant length was reduced by 20% after grown under 15% fc [23].

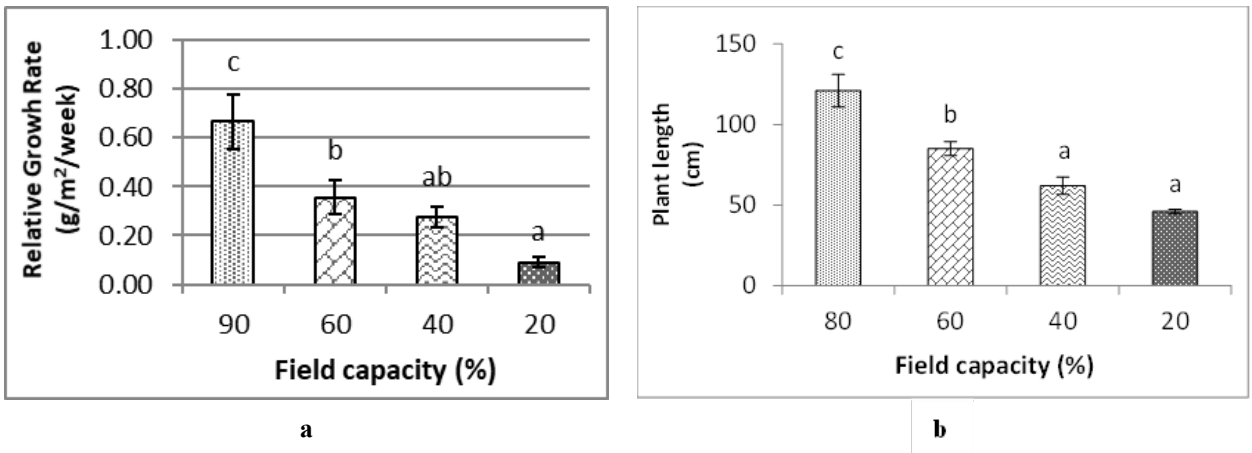

Figure 1. (a) RGR (g/m^2 week), and (b) plant length of SPC after 10 weeks exposure to drought stress. Different letters represent significant compared to control ($p < 0.05$).

Drought stress 20% fc reduced number of leaves of the plants by 56.67% (Figure 2a). Reduction in number of leaves under water stress has been reported in some sweet potato varieties [24]. Similarly, the leaf area in SPC reduced significantly by 48.7% at 20% fc (Figure 2b), this was similar to the result found by [25]. The reduction in number of leaves and leaf area showed plant strategy to adapt to drought stress by reducing water loss from transpiration. Drought causes reduction in absorption of water and nutrients by the roots so that plants experience deficiencies of water and nutrition supply [26].

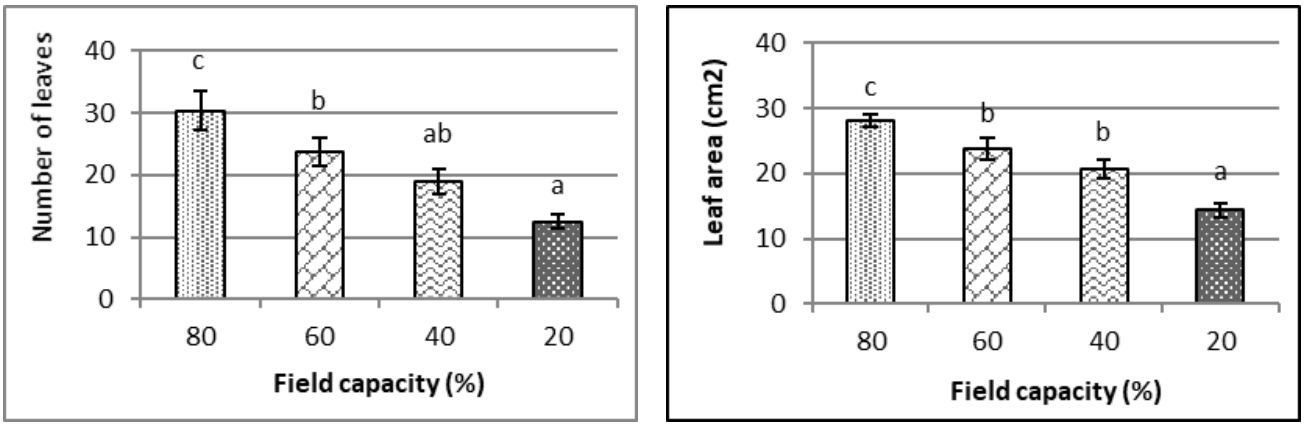

Figure 2. (a) Number of leaves and (b) leaf area of sweet potato plant var. Cilembu after 10 weeks exposure to drought stress. Different letters represent significance compared to control ($p < 0.05$).

Dry Weight

Drought stress decreased plants dry weight, both in shoot and in root (Table 1). Drought stress affect more on shoot dry weight than on root, as root:shoot ratio increased almost by two times as the drought level increased to 20% fc. An increase in root:shoot ratio in rice after drought treatment was reported [26]. An increase in root:shoot ratio indicates that shoot is more affected by drought stress than roots [27].

Tabel 1. Root dry weight, shoot dry weight and total dry weight of SPC after 10 weeks treatment to drought stress (average ±standard error, $p< 0.05$).

Field capacity (%)	Root (g)	Shoot (g)	Total (g)	Root/Shoot Ratio
80	0.60 ± 0.07^{c}	8.19 ± 1.09^{c}	8.79 ± 1.15^{c}	0.07
60	0.35 ± 0.06^{b}	5.36 ± 0.62^{b}	5.71 ± 0.65^{b}	0.07
40	0.28 ± 0.04^{ab}	2.63 ± 0.48^{a}	2.91 ± 0.52^{a}	0.12
20	0.18 ± 0.01^{a}	1.38 ± 0.12^{a}	1.56 ± 0.11^{a}	0.13

Relative Water Content of Leaf

Relative water content (RWC) of the plant was slightly reduced after 2 and 10 weeks exposure to drought stress (Figure 3). This indicates plant ability to retain water in its tissue. A decrease in leaf RWC is related to a reduction in water absorption by roots, followed by a decrease in stomata ability in retaining water [28]. A reduction in leaf RWC has also reported in several cultivars of potato and rice plants [29].

Leaf RWC is an indication of water status in plants [25]. The impact of drought on crop water status is influenced by several factors such as genotype, intensity and duration of drought. A slight decrease (15%) in leaf RWC of sweet potato var Cilembu after exposure to drought stress (40 and 20% fc) indicates plant ability to withstand drought stress.

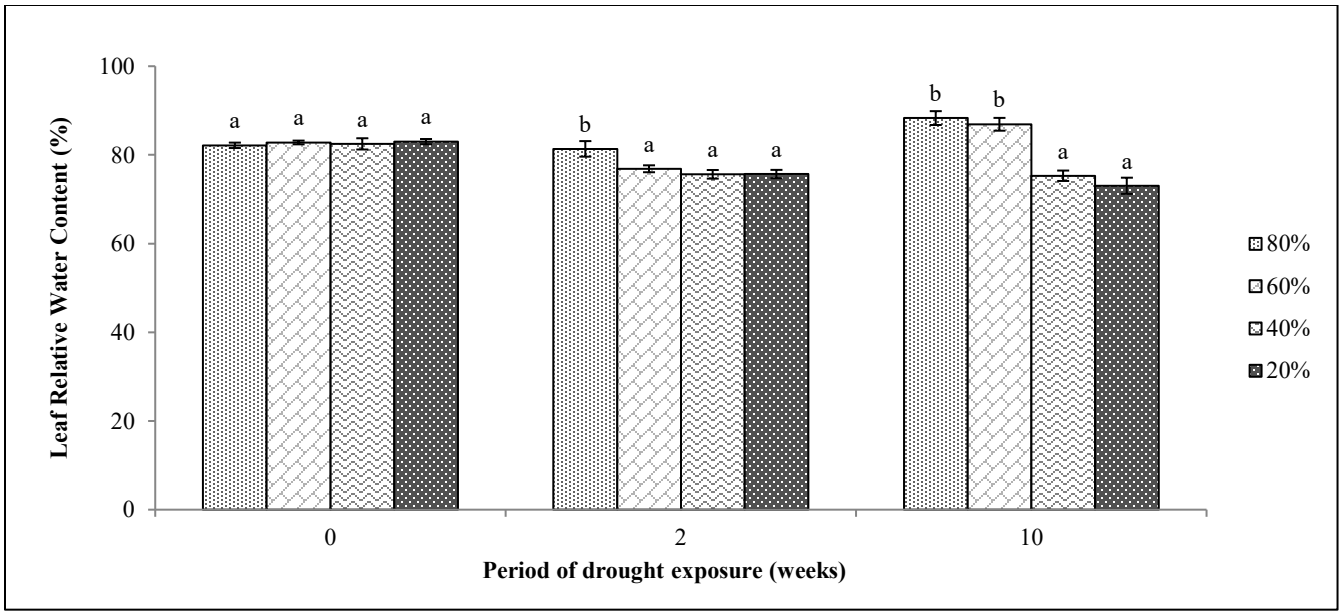

Figure 3. RWC (%) of SPC leaves after 10 weeks exposure to drought stress. Different letters represent significant compared to control (p <0.05).

Chlorophyll Content

There was a significant reduction in chlorophyll content in plant leaves after 10 weeks exposure to 20% and 40% fc, but at 60% fc chlorophyll content was not affected (Figure 4).

These results are in line with previous studies where there was a decrease in chlorophyll content in some sweet potato varieties after being subjected to drought stress with a decrease of 50% to 70% in chlorophyll content [30]. A decrease in chlorophyll content due to drought stress has also been reported in some rice cultivars, tomatoes and potatoes [22]. In potato leaf (Marfuna cultivar), there was no significant reduction in chlorophyll content after being exposed to drought stress [31].

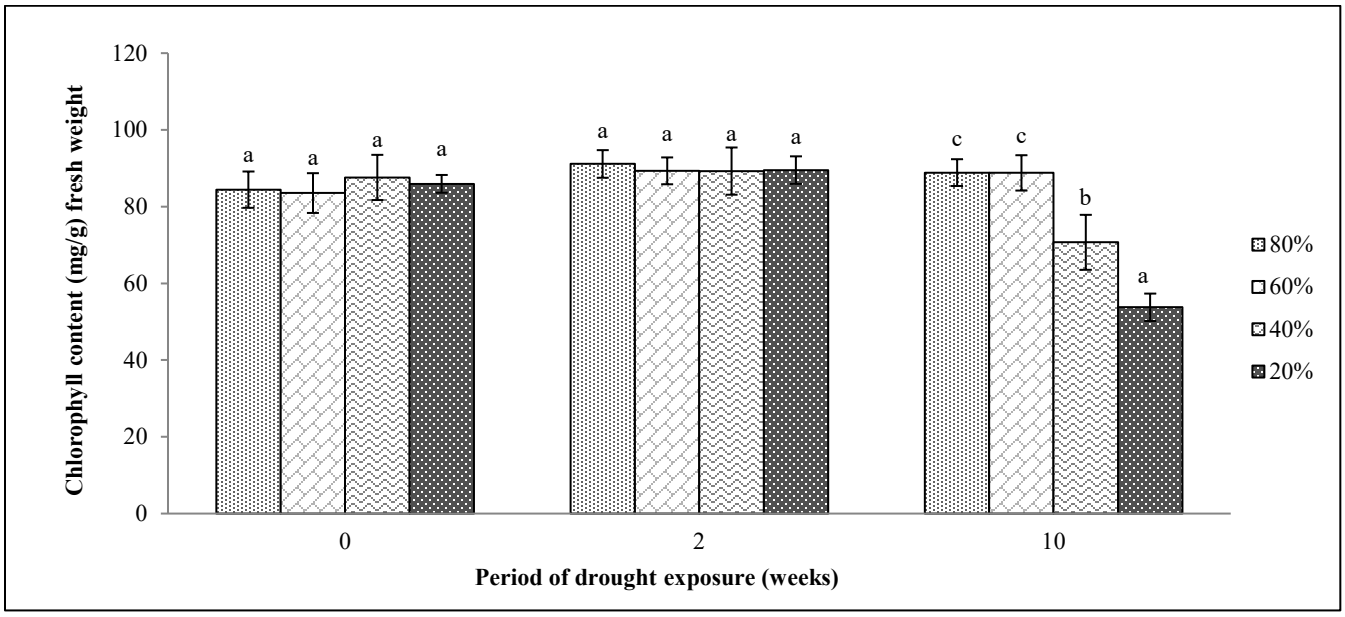

Figure 4. Chlorophyll content (mg/g fresh weight) of SPC plant after 10 weeks exposure to drought stress. Different letters represent significant compared to control (p < 0.05).

Proline content in Roots and Leaves

Drought stress increased proline content in the leaves and roots of SPC plants (Figure 5). The increase in proline content was higher in the leaves than in the roots.The increase of proline content especially in plant leaves was significant when the plants grown at 20% and 40% of fc. This result is similar to other experiment in sweet potato [32], and in maize [33]. An increase in proline content was also found in SPC after a short-term exposure to elevated CO_2 [16].

An accumulation of compatible solutes when the plant was subjected to drought stress is an osmotic adjustment that depends on the level of water stress of the plant [33]. An increase in proline content in the cells are also associated with its role in preventing protein denaturation, maintaining the structure and activity of enzymes and maintaining cell membranes from damage caused by increased production of Reactive Oxygen Species (ROS) during stress [34].

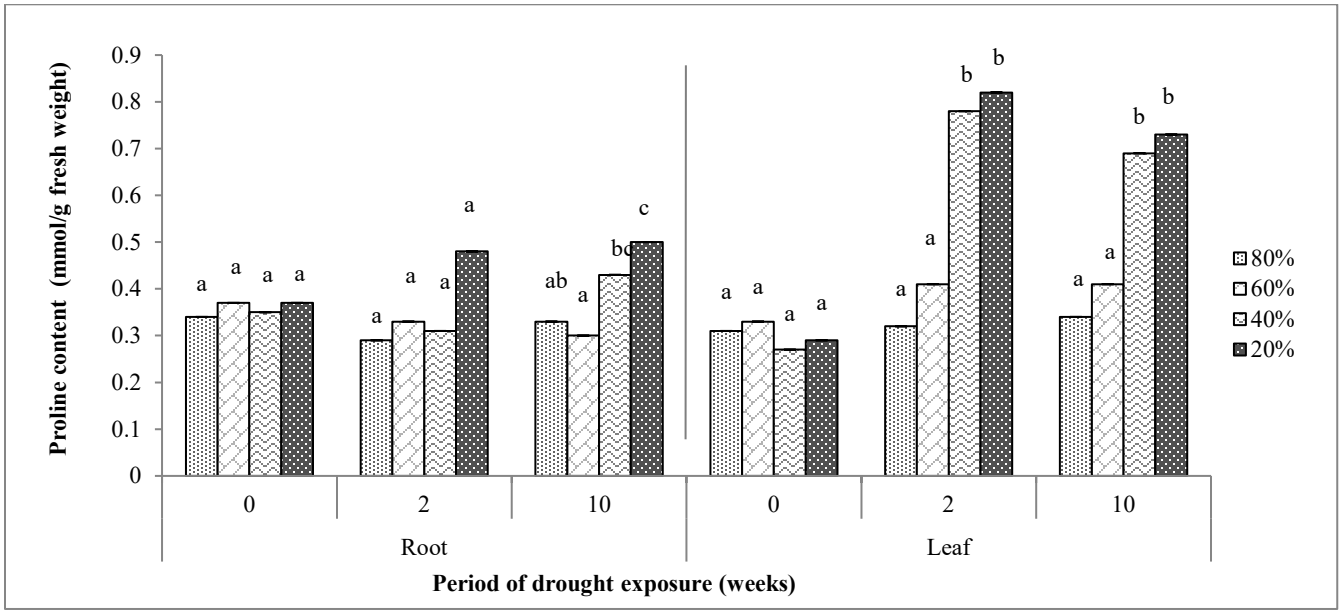

Figure 5. Proline content in roots and leaves of SPC after 10 weeks treatment to drought stress. Different letters represent significant compared to control (p <0.05).

Glycine Betaine Content in Roots and Leaves of SPC

Glycine betaine (GB) content in plant leaves was increased significantly after the plants being exposed to 20 % fc for a period of 2 and 10 weeks. In root, a slight incease in GB content was observed after exposure to drought stress for 10 weeks (Figure 6).

GB content in the leaves was higher than in the roots. It has also been reported in sugar beet, that GB biosynthesis is more prevalent in leaves especially in young leaves than in root [35]. An increase in GB content due to drought stress was also reported in corn seed cultivars MO17 and B73 as much as 10.6% and 5.9% respectively [36]. However, in some plants such as potatoes, tomatoes and rice, it was reported that drought stress did not cause an increase in levels of GB [37].

GB plays a role in plant adaptation to drought stress. Similar to proline, accumulation of GB in plants indicates a plant defence mechanism to drought stress [12]. GB is able to maintain CO_2 assimilation as well as maintain photosystem II which is a component of photosynthesis susceptible to various types of abiotic stress [38].

Besides that, GB plays as osmoregulator, and its level varies considerably among different plants. GB activates the adjustment of glutathione reductase (GR), ascorbic acid (AsA) and glutathione (GSH) contents in plants under heavy metal stress [39].

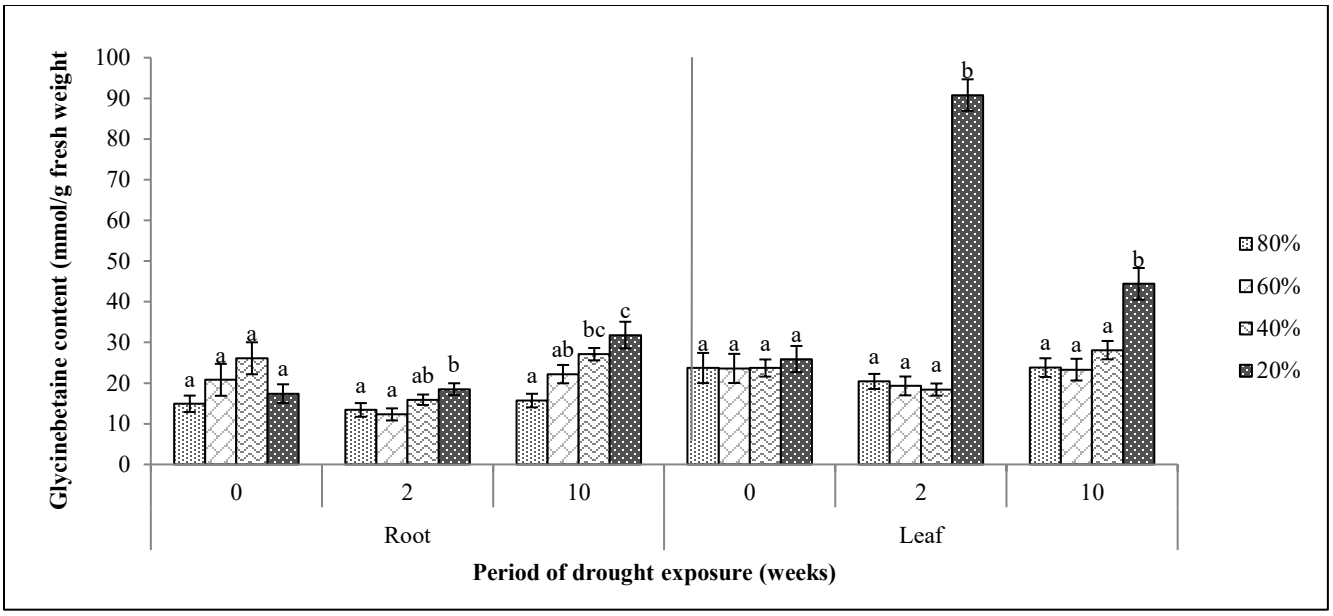

Figure 6. GB content (mmol/g fresh weight) in root and leaves of SPC plant after 10 weeks exposure to drought stress. Different letters represent significant compared to control (p <0.05).

Malondialdehyde Content in Root and Leaves of SPC Plant

There was an increase in Malondialdehyde (MDA) content in roots and leaves of the plant after exposure to 20% and 40% fc for two weeks and ten weeks (Figure 7). The content of MDA in leaves is 3 to 4 times higher than the content of MDA in roots.

MDA is the final product of membrane lipid peroxidation which is an indicator of oxidative damage to cell membranes caused by increased production of ROS [40]. Increased MDA content after exposure to drought stress indicates that SPC plants experienced an oxidative stress [40].

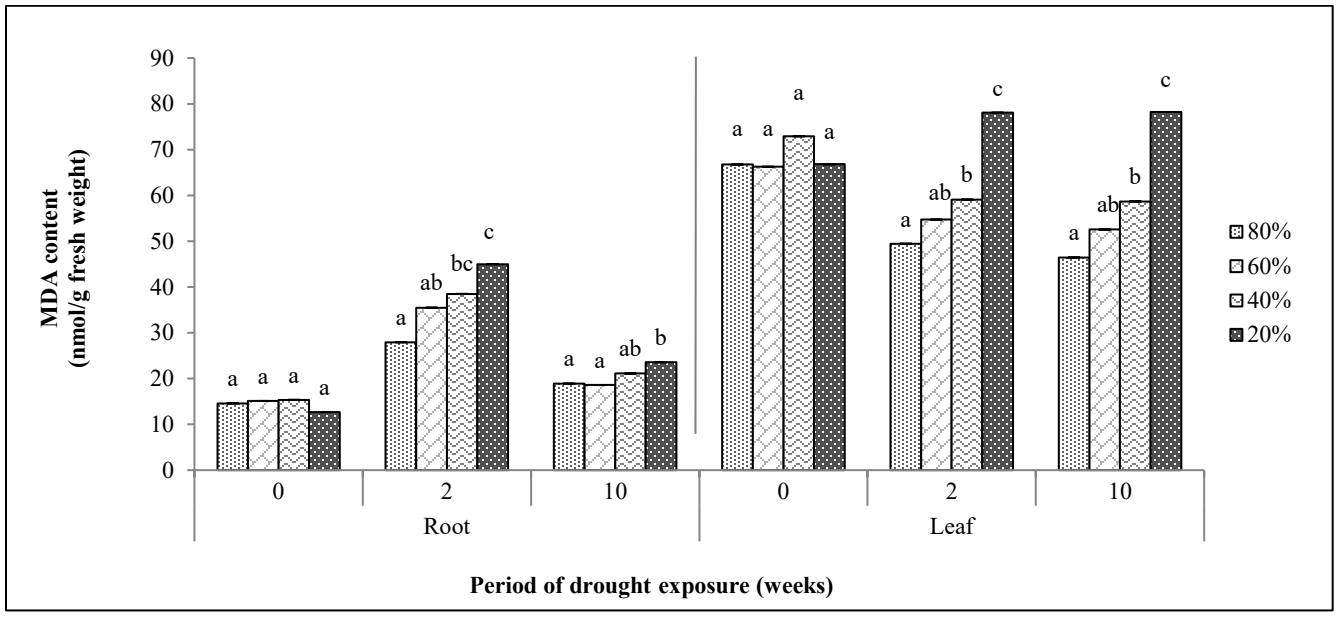

Figure 7. Malondialdehyde (MDA) content (nmol/g fresh weight) in root and leaves of SPC plant after 10 weeks exposure to drought stress. Different letters represent significant compared to control (p < 0.05).

Antioxidant Enzymes Activity: Catalase and Ascorbate Peroxidase of SPC leaves

After two and ten weeks of exposure to 20% fc, Catalase (CAT) activity in plant leaves significantly increased, whereas at 40% fc the increase was not significant (Figure 8a). Ascorbate Peroxidase (Apx) activity however was not affected by drought exposure, although there was a tendency that APx activity increased as time of exposure become longer (Figure 8b). It has also reported that there was no significant changes in APx activity due to water deficit compared with control in barley (*Hordeum vulgare* L.) [41].

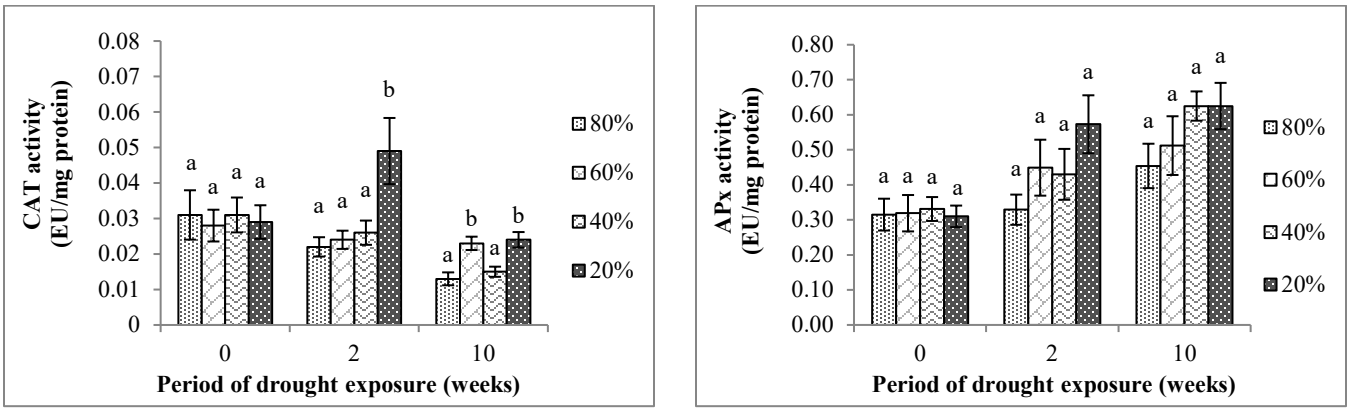

Figure 8. Antioxidant enzymes activity, a) CAT and b) APx in leaves of SPC plant after 2 weeks and 10 weeks exposure to drought stress. Different letters represent significant compared to control ($p < 0.05$).

CAT and APx are two enzymes that play a role in converting relatively stable H_2O_2 to H_2O and O_2 so as not to harm plant cells [42]. ROS can be in the form of hydroxide (OH^-), superoxide (O_2^-) and hydrogen peroxide (H_2O_2).

Under drought stress, CAT play a role more than APx, especially at 20% FC. In general, CAT activity in 20% FC were higher than control. Higher CAT activity could be plant strategy in avoiding further damage due to high levels of H_2O_2 molecules caused by drought stress. CAT activity decreased at 10th week, this may be related to plant age and tolerance level. Differences in enzyme activity could be related to plant type and age, tolerance mechanism, duration and intensity of stress [3]. It was reported that the activity of CAT increased significantly in the first month of drought stress and decreased in subsequent months [43].

CONCLUSION

Sweet potato var. Cilembu (SPC) plant was able to withstand severe drought stress (20% of field capacity) within 10 weeks of exposure, at the cost of its growth. Under stress conditions, plant dry wight, relative water content, chloropyll content decreased significantly compared to control. Drought stress increased the content of proline and glycine-betaine in plant root and shoot. The content of proline and glicine betaine in the leaves was much higher than the content in roots. This indicates that plant leaves suffered more during drought stress than the roots. This could be related to the fact that drought stress affect more on shoot dry weight than on root, as root:shoot ratio increased almost by two times as the drought level increased to 20% of field capacity. The content of Malondialdehyde (MDA) in leaves also higher (3-4 times) than the content of MDA in roots. Catalase (CAT) activity in plant leaves also significantly increased after exposure to 20% of field capacity, whereas Ascorbate Peroxidase (APx) activity was not affected by drought exposure. Further study is required to understand the effect of drought to the quality and quantity of tuber production in the field scale.

ACKNOWLEDGMENT

We are grateful to ITB for PPMI research grant, and Ministry of Research and Higher Education of Indonesia for PPDUPT research grant.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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