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Comparative study of morphophysiological responses among cowpea and long beans plants under drought stress condition

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Abstract. *Syafriani E, Susilo KR, Sayekti RS, Widyawan MH, Khoirunnisa NS, Rahmadhani M. 2022. Comparative study of morphophysiological responses among cowpea and long beans plants under drought stress condition. Biodiversitas 23: 5507-5518.* Long bean (*Vigna unguiculata* (L.) Walp subspecies sesquipedalis (L.) Verdc.) plants are generally more susceptible to drought stress than cowpea plants (*Vigna unguiculata* (L.) Walp). One of the promising solutions is through plant breeding to produce genetically modified long bean plants resistant to drought stress by crossing with cowpeas. Parental selection is the initial stage of the plant breeding program. This stage was performed by selecting parental varieties based on the desired superior characteristics, including morphological and physiological characteristics, as well as, yield production. Unfortunately, the comparison between long bean plants and cowpea plants is not well-studied. Therefore, the aim of this study was to analyze the morphophysiological similarities and differences between long beans and cowpeas to withstand drought stress. A two-factorial randomized block design was used in this study and consisted of 2 factors (plant varieties and type of drought stress). The plant varieties used in this study are 7 long bean plants and 7 cowpea plants. While the level of drought stress were: T0 (watering every day), T1 (every 5 days), and T2 (every 10 days). The observational data from morphophysiological characters indicated that long bean plants were more tolerant to drought stress than cowpea plants. Interestingly, one of the cowpea varieties (C2) had the highest proline contents (20.27 µmol/gram of leaves sample) compared to other varieties. Hence, the C2 variety can be considered a candidate for further studies. Meanwhile, all the tested 7 varieties of long bean plants showed varying adaptability, possibly due to the genetic diversity of each variety against drought stress.

Keywords: Cowpea, drought stress, long beans, morphophysiological, proline

INTRODUCTION

Long bean plants (*Vigna unguiculata* (L.) Walp subspecies sesquipedalis (L.) Verdc.) and cowpea plants (*Vigna unguiculata* (L.) Walp) originated from the same species with different subspecies or cultivar groups. Despite originating from the same species, there are only a few studies comparing these two cultivars. A previous study reported that cowpea plants have broader adaptive mechanisms against drought stress, meanwhile, long bean plants tend to have decreasing productivity under drought stress.

According to the Central Bureau of Statistics Indonesia (2019), the production of long beans in Indonesia in 2015 was 395,524 tons, which then decreased in 2019 as it only reached 352,700 tons. The decline in long bean production is known to be partly due to the dry season, which results in a lack of water availability on the ground. Dry land has various problems, including lack of nutrients (fertility loss and low soil organic matter), a deficit of moisture, prone to erosion, salinization, and desertification (Reynold et al. 2007; Cowie et al. 2011).

Plants experiencing drought stress will adapt through some changes in their morphology, physiology, cellular, and molecular (Fang and Xiong 2015). Wang et al. (2015) stated that under drought stress, plant photosynthesis tends to decrease along with hormonal changes (auxin, cytokinin, and ABA), causing stomata to close and reducing both cell division and development, as an effort of adaptation. The plant biochemical response to drought stress is achieved through the increasing proline compounds as osmoprotectants to maintain osmotic potential in plants (Sharma et al. 2019). Riduan et al. (2005) reported that peanut cultivars resistant to drought stress are able to rapidly accumulate proline in leaves (from 177% to 242%) compared to other intolerant cultivars. Whereas a study on mung beans showed that drought stress that reaches up to 50% of field capacity has shown a decrease in the leaf area and plant dry weight without any effect on proline content (Purwanto et al. 2019).

The general initial response of long bean plants against drought stress is by flowering 12 days earlier, although there are several varieties that remain green for weeks under drought stress and will flower when the ideal climate is met (Fatokun et al. 2012). In addition, long beans also adapt to water-deprived conditions through the mechanism of stomata closure, paraheliotropism, and high root hydraulic conductivity (Agbicodo et al. 2009).

Previous study reported that cowpea shows some changes in their morphology under abiotic stress. The level of water content and cultivar significantly influence the dry seed weight of cowpea per plant and also for the other cowpea plant growth parameters (Karuwal et al. 2018;

Mousa and Al Qurashi 2017; Awosanmi et al. 2019). Cowpea has also been reported to be tolerant of drought stress and soil acidity, which makes them suitable to be developed and grown on suboptimal lands, such as dry land and land with high acidity (Sas et al. 2021; Karuwal et al. 2017). Cowpea exhibits wide adaptation mechanisms against drought, such as drought escape, drought avoidance by reducing leaf area, and dehydration avoidance. Even during their vegetative stage, cowpea exhibits a drought-tolerant mechanism by delaying leaf senescence (Hall 2004). A study conducted by Sayekti et al. (2012) described that Semin and Wates varieties are the promising line of cowpea due to the high yield per hectare.

The purpose of this study was to analyze the similarities and differences in the morphological and physiological characteristics between long beans and cowpeas under drought stress. This study is expected to contribute to the establishment of a database about the morphology and physiology of various long bean and cowpea varieties from the PIAT collection garden, especially under drought stress. Moreover, the result of this study is expected to be able to provide recommendations for varieties of long beans and cowpeas that are resistant to drought stress, to further be used as the parents for plant breeding or directly used as the seeds for farming.

MATERIALS AND METHODS

This study was conducted at the Agrotechnology Innovation Center (PIAT UGM), Kalitirto, Berbah, Sleman, Yogyakarta, Indonesia from August to December 2021. The experimental design applied in this study was a two-factorial randomized block design. The treatment consisted of 2 factors, namely plant varieties and type of drought stress. Drought treatments were designed as intermittent drought for 5 days, 10 days, and control. For the control treatment, watering was carried out every day (T0). Meanwhile, for the intermittent drought treatment, watering was carried out every 5 days (T1) and 10 days (T2). The long bean varieties used were FB-KP159 (LB1), FB-KP225 (LB2), FB-KP375 (LB3), FB-KP96 (LB4), FB-KP104 (LB5), FB-KP360 (LB6), and FB-KP111 (LB7). For cowpea varieties used in this study were FB-KT97 (C1), FB-KT141 (C2), FB-KT223 (C3), FB-KT266 (C4), FB-KT444 (C5), FB-KT466 (C6), and FB-KT1198 (C7). The amount of water was determined based on the field capacity measured by putting soil into a black polybag with a diameter of 40 cm, then watering it until the first drop of water came out from the polybag (approximately 1 L).

Procedure

Planting

Planting was carried out by filling the planting media into the polybags (40 cm x 40 cm) as much as 7/8 of the polybag height. The planting hole was made using a *tugal* with a depth of 5 cm. Each cowpea and long bean seed was planted for each polybag which was then covered with the planting media.

Plant raising

Plant raising in this study included watering that was adjusted to each treatment, stakes installation, weeding, fertilization and pest control. Watering was performed by using the same bucket with water volume adjusted to the field capacity of plants. Stakes installation was carried out at 14 DAP or when the plant height reached 15-25 cm. The length of the installed stakes was 1.5 m. Weeding was performed manually once a week. Fertilization was carried out using NPK Mutiara 16:16:16 fertilizer at a dose of 19 g/L and each plant was given 250 mL of fertilizer solution per polybag, 2 times after planting. Pest control was conducted both manually by collecting pests that attacked plants and chemically by using insecticides with a concentration of 25 mL/5 L of water.

Variables

Growth parameter

Plant height. Plant height was measured 3 times during the experiments: at 14 DAP (2 WAP), 5 WAP, and 8 WAP. Measurement was carried out by measuring from the base of the stem to the growing point of the plants.

Number of leaves. The number of leaves was counted after the plants reached 14 DAP (2 WAP), 5 WAP, and 8 WAP .

Stem diameter. Stem diameter was measured 3 times at different time points, specifically at 14 DAP (2 WAP), 5 WAP, and 8 WAP. Stems located 5 cm above the soil surface were measured by using a caliper.

Root fresh weight. The root fresh was weighed at 3 WAP by dismantling the plants from the polybag, cleaning the roots from the soil, then weighing it with a digital scale.

Stem fresh weight. The fresh stem was uprooted at 3 WAP and immediately weighed.

Leaf fresh weight. The fresh leaf was uprooted at 3 WAP and immediately weighed.

The flowering age. The flowering age was observed and counted from the time the plants were planted until the time the first flower appeared on each plant.

Biochemical parameter

Biochemical observation was carried out through analysis of proline content. The proline content was observed at 8 WAP from the youngest leaves that reached a complete development based on a method explained by Bates et al. (1973). The leaves were crushed using a mortar in 10 mL of 3% sulfosalicylic solution. The solution was filtered with Whatman paper. Further, ninhydrin solution was prepared by dissolving 1.25 g of ninhydrin in 30 mL of glacial acetic acid and 20 mL of 6 M phosphoric acid. Then, 2 mL of the filtrate was mixed with 2 mL of glacial acetic acid in a test tube and heated until boiling for 1 hour. The test tube was then put into cold water. The solution was further mixed with 4 mL of toluene and stirred with a stirrer for 15-20 seconds. The toluene solution containing red proline was sucked up and transferred into a cuvette. The cuvette was then mounted on Spectronic 21-D and the absorbance value was read at 520 nm. The obtained values were then converted to a standard curve. Proline contents were determined by the equation:

Proline content = proline content (mg.cm^{-3}) x 0.347 mol.g^{-1}

This proline content was converted into proline content per plant by multiplying the proline content by the plant's dry weight.

Physiological parameter

Physiological observations were carried out through stomatal morphology analysis (density and width of stomata openings). The density and width of stomata openings were measured on actively growing leaves located at the center of the canopy and exposed to sunlight. The stomatal openings along with the number of stomata were counted using the stomata printing method. The underside of the leaves was coated with transparent nail varnish. The dried layer of nail varnish was peeled off by using sellotape, then stuck onto an object glass. The stomatal openings were observed under a microscope using an ocular micrometer at 10x magnification. The ocular micrometer was calibrated with the objective lens at 40x magnification.

Data analysis

The obtained data were analyzed using analysis of variance with a significance level of 5%, then followed by a posthoc HSD Tukey test to find the significant differences between treatment groups.

RESULTS AND DISCUSSION

Morphological response of cowpeas and long beans

The morphological response of long bean and cowpea plants against drought stress could be observed through plant height, the number of leaves, stem diameter, root length, root fresh weight, fresh stem weight, fresh leaf weight, and flowering age. The measurement of plant height, number of leaves, and stem diameter were carried out at 2 WAP, 5 WAP, and 8 WAP. This interval was assumed to represent the trend of the effect of drought stress on both plants in the early, middle, and late stages of growth. Meanwhile, the variables of root length, root fresh weight, stem fresh weight, and fresh leaf weight were measured at 3 WAP.

Plant height

The plant height data showed that both long bean and cowpea plants had naturally different heights. As annual herbaceous plants, cowpea plant height ranges from 30 to 140 cm (Trustinah 1998), depending on the variety. Meanwhile, the height of a long bean plant can reach up to 250 cm (Hutapea 1994). The heights of the seven varieties tested in this study varied widely (Figure 1). The order of cowpea plant heights at 8 WAP on control treatment (P0), from the lowest to the highest, were C3, C2, C7, C1, C4, C6, and C5. Diverse heights were also recorded from 7 varieties of long bean plants with the order height, from the lowest to the highest, were LB2, LB3, LB5, LB6, LB4, LB7, and LB1. These results indicated that the plant height parameter is strongly influenced by the genetic factors of each plant variety. Moreover, the wide differences in plant height between each variety may indicate that the genetic variation of the selected variable is quite high.

Drought treatments T1 and T2 resulted in a significant decrease in plant height compared to control (T0). As explained in the previous study, lack of water in plants can be a major limiting factor in the growth process, indicating that the result of this study was significant to the previous study. In detail, the results indicated that cowpea plants were more susceptible to drought stress than long beans due to the highly significant differences in plant height between control and drought treatments (both T1 and T2). However, some varieties exhibited different outcomes as there were no wide differences in the plant height of long bean plants between T0, T1, and T2 treatments. In other words, some of the long bean varieties used in this study are more tolerant to drought stress than cowpea plants. The order of long bean varieties according to their tolerance against drought stress was LB7, LB3, LB5, LB6, and LB4.

Almost all cowpea varieties exhibited extremely low growth after T1 treatment, which then continued to decrease after T2 treatments. In comparison, the average height of some drought-tolerant long bean plants was not widely different among treatments (T1 and T2). This indicates that some of the drought-tolerant long bean varieties have a fairly wide range of resistance. The comparison of the effect of drought on the 3 different observation times illustrates that drought stress consistently has an effect on early growth (2 WAP), mid-growth (5 WAP), to early generative/flowering stage (8 WAP).

The number of leaves

The obtained data showed that the number of leaves for long bean plants was naturally greater than for cowpea plants (Figure 2). This might be related to the morphology of long bean plants which tend to be taller than cowpea plants, which allows long bean plants to have a higher number of petiole growth points. Therefore, in line with the higher number of leaf stalks on long bean plants, the number of leaves formed will also be higher compared to cowpea plants. Furthermore, the number of leaves also indicates that a high genetic diversity among varieties of 2 different plants will result in varied morphological responses against drought stress. This statement is supported by the significant differences in the number of leaves between the control treatment (T0) and drought treatment, especially for data collected at 8 WAP.

Drought stress treatments (T1 and T2) showed that the number of leaves on the tested plants was strongly influenced by water availability. The obtained data indicated that the number of leaves was significantly decreased in all tested varieties on both T1 and T2 treatments compared to the control (T0). Considering the importance of leaves as the source of energy production through photosynthesis, therefore, drought stress can influence the survival of these two plants. However, C6 and C1 varieties exhibited a more tolerant trait against drought stress at 8 WAP compared to other varieties of cowpea plants based on the number of leaves after T1 treatment. On the other hand, all varieties of cowpea plants had a similar number of leaves in the T2 treatment.

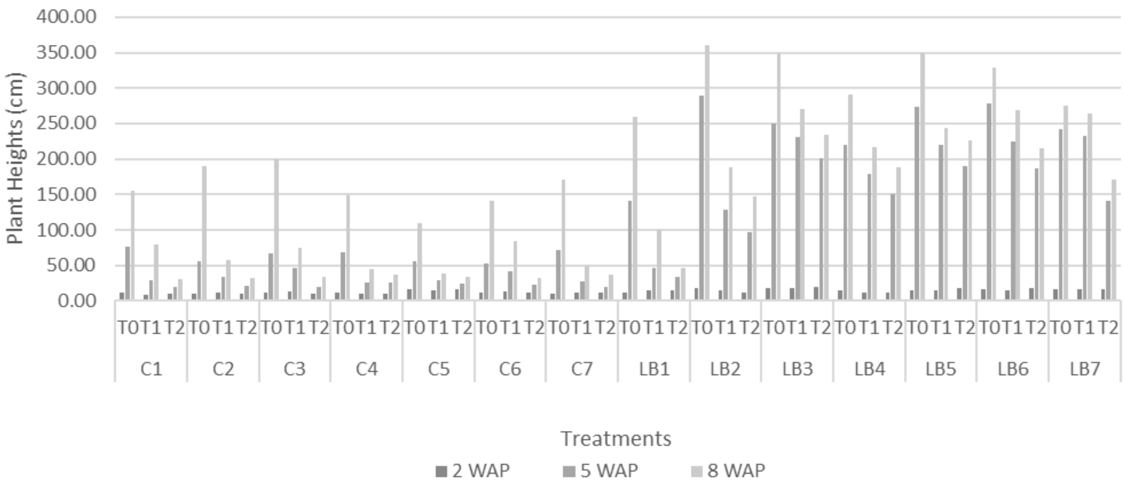

Figure 1. The average of cowpea and long bean plant's heights under different drought conditions

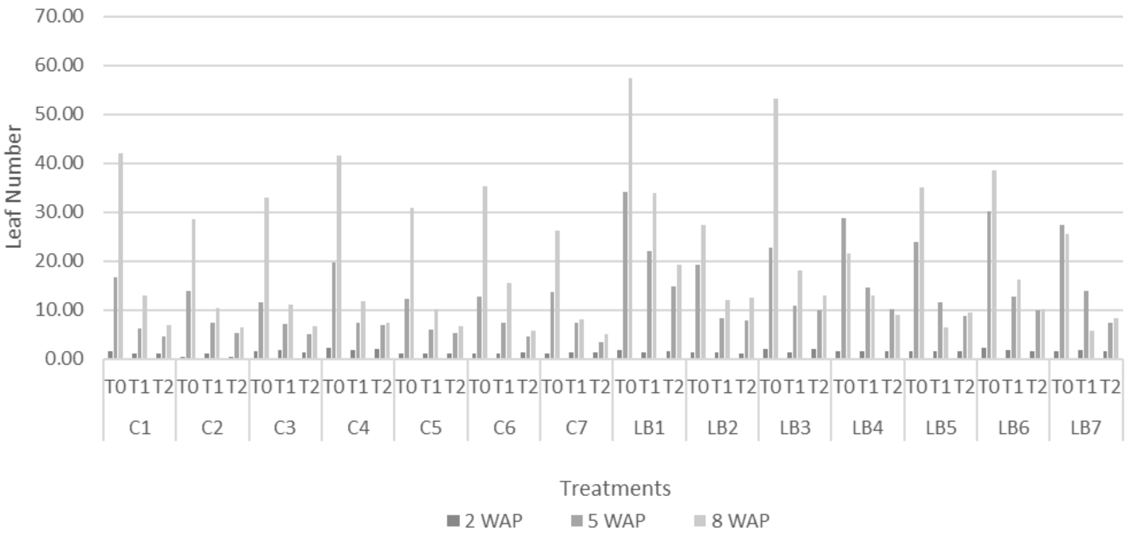

Figure 2. The average number of leaves of long bean and cowpea plants under different drought conditions

For long bean varieties, the obtained data showed that LB1 was the most tolerant to drought stress compared to others varieties in both T1 and T2 treatments. We also detected a decreasing trend in the number of leaves according to the data collected at 5 WAP and 8 WAP in both T1 (specifically LB4, LB5, and LB7 varieties) and T2 treatments (LB4), which implied that the three varieties are the most susceptible to drought stress. Generally, one of the plant defense mechanisms facing drought stress is leaf shedding in order to reduce evapotranspiration. This specific trait in responding to drought stress was also recorded in our experiments, which resulted in a decrease in plant growth. To add, Ali et al. (2021) stated that during drought stress, plants tend to reduce leaf area and limit the growth of new leaves.

The drought treatments at 2 WAP were found to have no significant effect on the number of leaves on all varieties of long bean and cowpea plants. The effect of drought stress on all varieties began to express clearly at 5 WAP and 8 WAP which implied significant differences between control and drought treatments.

Stem diameter

The stem diameter data implied that drought stress treatments had significant effects on all tested plant varieties (Figure 3). The results showed a great variety of stem diameters on both plants in the control treatment (T0), especially at 5 WAP and 8 WAP, which then strengthened the assumptions that the selected varieties have a fairly high genetic diversity. Cowpea plants showed a highly susceptible trait to drought stress as implied by the smaller stem diameters on all varieties under T1 and T2 treatments at all observation times (2 WAP, 5 WAP, and 8 WAP). However, different results were recorded in long bean plants as drought stress did not give a significant effect on all tested plant varieties. This result might suggest that long bean plants are more resistant to drought stress based on the stem diameter parameter.

The decrease in stem diameter of plants under drought treatments is associated with the plant water contents. Medeiros et al. (2012) reported that declining plant growth under drought stress is associated with low turgor pressures due to low-soil water availability, especially when water is essential in cell division and elongation.

Root length and fresh weight

The collected root length data indicated that there was no significant difference between varieties of both plants at 3 WAP (Figure 4). This indicates that the roots were not yet affected by the drought stress at the early stages of growth. However, the result of our measurement found that there was a length variation among the tested varieties between control (T0) and drought stress treatment (T1 and T2) plants.

The longest roots of the cowpea plants were recorded to be C6 in the control treatment, C1 in T1 treatment, and C1 in T2 treatment. Meanwhile, the shortest roots were C2 in the control treatment, C3 in T1 treatment, and C7 in T2 treatment. On the other hand, LB2 variety was reported to have the longest roots among long bean plant varieties in the control treatment, LB2 in T1 treatment, and LB3 in T2 treatment. Meanwhile, the shortest roots were recorded to be LB6 in the control treatment, LB5 in T1 treatment, and LB5 in T2 treatment. This data variation implies a diverse response to drought stress among different plant varieties. Kusvuran (2012) described that the response of plants to drought stress varies depending on the duration, stress intensity, plant species, and plant growth stage.

The root fresh weight data implied that drought stress treatments did not significantly affect both cowpea and long bean plants at the early stages of growth (3 WAP). However, Figure 5 showed that 2 varieties of cowpea plants, C5 and C6 varieties, were significantly better than other varieties in the control treatment. On the other hand, among long bean varieties, LB6 was reported to have the heaviest root fresh weight in the control treatment. This result supported the idea that the tested varieties had genetic diversity among them.

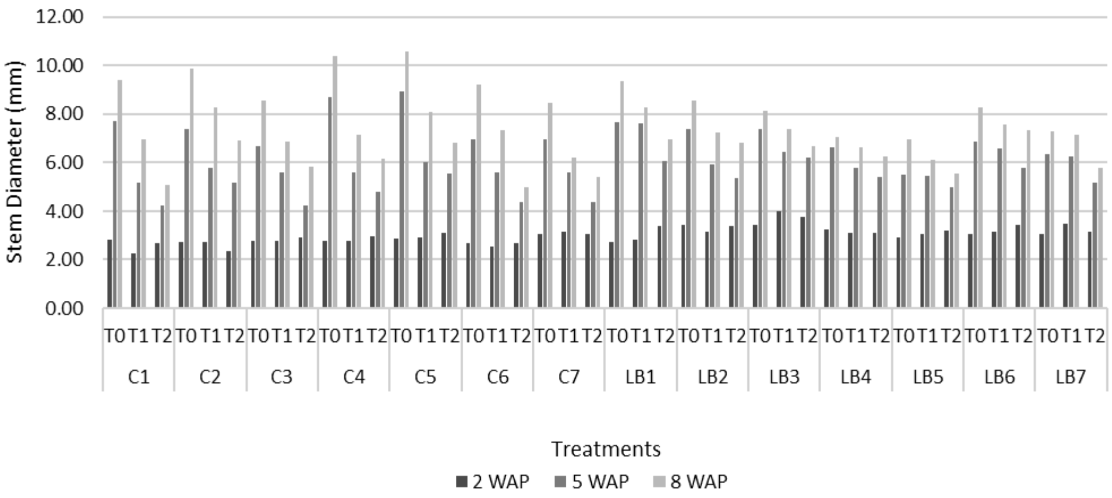

Figure 3. The average stem diameter of cowpea and long bean plants under different drought stress conditions

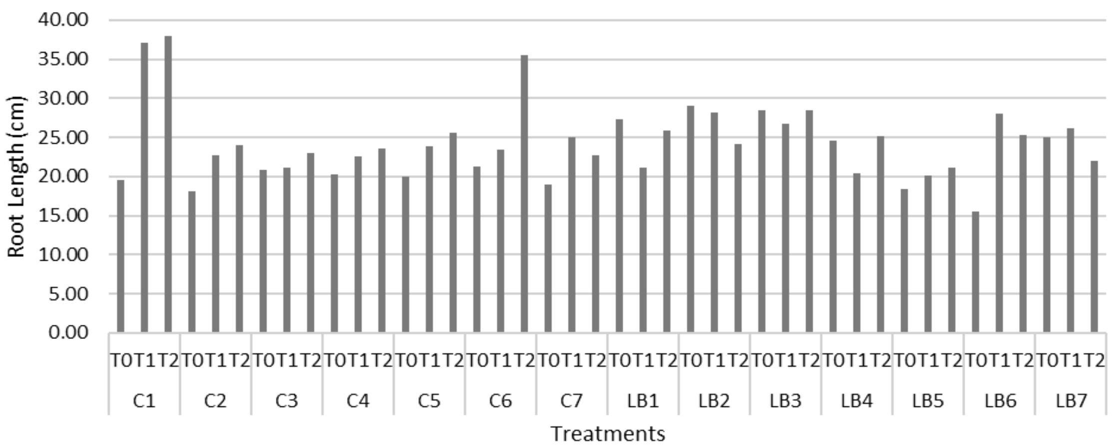

Figure 4. The average root length measured from cowpea and long bean plants under different drought stress conditions

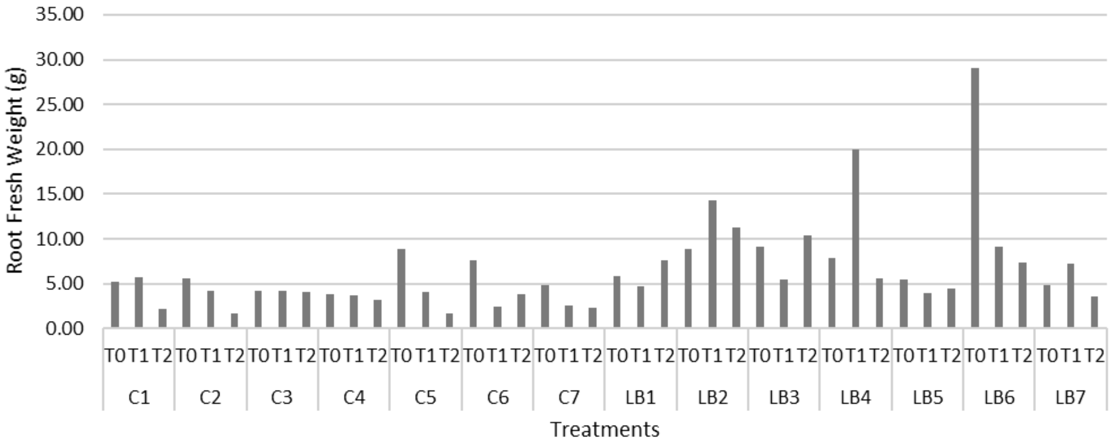

Figure 5. The average of root fresh weight measured from cowpea and long bean plants under different drought stress conditions

The data presented in Figure 5 indicated that the significant effect of drought stress on root fresh weight was found in some varieties of long bean plants, but not in cowpea plants, implying that long bean plants were more responsive than cowpea plants. Among the seven varieties of long bean plants, three of them (LB4, LB2, and LB7) had the opposite trend (heavier root fresh weight in drought stress treatments compared to the control treatment) compared to the other four in T1 treatment. This trend was also found in T2 treatment, where 3 varieties (LB2, LB3, and LB1) had heavier root fresh weight in the control treatment than drought stress treatment. On the contrary, the other four varieties of both plants exhibited lighter root fresh weight in T1 and T2 treatments in comparison to the control treatment. This result suggested that the variation in response to drought stress found in different varieties of long bean plants was also influenced by genetic factors, specifically by increasing the root fresh weight. Roots are the main organ of plants in absorbing water and nutrients contained in the soil, therefore, the increasing root fresh weight may imply a morphological response of the plants in expanding the absorption area through the increasing number of root hairs which indirectly increases the root fresh weight. During drought stress, plants tend to expand their root systems by undergoing some changes in the number and size of root cells (Lynch 2007; Siregar et al. 2021). Therefore, the results of this study are in line with the previous study, as there were an increase or decrease in root length and fresh weight in several plant varieties after drought stress treatments as compared to the control treatment.

Stem fresh weight

The obtained data on stem fresh weight showed that drought stress treatments had a significant effect on several plant varieties (Figure 6). The heaviest stem fresh weight of cowpea plants in the control treatment was C6 (19.44 gr), meanwhile, the lightest stem fresh weight was C2 variety (11.3 gr). This result might indicate that C6 variety had the capability of having high assimilated and water storage in the stems. All tested varieties from both cowpea and long bean plants exhibited similar responses to drought stress, specifically a decrease in the stem fresh weight. An exception was found in LB6T1 variety which exhibited a slightly different response to drought stress as it showed a non-significant increase in stem fresh weight obtained from drought stress treatments, as compared to control treatment. Additionally, C2T1 variety also showed no differences in stem fresh weight between stems under drought stress with the control plant (C2T0). Furthermore, the average of stem fresh weight of both cowpea and long bean plants was quite similar. However, a significant response through reduced stem fresh weight due to drought stress treatments was more apparent in cowpea plants. Therefore, based on the stem fresh weight parameter, long bean plants implied a more resistant trait against drought stress compared to cowpea plants.

Leaf fresh weight

The obtained data indicated that drought stress treatment had a significant effect on some of the tested plant varieties (Figure 7). Both cowpea and long bean plants showed no significant differences in the average leaf fresh weight. In T1 treatment, drought stress treatment caused a significant effect on the leaf fresh weight of the long bean LB2 variety only. In T2 treatment, only 4 varieties (C1, C2, C3, and C5) exhibited a significant decrease in leaf fresh weight, meanwhile the other varieties had no significant differences as compared to the control and T1 treatment. Meanwhile, the other varieties (C4, C6, and C7) exhibited a decrease in the leaf fresh weight on drought stress treatments, although the differences between T1 and T2 treatments were not significant, indicating that these varieties were quite susceptible to drought stress. In general, both cowpea and long bean plants showed a decreasing trend in the number of fresh leaf weights under drought stress treatments and only a few varieties implying the opposite response with no significant differences as compared to controls. This result indicated the presence of genetic diversity among the tested plant varieties. To conclude, cowpea plants were more susceptible to drought stress than long bean plants based on the leaf fresh weight data. However, it is important to take note that the data was collected only at 3 WAP, which makes it possible that the data might be different if it is collected at different time points (such as 5 WAP and 8 WAP).

The flowering age

One of the plant mechanisms in dealing with drought stress is by accelerating the flowering period. Cowpea plants are reported to have an average age of normal flowering at around 45-50 days after planting, meanwhile long bean plants at around 35 days after planting. The flowering age is influenced by the environment and plant varieties (genetic factors). The result of this study (Figure 8) exhibited that drought stress treatments (T1 and T2) significantly influenced some cowpea varieties compared to control and long bean plants. Under drought stress, the flowering age of several cowpea varieties (C3T1, C1T1, and C6T1) was longer than the other varieties as their first flower appeared at more than 50 DAP. The results of this study were in contrary to the previously reported studies where plants tend to accelerate the flowering period under drought stress treatments. The delay in the flowering period indicates that lack of water in cowpea plants inhibits the enzyme and hormone activities that induce flowering.

Different long bean varieties gave a different response to drought stress, indicating the influence of genetic factors aside from environmental factors. For example, some varieties had a normal flowering age (group 1), some were faster than the normal flowering age (group 2), and some took a longer time than the normal flowering age (group 3). The varieties of long bean plants that belong to group 1 were LB2T0, LB6T1/T2, LB3T1, LB2T1, LB4T2, LB3T2, and LB2T2. Meanwhile, group 2 consisted of LB7T0/T1/T2, LB4T0/T1, LB3T0, LB5T0/T1/T2, and LB6T0. Group 2 was dominated by long bean varieties in the control treatment (T0), indicating that these varieties naturally had an early flowering age compared to the normal flowering age. However, the flowering age of group 2 in drought stress treatments was not significantly different with the control treatment (T0), meaning that drought stress had no significant effect on group 2. Group 3 consisted of LB1T0/T1/T2.

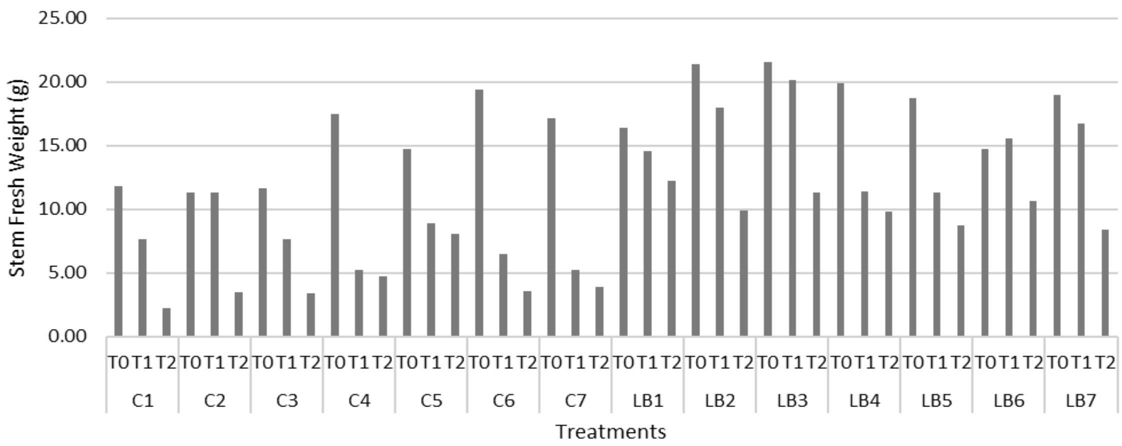

Figure 6. The average of stem fresh weight measured from long bean and cowpea plants under different drought stress conditions

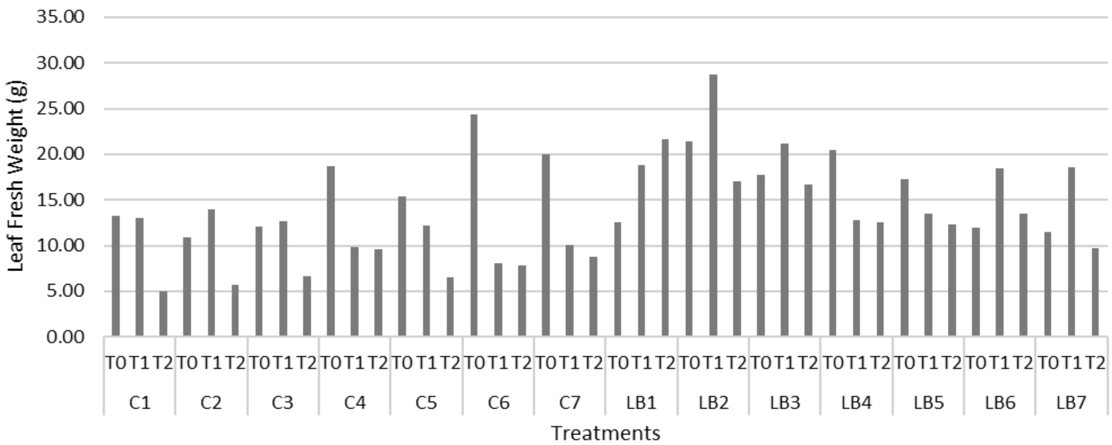

Figure 7. The average of leaf fresh weight recorded from both cowpea and long bean plants under drought stress treatments

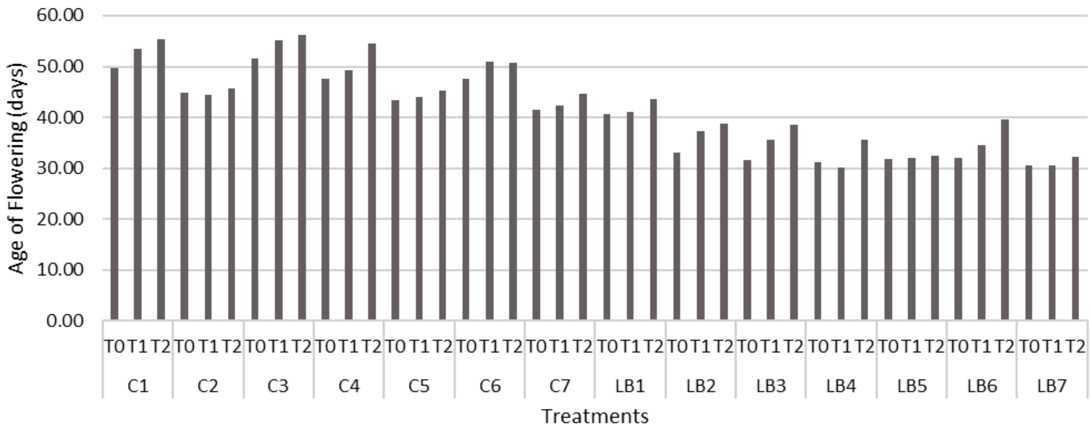

Figure 8. The average flowering age recorded from cowpea and long bean plants under different drought stress conditions

Physiological response of cowpea and long bean plants in drought conditions

Analysis of proline level

The analysis of proline level showed that there was no increase in proline level in T1 treatment for both cowpea and long bean plants (Figure 9). The increase in proline level has mostly occurred in T2 treatment for both tested plants (C1T2, C2T2, C4T2, C5T2, LB1T2, LB2T2, LB3T2, LB4T2, LB5T2, LB6T2, and LB7T2). The increased levels of proline are one of the plant mechanisms in dealing with drought stress. Plants have the ability to adapt to drought conditions and produce non-toxic dissolved compounds in order to reduce the osmotic potential in water deficit conditions, one of the mechanisms is through the production of proline compounds. The role of proline in drought stress is as a defense mechanism in keeping the turgor pressure of plant cells to avoid plasmolysis (Sanders and Arndt 2012). The order of varieties that showed significant increasing proline levels were C2T2 and C1T2 for cowpea plants, and LB2T2, LB1T2, as well as LB3T2 for long bean plants. Therefore, these varieties were considered to be physiologically responsive to drought stress.

Morphosiological analysis of plant stomata

A recent study showed that the drought stress treatments (T1 and T2) on both cowpea and long bean plants caused a significant narrowing in stomatal openings as compared to control treatments (Figure 10). Generally, the average width of stomatal openings between cowpea and long bean plants was not significantly different in the control treatment (T0), which ranged from 7.34 to 11.41 µm. The widest stomatal opening was recorded in LB3 from one of the long bean varieties in the control treatment, with a width of 20.81 µm. The extreme narrowing of the stomatal opening was found in T2 treatments. However, the average width narrowing of stomatal opening recorded in T1 treatments could reach up to 50% compared to control treatments.

Stomata are microscopic pores formed from pairs of guard cells located in the leaf epidermis. Stomata is a key process involved in the regulation of photosynthetic capacity in plants under stress conditions, mainly due to its role as the main route of CO_2 entry into the leaves (Perez-Martin et al. 2014). Stomata are also known to play an essential role in plant adaptation against drought stress as they can control water loss (Juairiah 2014; Zeng et al. 2010). The narrowing of stomatal openings on all plant varieties indicated a strong response to drought stress (Figure 10). This response aims to reduce the diffusion of water loss by evaporation through the micro pores of the stomatal complex. Physiologically, various chemical signals, such as abscisic acid (ABA) production in dehydrated roots, are the main regulators of stomata. According to the obtained data, the sensitivity towards drought stress tends to be higher in various long bean varieties compared to cowpea plants. LB3 variety was a variety that showed a highly significant narrowing of the stomatal opening in T1 and T2 treatments as compared to the control treatment. Just like any other morphological parameter, a diverse response of stomatal opening against drought stress exhibited by different plant varieties indicated a strong influence of genetic factors. The effect of genetic factors is assumed to be closely related to the expression of genes responsible for the production of chemical compounds, such as ABA which is the main regulator of stomatal regulation, including the opening and closing of stomata, the width of stomatal openings, and the stomatal density.

Furthermore, the data on stomatal density (Figure 11) indicated that cowpea plants were relatively more sensitive to drought stress than long bean plants. Three out of seven tested cowpea varieties were found to be adaptive to drought stress, namely C2, C3, and C7. And only one out of seven tested long bean varieties (LB7) was sensitive to drought. According to the classification of stomatal density (Juairiah 2014), a stomatal density of <300/mm^2 is classified as low-density, a stomatal density of 300-500/mm^2 is classified as medium-density, and a stomatal density of >500/mm^2 is classified as high-density. The result in Figure 11 showed that all tested varieties had low densities which varied between treatments and between varieties in both cowpea and long bean plants. This suggested that drought stress was able to reduce the stomatal density and the stomatal width on all tested varieties compared to control treatments, as an effort to reduce or maintain a high transpiration rate. Additionally, we also found that the higher the drought stress, the higher the decrease in stomatal density.

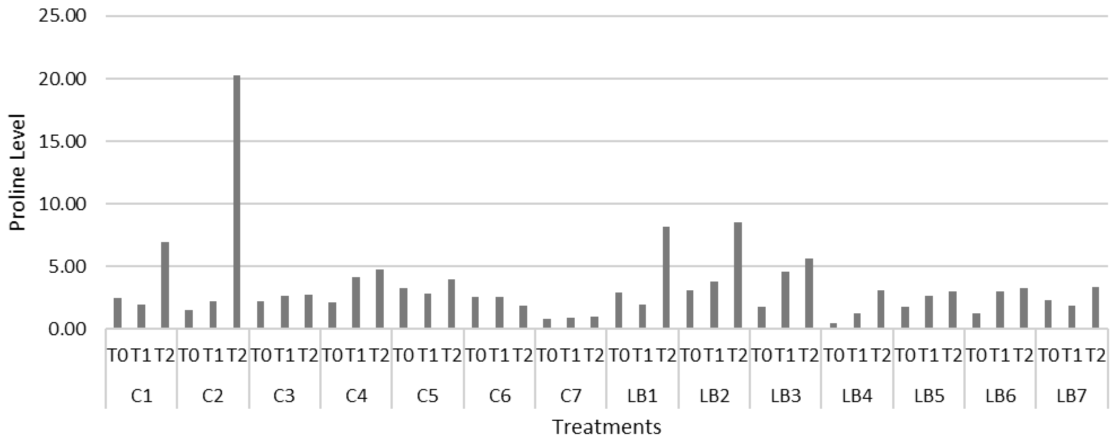

Figure 9. The proline level recorded from cowpea and long bean plants under different drought stress conditions (8 WAP)

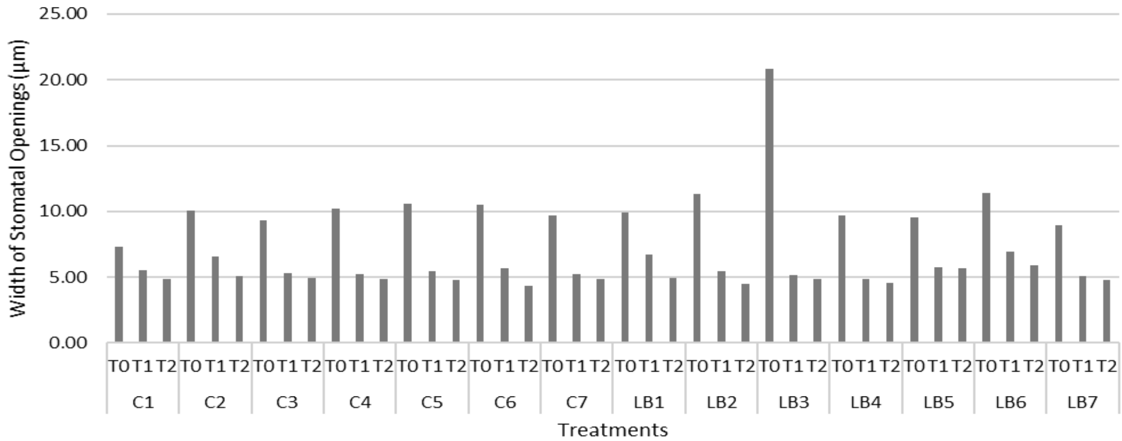

Figure 10. The average width of stomatal openings recorded from cowpea and long bean plants at 3 WAP

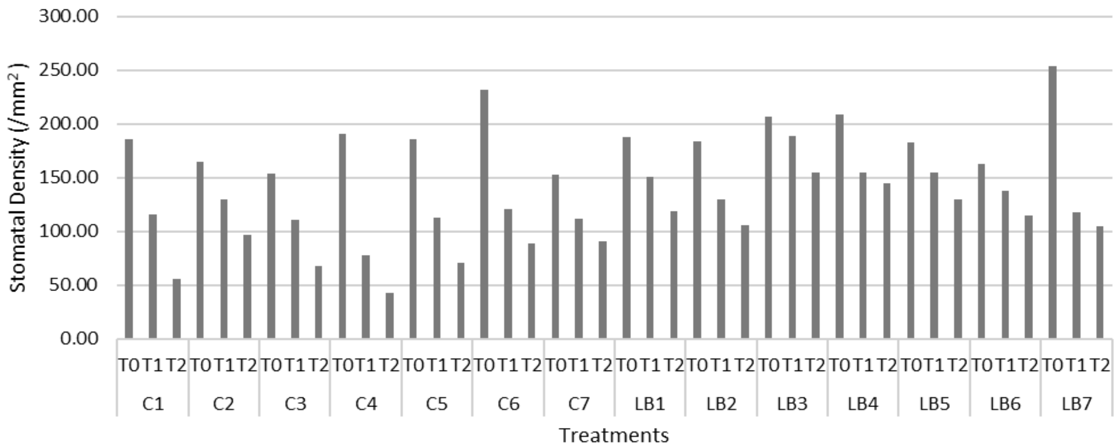

Figure 11. The average of stomatal density recorded from cowpea and long bean plants at 3 WAP

Discussion

The results of this study suggest that drought stress significantly affects several observed morphological and physiological parameters in both cowpea and long bean plants. Although both cowpea and long bean plants belong to the same subspecies, however, there are clear differences that make these two plants classified into different cultivars. Based on the pod and seed characteristics, the subspecies of *Vigna unguiculata* is classified into five cultivar groups: (i) "Unguiculata" cultivar (consists of cowpea and black-eyed pea); (ii) "Melanophthalmus" cultivar; (iii) "Biflora" cultivar; (iv) "Sesquipedalis" cultivar (consists of long bean and asparagus bean); and (v) "Textilis" cultivar (Fang et al. 2007 cit Milosevic 2013). This recent study showed that in control plants, the morphological characters of cowpea plants tended to be shorter than long bean plants with an average height of 159.18 cm (at 8 WAP), the average number of leaves was 33.95 leaves (at 8 WAP), the average stem diameter was 9.51 mm (at 8 WAP), the average root length was 19.84 cm (at 3 WAP), the average of root fresh weight was 5.75 gram (at 3 WAP), the average of stem fresh weight was 14.80 gram (at 3 WAP), the average of leaf fresh weight was 16.38 gram (at 3 WAP), and the average of flowering age was 46.57 days after planting. On the other hand, the characteristics of tested long bean plants in this study had an average height of 316.09 cm (8 WAP), the average number of leaves was 36.97 leaves (8 WAP), the average stem diameter was 7.95 mm (at 8 WAP), the average root length was 24.04 (at 3 WAP), the average of root fresh weight was 10.16 gram (3 WAP), the average of stem fresh weight was 18.8 gram (3 WAP), the average of leaf fresh weight was 16.1 gram (3 WAP), and the average of flowering age was 33 days after planting (DAP).

Cowpeas have an upright growth type, while long beans have a creeping growth type. This study recorded that cowpea plants were smaller than long bean plants and drought stress was able to reduce the height of both plants. Despite being reported to be tolerant to drought stress (Mahalakshmi et al. 2007), this study found that cowpea plants were more susceptible to drought stress compared to long bean plants. The result of this study can be further used as the source to select the candidate parents for plant breeding purposes. According to the result of this study, the selected seven varieties of cowpea plants are not suggested to be used as the parents for producing plants resistant to drought stress. However, the varieties of long bean plants used in this study exhibited a more tolerant morphological response against drought stress which is good to be used as candidate parents for plant breeding. One of the cowpea plant variety (C2) showed a high level of proline in T2 treatments (20,27 µmol/gram of leaves sample) compared to other varieties from both plants. This result indicates that C2 variety was physiologically more adaptive to drought stress compared to other varieties as proline is essential in maintaining plant cells to remain turgor and avoid plasmolysis. There are two proline biosynthetic pathways in plants, and the one that is preferred by plants involves the conversion of glutamate to proline by two successive chemical reactions catalyzed by Δ^1-pyrroline-5-carboxylate synthetase (P5CS) and Δ^1-pyrroline-5-carboxylate reductase enzymes (Hu et al. 1992 cit Vukovic et al. 2022; Meena et al. 2019). The high level of proline detected in C2 variety was expected to be the result of the over-expression of the P5CS genes. However, further study is required to confirm this assumption by analyzing the expression of the P5CS gene in C2 variety under drought stress. Hopefully, the result can then be transformed and applied to other plants through genetic engineering in order to produce plants resistant to drought stress.

Aside from producing proline, the other mechanism used by plants to deal with drought stress is through the expression of genes encoding protective proteins, such as dehydrin. Dehydrins are an essential group of proteins that are abundant in the late stages of embryogenesis (Murray and Graether 2022). The accumulation of dehydrin is induced by different developmental stages and abiotic stress factors. Although we did not measure the dehydrin level, however, we assume that the parameters tested in this study (plant height, number of leaf, and stem diameter) might be influenced by the ability of each variety to produce dehydrin at each growth phase (2 WAP, 5 WAP, and 8 WAP). The plant height, number of leaf, and stem diameters (Figures 1, 2, and 3) of cowpea and long bean plants measured at 2 WAP were not significantly different between each treatment. This might be because the production of dehydrin by DHNs gene (Khan et al. 2020) in the late stage of embryogenesis is still sufficient to protect plants from drought stress at 2 WAP. These results were supported by the root length data (Figure 4) which showed no significant difference between control and drought stress treatments (T1 and T2) at 3 WAP. Response to drought stress started to show significant variations at 5 and 8 WAP. This is presumably because of the interaction between dehydrin and proline proteins as described in the Arabidopsis plant. A previous study in Arabidopsis found that over-expression of the dehydrin gene (DHN5) increases tolerance to salt and osmotic stress associated with regulatory responses of proline and antioxidant metabolism (Brini et al. 2007). Therefore, the adaptability of each plant variety against drought stress is strongly influenced by its genetic factors.

Contradictory results were found in root length and root fresh weight. For example, the root length between control and drought stress treatment was not significantly different in all tested varieties, meanwhile, there was one long bean variety (LB4) that showed a significantly different result in T1 treatment compared to other varieties. Although LB4 variety in the control treatment (T0) had a longer root length compared to the drought treatment (T1), however, LB4 variety (LB4T1) had a heavier root weight in the drought treatment compared to the control treatment (LB4T0). This indicates that LB4 variety has the ability to effectively absorb and store water as well as photosynthate compared to other varieties. However, this ability is influenced by the level of drought stress that has been given. Therefore, in the 10-day watering (P2), the ability to absorb and store water by KP4 accessions began to decrease and was not even heavier than the control plants. One of the plant mechanisms in response to drought stress is to expand the area of soil water absorption, such as by increasing the root length or the density of root hairs which indirectly increasing the root fresh weight. Therefore, it can be temporarily concluded that LB4 variety tends to increase the density of root hairs in response to drought stress instead of increasing the root length. Similar results were also recorded on LB6 variety in control treatment where the root length was shorter in control than in drought stress treatments (T1 and T2), but the root fresh weight was significantly heavier in control than in drought stress treatments. Unfortunately, LB6 variety loses its ability to increase the number of root hairs after experiencing drought stress. Hence, the root fresh weight was lighter in the drought stress treatment than in the control treatment. This might happen due to the changes in genetic expression under drought stress, which then inhibits the production of growth hormones (auxins and cytokinins) important for increasing the length and the number of root hairs (Zhang et al. 2016).

Water availability as the main limiting factor can inhibit a number of chemical reactions (biosynthesis) in plant cells, which then significantly decrease the plant growth ability (as shown in almost all parameters observed in this study). The stem fresh weight (Figure 6) and leaf fresh weight (Figure 7) at 3 WAP exhibited a significant weight reduction trend in several varieties of both cowpea and long bean plants. However, there was an increase in both stem and leaf fresh weights in some varieties from drought stress treatments, indicating the adaptability owned by certain varieties in dealing with drought stress. However, we should take note that the increased fresh weights were not significantly different as compared to the control treatment. Therefore, it can be concluded that some varieties have a relatively low range of tolerance against drought stress. Even so, LB6 variety is recommended to be analyzed further based on the observed morphological parameters which consistently showed a better result in drought stress (T1) compared to control treatments (T0). Interestingly, the adaptability decreased under a higher level of drought stress treatment (T2), indicating that LB6 will be able to grow optimally in a quite dry environment (not too extreme). It is also assumed that LB6 will be able to survive under extreme drought stress although it will not be optimal.

Another morphological parameter observed in this study was the flowering age. This study found that cowpea plants responded to drought stress by taking longer time than normal flowering age. On the contrary, some varieties of long bean plants experienced accelerated flowering and some experienced delayed flowering. These results are considered normal considering that grain crops have varying degrees of sensitivity to drought stress. In general, legume plants, especially cowpea and long bean plants, tend to carry out an "escape" mechanism under drought stress (known as drought escape). A drought escape mechanism is a plant's response to drought stress by shortening its growth period, such as earlier flowering age compared to normal flowering age (Shavrukov et al. 2017). The delayed or accelerated flowering is expected to be influenced by the production of the bioactive gibberellin hormone (GA) which increases or decreases depending on the water content. GA is a hormone that plays an essential role in flowering (Gupta and Chakrabarty 2013). Generally, a high GA level will result in delayed flowering, and on the contrary, low GA levels will induce flowering. However, this regulation can not be applied to all plants since some plants need a high GA level to induce flowering. Lack of water can inhibit the expression of genes that synthesize GA, namely GA20 oxidase1 (GA20ox1) and GA20ox2, also induce GA biosynthesis and deactivation genes (GA20x7) in guard cells and leaf tissue (Shohat et al. 2021; Castro-Camba et al. 2022). As a result, the levels of bioactive GA in plants decreased and caused an early flowering under drought stress which was found in some long bean varieties tested in this study. Different responses were shown by cowpea plants in this study which experienced delayed flowering. This different response might be caused by the increase in GA bioactive during drought stress which delayed the flowering process. Another possibility is that the cowpea plants tested in this study need high GA levels to induce flowering. Therefore, although the possible mechanism of cowpea plants under drought stress was to reduce GA levels, but as flower formation requires high GA levels, then drought stress would result in delayed flowering. Further study on the GA content is required to confirm the result of this study.

GA is also reported to influence the width of stomatal openings and stomatal density. Inhibition of the GA20ox7 gene expression, which is responsible for the production of GA bioactive, due to drought stress might attenuate the stomatal response. The gibberellin-Insensitive Dwarf 1 (GID1) receptor gene is known to play an important role in the stomatal response to drought stress through GA and ABA signaling pathways (Shohat et al. 2021). Abscisic acid (ABA) is a hormone associated with the formation and narrowing of stomata. To add, hydraulic signals will induce ABA synthesis in plants when the soil is dry. ABA in the roots will be brought up to the stomatal guard cells and induce the closing of stomata (Bharath et al. 2021). Drought stress also causes a decrease in the water distribution to the guard cells, resulting in a decrease in turgor pressure and stomatal closing/narrowing. A decrease in turgor pressure along with the increase in free abscisic acid in leaves will further increase stomatal narrowing. Furthermore, another factor influencing stomatal density is the level of certain protein groups in the late stages of embryogenesis (LEA). López-Cordova et al. (2021) explained that LEA (Late Embryogenesis Abundant) protein is involved in the tolerance mechanism against drought stress and stomatal density. The result of this study found that both cowpea and long bean plants had a similar response by reducing the number of stomata (stomatal density) and the width of the stomatal opening under drought stress. Most of the cowpea varieties were more susceptible to drought stress characterized by the width of the stomatal opening and lower stomatal density compared to the long bean varieties. These results further confirm that almost all morphophysiological data showed that long bean plants were more adaptive/tolerant to drought stress than cowpea plants.

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