

## Morphological and Biochemical Responses of Glagah (*Saccharum spontaneum* L.) Accessions to Drought Stress

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### ABSTRACT

Glagah (*Saccharum spontaneum* L.) has potential value as a crop species and may also be used in sugarcane breeding programs; however, this germ-plasm has not been extensively used in breeding programs, primarily in relation to improve drought tolerance. The objectives of this experiment were to evaluate the effect of drought stress initiated at vegetative growth stage on morphological (plant height, stalk diameter, green leaf number) and biochemical (leaf proline content and protein pattern) responses of seven glagah accessions (BOT-53, BOT-54, BOT-60, BOT-77, BOT-78, BOT-84, and BOT-88). The plants were propagated from single node stalk segments in polybag in the field under non-stress condition for two months. The two-month-old plants were then subjected to drought stress by withholding watering for eight weeks. Untreated control plants were watered every two days. Results indicated that drought stress reduced plant height, stalk diameter and green leaf number. On the other hand, there was a little difference between drought-stressed and control plants in terms of proline content. The protein pattern showed that drought stress caused a change in gene expression in the form of induction or repression of protein expression. A specific protein with a low range of molecular weight (Rf value about 0.647) showed constitutively expressed in accession BOT-53 but drought-inducible expressed in BOT-54.

**Keywords:** *Drought stress, glagah, proline, protein pattern, Saccharum spontaneum*

### INTRODUCTION

Sugarcane (*Saccharum officinarum* L.) is an important source of sucrose and ethanol; however, drought stress often restricts sugarcane production [1, 2]. Drought stress affects morphological, physiological, biochemical and molecular processes, so limits the plant growth and development prior to the loss of production [3, 4]. Plant's behavior response to drought seems to be complex and has different mechanisms. One of the mechanisms might be via accumulation of proline [5] that is not only used for osmotic adjustment but also for scavenging and detoxifying oxidants [6, 7] and for preventing membrane dema-

-age and protein denaturation during severe drought stress [8, 9]. Generally, drought induces alterations in protein synthesis [3]. Maintaining proteins in their functional conformations and preventing the aggregation of non-native proteins are particularly important for cell survival under stress [10, 11].

Glagah (wild sugarcane, *S. spontaneum* L.) is a grass that can grow well in marginal soils [12]. It has also a high carbohydrate and fiber content, making it a suitable for ethanol production and paper industry [12, 13]. Glagah has better tolerant to drought stress than sugarcane [14] and cross compatible to sugarcane, therefore it may also be used in sugarcane breeding programs to improve drought tolerance. However, the genetic resources of glagah, especially those related to drought tolerance, have not been extensively utilized. Therefore, in this study, the effect of drought stress on plant height, stalk diameter and green leaf number of seven accessions of glagah (BOT-53, BOT-54, BOT-78, BOT-84, BOT-77, BOT-88 and BOT-60) is evaluated to

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estimate their drought tolerance. Leaf proline contents and leaf protein pattern were also examined to determine a possible involvement of these metabolites in drought tolerance and to learn the possibility of a specific proteins induced by drought stress.

## MATERIALS AND METHODS

### *Plant material and experimental conditions*

The experiment was carried out with seven glagah accessions (BOT-53, BOT-54, BOT-78, BOT-84, and BOT-77, BOT-88, and BOT-60). The accessions were kindly provided by the Indonesian Sugar Research Institute (ISRI/ P3GI), Pasuruan, East Java, Indonesia. The accessions were propagated from single node stalk segments (bud sets) in polybag ( $400 \times 200 \times 400 \text{ mm}^3$ ) containing 10 kg of a mixture of soil and manure (2:1) for two months before being subjected to drought stress. The plants were watered every two days until the treatments were imposed and were fertilized three times (1 day, 1 week, and 1 month after planting) with 2 g of ammonium sulphate and 1 g of triple superphosphate per polybag. The two-month-old plants were then subjected to drought stress by withholding watering for 8 weeks. Untreated, control plants were watered every two days. The experiment was performed in the field.

### *Growth measurement*

Plant height, stalk diameter and green leaf number were measured eight weeks after commencement of the treatment for both control and drought-stressed plants of each accession. Plant height was measured from the soil surface to the first fully-expanded leaf. Green leaf number was calculated from number of green leaves divided by total number of leaves; stalk diameter was measured at 50 mm above the soil surface.

### *Estimation of proline accumulation*

Assessment of proline content was performed in Lab. of Plant Physiology, Dept. of Biology, Fac. of Mathematics and Natural Sciences, Brawijaya University. Proline was assayed in the youngest fully-expanded leaves from both stressed and control plants. Proline content was determined using the colorimetric method of Bates et al. (1973) [15]. DL-proline (Sigma) was used as a standard.

### *Protein analysis*

Protein analysis was done in Lab. of Molecular Biology, Dept. of Biology, Fac. of Mathematics and Natural Sciences, Univ. of

Brawijaya. Protein was assayed in the youngest fully-expanded leaves from both stressed and control plants. Leaf samples were pulverized to a fine powder in liquid nitrogen with a mortar and pestle. Protein extraction was performed according to the methods as originally described by Stacy and Aalen [16]. Protein were separated in 12% SDS-PAGE following the method of Laemmli [17].

### *Experimental design and data analysis.*

The experiment was set out using a completely randomized design with three replications. The SPSS17 software package was used to analyze data of growth and proline. Means were separated by the Tukey HSD (Honestly Significant Different) test at  $P < 0.05$ .

## RESULTS AND DISCUSSION

In this study, there were significant effect due to accession, treatment and the accession treatment interaction on plant height (Figure 1). The least reduction on plant height was observed on BOT-54 (1.97 %), followed by BOT-53 (2.05 %). Thus, accessions BOT-53 and BOT-54 appear to be the most drought-tolerant and the tallest.

There were also significant effect due to treatment and the accession treatment interaction on stalk diameter (Figure 1b). Accessions BOT-53, BOT-54, BOT-78, BOT-84 and BOT-88 appeared to show the same degree of drought tolerance. The least reduction was also found on BOT-53 (2.58 %), even on BOT-54 showed an increase on stalk diameter about 0.63 %. Therefore, accessions BOT-53 and BOT-54 also show the most drought-tolerant.

For green leaf number, there were significant effect due to treatment and the accession treatment interaction (Figure 2a). The decrease on the leaves number which remain green of six accessions (BOT-53, BOT-54, BOT-78, BOT-84, BOT-88 and BOT-60) were not significant among accessions and treatments, while accession BOT-77 showed the greatest reduction on the leaves which remain green (26.61 %) though its response did not look different to any other accessions.

There were significant effect due to interaction between accession and treatment in term of proline content (Figure 2b). The proline content of leaves of the watered plants (control) did not differ significantly among the accessions, except for BOT-88 accession showed the highest value. The leaf proline content increased under

drought stress in BOT-53, BOT-54, BOT-78, BOT-77, and BOT-60 accessions, but slightly decreased in BOT-84 accession. On the contrary, the leaf proline content significantly decreased in BOT-88 under drought stress. Accession BOT-53 showed the greatest increase in concentration (80.63 %).

Results of protein electrophoresis by SDS-PAGE showed the presence of four types of changes in protein synthesis after eight weeks imposition of drought stress, i.e. the discovery of a new protein, not synthesized of certain protein, increasing and decreasing the synthesis of certain protein. Newly synthesized proteins with low molecular weight (MW) range (Rf value = 0.647) is found in BOT-54 accession (Figure 3, a).

This protein is synthesized constitutively in accession BOT-53, meaning that the protein is

constantly synthesized both in control and drought stress conditions, while in the other five accessions this protein is not synthesized in both conditions.

In BOT-77 accession appears to have also contained two new proteins with high MW range (Rf = 0,224 & 0,235) after experiencing drought stress (Figure 3 b,c). The while, in both BOT-88 and BOT-60 accessions were not found any more protein with high MW range (Rf = 0.224 and 0.235, respectively) in drought stressed condition (Figure 3d,e). Increased in protein sythesis with medium MW range (Rf = 0.471) was found in the five accessions, BOT-53, BOT-78, BOT-84, BOT-88 and BOT-60 (Figure 3, f), whereas a decrease in protein synthesis with high MW range was found in both BOT-78 and BOT-77 accessions (Figure 3g).

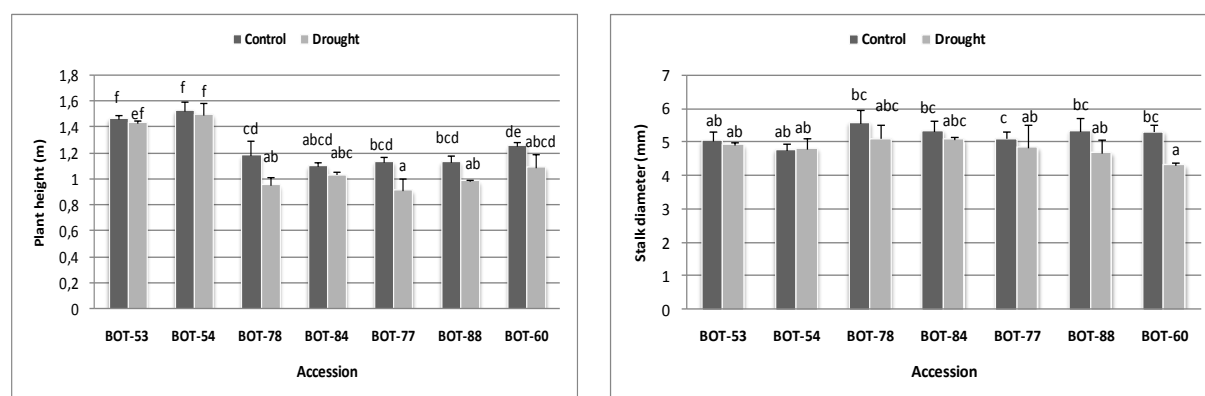


Figure 1 Effect of drought stress treatment on (a) plant height of seven glagah accessions. Each value is the mean of three independent samples. For each panel, means associated with the same letter are not statistically different from each other according to Tukey's HSD test at  $P = 0.05$ . (b) stalk diameter of seven glagah accessions. Each value is the mean of three independent samples. For each panel, means associated with the same letter are not statistically different from each other according to Tukey's HSD test at  $P = 0.05$ .

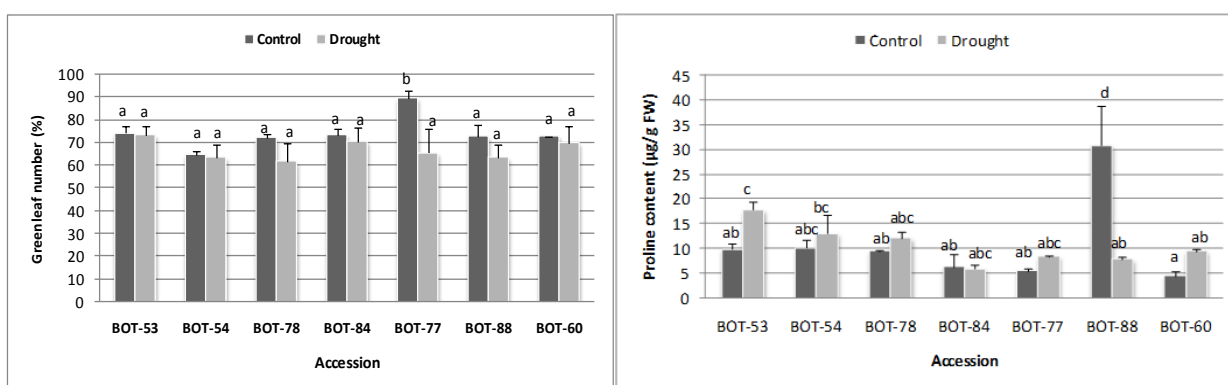


Figure 2 Effect of drought stress treatment on (a) green leaf number of seven glagah accessions. Each value is the mean of three independent samples. For each panel, means associated with the same letter are not statistically different from each other according to Tukey's HSD test at  $P = 0.05$ . (b) proline content of seven glagah accessions. Each value is the mean of two independent samples. For each panel, means associated with the same letter are not statistically different from each other according to Tukey's HSD test at  $P = 0.05$ .

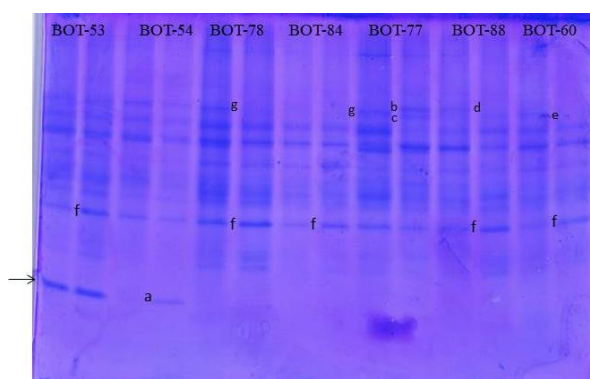


Figure 3 Effect of drought stress treatment on protein pattern of seven glagah accessions. New protein with Rf value 0.647, 0.224 and 0.235 (a, b, c, respectively), protein disappeared (d,e), protein increased with Rf value 0.471 (f), protein decreased (g). C= control, D= drought.

Of these changes, the protein with a low MW range in both BOT-53 and BOT-54 accessions is very interesting to further study because the protein is synthesized constitutively in accession BOT-53 but its synthesis is induced by drought stress condition in BOT-54 accession, while in the five other accessions, these proteins are not synthesized. Of these changes, the protein with a low MW range in both BOT-53 and BOT-54 accessions is very interesting to further study because the protein is synthesized constitutively in accession BOT-53 but its synthesis is induced by drought stress condition in BOT-54 accession, while in the five other accessions, these proteins are not synthesized.

Growth is the result of meristematic cell divisions and subsequent expansion of the young cells [8]. Under drought stress, cell elongation of higher plants can be inhibited by interruption of water flow from the xylem to the surrounding elongating cells. The cell elongating rate is primarily associated with how much water can be absorbed by elongating cells under water deficit [19]. In this study, drought stress decreased plant height, stalk diameter and green leaf number in the seven accessions of glagah studied. The reduction in plant height and stalk diameter may be due to a decline in the cell enlargement and greater leaf senescence under drought stress. Similar results were found in rice [20], sunflower [21] and apple [22]. Drought stress also decreased green leaf number which may be caused by a decrease in chlorophyll content. Low concentrations of photosynthetic pigments can directly limit photosynthetic potential and, hence, primary production.

The data for three morphological characteristics (plant height, stalk diameter and green leaf number) suggested that the accessions

BOT-53 and BOT-54 appear to be the most drought-tolerant. Plants of these two accessions were the tallest at the end of the drought period.

Previous research on proline content for marker of drought stress showed different results. Zhao et al. [23] suggested that proline was not a sensitive water stress indicator; whereas Rao and Asokan [24] found that drought-tolerant varieties of sugarcane accumulated more proline than susceptible ones and suggested that proline accumulation could be used as an index of drought tolerance. In this study, it is likely that proline involved in drought tolerance and may be able to act as a marker of drought tolerance in glagah as a mechanism for tolerance which has been suggested by Molinari et al. 2007 [25] for sugarcane. Nevertheless, this needs to be further confirmed with a wider range of genotypes and drought stress intensities.

By studying the protein pattern, expression of different polypeptides were found among glagah accessions. Appearance or disappearance of the protein band marks a change in gene expression. A number of proteins, whose accumulation level increased, were detected. The amount of some proteins decreased during drought stress. Such change in protein expression suggested that the gene involved the synthesis of this protein differentially activated under specific conditions. The synthesis of low molecular weight stress protein was specific for plants. Stress proteins are critical for maintaining homeostasis under stress [26].

In this glagah study showed earlier leaf senescence. It can be caused by the expression of proteins involved in photosynthesis has decreased, as the result of research of Bazargani et al 2011 [27] on wheat that discover changes in

protein expression coordinately involved in the oxidative stress defense, signal transduction, metabolism and photosynthesis.

Protein pattern changes in accessions BOT-53 and BOT-54, particularly which constitutively and drought-inducible expressed, respectively could be the focus on next research to identify it.

## CONCLUSION

The present study concluded that drought stress reduced plant height, stalk diameter and green leaf number. There were significant effect due to interaction between accession and treatment in term of proline content. Accession BOT-53 showed the greatest increase in proline concentration. Drought stress also caused induction and repression of certain protein. A specific protein with low molecular weight showed constitutively expressed in BOT-53 but drought-inducible expressed in BOT-54.

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