JERAMI (Indonesian Journal of Crop Science)
Volume 2, Issue 1, August 2019
http://jerami.faperta.unand.ac.id/index.php/Jerami-JIJCS

Research Article

Physiological Differential Response Of Sugar Cane (Saccharum Officinarum L.) On Water Deficit Condition

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Abstract

Sugarcane is the main sugar-producing plant in the world and also plays an important role as a raw material for bioethanol production. Productivity improvement of the plant is exposed to environmental stress ie: water shortage which is currently a serious problem associated with the global climate change phenomenon. Understanding plant responses to environmental stress are one of the keys to be able to resolve the issue. In this regard, the fundamental studies related to the sugarcane plant responses to water stress is very important. This study consists of a combination of two factors, namely the type of clones consisting of PS.864, PSJT.941, and VMC.76-16, 851 as tolerant group clones, PS.862, PS.882, and PS.851as non-tolerant clones group and lack of water stress treatment for 5 days. The data were analyzed further using DNMRT at 5% significance level. Observations showed that tolerant clones, as well as non-tolerant clones PS.862, showed better resistance response than non-tolerant groups. The indication was shown by the value of the Relative Water Content (RWC), Specific leaf area (SLA) and Water Deficit Value (WDV). Total protein profiling of sugarcane grown under water deficit and its counterpart differentially distinguished by suppression of protein expression of about 35 kDa in all clones. While in the water deficit condition expression of a protein with a size of 25 kDa is remarkably expressed.

Keywords: Saccharum officinarum, Differential response, drought, tolerant, physiological response

Citation: Renfiyeni 2019. Physiological Differential Response Of Sugar Cane (Saccharum Officinarum L.) On Water Deficit Condition. Jerami Indonesian J. Crop Sci., 2 (1): 1-13

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Received: November 20, 2018 **Accepted:** February 04, 2019 **Published:** August 31, 2019

Competing Interest: The authors have declared that no competing interest exists.

Introduction

Sugarcane (Saccharum officinarum L.) is the main source of sugar raw material in the world which is planted from the tropics to the sub-tropics (Waclawovsky, et al., 2010) including Indonesia. Besides that, sugar cane is currently the main choice as a source of bioenergy raw materials (bioethanol) as done by Brazil (da Graça, et al., 2010), United States of America (FAOSTAT, 2008), India (Suprasanna, 2010) and Thailand (Ngamhui, et al., 2012). In Indonesia alone, the area of sugarcane planting until 2011 reached 473,923 hectares with total production reaching 3,159,836 tons (Dirjen Perkebunan, 2011).

Nationally the productivity of sugar cane is still very low. Although many sugarcane superior clones have been successfully developed, the yield results obtained are still below the potential. This condition has become one of the obstacles to efforts to achieve the 2014 National Sugar Self-Sufficiency Program which is one of the National Programs of the Ministry of Agriculture. One factor that is considered to have an important role in this regard is the change in agro-climate conditions and weather anomalies as one of the abiotic stresses that have developed lately on a global scale. (Bray, et al., 2000), thus resulting in "leveling off" the productivity of sugarcane crops.

Among the various agro climate issues that have a very large influence on the productivity of sugarcane crops is the availability of water (Sugiharto, et al. 2002; Prabu, et al. 2011). Although sugar cane is included in the C4 plant class which is known to have an efficient photosynthesis system (Lopes, et al., 2011), the rate of photosynthesis in sugarcane plants continues to decline with drought stress (Carmo-Silva, et al., 2008). Gardner, et al., (1984) suggested that a number of physiological processes that would be disrupted included stomatal conductance, transpiration rate, leaf temperature, photochemical electron transport, photosynthesis, respiration, and assimilate partitioning.Some other researchers, like.

Ramesh and Mahadevaswamy, (2000); Robertson, et al., (1999); Da Silva and Da Costa, (2004); Singh and Reddy, (1980) and Soares, et al., (2004) stated, that some important agronomic characters that will be observed in the growth of sugarcane plants when faced with stress drought, among others, is a decrease in the diameter of the segment (Da Silva and Da Costa, 2004), stem height (Inman-Bamber and Smith, 2005; Ramesh and Mahadevaswamy, 2000; Da Silva and Da Costa, 2004; Singh and Reddy, 1980; Soares, et al., 2004). Emphasized by Domaingue (1995) and Soares, et al., (2004), that the height of the stem is the most affected parameter if sugar cane is in a condition of lack of water. With this basis, usually, the search for tolerant genotypes against water shortages is often based on the high resistance character of the stem. Although research on the response of sugar cane to water stress has been widely carried out, studies on physiological and morphological aspects associated with improved yields, and the content of sucrose are still little understood (Edmeades et al., 2004; Inman-Bamber et al. , 2005; Zhao, et al., 2010). Whereas a comprehensive understanding of these aspects is very important in the development of the selection program and at the same time and in order to improve the resistance of sugarcane crops to stress water shortages. In this regard, the research was carried out. In this manuscript, the initial results of the study describe the morphological and physiological differential responses of 3 tolerant clones and 3 non-tolerant clones.

Materials and Methods

Plant Material and Time of Research

The plant material used in this study were 6 sugarcane clones obtained from the Indonesian Sugar Research and Development Center (P3GI) Pasuruan. The clones consisted of three tolerant plant clones namely PS.864, PSJT.941, and VMC.76-16 and three intolerant plant clones consisting of PS.862, PS.882, and PS.851. The study was conducted at the Greenhouse of the Faculty of Agriculture, Andalas University, Padang, from May to October 2014.

Research Methodology and Statistical Analysis

The two experimental factors used in this study were: A stress factor lacking water. This factor consists of 2 levels, namely: A1, Water supply at field capacity and A2, without giving water for 5 days. While the second factor is the type of clone consisting of 6 levels (6 clones), namely: PS.864, PSJT.941, VMC.76-16 and PS.862, PS.882 and PS.851. The first three clones are described as tolerant clones while the last three clones are drought-sensitive clones according to P3GI claims. The two factors used are combined and are considered as treatment combinations. Thus there are 12 treatment combinations. Each trial unit is repeated 5 times. Thus 60 test units are used. Observation data were analyzed statistically using the F test. If the value of the F treatment was greater than the 5% F table, then the Duncan's New Multiple Range Test (DMNRT) was continued at the 5% level.

Planting and Plant Maintenance

The seeds used are sown first. The number of seeds from each clone was 25. Before sowing the seeds are soaked for one hour with a ZA fertilizer solution with a concentration of 25 g / liter. Nursery is carried out on a flat container containing a mixture of soil and sand with a composition of 1: 1 for one week. Seedlings with a height of about 20 cm are selected to be moved to the planting site. Planted seeds amounted to 60 seeds consisting of 10 seeds per each clone. The seeds are then planted in a bucket that has been filled with sand, soil and manure media with a ratio of 1: 2: 1 respectively. Plants are maintained according to recommendations by watering, weeding and tillers at the beginning of planting until before treatment. At the age of 3 months fertilization was carried out at a dose of 33 grams / NPK fertilizer plants into a pot encircling sugar cane.

Stress Treatment

The stress treatment for all experimental units is given after the plant is 3 months old. Treatment was given with two levels, namely level A1 (watering with field capacity) and level A2 (without watering for 5 days). As a result of the treatment can be observed in the form of leaves of the upper part of the plant and rolling (Widyasari et al., 2004).

Observation of Supporting Parameters

Two physiological parameters namely Relative Water Content (RWC) and Specific Leaf Area (SLA) were observed using leaf samples from the bottom 3 leaves. Samples were taken on days 3 and 5 after stress treatment. The measurement of relative water content (RWC) was carried out by weighing 1 cm × 1 cm fresh leaves as a Fresh Weight (FW), then saturated with aquadest for 24 hours to get the weight of turgid (WT) then dried with an oven for 2 ×

24 hours at 70ºC as dry weight (DW). Relative water content values are calculated by the formula:

$$RWC = \frac{FW - DW}{WT - DW} x100\%$$

Specific leaf area measurement (SLA) is calculated by measuring the area of leaf pieces (ALP), namely 1 cm x 1 cm multiplied by the number of pieces of leaves (NPL) by 10 pieces and divided by dry weight (DW) by the formula:

$$SLA = \frac{ALPxNPL}{DW}$$

The results obtained are recorded in units of m² / gr. While the water deficit measurement (WD) was carried out using heavy data of turgid, fresh weight and dry weight, and calculated by the formula:

$$WD = \frac{WT - FW}{WT - DW} x100\%$$

Isolation of Sugar Cane Protein

Protein isolation was carried out by modification of the TCA method (Almaraj et al., 2010). Sugarcane leaf samples are taken from the top 3 leaves. Leaves weighed as much as 500 mg, then sliced, then put into mortar and mixed with liquid nitrogen to be smoothed. Scouring results are transferred to the falcon tube and 2 ml of trichloroacetic acid (TCA) is added and then allowed to stand for one hour at -20ºC. Then the sample was centrifuged for 20 minutes at a temperature of -4ºC with a speed of 12,000 g. After centrifugation, the supernatant is removed. Pellets were added with 2 ml of cold acetone mixed with 0.014 grams of DTT. Then the mixture was centrifuged for 20 minutes at a speed of 12,000 g at -4ºC. After centrifuging the supernatant is removed. The washing process with aceton and DTT is repeated 3 times until the pellets are white. Then the pellets are dried and then stored at -80ºC until they are used in the next stage.

Before being analyzed, pellets are first dissolved in protein solvents. A total of 100 mg of dry protein pellets were weighed and put into a 2 ml eppendorf tube. Then 1 ml of saline buffer protein (SBP) was added and incubated for 1 hour at 37 ° C. Every 10 minutes once the sample is homogenized using vortex. Then the sample was centrifuged for 20 minutes at a speed of 12,000 g at -4ºC. Then the supernatant was transferred to 1.5 ml eppendorf and ready for analysis.

Visualization of Protein Profiles with SDS-PAGE

Protein visualization was carried out using a stacking gel consisting of Buffer B, 2.5 ml; Acrylamid 30%, Aquabidest, 10% APS, and 2.5 mL TEMED. Gel for separation (Separating gel) consists of: Buffer A, Acrylamide 30%; Aquabidest, 10% APS, and 5µl TEMED. Material mixture is put into the mold and left to harden. Then the gel is placed into an electrophoresis bath. A total of 100 ml of SDS running buffer is inserted into an

electrophoresis bath. Then, 20 µl of protein sample was put into 1.5 ml eppendorf, and added with 5µl SDS and then heated for 5 minutes at 95oC. The sample is then put into the gel well. Then SDS-PAGE is run with a current setting of 100 Ampere for 30 minutes. The gel is then placed in a plastic box, filled with aquadest and heated in an oven with medium high temperature for 1 minute. Aquadest is removed and the box is replenished with distilled water and shaker at a speed of 75 rpm for 15 minutes. The process is repeated 2 times to remove the remaining SDS buffer. Then the aquadest was discarded and the gel was soaked with coommassie dye solution and shaker at a speed of 75 rpm overnight. After overnight incubation, the coommassie solution was removed and the gel was washed again with aquadest 3 times. The gel is then documented using a scanner.

Results and Discussion

Relative Water Content (RWC)

The relative water content testing was carried out to determine the percentage of relative water content on the leaves of the six clones tested during stress that is on the 3rd and 5th days after the treatment was given. Observation data are presented in Table 1.

Table 1. Relative moisture content of 6 sugarcane clones after drought stress treatment on the 3rd and 5th days.

Klon	Relative water content(%)	
	Day 3	Day 5
PS.864 (T)	84,81 a	78,82 a
PSJT.941 (T)	86,47 a	80,27 a
VMC.76-16 (T)	81,94 a	75,65 a
PS.862 (NT)	88,90 a	81,93 a
PS.882 (NT)	74,21 c	69,21 c
PS.851 (NT)	81,17 b	74,43 b
	CC = 5,14%	CC = 6,42%

The numbers followed by the same letter in the same column are not significant according to the Duncan test of 5%.

Drought stress for 5 days applied gives a significantly different effect. KAR measurements on the 3rd day showed that PS.882 (non-tolerant) clones experienced a decline in RWC to 74.21%. This value was significantly different from the other five clones which on average still had RWC values above 80%, including the other two non-tolerant clones namely PS.862 and PS 851. Clones Even PS.862 clones claimed as non-tolerant clones on the third day after stress treatment still has the highest water content value of 88.90%. The RWC value of the PS.882 Clone in observing day 5 even only reached 69.21%. This figure is significantly different compared to the other five clones. The PS.862 clone also shows the highest RWC figure compared to the other five clones although it is claimed to be a non-tolerant clone to drought stress.

The decrease in the relative water content began to occur from the 3rd day and decreased on the 5th day since the treatment of stresses on all the clones treated. The lowest decrease in water content occurred in PS 862 clone of 18.07% while the highest decrease in water content

occurred in PS.882 which reached 31.79%. Other clones PSJT.941, PS.864, and VMC.76-16 experienced a decrease in water content of 20.73%, 21.18% and 24.35% respectively.

The clones used in this study consisted of tolerant clones (PS 864, PSJT 941, and VMC 76-16) and intolerant clones (PS 862, PS 882 and PS 851). The interesting thing happened in the PS 862 clone which was claimed to be an intolerant clone but still had the highest RWC on the 3rd and 5th days after drought stress. Data presented by Khozin, (2013) indicated that PS 862 clones were indeed susceptible to abiotic stress, but their susceptibility was more likely to inundation stresses so that the drought stresses could be more resistant. PS 864 and PSJT 941 clones have better stress resistance values on Ultisol, Vertisol, and Inceptisol soil types. The ability of PS 864 clones was also observed to be equally good in callus growth compared to PS 862 clones in stress conditions (Fiah, et al., 2014). PS 851 clones do not have stress resistance from the results of testing some character growth, sucrose and proline content (Rinanto, 2010). VMC 76-16 clones have tolerant values and also have drought stress resistance values (Directorate of Seed and Production Facilities, 2010). In the same study also examined PS 864 and VMC clones 76-16. The two clones appear to have good resistance to inundation stresses (Khozin, 2013). PS clone 851 in previous studies also examined its resistance to drought stress. As a result the 851 PS clone was concluded to be intolerant of drought stress.

Decrease in water content is a natural response shown by plants when facing drought stress. A decrease in water content occurs due to evaporation of the transpiration process which is not accompanied by a supply of water. Clones that have drought stress resistance have a way to maintain water levels in cell turgor in order to maintain metabolic sustainability. A good resistant clone will be able to maintain the amount of water in the body when compared to clones that cannot stand.

When plants experience drought stress, the adaptation response is to regulate the water status in their body. The ability to regulate the status of water is largely determined by the tolerance properties of plants to drought stress, one of which is through adjustments of osmotic pressure in plant cells (Kirkham, 1990). Plants make some changes as an adaptation response when water loss due to drought stress. These changes include closing the stomata, rolling the leaves, aborting leaves, reducing the growth rate or by maintaining the water supply with osmotic adjustments and increasing the root / crown ratio (Levitt, 1980).

Specific Leaf Area

The measurement of specific leaf area is used to determine the decrease in leaf formation and expansion during stress treatment. The measurement of specific leaf area is carried out on the 3rd and 5th days after the treatment is given. The results of the observations show a significantly different effect, as shown in table 2.

The morphological characteristics of the leaves act as the main indicator of tolerant plant groups and are not tolerant of stress water shortages. The RWC measurements on day 3 showed a non-significant difference with the SLA range between 0.126 m2 / g (PS.882) to 0.143.m2 / g bk (PSJT.941). Although the SLA statistic between the tolerant and intolerant

groups was not significantly different, in general the tolerant group at day 3 after stress administration had a high SLA rate compared to the non-tolerant group.

Table 2. Specific leaf area (m2 / g) in 6 sugarcane clones after drought stress on the 3rd and 5th days.

Klon	Specific leaf area		
	Day-3	Day-5	Difference
PS.864 (T)	0,137 a	0,140 a	0,003
PSJT.941 (T)	0,143 a	0,146 a	0,003
VMC.76-16 (T)	0,130 a	0,135 a	0,005
PS.862 (NT)	0,133 a	0,139 a	0,006
PS.882 (NT)	0,126 a	0,119 c	-0,007
PS.851 (NT)	0,128 a	0,121 c	-0,007
	CC = 9,27%	CC = 9,31%	

The numbers followed by the same letter in the same column are not significant according to the Duncan test of 5%.

SLA measurements on day 5 after stress reduction indicated the presence of SLA value-added differentiation from the tested clones. PS.864, PSJT.941, and VMC.76-16 tolerant clones and PS.862 non-tolerant clones still show an increase in LDS with a range between 0.003 to 0.006. m2 / g bk While two non-tolerant clones PS.882, PS.851 has decreased by 0.007 m2 / g.

One of the tolerant clones namely PSJT 941 clone has buliform cells whose rows of cells are more varied than other clones (Cholid, et al., 2014). Buliform cells are cells that are larger in size than epidermal cells, the function of these cells is to adapt by rolling the leaves when plants experience drought stress (Price and Courtois, 1991). Whereas PS 864 and PS 882 clones have thicker lamina than other varieties.

According to Shield (1950) in Sulistyaningsih (1994) thick lamina is an indication of xerophytic plants. VMC 76-16 clones have a non-glandular trichome that is flexible, not easily broken, and a longer size than other clones. Kebede et al., (1994) in Sulistyaningsih (1994) explained that the results of research on the Lycopersicon genus showed that Lycopersicon pennelii which was more resistant to drought than Lycopersicon esculentum turned out to have fewer trichomes but a longer size. Trichome size plays a role in reducing transpiration. From this theory, PSJT 941, PS 862, VMC 76-16 clones also have a tendency to be resistant to drought. With these considerations, it can be explained that the PS 862 clone has a tendency to adapt to drought stresses.

Canopy development, is very sensitive to drought stress, which results in a decrease in leaf formation and expansion. Higher specific leaf area values indicate that tolerant clones have

better adaptation with lower wilt symptoms compared to non-tolerant clones (Mathius et al., 2001). Abayomi (2002) reported that in sugarcane plants that experienced drought stress there was a decrease in leaf growth, rate of addition of leaf area, leaf area, and leaf area index.

Water Stress Value

Water deficit testing is used to determine the decrease in water content of plants due to stress. This parameter will provide an overview of the plant's ability to withstand water availability during a period of water shortage. The results of the water deficit measurement are shown in Table 3.

Table 3. Water deficit in 6 sugarcane clones after drought stress treatment on the 3rd and 5th days.

Klon	Water Deficit (%)		Difference
	Day-3	Day-5	
PS.864 (T)	18,06 b	21,17 b	3,11
PSJT.941 (T)	13,53 c	19,73 b	6,20
VMC.76-16 (T)	16,18 b	24,35 b	8,17
PS.862 (NT)	12,52 c	18,07 c	5,55
PS.882 (NT)	25,79 a	30,79 a	5,00
PS.851 (NT)	18,83 b	25,57 a	6,74
	CC = 20,67%	CC = 18,64%	

The numbers followed by the same letter in the same column are not significant according to the Duncan test of 5%.

Observation of water deficit on the 3rd and 5th days showed a significantly different effect. The lowest water deficit on day 3 was experienced by the PS.862 clone which was 12.88%. This figure is not significantly different from PSJT.941 tolerant clones of 13.53%. The intolerant clone of PS.882 has the highest water deficit value of 25.79% which is statistically significantly different from other clones.

The test results on day 5 showed the differentiation in holding the water deficit. The lowest water deficit was shown by the PS.862 clone (18.07%), while the highest water deficit was still shown by the intolerant clone group, namely PS.882 and PS.851 with 30.79 and 25.57 respectively. While clones classified as resistant have a water deficit value between the range of 19.73 to 24.35. If an increase in the water deficit value is calculated, in general all the clones tested experience an increase in the water deficit value. When viewed in detail the clone VMC.76-16 is the clone that has the highest increase in water deficit value of 8.17 while the clone with the lowest increase in water deficit is the PS.864 clone.

The water deficit value (DA) shows the amount of water in the lost network compared to the water content in full turgor conditions. The greater the deficit of water, the lower the water available for metabolism. This very important role of water causes direct or indirect consequences that the plant water deficit will affect all metabolic processes in plants that result in disruption of the growth process (Gardner et al, 1991). Prawiranata et al added. (1992) that large DA values, especially in plants that are less tolerant, will significantly affect all metabolic processes, so that the rate of plant growth decreases and if prolonged can lead to death.

Protein Profile

To obtain a protein profile, the total protein isolation of sugarcane leaves was carried out using the modified TCA method (Almaraj et al., 2010) as described in the methodology. The description of protein profiles was obtained through separation using acrylamide gel with a concentration of 12% (Figure 1). The results of visualization show the differentiation of the patterns between the leaves of sugarcane proteins from clones that are not treated with stress water shortages with clones that are treated with stress water shortages. Differentiation is characterized by the presence of a 25 kDa protein band that appears on plant clones that are treated with drought stress, where the band does not appear on clones that are not treated with stress water shortages.

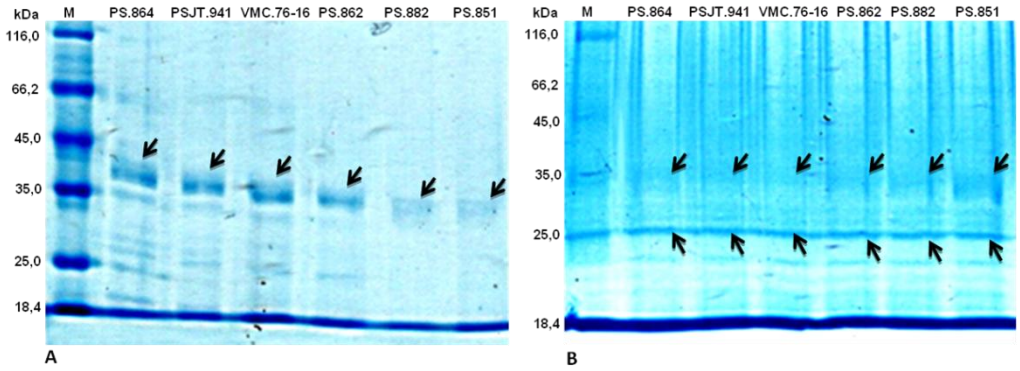

Figure 1. Differential protein profiles between (A) untreated clones and (B) treated clones.

On the other hand, the group of plants that were not treated with drought stress showed the appearance of protein bands with a size of about 35 kDa. The tape experienced a decrease in concentration seen from the thinning of the tape when treated with water drought stress. The low concentration is interpreted as a decrease in the expression of proteins at that size, or in other words experiencing down regulated. Protein bands with a size of about 25 kDa actually on the contrary experience an increase when treated with stress water shortages. Thus it can be interpreted that the groups of these proteins experience an increase in expression when experiencing stress water shortages (up regulated)

The possibility of these two protein bands is a group of proteins involved in metabolism that responds when sugar cane experiences stress water shortages. Unfortunately the results of this study cannot identify in more detail the types and identities of proteins expressed in both conditions.

In conditions without stress water shortages, PS.864, PSJT.941, VMC.76-16 and PS.862 clones produced a protein band measuring 35 kDa with a higher concentration than the two non-tolerant clones namely PS.882 and PS. 851. Almaraj et al. (2010) stated that the proteins produced in the isolation of sugar cane protein when analyzed will obtain several types of proteins. First, as much as 23% of protein is a protein that functions as a mechanism for self-defense from interference from fellow creatures or the environment. Then another 23.33% is a protein that acts as a catalyst for phosphate and sugar metabolism. The most important group that is as much as 20% is a protein protein producing sugar. Whereas 13.33% is responsible for nucleic acid metabolism and the other 10% act as initiators of cell growth and development plus 6.67% respectively as structural baselines and secondary metabolics. While the remaining 16.67% is the unknown protein.

In general, drought stress has other effects on the physiological activity of sugarcane crops such as protein degradation, photosynthesis, metabolism and antioxidant activity. This is characterized by an increase and decrease in protein expression in drought stress conditions which indicate that there are adaptation efforts and changes made by sugar cane in the face of drought stress (Xiang et al., 2010).

Zhou et al. (2012) stated that ATP and Isoflavone reductase-like proteins (IRL) are some examples of metabolic-related proteins that have decreased expression due to drought stress. These changes are related to the adaptation of sugar cane in the face of drought stress. Sabehat et al. (1998) states that in general total protein in a plant will experience a decrease if the plant experiences drought stress. This is a result of conditions that are less supportive for optimum plant metabolism. However, there is generally an increase in accumulation of proteins with low molecular weights.

Conclusions

The response of sugarcane clones of tolerant groups: PS.864, PSJT.941, VMC.76-16 and PS.862 was proven to have better drought stress resistance compared to stress tolerant PS.882 and PS.851 groups significantly. There are differences in protein profiles of 6 sugarcane clones during normal conditions and when experiencing drought stress. This difference is seen in the expression of a 25 kDa protein band that does not appear in normal conditions.

Acknowledgment

Thank you to the Agricultural Research and Development Agency of the Ministry of Agriculture of the Republic of Indonesia for fully funding this research through the KKP3N research grant scheme for the 2013 budget year with the contract number: 726 / LB.620 / I.1 / 2/2013, February 25, 2013.

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