Effect of water stress on growth, yield, proline and soluble sugars contents of Signal grass and Napier grass species

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ABSTRACT

The research was conducted at the greenhouse of the Faculty of Animal Science, Hasanuddin University, Makassar, Indonesia during the two months to investigate the effect of water stress treatments (W0 = Field capacity (FC), no water stress), W1 (50% FC, for 1 week), W2 (50% FC, for 2 weeks), and W3 (50% FC, for 3 weeks) on growth, yield, proline, and total soluble sugars contents of two grass species (Signal grass and Napier grass). Results showed that increasing water stress duration induced reduction in plant height, number of tillers, and dry matter yield but decreased the proline, and total soluble sugars content. Prolonged water stress led to increases in the proline and soluble sugars both grass species. Species variation was observed were Signal grass species had the highest number of tiller and proline content compare to Napier grass species, while Napier grass species was higher in plant height, yield and soluble sugar contents. This indicates variation between the two species grass in growth, yield, proline and soluble sugars, could be altered by duration of water stress.

KEY WORDS
Grass species, proline, soluble sugars, water stress, yield

INTRODUCTION

One of the keys to increasing the productivity of livestock in pastureland are introduces and developing grass that is able to produce high forages. During the past few years Signal grass (Brachiaria decumbens) and Napier grass (Pennisetum purpureum) have been introduced and planted by farmers in South Sulawesi which utilizes of dry land. On dry land, the water is very limited so that the development of forage often experience water stress. Statement from (Mahdava Rao, K.S., 2006), that water deficits result from low and erratic rain fall, poor soil water storage and when the rate of transpiration exceeds water uptake by plants.

Water stress is considered as one of the abiotic stress factors that restrict the growth and plants production (Keyvan, S., 2010; Sarker, B.C. and M. Hara, 2011; Grand, K., et al., 2014), as well as physiological and biochemical processes of plants (Shinozaki, K. and K. Yamaguchi-Shinozaki, 2007; Anjum, S.A., et al., 2012). Plants respond and adapt to the pressure to defend against abiotic stress (Shinozaki, K. and K. Yamaguchi-Shinozaki, 2007). The reaction of plants to water stress significantly different at various levels depending on the intensity and duration of the stress itself, and also species, cultivars and growth stage (Chaves, M.M., et al, 2002 Sofo, A., et al., 2004; Abbasi, A.R., et al., 2014). Plants adaptation mechanisms to cope with drought stress are with
the cell osmotic regulation. In this mechanism occurs synthesis and accumulation of organic compounds that can degrade osmotic potential resulting in lower water potential in cells without limiting enzyme function and maintain cell turgor (Wang, Z, et al., 1995). Water stress tolerance mechanisms can be attributed to the accumulation of osmotic protectant such as proline and soluble sugars (Fitter, A.H. and R.K M. Hay, 2002; Mohammadkhani, M. and R. Heidari, 2008). Proline can serve as a source of energy, nitrogen and carbohydrates and as osmolit, as a response to the drought (Hong, Z., et al., 2000). Accumulation of soluble carbohydrates increased resistance to the drought on the plant [14]. The role of carbohydrates may rather be as carbon sources for proline synthesis. The parallelism between the accumulation of soluble carbohydrates and proline accumulation may be a consequence of conjugation of the synthetic reactions of carbohydrates and proline with the accelerated hydrolytic reactions of insoluble sugars and protein (Fukutoku, Y. and Y. Yamada, 1982).

Plants that are suffered water stress caused decrease in leaf extension level and closure stomatal to reduce the water consumption through transpiration (Rhodes, D. and Y. Samaras, 1994), because of reduce turgor pressure, cell enlargement is reduce (Fales, S.L and J.O. Fritz, 2007) as well as reduce the magnification and leaf area with the accelerate the rate of leaf senescence (Begg, J.E., 1980). In wheat plants, water stress at tillering stage reduces the number of productive tillers (Nagarajan, S. and S. Nagarajan, 2010).

Although Signal grass and Napier grass has been widely cultivated in dryland South Sulawesi, but very little knowledge about the plant responses to water stress. Therefore, this study aims to study the effect of water stress on growth, proline, total sugars content of the two grass species.

MATERIALS AND METHODS

Plant Materials and treatments:

The material used were two species of Signal grass and Napier grass. The research was arranged based on completely randomized design factorial 2 × 4 with four replications. Two species of grass as the first factor and duration of water stress (W) as the second factors: W0 (Field capicity, FC, no water stress), W1 (50% FC, 1 week), W2 (50%FC, 2 weeks), W3 (50%FC, 3 weeks).

Determination of treatment 50% of field capacity (50% KL) is calculated by using the examples of plants grown in pots, then placed on a bench underneath was given a bucket to accommodate the excess water can not be absorbed by the soil or plants. Plants watered with a certain volume preset (initial volume), then the volume of water that has been accommodated measured (final volume). The difference between the initial volume of the final volume is the amount of water supplied to the plant with 100% field capacity.

\[
50\% \text{ FC} = \frac{(\text{initial volume} - \text{Final volume})}{2}
\]

Plant culture:

Each grass species were planted in pots containing soil weighing 10 kg. In this study used 24 pots, each species requires 12 pots. Each species planted three seedlings in one pot. During the first three weeks all the plants receive enough water (field capacity) and then thinning the plants by eliminating partly the plant by means of leaving a one plants in one pot, then performed the cutting plants with the same high so that to grow a more uniformly. Application of water stress treatment performed after five weeks later growth uniformity and random placement of the pot. Application of treatment lasts up to three weeks, and then the plants were harvested.

Growth and yield:

Growth parameters (plant height and tiller number). Plant height was measured using a ruler. Measurement started from ground level in the pot until to the tip the plant. To determine number of tiller, we counted the numbers of tiller per pot at each replication. Fresh matter weight of harvested plants was recorded and dried at 70°C until reaching a constant weight to determine dry matter weight. Percentage dry matter was as dry matter weight divided by fresh matter weight x 100.

Proline: Proline content of the leaves was measured according to Bates (Bates, L.S., et al., 1993):

Proline was extracted from 0.5 g of leaf sample by grinding in 10 ml of 3% sulphosalicylic acid and the mixture was then centrifuged at 10000 g for 10 min. Two ml of the supernatant was then added into test tubes to which 2 ml of freshly prepared acid-ninhydrin solution and 2 ml of glacial acetic acid were mixed. The tubes were placed in a water bath for 1 h at 90°C and the reaction was terminated in ice-bath. The mixture was then extracted with 5 ml toluene and vortexed for 15 sec. After allowing standing at least for 20 min in darkness at room temperature to
separate the toluene and aqueous phase, the toluene phase was then carefully collected into test tubes and the absorbance of the fraction was read at 520 nm with a Shimadzu UV-1700. The proline content in the sample was expressed as $\mu g$ FW. The standard curve was prepared by employing L-proline.

**Total soluble sugars:**

Total soluble sugars content was measured based on the Anthrone method (Irigoyen, J.J., et al., 1992). 0.5 g of the fresh leaf was crushed in a mortar and 5 ml of 80% hot alcohol was added to it. The mixture was centrifuged at 9000 g for 15 min (6000 rpm). The supernatant obtained was separated into another test tube and 12.5 ml of 80% alcohol was added to it. 1 ml of the solution was taken and 1 ml of 0.2% anthrone was added. The mixture was heated in a waterbath at 100°C for 10 min. The reaction was terminated by incubating the mixture on ice for 5 min. Total soluble sugars content was determined using a spectrophotometer at 620 nm. Calculation of the total soluble sugars was done by creating a standard curve using a standard glucose and was expressed in $\mu g$/g freshweight ($\mu g$ FW).

**Statistical analysis:**

Different experimental treatment (for all parameters) were compared with the Univariate ANOVA followed by DMRT test for comparisons post hoc. A probability level of $P \leq 0.05$ was considered to be statistically significant. The SPSS software package (SPSS Ver. 16.0, SPSS Inc., Chicago, Illinois) was used for all tests.

**RESULTS AND DISCUSSION**

**Effect of water stress on plant growth and dry matter yield:**

Water stress had a significant effect on plant height. Plant height in response to water stress was decreased to 19% compared to well-watered conditions (Table 1). A significant variation in plant height between species was obtained where Napier grass had more plant height than Signal grass species (Table 2). Plants were usually tallest when they were grown without water stress. The differences between species were decreased with the progress of water stress treatments (Fig. 1). The difference in plant height between the species could be due to effects of water stress on vegetative growth. Drought stress can reduce the growth of stems and plant height (Prasad, P.V.V. and S.A. Staggenborg, 2008). Plant height decreased with increasing water stress (Bouazzama, B. et al., 2012; Hussein, M.M. and A.K. Alva, 2014) and related with a decrease in cell enlargement (Bhatt, R.M. and N.K. Srinivasa-Rao, 2005).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Duration of water stress (Weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant height (cm)</td>
<td>FC, (Control) 150.389 149.639 133.501 122.380</td>
</tr>
<tr>
<td></td>
<td>50%FC, (1 week) 149.639 133.501 122.380</td>
</tr>
<tr>
<td></td>
<td>50%PC, (2 weeks) 133.501 122.380</td>
</tr>
<tr>
<td></td>
<td>50%PC, (3 weeks) 122.380</td>
</tr>
<tr>
<td>Number of tillers</td>
<td>50.63^a 49.88^a 46.38^b 44.13^c</td>
</tr>
<tr>
<td>Yield (g dry wt)</td>
<td>276.75^a 275.63^ab 239.00^b 207.00^c</td>
</tr>
<tr>
<td>Proline (ug dry wt)</td>
<td>2.93^a 11.68^b 10.62^c 9.68^d</td>
</tr>
<tr>
<td>Total soluble sugars (g dry wt)</td>
<td>3.08^a 4.98^c 6.12^b 7.02^c</td>
</tr>
</tbody>
</table>

Means in each row followed by the same letter are not significantly different ($P \leq 0.05$).

**Table 2:** Plant height, tiller, yield, proline, total sugars and crude protein contents of Signal grass and Napier grass species

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Signal grass</th>
<th>Napier grass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant height (cm)</td>
<td>130.06^a 147.89^b</td>
<td></td>
</tr>
<tr>
<td>Number of tillers</td>
<td>71.82^a 23.69^b</td>
<td></td>
</tr>
<tr>
<td>Yield (g dry wt)</td>
<td>162.25^a 336.94^c</td>
<td></td>
</tr>
<tr>
<td>Proline (ug dry wt)</td>
<td>11.75^a 5.70^b</td>
<td></td>
</tr>
<tr>
<td>Total soluble sugars (mg dry wt)</td>
<td>41.55^a 64.48^c</td>
<td></td>
</tr>
</tbody>
</table>

Means in each row followed by the same letter are not significantly different ($P \leq 0.05$).

The amount of tiller Signal grass was significantly higher compared to Napier grass (Table 1). Number of tiller were reduced by water-stressed treatments (Fig. 2). The reductions were 1%, 8% and 12%, respectively. Number of tillers been reduced because some tillers died as a result water shortages. Reduction in the number of tillers due to water stress has been reported by several researchers such as Bermudagrass and kleingrass (Bade, D.H.,

The dry matter yield response to water stress of Napier grass and Signal grass is given in Tables 2. Dry matter yields for both grass species were affected by water-stressed. Under water stress conditions both grass species declining productivity. Dry matter yield were reduced by water-stressed treatments (Fig. 3). The losses in yield in response to water stress treatment were 0.5%, 15% and 26%, respectively. A decrease in dry matter yield of the plants grown under drought conditions is largely due to old leaves quickly die, and some tillers die and slow growth due to lack of water. Dry matter yield was sharply decreased as duration of drought stress increased. The results of this study supports results of (Bouazzama, B. et al., 2012; Hussein, M.M. and A.K. Alva, 2014; Kalamian, S., et al., 2006; Jasso de Rodriguez, D., et al., 2002), who also showed decreasing dry matter yield because of drought stress.

**Fig. 1:** Effect of water stress on plant height of Signal grass and Napier grass

**Fig. 2:** Effect of water stress on number tillers of Signal grass and Napier grass
Effect of water stress on proline and total sugars contents:

Proline content is another component of osmotic regulation in plants. Plants species and duration of the stress were found to affect proline contents (Table 1-2). Response of Signal grass and Napier grass to water stress was affected by duration of the stress. Interactions between grass species with water stress showed that the proline content of the Signal grass and Napier grass species increased (Fig. 4). The increase were 415%, 411% and 351% for Signal grass and 177%, 109 and 106% for Napier grass, respectively. Response of Signal grass on water stress more than Napier grass. A decreased in the accumulation of proline on both grass species on duration of water stress treatments for three weeks. This suggests that both of grass species have started adapt to water stress. Statements from (Heuer, B., 2011) that the plants are experiencing recovery after stress will decrease the accumulation of proline. Plants have the ability to accumulate non-toxic compounds such as proline which protects cells damage due to low water potential of cells (Umezawa, T., et al., 2006; Krazsenky, J. and C. Jonak, 2012). The accumulation of proline plays an adaptive role (Verbruggen, N. and C. Hermans, 2008) and the main strategy of plants to avoid detrimental effects (Vendruscolo, A.C.G., et al., 2007) and as well as is one of the key adaptations for successful growth under acute water stress (Akram, N.A., et al., 2007). Effect of water stress treatments increased proline accumulations was previously reported in several species such as Cynodon dactylon and Cenchrus ciliaris (Akram, N.A., et al., 2007), Festuca rubra and Lolium perenne (Bandurska, H. and W. Jozwiak, 2010), wheat (Keyvan, S., 2010; bbasi, A.R., et al., 2014; Pireivatlou, J., et al., 2010; Nazarli, H. and F. Faraji, 2011), Rice (Mostajeran, A. and V. Rahimi-Eichi, 2009), upland rice (Lum, M.S., et al., 2014), and in wild plants proline content increased about 80 mg proline/g of fresh leaves before stress to 3000 mg/g after stress (Kishor, P.B.K., et al., 1995).

Total soluble sugars:

Total soluble sugars in response to water-stress was increased by up to 126% compared to well-watered conditions (Table 1). A significant variation in total soluble sugars between species was obtained where Napier grass had more total soluble sugars than Signal grass species (Table 2). Interactions between grass species with water stress showed that the total sugars contents of the Signal grass and Napier grass species increased (Fig. 5). The increase were 42%, 53% and 70% for Signal grass and 81%, 141 and 181% for Napier grass compared to control, respectively. This suggests that sugars play an important role in Osmotic Adjustment in grasses (Homayouni, H. and V. Khazarian, 2014). Accumulations of soluble carbohydrates increase the resistance to drought in plant (Kameli, A and D.M. Losel, 1993). Effect of water stress treatments increased soluble sugars was previously reported in several plants such as What cultivars (Al-Tabbal, J. A and O. M. Kafawin, 2005; Nazarli, H. and F. Faraji, 2011); Rice cultivars (Mostajeran, A. and V. Rahimi-Eichi, 2009; Zain, N.A.M., et al., 2014); Corn (Homayouni, H. and V. Khazarian, 2014); Potato (Farhad, M.S., et al., 2011); Canola cultivars(Nosrati, S., et al., 2014).
Conclusion:

All water stress treatments decrease to growth and yield for both species but increase of proline and soluble sugars contents. Signal grass species had the highest number of tiller and proline content compared to Napier grass species, while Napier grass species was higher in plant height, yield and soluble sugar contents.

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REFERENCES


