Drought tolerant screening of 20 indonesian sorghum genotypes through leaf water potential measurements under water stress

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Abstract. Understanding drought tolerance status in sorghum (Sorghum bicolor) is very important for the development of sorghum varieties suitable for sub-optimal, drought prone areas in Indonesia. We estimated drought tolerance status of 20 Indonesian sorghum genotypes by observing their leaf water potential under glasshouse condition. Research design was randomized complete block design with 20 sorghum genotypes, 2 water treatments (control and water stress), and 2 replicates. The control plants were irrigated under field capacity, while the water stress treated plants were sown under field capacity followed by drought treatment without watering for one month. Sorghum seeds were cultivated in soil medium containing top soil, organic fertilizer and sand (50:20:30) in four 1x1.2x1 m³ containers. Seeds were sown in soil media pre-treated with tap water under field capacity. Leaf water potential was observed one month after planting by using WP 4 Dew Point. Plant growth performances, including plant height and leaf width were observed. Leaf water potential observation of the 20 sorghum genotypes showed that 2 sorghums genotypes, KLR and KS, had leaf water potential of -2.43 Mpa and -2.455 Mpa respectively, which were categorized as tolerance to water stress. Four sorghum genotypes, Buleleng Empok, UPCA, Kawali and WHP, had leaf water potential of -3.7275 MPa, -3.7650, -3.7700 and -3.7950 Mpa respectively, were classified to be very sensitive to drought stress. The rest of the sorghum genotypes were classified as medium tolerance with leaf water potential between -2.5200 Mpa and 3.6550 Mpa. Although it is a preliminary results and needs to be combined with field experimental data, the results obtained was an important step in determining sorghum genotypes which was best suited to be cultivated in drought prone areas and also to identify sorghum genotypes suitable to be used as drought tolerant trait donor.

1. Introduction
Sorghum (Sorghum bicolor [L] Moench) was known as sugar-producing crop, and tolerance to the drought [1, 2,3]. Selection of the most drought tolerant sorghum is very important in agriculture development concerning to the climate change that affects to the water deficit and environment in some areas of Indonesia [4,5,6]. Research issues about water use efficiency (WUE) of crop, was very relevant to solve this problem [7, 8].

Study on WUE can be conducted by some methods such as instantaneous of WUE, intrinsic of WUE and relationship between dry weight of biomass yield, and gas exchange (photosynthetic and transpiration rate) [9, 10, 11]. These methods are already applied to analyse WUE of 30 Indonesian sorghum accessions in field [12].
Due to WUE was very close relation to water stress, the other physiological parameters can be observed to understand plant response to water stress. Water potential ($\Psi$), together with relative water content, RWC [7, 13, 14, 15] leaf thickness, stem diameter, and visual wilting [16, 17] were the most important methods to indicate water status. $\Psi$ mathematically as the effect of pressure ($\Psi_p$), of solute ($\Psi_s$), and matric ($\Psi_m$). Leaves and roots of herbaceous plants commonly consist of more than 80% water when turgid. As tissue water content decreases, change in $\Psi$, $\Psi_p$, and $\Psi_s$. In the case of fully turgid tissue, the initial decreases in tissue water content cause large decreases in $\Psi$ (becoming more negative). After more water lost and $\Psi_p$ falls to a negligible level (at $\Psi$ more or less -12 to -16 bars for some crop plants). Decreasing $\Psi$ was also followed by decreasing of RWC.

Plant response to water stress can be observed by photosynthetic and transpiration rate, and stomata conductance [16, 18, 19, 20, 21]. When water stress develops rather gradually in the plant, it would affect to the reduction of photosynthetic, metabolic process (probably abscisic acid hormone, and proline begin to accumulate) [22], lower of leave area index, old leave may shed soon, that effect to dry matter yield.

The loss of tissue water may be expected to have the following physical and chemical effects, i.e.: The chemical potential ($\Psi$) or activity of cellular water is reduced, turgor pressure decreases in the cell, small molecules and macromolecules become more concentrated as cell volume is reduced by water loss, spatial relations in plasmalemma, tonoplast, and membranes of organelles are altered by volume changes, and macromolecules may be affected, through removal of water of hydration or through modification of the structure of adjacent water [16, 22, 23]. This study aimed to select 20 sorghum accessions for trait relatives with water stress tolerant.

2. Material and Methods
2.1. Material
A total of 20 sorghum genotypes were obtained from Indonesian Cereals Research Institute, Ujung Pandang, South Sulawesi, Indonesian Agency for Agricultural Research and Development (IAARD).

2.2. Drought treatment
Research was conducted in the Green House of the Research Center for Biotechnology, the Indonesian Institute of Sciences, from October to November 2016. Research design used was randomized complete block design with 20 sorghum genotypes, 2 water treatments (control and water stress), and 2 replications. The control plants were irrigated under field capacity, while the water stress treated plants were sown under field capacity followed by drought treatment without watering for one month. Sorghum seeds were cultivated in soil medium containing top soil, organic fertilizer and sand.
(50:20:30) in four 1 x 1.2 x 1 m³ containers. Seeds were sown in soil media pre-treated with tap water under field capacity. Leaf water potential was observed one month after planting by using WP 4 Dew Point. Plant growth performances, including plant height, stem diameter, number of leaf and total dry weight were observed.

2.3. Water potential observation

Water Potential measures the potential energy of water per unit mass of water in the system [16, 21, 22]. The total water potential of a sample is the sum of four component potentials: gravitational, matric, osmotic, and pressure. Leaf water potential was measured by WP4 that gave readings directly in MegaPascals within five minutes. It measures water potential from 0 to -300 MPa with an accuracy of ±0.1 MPa from 0 to -10 MPa and ±1% from -10 to -300 MPa.

Operational procedure to observe WP4, i.e.: (a) The leaf sample was taken in the morning before evapotranspiration happened. To minimize water loss from the sample, immediately seal the sample with tissues paper a plastic bag for transport; (b) Cut the leaf with 1 cm x 1 cm size; (c) Turn the sample drawer knob to the OPEN/LOAD position and pull the drawer open; (d) Access the sample temperature menu (press lower right button) to watch the temperature difference between the sample and the instrument; (e) Place the sample in a disposable sample cup quickly and close the chamber; (f) Once you have prepared your sample, you are ready to take readings. Turn the sample drawer knob to the READ position to seal the sample cup with the chamber. The instrument will beep once, and the green light will flash once to indicate that the reading cycle has started. In about 40 seconds, the first measurement will be displayed [24].

3. Results and Discussion

3.1. Plant height and leaf width

Plant heights of some sorghum accessions under water stress were reduced (Figure 1, Figure 2). It was also indicated by the positive correlation between plant height of sorghum under water stress and control, with the $y = 0.9797x$, and $R^2 = 0.8444$ (Figure 3). This data was also supported by the positive correlation between leaf width of sorghum under water stress and control, with $y = 0.9915x$, and $R^2 = 0.9694$ (the figure was not displayed). ANOVA statistical analysis indicated that the plant height of Kawali was the shortest, while Jagung Rote was the highest among the 20 sorghum accessions (Table 1).
Figure 1. Sorghum under water stress (a) and sorghum control (b).

Figure 2. Plant height of sorghum under water stress and controls (no water stress) 3 weeks after sowing.
Figure 3. Relationship between plant height under water stress and control

Table 1. Plant height of 20 sorghum accessions under water treatment

| Accessions     | N  | Subset for alpha = 0.05 |
|----------------|----|------------------------|
|                |    | 1         | 2         | 3         | 4         | 5         | 6         |
| Kawali         | 4  | 116,75    |           |           |           |           |           |
| Pahat          | 4  | 132,50    | 132,50    |           |           |           |           |
| Upca Merah     | 4  | 158,50    | 158,50    | 158,50    |           |           |           |
| Super 2        | 4  | 161,75    | 161,75    | 161,75    |           |           |           |
| SMR 1          | 4  | 176,00    | 176,00    | 176,00    | 176,00    |           |           |
| Samurai 2      | 4  | 176,50    | 176,50    | 176,50    | 176,50    |           |           |
| 174.66.1       | 4  | 184,75    | 184,75    | 184,75    | 184,75    | 184,75    |           |
| N.6.1.2        | 4  | 189,50    | 189,50    | 189,50    | 189,50    | 189,50    |           |
| WR             | 4  | 194,50    | 194,50    | 194,50    | 194,50    | 194,50    |           |
| KS             | 4  | 197,75    | 197,75    | 197,75    | 197,75    | 197,75    |           |
| Suri 3         | 4  | 201,50    | 201,50    | 201,50    | 201,50    | 201,50    |           |
| Numbu          | 4  | 209,50    | 209,50    | 209,50    | 209,50    |           |           |
| 172.64.1       | 4  | 217,00    | 217,00    | 217,00    | 217,00    |           |           |
| N.6.1.1        | 4  | 217,50    | 217,50    | 217,50    | 217,50    |           |           |
| WHP            | 4  | 242,00    | 242,00    | 242,00    |           |           |           |
| Buleleng       | 4  | 243,50    | 243,50    | 243,50    |           |           |           |
| KLR            | 4  | 248,25    | 248,25    | 248,25    |           |           |           |
| Super 1        | 4  | 249,50    | 249,50    |           |           |           |           |
| Suri 4         | 4  | 251,00    | 251,00    |           |           |           |           |
| Jagung Rote    | 4  |           |           |           | 274,50    |           |           |
| Significance   |    | 0.056     | 0.059     | 0.112     | 0.052     | 0.075     | 0.074     |

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 4.000.
3.2. Leaf water potential

Research resulted Data and Figure reported in Table 2 and Figure 4.

Table 2. Leaf water potential of 20 sorghum accession under control and water stress (in -Mpa)

| Nu. | Accessions | Water stress, Replication 1 | Water stress, Replication 2 | Control 1 | Control 2 |
|-----|------------|-----------------------------|-----------------------------|------------|------------|
| 1   | UPCA       | -4.58                       | -3.93                       | -3.28      | -3.27      |
| 2   | 172.641    | -3.36                       | -3.59                       | -3.11      | -3.11      |
| 3   | SURI 3     | -4.41                       | -3.53                       | -3.32      | -3.32      |
| 4   | SAMURAI 2  | -3.42                       | -3.99                       | -2.89      | -3.27      |
| 5   | Jogung Rote| -3.28                       | -3.87                       | -2.79      | -2.96      |
| 6   | 174.661    | -3.73                       | -3.21                       | -3.11      | -3.11      |
| 7   | Buleleng   | -4.58                       | -3.72                       | -2.63      | -2.96      |
| 8   | WHP        | -4.06                       | -4.06                       | -3.46      | -3.6       |
| 9   | WR         | -4.7                        | -3.72                       | -3.05      | -3.44      |
| 10  | SUPER 1    | -3                         | -4.07                       | -2.69      | -2.69      |
| 11  | KS         | -3.24                       | -3.24                       | -1.67      | -1.67      |
| 12  | SMR 1      | -3.03                       | -3.88                       | -2.59      | -2.59      |
| 13  | SURI 4     | -2.91                       | -2.87                       | -2.73      | -2.73      |
| 14  | KLR        | -2.69                       | -2.69                       | -2.17      | -2.17      |
| 15  | PAHAT      | -2.91                       | -2.91                       | -2.13      | -2.13      |
| 16  | KAWALI     | -3.23                       | -5.64                       | -2.71      | -3.5       |
| 17  | N. 611     | -3.16                       | -5.37                       | -3.05      | -3.04      |
| 18  | SUPER 2    | -3.71                       | -3.84                       | -2.53      | -2.64      |
| 19  | N. 612     | -2.58                       | -4.34                       | -2.51      | -2.51      |
| 20  | NUMBU      | -2.87                       | -3.93                       | -2.67      | -2.67      |
Figure 4. Water potential of 20 sorghum accessions under water treatment on 3 WAP

Figure 4 illustrates that the more negative value, the more sensitive of sorghum to water stress. It happened in sorghum under water stress treatment, with range of water potential value -4.435 Mpa to -2.69; However, sorghum control have water potential less negative, with range of -3.53 Mpa to -1.67 Mpa.

Table 3. Anova of Sorghums under controle and water stress

| Source                | DF | Adj SS  | Adj MS  | F-Value | P-Value |
|-----------------------|----|---------|---------|---------|---------|
| Water Treatment       | 1  | 13.93   | 13.9278 | 38.59   | 0.000   |
| Error                 | 78 | 28.15   | 0.3609  |         |         |
| Total                 | 79 | 42.08   |         |         |         |

Note: Sorghum under water stress and controle were significantly different with P-Value 0.000 < 0.05.

Table 4. Mean value grouping under water stress and controle in MPa

| Water Treatment | N  | Mean   | Grouping |
|-----------------|----|--------|----------|
| Under Water Stress Tercekam | 40 | 3.646  | A        |
| Controle No Water Sressl      | 40 | 2.8118 | B        |

Note: Grouping information using the Tukey Method, with 95% confidence level.

According to group, the water potential mean value of sorghum under water stress was -3.646 Mpa, and sorghum control was -2.8118 Mpa, and both of them were significantly different (Table 4). Meanwhile, according to ANOVA of 20 sorghums under water treatment, we have 5 subset of
sorghum group. Moreover, sorghum KLR and KS had water potential less negative with value of -2.4300 Mpa and -2.4550 Mpa, respectively, were significantly different with the other accessions. On the other hand, WHP sorghum had water potential more negative with value -3.7950 Mpa, was significantly different with the other accessions (Table 5).

**Table 5.** Water potential mean value of 20 sorghum accessions under water treatment

| Accessions Number | Sorghum Name | N  | 1     | 2     | 3     | 4     | 5     | Subset |
|-------------------|--------------|----|-------|-------|-------|-------|-------|--------|
| 14.00             | KLR          | 4  | 2.4300|       |       |       |       |        |
| 11.00             | KS           | 4  | 2.4550|       |       |       |       |        |
| 15.00             | PAHAT        | 4  | 2.5200| 2.5200|       |       |       |        |
| 13.00             | SURI 4       | 4  | 2.8100| 2.8100| 2.8100|       |       |        |
| 19.00             | N. 612       | 4  | 2.9850| 2.9850| 2.9850| 2.9850|       |        |
| 12.00             | SMR 1        | 4  | 3.0225| 3.0225| 3.0225| 3.0225|       |        |
| 20.00             | NUMBU        | 4  | 3.0350| 3.0350| 3.0350| 3.0350| 3.0350|        |
| 10.00             | SUPER 1      | 4  | 3.1125| 3.1125| 3.1125| 3.1125| 3.1125|        |
| 18.00             | SUPER 2      | 4  | 3.1800| 3.1800| 3.1800| 3.1800| 3.1800|        |
| 5.00              | Jagung Rote  | 4  | 3.2250| 3.2250| 3.2250| 3.2250| 3.2250|        |
| 6.00              | 174.6.61     | 4  | 3.2900| 3.2900| 3.2900|       |       |        |
| 2.00              | 172.6.41     | 4  | 3.2925| 3.2925| 3.2925|       |       |        |
| 4.00              | SAMURAI      | 4  | 3.3925| 3.3925| 3.3925|       |       |        |
|                  | 2            |    |       |       |       |       |       |        |
| 7.00              | Buleleng     | 4  | 3.4725| 3.4725| 3.4725|       |       |        |
| 3.00              | SURI 3       | 4  | 3.6450| 3.6450|       |       |       |        |
| 17.00             | N. 611       | 4  | 3.6550| 3.6550|       |       |       |        |
| 9.00              | WR           | 4  | 3.7275| 3.7275|       |       |       |        |
| 1.00              | UPCA         | 4  | 3.7650| 3.7650|       |       |       |        |
| 16.00             | KAWALI       | 4  | 3.7700| 3.7700|       |       |       |        |
| 8.00              | WHP          | 4  | 3.7950|       |       |       |       |        |
| Sig.              |              |    | 0.055 | 0.069 | 0.095 | 0.052 | 0.056 |        |

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = .214.
Duncan:
a. Uses Harmonic Mean Sample Size = 4.000.
b. Alpha = .05.

Our research is concurrence with the previous research [18]. It was reported that the effects of water stress on water relation and stomatal conductance of apple trees, indicated that during heavy
water stress treatment, observed in predawn, leaf water potential decreased to -1.27 MPa and some leaves turned yellow and shed. In moderate water stress treatment, predawn water potential was reduced to -0.78 MPa, and then decreased again to -0.81 MPa. In non-stressed control treatment, plants were well watered and predawn leaf water potential was maintained higher than -0.38 MPa throughout the experimental period.

Study on relationship between RCW and water potential has been reported by [15]. The research was the effect of water supply to the physiological characteristic and production of Basil (*Ocimum basilicum* L.). The research was comparation of the control plants (water supply to 70% soil water content) and the driest condition (30% soil water content). The research showed that the driest condition effect to the relative water content (RWC) in the plants was reduced by 20%, and water potential was reduced to 45% as compared the control plants.

Research on the effect of water stress to the growth and water use efficiency (WUE) of some wheat cultivars (*Triticum durum*) grown in Saudi Arabia has been reported by Boutraa et al [7]. The three level of soil humidity was Mild (50% of soil field capacity), severe (30% of soil field capacity), control (80% of soil field capacity). The observation used parameters of plant height, leaf area, dry weights of roots, shoots and whole plant, Relative Water Content (RWC) and Water Use Efficiency (WUE). The results indicated that the effect of water deficit on growth depending on the combination of water stress regime and wheat cultivars.

Water stress affects the other physiological character, indicated by [19], reported that the stomatal density correlated to leaf water status, and photosynthetic rate in a perennial grass, *Leymus chinensis*, subjected to different soil moisture contents. In moderate water deficits, it had positive effects on stomatal number, but more severe deficits led to a reduction. The stomatal size obviously decreased with water deficit, and stomatal density was positively correlated with stomatal conductance (gs), net CO2 assimilation rate (An), and water use efficiency (WUE). Leaf water relations, osmotic adjustment, cell membrane stability, epicuticular waxload and growth as affected by increasing water deficits in sorghum [25].

4. Conclusion

Leaf water potential can be used to efficiently screen sorghum genotypes that sensitive to water stress. This result was supported by their growth performance, such as plant height and leaf width. However, this method needs to be combined with the other methods, such as measurement of gas exchange and biomass dry weight.
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