Physiological Characteristics to Indicate Water Use Efficiency and Drought Tolerance of 30 Indonesian Sorghum [Sorghum bicolor (L.) Moench] Accessions

Wahyu Widiyono*, Satya Nugroho

1Research Center for Environmental and Clean Technology, Research Organization of Life Sciences and Environment, National Research and Innovation Agency (BRIN), Kawasan Sains dan Teknologi B.J. Habibie, Gd. 820 Serpong, Tangerang Selatan, Indonesia
2Research Center for Genetic Engineering, Research Organization of Life Sciences and Environment, National Research and Innovation Agency (BRIN), Kawasan Sains dan Teknologi Soekarno, Cibinong, Bogor, Indonesia

1. Introduction

Sorghum (Sorghum bicolor [L.] Moench), a crop indigenous to Africa, is widely cultivated in drought-prone areas around the world (Food Security Department-National Resources Institute 1999). However, it would require improved varieties which are tolerant to water stress (Mathur et al. 2017; Steduto et al. 2012). In Indonesia, there are at least 15 sorghum varieties and 32 germplasm from Sorghum (Mukkun et al. 2018; Sumarno et al. 2013). Water is vital in photosynthesis and biomass production (Leakey et al. 2006). Water stress is the major environmental stress (Boutraa et al. 2010). When plants do not receive sufficient water, they are subjected to water deficits (Bray 2001).

The plant response to water stress includes reducing water potential, relative water content and stomatal conductance (Hsiao 1973). The ability of plants to meet the need for water depends on their water balance mechanism (Blum 2011; Comic and Massaci 1996). The variation in soil moisture significantly affects all sorghum traits (Sher et al. 2013). Food production and water use are closely related (Fracasso et al. 2017; Steduto et al. 2012). FAO informed that the relative yield reduction is related to the relative reduction in water use (Steduto et al. 2012).

Kapanigowda et al. (2012) mentioned that it was important to increase and improve crop water use efficiency (WUE). Different approaches can observe WUE measurement at the levels of leaf canopy (Medranoa et al. 2015; Steduto et al. 1997) and crop or biomass (Curt et al. 1995). Observation using portable equipment for measuring leaf gas exchange...
rates is convenient (Cheeseman and Lexa 1996; Long and Hällgren 1993). However, when the leaf level measurements are compared with the integrals or whole-plant estimates of WUE, the two methods sometimes do not fit (Medranoa et al. 2015). Research on crop ability to optimize water use and biomass production has been reported (Curt et al. 1995). WUEs of Indonesian sorghum accessions related to observations of gas exchange (photosynthetic and transpiration rates, including stomatal conductance (GSW)). The leaf area index (LAI) and biomass yield have not been described. In this research, the physiological characteristic of 30 Indonesian sorghum accessions related to their WUEs and drought tolerance at leaf and plant levels were measured by observing single leaf gas exchange parameters, LAI, and biomass yield. The information obtained was used to estimate their WUE and drought-tolerant status.

2. Materials and Methods

Thirty sorghum accessions used in this experiment were obtained from the Cereal Research Center in Maros, South Sulawesi, Indonesia (Table 1). Culturing was performed in the field trial at the Cibinong Science Center, National Research and Innovation Agency (BRIN), using a randomized block design with 3 replication plots (2 m x 3 m) containing 40 plants (70 cm x 25 cm spacing) per plot. Sorghum cultivation was done using the standard protocols. Physiological characters observation were conducted in the experimental field from May to August 2017. Photosynthetic rate (A), transpiration rate (E), and Leaf Area Index (LAI) were measured 2 weeks before harvesting on the third leaves from the top of 2 plants per plot with 3 replicates. Biomass production was measured after harvest.

Gas exchanges, including photosynthetic rate (A), transpiration rate (E), and stomatal conductance to water vapor (GSW), were measured using the LI-COR Li-6800 Portable Photosynthesis System (LI-COR, USA) under the photosynthetic photon flux density (PPFD) or Qinleaf from the ambient sunlight as the light source. The measurements were made from the 1st to 2nd of August 2017 between 10:57:00 a.m. to 12:49:04 p.m.

The settings of the readings were as follows: flow rate: 500 µmol s⁻¹, RH: 50 %, CO₂ reference: 400-500 µmol⁻¹, fan speed: 8,000 rpm, control temperature/ TxCh: 27-28°C, leaf constant: 3 cm x 3 cm, and PPFD from natural sunlight. The Li-3000 C Leaf Area Meter (LI-COR, USA) was used to observe LAI (leaf area, length, and width). The data was analyzed to understand the relationship between PPFD and A, PPFD and E, and biomass yield (BDW) and A, and BDW and E. DMRT was used to analyze A, instantaneous WUE, intrinsic WUE, BDW:A and BDW:E.

3. Results

3.1. Effect of PPFD and Stomatal Conductance (GSW) on Photosynthetic (A) and Transpiration (E) Rates

To understand the effect of PPFD and stomatal conductance (GSW) on photosynthetic (A) and transpiration (E) rates, we reported observation data in Table 2, and the average, maximum, and minimum transpiration rate (E), photosynthetic rate (A), stomatal conductance (GSW), and PPFD of 30 sorghum accessions in Table 3. We also reported the relationship between PPFD and photosynthetic rate, the relationship of PPFD with transpiration rate, the relationship between photosynthetic and transpiration rates, and between transpiration rate and stomatal conductance available in Table 4.

Table 1. Sorghum accessions used in this experiment

| Accessions | Accessions | Accessions |
|------------|------------|------------|
| Suri 3*    | Buleleng Empok | N6.1.2     |
| KLR        | Super 2-300 | WHP        |
| Kawali *   | 15105 D    | 172.64.1.1 |
| 1503 A     | KS         | WR         |
| Suri 4*    | UPCA*      | N 6.1.1    |
| Samurai 1* | Numbu*     | Super 2B*  |
| 181.73.1.1 | WHP 300    | 174.6.6.11 |
| JP         | 4183 A     | Super 1*   |
| 1090 A     | Sorgum Malai Mekar | Jagung Rote |
| Super 2A   | 1115 C     | Pahat*     |

*) Released varieties
| Sorghum accessions | E (mmol m⁻² s⁻¹) | A (µmol m⁻² s⁻¹) | GSW (mmol m⁻² s⁻¹) | PPFD (µmol m⁻² s⁻¹) |
|-------------------|------------------|------------------|---------------------|---------------------|
| SURI 3            | 1.55             | 27.4             | 0.08                | 990.22              |
| KLR               | 3.14             | 45.13            | 0.19                | 1806.33             |
| KAWALI            | 2.56             | 30.68            | 0.13                | 1698.82             |
| 1503 A            | 3.09             | 37.56            | 0.21                | 1648.77             |
| SURI 4            | 2.4              | 30.85            | 0.18                | 1457.2              |
| SAMURAI 1         | 2.98             | 39.1             | 0.21                | 1830.98             |
| 181.73.1.1        | 0.76             | 23.62            | 0.04                | 1410.91             |
| JP                | 2.48             | 20.48            | 0.17                | 1474.96             |
| 1090 A            | 2.25             | 34.96            | 0.14                | 1779.11             |
| SUPER 2           | 3.04             | 36.45            | 0.18                | 1748.6              |
| BULELENG EMPOK    | 2.66             | 29.58            | 0.18                | 1595.75             |
| SUPER 2-300       | 3.1              | 26.65            | 0.14                | 1759.11             |
| 15105 D           | 3.06             | 43.44            | 0.16                | 1646.56             |
| KS                | 3.38             | 49.29            | 0.2                 | 1722.91             |
| UPCA              | 3.11             | 36.19            | 0.17                | 1687.1              |
| NUMBU             | 3.23             | 36.15            | 0.17                | 1713.84             |
| WHP 300           | 1.49             | 21.92            | 0.06                | 1830.39             |
| 4183 A            | 3.47             | 47.73            | 0.2                 | 1746.61             |
| SORGUM MALAI M    | 3.38             | 40.95            | 0.19                | 1732.9              |
| 1115 C            | 2.46             | 20.67            | 0.13                | 1464.43             |
| N6.1.2.           | 3.24             | 43.89            | 0.17                | 1777.25             |
| WHP               | 2.54             | 29.95            | 0.14                | 1859.04             |
| 172.64.1.1        | 320              | 37.49            | 0.22                | 1884.48             |
| WR                | 2.13             | 25.92            | 0.11                | 1927.32             |
| N 6.1.1           | 3.3              | 41.99            | 0.18                | 1729.24             |
| SUPER 2           | 3.39             | 38.72            | 0.21                | 1695.31             |
| 174.6.6.1.1       | 2.94             | 32.78            | 0.15                | 1707.93             |
| SUPER 1           | 2.6              | 18.85            | 0.14                | 1133.3              |
| JAGUNG ROTE       | 2.65             | 30.81            | 0.17                | 1764.14             |
| PAHAT             | 2.96             | 47.72            | 0.18                | 1775.73             |
| SURI 3            | 2.47             | 24.47            | 0.14                | 1442.07             |
| KLR               | 3.08             | 23               | 0.25                | 1160.42             |
| KAWALI            | 2.53             | 33.95            | 0.16                | 1608.23             |
| 1503 A            | 1.62             | 11.89            | 0.12                | 350.8               |
| SURI 4            | 2.79             | 32.13            | 0.18                | 1653.85             |
| SAMURAI 1         | 1.73             | 22.39            | 0.1                 | 1686.32             |
| 181.73.1.1        | 1.3              | 27.39            | 0.09                | 736.75              |
| JP                | 1.85             | 27.18            | 0.12                | 1239.94             |
| 1090 A            | 1.64             | 28.71            | 0.11                | 697.36              |
| SUPER 2           | 3.13             | 27.08            | 0.12                | 1434.56             |
| BULELENG EMPOK    | 3.14             | 38.2             | 0.22                | 1541.16             |
| SUPER 2-300       | 3.13             | 30.48            | 0.21                | 1301.07             |
| 15105 D           | 2.82             | 27.75            | 0.16                | 1494.37             |
| KS                | 3.06             | 30.53            | 0.17                | 1598.89             |
| UPCA              | 3.7              | 37.62            | 0.25                | 1637.05             |
| NUMBU             | 2.98             | 28.27            | 0.18                | 1725.29             |
| WHP 300           | 4.33             | 25.59            | 0.27                | 1422.4              |
| 4183 A            | 3.4              | 20.34            | 0.21                | 1271.59             |
| SORGUM MALAI M    | 4.28             | 26.72            | 0.27                | 1476.21             |
| 1115 C            | 4.46             | 25.1             | 0.28                | 1590.25             |
| N6.1.2.           | 4.92             | 32.06            | 0.29                | 1680.12             |
| WHP               | 3.68             | 24.89            | 0.21                | 705.17              |
| 172.64.1.1        | 4.65             | 23.09            | 0.35                | 957.52              |
| WR                | 3.46             | 23.59            | 0.19                | 884.62              |
| N 6.1.1           | 2.87             | 19.12            | 0.12                | 1346.54             |
| SUPER 2           | 2.67             | 28.8             | 0.15                | 1522.24             |
Table 2. Continued

| Sorghum accessions | E (mmol m$^{-2}$ s$^{-1}$) | A (µmol m$^{-2}$ s$^{-1}$) | GSW (mmol m$^{-2}$ s$^{-1}$) | PPFD (µmol m$^{-2}$ s$^{-1}$) |
|--------------------|-----------------------------|----------------------------|----------------------------|-------------------------------|
| 174.6.6.1.1.       | 4.05                        | 30.01                      | 0.31                       | 1295.46                       |
| SUPER 1             | 2.57                        | 22.45                      | 0.14                       | 1348.87                       |
| JAGUNG ROTE        | 4.5                         | 34.15                      | 0.35                       | 1426.44                       |
| PAHAT               | 3.81                        | 33.92                      | 0.25                       | 1364.17                       |
| SURI 3              | 1.08                        | 20.03                      | 0.08                       | 509.58                        |
| KLR                | 1.51                        | 15.38                      | 0.1                        | 479.98                        |
| KAWALI              | 1.79                        | 17.61                      | 0.14                       | 478.28                        |
| 1503 A              | 2.42                        | 13.95                      | 0.23                       | 421.66                        |
| SURI 4              | 0.7                         | 13.82                      | 0.05                       | 424.53                        |
| SAMURAI 1           | 0.29                        | 16.22                      | 0.02                       | 515.14                        |
| 181.73.1.1          | 0.51                        | 14.09                      | 0.03                       | 512.14                        |
| JP                 | 1.08                        | 15.5                       | 0.08                       | 420.72                        |
| 1090 A              | 0.9                         | 15.86                      | 0.06                       | 539.83                        |
| SUPER 2             | 1.41                        | 24.09                      | 0.09                       | 843.95                        |
| BULELENG EMPOK      | 1.68                        | 26.69                      | 0.11                       | 1223.4                        |
| SUPER 2-300         | 2.32                        | 24.55                      | 0.16                       | 787.53                        |
| 15105 D             | 1.46                        | 18.08                      | 0.11                       | 596.43                        |
| KS                 | 2.06                        | 23.84                      | 0.17                       | 629.27                        |
| UPCA               | 1.84                        | 22.18                      | 0.16                       | 492.11                        |
| NUMBU              | 1.83                        | 20.24                      | 0.16                       | 638.08                        |
| WHP 300            | 2.21                        | 19.34                      | 0.19                       | 724.04                        |
| 4183 A             | 2.98                        | 30.17                      | 0.17                       | 1482.56                       |
| SORGUM MALAI M      | 3.68                        | 38.79                      | 0.24                       | 1449.27                       |
| 1115 C             | 2.3                         | 25.75                      | 0.12                       | 1302.34                       |
| N6.1.2.             | 2.51                        | 23.3                       | 0.21                       | 513.23                        |
| WHP                | 1.68                        | 13.83                      | 0.14                       | 436.12                        |
| 172.64.1.1         | 1.34                        | 14.48                      | 0.11                       | 399.25                        |
| WR                 | 1.04                        | 15.2                       | 0.08                       | 407.82                        |
| N 6.1.1            | 1.42                        | 18.8                       | 0.11                       | 457.81                        |
| SUPER 2            | 1.11                        | 16.79                      | 0.1                        | 441.91                        |
| 174.6.6.1.1        | 1.14                        | 15.48                      | 0.1                        | 412.83                        |
| SUPER 1            | 1.22                        | 15.41                      | 0.11                       | 452.19                        |
| JAGUNG ROTE       | 0.86                        | 13.99                      | 0.07                       | 375.49                        |
| PAHAT              | 0.25                        | 10.45                      | 0.02                       | 239.76                        |

Table 3. Average, maximum, and minimum transpiration rate (E), photosynthetic rate (A), stomatal conductance (GSW), and PPFD of 30 sorghum accessions

| Sorghum accessions | E (mmol m$^{-2}$ s$^{-1}$) | A (µmol m$^{-2}$ s$^{-1}$) | GWS (mol m$^{-2}$ s$^{-1}$) | PPFD (µmol m$^{-2}$ s$^{-1}$) |
|--------------------|-----------------------------|----------------------------|----------------------------|-------------------------------|
| Max                | 4.92                        | 49.29                      | 0.35                       | 1927.32                       |
| Min                | 0.25                        | 10.45                      | 0.02                       | 239.76                        |
| Average            | 2.47                        | 26.91                      | 0.16                       | 1202.29                       |

Table 4. Relationship between photosynthetic rate, transpiration rate, PPFD, and stomatal conductance parameters

| Parameters                      | Relationship   | R²  |
|--------------------------------|----------------|-----|
| Relationship between PPFD and photosynthetic rate | y = 0.021x | 0.94 |
| Relationship between PPFD and transpiration rate | y = 9 * 10^-5x | 0.91 |
| Relationship between photosynthetic and transpiration rates | y = 10082x | 0.91 |
| Relationship between transpiration rate and stomatal conductance | y = 0.0153x | 0.98 |
3.2. Effect of Leaf Area Index (LAI) on PPFD and Photosynthetic Rate (A)

To understand the effect of leaf area index (LAI) on PPFD and photosynthetic rate (A), we reported the statistical analysis of 30 sorghum accessions LAI (Table 5), a histogram of the LAI components (Figure 1), and average data on transpiration rate (E), assimilation rate (A), Biomass Dry Weight, Biomass Dry Weight: A, and Biomass Dry Weight: E (Table 6). Moreover, the relationship of LAI to PPFD and photosynthetic rate and the relationship of LAI to PPFD and transpiration rate are available in Table 7.

3.3. Instantaneous, Intrinsic, and Biomass Relationship to WUE

To analyze instantaneous, intrinsic, and biomass relation to WUE, we used data of the average transpiration rate (E), and assimilation rate (A). Biomass Dry Weight to calculate instantaneous, intrinsic, and biomass relation to WUE (Table 6). The relationship between intrinsic and instantaneous WUE and biomass dry weight to photosynthetic and transpiration rates (Table 8). The DMRT of instantaneous WUE, intrinsic WUE, Biomass Dry Weight: A, and Biomass Dry Weight: E and WUE significance scoring of 30 sorghum accessions are available in Tables 9 and 10.

4. Discussion

The ambient light was used as a light source to determine photosynthesis in actual sunlight conditions in the field. Similar studies on photosynthetic rates using ambient light under the sun have also been conducted earlier. Tsuji et al. (2003) conducted the experiments on sorghums on clear sunny days between 10.00 and 15.00. Du et al. (2020) researched to investigate the influences of sampling time on rice photosynthesis. They found that the tillers sampled in the early morning had the highest A and stomatal conductance to vapor (GSW). Moreover, the variabilities of A and GSW were lower in the tillers sampled early morning and at the end of the day (6:00 and 18:00) than in that sampled midday. Tatsumi et al. (2020) conducted a study of ambient light sources to determine the photosynthetic response of rice plants under conditions of continuously fluctuating light intensity. Lee et al. (2021) conducted a study using LICOR-6800 to observe the parameters of physiological foliage parameters and the photosynthesis rate of Acacia.

Our observation of gas exchange of a single leaf of Sorghum was more likely similar to previous observations by Bruns (2016). Bruns stated that different intensities of PPFD of 150, 650, 1,150,
Figure 1. The leaf area index (LAI) consisted of leaf number (A), total leaf area (B), average leaf area (C), average leaf length (D), average leaf width (E), and average maximum width.
## Table 6. Average transpiration rate (E), assimilation rate (A), biomass dry weight, biomass dry weight/A, and biomass dry weight/E of 30 sorghum accessions

| Sorghum genotypes | E (mmol m⁻² s⁻¹) | A (µmol m⁻² s⁻¹) | Biomass dry weight (g plant⁻¹) | Biomass dry weight/A | Biomass dry weight/E |
|-------------------|-----------------|-----------------|--------------------------------|---------------------|---------------------|
| SURI              | 1.55            | 27.40           | 22.40                          | 0.82                | 14.42               |
| KLR               | 3.14            | 45.13           | 32.48                          | 0.72                | 10.33               |
| KAWAJI            | 2.56            | 30.68           | 32.76                          | 1.07                | 12.82               |
| 1503 A            | 3.09            | 37.56           | 22.62                          | 0.60                | 7.31                |
| SURI 4            | 2.40            | 30.85           | 24.92                          | 0.81                | 10.38               |
| SAMURAI I         | 2.98            | 39.10           | 14.56                          | 0.37                | 4.88                |
| 181.73.1.1        | 0.76            | 23.62           | 35.56                          | 1.51                | 47.02               |
| JP                | 2.48            | 20.48           | 67.48                          | 3.29                | 27.17               |
| 1090 A            | 2.25            | 34.96           | 15.96                          | 0.46                | 7.10                |
| SUPER 2           | 3.04            | 36.45           | 72.80                          | 2.00                | 23.94               |
| BULELENG EMPOK    | 2.66            | 29.58           | 33.60                          | 1.14                | 12.65               |
| SUPER 2-300       | 3.10            | 26.65           | 39.20                          | 1.47                | 12.63               |
| 15105 D           | 3.06            | 43.44           | 20.16                          | 0.46                | 6.58                |
| KS                | 3.38            | 49.29           | 34.44                          | 0.70                | 10.19               |
| UPCARI            | 3.11            | 36.19           | 56.00                          | 1.55                | 18.01               |
| NUMBU             | 3.23            | 36.15           | 71.68                          | 1.98                | 22.17               |
| WHP 300           | 1.49            | 21.92           | 95.20                          | 4.34                | 64.11               |
| 4183 A            | 3.47            | 47.73           | 22.96                          | 0.48                | 6.62                |
| SORGUM MALAI MEKAR| 3.38            | 40.95           | 13.44                          | 0.33                | 3.97                |
| 1115 C            | 2.46            | 20.67           | 22.96                          | 1.11                | 9.35                |
| N6.12             | 3.30            | 41.99           | 70.84                          | 1.69                | 21.44               |
| WHP               | 2.54            | 29.95           | 56.00                          | 1.87                | 22.04               |
| 172.64.1.1        | 3.20            | 37.49           | 42.00                          | 1.12                | 13.12               |
| WR                | 2.13            | 25.92           | 70.84                          | 2.73                | 33.28               |
| N6.11             | 3.30            | 41.99           | 70.84                          | 1.69                | 21.44               |
| SUPER 2           | 3.39            | 38.72           | 25.87                          | 0.67                | 7.62                |
| 174.6.6.1.1.      | 2.94            | 32.78           | 61.60                          | 1.88                | 20.96               |
| SUPER 1           | 2.60            | 18.85           | 28.00                          | 1.49                | 10.79               |
| JAGUNG ROTE       | 2.65            | 30.81           | 66.36                          | 2.15                | 25.01               |
| PAHAT             | 2.96            | 47.72           | 59.53                          | 1.25                | 20.09               |

## Table 7. Relationship between LAI*PPFD to LAI* A, and the relationship between LAI*PPFD to LAI* E of 30 sorghum accessions

| Relationship | y   | R²  |
|--------------|-----|-----|
| LAI*PPFD to LAI* A | y = 21.88x + 1E + 06 | 0.57 |
| LAI*PPFD to LAI* E | y = 2E - 06x + 0.37 | 0.68 |

## Table 8. Relationship between A/GSW to A/E, and the relationship between BDW/A to BDW/E of 30 sorghum accessions

| Relationship | y   | R²  |
|--------------|-----|-----|
| A/GSW to A/E | y = 0.02x - 6.93 | 0.95 |
| BDW/A to BDW/E | y = 12183x + 628.97 | 0.74 |

## Table 9. DMRT of instantaneous WUE, intrinsic WUE, Biomass dry weight/A, and biomass dry weight/E

| a. Instantaneous WUE (A/E) | b. Intrinsic WUE (A/GSW) | c. BDW/A | d. BDW/E |
|----------------------------|--------------------------|----------|----------|
| Sorg. No.                  | Mean                     | Sorg. No. | Mean     | Sorg. No. | Mean     | Sorg. No. | Mean     |
| 20                         | 7.54a                    | 11        | 105.23a   | 24        | 1.19a     | 24        | 16.06a    |
| 11                         | 7.60a                    | 20        | 117.37a   | 25        | 1.38a     | 3         | 17.22a    |
| 16                         | 8.30a                    | 16        | 126.41a   | 9         | 1.52a     | 9         | 17.36a    |
| 12                         | 8.71a                    | 29        | 140.84a   | 23        | 1.55a     | 23        | 18.36a    |
| 4                          | 9.65a                    | 12        | 143.26a   | 5         | 1.56a     | 11        | 18.82a    |
| 28                         | 10.27a                   | 4         | 145.75a   | 3         | 1.56a     | 11        | 18.82a    |
| 29                         | 10.60a                   | 28        | 151.76a   | 2         | 1.79a     | 2         | 20.05a    |
| 27                         | 10.67a                   | 8         | 164.10a   | 27        | 1.89a     | 25        | 20.33a    |
| 14                         | 10.86a                   | 14        | 165.65a   | 10        | 2.02b     | 27        | 20.48a    |
| 3                          | 10.90a                   | 3         | 166.13a   | 29        | 2.07b     | 16        | 21.88a    |
| 8                          | 11.07a                   | 5         | 169.98a   | 30        | 2.12b     | 30        | 22.47a    |
| 30                         | 11.39a                   | 23        | 172.92a   | 14        | 2.12b     | 14        | 22.80a    |
| 2                          | 11.66b                   | 27        | 173.47a   | 13        | 2.26b     | 4         | 22.83a    |
Table 9. Continued

| Sorg. No. | a. Instantaneous WUE (A/E) | b. Intrinsic WUE (A/GSW) | c. BDW/A | d. BDW/E |
|-----------|---------------------------|---------------------------|----------|----------|
|           | Mean                      | Mean                      | Mean     | Mean     |
| 23        | 11.66<sup>a</sup>         | 174.32<sup>a</sup>        | 4        | 2.39<sup>a</sup> | 10        | 23.49<sup>a</sup> |
| 5         | 11.85<sup>a</sup>         | 177.16<sup>a</sup>        | 28       | 2.47<sup>b</sup> | 29        | 23.63<sup>a</sup> |
| 9         | 11.87<sup>a</sup>         | 184.89<sup>a</sup>        | 1        | 2.49<sup>b</sup> | 28        | 24.79<sup>a</sup> |
| 10        | 11.99<sup>a</sup>         | 199.05<sup>b</sup>        | 26       | 2.59<sup>b</sup> | 13        | 30.06<sup>b</sup> |
| 15        | 12.73<sup>a</sup>         | 199.42<sup>b</sup>        | 11       | 2.67<sup>b</sup> | 20        | 31.37<sup>b</sup> |
| 18        | 12.89<sup>a</sup>         | 199.66<sup>b</sup>        | 16       | 2.75<sup>b</sup> | 12        | 32.90<sup>b</sup> |
| 13        | 12.91<sup>a</sup>         | 208.15<sup>b</sup>        | 15       | 2.80<sup>b</sup> | 15        | 41.26<sup>b</sup> |
| 19        | 13.04<sup>a</sup>         | 212.86<sup>b</sup>        | 6        | 3.14<sup>b</sup> | 19        | 44.66<sup>b</sup> |
| 24        | 13.14<sup>a</sup>         | 216.10<sup>b</sup>        | 21       | 3.19<sup>b</sup> | 21        | 45.37<sup>b</sup> |
| 21        | 13.88<sup>a</sup>         | 216.97<sup>b</sup>        | 7        | 3.24<sup>b</sup> | 7         | 46.20<sup>b</sup> |
| 17        | 14.15<sup>a</sup>         | 238.42<sup>b</sup>        | 19       | 3.35<sup>b</sup> | 1         | 53.46<sup>b</sup> |
| 25        | 14.63<sup>a</sup>         | 243.53<sup>b</sup>        | 22       | 3.38<sup>b</sup> | 17        | 54.42<sup>b</sup> |
| 6         | 16.38<sup>a</sup>         | 250.35<sup>b</sup>        | 12       | 3.51<sup>b</sup> | 8         | 55.28<sup>b</sup> |
| 7         | 17.06<sup>a</sup>         | 275.59<sup>b</sup>        | 17       | 3.80<sup>b</sup> | 6         | 55.63<sup>b</sup> |
| 1         | 18.26<sup>a</sup>         | 301.09<sup>b</sup>        | 20       | 4.12<sup>b</sup> | 18        | 67.09<sup>b</sup> |
| 26        | 19.86<sup>a</sup>         | 304.37<sup>b</sup>        | 8        | 4.45<sup>b</sup> | 26        | 79.86<sup>b</sup> |
| 22        | 25.72<sup>c</sup>         | 416.64<sup>c</sup>        | 18       | 5.18<sup>c</sup> | 22        | 119.20<sup>c</sup> |

Table 10. WUE significance scoring of 30 sorghum accessions

| Accessions instantaneous | b. WUE intrinsic (A/E) | c. WUE (A/GSW) | a. BDW/A | d. DW/E | Sig. | Non-Sig. |
|--------------------------|------------------------|----------------|----------|----------|------|----------|
| SURI 3                   | s                      | s              | s        | 4        | 0    |          |
| KLR                      | s                      | ns             | ns       | s        | 2    | 2        |
| Kawali                   | ns                     | ns             | ns       | 0        | 4    |          |
| 1503 A                   | ns                     | ns             | s        | ns       | 1    | 3        |
| Suri 4                   | s                      | ns             | s        | 2        | 2    |          |
| Samurai 1                | s                      | s              | s        | 4        | 0    |          |
| 181.73.1.1               | s                      | s              | s        | 4        | 0    |          |
| JP                       | ns                     | ns             | s        | 2        | 2    |          |
| 1090 A                   | s                      | s              | ns       | ns       | 2    | 2        |
| Super 2A                 | s                      | ns             | s        | ns       | 2    | 2        |
| Buleleng E               | ns                     | ns             | s        | ns       | 1    | 3        |
| Super 2-300              | ns                     | ns             | ns       | s        | 1    | 3        |
| 15105 D                  | s                      | s              | s        | 3        | 1    |          |
| KS                       | ns                     | ns             | s        | ns       | 1    | 3        |
| UPCA                     | s                      | s              | s        | 4        | 0    |          |
| Numbu                    | ns                     | ns             | s        | ns       | 1    | 3        |
| WHP 300                  | s                      | s              | s        | 4        | 0    |          |
| 4183 A*                  | s                      | s              | vs       | s        | 4    | 0        |
| Sorgum M.                | s                      | s              | s        | 4        | 0    |          |
| 1115 C                   | ns                     | ns             | s        | 2        | 2    |          |
| N6.1.2.                  | s                      | s              | s        | 4        | 0    |          |
| WHP*                     | vs                     | vs             | s        | 4        | 0    |          |
| 172.64.1.1               | s                      | ns             | ns       | 1        | 3    |          |
| WR                       | s                      | s              | s        | 3        | 1    |          |
| N 6.1.1                  | s                      | s              | ns       | 2        | 2    |          |
| Super 2B                 | s                      | s              | s        | 4        | 0    |          |
| 1746.6.1.1.              | ns                     | ns             | ns       | 0        | 4    |          |
| Super 1                  | ns                     | ns             | s        | 1        | 3    |          |
| Jagung Rote              | ns                     | ns             | ns       | 1        | 3    |          |
| Pahat                    | s                      | ns             | s        | 2        | 2    |          |

<sup>v</sup> = very significant (most efficient), <sup>s</sup> = significant (efficient), <sup>ns</sup> = not significant, based on DMRT analyses
1,650, and 2,150 µmol m⁻² s⁻¹ produced significantly different effects on A, E, and GSW of Sorghum during the anthesis and milk to the early dough. In our observation, the A in the reproductive growth stage of anthesis declined fast when PPFD decreased. Previously, Subramanian et al. (1993) reported a similar phenomenon in rainfed grain sorghum in the Asian subcontinent of India; when PPFD exceeded 1,300 µmol m⁻² s⁻¹, they observed a decrease in A, both before anthesis and during grain filling. In their observation, LAI and GSW were also decreased with increasing PPFD.

Our data also showed a strong relationship between photosynthetic and transpiration rates with R² = 0.91, and E was highly correlated with GSW (R² = 0.98) (Table 4). So then, we conclude that an increase in stomatal conductance was followed by an increase in transpiration rate. This result was similar to the observation by Bruns (2016), who observed a higher transpiration rate during higher stomatal conductance. He also observed that during the reproductive growth stage of anthesis, the decline in GSW, A, and E was positively correlated to the decline of PPFD.

We then further analyzed the LAI and its histogram, which indicated a normal distribution frequency. However, each observed parameter showed different distribution characteristics (Figure 1). In addition, LAI, leaf area duration (LAD), known to be related to light absorption, and A are important to produce biomass dry weight (BDW) (Lawlor 1995). Although PPFD had a low correlation to A, LAI directly affected Q-leaf in from PPFD and A, and Q-leaf in from PPFD and E. This assumption was supported by the high correlation between LAI*A and LAI*PPFD (R² = 0.57), and LAI*E (R² = 0.68) (Table 7). Based on that, it can be assumed that LAI is essential in determining A and yield productivity. The capacity of each leaf in one plant is different, depending on its leaf area and position in the plant.

We could cluster the sorghum accessions based on WUE analysis (Table 9, 10). Group I consisted of sorghum accessions most efficient in water use based on four WUE measurement methods (A/E, A/GSW, BDW/A, and BDW/E) or based on its appearance 3 times under very significant categories (instantaneous, intrinsic, and BDW/E) and 1 time under the significant category (BDW/A). Group I include Suri 1, Samurai 181.73.1.1, UPCA, WHP, 4183 A, Sorghum Malai Mekar, N6.1.2, WHP, and Super 2B.

Group II is clustered based on their significant scores under three WUE measurement methods (A/E, A/GSW, and BDW/A), which include
15105 D and WR. Group II is considered less efficient in WUE due to BDW/E measurement results, which were not significant with low BDW and high transpiration rate (E), which indicated inefficiency in water use.

Based on the significant scores under two WUE measurement methods (A/E and BDW/A), JP, 1090 A, Super 2A, 1115 C, N6.1.1, and Pahat are listed as Group III. Group III is considered less efficient in water usage based on two not significant WUE measurement results (A/GSW and BDW/E), due to their high stomatal conductance (GSW); therefore, this group shows high transpiration rates (E).

Then, Group IV is clustered based on the significant score from one WUE measurement method (BDW/A), including KLR, 1503A, Suri 4, Buleleng Empok, Super 2-300, KS, Numbu, 172.64.1.1, Super 1, Jagung Rote and considered less efficient in water use. Although the member of this group exhibited high photosynthetic rate (A) and biomass dry weight (BDW), they, however, also showed high stomatal conductance (GSW) and high transpiration rate (E).

Lastly, Group V consists of sorghum accessions not efficient in water use based on their nonsignificant score under 4 WUE measurement methods. The last group consisted of Kawali and 174.6.6.1.1.

The results of WUE measurements using 4 different approaches indicated that for some accessions, measurement of WUE using single leaf gas exchange represented whole plant WUE measurements. In the accessions to a case of the Sorghum categorized as Group I, the most efficient water usage, it can be assumed that WUE measurements can be done by measuring the instantaneous or intrinsic WUE without considering other measurements. Since BDW is the product of carbon assimilation, A/E can be considered identical to BDW/E. A/E can also be considered identical to A/GSW because the stomatal opening and closing influence the transpiration rate (E). During water deficit, stomata are close to avoiding transpiration; therefore, E is small. Our data suggest that BDW/A is highly correlated with BDW/E (R^2 = 0.74), while A/GSW is highly correlated with A/E (R^2 = 0.95). So, BDW/A is highly correlated with BDW/E, while it is also understood that the transpiration rate (E) depends on the stomatal conductance (GSW).

Based on the above findings, it can be suggested that single leaf-based WUE measurements can be used instead of the whole plant-based WUE measurements for specific accessions in limited experimental equipment. However, some of our observation data showed that the results of WUE measurements using the 4 approaches were consistent, but not in other data. It may be due to the different physiological characteristics of sorghums concerning gas exchange, which can be affected by external stimuli such as light intensity (PPFD).

In conclusion, based on the 4 approaches of WUE’s analyses, the 30 Indonesian sorghum accessions can be classified into 5 groups ranging from the most efficient to not efficient in utilizing water. The efficiency in using water correlated with the sorghum cultivars to tolerate drought stress. The finding was crucial in determining whether Sorghum’s accession can be cultivated or used as a donor of tolerance traits in breeding strategies. We also found that WUE based on the measurement of a single leaf often had a positive correlation with WUE measurement based on biomass, which means that in some instances, single leaf measurement is sufficient to determine the WUE status of Sorghum.

**Acknowledgements**

This research was supported by the JICA-JST SATREPS Project: The Project for Producing Biomass Energy and Material through the Revegetation of Alang-alang (Imperata Cylindrica) Field to the Research Center for Plant Conservation and Botanical Gardens, Research Organization of Life Sciences and Environment, National Research and Innovation Agency (BRIN).

**References**

Anyia, A.O., Herzog, H., 2004. Water-use efficiency, leaf area and leaf gas exchange of cowpeas under mid-season drought. *Europ. J. Agronomy*. 20, 327-339.

Blum, A., 2011. Plant water relations, plant stress, and plant production. In: *Plant Breeding for Water-Limited Environments*. Springer, New York.

Boutraa, T., Akhkha, A., Al-Shoaibi, A.A., Alhejeli, A.M., 2010. Effect of water stress on growth and water use efficiency (WUE) of some wheat cultivars (*Triticum durum*) grown in Saudi Arabia. *JTUSCI*. 3, 39-48.

Bray, E.A., 2001. Plant response to water-deficit stress. In: *Encyclopedia of Life Sciences*. Nature Publishing Group, Wiley.

Bruns, A.H., 2016. Flag leaf photosynthesis and stomatal function of grain sorghum as influenced by changing photosynthetic photon flux densities. *International Journal of Agronomy*. 2016, 1-6. https://doi.org/10.1155/2016/1363740
Cheeseman, J.M., Lexa, M., 1996. Gas exchange: Models and measurement. in: Baker, N.R. (Eds.), Photosynthesis and Environment. The Netherlands: Kluwer Academic Publisher. pp. 223-240.

Cornic, G., Massacci, A., 1996. Leaf photosynthesis under drought stress. in: Baker, N.R. (Eds.), Photosynthesis and Environment. Kluwer Academic Publisher, Dordrecht. pp. 347-366.

Curt, M.D., Fernandez, J., Martinez, M., 1995. Productivity and water-use efficiency of sweet sorghum [Sorghum bicolor (L.) Moench] cv. “Keller” in relation to water regime. Biomass and Bioenergy. 8, 401-409. https://doi.org/10.1016/0961-9534(95)00036-4

Du, T., Meng, P., Huang, J., Peng, S., Xiong, D., 2020. Fast photosynthesis measurements for phenotyping photosynthetic capacity of rice. Plant Methods. 16, 6.

Food Security Department-National Resources Institute, 1999. Sweet sorghum [Sorghum bicolor (L.) Moench] cv. “Keller” in relation to water regime. Biomass and Bioenergy. 8, 401-409.

Fracasso, A., Magnanini, E., Marocco, A., Amaducci, S., 2017. Real-Time determination of photosynthesis, transpiration, water-use efficiency and gene expression of two Sorghum bicolor (Moench) genotypes subjected to dry-down. Frontiers in Plant Science. 8, 932.

Hsiao, T.C., 1973. Plant responses to water stress. Ann. Rev. Plant Physiol. 24, 519-570.

Kapanggowda, M.H., Payne, W.A., Rooney, W.L., Mullet, J.E., 2012. Transpiration ratio in sorghum [Sorghum bicolor (L.) Moench] for increased water-use efficiency and drought tolerance. Journal of Arid Land Studies. 22, 175-178.

Lawlor, D.W., 1995. Photosynthesis, productivity and environment. Journal of Experimental Botany. 46, 1449-1461.

Leakey, A.D.B., Uribelarrea, M., Ainsworth, E.A., Naidu, S.L., Rogers, A., Ort, D.R., Long, S.P., 2006. Photosynthesis, productivity, and yield of maize are not affected by open-air elevation of CO₂ concentration in the absence of drought. Plant Physiology. 140, 779-790.

Lee, C.H.Y., Lee, C.H.Y., Tang, A.M.C., Lai, D.Y.F., Tai, A.P.K., Leung, A.S.L., Tao, D.K.C., Leung, F., Leung, S.S.M., Wu, C., Sandy C.S. Tong, S.C.S., Kathy T.K., Ng, K.T.K., 2021. Problems and management of acacia-dominated urban forests on man-made slopes in a subtropical high-density city. Forests. 12, 323.

Long, S.P., Hallgren, J.E., 1993. Measurement of CO₂ assimilation by plants in the field and the laboratory. in: Hall, D.O., Scurlock, J.M.O., Bolhar-Nordenkampf, H.R., Leegod, R.C., Long, S.P. (Eds.), Photosynthesis and production in a changing environment. A Field and Laboratory Manual. New York: Springer. pp. 129-165.

Mathur, S., Umakant, A.V., Tonapi, V.A., Sharma, R., Sharma, M.K., 2017. Sweet Sorghum as biofuel feedstock: recent advances and available resources. Biotechnol Biofuels. 10, 146-165.

Medranoa, H., Tomáša, M., Martorella, S., Flexas, J., Hernández, E., Rosselló, J., Poub, A., Escalona, J.M., Bota, J., 2015. From leaf to whole-plant water use efficiency (WUE) in complex canopies: Limitations of leaf WUE as a selection target research group on plant biology under mediterranean conditions. The Crop Journal. 3, 220-228.

Mukkun, L., Lalad, H.J.D., Richana, N., Pabendon, M.B., Kleden, S.R., 2018. The diversity of local Sorghum (Sorghum bicolor (L) Moench) in Nusa Tenggara Timur province. IOP Conf. Series: Earth and Environmental Science. 144, 1–7. DOI:10.1088/1755-1315/144/1/012065

Sher, A., Barbanti, L., Ansar, M., Malik, M.A., 2013. Growth response and plant water status in forage sorghum [Sorghum bicolor (L) Moench] cultivars subjected to decreasing levels of soil moisture. Australian Journal of Crops Science. 7, 801-808.

Steduto, P., Katerji, N., Puertos-Molina, H., Unlu, M., Mastrorilli, M., Kana, G., 1997. Water-use efficiency of sweet Sorghum under water stress conditions Gas-exchange investigations at leaf and canopy scales. Field Crops Research. 54, 221-234.

Steduto, P., Hsiao, T.C., Fereres, E., Dirk Raes, D., 2012. Crop Yield Response to Water. FAO Irrigation and Drainage Paper 66. Food And Agriculture Organization of The United Nations, Rome.

Subramanian, V.B., Venkateswarlu, S., Maheswari, M., Reddy, M.N., 1993. Influence of solar radiation and vapour pressure deficit on transpiration efficiency of rainfed Sorghum. Journal of Agronomy and Crop Science. 171, 336–342.

Sumarno, Damardjati, D.S., Syam, M., Hermanto, 2013. Innovation Technology and Development. IAARD Press, Jakarta.

Tatsum, K., Kuwabara, Y., Motobayashi, T., 2020. Photosynthetic light-use efficiency of rice leaves under fluctuating incident light. Agrosyst Geosci Environ. 3, e20003.

Tsui, W., Ali, M.E.K., Inanaga, S., Sugimoto, Y., 2003. Growth and gas exchange of three sorghum cultivars under drought stress. Biologia Plantarum. 46, 583-587.