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

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ABSTRACT

Water use efficiency (WUE) is an essential subject in drought-restricted agricultures. Physiological parameters can be used to understand plant efficiencies in water usage. This research aimed to understand the physiological characteristics of 30 Indonesian sorghum accessions (*Sorghum bicolor* (L.) Moench), which indicate WUE and drought tolerance. Field research was conducted at Cibinong Science Center, National Research and Innovation Agency (BRIN) using Randomized Block Design with 30 sorghum accessions in 3 replicates. Experimental parameters included photosynthetic photon flux density (PPFD), stomatal conductance (GSW), photosynthetic (A) and transpiration (E) rates, and Leaf Area Index (LAI). The WUE was measured using four different approaches: instantaneous WUE (A:E), intrinsic WUE (A:GSW), and the relation between the biomass dry weight (BDW)/A and BDW/E. Based on WUE measurements, we concluded that sorghum accessions could be clustered into five groups, from the most efficient to inefficient water use. We also found that in some cases, WUE based on single leaf measurement had a positive correlation with WUE based on biomass, indicating its sufficiency in determining WUE status. The inconsistencies may be due to different sorghum physiological characteristics regarding gas exchange due to external stimuli (PPFD).

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

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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.

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	174.6.6.1.1.
JP	4183 A	Super 1*
1090 A	Sorgum Malai Mekar	Jagung Rote
Super 2A	1115 C	Pahat*

*) Released varieties

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 %, CO_2 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, A and E, and E and GSW. LAI*PPFD (LAI multiplied by PPFD) and LAI*A, LAI*PPFD, and LAI*E (Bruns 2016). Instantaneous WUE (A:E, photosynthetic rate divided by transpiration rate) (Bruns 2016; Kopanigowda *et al.* 2012), intrinsic WUE (A:GSW) (Bruns 2016; Kopanigowda *et al.* 2012; Medranoa *et al.* 2015) and the biomass relation to WUE (BDW:A and BDW:E) (Medranoa *et al.* 2015) were calculated. Statistical descriptions were used to analyze LAI. A statistical correlation was used to analyze the relationship between PPFD and A, PPFD and E, A and E, E and GSW, intrinsic and instantaneous WUEs, 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 2. Data of 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})	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 PPFD 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	R^2
Relationship between PPFD and photosynthetic rate	$y = 0.021x$	0.94
Relationship between PPFD and transpiration rate	$y = 9 * 10^{-5}x$	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,

Table 5. Leaf Area Index (LAI) of 30 sorghum accessions

	Leaf area statistical description					
	Leaf number area	Total leaf area (cm^2)	Average length (cm)	Average width (cm)	Average Maximum Width (cm)	Average (cm)
N Valid	30.00	30.00	30.00	30.00	30.00	30.00
Mean	7.10	2201.18	307.59	69.69	5.07	7.48
Std. Error of mean	0.39	157.02	14.23	3.87	0.16	0.19
Median	6.90[a]	2238.50[a]	313.21[a]	66.57[a]	4.93[a]	7.28[a]
Mode	7.00	664.53[b]	132.91[b]	36.15[b]	4.28	7.21[b]
Std. Deviation	2.18	860.04	77.95	21.21	0.88	1.07
Variance	4.78	739669	6077.10	450.03	0.77	1.15
Skewness	0.58	0.12	-0.009	1.62	0.04	-0.08
Std. Error of skewness	0.43	0.43	0.43	0.43	0.43	0.43
Kurtosis	0.16	-0.68	-0.05	3.74	0.02	-0.57
Std. Error of kurtosis	0.83	0.83	0.83	0.83	0.83	0.83
Range	9.00	2987.80	334.20	106.4	3.77	4.30
Minimum	4.00	664.53	132.91	36.15	2.93	5.40
Maximum	13.00	3652.33	467.11	142.55	6.70	9.70
Sum	213.00	66035.40	9227.95	2090.71	152.32	224.40

[a]Calculated from grouped data, [b]multiple modes exist. The smallest value is shown

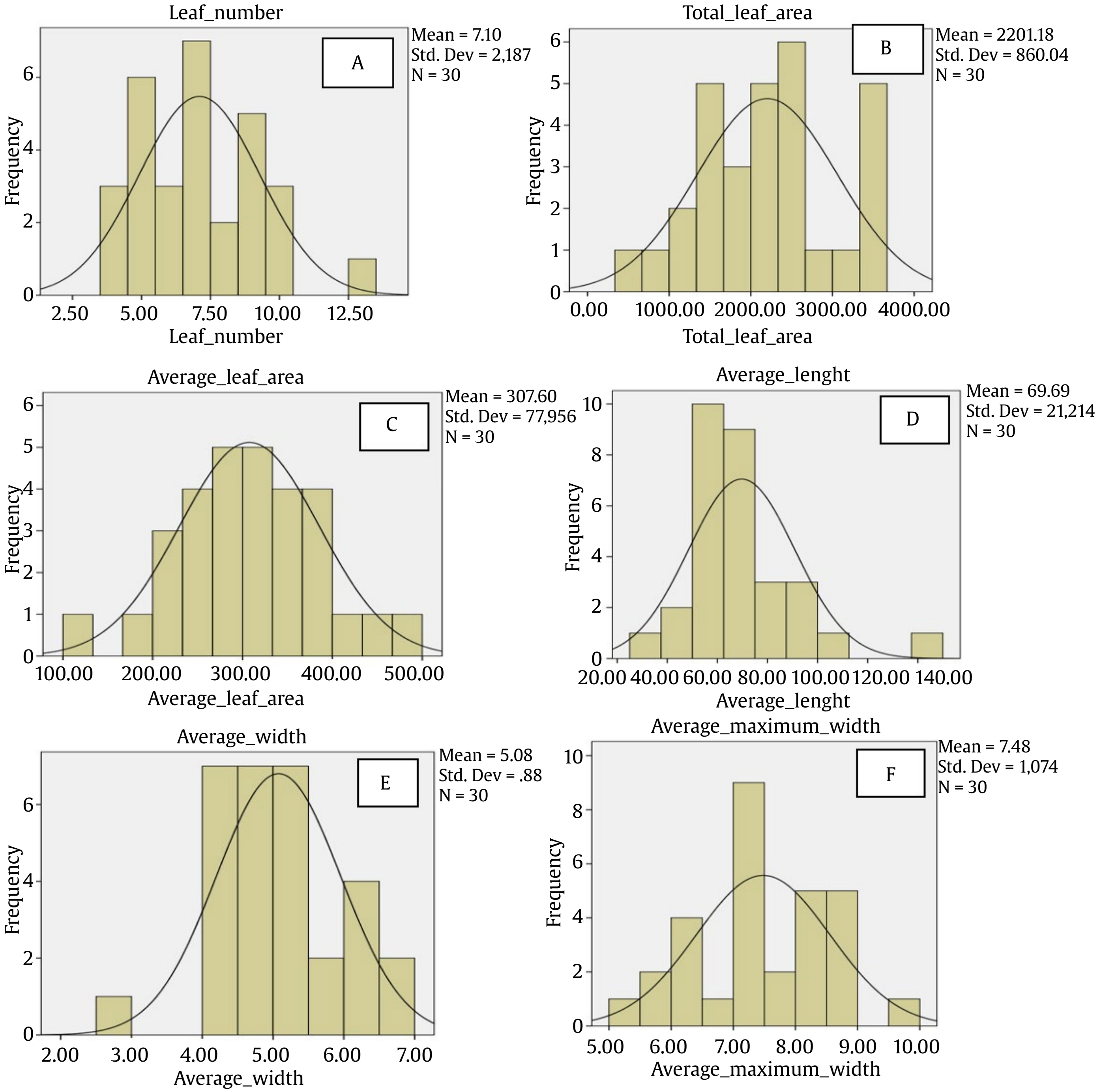

Figure 1. The leaf area index (LAI) consisted of leaf number (A), total leaf area (B), average leaf area (C), average leaf length (D), average leaf width (E), and average maximum width

Table 6. Average transpiration rate (E), assimilation rate (A), biomass dry weight, biomass dry weight/A, and biomass dry weight/E

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
KAWALI	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
UPCA	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
N6.1.2	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
N6.1.1	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 accession

Relationship	y	R^2
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	R^2
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	7.54^a	11	105.23^a	24	1.19^a	24	16.06^a
11	7.60^a	20	117.37^a	25	1.38^a	3	17.22^a
16	8.30^a	16	126.41^a	9	1.52^a	9	17.36^a
12	8.71^a	29	140.84^a	23	1.55^a	23	18.36^a
4	9.65^a	12	143.26^a	5	1.56^a	11	18.82^a
28	10.27^a	4	145.75^a	3	1.56^a	11	18.82^a
29	10.60^a	28	151.76^a	2	1.79^a	2	20.05^a
27	10.67^a	8	164.10^a	27	1.89^a	25	20.33^a
14	10.86^a	14	165.65^a	10	2.02^b	27	20.48^a
3	10.90^a	3	166.13^a	29	2.07^b	16	21.88^a
8	11.07^a	5	169.98^a	30	2.12^b	30	22.47^a
30	11.39^b	23	172.92^a	14	2.12^b	14	22.80^a
2	11.66^b	27	173.47^a	13	2.26^b	4	22.83^a

Table 9. Continued

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
23	11.66^{b}	30	174.32^{a}	4	2.39^{b}	10	23.49^{a}
5	11.85^{b}	10	177.16^{a}	28	2.47^{b}	29	23.63^{a}
9	11.87^{b}	2	184.89^{a}	1	2.49^{b}	28	24.79^{a}
10	11.99^{b}	19	199.05^{b}	26	2.59^{b}	13	30.06^{a}
15	12.73^{b}	18	199.42^{b}	11	2.67^{b}	20	31.37^{b}
18	12.89^{b}	9	199.66^{b}	16	2.75^{b}	12	32.90^{b}
13	12.91^{b}	13	208.15^{b}	15	2.80^{b}	15	41.26^{b}
19	13.04^{b}	15	212.86^{b}	6	3.14^{b}	19	44.66^{b}
24	13.14^{b}	21	216.10^{b}	21	3.19^{b}	21	45.37^{b}
21	13.88^{b}	24	216.97^{b}	7	3.24^{b}	7	46.20^{b}
17	14.15^{b}	6	238.42^{b}	19	3.35^{b}	1	53.46^{b}
25	14.63^{b}	25	243.53^{b}	22	3.38^{b}	17	54.42^{b}
6	16.38^{b}	17	250.35^{b}	12	3.51^{b}	8	55.28^{b}
7	17.06^{b}	26	275.59^{b}	17	3.80^{b}	6	55.63^{b}
1	18.26^{b}	7	301.09^{b}	20	4.12^{b}	18	67.09^{b}
26	19.86^{b}	1	304.37^{b}	8	4.45^{b}	26	79.86^{b}
22	25.72^{c}	22	416.64^{c}	18	5.18^{c}	22	119.20^{c}

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.
SURI3	s	s	s	s	4	0
KLR	s	ns	ns	s	2	2
Kawali	ns	ns	ns	ns	0	4
1503 A	ns	ns	s	ns	1	3
Suri 4	s	ns	s	ns	2	2
Samurai 1	s	s	s	s	4	0
181.73.1.1	s	s	s	s	4	0
JP	ns	ns	s	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	ns	3	1
KS	ns	ns	s	ns	1	3
UPCA	s	s	s	s	4	0
Numbu	ns	ns	s	ns	1	3
WHP 300	s	s	s	s	4	0
4183 A*	s	s	vs	s	4	0
Sorgum M.	s	s	s	s	4	0
1115 C	ns	ns	s	s	2	2
N6.1.2.	s	s	s	s	4	0
WHP*	vs	vs	s	vs	4	0
172.64.1.1	s	ns	ns	ns	1	3
WR	s	s	s	ns	3	1
N 6.1.1	s	s	ns	ns	2	2
Super 2B	s	s	s	s	4	0
174.6.6.1.1.	ns	ns	ns	ns	0	4
Super 1	ns	ns	s	ns	1	3
Jagung Rote	ns	ns	s	ns	1	3
Pahat	s	ns	s	ns	2	2

v = very significant (most efficient), s = significant (efficient), ns = not siginificant, 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 $R^2 = 0.91$, and E was highly correlated with GSW ($R^2 = 0.98$) (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 ($R^2 = 0.57$), and LAI*E ($R^2 = 0.68$) (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. Comic and Massaci (1996) observed gas exchange measurements of 14 different leaf positions in the grapevine canopy (lower, medium, and upper). The effect of leaf positions on daily carbon gain showed large variation from top layers of the canopy to lower positions. The differential light and microclimate environment caused significant A changes in the daily time course.

Furthermore, based on the measurements of instantaneous and intrinsic WUEs, both methods gave consistent results for all sorghum accessions. Therefore, we concluded that instantaneous and intrinsic WUEs could be used as indicators of WUE, indicated by their positive correlation ($R^2 = 0.95$) (Table 8). The results also indicated that GSW was positively correlated with E, and both were positively correlated with A.

It was found that WUEs analyzed using the relationship between instantaneous (A/E) and intrinsic (A/GSW) WUEs were following WUEs analyzed using the relationship between the biomass dry weight to photosynthetic (BDW/ A) and biomass dry weight to transpiration (BDW: E) rates ($R^2 = 0.74$) (Table 8). Therefore, it can be concluded that single leaf-based measurements using intrinsic and instantaneous WUEs can be used to describe WUE-based measurement on whole plant biomass represented by BDW. Table 9 shows the results of WUE's analyses using the 4 different methods. The higher the numbers obtained, the better the WUE. Using these approaches, we could determine the WUEs of the sorghum accessions.

The higher the values of WUE (instantaneous (A/E), intrinsic (A/GSW), and biomass relationship (BDB/A and BDB/E), the more efficient sorghums use water for photosynthesis and biomass production. The results are also consistent with the previous experiment that showed biomass production positively correlated with leaf gas exchange and leaf area during water deficit in cowpeas (Anyia and Herzog 2004). Our data also showed that the WHP and 4183A accessions could be considered the most efficient in the use of water, indicated by their highest number in 3 (instantaneous, intrinsic, and BDW/E) and 1 (BDW/A) WUE analyses, respectively (letter c). Meanwhile, the accession considered WUE efficient is indicated with the letter b, and not efficient is indicated with the letter a.

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 1. 181.73.1.1, UPCA, WHP 300, 4183 A, Sorghum Malai Mekar, N6.1.2, WHP, and Super 2B.

Next, 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.

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