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EVALUATION OF BAMBARA GROUNDNUT (*Vigna subterranea* L. Verdc.) GENOTYPES FOR DROUGHT TOLERANCE AT GERMINATION STAGE

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SUMMARY

The identification of drought-tolerant Bambara groundnut genotypes at the germination stage can provide important basic information to develop this crop. This study aimed to identify drought-tolerant genotypes and determine the appropriate parameters for screening drought-tolerant genotypes at the germination stage by using PEG 6000. Screening was carried out on 10 genotypes of Bambara groundnut (six Indonesian genotypes and four collection genotypes from Crop For the Future, Malaysia) under two different germination conditions: nonstress condition (0% PEG) and drought stress condition (10% PEG). This experiment was arranged in a randomized complete block design with four replications. Several parameters, such as seed germination percentage, uniformity of germination, speed of germination, root length, shoot length, seedling length, root-to-shoot length ratio, root dry weight, shoot dry weight, seedling dry weight, and drought sensitivity index (DSI), were observed. Results showed that all genotypes had significantly different responses under two germination conditions. All genotypes showed a reduction in all parameters except for root-to-shoot length ratio, which increased under 10% PEG treatment. Drought-tolerant genotypes were selected by using the DSI values of the speed of germination, shoot length, shoot dry weight, and seedling dry weight based on correlation and principal component analysis. The average DSI values from these parameters revealed that brown Sumedang (1.18), black Sumedang (1.09), black Gresik (1.01), black Tasikmalaya (1.02), and Uniswa Red (1.08) genotypes were drought-sensitive, whereas black Madura (0.98), black Sukabumi (0.97), IITA 686 (0.93), S 19-3 (0.87), and DODR (0.87) genotypes belonged to the medium tolerant category.

Keywords: Correlation, drought sensitivity index, drought-tolerant genotype, germination, polyethylene glycol, principal component analysis, screening

Key findings: This experiment was about a screening method for the early evaluation of drought-tolerant genotypes at the germination stage using PEG 6000. This experiment also determined the appropriate germination parameters that can be used as selection characters for screening genotypes under drought simulation at the germination stage. Based on this study, we could use this screening method (10% PEG) and selected parameters (based on correlation and PCA) to differentiate the drought-tolerance levels of Bambara groundnut genotypes.

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INTRODUCTION

Bambara groundnut (*Vigna subterranea* L.) is one of the legume plants with the potential to be developed to increase yield in the effort for local food diversification. This crop can be used as a source of proteins and carbohydrates. Bambara groundnut seeds contain 18%–20% proteins and 52%–60% carbohydrates (Adeleke *et al*., 2017). According to Amarteifio *et al*. (2006), Bambara groundnut seed is a good source of macro and micro minerals, especially Ca (37–128 mg), K (1545–2200 mg), Mg (159–335 mg), P (313--563 mg), and Fe (2.3–15.0 mg), per 100 g dry matter. Bambara groundnut seeds are also rich in lysine, which is an essential amino acid for protein synthesis in the body (Massawe *et al.,* 2005). Besides, Bambara groundnut pods can be used as animal feed (Mahala and Mohammed, 2010), and its seed oil can be used as raw material for the manufacture of paint, liquid soap, and shampoo (Aremu *et al.*, 2013). Given this information, this crop has the potential to be developed in the future in terms of cultivation technology, production, seed production, seed quality, and plant breeding activity to obtain superior genotypes, such as drought-tolerant genotypes.

One of the main problems that can disrupt agricultural activities is climate change. Climate change affects the availability of groundwater in agricultural land such that the land quality worsens. The lack of water can cause drought stress in plants. A strategy that can be used to deal with this problem is the use of drought-tolerant genotypes.

The Bambara groundnut plant is known to have drought-tolerant traits (Collinson *et al.*, 1997) and can adapt to marginal land compared with other legumes (Kay, 1979). Although this plant is known to be drought-tolerant, its tolerance to drought varies and is strongly influenced by its adaptation area and testa color. Bambara groundnut seeds from dry areas have better tolerance to drought than those from wet areas (Jorgensen *et al.,* 2011). Mabhaudi and Modi (2013) stated that Bambara groundnut seeds with dark-colored testa (dark brown and red) have a better adaptation response to drought stress than those with light-colored testa (light brown). Previous works show that information about the ability or the tolerance of Bambara groundnut genotypes to drought stress remains varied. Thus, the genetic potentials of Bambara groundnut genotypes still need to be identified further to obtain information about the drought tolerance of various genotypes.

Drought-tolerant genotypes can be identified at the germination stage as an early evaluation for genotype screening. Evaluation is performed by decreasing the external water potential of the germination medium. Lower water potential causes a lower amount of available water for seed. Decreasing the amount of water that is absorbed by the seed reduces cell division activities; affects the germination process; and consequently decreases germination percentage, root length, and shoot length (Zaefizadeh *et al.,* 2011).

Drought conditions can be simulated on the germination medium by using polyethylene glycol (PEG) solution to decrease the external water potential. Three commonly used osmoticum compounds are melibiose, mannitol, and PEG, but the best compound in controlling the potential of water that cannot be absorbed by the plant is PEG (Verslues *et al.,* 2006). PEG has a large molecular weight and cannot be absorbed by seed or plant cell walls (Chazen and Neumann, 1994). Thus, this compound can hold water, which becomes unavailable to seeds or plants (Michel and Kaufmann, 1973). Verslues *et al.* (2006) stated that PEG with a molecular weight of 6000 (PEG 6000) is suitable for screening genotypes for drought tolerance at the germination stage because it can simulate the groundwater potential or drought stress levels in the soil.

PEG has been widely used for simulating drought stress conditions at the germination stage. This method had been used in many studies on several agricultural crops such as rice (Afa *et al.,* 2013; Widyastuti *et al.,* 2016; Akbar *et al.,* 2018), soybean (Kosturkova *et al.,* 2008; Vijay *et al.,* 2018), peanut (Adisyahputra *et al.,* 2004; Kasno and Trustinah 2009), mungbean (Swathi *et al.,* 2017; Saima *et al.,* 2018), cowpea (Carvalho *et al.,* 2019), corn (Efendi *et al.,* 2009; Queiroz *et al.,* 2019), sorghum (Queiroz *et al.,* 2019), chili (Molla *et al.,* 2019), tomato (Florido *et al.,* 2018), and sunflower seed (Razzaq *et al.,* 2017; Toscano *et al.,* 2017).

Screening genotypes using PEG requires the appropriate concentration and germination parameters that can be used to differentiate the responses and tolerance of the tested genotypes to drought stress. A PEG concentration of 25% (W/V) was used for the early evaluation of drought-tolerant rice genotypes with seedling dry weight (Afa *et al.*, 2013), germination percentage, seed vigor, seminal root length, seedling length, and seminal root dry weight (Widyastuti *et al.*, 2016; Akbar *et al.,* 2018) as major parameters. Vijay *et al.* (2018) stated that drought-tolerant soybean genotypes can be screened using PEG 6000 at −0.3 MPa osmotic potential with germination percentage, vigor index, and stress tolerance index as major parameters. Kasno and Trustinah (2009) proved that PEG 6000 solution at −0.3 MPa osmotic potential can be used to differentiate the responses of peanut genotypes to drought stress with root length, the root number, root weight, and seedling dry weight as major parameters. Carvalho *et al.* (2019) reported that PEG 6000 solution at −0.15 MPa osmotic potential is effective in determining the drought tolerance of cowpea genotypes at the germination stage using root length, vigor index, and proline content as major parameters. Molla *et al.* (2019) stated that drought stress simulated using 12.5% PEG 6000 at the germination stage significantly affected the growth of root and shoot of chili. Efendi *et al.* (2009) used 10% PEG concentration for the early screening of drought-tolerant maize genotypes with root dry weight as a predictor parameter. In tomato, drought stress at the germination stage using PEG 6000 at −0.5 MPa was used to screen genotypes with germination percentage as a major parameter (Florido *et al.,* 2018).

Even though PEG has been used to screen the various genotypes of different crop species, there is no such information for Bambara groundnut. Therefore, this research aimed to simulate drought stress at the germination stage using PEG and screen Bambara groundnut genotypes for drought tolerance.

MATERIALS AND METHODS

This experiment was conducted at Leuwikopo Experiment Station Greenhouse, IPB University, Bogor, Indonesia, from December 2018 to February 2019. The temperature and relative humidity of the greenhouse ranged from 22.9 °C to 30.0 °C and from 55% to 98%, respectively. The experiment was conducted with a randomized complete block design with two factors and four replications. The first factor was 10 genotypes of Bambara groundnut seeds (six Indonesian genotypes and four collection genotypes from Crop for the Future), Malaysia): brown Sumedang, black Sumedang, black Gresik, black Madura, black Tasikmalaya, black Sukabumi, IITA 686, S 19-3, DODR, and Uniswa Red. The second factor was two conditions of germination: nonstress condition (without PEG solution, 0%) and drought stress condition (with 10% PEG solution) equivalent to 0.0 and −0.19 MPa water potential in the field, respectively (Mexal *et al.*, 1975). The determination of PEG concentration for drought stress conditions was based on the results of the preliminary experiment and consideration from some literature on peanut and soybean.

Procedures

Bambara groundnut seeds were germinated in containers (25 cm × 20 cm) that contained 4 kg of sterilized sand (ca. 0.8 mm) as a medium for germination. The seeds were germinated for 14 days (Ilyas and Sopian, 2013). Twenty-five seeds were germinated in each container. Beforehand, the seeds were surface-sterilized with 70% ethyl alcohol for 30 s then with 7% calcium hypochlorite solution for 30 min and subsequently rinsed with distilled water three to four times (Kone *et al.,* 2015).

Germination condition treatment was performed by watering the germination medium with distilled water for the nonstress condition and with 10% (W/V) PEG solution (100 g/L solution) to simulate the drought stress condition. Initially, the germination medium was watered with 450 ml of distilled water or PEG solution to reach the field capacity water content. This volume was determined from the deviation between the field capacity water content (12.44%) and initial water content (1.28%) of the germination medium. The water content was obtained from the preliminary experiment that determined the field capacity and initial water content of sand medium based on the gravimetric method following Haridjaja *et al*. (2013) and Reynolds (1970). During the germination period, the moisture content of the medium was maintained by watering the medium with 130 ml of distilled water or 10% PEG solution. The maintenance volume was based on a preliminary experiment by calculating the average of daily water loss from the sand medium for 5 days.

Several parameters, such as seed germination percentage, uniformity of germination, speed of germination, root length, shoot length, seedling length, root-to-shoot length ratio, root dry weight, shoot dry weight, seedling dry weight, and drought sensitivity index (DSI), were observed (Fischer and Maurer, 1978) to categorize the drought tolerance of 10 genotypes. Seed germination percentage was calculated by observing seedling numbers on the 7th day (first count) and 14th day (final count) after planting in accordance with Ilyas and Sopian (2013). The uniformity of germination was observed on the 10th day after planting. The speed of germination was observed daily by accumulating the normal seedling percentage per 24 h. Root length, shoot length, seedling length, and root-shoot length ratio were observed on the final day of the germination period. The dry weights of roots, shoots, and seedlings were measured after the samples were oven-dried at 60 °C for 72 h.

The DSI of all germination parameters was calculated from the original observation values using the formula of Fischer and Maurer (1978):

$$DSI = \frac{1 - \left(\frac{Y}{Yp}\right)}{1 - \left(\frac{X}{Xp}\right)},$$

where

DSI	=	Drought sensitivity index
Y	=	Average observation of one genotype under drought stress condition
Yp	=	Average observation of one genotype under nonstress condition
X	=	Average observation of all genotypes under drought stress condition
Xp	=	Average observation of all genotypes under nonstress condition

The final criteria for DSI values can be used to differentiate the tolerance of various Bambara groundnut genotypes to drought stress. The genotypes belong to the tolerant category if DSI value ≤ 0.5, to the moderately tolerant category if DSI value 0.5 < DSI ≤ 1, and to the sensitive category if DSI > 1.

Data analysis

Data were analyzed using the Statistical Analysis System (SAS) 9.1 program and Microsoft Excel. The differences between treatments were tested by Duncan's multiple range test (DMRT). Correlation analysis between all parameters and principal component analysis (PCA) were conducted to determine the important or major parameters that can be used as selection criteria on the basis of DSI value (Widyastuti *et al.*, 2016; Akbar *et al.,* 2018). This analysis was carried out using Minitab 16 program.

Table 1. Mean squares of variance analysis for all germination parameters of 10 Bambara groundnut genotypes under nonstress and drought stress conditions.

Germination parameters	Block (d.f.= 3)	Treatment (T) (d.f.= 1)	Genotypes (G) (d.f.= 9)	Interactions (T x G) (d.f= 9)	Error (d.f.= 57)	CV (%)
Germination percentage	365.33**	16820.00**	554.76**	510.22**	74.95	10.28
Uniformity of germination	274.20	106872.20**	964.56**	909.98**	44.52	10.81
Speed of germination	3.07*	1181.49**	15.07**	2.33**	0.84	8.99
Root length	4.65**	1290.82**	9.09**	2.95**	0.65	6.01
Shoot length	1.40	1982.84**	12.97**	7.28**	0.73	8.23
Seedling length	10.43**	6473.34**	33.28**	15.63**	1.76	5.58
Root-shoot length ratio	0.13	20.14**	0.47**	0.41**	0.05	13.82
Root dry weight	0.14**	44.94**	0.30**	0.25**	0.03	13.89
Shoot dry weight	0.43**	558.84**	0.97**	1.28**	0.10	9.94
Seedling dry weight	1.04**	920.73**	1.84**	2.40**	0.20	10.06

**,* = Significant at 1 and 5% probability levels, CV = Coefficient of variance, d.f. = Degree of freedom.

RESULTS

Analysis of variance showed the significant effects of genotypes and germination conditions (nonstress condition and drought stress simulation using 10% PEG 6000) on all of the germination parameters (Table 1). The analysis also showed significant interactions between genotypes and germination conditions on all observed parameters. The interactions showed that the genotypes had different responses under two different germination conditions.

Response of genotypes to drought based on germination parameters

The responses of Bambara groundnut genotypes to drought stress at the germination stage were evaluated using 10% PEG solution to simulate drought. Significantly different responses to drought stress were observed among Bambara groundnut genotypes. Generally, all genotypes showed a decrease in germination percentage under 10% PEG treatment compared with that under nonstress condition (Figure 1) except that the root-to-shoot length ratio increased. The means of the observed parameters of the 10 Bambara groundnut genotypes under two different germination conditions are presented in Table 2, 3, and 4.

All genotypes had a high germination percentage and uniformity of germination under nonstress germination conditions (>96%) and were not statistically different from each other (Table 2). These parameters decreased in all genotypes under 10% PEG treatment as can be seen from the reduction percentages, which ranged from 9.0%–63.9% in germination and 32.0%–99.0% in the uniformity of germination. The DODR genotype maintained high germination

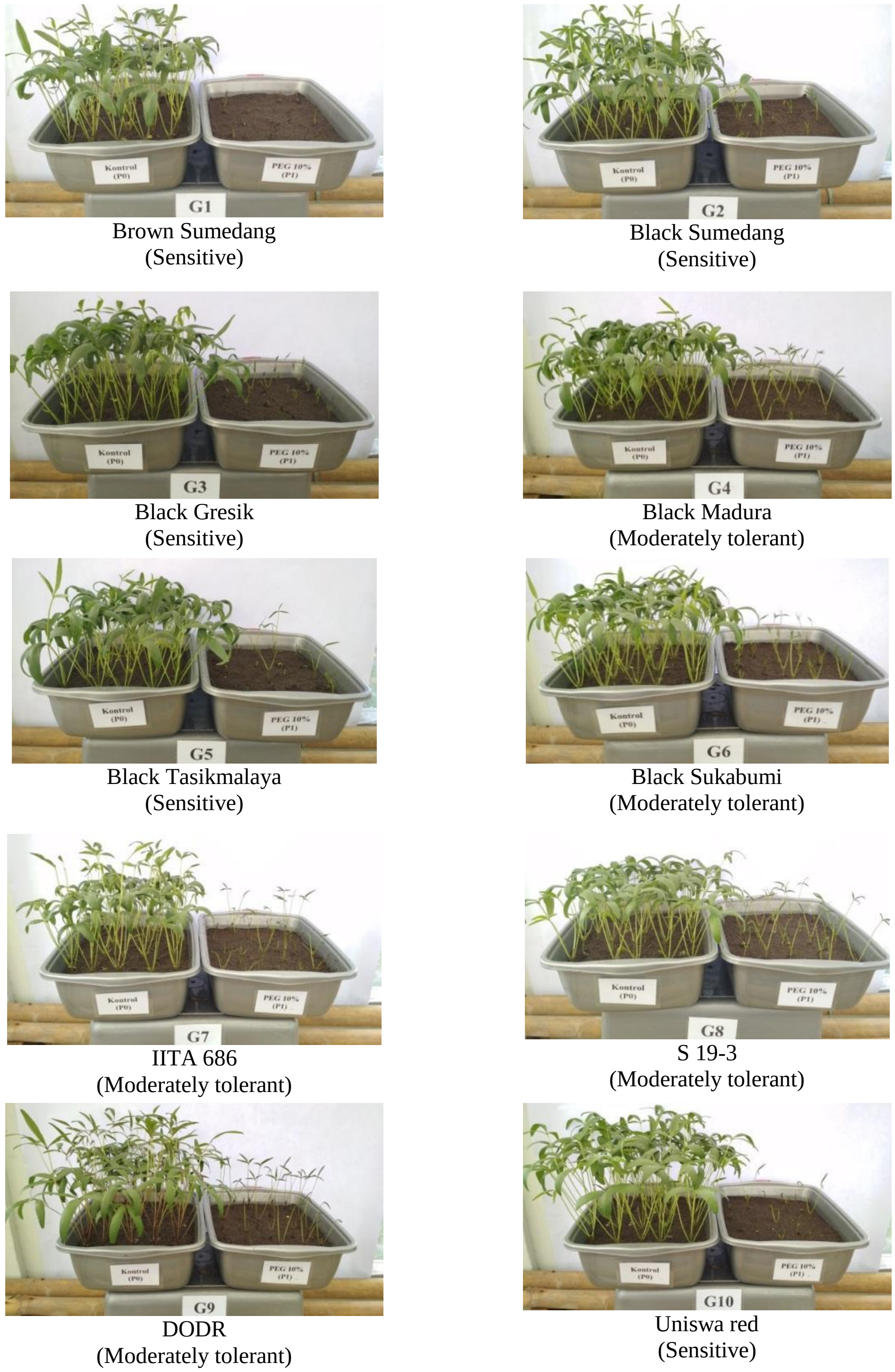

Figure 1. Drought tolerance levels of Bambara groundnut genotypes at the germination stage using 10% PEG. Seedlings at 14^{th} (final) day of germination: left container (nonstress condition), right container (drought stress condition).

Table 2. Mean of germination percentage, uniformity, and speed of germination of 10 Bambara groundnut genotypes under nonstress and drought stress conditions.

Genotypes	Germination percentage (%)			Uniformity of germination (%)			Speed of germination (% day^{-1})		
	Non stress	Drought stress	Reduction (%)*	Non Stress	Drought stress	Reduction (%)*	Non stress	Drought stress	Reduction (%)*
Brown Sumedang	97.0 a	35.0 g	63.9	96.0 a	1.0 g	99.0	12.1 d	2.8 i	76.7
Black Sumedang	99.0 a	54.0 f	45.5	98.0 a	3.0 g	96.9	13.1 cd	4.5 h	65.8
Black Gresik	99.0 a	66.0 def	33.3	99.0 a	18.0 ef	81.8	14.2 abc	5.9 g	58.8
Black Madura	100.0 a	77.0 cd	23.0	100.0 a	19.0 ef	81.0	14.3 abc	6.8 fg	52.5
Black Tasikmalaya	99.0 a	69.0 cde	30.3	99.0 a	10.0 fg	89.9	13.6 bc	5.9 g	56.5
Black Sukabumi	98.0 a	80.0 bc	18.4	98.0 a	16.0 f	83.7	14.0 bc	7.0 fg	50.0
IITA 686	96.0 a	80.0 bc	16.7	97.0 a	52.0 c	46.4	15.0 ab	8.0 f	46.8
S 19-3	99.0 a	82.0 bc	17.2	97.0 a	38.0 d	60.8	13.8 bc	7.7 f	44.7
DODR	100.0 a	91.0 ab	9.0	100.0 a	68.0 b	32.0	15.7 a	9.3 e	40.5
Uniswa Red	100.0 a	63.0 ef	37.0	99.0 a	27.0 e	72.7	14.7 ab	5.9 g	60.3

Numbers followed by the same letters on the same variable show no significant difference based on the DMRT test at the 5% probability level. Values are the percentage of reduction compared with the nonstress condition. ** Values are the percentage of increase compared with nonstress condition.

Table 3. Means of root length, shoot length, seedling length, and root-to-shoot length ratios of 10 Bambara groundnut genotypes under nonstress and drought stress conditions.

Genotypes	Root length (cm)			Shoot length (cm)			Seedling length (cm)			Root-shoot length ratio		
	Non stress	Drought stress	Reduc - tion (%)*	Non stress	Drought stress	Reduc-tion (%)*	Non stress	Drought stress	Reduc-tion (%)*	Non stress	Drought stress	Increa-se (%)**
Brown Sumedang	17.01 cd	9.49 efg	44.2	15.41 bc	3.49 h	77.4	32.43 cd	12.99 i	59.9	1.13 fg	2.99 a	164.6
Black Sumedang	16.76 cd	9.58 efg	42.8	15.15 bc	4.35 gh	71.3	31.91 cd	13.94 ghi	56.3	1.12 fg	2.51 b	124.1
Black Gresik	16.19 d	9.11 fg	43.7	14.38 c	5.43 fg	62.2	30.58 de	14.54 ghi	52.5	1.13 fg	1.97 cd	74.3
Black Madura	16.91 cd	8.84 g	47.7	14.44 c	5.22 fg	63.9	31.35 cde	14.06 ghi	55.2	1.19 fg	1.99 cd	67.2
Black Tasikmalaya	16.53 cd	8.84 g	46.5	14.41 c	4.84 fg	66.4	30.94 cde	13.68 hi	55.8	1.16 fg	2.15 c	85.3
Black Sukabumi	16.62 cd	8.90 fg	46.5	14.62 c	5.37 fg	63.3	31.25 cde	14.27 ghi	54.3	1.15 fg	1.91 cd	66.1
IITA 686	16.98 cd	9.00 fg	47.0	15.96 b	6.09 f	61.8	32.94 c	15.09 ghi	54.2	1.07 g	1.77 de	65.4
S 19-3	17.55 bc	9.87 efg	43.8	12.22 d	6.05 f	50.5	29.77 e	15.93 g	46.5	1.46 ef	1.92 cd	31.5
DODR	18.24 b	10.19 ef	44.1	18.65 a	8.15 e	56.3	36.90 b	18.34 f	50.3	0.97 g	1.62 de	67.0
Uniswa Red	21.82 a	10.46 e	52.1	18.06 a	4.73 fgh	73.8	39.87 a	15.19 gh	61.9	1.21 fg	2.79 ab	130.6

Numbers followed by the same letters on the same variable show no significant difference based on the DMRT test at 5% probability level. ☐Values are the percentage of reduction compared with nonstress condition. ** Values are the percentage of increase compared to nonstress condition.

Table 4. Means of root dry weight, shoot dry weight, and seedling dry weight of 10 Bambara groundnut genotypes under nonstress and drought stress conditions.

Genotypes	Root dry weight (mg)			Shoot dry weight (mg)			Seedling dry weight (mg)		
	Non stress	Drought stress	Reduction (%)*	Non stress	Drought Stress	Reduction (%)*	Non stress	Drought stress	Reduction (%)*
Brown Sumedang	2.44 a	0.36 e	85.3	6.03 bc	0.23 g	96.2	8.46 b	0.59 h	93.0
Black Sumedang	2.05 b	0.48 de	76.6	5.59 c	0.40 fg	92.8	7.64 cd	0.88 gh	88.5
Black Gresik	2.17 ab	0.58 de	73.3	5.97 bc	0.51 fg	91.5	8.14 bc	1.09 gh	86.6
Black Madura	2.30 ab	0.62 de	73.0	6.15 b	0.57 fg	90.7	8.44 b	1.19 gh	85.9
Black Tasikmalaya	2.25 ab	0.55 de	75.6	6.13 b	0.53 fg	91.4	8.37 b	1.08 gh	87.1
Black Sukabumi	2.23 ab	0.66 d	70.4	6.17 b	0.62 fg	90.0	8.40 b	1.28 gh	84.8
IITA 686	1.51 c	0.40 de	73.5	5.00 d	0.65 fg	87.0	6.51 e	1.05 gh	83.9
S 19-3	1.49 c	0.47 de	68.5	4.38 e	0.67 fg	84.7	5.87 f	1.14 gh	80.6
DODR	1.56 c	0.58 de	62.8	5.90 bc	0.88 f	85.1	7.46 d	1.46 g	80.4
Uniswa Red	2.07 b	0.37 de	82.1	7.08 a	0.46 fg	93.5	9.15 a	0.83 gh	90.9

Numbers followed by the same letters on the same variable show no significant difference based on the DMRT test at 5% probability level. * Values are the percentage of reduction compared with nonstress condition.

Table 5. Drought sensitivity indexes for all of the germination variables of 10 Bambara groundnut genotypes.

Genotype	GP	UG	SG	RL	SL	SDL	RSLR	RDW	SDW	SDDW
Brown Sumedang	2.18	1.33	1.40	0.96	1.19	1.09	1.90	1.14	1.06	1.08
Black Sumedang	1.55	1.30	1.20	0.93	1.10	1.03	1.43	1.02	1.03	1.02
Black Gresik	1.13	1.10	1.08	0.95	0.96	0.96	0.86	0.98	1.01	1.00
Black Madura	0.78	1.09	0.96	1.04	0.98	1.01	0.78	0.98	1.00	0.99
Black Tasikmalaya	1.03	1.21	1.03	1.01	1.02	1.02	0.99	1.01	1.01	1.01
Black Sukabumi	0.63	1.13	0.91	1.01	0.97	0.99	0.76	0.94	0.99	0.98
IITA 686	0.57	0.62	0.85	1.02	0.95	0.99	0.76	0.98	0.96	0.97
S 19-3	0.58	0.82	0.82	0.95	0.78	0.85	0.36	0.92	0.94	0.93
DODR	0.31	0.43	0.74	0.96	0.87	0.92	0.77	0.84	0.94	0.93
Uniswa Red	1.26	0.98	1.10	1.13	1.14	1.13	1.51	1.10	1.03	1.05

GP = Germination percentage, UG = Uniformity of germination, SG = Speed of germination, RL = Root length, SL = Shoot length, SDL = Seedling length, RSLR = Root-shoot length ratio, RDW = Root dry weight, SDW = Shoot dry weight, SDDW = Seedling dry weight.

percentage (91.0%) and uniformity of germination (68.0%) under 10% PEG treatment compared with other genotypes, whereas the brown Sumedang genotype had the lowest germination percentage under both conditions (35.0% and 1.0%). Under nonstress condition, the DODR genotype had the fastest speed of germination (15.7% day^{-1}) but it was not significantly different from IITA-686, Uniswa Red, black Madura, black Sukabumi, and black Tasikmalaya, whereas brown Sumedang had the lowest value (12.1% day^{-1}) that was similar to black Sumedang (Table 2). DODR and brown Sumedang were also the genotypes with the fastest and the slowest speed of germination values of 9.3 and 2.8% day^{-1} under 10% PEG treatment, respectively, with the lowest and the highest reduction (40.5% and 76.7%).

Observations on root length, shoot length, seedling length, and root-shoot length ratio (Table 3) were carried out at the end of the germination period (14 DAP). Uniswa Red had the longest root length both under nonstress (21.82 cm) and drought stress conditions using 10% PEG (10.46 cm). However, the root length of Uniswa Red was not significantly different from that DODR, S19-3, brown Sumedang, and black Sumedang under 10% PEG treatment. All genotypes had almost the same values of reduction that ranged from 43.7% to 52.1%. This parameter is presumed to be less sensitive in differentiating the responses of Bambara groundnut genotypes to drought.

All genotypes were unable to maintain their canopy growth, as exemplified by shoot length, under 10% PEG treatment. DODR had the longest shoot length (18.65 cm), which was not significantly different from that of Uniswa Red, whereas S19-3 had the shortest shoot length (12.22 cm) under nonstress conditions (Table 3). Under 10% PEG treatment, the DODR genotype had the longest shoot length (8.15 cm), and brown Sumedang had the shortest shoot length (3.49 cm), which was not significantly different from that of black Sumedang (Table 3). Brown Sumedang, black Sumedang, and Uniswa Red had the largest decrease in shoot length compared with other genotypes.

Uniswa Red and S 19-3 showed the longest (39.87 cm) and the shortest (29.77 cm) seedling lengths, respectively, under nonstress condition. Under 10% PEG treatment, DODR and brown Sumedang showed the longest and the shortest seedling lengths, which were not significantly different from those of other genotypes, except for S 19-3, DODR, and Uniswa Red. Given that Uniswa red had the longest root, shoot, and seedling lengths under the nonstress condition but generally had the largest reduction percentage under 10% PEG treatment, this genotype was assumed to be the most sensitive to drought stress (Table 3).

The root-to-shoot length ratio of seedlings increased under 10% PEG treatment in all genotypes. This increase ranged from 31.5% to 164.6% (Table 3). The brown Sumedang, black Sumedang, and Uniswa Red genotypes had the largest increase (>100%). Root and shoot length observations (Table 3) showed that shoot length had a high reduction (50.5%–77.4%) compared with root length (42.8%–52.1%). Generally, the shoot and seedling dry weight of the DODR genotype was significantly higher than that of brown Sumedang under drought conditions but was not significantly different from that of other genotypes (Table 4). The root dry weight of black Sukabumi was significantly higher than that of brown Sumedang but was not significantly different from that of other genotypes. Based on the reduction percentage, brown Sumedang and Uniswa Red genotypes had a higher reduction than others.

DSI of all the germination parameters of genotypes

DSI values were used to determine the drought tolerance levels of various Bambara groundnut genotypes to drought stress by 10% PEG simulation. S19-3 and DODR had DSI values that were consistently below 1.0 (<1.0) for all parameters (Table 5). This result indicated that these genotypes were relatively drought-tolerant. The DSI for nine parameters of brown Sumedang, black Sumedang, black Tasikmalaya, and Uniswa Red genotypes were almost consistently above 1.0 (>1.0), indicating that these genotypes can be assumed to be sensitive to drought stress. Each parameter of each genotype showed different DSI values and tolerance levels. Therefore, major parameters with a high contribution to the variance in DSI values must be selected.

Correlation analysis

Correlation analysis (Table 6) showed that germination percentage was positively and significantly correlated with uniformity of germination, speed of germination, shoot length, seedling length, root dry weight, shoot dry weight, and seedling dry weight. However, root length was not correlated with other parameters. Based on the analysis, it was assumed that all germination parameters, except root length, can be used as basic references to determine the drought tolerance level of the genotypes. However, further analysis, such as PCA, is needed to select the major parameters in determining the drought tolerance levels of genotypes with high validity (Afa *et al.,* 2013; Widyastuti *et al.,* 2016).

PCA

PCA (Table 7) showed that the first main component (PC1) explained 79.1% of the total variance. PC selection was based on the criterion of 70% minimum cumulative percentage of total variation (Jolliffe, 2002; Mattjik and Sumertajaya, 2011). Therefore, the major parameters that can be used in determining the drought tolerance levels of Bambara groundnut genotypes were selected in PC1. PCA included parameters with significant interactions between genotypes and germination condition treatments and correlations between parameters. The results showed that all parameters, except uniformity of germination and root length, had the same PC value. The ranking of PC values of all parameters from the highest to the lowest was as follows: seedling dry weight (0.354), shoot dry weight (0.348), shoot length (0.345), speed of germination (0.340), root dry weight (0.338), germination percentage (0.332), root-to-shoot length ratio (0.331), seedling length (0.319), uniformity of germination (0.276), and root length (0.079). Based on the highest PC values, seedling dry weight, shoot dry weight, shoot length, and speed of germination were selected as major

Table 6. Correlation analysis between all germination parameters.

	GP	UG	SG	RL	SL	SDL	RSLR	RDW	SDW
UG	0.770**								
SG	0.992**	0.820**							
RL	-0.094	-0.068	-0.049						
SL	0.851**	0.673*	0.883**	0.307					
SDL	0.694*	0.535	0.736**	0.564	0.957**				
RSLR	0.900**	0.566	0.895**	0.136	0.947**	0.861**			
RDW	0.884**	0.682*	0.894**	0.334	0.901**	0.864**	0.854**		
SDW	0.901**	0.837**	0.943**	0.200	0.943**	0.866**	0.874**	0.893**	
SDDW	0.912**	0.751*	0.940**	0.286	0.966**	0.911**	0.916**	0.958**	0.979**

GP = Germination percentage, UG = Uniformity of germination, SG = Speed of germination, RL = Root length, SL = Shoot length, SDL = Seedling length, RSLR = Root-shoot length ratio, RDW = Root dry weight, SDW = Shoot dry weight, SDDW = Seedling dry weight, **,* = Significant correlations at 1% and 5% probability levels, respectively.

Table 7. PCA of tested genotypes.

Germination parameters	PC1[a]	PC2
Germination percentage	0.332	0.264
Uniformity of germination	0.276	0.295
Speed of germination	0.340[b]	0.235
Root length	0.079	−0.803
Shoot length	0.345[b]	−0.111
Seedling length	0.319	−0.346
Root-shoot length ratio	0.331	0.007
Root dry weight	0.338	−0.094
Shoot dry weight	0.348[b]	0.032
Seedling dry weight	0.354[b]	−0.052
Eigen values	7.909	1.404
The proportion of variance (%)	79.1	14.0
Cumulative proportion (%)	79.1	93.6

PC = Principal component, [a] = Selected PC, [b] = Selected variables with high values.

parameters to determine the drought tolerance levels or categories of the tested Bambara groundnut genotypes.

Drought tolerance of tested genotypes

Drought tolerance levels or categories can be obtained by using the averaged data of DSI values from four selected parameters based on correlation analysis and PCA. On the basis of average DSI values (Table 8), 10 Bambara groundnut genotypes were categorized as sensitive and moderately drought tolerant. The category of tolerance was determined by using DSI criteria based on Fisher and Maurer (1978). The results showed that brown Sumedang, black Sumedang, black Gresik, black Tasikmalaya, and Uniswa Red were categorized as sensitive to drought with average DSI values > 1.0, whereas black Madura, black Sukabumi, IITA 686, S 19-3, and DODR were categorized as moderately tolerant with average DSI values 0.5 < DSI ≤ 1.0.

Table 8. DSI values of shoot length, shoot dry weight, and seedling dry weight for selecting drought tolerance of tested genotypes.

Genotypes	Speed of germination	Shoot length	Shoot dry weight	Seedling dry weight	Average DSI	Drought tolerance
Brown Sumedang	1.40	1.19	1.06	1.08	1.18	Sensitive
Black Sumedang	1.20	1.10	1.03	1.02	1.09	Sensitive
Black Gresik	1.08	0.96	1.01	1.00	1.01	Sensitive
Black Madura	0.96	0.98	1.00	0.99	0.98	Medium tolerant
Black Tasikmalaya	1.03	1.02	1.01	1.01	1.02	Sensitive
Black Sukabumi	0.91	0.97	0.99	0.98	0.97	Medium tolerant
IITA 686	0.85	0.95	0.96	0.97	0.93	Medium tolerant
S 19-3	0.82	0.78	0.94	0.93	0.87	Medium tolerant
DODR	0.74	0.87	0.94	0.93	0.87	Medium tolerant
Uniswa Red	1.10	1.14	1.03	1.05	1.08	Sensitive

DISCUSSION

The evaluation and estimation of Bambara groundnut genotype tolerance to drought stress at the germination stage can be done using 10% PEG solution (−0.19 MPa) to simulate drought. This concentration can be used to differentiate responses among Bambara groundnut genotypes and categorize genotypes into tolerant and moderately tolerant categories in this study. Our finding supports previous studies on peanut (Adisyahputra *et al.* 2004) and maize (Efendi *et al.* 2009) showing that 10% PEG 6000 treatment is effective for evaluating genotypes to drought stress conditions at the germination stage. The use of concentrations above 10% in the preliminary experiment did not show any progress on the germination process. The seeds remained intact and fresh until the final day of observation. The ability of seeds to germinate under drought stress (lower osmotic potential) differs across species. Germination does not occur at <−0.2 MPa for cowpea seeds (Murillo-Amador *et al.,* 2002; Carvalho *et al.,* 2019), −1.76 MPa for corn seeds (Mohammadkhani and Heidari, 2008), −0.9 MPa for soybean seed (Wijewardana *et al.,* 2018), and −1.2 MPa for sunflower seeds (Kaya *et al.,* 2006).

The lack of water inhibits seed imbibition, reduces water uptake by the seed, reduces cell division activities, and then decreases the observed germination parameters (Zaefizadeh *et al.,* 2011). Drought stress simulated by using an osmotic compound, such as PEG, on germination medium can decrease seed germination through damaging biochemical processes inside the seeds (Dell'Aquila and Spada, 1992). Water availability in the germination medium must be sufficient for enzyme activation to begin the hydrolysis process of food reserves and finally initiating the germination process (Marcos-Filho, 2016).

Water is one of the limiting factors affecting the germination percentage, uniformity, and speed of seed germination (Marcos-Filho, 2016). Drought stress simulation using PEG 6000 (−0.3 MPa) at the germination stage reduced germination percentage by 44% and uniformity of germination by 46% of 200 genotypes and 25 Indonesian varieties of groundnut (Kasno and Trustinah, 2009). Drought stress simulation using 25% PEG treatment decreased the germination percentage (0%–72%) and root length (56.6%–77.2%) of wheat cultivars (Jatoi *et al.,* 2014). The reduction in germination percentage showed that the seeds need more time to germinate under drought stress (Carvalho *et al.,* 2019). Seedlings that emerged more quickly have a longer time to increase their seedling length and dry matter accumulation than late-emerging seedlings (Queiroz *et al*., 2019). Generally, the application of 10% PEG in the germination medium was followed by a reduction in germination parameters compared with nonstress conditions. Molla *et al.* (2019) showed the difference of germination potency between nonstress and PEG treatment as drought simulation divided by the germination potency under nonstress condition, which is termed as relative PEG injury, was a good parameter to show the effect of PEG treatment on chili at the germination stage. The germination potency of seeds under drought stress conditions also decreased in several other crops, such as corn (Khodarahmpour, 2011; Khayatnezhad *et al*., 2010), oat (Mut *et al.,* 2010), wheat (Zaefizadeh *et al.,* 2011), sorghum (Queiroz *et al.,* 2019), sesame (Keshavarzi, 2012), mungbean (Saima *et al.,* 2018), and cowpea (Murillo-Amador *et al*., 2002).

The reduction in root, shoot, and seedling lengths was due to the disruption of metabolic processes in seeds. Seeds need water to activate enzyme activities and growth regulators, mobilize food reserves, and release energy to start embryonic growth, such as the root and shoot part of seedling (Marcos-Filho, 2016). Seedling growth is inhibited by the presence of PEG in germination medium because of the delay in the water imbibition process, the decomposition process of cotyledon reserves, and the mobilization process of reserves to the embryonic axis of the seeds (Murillo-Amador *et al.,* 2002). The lack of water will delay all metabolic processes in the seed and inhibit the growth of the seed. The reduction in root, shoot, and seedling growth due to drought stress has also been found in several other crops, such as rice (Widyastuti *et al.,* 2017), groundnut (Kasno and Trustinah, 2009), soybean (Kosturkova *et al.,* 2008), maize (Khodarahmpour, 2011), sorghum (Queiroz *et al.,* 2019), chili (Molla *et al.,* 2019), and pea (Okcu *et al.,* 2005).

The increase in root-to-shoot length ratio may be caused by the greater inhibition in shoot length than in root length. This inhibition was caused by the limited transfer of water and nutrients to the shoot and the increased transfer of nutrients to the root, which is the part of the seedling that must grow to reach water. The results of this study showed that the reduction in shoot length was higher than that in root length and fell in the ranges of 50.5%–77.4% and 42.8%–52.1%, respectively (Table 3). Similar results were found for other crops. In corn seedlings, the reduction in shoot length (89.8%) was higher than that in root length (60.0%) under drought stress simulation using PEG 6000 at −1.2 MPa osmotic potential (Khodarahmpour, 2011). In cowpea, the reduction in shoot length (59.0%) was higher than that in root length (30.0%) under drought stress simulation using PEG 6000 at −0.15 MPa osmotic potential (Carvalho *et al.,* 2019). Sunflower seeds germinated under −0.9 MPa did not form radicles, and seeds germinated under −0.6 MPa did not show shoot growth, indicating that the shoots were more sensitive to drought compared to roots (Kaya *et al.,* 2006). In kenaf seed, lateral root did not grow and cotyledons did not expand at 20% PEG 6000 concentration (Tang *et al.,* 2019). Increased root-to-shoot length ratio caused by the greater reduction in shoot length than in root length was also found in wheat (Dhanda *et al.,* 2004) and pea (Okcu *et al.,* 2005). Therefore, shoot length was assumed to be a more sensitive character that was inhibited by drought stress compared with the root.

Farooq *et al.* (2009) stated that plants had several morphological mechanisms against drought stress conditions, such as an escape mechanism by shortening their life cycle and accelerating the reproductive stage and an avoidance mechanism by reducing leaf area to suppress transpiration. Besides, Bambara groundnut plants are known to have several mechanisms of adaptation to drought, such as reducing the canopy growth and leaf area, and increasing root-to-shoot ratio (Chibarabada *et al.,* 2015; Chai *et al.,* 2015). This information showed that Bambara groundnut could survive under drought conditions by reducing the growth of surface area (shoot growth in this study) instead of the extent of the root growth.

The determination of germination parameters that highly contribute to the diversity of the drought tolerance levels of rice genotypes can be carried out using correlation and PCA among all parameters using DSI values (Widyastuti *et al.,* 2016; Akbar *et al.,* 2018). Correlated parameters can then be used in the main component analysis (Afa *et al.*, 2013; Widyastuti *et al.*, 2016). The parameters that are significantly correlated with each other can be used in the evaluation and determination of the drought tolerance levels of the various genotypes. Saima *et al.* (2018) stated that cowpea genotypes can be screened at germination stage by using correlated germination parameters, biomass, and various stress indexes. However, the major parameters must be selected to ensure that the drought tolerance levels have high validity through further analysis using PCA. In this experiment, all parameters, except for root length, showed a significant and positive correlation with each other (Table 6). Therefore, root length was not predicted as a major parameter determining the drought tolerance levels of Bambara genotypes.

PCA is the simple analytical method for reducing datasets that have a large number of parameters while maintaining the diversity of information in the dataset as much as possible (Jolliffe, 2002). PCA has been used to determine major parameters and DSI has been applied to estimate the drought tolerance levels of various genotypes at the germination stage in rice (Afa *et al.*, 2013), corn (Efendi *et al.,* 2009) and lettuce (Liu *et al.,* 2017). Five selected parameters (germination percentage, seed vigor, seminal root length, seedling length, and seminal root dry weight) based on PCA were used to estimate the drought tolerance levels of rice genotypes, and 7 out of 16 genotypes were categorized as tolerant to drought (Widyastuti *et al.*, 2016). Root dry weight was chosen to estimate the drought tolerance levels of 15 corn genotypes under 10% PEG 6000 treatment based on PCA: two genotypes were categorized as drought tolerant, 10 genotypes as drought sensitive, and three genotypes as moderately drought tolerant (Efendi *et al.,* 2009). Liu *et al.* (2017) stated that the three best indicators for identifying the drought-tolerant genotypes of lettuce under 20% PEG 6000 treatment are final germination percentage, relative germination rate, and relative sprout potential. These indicators can be used to categorize the genotypes as tolerant, moderately tolerant, and sensitive to drought.

In this experiment, four parameters (seedling dry weight, shoot dry weight, shoot length, and speed of germination) were chosen to estimate the drought tolerance levels of 10 Bambara groundnut genotypes based on correlation analysis and PCA and resulted in two categories or levels of drought tolerance. Five genotypes (brown Sumedang, black Sumedang, black Gresik, black Tasikmalaya, and Uniswa Red) were sensitive to drought, whereas five other genotypes (black Madura, black Sukabumi, IITA 686, S 19-3, and DODR) were moderately tolerant to drought. The responses and drought tolerance of Bambara groundnut seeds at the germination stage can be differentiated by using 10% PEG 6000 solution in germination medium as the drought simulation condition.

CONCLUSION

Bambara groundnut genotypes can be evaluated and screened at the germination stage using 10% PEG 6000 solution in the germination medium. Through this evaluation, five genotypes were categorized as sensitive to drought (DSI > 1.0) and five genotypes were categorized as moderately drought tolerant (0.5 < DSI ≤ 1) by using seedling dry weight, shoot dry weight, shoot length, and speed of germination as major parameters through PCA. These genotypes need to be evaluated further to know their responses to drought stress at specific growth stages and the suitability of the predicted drought tolerance levels at the germination stage with the ones at the growth stage.

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