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Tolerance and determinants of drought character descriptors of the Madurese landrace bambara groundnut (*Vigna subterranea*)

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Abstract. *Fatimah S, Ariffin, Rahmi AN, Kuswanto. 2020. Tolerance and determinants of drought character descriptors of the Madurese landrace bambara groundnut (*Vigna subterranea*). Biodiversitas 21: 3108-3116.* Bambara groundnut (*Vigna subterranea* L. Verdc.) is legume of African origin overlooked in Indonesia. It has underdeveloped in Indonesia; for example, in East Java, it is only cultivated in Gresik, Lamongan, and Bangkalan of Madura. This plant can potentially be developed in dry lands, such as Madura as it has the ability to grow and develop well in a dry environment with low nutrient level. At present, there are sparse researches on the selection and determination of the drought-tolerant character descriptors of bambara groundnut in Indonesia. The present study used the expected lines of bambara groundnut selected from local lines of various regions in Indonesia using the nested design. The results showed that the drought stress treatment led to stunted growth of 12 bambara groundnut genotypes, including the number of leaves, plant height, canopy diameter, leaf thickness, number of flowers, number of stems or branches, number of internodes, length of leaf stems, root length, root wet weight, canopy wet weight, canopy dry weight, root dry weight, and leaf chlorophyll content. However, drought leads to a slight increase in the width and length of the stomata opening and leaf proline contents. The cluster analysis based on stress index and sensitivity index can classify 3 expected lines originating from Gresik Regency (G1, G2, G3), falling into the drought stress-tolerant category. Accumulated proline contents cannot be used as a descriptor of tolerance to drought stresses in bambara groundnut since the expected lines with an increase in proline contents in leaves during drought stresses based on the cluster analysis do not fall into the genotypic cluster tolerant to drought stresses.

Keywords: Bambara groundnuts, drought stresses, Madura, *Vigna subterranea*

INTRODUCTION

Bambara groundnut (*Vigna subterranea* L. Verdc.) is among the legumes of African origin. In Bogor area of West Java this plant is known as Bogor nut and in Gresik (East Java) it is called peases (Redjeki 2007; Kuswanto et al., 2012). At the "A Field Workshop on bambara groundnut" at the University of Trunojoyo Madura in 2017 the name of "*kacang bambara*" (Bambara groundnut) was agreed for use in research activities. According to Mabhaudhi et al. (2013), this plant has the potential as a substitute for soybeans since its dry seeds contain 17-25% of protein and 46-65% of carbohydrate.

Additionally, this plant also has the potential for cultivation in such dry land as Madura due to its ability to grow in dry environments with low levels of nutrients. According to Berchie et al. (2012), bambara groundnut is able to grow in dry environments with low levels of nutrients. A number of literature describes that bambara groundnut is different from other legumes; this plant is more adaptive and tolerant in less fertile and less watery areas. According to Nakano (2002), bambara groundnut has the best drought resistance among nuts and fruits growing underground.

Until recently, bambara groundnut cultivation in Indonesia has not been widespread and this plant remains underutilized, one of the causes being the low productivity at the farmers' level. Redjeki's study (2003) showed that planting different colored grains resulted in dry seeds of 0.7 to 2.0 tons per hectare, despite this plant's capability of potential yield of 4.0 tons per hectare of dried grains in optimal growing environmental conditions (Kouassi and Zoro-Bi, 2010).

The low productivity at the farmers' level is due to the fact that the local lines are still planted with a high degree of diversity. A study by Kuswanto et al. (2011) showed that of the 50 local lines of bambara groundnut originating from East Java and West Java there were both high inter-line and within-line diversities tested, including the characters of plant growth types, leaf shapes and hairs on the stems. Thus, plant breeding activities are required immediately to improve the local lines, for example by means of purifying potential local lines, followed by the selection program. Furthermore, these lines are expected to be developed into new varieties or as cross parents.

The breeding activities of bambara groundnut through the method of selection of local lines from various regions in Indonesia, including Madura Island, were started since 2012. Those activities began with the collection of local lines, followed by purification activities based on the character of the pods and grains. There was a report of phylogenetic relationships among the local lines of Bogor groundnut (*Vigna subterranea* (L.) Verdc.) and their implications in selection (Nuryati, 2014). Evaluation of the genetic purity of 20 genotypes of Bogor groundnut (*Vigna Subterranea* L. Verdcourt) from the selection of first-stage Single Seed Descent based on the morphological appearance has also been reported (Ainin et al. 2016). The second-stage selection resulted in 17 expected lines with good within-line uniformity (similarity of 80%– 90%) and one line with an excellent within-line uniformity line (similarity of over 90%) (Nugraha, 2015). This indicates that the expected lines from the selection of the second-stage Single Seed Descent can be categorized as pure lines.

Drought evaluation of the expected lines produced is crucial to collect information on their capability to adapt to drought conditions. The morphological and physiological aspects in drought conditions need to be identified in order to determine which expected lines will produce better under drought conditions. The purpose of the present study was to evaluate the potential of the expected lines of bambara groundnut selected in drought conditions.

MATERIALS AND METHODS

The present study was conducted at the Greenhouse of the Faculty of Agriculture, Trunojoyo University Madura from December 2016 to May 2017. It was performed using the nested design with watering as the main plot and the expected lines of bambara as the subplots. Watering as the main plot (P) consisted of 3 levels: (1) 100% field capacity as the control (P0), (2) 75% field capacity (P1), and (3) 50% field capacity (P2). The subplots were the expected lines of bambara groundnut (G) with 12 (twelve) bambara groundnut genotypes (11 expected lines of bambara groundnut selected from the local lines from various regions in Indonesia and one comparison line being a collection of the Plant Breeding Laboratory of the Agriculture Faculty of Brawijaya University Malang (UB Cream) (Table 1). The subplots in each watering stage were replicated three times, so that there were 108 experimental units (12 genotypes x 3 replications x 3 watering levels). Each experiment unit was planted with 6 sample plants, resulting in a total of 648 plant samples being planted.

The characters for observation included morphology, anatomy, and physiology. The morphological characters were the number of leaves (70 days after planting), height of canopy (70 days after planting), diameter of canopy (70 days after planting), leaf thickness (70 days after planting), number of stems or branches (at harvest), number of internodes per stem (at harvest), diameter of internode (the 4^{th} internode 70 days after planting), total number of flowers, petiole length (the 4^{th} internode 70 days after planting), root length (at harvest), canopy wet weight (at harvest), root wet weight (harvest), root dry weight and canopy dry weight. The anatomical characters were stomata density, width of stomata opening, and length of stomata opening. The physiological characters were proline and chlorophyll contents. The data obtained were analyzed by the use of the Analysis of Variance (ANOVA) at the 5% level by using the SAS 9.1 and Microsoft Excel software based on the nested design. In case of any significant effect, the test is continued using the DMRT (Duncan Multiple Range Test) at the 5% level.

Tolerance of bambara groundnut genotypes to drought stresses was calculated based on stress index and sensitivity index values on several morphological characters. The stress sensitivity index values were calculated based on the formula of Fischer & Maurer (1978): $IS = (1-Y / Yp)/(1-X/Xp)$. The sensitivity index criteria could be grouped as $IS \leq 0.5$ (tolerant), $0.5 \leq IS \leq 1$ (moderate), and $IS \geq 1$ (sensitive). The stress index values were calculated using the formula of the observed value under stress conditions divided by the observed value under control conditions. The stress index criteria were $IC > 0.75$, high tolerance (HT); $0.5 \leq IC \leq 0.74$, moderate tolerance (MT) and $IC < 0.5$, low tolerance (LT).

Drought-stress tolerance of the expected lines of bambara groundnut was subjected to cluster analysis based on the Simple Matching Coefficient using the MVSP (Multivariate Statistical Package) 3.22 software.

Table 1. List of the expected lines of bambara groundnut

Code	Names of the expected line	Line origins	Seed colors
G1	GSG 1.1.1	Gresik	Purplish black
G2	GSG 2.4	Gresik	Purplish black
G3	GSG 3.1.2	Gresik	Purplish black
G4	BBL 2.1.1	Lamongan	Purplish black
G5	BBL 6.1.1	Lamongan	Purplish black
G6	CCC 2.1.1	Cianjur	Purplish black
G7	GSG 2.1.1	Gresik	Purplish black
G8	JLB 1	Madura	Purplish black
G9	CKB 1	Madura	Purplish black
G10	TKB 1	Madura	Purplish black
G11	PWBG 5.3.1	Gresik	Purplish black
G12	UB Cream	Collection of the Breeding Lab of the Faculty of Agriculture of Brawijaya University, Malang, Indonesia	Cream

RESULTS AND DISCUSSION

Results of ANOVA showed that drought treatments with different watering (P) significantly affected all observed characters, except for the stomata density and length of stomata opening (Table 2). There were differences in growth among the 12 genotypes (G) in terms of the characters of the number of stems, number of flowers, petiole length, width of stomata opening, length of stomata opening, chlorophyll contents, and leaf proline contents. There was an interaction of genotypes and water treatment (GP) with the characters of the number of branches, number of flowers, width of stomata opening, length of stomata opening, chlorophyll contents, and leaf proline contents.

Drought treatments inhibited the growth of the 12 bambara groundnut genotypes, including the number of leaves, plant height, canopy diameter, leaf thickness, number of flowers, number of stems, number of internodes, petiole length, root length, root wet weight, canopy wet weight, canopy dry weight, canopy dry weight, root dry weight, and leaf chlorophyll contents. However, drought led to an increase in leaf thickness, length and width of stomata opening and leaf proline contents.

Response of bambara groundnut genotypes to drought stresses

Responses of bambara groundnut genotypes to drought stresses were evaluated through different watering (P) with regard to growth and the results served as a drought simulation. The results showed that there were differences in responses to drought stresses among the bambara groundnut genotypes tested, among others in the characters of petiole length, number of stems, number of flowers, length of stomata opening, width of stomata opening, leaf chlorophyll and proline contents. Data on the differences in the growth of the number of branches and flowers among the 12 bambara groundnut genotypes tested are shown in Table 3.

In general, drought stresses inhibited almost all of the observed growth characters. A drought stress treatment by watering 50% of field capacity (P2) led bambara groundnut plants to be shorter; the number of leaves, flowers, stems, and internodes to be smaller; canopy diameter, internode diameter, stem length, and leaf length to be shorter; canopy wet weight and dry weight and root wet weight and dry weight to be lower. Only the character of leaf thickness showed a slight increase. The percentage decrease in the mean value of agronomic characters observed from the 12 bambara groundnut genotypes due to drought stresses ranged from 16.28 to 94.92% (Tables 4 and 5).

Accumulated chlorophyll and proline contents

Measurement of such physiological characteristics as chlorophyll contents is among the approaches to study the effect of water shortages on plant growth since this parameter is closely related to the rate of photosynthesis (Li et al., 2006). Biosynthesis of chlorophyll constitutes one aspect of photosynthesis that is very sensitive to water shortages is (Salisbury and Ross, 1995). Another physiological character frequently used to study plant responses to water shortages is to calculate the accumulated proline contents in leaves. According to Ferrat and Lovatt (1999), accumulated proline contents in plants indicate plants experiencing drought stresses.

Table 2. Results of ANOVA and mean of some quantitative characters of bambara groundnut under different water availability

Characters	Analysis of Variance			Means			Decrease in P2 relative to P0
	Watering (P)	Genotypes (G)	Interactions (PxG)	100% field capacity (P0)	75% field capacity (P1)	50% field capacity (P2)	
Plant height (cm) (70DAP)	**	ns	ns	28.16	21.92	22.83	18.93
Number of leaves (70 DAP)	**	ns	ns	49.58	22.75	19.53	60.61
Canopy diameter (cm) (70 DAP)	**	ns	ns	55.38	44.27	39.29	29.05
Flowers number	**	*	*	154.65	15.35	8.2	94.81
Root length (cm)	**	ns	ns	28.67	23.47	22.55	21.35
Petiole length (cm)	**	*	ns	19.23	17.77	16.07	16.44
Internode diameter (cm)	**	ns	ns	1.49	1.19	1.6	21.99
Internode number per stem	**	ns	ns	17.65	15.69	10.25	41.93
Stems number	**	*	**	10.18	8.59	6.4	31.80
Leaf thickness (mm)	**	ns	ns	0.87	0.97	0.2	5.79#
Root wet weight (g)	**	ns	ns	3.21	0.94	0.9	81.49
Canopy wet weight (g)	**	ns	ns	68.93	33.22	19.42	71.82
Root dry weight (g)	**	ns	ns	1.49	0.82	0..6	68.96
Canopy dry weight (g)	**	ns	ns	27.74	14.83	7.4	73.19
Pod weight (g)	**	ns	ns	3.24	0.18	0.3	99.19
Stomata opening length	**	**	**	4.89	4.38	5.4	5.11#
Stomata opening width	ns	**	**	6.13	6.09	6.2	1.53#
Stomata density	ns	ns	ns	347.58	296.33	303.75	12.61
Chlorophyll (mg g^{-1})	**	**	**	0.18	0.14	0.9	46.96
Proline (μ mol g^{-1})	**	**	**	16.62	16.37	24.51	47.48#

Note: **, * = significant at 1% and 5% levels, ns = not significant, # = increasing

Results of ANOVA in the present study showed that the drought treatment and genotypic treatment affected the leaf chlorophyll and proline contents. This means that there are differences in leaf chlorophyll and proline contents among the 12 genotypes of bambara groundnut. There was an interaction of genotypic and watering treatments (GP) with the characters of leaf chlorophyll and proline contents (Table 6). The drought-stress condition led to a decrease in the leaf chlorophyll contents of bambara groundnut, but the opposite occurred for the leaf proline contents, in which the drought stresses precisely led to an increase in the leaf proline contents of bambara groundnut.

Drought tolerance of bambara groundnut genotypes

Tolerance of bambara groundnut genotypes to drought stress was calculated based on the stress index and sensitivity index values for some of the observed morphological characters. Results of the calculation of the stress index values for characters of root dry weight and canopy dry weight showed that the 12 expected lines of bambara groundnut had the same tolerance to drought stresses, categorized as low resistance. However, calculation of the tolerance index with regard to the characters of root length, number of internodes and canopy diameter showed that the 12 expected lines of the tested bambara groundnut had different tolerances to drought stresses as shown in Table 7.

Calculation of stress index values for the root length character showed that the expected lines originating from Madura (G9 and G10), Gresik (G1 and G11), Lamongan (G5) and Cianjur (G6) had a high tolerance to drought stresses, while others had a moderate tolerance to drought stresses. Calculation of the stress index for the character of internode number showed that the expected lines originating from Madura (G8) and Gresik Regency (G1) had a high tolerance, while others had a moderate and low tolerance. Calculation of the stress index for the character of canopy diameter showed that the expected lines originating from Madura (G9) and Gresik (G2 and G3) had a high tolerance, while others had a moderate tolerance.

Tolerance of bambara groundnut genotypes to drought stresses was also calculated on the basis of stress sensitivity index values for some characters of plant morphology observed. Similar to the results of the stress index calculation, on the basis of the characters of root length, number of internodes, canopy dry weight, root dry weight and canopy diameter, the 12 expected lines of bambara groundnut tested had different tolerances to drought stresses. Results of the calculation of sensitivity index values for the 12 bambara groundnut genotypes for some of the morphological characters observed are shown in Table 8.

The stress index and stress sensitivity index values for some morphological characters of bambara groundnut were subjected to a cluster analysis based on the Simple Matching Coefficient using the MVSP (Multivariate Statistical Package) 3.22 software. The results showed that the expected lines originating from Gresik Regency (G1, G2, and G3) were in the same tolerant cluster. The expected lines originating from Gresik (G11) and (G5) and 2 (two) expected lines originating from Madura (G9 and G10) had a moderate tolerance to drought stresses. The remaining 5 (five) expected lines originating from Lamongan Regency (G4), Cianjur (G6), Gresik Regency (G7), Madura (G8) and from the collection of the Plant Breeding Laboratory of the Faculty of Agriculture of Brawijaya University (G12) were categorized as a sensitive cluster (Figure 1).

Table 3. Number of stems and flowers of the 12 bambara groundnut genotypes under environmental conditions of P0 (100% field capacity), P1 (75% field capacity) and P2 (50% field capacity)

Genotypes	Number of stems			Decrease in P2 relative to P0	Number of flowers			Decrease in P2 relative to P0
	P0	P1	P2		P0	P1	P2	
G1	9.89 e-i	9.00 d-h	7.06 a-d	28.65	191.61 cd	9.94 a	8.00 a	95.82
G2	8.78 c-g	8.44 a-f	6.50 a	25.95	204.83 cd	6.39 a	16.00 a	92.19
G3	10.78 g-i	8.89 c-g	6.78 a-c	3..11	153.72 b-d	13.89 a	14.61 a	90.50
G4	9.83 e-i	8.83 c-g	6.94 a-d	29.38	169.22 b-d	28.39 a	12.39 a	92.68
G5	10.56 f-i	9.78 e-i	6.56 ab	37.89	206.72 cd	27.44 a	10.83 a	94.76
G6	8.06 a-e	8.44 a-f	7.50 a-d	6..0	160.11 b-d	18.50 a	12.83 a	91.98
G7	9.94 e-i	8.06 a-e	7.61 a-d	23.46	228.00 d	15.83 a	1.67 a	99.27
G8	11.22 i	8.78 c-g	6.78 a-c	39.60	146.39 b-d	8.89 a	3.17 a	97.84
G9	11.05 hi	8.72 b-g	6.72 a-c	39.20	166.33 b-d	18.28 a	3.50 a	97.90
G10	14.00 j	8.06 a-e	6.44 a	53.97	118.83 bc	24.56 a	7.33 a	93.83
G11	9.94 e-i	8.06 a-e	7.22 a-d	27.37	88.67 ab	9.39 a	5.67 a	93.61
G12	8.06 a-e	8.11 a-e	7.17 a-d	11.03	21.39 a	2.67 a	0.28 a	98.70

Note: Numbers followed by the same letters on the same variable show no significant difference based on the DMRT test (5%).

Table 4. Percentage decrease in the mean value of agronomic characters of the expected lines of bambara groundnut due to drought stresses (%)

Genotype	Line origin	Plant height	Number of leaves	Lenght of petioles	Canopy diameter	Thickness of leaves*)	Number of stalks	Number of internodes
G1	Gresik	14.85	45.25	9.78	29.86	31.62	28.65	21.87
G2	Gresik	17.69	58.90	7.16	23.81	8..7	25.95	39.50
G3	Gresik	17.99	67.70	4.13	23.78	14.06	37.11	44.55
G4	Lamongan	23.94	51.94	1.85	31.47	12.98	29.38	38.28
G5	Lamongan	15.11	64.87	1.97	24.57	12.73	37.89	63.43
G6	Cianjur	32.22	51.41	1.08	30.87	18.63	6.90	35.17
G7	Gresik	21.17	63.19	2.06	34.62	20.78	23.46	41.95
G8	Madura	15.91	40.84	2.42	33.50	3.5	39.60	11.71
G9	Madura	18.98	70.49	1.29	23.29	0.1	39.20	35.91
G10	Madura	13.98	67.17	1.07	28.86	2.44	53.97	33.37
G11	Gresik	9.5	71.63	1.19	27.90	6.88	27.37	56.45
G12	Lab UB	23.06	60.86	2.37	35.05	2.90	11.03	57.23
Mean		18.69	59.48	1.28	28.97	6.74	30.04	39.95

Note: *) an increase

Table 5. Percentage decrease in the mean value of agronomic characters of the expected lines of bambara groundnut due to drought stresses (%)

Genotipa	Line origins	Internode diameter	Number of flowers	Root length	Root wet weight	Canopy wet weight	Root dry weight	Canopy dry weight
G1	Gresik	19.03	95.82	12.34	86.96	67.28	68.92	65.86
G2	Gresik	1.24	92.19	32.06	74.95	67.36	69.48	63.34
G3	Gresik	18.19	90.50	28.00	86.23	72.81	76.98	71.62
G4	Lamongan	25.60	92.68	30.90	80.49	70.74	60.99	70.28
G5	Lamongan	12.85	94.76	20.27	76.88	75.55	62.71	72.24
G6	Cianjur	24.30	91.98	12.44	69.28	72.85	56.99	74.71
G7	Gresik	28.22	99.27	27.16	84.77	72.49	69.99	73.92
G8	Madura	34.20	97.84	32.73	83.05	68.94	67.27	79.04
G9	Madura	27.10	97.90	3.32	87.62	72.66	78.61	77.62
G10	Madura	19.87	93.83	6.40	80.24	66.58	70.88	72.30
G11	Gresik	17.25	93.61	1.61	72.95	75.05	63.38	75.82
G12	Lab UB	30.70	98.70	36.33	82.40	76.79	73.70	78.41
Mean		21.55	94.92	20.03	80.48	71.59	68.32	72.93

Discussion

In the present study, almost all plants were unable to produce pods, despite the large number of flowers formed (the mean number of flowers was 154.65). According to Berchie et al. (2012), drought tolerances of bambara groundnut are considered as a result of their adaptation to the environmental conditions in which they grow. Although bambara groundnut is a drought-resistant plant, temperatures of above 38°C and a relatively low humidity can disturb the formation of pods, despite the irrigation availability. High temperatures and relatively low humidity are thought to create dry conditions, which can cause flower drying, further inhibiting the growth of pollen tubes and pollen germination. This condition is capable of inhibiting pod production. According to Berchie et al. (2012), bambara groundnut under drought-stress and high-temperature conditions produce few pods.

Results of ANOVA show that genotypic treatments had a significant effect on the characters of the number of stems and flowers. This shows that there are variations in the characters of the number of stems and flowers among the 12 expected lines tested. Under the P0 field capacity condition, the expected lines from Madura (G8, G9, and G10) showed the largest number of stems growing. But under environmental conditions of drought stresses, the expected lines originating from Gresik (G7) and Cianjur (G6) showed the highest number of stems. This means that under environmental conditions of drought stresses the G7 and G6 lines are more adaptable than those expected lines originating from Madura. According to Kusvuran (2012), plant responsiveness to drought stresses varies depending on the stress duration, stress intensity, plant species, and plant growth stage.

Results of the present study indicate that drought stresses lead to a decrease in the mean values of agronomic characters of bambara groundnut. The characters of the number of leaves, number of flowers, root wet weight, canopy wet weight, root dry weight and canopy dry weight are those characters with a quite large decrease (>50%) due to drought stresses. Taiz and Zeiger (2002) argue that the decrease in leaf formation under conditions of drought stresses constitutes a plant mechanism to avoid water loss that would occur when the plants have a large number of leaves. Drought stresses lead to inhibition of division, enlargement and elongation of cells due to the low availability of water contents. This condition causes a decrease in plant water potential due to reduced water diffusion from the soil solution into the plant body, thereby reducing the cell turgor. Similarly, a study by Mahamood et al. (2008) showed that drought stresses at the vegetative phase caused a very large decline in growth and development of soybean plants. In green bean plants (Ranawake et al. 2011) drought stresses at the beginning of the vegetative phase lead to a decrease in plant height, primary root length, number of leaves, number of lateral roots, leaf dry weight, root dry weight and a decrease in plant yield components.

In general, drought inhibited the growth of bambara groundnut. The treatment of watering 50% of field capacity (P2) led the growth of bambara groundnut to be shorter; the number of leaves, flowers, stems, and internodes to be smaller; canopy diameter, internode diameter, leaf stem length and root length to be shorter. Additionally, drought also led canopy wet weight, canopy dry weight and root dry weight to be lower. The decrease in the growth of bambara groundnut due to drought stresses ranged from 16.44% to 94.81%

Salisbury and Ross (1992) argued that water stress would disrupt all plant growth activities, including those physiological, biochemical, anatomical, and morphological activities of the plant. Water stresses lead to the closing of leaf stomata, preventing the entry of CO_2 into the leaves and decreasing photosynthetic activity. Additionally, water stresses also inhibit synthesis of proteins and cell walls. Plants subject to water stresses are generally smaller than those growing normally (Kurniasari et al., 2010). According to Astanto and Trustinah (2009), groundnuts subjected to drought stresses grow shorter and the number of their leaves and filled pods is reduced. According to Khan et al. (2012), the chili plants (*Capsicum annuum* L.) subjected to drought stresses also show a decrease in canopy dry weight and total plant dry weight. The present study showed that the height of bambara groundnut subjected to drought stresses was reduced by 18.93%, the number of leaves decreased by 60.61%, the width of canopy diameter decreased by 28.91% and the number of flowers decreased by 94.81%.

Table 6. Means of chlorophyll and proline contents of the 12 bambara groundnut lines under environmental conditions of P0 (100% of field capacity) and P2 (50% of field capacity)

Genotypes	Chlorophyll contents		Reduction (%)*	Proline contents		Increase (%)**
	Non-stress (100% FC)	Drought stresses (50% FC)		100% of field capacity (P0)	50% of field capacity (P2)	
G1	0.187	0.129	30.80	19.08	21.61	13.27
G2	0.203	0.119	41.57	20.06	20.74	3.2
G3	0.151	0.109	27.79	24.99	23.18	7.1
G4	0.169	0.075	55.35	13.26	21.50	62.16
G5	0.216	0.087	59.63	14.63	26.03	77.91
G6	0.162	0.084	48.36	11.32	26.14	13..83
G7	0.176	0.074	58.20	17.35	28.61	6.86
G8	0.214	0.078	63.65	18.17	22.29	22.71
G9	0.193	0.140	27.17	12.59	21.70	72.38
G10	0.184	0.079	57.04	18.13	19.95	10.06
G11	0.097	0.092	5.44	12.72	21.73	70.80
G12	0.185	0.068	63.45	17.14	40.65	13.18

Note: *Values are the percentage of reduction compared to non-stress conditions; ** Values are the percentage of reduction compared to non-stress conditions

Table 7. Drought tolerance of bambara groundnut based on stress index values

Lines	Line code	Root dry weight		Canopy dry weight		Root length		Internode number		Canopy diameter	
G1	GSG 1.1.1	0.31	LT	0.34	LT	0.88	HT	0.78	HT	0.70	MT
G2	GSG 2.4	0.31	LT	0.37	LT	0.68	MT	0.61	MT	0.76	HT
G3	GSG 3.1.2	0.23	LT	0.28	LT	0.72	MT	0.55	MT	0.76	HT
G4	BBL 2.1.1	0.39	LT	0.30	LT	0.69	MT	0.62	MT	0.69	MT
G5	BBL 6.1.1	0.37	LT	0.28	LT	0.80	HT	0.37	LT	0.75	MT
G6	CCC 2.1.1	0.43	LT	0.25	LT	0.88	HT	0.65	MT	0.69	MT
G7	GSG 2.1.1	0.30	LT	0.26	LT	0.73	MT	0.58	MT	0.65	MT
G8	JLB 1	0.33	LT	0.21	LT	0.67	MT	0.88	HT	0.67	MT
G9	CKB 1	0.21	LT	0.22	LT	0.97	HT	0.64	MT	0.77	HT
G10	TKB 1	0.29	LT	0.28	LT	0.94	HT	0.67	MT	0.71	MT
G11	PWBG 5.3.1	0.37	LT	0.24	LT	1.02	HT	0.44	LT	0.72	MT
G12	UB Cream	0.26	LT	0.22	LT	0.64	MT	0.43	LT	0.65	MT

Note: Stress index criteria: IC> 0.75 high tolerance (HT), 0.5≤ IC≤0.75 moderate tolerance (MT), IC <0.5 low tolerance (LT). The stress index is calculated by the observation value formula under stress conditions divided by the observed value under control conditions

Sinaga (2008) argues that the responses of plants to water stresses are largely determined by the stress levels and the phase of plant growth under stresses. Responses of plants to drought stresses include changes at the cellular and molecular levels, such as changes in plant growth, smaller cell volume, decreased leaf area, thick leaves, presence of hair on leaves, increased root-to-canopy ratio, stomata sensitivity, decreased rate of photosynthesis, changes in carbon and nitrogen metabolism, changes in enzyme and hormone production activity, and changes in expression. Results of the present study indicate that bambara groundnut from Gresik Regency (G11) had the highest decrease in leaf number (71%) and the lines from Madura (G8) showed the lowest decrease in leaf number by 40.84% when the plant was subjected to water stresses (Figure 1). However, the canopy dry weight of lines from Madura (G8) showed the greatest decline in growth (79%) and the lines from Gresik (G2) showed the lowest decline in growth. Underwater stress conditions, the leaves of all the expected lines of bambara groundnut tested were thicker, except for the line from Cianjur (G6) and a line from the collection of the Plant Breeding Laboratory of the Faculty of Agriculture of Brawijaya University (G12). The expected line showing the highest leaf thickening response was the line from Gresik (G1). The responses of the 12 lines of bambara groundnut to water stresses included the number of leaves, canopy dry weight and leaf thickness shown in Figure 2.

Mild to severe drought stresses affect the biochemical processes in cells. Drought stresses can reduce the rate of plant photosynthetic biochemical reactions. One of the most sensitive effects of a decreased plant photosynthesis reaction is a decrease in chlorophyll biosynthesis (Fitter and Hay, 1981). Results of the present study indicate that drought stresses lead chlorophyll contents in bambara groundnut leaves to decrease by 46.96%, but the leaf proline contents increase by 47.487%.

These results are similar to those of Ai (2011) in ginger (*Zingiber officinale* L.), that drought stresses can reduce total chlorophyll contents. According to Kurniawati et al. (2014), water stresses lead to an increase in proline contents in eggplants (*Solanum* spp.). A study by Rosawanti (2015) on soybean plants (*Glycine max* (L.) Merr.) showed that drought stresses lead to lower leaf chlorophyll contents, but higher proline contents. The graph of accumulated leaf chlorophyll and proline contents in 12 lines of bambara groundnut under environmental conditions of watering 50% and 100% of field capacity is shown in Figure 3.

Table 8. Drought tolerance of bambara groundnut based on drought sensitivity index (DSI) values for some morphological characters

Lines	Root dry weight		Canopy dry weight		Root length		Internode number		Canopy diameter	
G1	1.00	Moderate	0.90	Moderate	0.58	Moderate	0.52	Moderate	1.03	Sensitive
G2	1.01	Sensitive	0.87	Moderate	1.50	Sensitive	0.94	Moderate	0.82	Moderate
G3	1.12	Sensitive	0.98	Moderate	1.31	Sensitive	1.06	Sensitive	0.82	Moderate
G4	0.88	Moderat	0.96	Moderate	1.45	Sensitive	0.91	Moderate	1.08	Sensitive
G5	0.91	Moderate	0.99	Moderate	0.95	moderate	1.51	Sensitive	0.85	Moderate
G6	0.83	Moderate	1.02	Sensitive	0.58	moderate	0.84	Moderate	1.06	Sensitive
G7	1.01	Sensitive	1.01	Sensitive	1.27	Sensitive	1.00	Moderate	1.19	Sensitive
G8	0.98	Moderate	1.08	Sensitive	1.53	Sensitive	0.28	Tolerant	1.15	Sensitive
G9	1.14	Sensitive	1.06	Sensitive	0.16	Tolerant	0.86	Moderate	0.80	Moderate
G10	1.03	Sensitive	0.99	moderate	0.30	Tolerant	0.80	Moderate	0.99	Moderate
G11	0.92	Moderate	1.04	Sensitive	-0.08	Tolerant	1.35	Sensitive	0.96	Moderate
G12	1.07	Sensitive	1.07	Sensitive	1.70	Sensitive	1.36	Sensitive	1.21	Sensitive

Note: Drought sensitivity index by Savitri (2010): ICS <0.5 tolerant, 0.5 ≤ ICS≤ 1 moderate, ICS> 1 sensitive. The Sensitivity Index of stress is calculated based on Fernandez (1992):

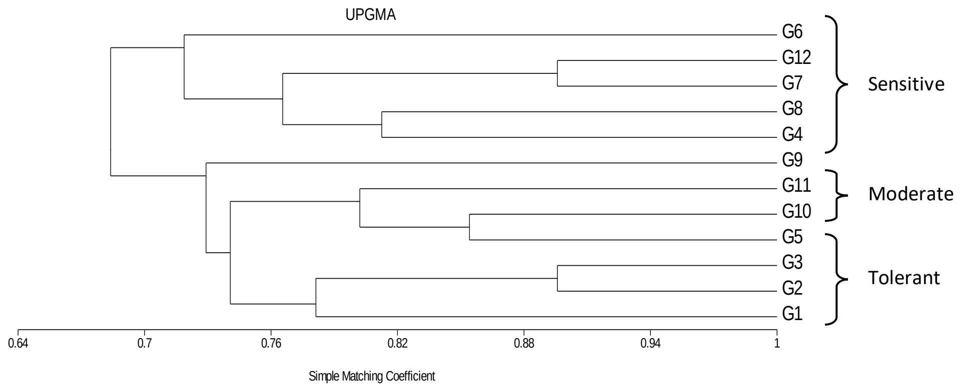
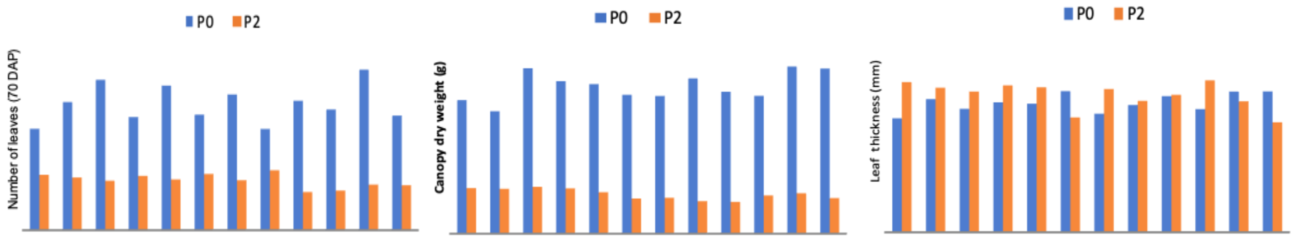
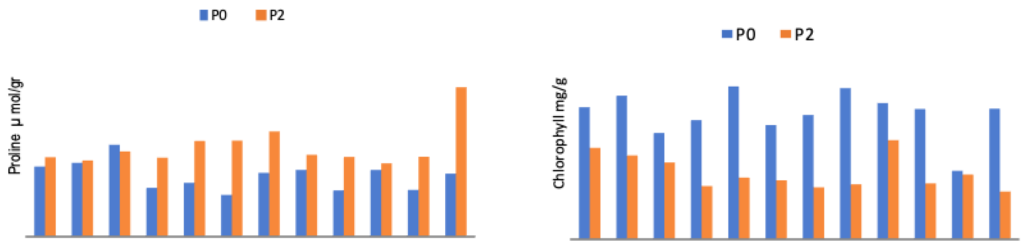


Figure 1. Dendrogram of cluster analysis of the 12 expected lines of bambara groundnut based on the stress index and sensitivity index values



A B C

Figure 2. Growth of leaf number 10 wap (A), dry weight of canopy per plant (B) and leaf thickness (C) of the 12 bambara groundnut genotypes for watering 100% of FC (P0), 75% of FC (P1) and 50% of FC (P2).



A B

Figure 3. Accumulated chlorophyll and proline contents in the 12 genotypes of bambara groundnut under conditions of watering 100% of FC (P0), 75% of FC (P1) and 50% of FC (P2)

The response of bambara groundnut to water stresses is shown by an increase in proline compounds serving as osmoprotectants to maintain osmotic potential in plants. According to Yoshida et al. (1997), water stresses lead to proline contents in tolerant plants to increase higher than those in sensitive plants. Results of the present study showed that the line from the collection of the Plant Breeding Laboratory of the Faculty of Agriculture of Brawijaya University (G12) had the highest increase in proline contents, water stresses (P2) increased proline content by 137.18% relative to those plants without drought stresses (P0). However, drought stresses led the proline contents in the line from Gresik Regency (G3) to decrease by 7.2% relative to those plants with no drought stresses (Table 6).

According to Riduan et al. (2005), tolerance of a number of groundnut cultivars to drought stress varies. Results of the analysis of tolerance to drought stresses based on the calculation of stress index values showed that there were differences in tolerability among the 12 expected lines of bambara groundnut tested (Tables 7 and 8).

Suryanti et al. (2015) argue that cluster analysis can be used to classify plant resistance to drought stresses. Results of their study showed that cluster analysis is able to group 18 soybean cultivar resilience to drought stress into 3 clusters of criteria: lines resistant, moderately resistant, and not resistant to drought stresses.

The present study used cluster analysis to determine the clustering of bambara groundnut line tolerance to drought stresses based on the stress index and stress sensitivity index values for several morphological characters. The cluster analysis could group the 12 bambara groundnut lines into 3 groups: tolerant, moderate, and sensitive. Five (5) expected lines (G4, G6, G7, G8, and G12) fall into the sensitive category and four (4) expected lines fall into the moderate, namely those lines from Lamongan Regency (G5), two lines from Madura (G9 and G10) and one line from Gresik Regency (G11). The expected lines of bambara groundnut falling into the drought-tolerant category are all those lines that come from Gresik Regency (G1, G2, and G3).

Results of the cluster analysis also show that the expected lines with high proline content, when exposed to drought, do not belong to the cluster of tolerant lines. The proline contents of the expected lines from Lamongan (G4) increased by 62.16%, the lines from Cianjur (G6) increased by 130.87%, the lines from Gresik Regency (G7) increased by 64.86%, and the lines from the collection of the Plant Breeding Laboratory of the Faculty of Agriculture of Brawijaya University (G12) increased by 137.18%. This shows that the accumulated proline contents in bambara groundnut leaves cannot be used as a descriptor of tolerance to drought stresses since the expected lines with an increase in proline contents in leaves during drought stresses, based on the cluster analysis, fall into the drought stress-sensitive cluster, rather than falling into the drought stress-tolerant cluster.

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