ISSN : 1812-5379 (Print)
ISSN : 1812-5417 (Online)
http://ansijournals.com/ja

JOURNAL OF AGRONOMY

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS

Journal of Agronomy

ISSN 1812-5379
DOI: 10.3923/ja.2018.136.146

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Research Article

Genotypic Responses of Brachiaria Grass (*Brachiaria* spp.) Accessions to Drought Stress

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Abstract

Background and Objective: Brachiaria, a warm season C4 grass, is rapidly gaining popularity as fodder crop in Africa where it is also used as a component of a habitat management strategy for maize stem borers. However, increasing drought limits productivity of this grass species. The aim of this study was to evaluate and select high yielding brachiaria genotypes under simulated drought conditions. **Materials and Methods:** The morphological and physiological performance of 18 apomictic accessions of brachiaria in simulated drought conditions in a screen house were evaluated. Plants were exposed to different watering regimes. Well-watered (control) plants were watered every 48 h to 100% field capacity while drought was simulated by suspending watering for 14 and 28 days, representing moderate and severe drought conditions, respectively. Shoot length, leaf length and width, number of tillers, leaf relative water content, chlorophyll content and above ground biomass were studied. **Result:** Water stress had negative effects on the morphological and physiological traits, with the effects being more pronounced under severe drought stress. Based on the drought stress index (DSI) values for the measured parameters and Principal Component of Analysis (PCA) biplots, the following accessions were least affected under severe drought stress: 'Xaraes', 'Piata', 'Marandu', 'CIAT 679', 'Mulato II' and 'Mulato I'. **Conclusion:** Under increasing drought conditions, biomass yield was an accurate predictor of drought tolerance of the genotypes. 'Piata' and 'Xaraes' combined both drought tolerance and biomass yield. These two accessions were proposed as of value in improvement of the sustainability of cereal-livestock farming systems under conditions of increasing aridification.

Key words: Brachiaria, drought stress index, drought tolerance, genotypic variation

Citation: Duncan Cheruiyot, Charles Aura Odhiambo Midega, Johnnie Van den Berg, John Anthony Pickett and Zeyaur Rahman Khan, 2018. Genotypic responses of brachiaria grass (*Brachiaria* spp.) accessions to drought stress. J. Agron., 17: 136-146.

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Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Drought poses one of the most important environmental constraints to plant growth and productivity[1,2]. Plants primarily respond to drought by arresting growth. This reduces metabolic demands and mobilizes metabolites for the synthesis of protective compounds[3,4]. In some plants, exposure to drought stress leads to changes in carbon partitioning between the source and the sink, resulting in reduced photosynthesis and an associated decrease in chlorophyll content[5-7]. Numerous metabolic and physiological processes within the plant are also affected[8]. For cultivated plants, tolerance to drought is generally considered as the potential of a species or variety to yield more in comparison to others under limited water conditions[2]. Drought tolerance is a highly complex trait that involves multiple genetic, morphological, physiological and biochemical mechanisms[9,10].

Brachiaria spp. are perennial C4 plants, native to Africa[11]. There are more than 14 species in the world, mostly tetraploid ($2n = 4x = 36$) and apomictic. Their progenies are uniform, produce high dry matter and persist on poor acid soils[12,13]. They form natural constituents of grasslands in eastern, central and southern Africa[14,15]. These grasses also play an important role in cultivated pastures in tropical America[16], South-east Asia[17] and East Africa[15,18]. In addition to its use as a forage crop, *B. brizantha* cv Mulato II, henceforth referred to as 'Mulato II', has gained large uptake in East Africa where the grass has been incorporated as a trap plant in the 'push-pull' pest management system[19,20]. This system was developed for management of cereal stem borers by exploiting behaviour-modifying stimuli to manipulate pest and natural enemy behavior and reduce pest infestations[21-24].

According to Guenni *et al.*[25], most *Brachiaria* spp. respond to induced mild drought conditions through adjusted growth and biomass allocation, leaving the total plant yield relatively unaffected. In previous studies, 'Mulato II' has been observed to tolerate extended periods of drought of up to three months with limited water availability and temperatures of 30°C and higher[26]. Their apomictic nature enables brachiaria to produce seeds which are true to type and can colonize a wide range of habitats[27]. This phenomenon preserves the vigor of the plant across environments. Such C4 plants possess greater competitive ability than their C3 counterparts under dry and high irradiance conditions such as those that are common in tropical grasslands and savannas[28-30]. This competitive advantage is brought about by the ability of C4 species to maintain greater photosynthetic rates per unit of water loss than C3 species[30,31]. Nevertheless, water availability still dictates the maximum yields achieved by C4 plants such as brachiaria.

The purpose of this study was to provide an understanding of the morphological and physiological responses of different brachiaria accessions to different drought stress regimes. The aim of the study was to identify putative drought tolerant brachiaria genotypes for utilization in specifically improving cereal-livestock productivity through management of stem borers amid the increasing threat of climate change.

MATERIALS AND METHODS

Plant materials: Seeds of brachiaria plants were sourced from International Center for Tropical Agriculture (CIAT), Columbia and International Livestock Research Institute (ILRI), Ethiopia. These accessions were grown in an on-station nursery at the International Center of Insect Physiology and Ecology-Thomas Odhiambo Campus (ITOC), Mbita Point (0°25 S, 34°12 E, 1200 m above sea level) in western Kenya for observation and pre-selection of candidate accessions. Eighteen accessions were selected for further evaluation based on desirable agronomic performance. These accessions were *Brachiaria decumbens* cv Basilisk, *B. brizantha* cv Cayman, *B. brizantha* cv Marandu, *B. brizantha* cv Piata, *B. brizantha* cv Xaraes, *B. brizantha* cv Mulato II, *B. brizantha* cv Mulato I, *B. brizantha* (ILRI 11553), *B. brizantha* (ILRI 12991), *B. brizantha* (ILRI 12995), *B. brizantha* (ILRI 13344), *B. brizantha* (13368), *B. brizantha* (13648), *B. humidicola* (CIAT 679), *B. brizantha* (ILRI 13497), *B. brizantha* (ILRI 13810), *B. brizantha* (ILRI 13545) and *B. brizantha* (ILRI 14807). The commercial hybrid, 'Mulato II' was included as control treatment due to its previous use as a trap crop in a 'push-pull' strategy and due to the fact that it had been reported to produce comparatively high fodder yield under conditions of drought stress[19,24].

Experimental site and procedure: The experiment was conducted in a screen house at ITOC in the year 2016. Over the period during which the experiment was conducted, mean minimum and maximum daily temperatures in the screen house were 18 and 35°C, respectively. The soil used in the experiment was well drained alluvial and sandy loam classified as Chronic Vertisols[32]. Before planting, field capacity of potting soils was determined as described by Somasegaran and Hoben[33].

The different accessions were planted in plastic bags measuring 60 cm deep and 26 cm wide with holes at the bottom. Bags were filled with fine air-dried soil leaving a space of 5 cm from the top. The bags were then placed on 30 cm high benches covered with metallic mesh. The plastic bags provided a plant biomass to pot volume ratio of less than 2 kg m^{-3} as recommended by Poorter *et al.*[34]. This is crucial in minimizing both the risks of having reduced plant growth which may influence the relative differences between treatments. The experimental setup followed a complete randomized design (CRD) in a factorial arrangement (3×18) with three replicates.

Five seeds were planted in each bag and later thinned to two plants per pot when most of the seedlings had four expanded leaves. Watering was done by adding 244 mL of water to all plant bags every 48 h, to restore the soil moisture to 100% field capacity until the commencement of the water restriction period. At three weeks after planting (WAP), the plants were top dressed with 60 kg N ha^{-1} in the form of Calcium Ammonium Nitrate (CAN). Leaves of plants were also trimmed to standardize their heights at 10 cm. Application of the different watering treatments commenced 25 days after trimming. There were three treatments: Well-watered control, moderate drought stress and severe drought stress. The bags in the control group (without water restriction) continued to receive water to 100% field capacity, every 48 h throughout the experiment, while watering was not done for a period of 14 and 28 days for the moderate and severely stressed treatments, respectively.

Data collection: Data were collected 14 and 28 days after water restriction commenced to represent moderate and severe stress regimes. At each sampling period, one plant was randomly sampled from both stressed and control plants. The numbers of tillers per pot were counted. Shoot length (SL) was measured from the surface of the soil to the tip of the youngest fully expanded leaf. Leaf area of the second fully expanded leaf was measured from the tip to the junction of the petiole and the width of the leaf at its widest part. Leaf chlorophyll content was measured by means of a SPAD chlorophyll meter (SPAD-502 Plus, Konica Minolta Sensing Inc., Japan) and presented as SCMR index values. Ten measurements were done on the second fully emerged leaf of each plant. Leaf relative water content was estimated following the procedure used by Chen *et al.*[35]. The youngest fully expanded leaf was removed and weighed immediately to determine fresh weight (FW). Turgid weight (TW) was determined after leaf segments were immersed in distilled water for 6 h and dry weight (DW) was measured after leaf segments were dried at 70°C in an oven for 24 h. Each treatment was replicated three times. The relative water content (RWC) was calculated as follows:

$$\text{RWC} = \frac{\text{FW}-\text{DW}}{\text{TW}-\text{DW}} \times 100$$

The above ground dry biomass (BM) was determined after harvesting all the shoots per plant and drying it at 65°C for 48 h.

Statistical analysis: Data were checked for homogeneity of variance between treatments using Bartlett's test. A two-way ANOVA was used to test for treatment differences, genotypic effects and interactions for each stress regime. Significance of differences between the genotypes was tested by F-test, while the treatment means were compared by least significant differences (LSD) at $p = 0.05$. Simple correlation coefficients among traits were determined using mean trait values for genotypes, following Pearson's correlation method. These analyses were performed using R software (Version 3.3.1)[36]. Drought Stress Index (DSI) values were used to compare the responses between individual accessions, based on the difference between stress treatments and the control plants. The values were calculated as follows:

$$\text{DSI} = \frac{\text{Value of trait under stress condition}}{\text{Value of trait under control condition}} \times 100$$

According to Wojcik-Jagla *et al.*[37], this equation removes the effect of germplasm variation from the drought stress treatment and can therefore be used to assess a large collection of germplasm simultaneously. Biplots of principle components derived from DSI values of each trait were used to comprehensively identify stress tolerant accessions, i.e., those that were least affected by the stress treatments. This was computed by use of Microsoft XLSTAT software[38].

RESULTS

At moderate drought conditions, effects of soil moisture regimes were significant ($p \leq 0.05$) for all the traits except shoot length and tiller numbers. Genotypic effects were significant for all the traits, while interactions between soil moisture and genotype was only significant ($p \leq 0.05$) for relative water content and plant biomass. There was a general decrease in mean values of traits between the control and stressed plants for both stress regimes (Table 1). Genotypic effects were also more pronounced except for chlorophyll content, while genotype x treatment effects were only significant ($p \leq 0.05$) for number of tillers and relative water content. Exposure to severe stress resulted in the highest percentage reduction in relative water content (55.8%) and the lowest percentage reduction in SPAD readings (13.7%) (Table 1).

Table 1: Significance of treatment, genotype and genotype-treatment effects for traits in 18 brachiaria accessions grown under moderate and severe drought stress conditions in a screen house conditions

						Average		Minimum		Maximum		
Traits	T (df = 1)	G (df = 17)	G×T (df = 17)	CV (%)	R^2	Stress	Control	Stress	Control	Stress	Control	Reduction (%)
Moderate stress												
Shoot length (cm)	ns	**	ns	12.6	83	77.5^{a}	80.6^{a}	35.6	39.9	125.6	136.5	3.8
Leaf surface area (cm^2)	**	**	ns	18.3	75	55.3^{a}	64.0^{b}	14.5	15.0	100.2	106.5	13.7
Tiller number	ns	**	ns	20.5	76	9.2^{a}	9.3^{a}	3.0	4.0	17.0	20.0	1.1
SPAD readings	**	**	ns	11.8	61	36.0^{a}	38.5^{b}	23.5	27.3	51.0	50.9	6.2
Relative water content	**	**	**	8.4	58	77.0^{a}	81.7^{b}	50.7	61.6	93.8	97.6	5.1
Biomass (g)	*	**	**	20.9	86	10.6^{a}	11.6^{a}	2.5	3.1	20.2	25.3	8.6
Severe stress												
Shoot length (cm)	**	**	ns	11.6	88	83.9^{a}	98.1^{b}	56.2	66.7	154.4	160.7	14.4
Leaf surface area (cm^2)	**	**	ns	25.1	74	48.7^{a}	61.5^{b}	29.4	35.5	123.2	128.2	20.9
Tiller number	**	**	*	23.0	83	10.6^{a}	17.2^{b}	5.0	7.3	17.7	30.7	38.0
SPAD readings	**	ns	ns	19.1	44	38.6^{a}	44.7^{b}	28.3	34.1	45.6	58.8	13.7
Relative water content	**	**	*	17.0	89	34.8^{a}	78.6^{b}	21.6	63.8	57.8	89.3	55.8
Biomass (g)	**	**	ns	23.0	79	18.5^{a}	29.7^{b}	9.7	14.7	30.7	41.6	37.8

*Significant at $p \leq 0.05$, **Significant at $p \leq 0.01$, Abbreviations: T, treatment, G, genotype, CV, coefficient of variation, R^2, coefficient of determination, ns, non-significant. Means followed by the same letters within a row are not significantly different (Fisher's LSD $p \leq 0.05$)

Morphological and physiological characteristics of brachiaria genotypes: Mean values for each measured trait of the different accessions under the different stress treatments were presented in Table 2. Under moderate stress, the shortest shoots were recorded in 'Mulato II' (42.8 cm), 'Mulato I' (50.9 cm) and 'Cayman' (56.5 cm), whereas CIAT 679 recorded the longest shoots with 111.9 cm, followed by ILRI 11553 (92.7 cm) and 'Xaraes' (90.6 cm). The lowest ranking accessions under severe stress conditions were ILRI 13648 (56.2) and 'Mulato II', while CIAT 679 maintained its rank having the longest shoots (154.4 cm). Although there was a general decrease in shoot length due to drought stress in comparison with control plants, 'Basilisk', CIAT 679 and ILRI 13545 continued to grow despite the moderate drought conditions.

Leaf area of all the genotypes was reduced under conditions of both moderate and severe stress (Table 2). There was a clear variation in leaf area with 'Xaraes' having the largest leaves (100.2 cm^2) followed by 'Piata' (75.2 cm^2) and ILRI 13810 (71.3 cm^2). The smallest leaf areas were recorded for CIAT 679 (14.5 cm^2) and ILRI 14807 (34.6 cm^2). Under severe stress, 'Xaraes' maintained its rank with a leaf area size of 123.3 cm^2 followed by 'Mulato II' (74.7 cm^2) and 'Piata' (72.7 cm^2). The smallest leaves were observed in ILRI 13344 (29.4 cm^2) and CIAT 679 (30.7 cm^2).

The highest number of tillers per plant under moderate stress conditions was recorded in ILRI 13545 (16.0) (Table 2). CIAT 679 and 'Marandu' had the lowest numbers of 5.0 and 6.0 tillers, respectively. Notably, despite an overall decrease in the number of tillers under moderate drought stress, 44% of the genotypes displayed an increase in mean number of tillers per plant. 'Basilisk', 'Piata', 'Mulato II, ILRI 11553, ILRI 12995, ILRI 13648, CIAT 679 and ILRI 13497 produced more tillers when exposed to moderate stress than to severe stress. However, growth under severe drought conditions for 28 days resulted in reduced tillering in all accessions. ILRI 13497 (5.0) and CIAT 679 (6.0) had the lowest number of tillers while the high-ranking counterparts were ILRI 13368 (17.7), ILRI 13545(16.3) and 'Mulato II' (14.7).

Estimated chlorophyll content (SCMR index) was generally reduced when plants were subjected to moisture stress (Table 2). At moderate stress levels, the highest SCMR index values were recorded in 'Mulato I' (48.7). CIAT 679 had the lowest SCMR values of 28.3 under moderate stress conditions. Accessions that recorded the highest SCMR values under severe water stress were ILRI 14807 (45.6), 'Piata' (41.4) and 'Mulato I' (41.4), whereas ILRI 13545 (28.3) had the lowest.

Both regimes of moisture stress generally reduced the leaf relative water content (Table 2) of the leaves, but the levels of reduction varied with the genotypes. Among the genotypes that recorded high relative water content under moderate stress were ILRI 13344 (86.0), ILRI 13648 (85.9) and ILRI 13545 (85.5). On the other hand, ILRI 14807 and 'Basilisk' are among those that recorded low relative water content values of 68.6 and 68.9, respectively.

Severe drought induced a greater level of relative water content reduction in all the genotypes, with ILRI 13648 (57.8), ILRI 13497 (49.9), ILRI 13368 (21.5) and 'Mulato II' (22.7) recording the lowest relative water content (Table 2). Among the high biomass yielders at moderate stress levels were ILRI 13368 (18.1 g), ILRI 12995 (18.0 g) and 'Piata' (17.9 g).

Table 2: Means of traits in control and drought stressed brachiaria plants grown under moderate drought stress and severe drought stress under screen house conditions

	Shoot length (cm)		Leaf area (cm^2)		Number of tillers		SCMR index		Relative water content		Biomass (g)	
Accession	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress
Moderate drought stress												
Basilisk	59.1	61.0	73.4	66.0	9.3	10.7	36.7	35.5	73.7	68.9	7.5	5.3
Cayman	57.0	56.5	78.6	65.1	7.3	4.3	44.1	40.8	87.5	78.0	12.7	7.3
Marandu	64.0	61.8	78.7	69.2	10.0	6.0	40.3	38.1	77.2	76.4	21.2	12.3
Piata	80.7	79.8	80.3	75.2	7.67	8.0	39.4	39.3	77.8	75.0	14.2	17.9
Xaraes	91.9	90.6	106.5	100.2	10.0	10.0	34.4	33.6	87.3	82.7	14.1	13.8
Mulato II	43.9	42.8	66.6	57.8	10.7	11.0	35.1	33.6	88.2	83.3	16.4	13.0
ILRI 11553	96.5	92.7	53.9	50.7	8.7	11.3	33.4	32.9	81.6	80.2	7.3	15.0
ILRI 12991	92.8	90.2	61.9	44.0	10.7	9.3	37.3	34.3	88.0	85.5	17.6	7.0
ILRI 12995	86.1	82.9	48.9	38.0	9.3	10.3	35.7	33.9	77.1	74.4	14.1	18.0
ILRI 13344	111.8	87.4	72.9	56.9	12.0	10.0	39.1	35.3	86.9	86.0	19.4	14.5
ILRI 13368	86.7	84.5	59.2	50.0	12.0	10.7	36.6	33.0	75.2	73.4	10.9	18.1
ILRI 13648	93.4	82.2	64.2	53.2	6.3	10.0	39.3	35.2	93.6	85.9	6.7	9.9
CIAT 679	109.4	111.9	15.0	14.5	4.3	5.0	31.6	28.3	81.5	70.5	4.0	6.2
ILRI 13497	90.0	78.3	61.3	53.0	6.3	7.0	36.2	34.5	87.0	70.9	6.6	3.7
ILRI 13810	89.2	88.6	80.7	71.3	8.3	8.0	42.0	37.6	80.6	77.6	9.4	5.1
ILRI 13545	68.6	69.6	42.1	37.2	17.3	16.0	39.7	34.6	85.7	85.5	11.2	7.8
ILRI 14807	87.4	83.4	41.3	34.6	8.0	7.7	41.7	40.2	68.6	68.6	9.8	7.1
Mulato I	52.4	50.9	67.0	57.9	10.0	9.7	50.1	48.7	73.3	72.2	6.4	8.7
LSD (5%)	17.5	14.8	20.0	16.3	3.1	3.3	6.9	7.5	10.1	13.2	4.4	3.0
CV (%)	13.1	11.5	18.8	21.6	20.0	21.6	10.8	12.5	7.4	10.2	22.7	17.0
Severe drought stress												
Basilisk	97.2	87.5	61.6	49.1	20.3	17.0	38.8	38.8	81.9	30.4	31.3	12.7
Cayman	77.9	67.5	70.1	56.7	18.0	7.0	39.8	39.7	73.9	25.1	27.3	9.7
Marandu	78.8	68.7	61.2	49.4	13.0	9.0	40.6	37.6	77.9	38.9	25.0	13.3
Piata	102.1	94.5	80.8	72.7	10.0	7.0	44.1	41.4	88.7	35.5	38.3	30.7
Xaraes	99.1	89.2	128.2	123.2	17.7	13.7	40.4	36.8	89.3	46.0	41.7	30.0
Mulato II	66.7	57.0	46.9	40.2	19.3	14.7	40.9	39.4	63.8	22.7	33.3	19.7
ILRI 1553	111.2	87.8	50.5	41.0	16.3	12.7	40.0	36.9	77.9	29.6	36.3	22.0
ILRI 12991	108.1	77.9	83.4	36.0	13.3	8.3	54.7	39.9	76.8	37.1	29.7	23.0
ILRI 12995	99.0	86.2	50.0	38.2	23.0	9.0	45.6	40.9	72.2	40.3	25.0	14.3
ILRI 13344	100.6	91.4	46.9	29.4	22.7	14.3	49.0	40.0	83.0	23.0	33.7	19.3
ILRI 13368	116.0	100.1	50.9	34.3	24.7	17.7	44.3	37.9	75.0	21.5	33.7	26.7
ILRI 13648	71.0	56.2	68.7	59.5	9.3	7.0	45.0	34.9	68.4	57.8	16.7	15.3
CIAT 679	160.7	154.4	35.5	30.7	7.3	6.0	34.1	37.0	84.8	36.3	14.7	11.0
ILRI 13497	106.3	93.9	50.5	36.0	17.0	5.0	49.3	39.6	86.1	49.9	33.3	15.0
ILRI 13810	109.1	83.3	60.7	36.5	15.0	11.7	58.7	38.5	86.2	34.9	17.7	11.7
ILRI 13545	77.2	57.2	40.0	35.3	30.7	16.3	51.7	28.3	82.4	30.2	36.3	21.3
ILRI 14807	114.0	97.3	39.5	33.4	17.0	6.0	44.6	45.6	76.0	36.0	30.7	19.7
Mulato I	70.7	60.3	81.7	74.7	14.7	9.3	43.1	41.4	73.6	30.3	30.0	17.3
LSD (5%)	16.7	16.3	27.3	14.9	6.4	4.4	13.1	13.6	13.2	18.9	11.1	7.6
CV (%)	10.3	11.7	27.1	18.5	22.5	25.1	17.7	21.1	10.1	32.7	22.4	24.9

Under severe stress conditions, all the tested genotypes recorded lower biomass yield compared to their counterparts under no water stress. Nonetheless, outstanding accessions with regard to biomass yield under severe drought stress were 'Piata' (30.7 g) and 'Xaraes' (30.0 g), closely followed by ILRI 13368 (26.7 g).

Correlation analysis between traits: Under moderate stress, the only significant correlation was observed between SCMR index values (chlorophyll content) and shoot length ($r = -0.6$, $p<0.05$). Under severe stress, there was a significantly positive correlation between biomass yield and leaf area ($r = 0.5$, $p<0.05$) and a significant negative correlation between relative water content and the number of tillers per plant ($r = -0.6$, $p<0.05$). Correlations between other traits under both stress regimes were however not significant.

PCA analysis based on drought tolerance indices (DSI) values: Principal component analysis (PCA) based on DSI values of the traits were plotted in a Biplot to better

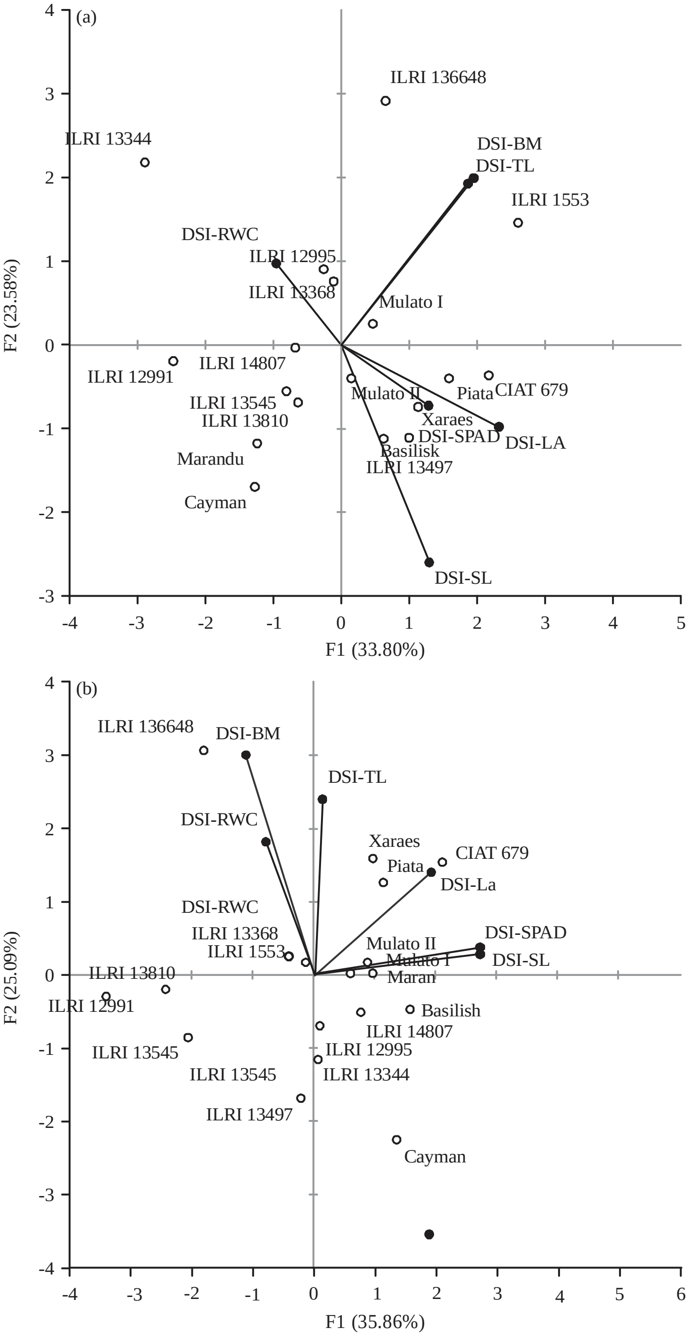

Fig. 1(a-b): Principle component analysis biplot of the drought stress index (DSI) of five physiological and morphological traits of 18 Brachiaria genotypes under, (a) Well-watered (control) vs moderate (14 days) drought stress and (b) Well-watered vs severe (28 days) drought stress. Arrows represent the traits with various length based on the impact of each trait on the discrimination between genotypes. The 18 Brachiaria grasses were categorized into three groups. Based on the DSI values, the best performers are in group I (upper right) and intermediates are in group III (lower right) while the poor performers (group III) are in both the upper and lower left. DSI, drought stress index, BM, biomass, LA, leaf area, SCMR, SPAD chlorophyll meter index, SL, shoot length, TL, tillers

understand the relationships among the drought stress indices and the levels of drought tolerance exhibited by different brachiaria accessions (Fig. 1). The PCA converted the traits into six different factors and Eigen values. Under moderate stress, factor 1 accounted for 33.80% of the variation and showed the largest loading values, followed by factor 2 with 23.58%. Under severe drought stress, factor 1 accounted for 35.86% of the variation while factor 2 accounted for 25.09%.

The relationships between indices are illustrated by arrows (axis). The cosine of the angle between the vectors of two indices approximates the correlation coefficients between them, which reflects on the interrelationships among the morpho-physiological indices. An acute angle depicts a positive correlation, while obtuse angle shows a negative correlation. The projection of the traits from the biplot origin shows the impact of that trait on separation of the accessions. The biplot of DSI traits under moderate stress (Fig. 1a) showed no outstanding trait that separated the genotypes. However, under severe stress (Fig. 1b), biomass yield made the largest contribution since it had the largest projection. With regard to genotypic performance under drought conditions, the 18 brachiaria grasses were categorized into three groups. The best performers are indicated in group I (Fig. 1b, upper right quadrant), intermediate performers in group II (Fig. 1b, lower right quadrant), while the poor performers (group III) grouped in the upper and lower left quadrants. Under moderate stress conditions, ILRI 13648, ILRI 11553 and 'Mulato I' emerged as the best performers, while the performance of CIAT 679, 'Piata', 'Xaraes', 'Mulato II', ILRI 13497 and 'Basilisk' can be described as intermediate. Under severe drought stress, the best performers were 'Xaraes', 'Piata', CIAT 679, 'Marandu', 'Mulato II' and 'Mulato I' while the intermediate performers were ILRI 14807, 'Basilisk', ILRI 12995, ILRI 13344 and 'Cayman'.

DISCUSSION

Drought stress is one of the most important factors that limit plant growth and reproduction. Although C4 grasses such as brachiaria show great adaptability to water stress conditions, water availability is still critical in determining the productivity of such grasses and wide variability has been found in their response to prolonged periods of drought[39]. The results of this study showed that drought stress had marked effects on morphological (shoot length, leaf area, number of tillers and biomass yield) and physiological (relative water content and chlorophyll content) characters of brachiaria grasses. Under moderate drought stress, the observed reductions in shoot length and number of tillers were not significant, which implies that even under conditions of low stress, it is still possible to select drought tolerant genotypes based on all traits except shoot length and tillering.

Drought stress resulted in a significant reduction in leaf area, though under moderate drought stress, the reduction in leaf area was largely ascribed to leaf rolling. Leaf rolling is a common symptom of drought stress and is an expression of leaf turgor and plant water content[40]. Stomatal opening and closure responses to evaporative demand (usually higher at noon) and soil water content, lead to changes in leaf turgor[41]. Results of this study which indicate reduced leaf area brought on by drought stress are similar to those reported by Santos *et al.*[42] for other brachiaria accessions. Leaf expansion generally depends on leaf turgor, temperature and assimilating supply for growth. Drought also suppresses leaf expansion by reducing photosynthesis[43]. Chlorophyll content is a sensitive and easily measurable trait that could be used to screen for stress tolerance among genotypes[44]. In the present study, reduction in chlorophyll content due to water stress was evident. Studies on barley showed that chlorophyll content was significantly reduced in plants exposed to drought stress[45]. Even though crucial plant processes such as cell division and cell expansion are the earliest to be affected by water deficit[46], degradation of chlorophyll may arise due to sustained photo-inhibition and photo-bleaching[7,47]. There were no effects of the moderate drought on shoot length but under severe drought, the reduction was significant. Reduction in shoot growth due to drought stress was also reported in another brachiaria accession, 'Marandu'[48]. This reduction may be attributed to progressive water stress that result in reduced plant height which is attributed to a decline in the cell enlargement[49] and other processes such as cell division and cell expansion[45]. Leaf relative water content in drought stressed brachiaria plants declined significantly compared to values recorded in control plants. Similar findings from studies with brachiaria have also been reported by Guenni *et al.*[25]. Maintenance of relative water content is essential in provision of turgor for cell enlargement and growth in plants[4]. Therefore, leaf relative water potential may serve as an indicator of plant water status, as well as the ability of a plant to maintain adequate water status which improves drought adaptability by enhancing drought tolerance[50,51].

Moreover, emphasis has been put on responsiveness of relative water content to drought stress and its reliability in distinguishing drought tolerant and susceptible genotypes[44]. Moderate drought stress was not sufficient to affect tillering since there was no significant difference between the numbers of tillers of plants under moderate stress and those of the control. Following severe stress, the number of tillers were significantly reduced. These results confirm those of El-Rawy and Hassan[52] who observed a reduced number of tillers in wheat (*Triticum aestivum*) in response to drought. According to De Barros Lima *et al.*[53], reduced tillering in plants exposed to water deficit conditions mainly occurs due the low immediate availability of nutrients for growth, because the nutrients are taken up by plants through the soil water solution. Plants subjected to drought stress, on the other hand, showed a significant decrease in above-ground biomass accumulation. Evidently, the severity of the adverse effects of drought stress on growth varied among the genotypes. The notable effect of drought stress on biomass production of brachiaria has been reported in previous studies which largely indicate drought stress reduces plant growth in brachiaria genotypes[25,42,48,54].

Because of the large genotypic variability in the studied traits of brachiaria genotypes in both non-stressed (control) and drought stressed plants, it is often difficult to assess drought tolerance of large germplasm collections, based only on data collected from drought stressed experiments. The DSI has therefore been used for example to evaluate the effect of drought stress on individual germplasm accessions based on the difference between drought stress treatments and control plants of switchgrass (*Panicum virgatum*)[55] and common bean (*Phaseolus vulgaris*)[56]. This approach removes the effect of germplasm variation from the drought stress evaluation and can therefore be used to assess a large collection of germplasm simultaneously[37,55].

The PCA biplots based on DSI values for each parameter grouped the genotypes and showed the relative contribution of different parameters in separating the accessions based on the projection of the traits from the biplot origin. This was sufficient to evaluate the genotypes taking into consideration all the traits that were evaluated. This study showed that biomass yield is a sensitive indicator of drought tolerance under severe drought stress since it produced the largest projection. Under moderate stress conditions, ILRI 13648, ILRI 11553 and 'Mulato I' emerged as the best performers, while intermediate performers were CIAT 679, 'Piata', 'Xaraes', 'Mulato II', ILRI 13497 and 'Basilisk'. Under severe drought stress, the best performers were 'Xaraes', 'Piata', CIAT 679, 'Marandu', 'Mulato II' and 'Mulato I', signifying that these genotypes are more tolerant to drought conditions. Biomass production can also be used as an accurate discriminator between drought tolerant and susceptible accessions, with the two accessions, 'Piata' and 'Xaraes', ranking the highest with 30.7 and 30.0 g biomass, respectively, under severe stress conditions. Despite a very low biomass yield of only 11.0 g, CIAT 679 was less affected by drought conditions. This is ascribed to its slow growing nature and high water-use efficiency that allows it to survive longer under conditions of prolonged drought[25].

CONCLUSION

In conclusion, there was wide variation in drought tolerance of the brachiaria genotypes examined in this study. Based on DSI values for the morphological and physiological parameters and PCA biplots, we conclude that genotypes 'Xaraes', 'Piata', CIAT 679, 'Marandu', 'Mulato II' and 'Mulato I' were similarly and more drought tolerant under severe drought stress. 'Piata' and 'Xaraes' emerged as candidate genotypes that would suffer lower yield penalties in arid and semi-arid areas that experience frequent and severe drought conditions. Their apomictic nature enables that true to type seeds are produced and utilized by farmers without losing the vigor of the plant. Utilization of these genotypes would renew confidence in cereal-livestock productivity through management of stem borers in smallholder farming systems in sub-Saharan Africa amid the increasing threat of climate change.

SIGNIFICANCE STATEMENT

This study highlights the potential sources of tolerance to drought among brachiaria genotypes. In the face of ever increasing climate change effects such as increased drought conditions, focus is shifting to utilization of climate change resilient crop varieties. The proposed candidate varieties of brachiaria are of value to small holder cereal-livestock farmers of sub-Saharan Africa and especially in areas that experience frequent and extreme droughts.

ACKNOWLEDGMENTS

We gratefully acknowledge the financial support for this research by the following organizations and agencies: European Union, Biovision foundation, Rothamsted Research UK, UK's Department for International Development (DFID), Swedish International Development Cooperation Agency (Sida), the Swiss Agency for Development and Cooperation (SDC) and the Kenyan Government. D. Cheruiyot was supported by a German Academic Exchange Service (DAAD) In-Region Postgraduate Scholarship ID 57221138. The views expressed herein do not necessarily reflect the official opinion of the donors. We also thank the International Center for Tropical Agriculture (CIAT) and the International Livestock Research Institute (ILRI) for provision of brachiaria accessions used in the study and screen house assistance by Danshem Simiyu.

REFERENCES

1. Carmo-Silva, A.E., A.J. Keys, M.H. Beale, J.L. Ward and J.M. Baker *et al*., 2009. Drought stress increases the production of 5-hydroxynorvaline in two C4 grasses. Phytochemistry, 70: 664-671.
2. Jones, H.G., 1992. Plants and Microclimate: A Quantitative Approach to Environmental Plant Physiology. 2nd Edn. Cambridge University Press, Cambridge, UK., Pages: 428.
3. Bhargava, S. and K. Sawant, 2013. Drought stress adaptation: Metabolic adjustment and regulation of gene expression. Plant Breed., 132: 21-32.
4. Hsiao, T.C. and L.K. Xu, 2000. Sensitivity of growth of roots versus leaves to water stress: Biophysical analysis and relation to water transport. J. Exp. Bot., 51: 1595-1616.
5. Roitsch, T., 1999. Source-sink regulation by sugar and stress. Curr. Opin. Plant Biol., 2: 198-206.
6. Souza, R.P., E.C. Machado, J.A.B. Silva, A.M.M.A. Lagoa and J.A.G. Silveira, 2004. Photosynthetic gas exchange, chlorophyll fluorescence and some associated metabolic changes in cowpea (*Vigna unguiculata*) during water stress and recovery. Environ. Exp. Bot., 51: 45-56.
7. Yang, X., X. Chen, Q. Ge, B. Li and Y. Tong *et al*., 2006. Tolerance of photosynthesis to photoinhibition, high temperature and drought stress in flag leaves of wheat: A comparison between a hybridization line and its parents grown under field conditions. Plant Sci., 171: 389-397.
8. Levitt, J., 1972. Responses of Plants to Environmental Stresses. Academic Press, New York, pp: 25-211.
9. Cushman, J.C. and H.J. Bohnert, 2000. Genomic approaches to plant stress tolerance. Curr. Opin. Plant Biol., 3: 117-124.
10. Renvoize, S.A., W.D. Clayton and C.H.S. Kabuye, 1996. Morphology, Taxonomy and Natural Distribution of Brachiaria (Trin.) Griseb. In: Brachiaria: Biology, Agronomy and Improvement, 1st Edn., Miles, J.W., B.L. Maass, C.B. do Valle and V. Kumble (Eds.). CIAT. Cali, Colombia, EMBRAPA/CNPGC, Campo Grande, Brasil, pp: 1-17.
11. Do Valle, C.B. and Y.H. Savidan, 1996. Genetics, Cytogenetics and Reproductive Biology of Brachiaria. In: Brachiaria: Biology, Agronomy and Improvement, 1st Edn., Miles, J.W., B.L. Maass, C.B. do Valle and V. Kumble (Eds.)., CIAT. Cali, Colombia, EMBRAPA/CNPGC, Campo Grande, Brasil, pp: 147-163.
12. Mattana, M., E. Biazzi, R. Consonni, F. Locatelli, C. Vannini, S. Provera and I. Coraggio, 2005. Overexpression of *Osmyb4* enhances compatible solute accumulation and increases stress tolerance of *Arabidopsis thaliana*. Physiol. Planta., 125: 212-223.
13. Vigna, B.B.Z., L. Jungmann, P.M. Francisco, M.I. Zucchi, C.B. do Valle and A.P. de Souza, 2011. Genetic diversity and population structure of the *Brachiaria brizantha* germplasm. Trop. Plant Biol., 4: 157-169.
14. Boonman, J.G., 1993. East Africa's Grasses and Fodders: Their Ecology and Husbandry. Kluwer Academic Publishers, Dordrecht, Netherlands, Pages: 343.
15. Maass, B.L., C.A. Midega, M. Mutimura, V.B. Rahetlah and P. Salgado *et al*., 2015. Homecoming of *Brachiaria*: Improved hybrids prove useful for African animal agriculture. East Afr. Agric. For. J., 81: 71-78.
16. Keller-Grein, G., B.L. Maass and J. Hanson, 1996. Natural Variation in Brachiaria and Existing Germplasm Collections. In: Brachiaria: Biology, Agronomy and Improvement, 1st Edn., Miles, J.W., B.L. Maass, C.B. do Valle and V. Kumble (Eds.)., CIAT. Cali, Colombia, EMBRAPA/CNPGC, Campo Grande, Brasil, pp: 16-42.
17. Hare, M.D., E.A. Pizarro, S. Phengphet, T. Songsiri and N. Sutin, 2015. Evaluation of new hybrid brachiaria lines in Thailand. 1. Forage production and quality. Trop. Grasslands-Forrajes Tropicales, 3: 83-93.
18. Ndikumana, J. and P.N. de Leeuw, 1996. Regional Experience with Brachiaria: Sub-Saharan Africa. In: Brachiaria: Biology, Agronomy and Improvement, 1st Edn., Miles, J.W., B.L. Maass, C.B. do Valle and V. Kumble (Eds.)., CIAT. Cali, Colombia, EMBRAPA/CNPGC, Campo Grande, Brasil, pp: 247-257.
19. Khan, Z.R., C.A.O. Midega, J.O. Pittchar, A.W. Murage, M.A. Birkett, T.J.A. Bruce and J.A. Pickett, 2014. Achieving food security for one million sub-Saharan African poor through push-pull innovation by 2020. Philos. Trans. R. Soc. London Ser. B: Biol. Sci., Vol. 369, No. 1639. 10.1098/rstb.2012.0284
20. Midega, C.A.O., T.J.A. Bruce, J.A. Pickett, J.O. Pittchar, A. Murage and Z.R. Khan, 2015. Climate-adapted companion cropping increases agricultural productivity in East Africa. Field Crops Res., 180: 118-125.
21. Cook, S.M., Z.R. Khan and J.A. Pickett, 2007. The use of push-pull strategies in integrated pest management. Annu. Rev. Entomol., 52: 375-400.
22. Bruce, T.J.A., C.A.O. Midega, M.A. Birkett, J.A. Pickett and Z.R. Khan, 2010. Is quality more important than quantity? Insect behavioural responses to changes in a volatile blend after stemborer oviposition on an African grass. Biol. Lett., 6: 314-317.
23. Khan, Z.R., C.A.O. Midega, T.J.A. Bruce, A.M. Hooper and J.A. Pickett, 2010. Exploiting phytochemicals for developing a 'push-pull'crop protection strategy for cereal farmers in Africa. J. Exp. Bot., 61: 4185-4196.
24. Midega, C.A.O., Z.R. Khan, J.A. Pickett and S. Nylin, 2011. Host plant selection behaviour of *Chilo partellus* and its implication for effectiveness of a trap crop. Entomol. Exp. Applicata, 138: 40-47.

25. Guenni, O., D. Marin and Z. Baruch, 2002. Responses to drought of five *Brachiaria* species. I. Biomass production, leaf growth, root distribution, water use and forage quality. Plant Soil, 243: 229-241.
26. Pickett, J.A., C.M. Woodcock, C.A.O. Midega and Z.R. Khan, 2014. Push-pull farming systems. Curr. Opin. Biotechnol., 26: 125-132.
27. Dall'agnol, M. and M.T. Schifino-Wittmann, 2005. Apomixia, genetica e melhoramento de plantas. Curr. Agric. Sci. Technol., 11: 127-133.
28. Edwards, E.J., C.P. Osborne, C.A. Stromberg, S.A. Smith and C_4 Grasses Consortium, 2010. The origins of C_4 grasslands: Integrating evolutionary and ecosystem science. Science, 328: 587-591.
29. Taylor, S.H., B.S. Ripley, F.I. Woodward and C.P. Osborne, 2011. Drought limitation of photosynthesis differs between C_3 and C_4 grass species in a comparative experiment. Plant Cell Environ., 34: 65-75.
30. Taylor, S.H., B.S. Ripley, T. Martin, L.A. De Wet, F.I. Woodward and C.P. Osborne, 2014. Physiological advantages of C_4 grasses in the field: A comparative experiment demonstrating the importance of drought. Global Change Biol., 20: 1992-2003.
31. Sage, R.F. and D.S. Kubien, 2003. Quo vadis C_4? An ecophysiological perspective on global change and the future of C_4 plants. Photosynth. Res., 77: 209-225.
32. Jaetzold, R. and H. Schmidt, 1983. Farm Management Handbook: Natural and Farm Management Information. Vol. II/B. Ministry of Agriculture, Nairobi, Kenya.
33. Somasegaran, P. and H.J. Hoben, 1985. Methods in legume-Rhizobium technology. University of Hawaii NifTAL Project and MIRCEN, Department of Agronomy and Soil Science, Hawaii Institute of Tropical Agriculture and Human Resources, College of Tropical Agriculture and Human Resources, Paia, Maui, pp: 365.
34. Poorter, H., J. Buhler, D. van Dusschoten, J. Climent and J.A. Postma, 2012. Pot size matters: A meta-analysis of the effects of rooting volume on plant growth. Funct. Plant Biol., 39: 839-850.
35. Chen, D., S. Wang, B. Cao, D. Cao and G. Leng *et al*., 2016. Genotypic variation in growth and physiological response to drought stress and re-watering reveals the critical role of recovery in drought adaptation in maize seedlings. Front. Plant Sci., Vol. 6. 10.3389/fpls.2015.01241.
36. R Core Team, 2016. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
37. Wojcik-Jagla, M., M. Rapacz, M. Tyrka, J. Koscielniak, K. Crissy and K. Zmuda, 2013. Comparative QTL analysis of early short-time drought tolerance in Polish fodder and malting spring barleys. Theor. Applied Genet., 126: 3021-3034.
38. Addinsoft, S., 2010. XLSTAT-software, version 10. Addinsoft, Paris, France.
39. Wedin, D.A., 2004. C_4 Grasses: Resource Use, Ecology and Global Change. In: Warm-Season (C_4) Grasses, Moser, L.E., B.L. Burson and E. Sollenberger (Eds.)., ASA-CSSA-SSSA., Madison, pp: 15-50.
40. Blum, A., 2011. Drought Resistance and its Improvement. In: Plant Breeding for Water-Limited Environments, Blum, A. (Ed.)., Springer, Berlin, Germany, pp: 53-152.
41. Martinez Vilalta, J. and N. Garcia Forner, 2017. Water potential regulation, stomatal behaviour and hydraulic transport under drought: Deconstructing the iso/anisohydric concept. Plant Cell Environ., 40: 962-976.
42. Santos, P.M., P.G. da Cruz, L.C. de Araujo, J.R.M. Pezzopane, C.B. do Valle and C.D.G. Pezzopane, 2013. Response mechanisms of *Brachiaria brizantha* cultivars to water deficit stress. Rev. Bras. Zootec., 42: 767-773.
43. Earl, H.J. and R.F. Davis, 2003. Effect of drought stress on leaf and whole canopy radiation use efficiency and yield of maize. Agron. J., 95: 688-696.
44. O'Neill, P.M., J.F. Shanahan and J.S. Schepers, 2006. Use of chlorophyll fluorescence assessments to differentiate corn hybrid response to variable water conditions. Crop Sci., 46: 681-687.
45. Zhao, J., H. Sun, H. Dai, G. Zhang and F. Wu, 2010. Difference in response to drought stress among Tibet wild barley genotypes. Euphytica, 172: 395-403.
46. Dale, J.E., 1988. The control of leaf expansion. Annu. Rev. Plant Physiol. Plant Mol. Biol., 39: 267-295.
47. Anjum, S.A., X.Y. Xie, L.C. Wang, M.F. Saleem, C. Man and W. Lei, 2011. Morphological, physiological and biochemical responses of plants to drought stress. Afr. J. Agric. Res., 6: 2026-2032.
48. De Araujo, L.C., P.M. Santos, F.C. Mendonca and G.B. Mourao, 2011. Establishment of *Brachiaria brizantha* cv. Marandu, under levels of soil water availability in stages of growth of the plants. Rev. Bras. Zootec., 40: 1405-1411.
49. Manivannan, P., C.A. Jaleel, B. Sankar, A. Kishorekumar, R. Somasundaram, G.M.A. Lakshmanan and R. Panneerselvam, 2007. Growth, biochemical modifications and proline metabolism in *Helianthus annuus* L. as induced by drought stress. Colloids Surf. B: Biointerfaces, 59: 141-149.
50. Altinkut, A., K. Kazan, Z. Ipekci and N. Gozukirmizi, 2001. Tolerance to paraquat is correlated with the traits associated with water stress tolerance in segregating F_2 populations of barley and wheat. Euphytica, 121: 81-86.
51. Keles, Y. and I. Oncel, 2004. Growth and solute composition in two wheat species experiencing combined influence of stress conditions. Russian J. Plant Physiol., 51: 203-209.

52. El-Rawy, M.A. and M.I. Hassan, 2014. Effectiveness of drought tolerance indices to identify tolerant genotypes in bread wheat (*Triticum aestivum* L.). J. Crop Sci. Biotechnol., 17: 255-266.
53. De Barros Lima, N.R.C., P.M. Santos, F.C. Mendonca and L.C. de Araujo, 2011. Critical periods of sorghum and palisadegrass in intercropped cultivation for climatic risk zoning. Rev. Bras. Zootec., 40: 1452-1457.
54. Cardoso, J.A., M. Pineda, J. de la Cruz Jimenez, M.F. Vergara and I.M. Rao, 2015. Contrasting strategies to cope with drought conditions by two tropical forage C_4 grasses. AoB Plants, Vol. 7. 10.1093/aobpla/plv107.
55. Liu, Y., X. Zhang, H. Tran, L. Shan and J. Kim *et al*., 2015. Assessment of drought tolerance of 49 switchgrass (*Panicum virgatum*) genotypes using physiological and morphological parameters. Biotechnol. Biofuels, Vol. 8, No. 1. 10.1186/s13068-015-0342-8.
56. Darkwa, K., D. Ambachew, H. Mohammed, A. Asfaw and M.W. Blair, 2016. Evaluation of common bean (*Phaseolus vulgaris* L.) genotypes for drought stress adaptation in Ethiopia. Crop J., 4: 367-376.