IDENTIFICATION OF DROUGHT TOLERANT KENYAN *BRACHIARIA* ECOTYPES USING DROUGHT TOLERANCE INDICES

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ABSTRACT

Drought is one of the major abiotic stress factors limiting agricultural productivity globally. *Brachiaria* also known as signal grass is a native of sub-tropical and tropical Africa and important in livestock production. The grass has many advantages including; high biomass, high nutritional value, adaptation to drought and low fertility soils, sequestration of carbon, enhanced nitrogen uses efficiency and low greenhouse gas emissions. Emergence of climate change with increased global temperatures has led to prolonged drought which has adversely affected the improved *Brachiaria* hybrids. Locally available ecotypes are a rich source of unique genes and characteristics that could be key in developing drought resilient hybrids. The objectives of this study were to i) assess the effectiveness of various indices in selection of drought tolerant Kenyan *Brachiaria* ecotypes, ii) evaluate the relationship between the indices and iii) to identify high yielding and stable ecotypes under stressed condition. The design of the experiment was completely randomized design (CRD) with three replications in a factorial arrangement (3 x 25). A total of 11drought tolerance indices; tolerance (TOL), stress Tolerance Index (STI), mean productivity (MP), yield stability index (YSI), Geometric Mean Productivity (GMP), stress susceptibility index (SSI), Yield Index (YI), harmonic Mean (HM), drought intensity index (DII), modified stress tolerance k1 and modified stress tolerance k2 were calculated based on shoot biomass production under non-stressed (Y_P) and stressed (Y_S) conditions. Rank means, rank sum and standard deviation were also used to identify the tolerant materials. In the previous experiment, various physiological parameters were scored which included; leaf relative water content, relative chlorophyll content using SPAD -502 Chlorophyll meter (Minota Co), leaf fresh weight, leaf dry weight and leaf relative water content. Relative water content was also estimated and comparative scores were done between control, medium and extreme or water deficit experiments. Based on all the indices and ranking, BrK 1, BrK 6, BrK 7, BrK13 and BrK 18 were the most tolerant in stressed condition. These ecotypes can be recommended for planting in areas prone to drought. More studies on the identified tolerant ecotypes are essential to ascertain whether the materials hold unique genes that could later be introgressed into various breeding schemes to confer tolerance.

Key words: *Brachiaria, drought, indices, tolerance*

INTRODUCTION

Drought is a climatic phenomenon described as reduced rainfall or soil moisture content that leads to reduced crop production and animal loss due to scarcity of pasture and fodder. These losses are directly related to economic loss and environmental degradation which are the consequences of prolonged drought. Other profound effects of drought include, induction of malnutrition, diseases and population migration. There is a cascading negative effect of climate change to food production which affects countries and their citizens who solely rely on rain fed agriculture.

Identification of tolerant/resistant plant genotypes for any trait is a critical process in plant breeding and genetics. With the many selection methods for different crops and traits, it is imperative to develop and identify an efficient selection criterion for developing drought tolerant *Brachiaria* ecotypes with high and stable yield under different water stress regimes (Blum, 2011, Pinter *et al*., 1990).

The most sustainable approach towards improvement of drought tolerance is to introgress genes responsible for resistance to drought and high temperature/heat into the desired germplasm with good agronomic traits

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and without compromising on the quantity, quality and stability of the yield (Akcura and Ceri, 2011). Continued introgression of target genes into local genotypes under both stress and non-stress conditions leads to further selection of tolerant genotypes. Among the selection methods available, use of drought indices, which are mathematical equations based on yield loss under drought conditions as compared to optimal conditions have been used extensively. Several drought indices based on drought resistance or susceptibility have been proposed which compare the yield variations between optimal and drought stressed genotypes .

The ability to clearly discriminate between drought tolerant and susceptible plant genotype using indices is one of the characteristics of a good drought tolerance index (Farshadfar *et al*., 2013). Various studies have evaluated the efficiency and effectiveness of different drought indices in genotype selection though selection of tolerant genotype(s) which is highly dependent on both stress and selection intensities (Gholinezhad *et al*., 2014). Among the drought indices used to discriminate genotypes, there exists a strong correlation between their outputs hence it is important to critically evaluate the available indices to avoid redundancy (Gitore *et al*., 2021). Identification of drought tolerant plants by use of indices is complex because the tolerance exhibited by the plant is a summation of genotypic expression and environmental interaction (Staniak and Koco, 2015).

Many studies have recommended the use of more than one index to evaluate and select for tolerance and to discriminate against susceptibility. This is because evaluation of one index would rank plants differently as compared to another different or closely related index. Use of more than one index then reduces chances of bias and would give more discriminating results to ensure a robust selection pressure. Some of these indices have been used to evaluate genetic variation in sorghum (*Sorghum bicolor*) (Menezes *et al*., 2014), soybeans (*Glycine max*) (Cabral *et al*., 2020), maize (*Zea mays*) (Naghavi *et al*., 2013), bread wheat (*Triticum aestivum*) (El-Rawy and Hassan, 2014), sunflower *(Helianthus annus L.)* (Gholinezhad *et al*., 2014) and common beans *(Phaseolus vulgaris L)*. In this study, 25 Kenyan *Brachiaria* ecotypes were evaluated for drought tolerance in two treatments; stressed and non-stressed using 11 different drought tolerance indices in a glass house. The indices used for evaluation included; tolerance (TOL) (Rosielle and Hamblin1981), mean productivity (MP) (Rosielle and Hamblin 1981), yield stability index (YSI) (Bouslama and Schapaugh, 1984), Stress tolerance index (STI) (Fernandez, 1992), drought stress/resistance index (DSI) (Blum, 1988), geometric mean productivity (GMP) index, yield index (YI) harmonic mean index (HM) (Basu *et al*., 2016), stress intensity index (SI) and stress susceptibility percentage index (SSPI) (Moosavi *et al*., 2008) were used to identify and select the tolerant genotype(s).

MATERIALS AND METHODS

Brachiaria ecotypes were collected from different parts of the country where 25 were selected and assembled at the Kenya Agricultural and Livestock Research Organization (KALRO) Kitale and KALRO Muguga. *Brachiaria* root splits were planted in standard plastic bags measuring 60 cm deep and 26 cm wide in the glass house at Muguga center, Food crops research Institute (latitude $1^0$13’ S, and longitude 36^0 38’ E and 1675 m asl) following completely randomized design (CRD) with three replicates in a factorial arrangement (3 x 25). Greenhouse condition was maintained at 15^0 C at night and 23^0 C daytime temperature, 16 hrs light and 8 hrs dark and humidity ranging from 40 to 50 %. Leaves were trimmed at 21 days after planting to a height of 10 cm for uniformity across the replicates. Each experimental replicate was divided into three water regimes; control, medium and extreme. Watering regime was initiated 4 weeks (28 days) after planting. Medium water experiment was watered after 14 days while the water deficit experiment was watered after every 28 days. The experiment was repeated for three cropping cycles each consisting of six months per cycle. In the previous experiment, various physiological parameters were scored which included; leaf relative water content, relative chlorophyll content using SPAD -502 Chlorophyll meter (Minota Co), leaf fresh weight, leaf dry weight and leaf relative water content. Relative water content was also estimated and comparative scores were done between control, medium and extreme or water deficit experiments. Data on biomass was collected for every harvesting and thereafter, drought indices were calculated.

Statistical analysis

The mean biomass yield for the ecotypes were recorded both for stressed and non-stressed environments and were subjected to analysis for various indices using Excel for rank mean, standard deviation-based ranking and rank sum. XLSTAT 2023 version 3 was used to calculate correlation between the indices and analysis of variance (ANOVA) for significant differences.

TABLE I-DROUGHT TOLERANCE INDICES AND THEIR RESPECTIVE FORMULAS USED FOR CALCULATION

Stress index	Equation
Tolerance	$TOL = Y_p – Y_s$
Mean productivity	$MP = (Y_s + Y_p)/2$
Yield stability index	$YSI = Y_s/Y_p$
Drought resistance index	$DI= [Y_S × (Y_S/Y_P)]/\tilde{Y}_S$
Stress tolerance index	$STI= (Y_S × Y_P)/(\tilde{Y}_P)^2$
Geometric mean productivity index	$GMP = \sqrt{(Y_S × Y_P)}$
Yield index	$YI = (Ys/ \bar{Y}s)$
Stress susceptibility percentage index	$SSPI = [(Yp − Ys)/2 × \bar{Y}s] × 100$
Harmonic mean	$HM = [2 × (Ys × Yp)]/ (Ys + Yp)$
Modified stress tolerance (k_1)	$k_1STI = Yp^2/\bar{Y} p^2$
Modified stress tolerance (k_2)	$k_2STI = Ys^2/\bar{Y} s^2$
Drought intensity index	$DII = 1-(Y_s/Y_p)$

Key: Ys and Yp represent biomass production of *Brachiaria* ecotypes grown under stress and non-stress condition respectively. $\bar{Y}s$ and $\bar{Y}p$ represent mean yield under stress and non-stress conditions for all the genotypes evaluated.

RESULTS

Yield potential (Y_P)

Though this is not an index *per se*, it is critical for identification of the high yielding ecotypes as compared to their tolerance. Yield potential is important in determination of the correlation between the stress intensity viz a viz yield. This means that the higher the Y_P, the higher the tolerance. In this study, ecotypes with the highest Y_P values were BrK 21, BrK 10, BrK 24, BrK 2 and BrK 18 weighing 34.49 g 33.18 g, 30.97 g, 228.49 g, 1827.60 g, respectively. The lowest was BrK 12 weighing 15.47 gms as shown in Table II.

Yield stability index (YSI)

For both stressed and non-stressed conditions, ecotypes with high YSI were considered stable while those with low YSI as non-stable. Stable ecotypes in this study were BrK 3, BrK 13 and BrK 19 while the non-stable was BrK 17 and BrK 21 as shown in Table II.

Tolerance (TOL) index

Tolerance (TOL) index is the differences in yield between the stress (Ys) and no stress (Yp) environments. The lower the TOL index, the more tolerant the ecotypes in both stressed and non-stressed environments. In this study, the following ecotypes were tolerant based on TOL index; BrK 12(3.66), BrK 3(3.70), BrK 9(4.22), BrK 25(4.55) and BrK 19 (4.90). The most susceptible to drought using TOL index was BrK 21 (13.95) (Table II).

Mean productivity (MP) index

This is the average biomass yield of a plant in both stress and non-stress environment. Ecotypes with high MP values are classified as tolerant and more desirable while those with low MP are considered susceptible. Ecotypes which had high MP values were; BrK 10 (27.60), BrK 21 (27.52), BrK 24 (25.86), BrK 18 (23.21) and BrK 2 (23.13). Susceptible ecotypes with low MP were BrK 12 (13.64), BrK 25 and BrK 19.

TABLE II- ESTIMATION OF STRESS TOLERANCE INDICES FOR *BRACHIARIA* ECOTYPES BASED ON MEAN BIOMASS FOR OPTIMAL AND WATER STRESS CONDITIONS

Genotype	Yp	TOL	MP	YSI	GMP	HMP	DSI	YI	STI	SSPI	k1 STI	k2 STI	ATI
1	23.74	7.13	20.18	0.70	19.86	0.07	0.75	1.07	0.75	15.56	1.08	1.15	95.88
2	28.49	10.73	23.13	0.62	22.50	0.06	0.71	1.15	0.97	23.44	1.55	1.31	163.57
3	16.88	3.70	15.03	0.78	14.92	0.10	0.66	0.85	0.42	8.08	0.54	0.72	37.38
4	25.80	9.35	21.13	0.64	20.60	0.07	0.68	1.06	0.81	20.42	1.27	1.12	130.52
5	22.90	9.19	18.31	0.60	17.72	0.08	0.53	0.88	0.60	20.07	1.00	0.78	110.33
6	23.16	7.14	19.59	0.69	19.27	0.07	0.71	1.03	0.71	15.60	1.02	1.07	93.21
7	25.37	7.49	21.63	0.70	21.30	0.07	0.81	1.15	0.87	16.36	1.23	1.33	108.13
8	19.92	7.97	15.93	0.60	15.43	0.09	0.46	0.77	0.45	17.41	0.76	0.59	83.29
9	17.84	4.22	15.74	0.76	15.59	0.09	0.67	0.88	0.46	9.21	0.61	0.77	44.55
10	33.18	11.17	27.60	0.66	27.03	0.05	0.94	1.42	1.39	24.41	2.10	2.01	204.61
11	18.92	5.58	16.13	0.71	15.89	0.09	0.61	0.86	0.48	12.18	0.68	0.74	60.04
12	15.47	3.66	13.64	0.76	13.51	0.11	0.58	0.76	0.35	8.00	0.46	0.58	33.54
13	24.22	5.58	21.43	0.77	21.25	0.07	0.93	1.20	0.86	12.18	1.12	1.44	80.30
14	20.56	6.18	17.47	0.70	17.20	0.08	0.65	0.93	0.56	13.50	0.81	0.86	71.99
15	21.17	6.69	17.83	0.68	17.51	0.08	0.64	0.93	0.59	14.61	0.86	0.87	79.37
16	21.71	6.09	18.66	0.72	18.41	0.08	0.72	1.01	0.65	13.29	0.90	1.01	75.93
17	24.29	9.98	19.30	0.59	18.64	0.08	0.54	0.92	0.66	21.80	1.13	0.85	126.09
18	27.60	8.78	23.21	0.68	22.79	0.06	0.83	1.21	0.99	19.19	1.45	1.47	135.65
19	16.62	4.90	14.17	0.71	13.95	0.10	0.53	0.76	0.37	10.71	0.53	0.57	46.34
20	20.84	6.36	17.67	0.70	17.38	0.08	0.65	0.93	0.58	13.88	0.83	0.87	74.83
21	34.49	13.95	27.52	0.60	26.62	0.05	0.79	1.32	1.35	30.46	2.27	1.75	251.53
22	22.86	7.67	19.03	0.66	18.64	0.08	0.65	0.98	0.66	16.75	1.00	0.96	96.83
23	18.95	6.26	15.82	0.67	15.51	0.09	0.55	0.82	0.46	13.68	0.69	0.67	65.80
24	30.97	10.23	25.86	0.67	25.35	0.06	0.90	1.34	1.23	22.35	1.83	1.79	175.72
25	16.25	4.55	13.98	0.72	13.79	0.10	0.54	0.75	0.36	9.93	0.50	0.57	42.50
Mean	**22.89**	**7.38**	**19.20**	**0.68**	**18.83**	**0.08**	**0.68**	**1.00**	**0.70**	**16.12**	**1.05**	**1.04**	**99.52**
SD	**5.03**	**2.56**	**4.00**	**0.05**	**3.86**	**0.02**	**0.13**	**0.19**	**0.30**	**5.59**	**0.48**	**0.41**	**54.11**
CV%	**21.98**	**34.68**	**20.84**	**8.02**	**20.52**	**19.69**	**18.98**	**19.25**	**42.16**	**34.68**	**46.14**	**39.42**	**54.37**

Yield stability (YSI) index

Yield stability index (YSI) compares the stability of plants in both stress and nonstress conditions. In this case, plants with high YSI index were considered stable hence more tolerant than those with low values. Tolerant *Brachiaria* ecotypes with high YSI values were; BrK 3 (0.78), BrK 13 (0.77) and BrK 9(0.76) while susceptible ecotypes were BrK 17 and BrK 21 as shown in Table II.

Geometric mean productivity (GMP) index

This index is used to compare the relative performance of plants due to the fact that, environmental fluctuations occur over time which adversely affect plant performance under stress conditions. Ecotypes in this study ecotypes with high GMP index thus tolerant were; BrK 10, BrK 21, BrK 2, BrK 18, BrK 13 and BrK 2 (Table II). The lowest (Susceptible) for this index was BrK 12 (13.51).

Harmonic mean (HM) index

High harmonic (HM) index indicated tolerance while low values ecotypes implied susceptibility to drought. In this study, high HM values were recorded in BrK12, BrK 25 and BrK 19 while the lowest were BrK 10, BrK 21 and Brk 24 as shown in Table II.

Drought stress index (DSI)

It has been shown that, drought stress index values > 1 is an indicator of above average susceptibility to drought stress. Values below 1 indicate tolerance to drought. The study calculations indicated variations among the ecotypes in terms of tolerance (Table II).

Yield index

Yield index is the yield performance of plants in stress environment, it compares the optimal plant yield viz a viz the average yield for the same plant in the stressed condition. Using this index, Brk 10 (1.42), BrK 24(1.34), BrK 21 (1.32), BrK 18 (1.21) and BrK 13 (1.20) were selected for tolerance while the lowest was BrK 25 (0.75) (Table II).

Stress tolerance index (STI)

Stress tolerance index (STI) is a modified yield index expression which compares yield under stress and non-stress condition as a ratio expressed in percentage. It is calculated as ratio of mean yield reduction of a plant sample in stressed condition as compared to all samples in the experiment. The index is used to select plant genotypes based on yield and drought tolerance. The higher the STI index, the stable the plant. Ecotypes that showed tolerance based on STI were; BrK 10 (1.39), BrK 21(1.35) and BrK 24 (1.23) while the lowest was BrK 12(0.35) as shown in Table II.

Modified stress tolerance index (k_1 STI)

The modified stress tolerance index compares the mean yield reduction in non-stressed condition. Stable or tolerant plants are identified by high k_1 STI values while low values indicate susceptibility. *Brachiaria* ecotypes selected under this index were; BrK 21 (2.27), BrK 10 (2.10) and BrK 24 (1.83) while the most to drought under non stress conditions was BrK 12 (Table II).

Modified stress tolerance index (k_2 STI)

This is a modified form of stress tolerance index that compares the mean yield reduction in stressed condition. Selected Brachiaria ecotypes with high k_2 STI values were; BrK 10 (2.01), BrK 24 (1.79) and BrK 21 while BrK 25 was susceptible under stressed condition.

Drought intensity index (DII)

This was used to quantify or to measure the magnitude of drought stress imposed on the plants throughout the experimental period. Average DII value for the experiment was 0.32 which according to Worku and Skjelvag *l* (2006), indicates a moderate stress. This could have been attributed to the fact that, the calculation for the general DII was based on the three treatments comprising of control, moderate and extreme. Ideally, this could be explained by the fact that plants recovered immediately after watering even after exposure for a long time, in this case, watering was done after 14 and 28 days for medium and extreme drought experiments respectively. This has been corroborated by studies on common bean by Asfaw and Blair (2014) and Darkwa *et al*., (2016) where they obtained a DII of 0.335 at vegetative stage of the crop.

Ranking of *Brachiaria e*cotypes based on index

The purpose of ranking ecotypes was to help identify stable ecotypes under both stress and optimal conditions. The various indices used for selection can be used for the same purpose though selection based on one index is not conclusive. In this study, Table III shows ranking based on rank mean, standard deviation, rank sum and overall ranking. The formula below was used to calculate the rank sum which was used to obtain the overall rank;

Rank Sum (RS) = Rank Mean (R) + Standard Deviation of Rank (SDR) (Farshadfar *et al*., 2011)

Depending on the index under consideration, the ranking was either in ascending or descending order. Overall rank shows that BrK 10, BrK 21, BrK 24, BrK 2 and BrK 18 were drought tolerant while BrK 12, BrK 25, BrK 3, BrK 19 and BrK 8 were drought susceptible as shown in Table III.

TABLE III- RANK OF **BRACHIARIA** ECOTYPES BASED ON DIFFERENT DROUGHT INDICES

Ecotype	Yp	Ys	TOL	MP	YSI	GMP	DSI	YI	STI	SSPI	k1	k2	ATI	HMP	MR	SD	SR	**OR**
BrK1	10	8	13	9	10	16	7	8	9	13	10	8	11	17	11	3	14	**4**
Brk2	4	7	23	5	21	5	10	7	5	3	4	7	4	21	9	7	16	**12**
BrK3	22	20	2	22	1	22	13	20	22	24	22	20	24	4	17	8	25	**22**
BrK4	6	9	20	8	20	8	11	9	8	6	6	9	6	18	10	5	15	**10**
BrK5	12	17	19	14	23	14	24	17	14	7	12	17	8	12	15	5	20	**17**
BrK6	11	10	14	10	13	10	9	10	10	12	11	10	12	16	11	2	13	**2**
BrK7	7	6	15	6	9	6	5	6	6	11	7	6	9	20	9	4	13	**1**
BrK8	18	22	17	19	22	21	25	22	21	9	18	22	13	7	18	5	24	**21**
BrK9	21	18	3	21	3	19	12	18	19	23	21	18	22	5	16	7	23	**20**
BrK10	2	1	24	1	19	1	1	1	1	2	2	1	2	25	6	9	15	**8**
BrK11	20	19	7	18	7	18	18	19	18	19	20	19	20	8	16	5	21	**18**
BrK12	25	23	1	25	4	25	19	23	25	25	25	23	25	1	19	9	29	**25**
BrK13	9	5	6	7	2	7	2	5	7	20	9	5	14	19	8	6	14	**5**
BrK14	17	15	9	17	11	17	16	15	17	17	17	15	18	9	15	3	18	**15**
BrK15	15	14	12	15	14	15	17	14	15	14	15	14	15	11	14	1	16	**11**
BrK16	14	11	8	13	6	13	8	11	13	18	14	11	16	13	12	3	15	**9**
BrK17	8	16	21	11	25	11	22	16	11	5	8	16	7	15	14	6	20	**16**
BrK18	5	4	18	4	15	4	4	4	4	8	5	4	5	22	8	6	14	**3**
BrK19	23	24	5	23	8	23	23	24	23	21	23	24	21	3	19	8	27	**23**
BrK20	16	13	11	16	12	16	15	13	16	15	16	13	17	10	14	2	16	**13**
BrK21	1	3	25	2	24	2	6	3	2	1	1	3	1	24	7	9	16	**14**
BrK22	13	12	16	12	18	12	14	12	12	10	13	12	10	14	13	2	15	**7**
BrK23	19	21	10	20	17	20	20	21	20	16	19	21	19	6	18	4	22	**19**
BrK24	3	2	22	3	16	3	3	2	3	4	3	2	3	23	7	8	14	**6**
BrK25	24	25	4	24	5	24	21	25	24	22	24	25	23	2	19	9	28	**24**

Key: OR -Overall rank, SR-Sum rank, SD -Standard deviation rank and MR-Mean rank

Correlation between indices

From the Pearson correlation analysis (Table IV), mean productivity index (MP) showed positive significant correlation with six other indices; TOL, GMP, HMP, STI, k1 STI and k2 STI at $p\leq 0.05$. There was however high significant negative correlation between HMP and five other indices; GMP, YI, STI, k1STI and k2 STI at $p\leq 0.05$. Though other correlations depicted in Table IV were high, they were not significant at $p\leq 0.05$..

TABLE IV- PEARSON CORRELATION MATRIX BETWEEN TOLERANCE INDICES FOR STRESSED AND NON-STRESSED ENVIRONMENTS AT P≤ 0.05

Variables	TOL	MP	YSI	GMP	HMP	DSI	YI	STI	SSPI	k1 STI	k2 STI
TOL	**1**	0.852	-0.822	0.833	-0.830	0.371	0.714	0.831	1.000	0.907	0.716
MP	0.852*	**1**	-0.424	0.999*	-0.977*	0.801	0.975	0.994*	0.852	0.984*	0.972*
YSI	-0.822	-0.424	**1**	-0.393	0.439	0.190	-0.216	-0.381	-0.822	-0.512	-0.216
GMP	0.833	0.999*	-0.393	**1**	-0.977	0.822NS	0.982	0.995*	0.833	0.979*	0.979*
HMP	-0.830	-0.977	0.439	-0.977*	**1**	-0.784	-0.953*	-0.950*	-0.830	-0.937*	-0.933*
DSI	0.371	0.801	0.190	0.822	-0.784	**1**	0.915	0.816	0.371	0.712	0.908
YI	0.714	0.975	-0.216	0.982*	-0.953	0.915	**1**	0.976	0.714	0.930NS	0.996*
STI	0.831	0.994*	-0.381	0.995*	-0.950	0.816	0.976	**1**	0.831	0.986	0.982
SSPI	1.000	0.852	-0.822	0.833NS	-0.830	0.371	0.714	0.831	**1**	0.907	0.716
k1 STI	0.907	0.984*	-0.512	0.979*	-0.937*	0.712	0.930	0.986*	0.907	**1**	0.938
k2 STI	0.716	0.972*	-0.216	0.979*	-0.933*	0.908	0.996*	0.982*	0.716	0.938	**1**

*= significant at P≤0.05, NS = non-significant. Key: TOL: Tolerance, MP: Mean productivity index, YSI: Yield stability index, GMP: Geometric mean productivity, HMP: Harmonic mean, DSI: Drought stress index, YI: Yield index, STI: Stress tolerance index, SSPI: Stress susceptibility percentage index, k_1 STI: Modified stress tolerance index 1and k_2 STI and: Modified stress tolerance index 2

Combined cluster analysis

Based on coefficient of variation CV, yield stability index (YSI), Harmonic mean (HMP), drought stress index (DSI) and yield index (YI) were the best indices to use in selection for drought. This is due to the low CV values which is an indicator of data dispersion from the mean, the higher the CV, the higher the dispersion hence low values below 20 are acceptable. Dendrograms were used to group the ecotypes using the selected indices. Combined cluster analysis showed two major clades with 10 minor groups. Ecotypes BrK 13, 21 and 8 were unique since they separated forming their unique groups (Figure 1).

DISCUSSION

Quantification of the magnitude of drought stress is key in the identification of tolerant plant materials for subsequent selection. Drought stress indices have been extensively used by many authors to quantify drought stress in addition to other methods. In this study, drought intensity index (DII) was used to quantify the magnitude of drought stress on the *Brachiaria* ecotypes. Average DII value for the experiment was 0.32 which according to Worku and Skjelvag (2006), indicates a moderate stress. This could have been attributed to the fact that, the calculation for the general DII was based on the three treatments comprising of control, moderate and extreme. Another reason could be that on re-watering, which could have triggered physiological processes that led to quick response in vegetative growth which was later observed in the yield (biomass). This has been corroborated by studies on common bean by Asfaw and Blair (2014), Darkwa *et al.* (2016) and Panthuwan *et al.* (2002) where they obtained a DII of 0.335 at vegetative stage of the crop.

Drought tolerance (TOL) index showed differences in yield between the stress (Ys) and non-stress (Yp) environments. In this study, the following ecotypes were

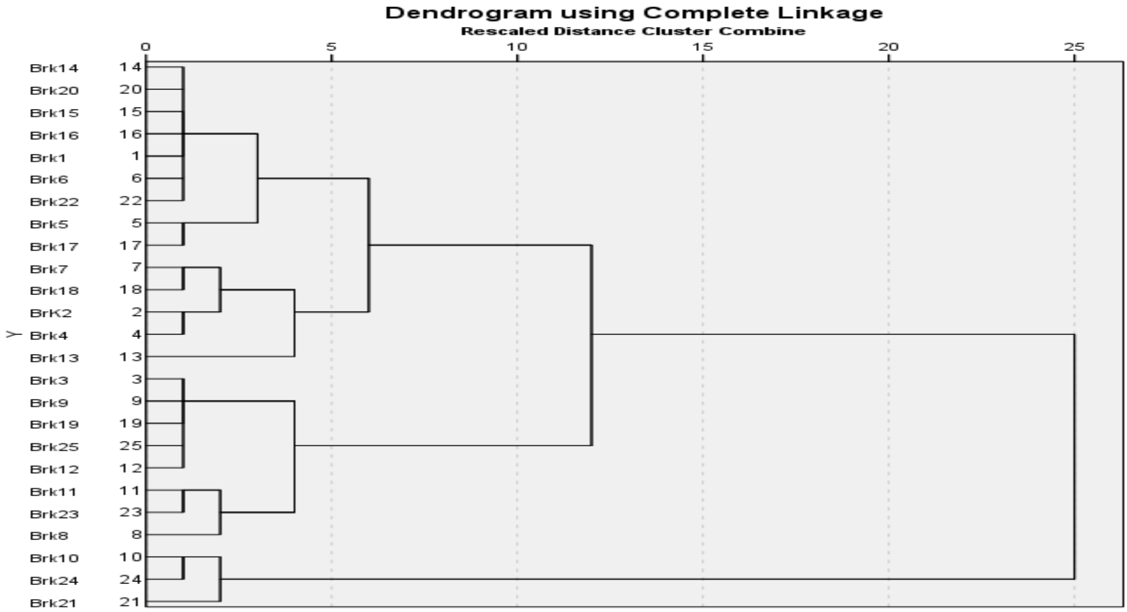

Figure 1. Scaled dendrogram depicting distance between different **Brachiaria** ecotypes

tolerant based on TOL index; BrK 12(3.66), BrK 3(3.70), BrK 9(4.22), BrK 25(4.55) and BrK 19 (4.90). Genotypic variability of the ecotypes is evident in response to TOL. This could be attributed to the fact that these ecotypes are more vigorous and are able to have a large soil cover within a short period of time hence reducing soil water evaporation. Similar results have been corroborated by Bennani *et al*. (2017), Darkwa *et al*. (2016) and Zarei *et al*. (2007).

Geometric mean productivity, harmonic mean and mean productivity indices clearly identified ecotypes that can be well adopted to both stressed and non-stressed environments. This means that the ecotypes are able to adjust to sudden cellular physiological changes including hormonal response due to drought. Hormonal changes are important in controlling signal pathways such as salicylic acid (SA), abscisic acid (ABA) and ethylene (Kurepin *et al*., 2017). During drought stress, plant vigor is severely affected and reduced leading to retarded growth. This is due to limited or controlled stomatal opening triggered by varied cellular ABA concentrations. Increased ABA concentration leads to regulation of stomatal opening and production of stress related genes; ABA1 and ABA2 (Kuromori *et al.,* 2018). Ecotypes with high MP, GMP and HMP were BrK 10, BrK 21 and BrK 24. Use of these indices in selection of tolerant plants in different stresses have been used in wheat (Farshadfar *et al*, 2013) and in maize (Moradi *et al*., 2012). Concurrent use of these indices indicate that they are important and can be used confidently in selection of plants in two contrasting environments. Other indices used in this study had contradicting results on selection of the tolerant ecotypes rendering them unreliable in making decision on selection.

Generally, there was high significant correlation at $p < 0.05$ between the biomass recorded in the non-tress environment as compared to the stressed environment. This correlation indicates that the biomass is greatly influenced by the genotypic potential of the ecotypes. This could indicate that there are different alleles that influence the biomass yield hence further elucidation of ecotype genetics is key to unravel selection based on different stress environments. This is in concurrence with studies by Naghavi *et. al.* (2013) on studies of maize on different stress environments.

Identification of drought tolerant ecotypes based on different indices resulted in varied conclusions on the selected ecotypes. Due to the huge variability, different authors have recommended use of ranking based on cumulative indices, rank mean, standard deviation and rank sum. Based on the ranking criterion, BrK 1, BrK 6, BrK 7, BrK 13 and BrK 18 were drought tolerant while BrK 12, BrK 25, BrK 3, BrK 19 and BrK 8 were drought susceptible. The use of ranking has also been supported by Awoke (2021) and Naghavi *et al*. (2013) to conclusively select drought tolerant plant genotypes on haricot bean and maize respectively.

Based on Pearson correlation at $p \leq 0.05$, there was both positive and negative correlation between various indices. Significant positive correlation between MP and GMP is an indicator that either of the two indices are can be used interchangeably to calculate tolerance or susceptibility of *Brachiaria* ecotypes against drought. This could reduce the number of indices used to make conclusive recommendations by narrowing the selection criteria further. Though not significant, the correlation between TOL and SSPI was 1 meaning that one can use either of the indices to calculate the same value. Significant negative correlation between HMP and GMP, YI, STI, k1STI and k2 STI at $p \leq 0.05$ is a pointer that these indices can also be used interchangeably to give the desired or almost similar results.

Clustering of ecotypes based on the selected indices concurred with biomass potential in the two stress environments. Combined cluster analysis showed 2 major clades with 10 minor groups. Ecotypes BrK 13, 21 and 8 were unique since they separated forming unique groups. The clustering of unique groups partially coincides with ranking which shows that BrK 8 is susceptible while BrK 21 is tolerant in both methods. This shows that clustering based on best selected indices acts as a complementary method to help in making conclusive selection decision in addition to another well-known criterion. This is equally supported by studies by Awoke (2021) and Kumar *et al*. (2016) in selection of maize genotypes using selection indices and clustering method. Clustering method based on the best indices and best performing ecotypes need further interrogation and validation in wide environments.

CONCLUSION AND RECOMMENDATION

This study showed that MP, GMP and HMP are the best biomass (yield) indices that could be used to select for drought tolerant and susceptible *Brachiaria* ecotypes in both stressed and non-stressed environments. It is also clear from the study that drought tolerance indices can also be used in selection of drought tolerant ecotypes in drought stressed environment in combination with the yield indices, ranking and clustering methods. Based on the ranking criterion, BrK 10, BrK 21, BrK 24, BrK 2 and BrK 18 were tolerant in drought condition and are recommended for planting in areas prone to drought. From combined cluster analysis, BrK 21, BrK8 and BrK13 were unique and clustered differently from the main clades. There is need to further investigate the identified drought tolerant ecotypes and the three unique ecotypes. This will help to understand whether the ecotypes possess unique genes and what the genes confer. In the event that the genes are responsible for tolerance/resistant to drought, they could be introgressed into breeding schemes to improve on drought tolerance.

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