

Studies

Morpho-physiological and yield traits for selection of drought tolerant *Urochloa* grass ecotypes

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Associate Editor: Abdelbagi Ismail

Abstract. Drought has become more recurrent and causes a substantial decline in forage yields leading to strain on feed resources for livestock production. This has intensified the search for drought-tolerant forages to promote sustainable livestock production. The objective of this study was to identify drought-tolerant *Urochloa* grasses and to discern their morpho-physiological and yield traits to water stress as well as the relationship between these traits and indices of drought resistance. The results showed that the ecotypes, water regimes and their interaction significantly influenced all the studied morpho-physiological and yield traits. There was a significant decrease in plant height, number of leaves and tillers, dry matter yield, relative water content, photosystem II and efficiency of photosystem II with an increase in non-photochemical quenching. The principal component analysis revealed that the performance of *Urochloa* grass ecotypes was different under water sufficient (WS) and water deficit conditions. Drought tolerance indicators (mean productivity, geometric mean productivity, tolerance index and stress tolerance index) were most effective in identifying *Urochloa* ecotypes with high biomass production under both water deficient and WS conditions. Ecotypes K17, K7, Kisii, Busia and Kakamega were the most drought tolerant, Basilisk, K6, K10, K19 and Toledo were moderately tolerant whereas, CIAT6385, CIAT16449, K13, K5 and K9 were drought sensitive. The five drought-tolerant *Urochloa* ecotypes should be tested for sustainable biomass production under field conditions and used in breeding programmes to develop high-yielding drought-tolerant varieties.

Keywords: Ecotypes; forage; principal component analysis; *Urochloa*; water stress.

Introduction

The genus *Urochloa* (syn. *Brachiaria*) has about 135 species that are members of the Poaceae family, Paniceae tribe and Melinidinae subtribe (Ferreira *et al.* 2021). *Urochloa brizantha*, *U. humidicola*, *U. decumbens* and *U. ruziziensis* are the most widely cultivated species in the tropics and sub-tropics because of their economic and agronomic value. *Urochloa* grass grows effectively on infertile acid soils, traps carbon in the soil, fixes nitrogen and minimizes greenhouse gas emissions and ground water pollution by reducing nitrate leaching from agricultural fields (Nandakumar *et al.* 2019; Njarui *et al.* 2020; Van Thanh Ho *et al.* 2020), and therefore, regarded as a climate-smart feed. It also generates a large amount of palatable and nutritious biomass for livestock (Mutimura and Ghimire 2021). *Urochloa* grass has been shown to improve livestock feed availability and thus enhance food and nutrition security (Njarui *et al.* 2020).

The demand for livestock products is high with the world's meat consumption projected to increase by 8 % and 21 % in developed and developing countries, respectively, by 2027 (OECD 2018). In Kenya, livestock contributes 12 % to the national gross domestic product, and provides food

security, income, manure and social-cultural function (GoK 2019; Njarui *et al.* 2020). Livestock production contributes to the realization of United Nations sustainable development goals 1 and 2 including no hunger, provision of food and nutritional security (Schneider and Tarawali 2021). The availability of quality and quantity forage resource is vital for sustainable livestock production (Negawo *et al.* 2017). However, drought reduces forage biomass yield resulting in widespread feed shortage and affect livestock productivity (Djikeng *et al.* 2014; Martin *et al.* 2016; Staver *et al.* 2019). Therefore, the selection of forages that produce high biomass yield under WD is paramount in the face of changing climates (Zuffo *et al.* 2022).

Climate change, especially drought stress, affects the efficiency and productivity of agriculture and subsequently exacerbates food insecurity globally (Dinar *et al.* 2019; Affoh *et al.* 2022; Rohde 2023). Water stress due to drought conditions results in a significant reduction in leaf expansion rate and photosynthesis rate, which inhibits plant development and reduces the biomass production of forage grasses including *Urochloa* grass (Cheruiyot *et al.* 2018; Thiana *et al.* 2020; Zuffo *et al.* 2022). Therefore, urgent screening of *Urochloa* grass ecotypes under water stress is of fundamental

Received: 2 July 2023; Editorial decision: 1 May 2024; Accepted: 4 June 2024

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importance in order to identify drought-tolerant varieties that can adapt to future drought conditions. This is critical, especially in arid and semi-arid regions of Kenya, where drought frequency is high. To achieve this, there is a need to comprehend the mechanisms of water stress tolerance in grasses. The identification of *Urochloa* ecotypes with tolerance to drought stress is the first step towards the development of drought-tolerant cultivars. Forage plants exposed to WD during their growth and development must adapt in order to cope with the environmental conditions. As a result, plants under water stress respond through morpho-physiology modifications to withstand the stress and avoid cell damage (Mastalerczuk and Borawska-Jarmulowicz 2021).

Morpho-physiological and yield responses to drought for each forage grass species or cultivar are dependent on the plant genetic characteristics and phenotype (Staniak and Kocoń 2015; Zahid *et al.* 2021; Zhang, *et al.* 2022). The photosynthetic parameters affected by drought stress include efficiency for photosystem II (F_v/F_m), relative chlorophyll content (SPAD), photosystem II photochemistry (Φ_2) and non-photochemical quenching (Φ_{NPQ} , Kuhlert *et al.* 2016; De Souza *et al.* 2021). WD significantly reduces photosynthesis that hinders plant growth and lowers the biomass yield of grasses (Staniak 2016; Fariaszewska *et al.* 2020). The associations between dry matter yield (DMY) and the morpho-physiological variables, however, are not always direct and clear. The adaptation of plants to drought is influenced by their ability to maintain normal chlorophyll fluorescence or photosynthetic features under drought stress (Chen *et al.* 2016). The effectiveness of breeding programmes in water-stressed areas can be increased by understanding the relationships between yield, morpho-physiological traits and selection indices within a plant ecotype. The aim of this study was to identify potential drought-tolerant *Urochloa* spp. germplasm and to discern their morpho-physiological and yield traits to water stress as well as the relationship between these traits and indices of drought resistance.

Materials and Methods

Experimental site and plant materials

The drought stress experiment was conducted in a screen house at Kenya Agricultural and Livestock Research Organization (KALRO), Katumani station (37° 28' E, 1° 58' S and 1600 m above sea level) in between April and August 2021. A total of 35 *Urochloa* ecotypes grass obtained from KALRO were used in this study [see Supporting Information—Table S1]. The ecotypes were originally obtained from different geographical locations in Kenya and preserved at KALRO—Katumani. The 33 ecotypes selected for use in this study were collected from semi-arid regions that are characterized by frequent droughts. Three ecotypes namely *Urochloa decumbens* cv. Basilisk, *Urochloa brizantha* cvs Toledo and Piata were included as controls due to the fact that they have been reported to produce comparatively high fodder yield under conditions of drought stress (Njarui *et al.* 2016; Cheruiyot *et al.* 2018).

Experimental design, water treatments and growing conditions

The design of the experiments was a randomized complete block in split-plot arrangement with five replications. Two water regime used in this study: water sufficient (WS) ~45 %

volumetric water content (VWC) and water deficit (WD) ~11 % VWC. The two WR were selected due to the fact that they have been reported to be efficient in the selection of drought-tolerant plants including *Urochloa* grass ecotypes (Cheruiyot *et al.* 2018; Marchin *et al.* 2020). We also performed preliminary trials at ~45 % and ~11 % VMC to test the efficacy of the two WR, which proved to be efficient in the classification of ecotypes into different categories of drought tolerance.

Because of limited seeds, each *Urochloa* grass ecotype was propagated using rooted tillers. The rooted tillers of approximately the same size and age from each ecotype were selected, and one tiller was transplanted per plastic pot (11.5 cm × 15 cm) with eight holes at the bottom for drainage. The pots were labelled by ecotype name, filled with 1 kg sterile forest soil (N 0.17, C 1.0, P 10, K 0.76, Mg 3.0, Ca 2.0, Mn 0.32 Cu 4.32, Fe 10.7, Zn 0.67 and Na 0.43) and sand mixed in the ratio of 3:2 to improve drainage. The plants were then watered daily to 100 % of the maximal VWC. After 4 weeks, a standardization cut was made at 5 cm above the soil. The plants were then maintained at 100 % field water capacity (FC) (~45 % VWC) for 21 days and developed between 3 and 4 leaves prior to water stress treatments. The plants were grown in the greenhouse under natural light. The temperature ranged from 17.2 °C to 18.5 °C, from 35.3 °C to 40.4 °C, and from 29.6 °C to 30.7 °C, while humidity ranged from 60 % to 75 %, 28.3–29.7 % and 31.5–33.2 % at 8.00 a.m., 12 p.m. and 5 p.m., respectively.

Plants under WS condition were watered daily to 45 % VWC. However, watering was stopped for the experimental plants under WD to enable progressive drying of the soil to achieve ~11 % VWC as described in Marchin *et al.* (2020). The soil VWC of each plastic pot in the WD treatments before and during the experimental period was measured daily using a soil moisture sensor (Procheck Decagon Device, Inc PC 157C with GS3). Pots exceeding the upper limit of the targeted drought intensity per day (>11 % VWC) were allowed time to drain to achieve the targeted intensity. If soil VWC surpassed the lower limit of the targeted intensity, water was added to the soil surface of the pot to maintain the targeted soil VWC. The specific soil VWC (~11 % VWC) was then maintained for a period of 28 days. Poorter *et al.* (2012) recommended drought treatment period of ~28 days as adequate to ensure total plant dry weight to pot volume less than 2 kg m³.

Measurement of morphological traits

After 28 days of water stress treatment, morphological traits were measured. In both the WD and WS conditions, three plants of each ecotype were randomly selected from each pot. The morphological data collected included the number of tillers (NT) per pot and plant height (PH) of tallest tiller in each pot measured above the soil using a wooden ruler. The number of leaves (NL) in the primary tiller was counted. The plants were uprooted, roots cleaned in tap water and root length (RL) measured.

Measurement of physiological traits

In order to assess the impact of the soil WD on plant physiological function, one plant per replication was selected and used for analysis. The youngest fully expanded leaf was used to determine the relative water content (RWC) as described by Chen *et al.* (2016). Fresh weight (FWT) was immediately

determined by weighing the youngest fully expanded leaf. Leaf segments were then rehydrated in distilled water for 6 h in a closed container in the dark to determine turgid weight while the DMY was measured after leaf segments is dried at 65 °C in an oven for 48 h.

Relative water content (RWC) =

Chlorophyll fluorescence-based photosynthetic parameters including photosystem II photochemistry (Phi2), non-photochemical quenching (PhiNPQ), relative chlorophyll content (SPAD) and quantum yield efficiency for photosystem II (F_v/F_m) were measured using photosynq multispeQ instrument (v1.0) linked to the PhotosynQ platform (<http://www.photosynq.com/technology>) (Kuhlgert *et al.* 2016; Putranto 2018). In each water treatment, one plant per ecotype in each replication was randomly sampled and chlorophyll fluorescence-based photosynthetic parameters determined from the centre of the last fully expanded leaf without altering the leaf angle. The multispeQ protocol used during this study was Photosynthesis RIDES 2.0.

Biomass yield and calculation of drought tolerance indices

All the plant leaves and stems of each ecotype per replication were cut and weighed using analytical balance to determine the FWT. DMY was determined after drying the shoot samples in the oven at 65 °C for 72 h. Drought tolerance indices (DTI) based on biomass yield under the two WR (WD and WS) were calculated as follows:

Mean productivity (MP) = $\frac{Y_s + Y_p}{2}$ (Naghavi *et al.* 2013)

Geometric mean productivity (GMP) = $\sqrt{Y_s \times Y_p}$ (Majidi *et al.* 2016)

Tolerance index (TOL) = $Y_p - Y_s$ (Menezes *et al.* 2014)

Yield stability index (YSI) = $\frac{Y_s}{\bar{Y}_p}$ (Naghavi *et al.* 2013)

Yield index (YI) = $\frac{Y_s}{\bar{Y}_s}$ (Naghavi *et al.* 2013)

Stress susceptibility index (SSI) = $\frac{1 - Y_s/Y_p}{1 - \bar{Y}_s/\bar{Y}_p}$ (Fischer and Maurer 1978)

Stress Tolerance Index (STI) = $\frac{Y_s \times \bar{Y}_p}{\bar{Y}_p^2}$ (Zuffo *et al.* 2022)

where Y_s is the ecotype yield under WD; Y_p is the ecotype yield under water sufficient; \bar{Y}_s is the mean yield of all ecotypes under WD; and \bar{Y}_p is the mean yield of all ecotypes under WS condition.

Statistical analysis

The General Linear Model procedure in R (version 4.1.2) (R Core Team 2021) was used for the analysis of variance (ANOVA) for the morpho-physiological and yield traits. Mean separation was done using the least significant difference (LSD) ($P \leq 0.05$) to test the genotypic difference, drought stress effect and to compare the phenotypic value of the genotype for specific trait and different WR. FactoMineR and Factoextra were used to create principal component analysis (PCA) biplots using all the measured and calculated variables (Kassambara and Mundt 2017; Chaouachi *et al.* 2023). Agglomerative hierarchical clustering was done using the Euclidean distance algorithm. Ranking using PCA was applied to assess drought tolerance level of each *Urochloa* ecotype. Ranking value was calculated using a formula given by Aghaie *et al.* (2018) and Ajtahed *et al.* (2021) as follows:

$$\begin{aligned} \text{Ranking value} = & [(\text{contribution of PC1}(\%) \times \text{PC1}) \\ & + (\text{contribution of PC2}(\%) \times \text{PC2}) \\ & + (\text{contribution of PC3}(\%) \times \text{PC3}) \\ & + (\text{contribution of PC4}(\%) \times \text{PC4})]. \end{aligned}$$

The contributions of the four primary components PC1, PC2, PC3 and PC4 were determined by PCA analysis and are represented as % in this formula. The PC1, PC2, PC3 and PC4 are the PCA loading of morpho-physiological and yield traits for 35 *Urochloa* ecotypes subjected to WD conditions. A numerical rank was calculated from the mean ranking values under WD conditions.

Results

Effects of drought stress on morpho-physiological and yield traits

The ANOVA revealed significant ($P < 0.001$) variation among *Urochloa* grass ecotypes for all the studied morpho-physiological and yield traits [see Supporting Information—Table S2]. The water regimes (WR) were the largest contributor to the variation observed across all the traits (see Supporting Information—Table S2; $P < 0.001$). Moreover, the interaction between Eco \times WR was significant for all the traits [see Supporting Information—Table S2].

Morpho-physiological traits and DM yield were significantly ($P < 0.05$) reduced in plants exposed to WD, while non-photochemical quenching (PhiNPQ) significantly increased under WD (Tables 1 and 2 and Supporting Information—Table S3). The percentage reductions varied in studied traits, with RWC, relative chlorophyll content (SPAD) and fresh weight (FWT) recording significantly high reductions of 85.9 %, 85.3 % and 84 %, respectively, under WD conditions. The NT per plant, followed by the NL, and RL, was the least affected by WD conditions (Table 1).

Drought stress led to a significant decrease in PH by 43.8 %. The highest decrease was observed for CIAT6399 (73.7 %), K10 (65.5 %) and K6 (64.4 %). Ecotypes K7 and K17 were significantly taller than all the other ecotypes under WS and WD conditions, respectively, (Table 1; $P \leq 0.05$). The NT was significantly reduced by WD for most of the studied ecotypes, except CIAT16514, CIAT6385, K2, K6, K15 and K22. Ecotypes K12 and K19 recorded significant NT decreases of 63.2 % and 61.9 %, respectively (Table 1; $P \leq 0.05$). Kisii, K15 and K23 had significantly higher NT than all the other ecotypes while CIAT6684 recorded the lowest NT under WD conditions. WD significantly reduced NL by 29.7 %. Ecotypes K1 and K15 recorded significantly high NL (Table 1). Decreased RL was observed under WD conditions ($P \leq 0.05$), with CIAT16514, K8, and Lanet recording high percentage reductions of 53.3 %, 56.5 % and 53.2 %, respectively.

The RWC significantly decreased under WD conditions (Table 2; $P \leq 0.05$). Ecotypes K5 (96.2 %) and K18 (94.1 %) recorded the highest percentage reductions. A significant high RWC was recorded by CIAT16449 under WD conditions, whereas K5 recorded the lowest value (Table 2; $P \leq 0.05$). In addition, a significant reduction of 50 % was observed in Phi 2 under WD conditions (Table 2). The highest decrease was recorded by Kisii (79.4 %) and K7 (76.6 %), whereas significant high values were observed in K22 and K9. WD

Table 1. Mean PH, NT, number of leaves and root length *Urochloa* grass ecotypes under WS and WD. CV, coefficient of variation; LSD, least significance difference. Values expressed as Mean \pm SEM (n = 3)

Traits	Plant height (cm)		Number of tillers		Number of leaves		Root length (cm)	
	WS	WD	WS	WD	WS	WD	WS	WD
CIAT16449	8.66 \pm 0.21	5.6 \pm 0.25	2.6 \pm 0.60	1.4 \pm 0.25	4.4 \pm 0.40	2.8 \pm 0.37	54.8 \pm 0.20	44.4 \pm 2.25
CIAT16514	25.04 \pm 0.16	10.4 \pm 0.25	4.6 \pm 0.25	6.20.37	4.2 \pm 0.37	3.2 \pm 0.80	90.8 \pm 0.20	42.4 \pm 5.06
CIAT 6384	11.00 \pm 0.32	8.4 \pm 0.25	3.0 \pm 0.45	2.8 \pm 0.37	5.4 \pm 0.51	2.8 \pm 0.37	85.0 \pm 1.67	57.4 \pm 5.99
CIAT 6385	8.60 \pm 0.25	7.8 \pm 0.26	1.2 \pm 0.20	2.0 \pm 0.00	3.2 \pm 0.20	3.0 \pm 0.45	111.2 \pm 0.58	55.2 \pm 13.9
CIAT6399	17.46 \pm 0.16	4.6 \pm 0.25	7.2 \pm 0.20	5.0 \pm 0.32	5.4 \pm 0.40	2.6 \pm 0.25	90.6 \pm 0.25	48.2 \pm 4.74
CIAT6426	15.7 \pm 0.26	6.5 \pm 0.22	2.2 \pm 0.49	1.8 \pm 0.37	4.4 \pm 0.24	3.2 \pm 0.20	76.6 \pm 0.93	49.2 \pm 6.06
CIAT6684	10.82 \pm 0.23	9.0 \pm 0.27	3.0 \pm 0.00	1.2 \pm 0.20	4.2 \pm 0.74	3.2 \pm 0.20	51.0 \pm 0.45	47.8 \pm 3.18
cv. Basilisk	18.5 \pm 0.22	7.8 \pm 0.30	11.8 \pm 0.20	4.8 \pm 0.20	5.8 \pm 0.37	2.8 \pm 0.20	79.6 \pm 4.89	57.0 \pm 4.09
Busia	13.9 \pm 0.19	7.9 \pm 0.18	7.6 \pm 0.25	6.6 \pm 0.25	6.2 \pm 0.49	4.0 \pm 0.00	55.4 \pm 0.25	54.8 \pm 5.42
K1	24.1 \pm 0.40	11.5 \pm 0.32	4.6 \pm 0.25	3.4 \pm 0.40	5.8 \pm 0.37	4.6 \pm 0.40	80.0 \pm 0.32	43.4 \pm 4.28
K2	16.60 \pm 0.37	12.2 \pm 0.52	6.6 \pm 0.25	6.8 \pm 0.37	4.4 \pm 0.25	2.8 \pm 0.37	77.0 \pm 0.45	43.6 \pm 9.41
K3	12.8 \pm 0.12	11.0 \pm 0.61	3.2 \pm 0.20	2.0 \pm 0.32	4.0 \pm 0.32	4.4 \pm 0.40	74.4 \pm 0.25	53.2 \pm 7.08
K4	15.46 \pm 0.17	11.6 \pm 0.29	3.4 \pm 0.25	2.4 \pm 0.25	4.2 \pm 0.37	3.6 \pm 0.25	82.0 \pm 0.00	50.2 \pm 6.64
K5	12.56 \pm 0.25	5.2 \pm 0.20	2.8 \pm 0.20	1.4 \pm 0.25	4.0 \pm 0.32	2.4 \pm 0.40	50.6 \pm 0.40	55.0 \pm 1.48
K6	18.00 \pm 0.27	6.4 \pm 0.25	4.2 \pm 0.86	5.0 \pm 0.32	5.2 \pm 0.92	3.0 \pm 0.45	72.6 \pm 4.37	53.0 \pm 4.72
K7	29.90 \pm 0.33	12.5 \pm 0.22	6.6 \pm 0.25	3.8 \pm 0.20	5.2 \pm 0.58	4.2 \pm 0.20	87.2 \pm 0.37	62.6 \pm 3.47
K8	12.54 \pm 0.23	6.6 \pm 0.25	5.0 \pm 0.71	4.2 \pm 0.20	6.0 \pm 0.55	2.8 \pm 0.20	97.0 \pm 0.55	51.0 \pm 2.30
K9	11.54 \pm 0.25	6.5 \pm 0.32	2.8 \pm 0.20	2.2 \pm 0.37	5.0 \pm 0.55	2.8 \pm 0.49	121.8 \pm 6.21	53.0 \pm 11.6
K10	22.0 \pm 0.55	7.6 \pm 0.25	5.6 \pm 0.25	4.4 \pm 0.25	5.8 \pm 0.37	3.6 \pm 0.40	68.2 \pm 0.49	69.2 \pm 4.22
K12	16.3 \pm 0.37	7.7 \pm 0.20	3.8 \pm 0.20	1.4 \pm 0.25	4.2 \pm 0.20	3.6 \pm 0.25	87.4 \pm 0.40	42.0 \pm 3.89
K13	7.08 \pm 0.11	5.2 \pm 0.37	2.2 \pm 0.20	2.2 \pm 0.20	4.6 \pm 0.25	2.8 \pm 0.37	53.0 \pm 6.20	55.4 \pm 8.62
K15	7.64 \pm 0.09	4.6 \pm 0.19	7.2 \pm 0.20	9.0 \pm 0.00	4.8 \pm 0.58	4.6 \pm 0.40	55.6 \pm 0.25	50.6 \pm 5.62
K16	11.62 \pm 0.18	8.8 \pm 0.12	3.0 \pm 0.71	2.0 \pm 0.45	4.8 \pm 0.66	3.2 \pm 0.49	84.8 \pm 1.24	49.0 \pm 7.63
K17	21.20 \pm 0.37	15.8 \pm 0.26	5.8 \pm 0.20	4.2 \pm 0.37	4.6 \pm 0.25	4.0 \pm 0.32	71.8 \pm 3.81	62.0 \pm 6.31
K18	14.72 \pm 0.19	5.7 \pm 0.30	4.0 \pm 0.00	1.6 \pm 0.45	5.0 \pm 0.32	3.4 \pm 0.25	68.6 \pm 0.25	63.2 \pm 10.9
K19	17.50 \pm 0.22	11.5 \pm 0.32	8.4 \pm 0.25	3.2 \pm 0.37	5.6 \pm 0.25	3.6 \pm 0.25	75.2 \pm 0.37	52.6 \pm 6.53
K20	7.80 \pm 0.20	6.4 \pm 0.25	2.4 \pm 0.25	1.8 \pm 0.37	5.0 \pm 0.32	3.8 \pm 0.37	62.2 \pm 0.20	53.6 \pm 8.39
K21	19.60 \pm 0.25	11.6 \pm 0.25	3.4 \pm 0.25	2.8 \pm 0.20	4.0 \pm 0.32	3.2 \pm 0.20	74.4 \pm 0.25	45.8 \pm 4.45
K22	24.50 \pm 0.22	11.6 \pm 0.25	5.0 \pm 0.95	5.2 \pm 0.20	5.6 \pm 0.25	3.4 \pm 0.25	58.4 \pm 0.25	47.8 \pm 2.60
K23	8.12 \pm 0.20	5.5 \pm 0.32	10.6 \pm 0.25	9.0 \pm 0.32	5.6 \pm 0.40	4.2 \pm 0.20	61.4 \pm 1.66	63.6 \pm 7.35
Kakamega	11.20 \pm 0.52	8.6 \pm 0.19	5.6 \pm 0.25	4.8 \pm 0.37	3.6 \pm 0.25	3.0 \pm 0.00	85.4 \pm 1.86	47.6 \pm 2.50
Kisii	11.90 \pm 0.19	6.8 \pm 0.26	11.6 \pm 0.40	9.0 \pm 0.32	5.2 \pm 0.37	3.6 \pm 0.25	69.0 \pm 0.20	42.2 \pm 1.24
Lanet	20.00 \pm 0.35	13.0 \pm 0.16	6.6 \pm 0.25	3.8 \pm 0.37	5.6 \pm 0.25	4.2 \pm 0.20	95.2 \pm 0.74	44.6 \pm 4.52
cv. Piata	6.40 \pm 0.29	4.6 \pm 0.25	4.2 \pm 0.20	3.0 \pm 0.32	4.0 \pm 0.95	3.6 \pm 0.25	69.8 \pm 0.20	36.0 \pm 1.26
cv. Toledo	11.0 \pm 0.16	6.4 \pm 0.24	5.8 \pm 0.97	4.2 \pm 0.37	3.6 \pm 0.40	2.8 \pm 0.20	67.4 \pm 2.94	63.4 \pm 3.41

Table 1. Continued

Traits	Plant height (cm)		Number of tillers		Number of leaves		Root length (cm)	
	WS	WD	WS	WD	WS	WD	WS	WD
Mean	14.9 ± 0.44	8.37 ± 0.22	5.07 ± 0.21	3.85 ± 0.17	4.82 ± 0.09	3.39 ± 0.07	75.53 ± 1.27	51.72 ± 1.09
% CV	38.0	34.7	53.70	59.3	24.5	26.6	22.3	27.8
LSD _{0.05}	0.76	0.79	1.13	0.85	1.25	0.95	5.94	17.31

significantly increased PhiNPQ by 28.3 % (Table 2; $P \leq 0.05$). CIAT6426 recorded the highest increase in PhiNPQ by 110.3 % and cv. Toledo the lowest (2.9 %). For Phi2, PhiNPQ, SPAD and F_v/F_m , significant high values were observed in ecotype K22, Kisii, K1 and K22, respectively, under WD conditions (Table 2; $P \leq 0.05$). The SPAD significantly declined under WD ($P \leq 0.05$), with K2 recording the highest value. Moreover, a significant decline of 43.9 % was observed in F_v/F_m ratio under water stress. Ecotype K22 and K9 recorded the highest F_v/F_m under WD. The average DMY decreased by 66.8 %, with the Kakamega ecotype recording a significantly higher DM yield under WD than the other ecotypes [see Supporting Table S3].

Association among morpho-physiological traits and biomass yield

Under WD conditions, DMY was significantly and positively correlated with NT ($P < 0.001$) and NL ($P \leq 0.05$; Fig. 1A). Additionally, NL was positively associated with PH and PhiNPQ but negatively correlated with RWC, Phi2 and F_v/F_m . Relative chlorophyll content (SPAD) was positively associated with PH and PhiNPQ but negatively correlated with Phi2 and F_v/F_m . A significant positive correlation was also observed between F_v/F_m with Phi2 while PhiNPQ was negatively associated with Phi2 and F_v/F_m under WD (Fig. 1A).

Under WS, morpho-physiological traits showed significant and positive correlations between PH with DMY, whereas, SPAD was negatively associated with PH ($P < 0.05$; Fig. 1B). Moreover, there was a significant positive association between DMY with NT and NL. RL was significantly and positively correlated with Phi2 and F_v/F_m ($P \leq 0.05$). The DYM and FWT; Phi2 and F_v/F_m ; RWC and SPAD were positively correlated under WS conditions (Fig. 1B).

Yield under WS (Yp) was significantly and positively associated with yield under WD (Ys) conditions [see Supporting Information Table S4]. The data showed that MP, GMP, TOL, STI and YI were positively associated with Yp and Ys. SSI was negatively correlated with Ys but not significantly associated with Yp. In contrast, YSI was negatively correlated with all the drought indices tested in this study [see Supporting Information Table S4].

Principal component analysis biplots for morpho-physiological and biomass yield traits

The first two PCA explained cumulative variance of 49.4 % and 58.9 % under WS and WD conditions, respectively (Fig. 2). It is evident that PH, NL, RL, NT, RWC, DMY, Phi2, F_v/F_m , PhiNPQ and SPAD parameters have a role in the variability of the *Urochloa* germplasm (Fig. 2). Under WS conditions, DMY, NT and PH showed significant positive contributions while PhiNPQ and SPAD contributed negatively in PC1 (Fig. 2A). The PC2 was associated with diversity among *Urochloa* ecotypes due to F_v/F_m , Phi2, RL and SPAD. Kisii and Busia cultivars had the highest biomass yield as well as RWC, which are related to the NT per plant. In addition to the RL, ecotypes K2, K8 and K6 were efficient in the photosynthetic traits Phi2 and F_v/F_m (Fig. 2A). Under WD, F_v/F_m and Phi2 showed positive contribution in PC1 whereas, PC2 was associated with diversity among ecotypes due to the positive contribution of NT, DMY, Phi2 and F_v/F_m (Fig. 2B). Ecotypes Kakamega, K15 and K23 inclined toward the direction of the NT and DM yield while K19, N3 and N1 clustered toward SPAD and PhiNPQ under WD conditions.

Table 2. Mean physiological traits for *Urochloa* grass ecotypes under WS and WD conditions.

Traits	Relative water content (RWC)		Photosystem II (Phi2)		Non-photochemical quenching (PhiNPQ)		Relative chlorophyll content (SPAD)		Efficiency of photosystem II (Fv/Fm)	
	WS	WD	WS	WD	WS	WD	WS	WD	WS	WD
CIAT16449	81.66 ± 1.60	24.59 ± 1.14	0.45 ± 0.01	0.21 ± 0.03	0.57 ± 0.06	0.74 ± 0.04	43.62 ± 3.02	2.14 ± 0.42	0.43 ± 0.04	0.26 ± 0.03
CIAT16514	73.66 ± 0.44	12.68 ± 0.65	0.33 ± 0.10	0.19 ± 0.04	0.56 ± 0.11	0.75 ± 0.05	33.42 ± 1.90	4.72 ± 0.31	0.44 ± 0.08	0.27 ± 0.05
CIAT6384	68.30 ± 3.41	21.50 ± 13.8	0.56 ± 0.06	0.20 ± 0.03	0.69 ± 0.05	0.75 ± 0.03	36.59 ± 0.81	3.46 ± 0.93	0.50 ± 0.06	0.24 ± 0.03
CIAT6385	75.14 ± 1.08	13.31 ± 1.42	0.42 ± 0.08	0.24 ± 0.04	0.59 ± 0.12	0.69 ± 0.05	26.98 ± 1.22	2.97 ± 0.66	0.48 ± 0.09	0.33 ± 0.04
CIAT6399	89.96 ± 1.69	6.77 ± 0.94	0.37 ± 0.05	0.23 ± 0.04	0.63 ± 0.03	0.71 ± 0.04	37.18 ± 1.03	2.74 ± 0.35	0.45 ± 0.04	0.27 ± 0.03
CIAT6426	70.23 ± 1.12	16.46 ± 0.44	0.45 ± 0.02	0.14 ± 0.02	0.39 ± 0.10	0.82 ± 0.02	37.46 ± 1.32	11.16 ± 1.12	0.46 ± 0.05	0.18 ± 0.02
CIAT6684	72.40 ± 0.66	17.72 ± 0.25	0.17 ± 0.03	0.15 ± 0.02	0.39 ± 0.09	0.81 ± 0.03	43.75 ± 0.86	3.38 ± 0.71	0.32 ± 0.04	0.20 ± 0.03
cv. Basilisk	80.74 ± 0.55	18.10 ± 0.64	0.54 ± 0.02	0.20 ± 0.04	0.70 ± 0.04	0.75 ± 0.03	35.13 ± 0.93	3.13 ± 0.67	0.45 ± 0.05	0.24 ± 0.04
Busia	86.63 ± 0.59	7.09 ± 0.61	0.26 ± 0.01	0.14 ± 0.00	0.64 ± 0.02	0.82 ± 0.01	42.75 ± 0.22	4.12 ± 0.56	0.47 ± 0.04	0.18 ± 0.01
K1	77.73 ± 0.43	9.96 ± 1.38	0.34 ± 0.01	0.12 ± 0.03	0.70 ± 0.04	0.84 ± 0.03	38.41 ± 0.16	13.70 ± 1.02	0.39 ± 0.03	0.17 ± 0.03
K2	93.62 ± 0.89	12.28 ± 0.22	0.53 ± 0.04	0.21 ± 0.01	0.45 ± 0.04	0.72 ± 0.01	38.76 ± 1.66	13.11 ± 0.34	0.46 ± 0.05	0.28 ± 0.01
K3	66.77 ± 0.43	5.08 ± 0.84	0.26 ± 0.02	0.14 ± 0.01	0.56 ± 0.06	0.82 ± 0.02	36.31 ± 0.59	12.08 ± 1.25	0.43 ± 0.01	0.18 ± 0.02
K4	75.62 ± 0.39	16.61 ± 61	0.32 ± 0.05	0.12 ± 0.01	0.65 ± 0.04	0.84 ± 0.02	40.54 ± 0.84	6.57 ± 1.86	0.41 ± 0.02	0.19 ± 0.02
K5	64.71 ± 0.43	2.46 ± 0.33	0.30 ± 0.05	0.21 ± 0.02	0.68 ± 0.03	0.74 ± 0.02	34.59 ± 0.19	2.48 ± 0.53	0.38 ± 0.06	0.20 ± 0.02
K6	63.79 ± 0.13	9.65 ± 1.04	0.56 ± 0.06	0.22 ± 0.04	0.71 ± 0.06	0.72 ± 0.03	30.70 ± 1.27	6.66 ± 0.23	0.44 ± 0.09	0.27 ± 0.02
K7	74.35 ± 0.82	9.61 ± 2.21	0.47 ± 0.01	0.11 ± 0.01	0.67 ± 0.06	0.86 ± 0.02	34.51 ± 1.70	12.12 ± 1.00	0.37 ± 0.02	0.15 ± 0.02
K8	76.58 ± 0.54	10.88 ± 1.81	0.40 ± 0.03	0.21 ± 0.01	0.67 ± 0.03	0.73 ± 0.01	32.43 ± 0.88	9.31 ± 0.29	0.47 ± 0.02	0.26 ± 0.01
K9	85.43 ± 0.90	11.56 ± 0.39	0.49 ± 0.04	0.32 ± 0.04	0.51 ± 0.09	0.57 ± 0.05	36.23 ± 0.48	1.92 ± 0.42	0.51 ± 0.03	0.44 ± 0.03
K10	74.18 ± 0.67	8.97 ± 2.31	0.68 ± 0.01	0.19 ± 0.02	0.64 ± 0.04	0.76 ± 0.03	36.45 ± 1.14	2.06 ± 0.37	0.55 ± 0.08	0.25 ± 0.03
K12	62.32 ± 1.05	8.99 ± 1.22	0.45 ± 0.01	0.17 ± 0.02	0.58 ± 0.02	0.78 ± 0.30	33.01 ± 1.50	5.88 ± 0.30	0.42 ± 0.04	0.22 ± 0.03
K13	64.27 ± 2.47	10.0 ± 0.62	0.23 ± 0.01	0.16 ± 0.01	0.72 ± 0.03	0.80 ± 0.02	33.62 ± 0.97	3.13 ± 0.53	0.30 ± 0.04	0.20 ± 0.01
K15	61.32 ± 0.57	5.75 ± 0.65	0.25 ± 0.03	0.16 ± 0.02	0.59 ± 0.06	0.79 ± 0.03	48.48 ± 0.20	1.44 ± 0.16	0.41 ± 0.03	0.21 ± 0.02
K16	74.41 ± 3.36	8.19 ± 0.50	0.24 ± 0.01	0.20 ± 0.05	0.59 ± 0.06	0.74 ± 0.07	34.60 ± 1.17	4.41 ± 1.82	0.36 ± 0.02	0.26 ± 0.05
K17	55.68 ± 1.06	6.74 ± 1.09	0.39 ± 0.02	0.14 ± 0.03	0.66 ± 0.01	0.82 ± 0.04	32.33 ± 0.52	8.33 ± 0.88	0.42 ± 0.04	0.18 ± 0.03
K18	55.68 ± 1.42	3.28 ± 0.81	0.16 ± 0.01	0.15 ± 0.02	0.75 ± 0.04	0.81 ± 0.03	42.10 ± 0.53	3.11 ± 0.37	0.37 ± 0.04	0.19 ± 0.03
K19	64.97 ± 0.91	5.51 ± 0.84	0.23 ± 0.01	0.16 ± 0.03	0.61 ± 0.06	0.79 ± 0.03	36.61 ± 0.52	2.85 ± 0.70	0.28 ± 0.04	0.21 ± 0.03
K20	68.46 ± 1.15	8.94 ± 0.54	0.37 ± 0.05	0.15 ± 0.01	0.41 ± 0.06	0.81 ± 0.01	33.92 ± 0.81	2.94 ± 0.46	0.37 ± 0.06	0.19 ± 0.01
K21	83.73 ± 1.33	13.36 ± 0.42	0.36 ± 0.03	0.17 ± 0.04	0.67 ± 0.03	0.78 ± 0.05	37.14 ± 0.65	11.30 ± 0.46	0.42 ± 0.05	0.23 ± 0.05
K22	68.11 ± 1.39	10.74 ± 1.32	0.27 ± 0.02	0.36 ± 0.09	0.46 ± 0.06	0.52 ± 0.12	36.83 ± 2.07	2.45 ± 0.08	0.38 ± 0.03	0.46 ± 0.09
K23	68.05 ± 0.36	4.18 ± 0.85	0.21 ± 0.02	0.18 ± 0.02	0.55 ± 0.02	0.77 ± 0.03	38.12 ± 2.16	1.45 ± 0.11	0.42 ± 0.05	0.24 ± 0.03
Kakamega	76.20 ± 0.32	6.72 ± 0.53	0.31 ± 0.02	0.16 ± 0.02	0.66 ± 0.03	0.79 ± 0.02	42.09 ± 0.74	5.28 ± 0.71	0.40 ± 0.02	0.21 ± 0.01
Kisii	91.56 ± 1.72	10.67 ± 0.38	0.34 ± 0.04	0.07 ± 0.00	0.52 ± 0.03	0.90 ± 0.00	37.89 ± 0.55	8.12 ± 1.98	0.47 ± 0.01	0.12 ± 0.01
Lanet	71.62 ± 0.29	5.61 ± 1.73	0.37 ± 0.04	0.17 ± 0.02	0.64 ± 0.04	0.78 ± 0.03	33.11 ± 1.80	4.90 ± 0.86	0.34 ± 0.09	0.22 ± 0.02
cv. Piata	88.96 ± 0.22	10.69 ± 1.11	0.25 ± 0.02	0.16 ± 0.01	0.63 ± 0.05	0.80 ± 0.02	45.11 ± 0.53	2.36 ± 0.36	0.26 ± 0.09	0.19 ± 0.01
cv. Toledo	92.97 ± 0.70	11.39 ± 0.23	0.19 ± 0.01	0.23 ± 0.02	0.68 ± 0.03	0.70 ± 0.03	39.37 ± 1.46	4.51 ± 0.85	0.40 ± 0.02	0.31 ± 0.02
Mean	74.27 ± 0.79	10.46 ± 0.54	0.36 ± 0.01	0.18 ± 0.01	0.60 ± 0.01	0.77 ± 0.01	37.14 ± 0.38	5.44 ± 0.30	0.41 ± 0.01	0.23 ± 0.00
%CV	14.05	68.05	35.25	44.05	24.06	13.48	13.53	73.89	28.91	39.76
LSD	3.45	7.12	0.104	0.082	0.16	0.10	3.46	2.31	0.14	0.27

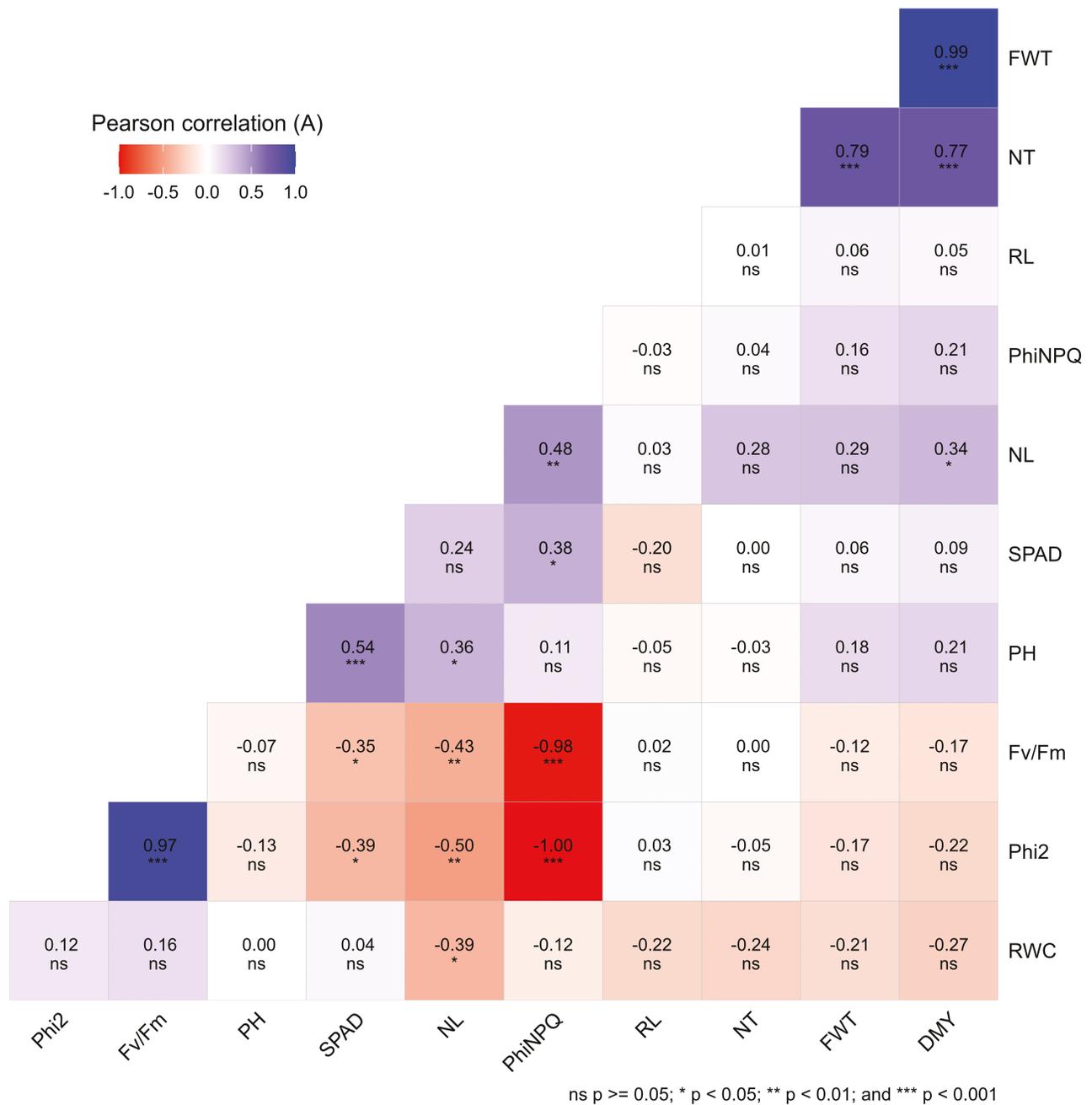


Figure 1. Correlation plot for morpho-physiological and yield traits under WD (A) and WS (B) conditions. NT, number of tillers; PH, plant height; NL, number of leaves; RL, root length; DMY, dry matter yield; FWT, fresh weight; RWC, relative water content; Phi2, photosystem II photochemistry; PhiNPQ, non-photochemical quenching, SPAD, relative chlorophyll content; F_v/F_m , efficiency of photosystem II.

For the DTI, PC1 represented 75.25 % of the total variation among the ecotypes and was positively attributed to variation in all the indices except YSI. However, PC2 contributed 21.5 % of the total variation due to the positive contribution of Ys, GMP, YSI, YI and STI (Fig. 2C). Strong positive associations were observed among YI, Ys, STI, GMP, MP, Yp and TOL in the PCA biplot. Moreover, negative association between YSI and SSI was observed (Fig. 2C).

Hierarchical cluster analysis

The 35 *Urochloa* ecotypes were grouped into five clusters: cluster I, cluster IIa, cluster IIb, cluster III and cluster IV,

each comprising 3, 10, 9, 8 and 5 ecotypes, respectively. Each cluster had closely related ecotypes (Fig. 3).

Cluster I accounted for 8.6 % of all the studied ecotypes that had higher Phi2 and F_v/F_m but recorded low values for all the other parameters and hence were considered susceptible to drought (Fig. 3, Table 3). Cluster II, comprised for 54.3 % of all ecotypes, which exhibited higher RWC and moderate chlorophyll content under WD and were considered moderately tolerant to drought stress (Table 3). Cluster IIb contained ecotypes with significantly longer roots but poor biomass yield. Cluster III had 37.1 % of the ecotypes, which were categorized as drought tolerant, with higher values of

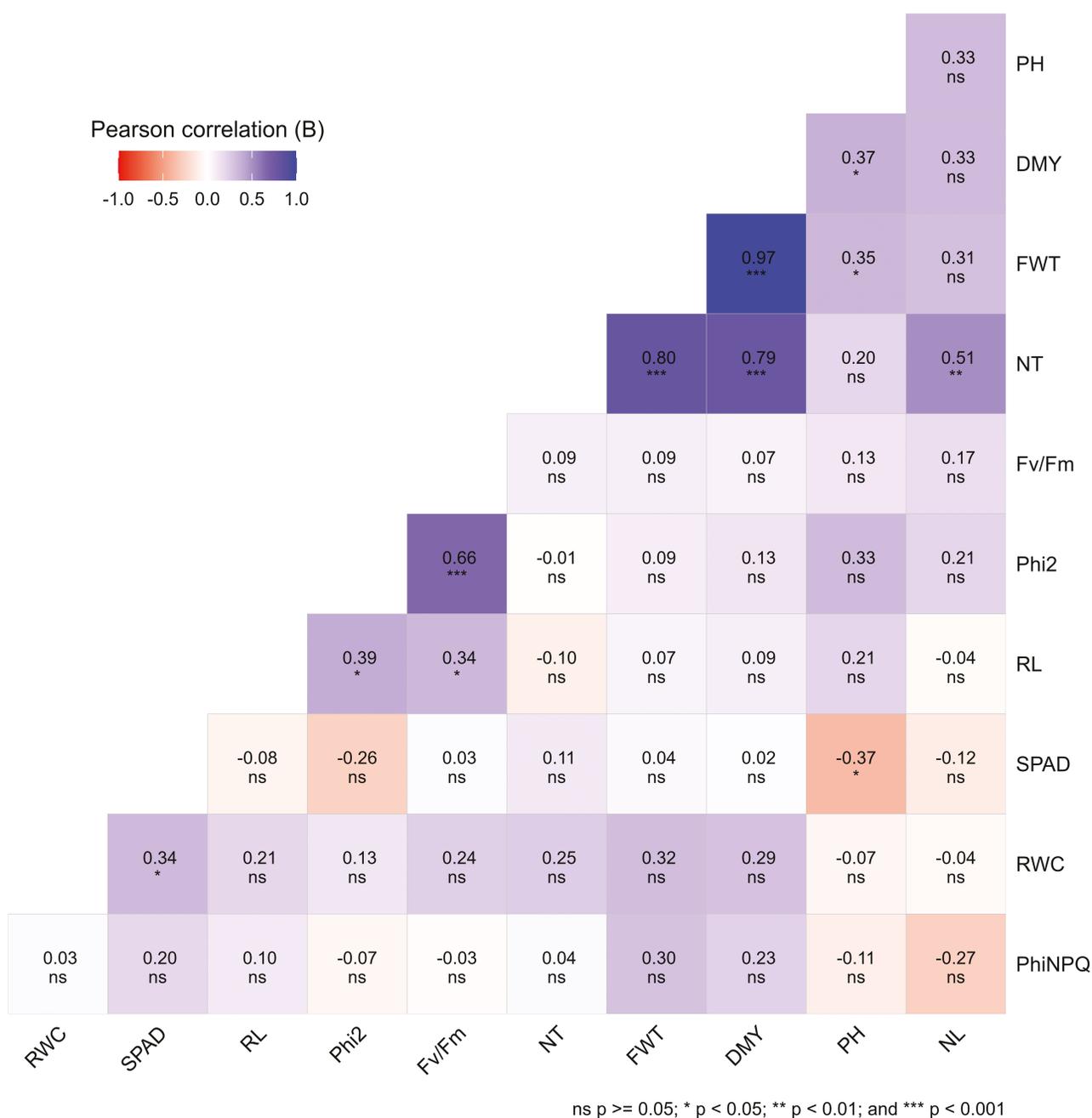


Figure 1. Continued

NT, PH, NL, Phi2 and SPAD under WD conditions. Cluster IV had mild tolerance to drought with higher Phi2 and F_v/F_m but recorded low values for all the other parameters (Fig. 3 and Table 3).

Table 4 shows the mean ranking value for *Urochloa* grass ecotypes. Ecotypes K17, Kisii, Busia, K7 and Kakamega had the highest mean ranking values while ecotypes K13, CIAT6385, CIAT16449, K5 and K9 had the lowest mean ranking.

Discussion

Forage breeders are constantly screening germplasm for selection of drought tolerant for utilization in advanced breeding

programmes (Liu *et al.* 2015; Cheruiyot *et al.* 2018; Ajtahed *et al.* 2021). Greenhouse screening of germplasm for ability to withstand drought is an artificially induced method and has been reported to be an efficient way to identify drought tolerant forages (Fariaszewska *et al.* 2017). This is due to ease in the generation of uniform environmental conditions resulting in controlled plant growth (Ma *et al.* 2019). *Urochloa* is one of the important tropical forage grasses as it produces high tonnage of foliage biomass (Njarui *et al.* 2016). It is known to exhibit drought tolerance, although water availability still dictates the maximum yields achieved by *Urochloa* plants. This requires studies on cultivar-specific responses for the identification of *Urochloa* ecotypes with potential drought tolerance and high biomass and DMY. Drought stresses can

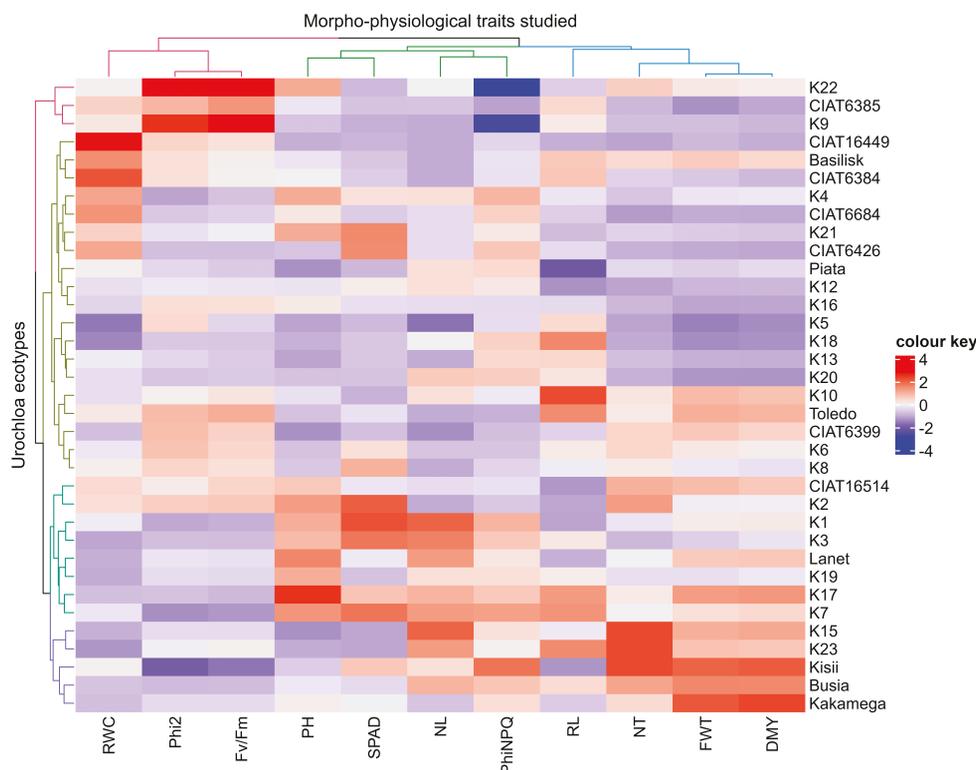


Figure 3. A heatmap with dendrogram showing hierarchical clustering of 35 *Urochloa* grass ecotypes and 11 studied traits under WD conditions. NT, number of tillers; PH, plant height; NL, number of leaves; RL, root length; FWT, fresh weight; DMY, dry matter yield; RWC, relative water content; Phi2, photosystem II photochemistry; PhiNPQ, non-photochemical quenching, SPAD, relative chlorophyll content; F_v/F_m , efficiency of photosystem II.

K6 and K22 did not change under WD conditions, an indication that the ecotypes gave priority to generating more tillers as a drought avoidance strategy. More tillers maximize plant use of available resources, such as water, capture more sunlight and thus increase photosynthesis efficiency. This is similar to tall wheatgrass (Borrajó *et al.* 2018) and perennial ryegrass (Turner *et al.* 2012) in which tillering reduction was not pronounced under water stress. Therefore, *Urochloa* ecotypes with the highest NT would be the most stable in WD conditions.

Ecotype K7, K10, K17, K18 and K23 had longer RL than other ecotypes under water stress. Longer RL improves plant water absorption, but elongated roots may hinder shoot growth as more photosynthetic products are translocated toward the roots (Zahid *et al.* 2021). The reduction in the NL under WD observed in this study is an adaptive strategy for *Urochloa* grasses to survive under drought stress conditions as reported by Staniak and Kocóń (2015).

Sufficient water is important in the plant's life cycle. Leaf water status, estimated by leaf RWC, is crucial for assessing plant drought tolerance in water stress conditions. WD conditions reduced RWC in all the ecotypes examined. Similarly, Mastalerczuk and Borawska-Jarmulowicz (2021) observed a reduction in the RWC in drought stress in *Festulolium braunii*, *Lolium perenne* and *Festuca arundinacea*. Such decrease in RWC hinder grass growth under WD conditions (Wang *et al.* 2017; Faraszewska *et al.* 2020). However, accession CIAT16449 had a significantly higher RWC suggesting that different ecotypes of *Urochloa* grass have varying capacities to absorb soil moisture as well as distinct survival strategy in WD conditions (Mukami *et al.* 2019).

Drought stress significantly impacted chlorophyll content and efficiency of photosynthesis as measured by the MultispeQ device. Photosystem II photochemistry reduced in most of the ecotypes under WD conditions. The same findings were observed by Singh *et al.* (2022) and Akello *et al.* (2023). On the contrary, the K22 ecotype recorded increased Phi2 under water stress as was previously observed in ryegrass (Cielniak *et al.* 2006), *Arabidopsis* (Chen *et al.* 2016) and barley (Fernández-Calleja *et al.* 2020). Ecotype K22 may have transpired more often under water stress, relieving the electron pressure and allowing Phi2 to function more effectively (Fernández-Calleja *et al.* 2020).

A significant increase in PhiNPQ was observed with decreasing Phi2 under water stress. Ben-Jabeur *et al.* (2021) and Madumane *et al.* (2024) also recorded increasing PhiNPQ with drought stress. Thus, the plants preferred light-dependent dissipative processes involving PhiNPQ (Gómez *et al.* 2018). Drought-susceptible ecotypes CIAT6426 and CIAT6684 had the highest percentage increase in PhiNPQ in comparison to others under WD conditions. Ecotype K6 had slight increase in PhiNPQ implying less photosystem damage and downregulation of photosynthesis hence more drought tolerant. This is similar to a study by Singh *et al.* (2022) in which drought tolerant maize genotype increases PhiNPQ marginally in comparison to drought susceptible genotypes under drought stress. However, other authors observed a decrease in PhiNPQ with increasing drought stress (Singh *et al.* 2022; Akello *et al.* 2023). This could be due to differences in the plant species studied and the drought tolerance period applied in the experiments. A significant negative association between the increase in PhiNPQ and the decrease in Phi2

Table 3. Mean \pm SEM values for all traits of 35 *Urochloa* grass ecotypes under different clusters. NT, number of tillers; PH, plant height; NL, number of leaves; RL, root length, DMY, dry matter yield; RCW, relative water content; Phi2, photosystem II photochemistry; F_v/F_m , efficiency of photosystem II; PhiNPQ, non-photochemical quenching; SPAD, relative chlorophyll content.

Trait/Cluster	PH	NT	NL	RL	RWC	Phi2	PhiNPQ	SPAD	F_v/F_m	DMY
I	8.63 \pm 1.53	3.13 \pm 1.03	3.07 \pm 0.18	52.00 \pm 2.19	11.87 \pm 0.76	0.31 \pm 0.04	0.59 \pm 0.05	2.25 \pm 0.30	0.41 \pm 0.04	0.66 \pm 0.25
IIa	8.24 \pm 0.64	2.48 \pm 0.38	3.12 \pm 0.01	50.62 \pm 2.11	15.69 \pm 1.66	0.18 \pm 0.01	0.77 \pm 0.01	5.59 \pm 1.03	0.22 \pm 0.01	1.61 \pm 0.26
IIb	6.01 \pm 0.31	3.31 \pm 0.51	3.02 \pm 0.16	56.96 \pm 2.31	8.04 \pm 1.07	0.19 \pm 0.01	0.75 \pm 0.15	4.10 \pm 0.80	0.24 \pm 0.01	0.87 \pm 0.22
III	12.24 \pm 0.57	4.18 \pm 0.56	3.88 \pm 0.22	50.55 \pm 2.95	8.43 \pm 1.10	0.16 \pm 0.01	0.80 \pm 0.02	8.98 \pm 1.53	0.21 \pm 0.02	1.33 \pm 0.14
IV	6.68 \pm 0.34	7.68 \pm 0.86	3.88 \pm 0.27	51.76 \pm 3.60	6.88 \pm 1.07	0.14 \pm 0.02	0.82 \pm 0.02	4.08 \pm 1.26	0.19 \pm 0.02	2.16 \pm 0.21

efficiency indicated that a larger proportion of the energy was thermally lost. This is due to PhiNPQ downregulation of photosynthesis by competing with photochemistry for absorbed energy (Singh *et al.* 2022). *Urochloa* grass ecotypes increased PhiNPQ either as a photoprotective response or non-regulated photoinhibition due to drought stress. Elevated PhiNPQ helps maintain photosynthesis through the dissipation of excess excitation energy as heat to protect photosynthetic apparatus (Phi2) from excessive exposure (Murchie and Ruban 2020; Ruban and Wilson 2021). Further studies are required to unlock the precise role of PhiNPQ under WD conditions in *Urochloa* grass ecotypes.

The F_v/F_m measures linear electron transport rate and shows the overall plant photosynthetic capability (Shariatipour *et al.* 2023). A decline in F_v/F_m was observed in all the ecotypes subjected to WD conditions. Similarly, Faraszewska *et al.* (2020) and Itam *et al.* (2024) previously reported a decrease in F_v/F_m when *F. arundinacea* varieties and Kentucky bluegrass were subjected to soil moisture stress respectively. A decline in F_v/F_m under drought indicates Photosystem II damage (Malan and Berner 2022; Shariatipour *et al.* 2023). The photosynthetic attributes showed genetic variation, with ecotype K22 and K9 having higher Phi2, PhiNPQ, SPAD and F_v/F_m . The high F_v/F_m demonstrates improved net photosynthetic rate and biomass production under stress, which is a desirable trait to improve performance. Water stress did not affect K22 and K9 photochemical system, making them suitable ecotypes with better physiological adaptation under WD conditions. The increased F_v/F_m was also associated with the efficiency of photosynthetic processes to use excess light energy, which was constrained by PhiNPQ under water stress (Sharma *et al.* 2015). Higher F_v/F_m has also been associated with higher leaf temperature depression and higher percent of transpiration cooling rate leading to greater thermal stability of thylakoid membranes and lesser inhibition of Phi2 (Sharma *et al.* 2015).

Chlorophyll is the main pigment for photosynthesis and is most sensitive to water stress. Under drought stress conditions, relative chlorophyll content (SPAD) decreased, which is consistent with previous findings (Badr and Brüggemann 2020; Fernández-Calleja *et al.* 2020; De Souza *et al.* 2021; Mastalerczuk *et al.* 2022; Chaouachi *et al.* 2023; Shariatipour *et al.* 2023). This could be due to chlorophyll degradation in WD conditions. Improved productivity in water-limited situations has been linked to the plant's capacity to maintain high chlorophyll concentrations (Zahid *et al.* 2021).

Significant positive correlation was observed between DMY, FWT, NT and RWC indicating that ecotypes with a high NT and the ability to maintain leaf water status (RWC) produced higher biomass yield (Zuffo *et al.* 2022). There was no correlation between the photosynthetic traits and yield components. This implies that exploiting photosynthetic features for indirect selection for yield under both WS and WD conditions is limited. Furthermore, compared to yield and morphological traits, photosynthetic attributes have distinct response pathways to drought, offering better insight into the physiological and mechanical elements of *Urochloa* grass's tolerance to WD. When considered collectively, significant negative and positive relationships were discovered between several variables under WD, and these relationships can be used to uncover potential drought tolerant traits.

Comparing the drought tolerance levels of *Urochloa* grass ecotypes using one criterion or tolerance index is contradictory. Zuffo *et al.* (2022) observed that a single drought

Table 4. Mean ranking values using PCA loading of morpho-physiological and yield traits for the 35 *Urochloa* grass ecotypes under WD conditions. Values are the mean of at least three independent replicates.

Ecotypes	PC1	PC2	PC3	PC4	Mean ranking value	Numerical rank
K17	2.89	0.74	1.00	1.88	159.94	1
Kisii	4.25	1.15	-0.10	-2.53	153.82	2
Busia	2.60	1.61	-0.85	-0.24	119.42	3
K7	3.02	-0.98	0.61	1.47	113.17	4
Kakamega	1.86	2.03	0.11	-1.02	107.16	5
K1	2.74	-1.39	1.78	0.49	98.43	6
Lanet	1.59	0.50	0.96	0.87	94.25	7
K23	1.27	2.43	-2.10	0.71	83.12	8
K15	2.01	1.27	-1.62	-0.47	75.91	9
K3	1.86	-1.56	0.68	1.82	61.60	10
CIAT16514	0.24	1.34	1.21	-1.04	46.66	11
K2	-0.24	0.52	2.72	-0.30	39.43	12
K10	0.29	1.52	-1.45	1.02	38.11	13
K19	0.55	-0.37	-0.27	1.06	19.57	14
K4	1.09	-1.59	0.84	-0.31	10.43	15
cv. Toledo	-1.10	2.09	-0.47	0.08	3.94	16
K21	0.07	-1.43	1.85	0.03	-4.84	17
K6	-0.87	0.93	-0.03	-0.20	-12.20	18
K22	-3.91	3.42	2.17	1.29	-15.59	19
cv. Basilisk	-0.62	0.85	-0.08	-1.11	-16.01	20
K8	-0.87	0.06	0.42	-0.39	-29.03	21
CIAT6399	-1.16	1.18	-0.79	-0.96	-36.72	22
K12	-0.39	-1.58	0.15	-0.07	-50.94	23
cv. Piata	-0.06	-1.03	-0.68	-1.60	-55.15	24
CIAT6426	0.04	-2.58	0.42	-0.84	-64.36	25
K16	-1.37	-1.00	-0.01	0.60	-67.74	26
K20	-0.18	-1.92	-1.42	0.47	-67.85	27
K18	-0.33	-1.70	-2.46	1.46	-71.98	28
CIAT6684	-0.62	-2.19	0.10	-0.80	-83.25	29
CIAT6384	-1.66	-0.89	0.13	-0.78	-89.45	30
K13	-0.82	-1.46	-1.60	-0.36	-92.26	31
CIAT6385	-2.89	-0.36	-0.01	0.56	-108.81	32
CIAT16449	-2.53	-1.36	0.36	0.32	-116.87	33
K5	-1.96	-1.30	-1.94	0.60	-124.42	34
K9	-4.80	1.50	0.40	0.76	-125.83	35

tolerance index did not precisely select maize genotypes for drought resistance. Ecotypes K17, Kisii, Busia, K7 and Kakamega were the most drought tolerant since they had the highest mean ranking values. The lowest mean rankings were observed in K13, CIAT6385, CIAT16449, K5 and K9 inferring susceptibility to drought.

Yield selection indices YI, GMP, STI, TOL and MP were useful in selection of *Urochloa* grass ecotypes that were high-yielding under both WD and WS conditions. DTI identified superior sorghum genotypes under well-watered and water stress environments (Abebe *et al.* 2020). The findings of this study also corroborated with Zuffo *et al.* (2022) who found STI, GMP and MP indices were useful in classification of forage grass cultivars to different drought tolerance levels. Ferede *et al.* (2020) and Mohammadi (2016) studied DTI in teff and wheat, respectively confirmed that high values of YI, STI, GMP, MP and YSI are the best indices for selection of

high-yielding genotypes under both WS and WD conditions. With regard to YSI, ecotypes K13 and 16514 were the most desirable *Urochloa* ecotypes. The SSI can be utilized to identify ecotypes with relatively low Yp but high Ys (Ferede *et al.* 2020). Zahid *et al.* (2021) selected drought-tolerant cotton genotypes using SSI. Thus, breeders should also consider YSI and SSI to characterize breeding lines for drought tolerance.

The high-yielding ecotypes under both WD and WS (K17, Basilisk, K7, CIAT6399, Lanet, K23, K10, Kisii, Kakamega, K23 and Busia) were inclined to the direction of YI, Ys, STI, GMP, MP, Yp and TOL. The upper left quadrant of the PCA biplot had ecotypes (K13 and CIAT 16514) which are drought tolerant and characterized by the least percentage reduction in biomass yield under water stress as they clustered to the direction of YSI. Significant and negative association between YSI and SSI was recorded in this study (Zuffo *et al.* 2022). TOL was significant and positively associated with Ys,

Yp, GMP and MP. However, TOL did not correlate with Ys in sorghum (Menezes *et al.* 2014) and soybean (Chiipanthenga 2020). Biplot reveals YI is optimal for evaluating ecotypes under drought stress, Kisii cultivar was the best suited to drought stress. SSI and YSI strongly negatively associate with Ys due to large obtuse angles. This result corroborates with other studies by Abebe *et al.* (2020) and Ferede *et al.* (2020). Furthermore, cultivars with high YSI cultivars yield less in non-stressed conditions and highest in stressed conditions (Ferede *et al.* 2020).

PCA biplot analysis shows *Urochloa* ecotypes near the origin and vector lines have superior breeding potential (Gedam *et al.* 2021). Under WD conditions, ecotypes K23, K15, Kakamega and Busia, were located near origin and along the FWT, DMY and NT vector line, can increase productivity in *Urochloa* breeding programmes.

Conclusions

Morpho-physiological and yield traits varied among all the studied *Urochloa* grass ecotypes under both WS and WD conditions. Drought tolerance indicators (mean productivity, geometric mean productivity, tolerance index and stress tolerance index) were most effective in the identification of drought-tolerant ecotypes. The study revealed variation among *Urochloa* grass ecotypes and provided a theoretical basis for improving tolerance of *Urochloa* grass ecotypes to drought stress. Ecotypes K17, Kisii, Busia, K7 and Kakamega depicted greater drought adaptation with higher biomass yield and mean ranking values under WD. The five drought tolerant ecotypes identified need to be tested further under field for sustainable forage grass production under drought stress conditions. These ecotypes with drought-adaptive traits could be utilized in breeding programs to develop high-yielding and drought-tolerant varieties. Further studies could elucidate the biochemical and molecular mechanisms behind tolerance to drought stress to achieve tangible progress in *Urochloa* grass breeding programmes.

Supporting Information

The following additional information is available in the online version of this article –

Table S1. List of *Urochloa* ecotypes used in the study.

Table S2. Mean squares values for the morpho-physiological and yield traits through Generalized Linear Model Analysis of Variance. *** and ** significant at $P < 0.001$ and $P < 0.01$, respectively. PH, plant height; NT, number of tillers; NL, number of leaves; RL, root length, DMY, dry matter yield; FWT, fresh weight; RCW, relative water content; Phi2, photosystem II photochemistry; PhiNPQ, non-photochemical quenching; SPAD, relative chlorophyll content; F_v/F_m , efficiency for photosystem II.

Table S3. Mean biomass yield for each ecotype under different WR. CV, coefficient of variation; LSD, least significance difference. Values expressed as Mean \pm SEM ($n = 5$).

Table S4. Correlation coefficients (r) between Biomass yield of *Urochloa* ecotypes under non-stressed and stressed conditions and among selected indices. Symbols ***, ** and * shows significant at 0.001, 0.01 and 0.05, respectively. NS, non-significant; MP, mean productivity; GMP, geometric mean productivity; TOL, tolerance index; YSI, yield stability

index; YI, yield index; Yp, mean Biomass yield under WS; Ys, mean Biomass yield under WD; STI, stress tolerance index; SSI, stress susceptible index.

Sources of Funding

This research was part of PhD project supported by Kenya Climate Smart Agriculture Project (KCSAP).

Conflict of interest

None declared.

Data Availability

All data generated and used is included in this article and supplementary file.

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