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Genotypic Yield Stability of Wild and Landrace Sorghum Species Under Drought Stress and Striga Infestation

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Abstract

Recurring drought stress cycles and widespread striga (*Striga hermonthica* (Del.) Benth) infestations are two of the major constraints of sorghum production in sub-Saharan Africa (SSA) where they cause a crop loss of about 60 billion US dollars and affect a population of about 100 M people annually. Plant breeders continue to employ conventional and molecular crop breeding strategies in the search for durable genetic resistance/tolerance mechanisms or for germplasm with genes against these two constraints. Crop wild relatives and landraces remain valuable resources of resistance/tolerance genes and have been utilized in the past to improve tolerance to drought stress and resistance to striga. The aim of this study was to assess the stability of performance of 64 sorghum wild relatives, landraces and progenies from some generation of crosses under striga infested and drought stress conditions in agroecological environments endemic for these two stresses. The performance of the genotypes under drought stress was assessed in well-watered and in water stressed conditions at the Kenya Agricultural Livestock Research Organization (KALRO) Kiboko Research Centre whereas the same set was evaluated under striga artificially infested field and potted trials at the KALRO Alupe Research Centre during 2018/2019 rainy seasons. Genotypes, B35 × ICSV III N, Macia, N13, ICSV 111 IN, F6YQ212 × B35, SRN39, GENO47293, ICSV 111 IN × B35, IS9830, Framida, GENO 45827, F6YQ212, B35 × AKUOR ACHOT were found to maintain stable high yields in both striga and drought conditions. The results here, showed that Genotype, Genotype × Environment (GGE) interaction partitioned genotypes in two of the four mega-environments according to their stability and mean grain yield (GY) and identified representative genotypes of the two traits that could be exploited to develop superior sorghum varieties adapted to drought and striga prone environments.

Keywords: drought stress, genotype, genotype environment (GGE) interaction, *Striga hermonthica*, yield stability

1. Introduction

Sorghum is the fifth most important cereal crop globally and is a staple food for more than 500 million people in the semi-arid tropics of Africa and Asia that constitute more than 80% of the World's production area (Beyene et al., 2015). Although sorghum has many biotic and abiotic production constraints drought stress and striga infestation rank as the two most crucial factors especially in SSA (Beyene et al., 2015). Drought stress is often the foundational cause of other constraints to production such as charcoal rot, Fusarium stalk, and sorghum ergot (Assefa et al., 2010). Intense drought stress cycles drastically reduce sorghum biomass and grain yields (Borrell et al., 2014). In the arid and semi-arid regions of Eastern Africa for instance, the average on farm grain yields of sorghum range between 0.6-1.5 t/ha against a worldwide average of 4.5 t/ha (Ngugi & Maswili, 2010) because of unpredictable and irregular rainfall. Under drought, sorghum genotypes develop drought avoidance or escape

mechanisms such as deeper roots, low osmotic adjustments or ability to maintain closed stomata. Selection for drought tolerance constitute use of indirect phenotypic traits such as stay-green and chlorophyll content. Drought stress occurs mostly during pre-flowering and post-flowering growth stages (Tuinstra et al., 2009) and affects the development of panicles and grain yield (Subudhi et al., 2000; Borell et al., 2014). Stay-green, a trait that allows genotypes under water stress to remain photosynthetically active is expressed after flowering, during grain filling period and has been positively associated with improved grain yields. Quantitative Trait Loci (QTLs) for stay-green and chlorophyll content been mapped to five chromosomal locations and have been utilized in several crop improvement programs to successfully select for drought tolerant genotypes (Subudhi et al., 2002; Prasad et al., 2006). Published sources of drought tolerance with stay-green QTLs include B35, E-36-1 (Haussmann et al., 2002; Kebede et al., 2001) and more recently novel sources have been found in landraces and wild relatives (Ochieng et al., 2020).

Striga hermonthica affects cereal crops such as maize, millets and sorghum in SSA on an area of over 21 million ha (Sauerborn, 1991) where farmers lose 20-80% of their yield, equivalent to 4.1 million tons of grain per year (Kanampiu et al., 2002). *Striga* species is an obligate parasitic weed that is a major biotic stress in sorghum cultivation especially in areas with poor soil fertility (Rodenburg et al., 2005). The weed germinates upon stimulation by strigolactones induced by the host, or in some cases, by non-host plants (Bouwmeester et al., 2019; Hausmann et al., 2002). The germinated *Striga* then attaches to the roots of the host plants, using a special invasive organ, the haustorium (Gurney et al., 2005). The haustorium enables uptake of water and nutrients from the host plants resulting in yield losses of up to 100% (Kim et al., 2002; Ejeta, 2007). An adult *Striga* plant can produce up to 100, 000 tiny seeds that can survive in the soil for over 20 years making conventional control measures difficult to implement (Pieterse & Pesch, 1983; Gurney et al., 2005).

Genetic diversity studies within *S. hermonthica* populations infesting cereal crops in Western, Eastern and Central Africa have reported existence of biotypes within the species (Ejeta, 2007). These biotypes are believed to be responsible for the breakdown of *Striga* resistance in previously resistant varieties (Doggett, 1988).

Striga populations are highly outcrossing, making the use of single resistance genes to manage infestations inadequate. Genotypes that possess multiple genes for striga resistance, are likely to have genetic resistance that is durable across several environmental conditions as well as across ecological variants of the parasite (Haussmann et al., 2002). Wild sorghum genotypes have demonstrated resistance to *Striga* over the years and are likely to harbor resistance genes which if exploited may assist in the improvement of adapted sorghum varieties (Muraya et al., 2011; Magomere et al., 2015). Known sources of resistance to striga, include N13, SRN 39, Framida, IS9830 (Rodenburg et al., 2005) landraces and wild relatives recently identified by Muchira et al. (2021).

Interaction between striga resistance and drought tolerance has been reported (Muchira et al., 2021) which suggested that most consistent top-yielding genotypes under natural and artificial striga infestation were from generation of crosses with drought-tolerant genotypes, such as LODOKA, B35, and E36-1 (Muchira et al., 2021).

Multi-environment trials data help to select the best environment for evaluating a genotype's adaptability and stability by the analysis of the genotype × environment (G × E) interactions (Gauch et al., 2008). The variation of yield stability and adaptability that determine a genotype's ability to thrive in a given environment is done through multivariate analyses such as Additive Main effects Multiplicative Interaction (AMMI) or Genotype plus Genotype Environment interaction (GGE) biplots (Gauch et al., 2008; Yan, 2001). Recent studies (Yan & Tinker, 2006; Yan et al., 2007) point to the simplicity and clarity of GGE analysis for G × E in its use of means versus stability graphical plots. GGE biplots provide the discriminating power and representativeness in a multi-environment analysis demonstrating "which-won-where" pattern (Yan et al., 2007; Angelini et al., 2019). By grouping target locations to one mega environment, the GGE Bi-plot analysis provides an important means of investigating the representativeness of the mega-region. Genotypic stability is evaluated with the aid of the Average Environment Coordination (AEC) (Yan & Rajcan, 2002) that considers the most stable genotypes to be the ones with the shortest vertical distances from the AEC. According to GGE analysis a superior genotype is the one with high mean yield and high stability for that environment (Yan, 2001). The objective of this work was to evaluate grain yield performance and estimate the stability using GGE biplot analysis from data of multi-environment trials conducted under drought stress and striga infestation conditions.

2. Method

2.1 Study Locations

The study was conducted at Kiboko, and Alupe research Centres of KALRO. Kiboko Research Centre is 975 m above sea level (m a.s.l.) lies between latitude 2.15° S and longitude 37.75° E, in agroecological zone 4, lower semi-arid, with a rainfall average of 250 mm per season. Alupe is located at 1189 meters above sea level and is situated at latitude 00.29° S and longitude 34.08° E (Haussmann et al., 2004). Alupe Research Centre is classified as being in the lower medium agro-ecological zone with an annual mean temperature that ranges from 20.5 to 21.7 °C and an annual rainfall of 1800-2000 mm. The soils at Alupe are shallow to deep, ferrallisols and the site is in a *Striga* hotspot zone making it appropriate for screening genotypes for *Striga* resistance.

2.2 Germplasm: Accessions and Generation of Crosses

64 sorghum diverse genotypes comprising of wild accessions, local landraces, improved varieties and F_4 segregating populations were selected (Table 1). Most wild relatives and landraces were sourced from Genetic Resources Research Institute (GeRRI) of the Kenya Agricultural and Livestock Research Organization (KALRO), University of Nairobi (UON) with some accessions obtained from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Nairobi. The F_4 populations were derived as generation of crosses between the 43 wild and cultivated accessions as shown in Table 1. This germplasm was chosen for evaluation of yield stability because it contains previously tested and proven sources of drought tolerance and striga resistance by different authors (Kebede et al., 2001; Hausmann et al., 2002; Rodenburg et al., 2005; Muchira et al., 2021; Ochieng et al., 2020).

Table 1. Sorghum genotypes used in this study, sourced from ICRISAT and University of Nairobi (UON) and classified according to species

Genotype	Source	Classification	Species
1. GBK 044058	GeRRI	Wild	*Sorghum* sp.
2. GBK 044336	GeRRI	Wild	*Sorghum* sp.
3. GBK 048922	GeRRI	Wild	*Sorghum* sp.
4. GBK 047293	GeRRI	Wild	*Sorghum arundinaceum (Desv.) Stapf*
5. GBK 048916	GeRRI	Wild	*Sorghum* sp.
6. GBK 016085	GeRRI	Wild	*Sorghum arundinaceum (Desv.) Stapf*
7. GBK 048917	GeRRI	Wild	*Sorghum* sp.
8. GBK 016114	GeRRI	Wild	*Sorghum sudanense (Piper) Stapf*
9. GBK 044063	GeRRI	Wild	*Sorghum* sp.
10. GBK 048156	GeRRI	Wild	*Sorghum arundinaceum (Desv.) Stapf*
11. GBK 016109	GeRRI	Wild	*Sorghum arundinaceum (Desv.) Stapf*
12. GBK 044120	GeRRI	Wild	*Sorghum* sp.
13. GBK 040577	GeRRI	Wild	*Sorghum arundinaceum (Desv.) Stapf*
14. GBK 048921	GeRRI	Wild	*Sorghum* sp.
15. GBK 044448	GeRRI	Wild	*Sorghum* sp.
16. GBK 045827	GeRRI	Wild	*Sorghum purpureosericeum* (Hochst. ex A. Rich.) Asch. &Schweinf.
17. GBK 048152	GeRRI	Wild	*Sorghum arundinaceum* (Desv.) Stapf
18. GBK 044065	GeRRI	Landrace	*Sorghum* sp.
19. GBK 043565	GeRRI	Landrace	*Sorghum arundinaceum* (Desv.) Stapf
20. GBK 044054	GeRRI	Landrace	*Sorghum almum* Parodi
21. OKABIR	ICRISAT	Landrace	*Sorghum bicolor*
22. IS 9830	ICRISAT	Landrace	*Sorghum bicolor*
23. IBUSAR	ICRISAT	Landrace	*Sorghum bicolor*
24. AKUOR-ACHOT	ICRISAT	Landrace	*Sorghum bicolor*
25. LODOKA	ICRISAT	Landrace	*Sorghum bicolor*
26. E36-1	ICRISAT	Stay-green source	*Sorghum bicolor*
27. B35	ICRISAT	Stay-green source	*Sorghum bicolor*
28. N13	ICRISAT	Landrace	*Sorghum bicolor*
29. SRN39	ICRISAT	Improved variety	*Sorghum bicolor*
30. KARIMTAMA-1	ICRISAT	Improved variety	*Sorghum bicolor*
31. GADAM	ICRISAT	Improved variety	*Sorghum bicolor*

32. F6YQ212	ICRISAT	Improved variety	*Sorghum bicolor*
33. MACIA	ICRISAT	Improved variety	*Sorghum bicolor*
34. FRAMIDA	ICRISAT	Improved variety	*Sorghum bicolor*
35. KAT/ELM/2016 PL82 KM32-2	ICRISAT	Improved variety	*Sorghum bicolor*
36. KAT/ELM/2016 PL1 SD15	ICRISAT	Improved variety	*Sorghum bicolor*
37. ICSV III IN	ICRISAT	Improved variety	*Sorghum bicolor*
38. HAKIKA	ICRISAT	Improved variety	*Sorghum bicolor*
39. B35 × AKUOR-ACHOT	UON	F4 Population	*Sorghum bicolor*
40. B35 × E36-1	UON	F4 Population	*Sorghum bicolor*
41. B35 × F6YQ212	UON	F4 Population	*Sorghum bicolor*
42. B35 × ICSVIII_IN	UON	F4 Population	*Sorghum bicolor*
43. B35 × LANDWHITE	UON	F4 Population	*Sorghum bicolor*
44. B35 × LODOKA	UON	F4 Population	*Sorghum bicolor*
45. E36-1 × MACIA	UON	F4 Population	*Sorghum bicolor*
46. F6YQ212 × B35	UON	F4 Population	*Sorghum bicolor*
47. F6YQ212 × LODOKA	UON	F4 Population	*Sorghum bicolor*
48. IBUSAR × E36-1	UON	F4 Population	*Sorghum bicolor*
49. IBUSAR × ICSVIII_IN	UON	F4 Population	*Sorghum bicolor*
50. IBUSAR × LANDWHITE	UON	F4 Population	*Sorghum bicolor*
51. ICSVIII_INxB35	UON	F4 Population	*Sorghum bicolor*
52. ICSVIII_INxE36-1	UON	F4 Population	*Sorghum bicolor*
53. ICSVIII_IN × LANDWHITE	UON	F4 Population	*Sorghum bicolor*
54. ICSVIII_IN × LODOKA	UON	F4 Population	*Sorghum bicolor*
55. ICSVIII_IN × MACIA	UON	F4 Population	*Sorghum bicolor*
56. LODOKA × ICSVIII_IN	UON	F4 Population	*Sorghum bicolor*
57. LODOKA × LANDWHITE	UON	F4 Population	*Sorghum bicolor*
58. LODOKA × OKABIR	UON	F4 Population	*Sorghum bicolor*
59. OKABIR × AKUOR-ACHOT	UON	F4 Population	*Sorghum bicolor*
60. OKABIR × B35	UON	F4 Population	*Sorghum bicolor*
61. OKABIR × ICSVIII_IN	UON	F4 Population	*Sorghum bicolor*
62. AKUOR-ACHOT × ICSVIII_IN	UON	F4 Population	*Sorghum bicolor*
63. LANDWHITE × B35	UON	F4 Population	*Sorghum bicolor*
64. LANDWHITE × MACIA	UON	F4 Population	*Sorghum bicolor*

2.3 Drought Stressed Trials

Drought tolerance evaluation experiment was sown at the KALRO Kiboko field station, Kenya for one season, in July 2017, in two blocks; the first block was irrigated throughout from the germination to physiological maturity whereas in the second block, water stress was strategically applied in three regimes so as to induce drought tolerance response: The well-watered trial was irrigated 3 times per week, each time receiving 3 hours of irrigation supplied at 25 mm per plot from sowing to physiological maturity stage. For the water stressed trial, irrigation was withdrawn at 14 days after sowing. From then on, water stress was maintained throughout until at 40 days and up to 60 days after sowing, when 25 mm of water per plot was applied twice a day at an interval of two days at each application period. Sixty days after sowing, water was again withdrawn, and drought stress maintained till physiological maturity.

Both irrigated and drought stressed blocks were laid out in a 12 × 8 alpha lattice design, replicated three times. The trial consisted of 2 row plots of 2 m length with an inter-row spacing of 0.75 m and intra-row spacing of 0.25 m. Diammonium Phosphate (DAP) fertilizer was applied during planting at a rate of 100 kg ha^{-1} and the crop was top-dressed with urea, 21 days after emergence, at a rate of 40 kg ha^{-1}, and then earthed up at 30 days after emergence. The crop was raised according to the standard agronomic practices recommended in the area.

2.4 Drought Tolerance Scoring

Data on agronomic traits were collected on 6 randomly selected plants in the 2 center rows following the methodology suggested by IBPGR and ICRISAT (1993). Data was scored on the following traits: days to 50% flowering (DFL; counts), number of green leaves at maturity (GLAM), Relative chlorophyll content (RCC), and

grain yield (GY; t ha ^{1}). Grain yield data was determined on plot basis as recommended by IBPGR and ICRISAT (1993).

2.5 Striga Trials and Data Scoring

For the assessment of the response to Striga, two sets of trials, one in the field and the other in pots were designed. Both field and pot trials were set up in one season of the long rains of 2017 at KALRO Alupe and as noted elsewhere, the site is a *Striga hermonthica* hotspot. Both experiments were laid out in a square lattice design with three replications, each block consisting of eight plots. The field experiment was planted in an artificially striga-infested field with a spacing of 75 cm between rows and 20 cm between plants in the row. Each row contained 21 plants. striga inoculum was prepared by mixing 5 kg of sand with 15 g of striga seeds that had been harvested from the same location in the previous season. A supplemental striga inoculum of 15 g was spread along each row during planting to improve the consistency of striga seed load across the plot. Phosphorus (P) was applied at the rate of 90 kg ha^{-1} after thinning, whereas nitrogen (N) was applied at the rate of 92 kg ha^{-1} when the plants were 45-50 cm tall, which was about 30 days after germination. Insect pests, especially fall armyworm (*Spodoptera frugiperda*) and cutworms (*Agrotis* spp., *Spodoptera* spp., and *Schizonycha* spp.), were controlled using Voliam TargoR SC (Syngenta Crop Protection AG, Switzerland) containing active ingredients, chlorantraniliprole and abamectin. The field experiment was rainfed and was sown at start of the rainy season.

For the pot experiment, jiffy pots measuring 30 cm diameter were filled with 20 kg of striga free soil obtained from a striga free field. Each pot contained four plants and was used to represent a plot. The pot experiment was set up in the field alongside the field experiment but not under any shelter. The pot experiment was rainfed for most of the growth period but due to the restricted pot size, supplemental irrigation was applied when deemed necessary. Fertilizer and insecticide applications were done as described earlier. Striga infestation count in the field and pot trials was recorded at two-week intervals from the 42nd day after sowing when the first striga germination was expected. The total striga counts (TSC) were used to calculate (Area under Striga Number Progressive Curve) ASNPC. Agronomic data scored included: Number of *Striga* forming capsules (NSFC), Days to 50% anthesis (DTF), Dry panicle weight (DPW), Grain yield (GY) and 100 seed weight (SWT). Grain yield per plot was calculated using the method on recommended by IBPGR and ICRISAT (1993).

2.6 Estimation of Genetic Diversity

DNA was extracted for genotyping from a total of 153 accessions and 6 randomly tagged F_4 plants using the method described by Ochieng et al. (2020) and quality and quantity was checked by running a gel electrophoresis and the samples were aliquoted into a 96 well plate. Samples were then sent to the Integrated Genotyping Service and Support (IGSS) at the Bioscience Eastern and Central Africa (BecA) Lab at the International Livestock Research Institute (ILRI) hub, for library construction and DArT-sequencing (DArTseq). Estimation of genetic diversity was performed using The TASSEL (Trait analysis by association, Evolution and Linkage) software. A total of 26,291 raw SNPs were generated for the 153 diverse genotypes in TASSEL software. Filtering was performed using a site minimum count of 70%, and a minimum allele frequency of 0.05. After filtering, 7,075 SNPs were recovered, and these were used to assess genetic diversity within the 153 genotypes with TASSEL software version 5.2.63 and the results were generated from the archaeopteryx tree.

2.7 Statistical Analysis

The META-R software was used to generate Best Linear Unbiased Estimates (BLUEs) for grain yield across the locations for the two traits. In each location the BLUEs statistics were then used for GGE biplot analyses to show, "Which Won Where "and to plot out "Mean vs. Stability". Stability of the genotypes for grain yield was assessed with the R software using means of grain yield, striga-related, and drought-related parameters measured. A selection intensity of 20% was imposed to identify the best genotypes.

3. Results

3.1 AMMI Analysis and Within Environment ANOVA

Table 2 shows the environment variance was highly significant, but genotype and genotype × environment interactions were not significant. PC1 (env) contributed 97.9% of the variation but PC2 (GY) contributed only 1.6%. In Table 3, the highest mean GY were from Env4 (potted striga trial) and Env3 (field striga trial). Env1 (drought stressed) had the highest cv for GY. GY had the highest narrow-sense heritability, h^2 (0.874) in Env3 followed by Env4 (0.780) and Env2 (0.689).

Table 2. Grain Yield (GY) AMMI-ANOVA

SOV	DF	MS	F-Value	Pr (> F)
Env	3	647.43	431.59	3.49***
Rep(env)	8	8.45	0.85	5.59*
Gen	76	73.38	7.38	9.93ns
Gen × Env	174	39.95	4.01	5.84ns
PC1	78	107.40	10.81	0.005**
PC2	76	1.85	0.19	1.00ns
PC3	74	0.56	0.06	1.00^{ns}
Error	497	9.93		
Total	986	37.56		

Note. ^{ns}, *, **, ***: not significant ($p > 0.05$), highly significant ($p < 0.05$), very highly significant difference ($p < 0.001$), respectively; SOV: Source of variation; DF: degrees of freedom; MS: Mean squares; F: Fisher value; P: Probability.

Table 3. Grain Yield (GY) within environment ANOVA

Env	Mean	DF	MSG	MSE	CV	h^2
Env1	1.46	63	1.08	1.18	74.5	-0.09
Env2	0.998	62	0.682	0.212	46.1	0.689
Env3	1.48	63	4.42	0.539	50.4	0.874
Env4	10.6	62	177	38.8	58.8	0.780

Note. SOV: Source of variation; DF: Degrees of freedom; MSG: Mean squares; MSE: Mean squares error; CV: Coeff of variation; h^2: narrow sense heritability.

3.2 Drought Tolerance Evaluation

Nine genotypes (IESV21400 DL, LODOKA, IESV23006 DL, IESV92043 DL, OKABIR, GBK 016109, GBK 048156, IESV23010 DL, AKUOR-ACHOT) outperformed the two widely known published sources of drought genes, in sorghum, namely , E36-1 and B35 with respect to relative chlorophyll content (RCC) at maturity (Figure 1), whereas 7 genotypes (LODOKA, OKABIR, IBUSAR, F6YQ212, AKUOR-ACHOT, GBK 047293, GBK 048917) had more green leaves at maturity (GLAM) than E36-1 and B35 under drought stress conditions (Figure 2).

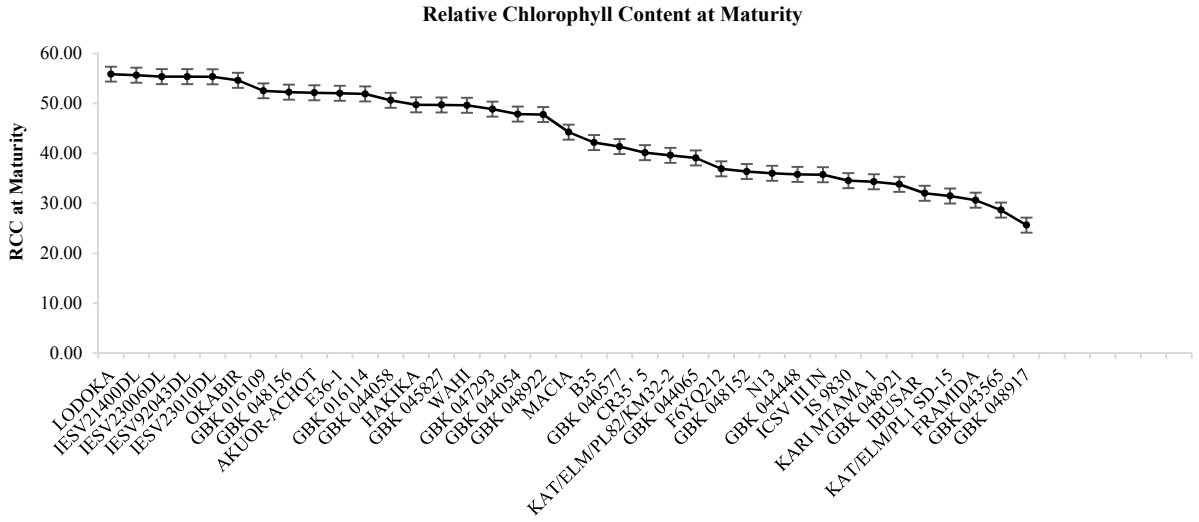

Figure 1. Relative chlorophyll content of genotypes under drought stress in comparison with known stay-green sources, E36-1 and B35

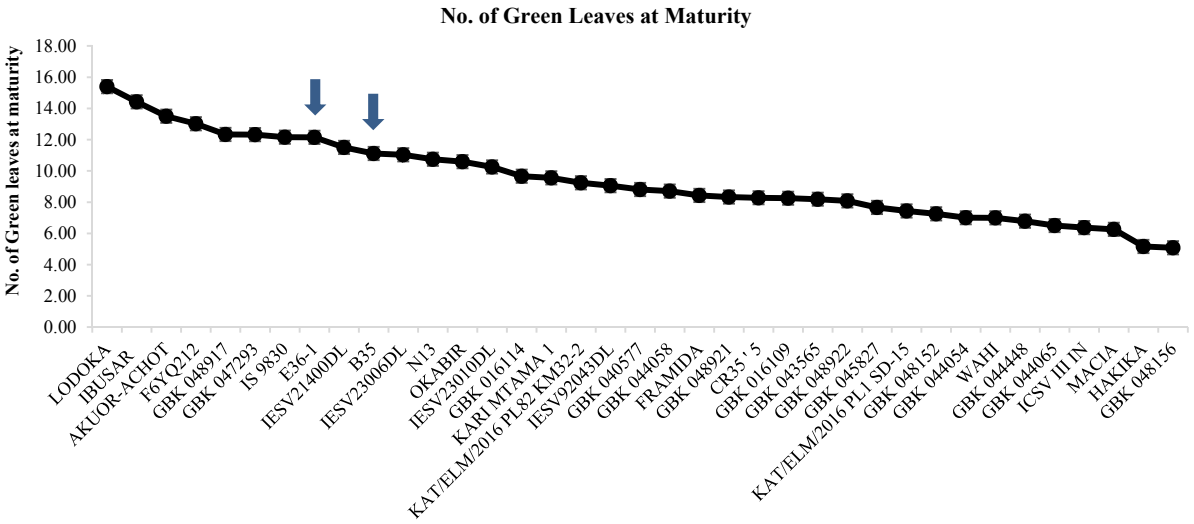

Figure 2. Number of green leaves at maturity of genotypes under drought stress conditions in comparison with known stay-green sources, E36-1 and B35

Ten (IESV23010 DL, IESV23006 DL, IESV92043 DL, AKUR-ACHOT, GBK 047293, LODOKA, WAHI, GBK 016114, GBK 045827, OKABIR) of the 18 genotypes that had outperformed B35 when ranked using RCC also yielded better than both E36-1 and B35 for green number of leaves at maturity (GLAM) (Figure 2). The landrace genotype, LODOKA, stood out as having the highest GLAM and RCC and was also a top yielder, with a yield of 2.2 tons ha^{-1} out of the highest recorded yield of 2.45 tons ha^{-1} (Figure 3). All the genotypes that yielded better than E36-1 were considered as potential sources of drought tolerance.

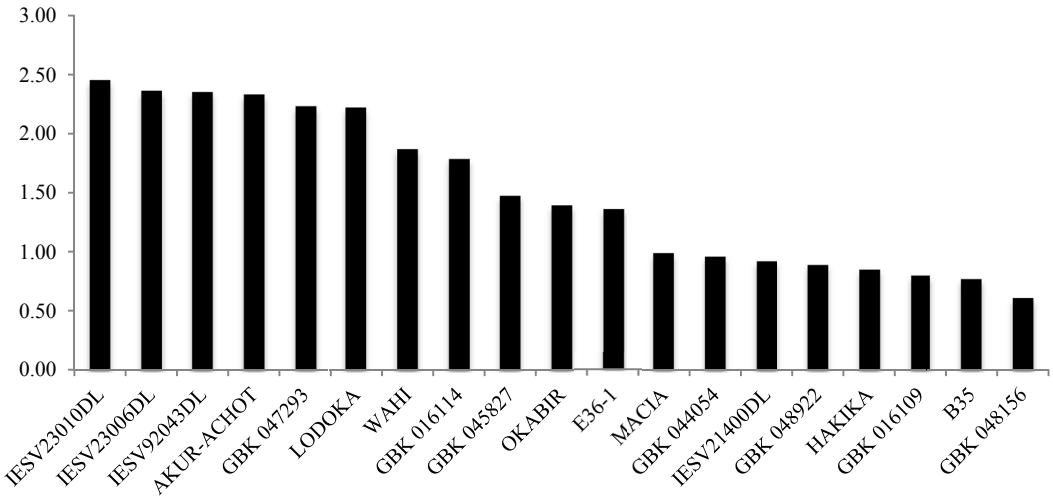

Figure 3. Grain yield of the most drought tolerant genotypes in comparison with known stay-green sources, B35 and E36-1

3.3 Genetic Relatedness

Three major clusters were observed with the F_4 populations being evenly distributed across the accessions (Figure 4). In cluster 1, *Striga* resistance donors SRN39, Framida, IS9830 and Hakika clustered together in a subcluster alongside the resistant F_4 cross F6YQ212 × B35 (Figure 4). Resistant F_4 crosses with B35 as the female parent B35 × ICSVIII_IN (field trial and potted trial), B35 × LODOKA (field plot), B35 × LANDIWHITE (sick plot) and LODOKA × LANDIWHITE (potted trial) clustered together with resistant donor line N13 in a subclade as shown in (Figure 4). In the second cluster, ICSVIII_IN × E36_1 which exhibited a

resistance response in the potted trial clustered with the *Striga* resistant improved line ICSVIII_IN. IBUSAR × E36_1 was the only *Striga* resistant cross that did not cluster with any known *Striga* resistance source (Figure 4). The genotype was found in the third cluster which had Macia as the only improved genotype. Macia together with ICSVIII_IN × Macia and B35 × Akuor-Achot exhibited high yields in the field trial. Another trend of clustering based on yielding capacity was observed in cluster 2 where high yielding genotypes, ICSVIII_IN, E36-1, ICSVIII_IN × Land white and ICSVIII_1N × E36-1 were found in the same cluster (Figure 4).

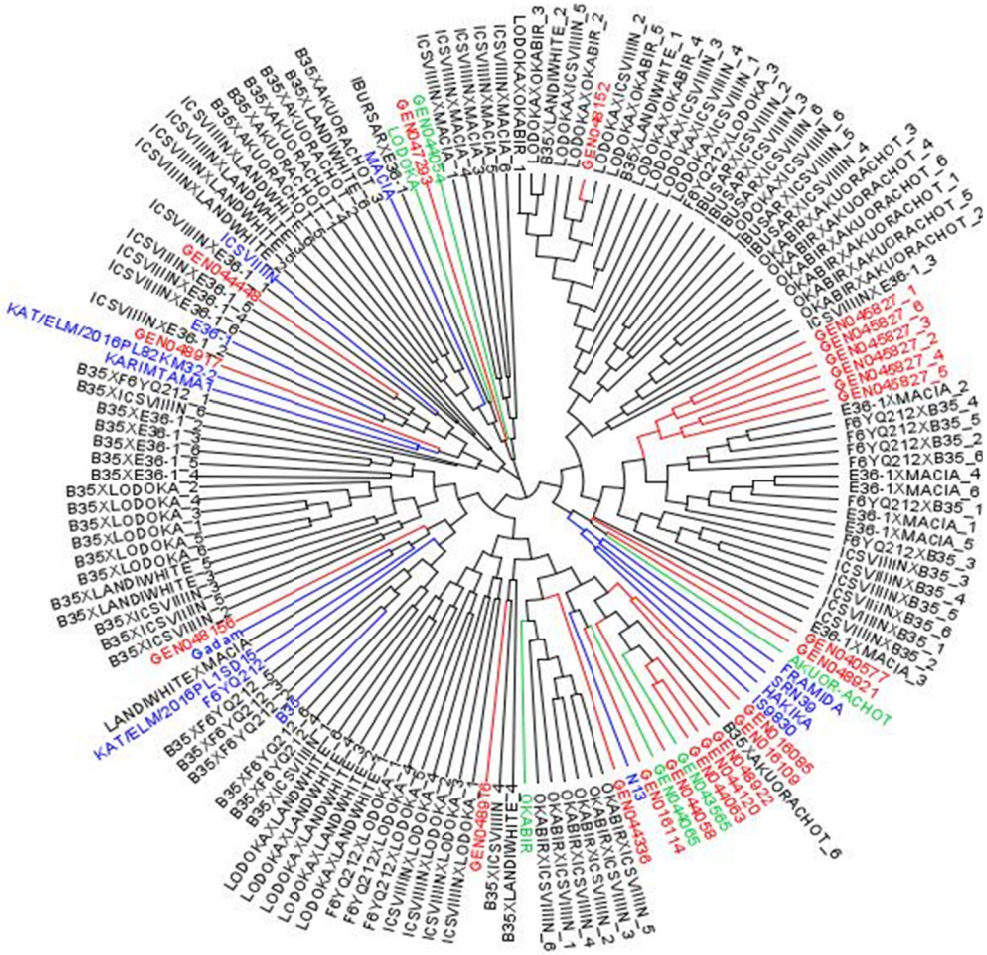

Figure 4. Dendrogram showing the clustering of 153 accessions used in this study. Red-wild accession, Green-landrace, Blue-improved variety and Black -F4 population

3.4 Response of Genotypes to Striga in the Field and Potted Experiment

Significant differences ($P < 0.001$) were observed between genotypes for all striga-related traits. Yield-related traits (*i.e.*, YLD and HGW) were consistently higher in the pot trial than in the field trial, whereas striga-related traits (for example ASNPC and NSmax) had lower values in the pot trial than in the field trial. F4 generation of crosses yielded higher than parental genotypes but parental genotypes were more striga resistant (Figure 5).

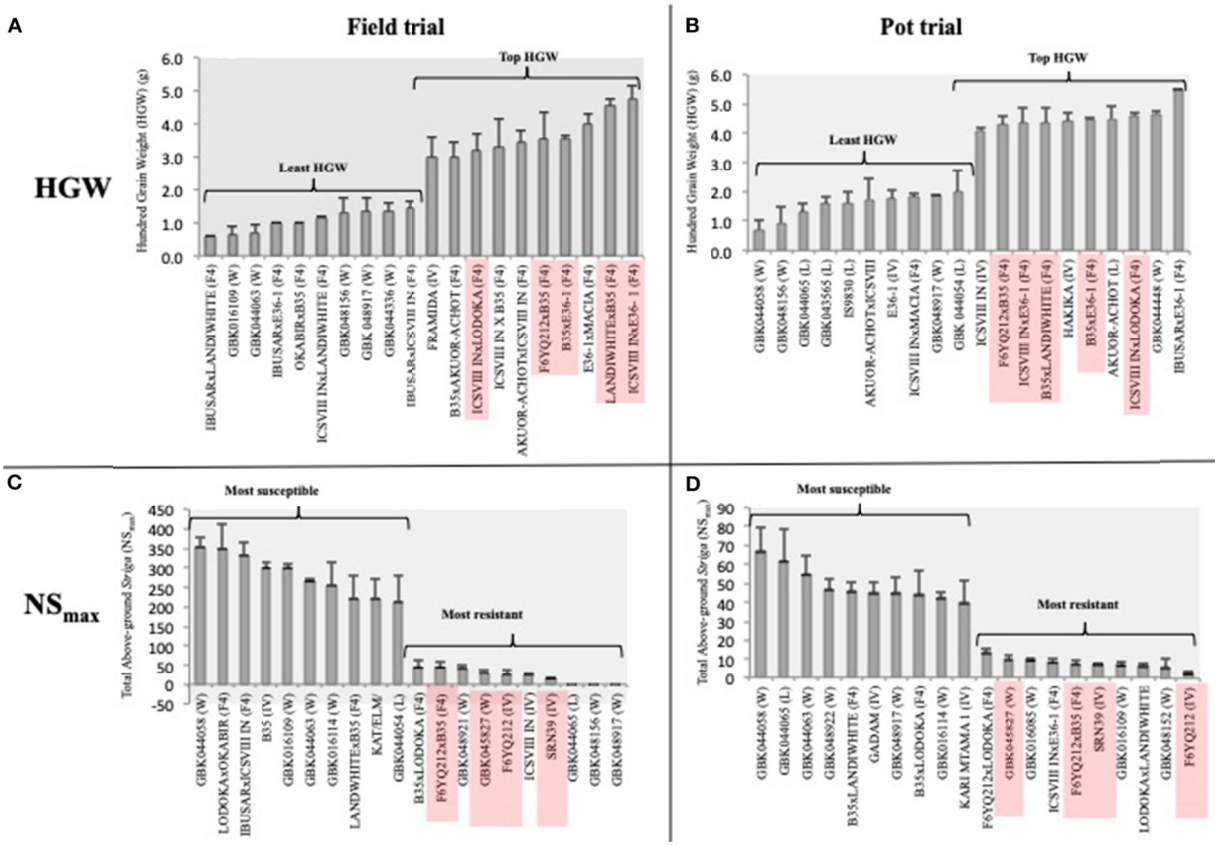

Figure 5. The top 10 highest and lowest performing genotype for HGW and *Striga* (NSmax) in the field trial (A, C) and in the pot trial (B, D), respectively. Genotypes with consistent performance across the two environments are highlighted in red

3.5 "Which-Won-Where" Patterns and Stability of Genotypes for Grain Yield

The Genotype and Genotype × Location interactions for the traits were visualized in "Which-Won-Where" GGE. The "which-won-where/what" graph is based on symmetrical scaling and represents which genotype won where pattern considering the test genotypes and the environments. As shown in Figure 6, genotypes located at the vertices of the polygon were identified as the winners in their respective environments. The GGE biplot explained 99.10% of the total interaction variations, distributed as 97.18% and 1.92% between principal component 1 (PC1) and PC2, respectively. With regards to grain yield, genotype, G39 (IBURSAR × LANDWHITE) was identified as the best performing genotype at Env1 (drought stressed), G62 (OKABIR × B35) was the most outstanding genotype at Env2 (well-watered), G45 (ICSVIIIN × B35) was the best performing at Env3 (striga field infested) and G55 (LODOKA × ICSVIIIN) was the best genotype at Env4 (striga pot infested) (Figure 6).

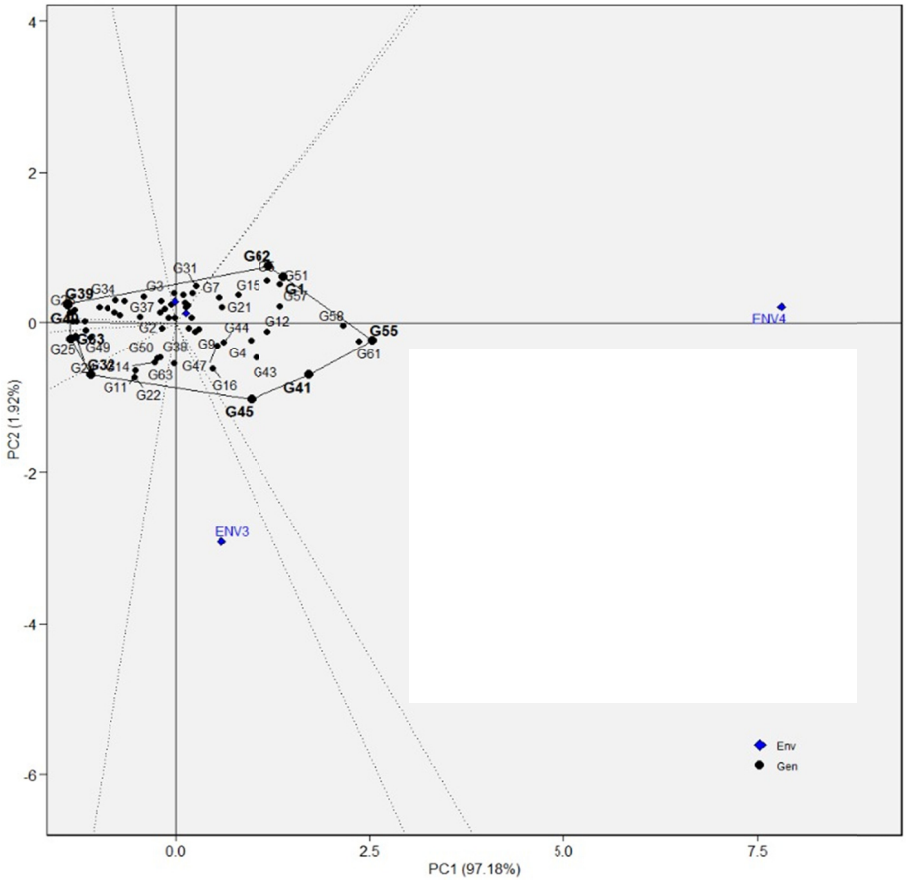

Figure 6. A GGE scatterplot based on symmetrical scaling for" which won where "pattern of the 64 sorghum genotypes evaluated in 4 environments

3.6 Mean Yield and Stability

Figure 7 shows the average-environment coordination (AEC) view of the GGE biplots in yield in which the single arrowed lines are regarded as the AEC abscissas and point towards higher mean yields across environments (Yan et al., 2000; Yan et al., 2007). Genotype, G45 yielded highest, as it was furthest from the mean along the "average environment axis". The AEC ordinate that passes the plot origin and is perpendicular to the AEC abscissa represents yield stabilities. The AEC ordinate points to larger variability (low stability) in either direction but even under these circumstances, genotypes, G45, G12 and G43 were highly stable for grain yield.

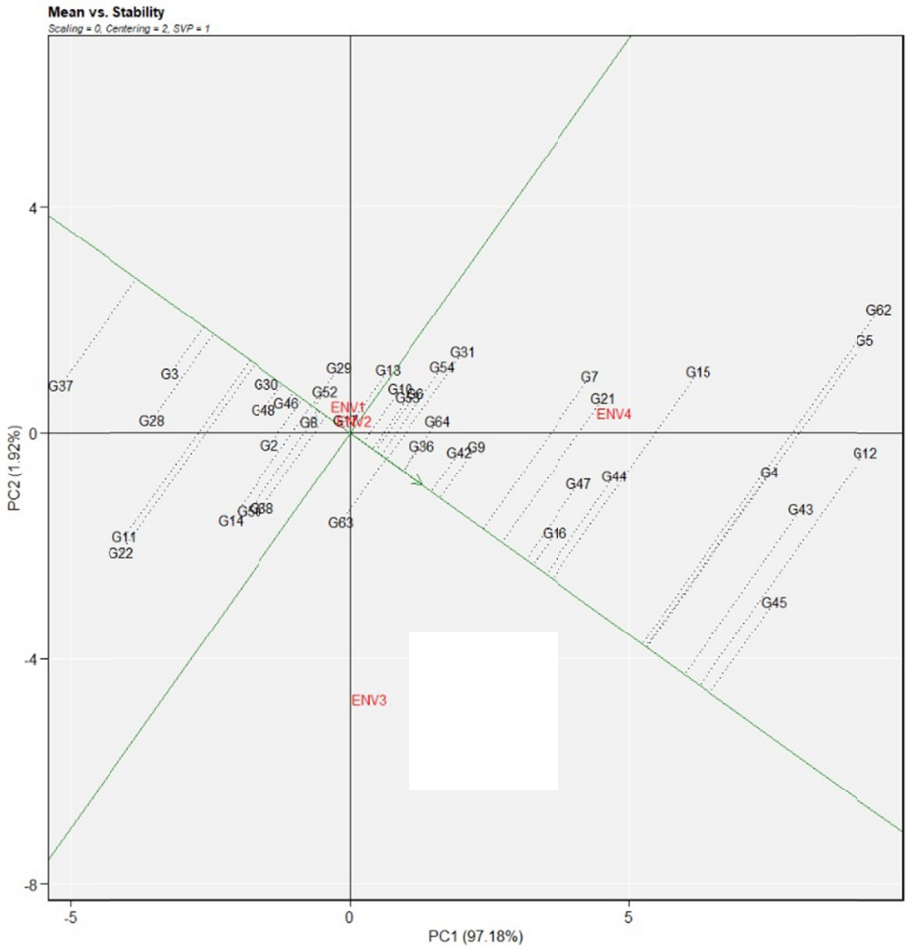

Figure 7. A GGE biplot showing the mean performance and stability for the grain yield of 64 sorghum genotypes evaluated in 4 environments

3.7 Ideal Genotypes in Response to Striga Infestation and Drought Stress

According to the biplots presented in Figs 6 and 7, ideal genotypes were the ones that yielded highest and were the most stable in those diverse environments. In Figure 8, those genotypes were plotted out from the four environments and identified as genotypes, B35 × ICSV III N, Macia, N13, ICSV 111 IN, FYYQ212 × B35, SRN39, GENO47293, ICSV 111 IN × B35, IS9830, Framida, GENO 45827, F6YQ212, B35 × AKUORACHOT, the best performers under both drought stress and striga prone conditions.

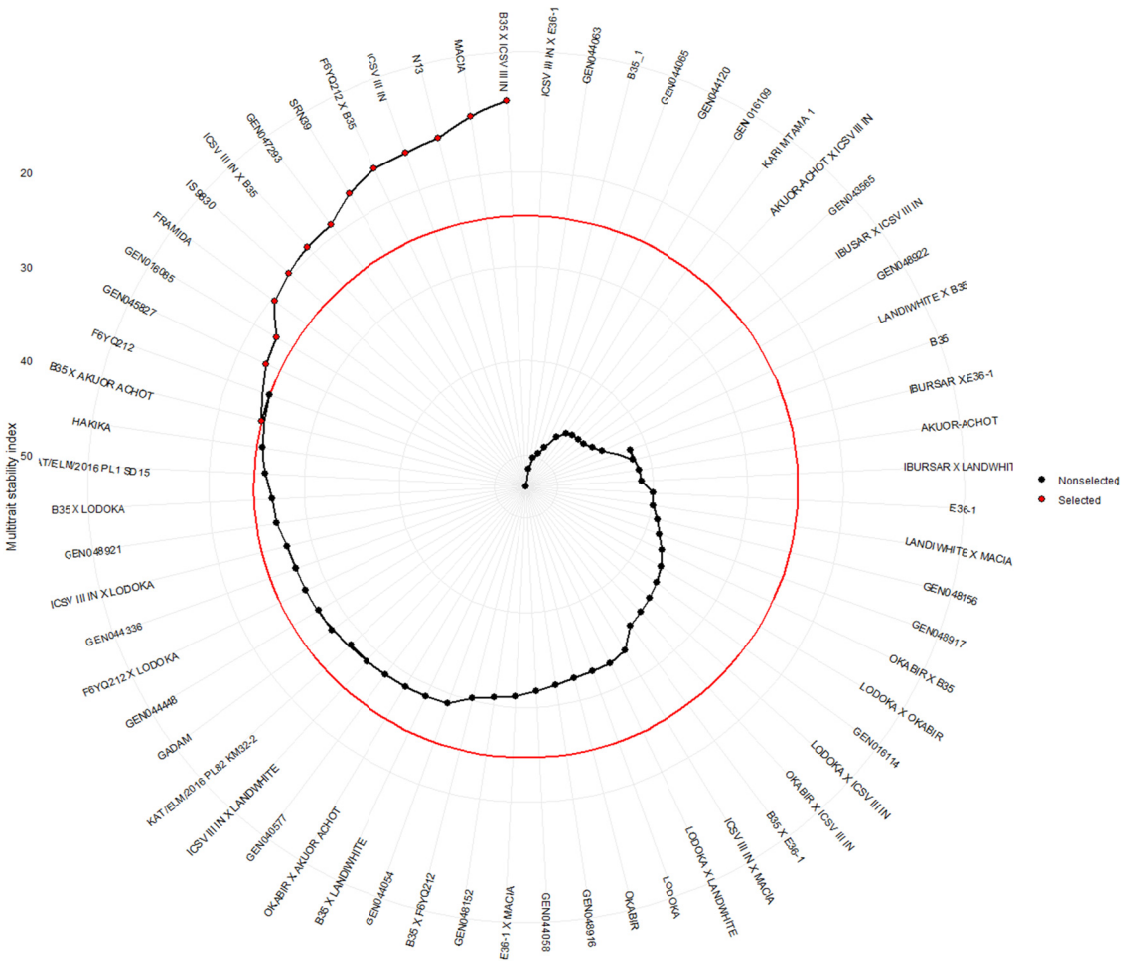

Figure 8. Ranking of the genotypes for their yield stability under drought stress and striga infestation conditions

4. Discussion

4.1 AMMI Analysis and Within Environments Variance

In considering the environments where these trials were conducted, some key points must be taken into account to explain the randomness of the GGE interactions and the lack of significance in some of the mega-environments such as in the drought-stressed and well-watered environments at Kiboko as shown in Table 2. Plot to plot variation was a source of environmental variation in defining mega environments 3 and 4. The drought stressed (Env1) and well-watered trials (Env2), had inherent large random variations, due to differences in evapotranspiration rates, soil structure and in the measurement of amounts of water applied that might have contributed to the lack of differentiation of the two as distinct mega-environment. Overall, the mega-environments defined here cannot be treated as being of fixed effects, and so the results must be treated with caution, since neither seasonal, annual rainfall, temperature or soil types can be classified as being constant in the two locations. These factors combined with the limited number of replications might have resulted into an error variation that introduced a random GGE interaction. Nevertheless, GY registered a high narrow-sense (h^2) heritability value in all the three environments except in the drought stressed one (Env1; Table 3) affirming that even where environmental variance was high, the GGE biplot was able to estimate the inherited or additive genetic variance for GY (Table 3).

4.2 Genotypes 'Which Won Where' in Biplots

Despite the forgoing observation, the AMMI analysis for GY showed that of the two main effects, environments were highly variable, but the genotype variance was not highly significant. The within environment variance, indicated that Env3 (striga infested field trial) was the most discriminative followed by Env4 (striga infested potted trial), whereas Env2 (well-watered) and Env1 (drought stressed) were the least (Figs 6 and 7). GGE

biplots have been used to analyze mean of yields, stability and adaptability performance of genotypes across environments (Yan et al., 2007). The GGE biplots sown here, showed not only the most stable and high yielding genotypes in the four mega-environments defined here but they also demonstrated 'which genotypes won where', which environments were most representative and discriminative. Genotypes, E36-1 × MACIA, B35 × LANDWHITE, GEN044120 in Env3, GEN016085, AKOUR ACHOR × ICSVIII.IN in Env4 were the most stable and had the highest mean GY (Figure 7). As confirmed by the 'which won where' biplot data in Figure 6, E36-1 × MACIA, B35 × F6YQ212 and ICSVIII.IN × MACIA were the best performers in Env3 whereas AKOUR ACHOR × ICSVIII.IN, ICSVIII.IN × B35 and OKABIR × AKUOR ACHOT were the most well adapted in Env4. Genotypes, ICSVIII, GEN048922 and GEN 016109 and IS9830 were the most unstable and low yielding in Env2 and Env1 respectively. The influence of the large environmental variation in the measurement of grain yield under drought stress conditions explains the low stability and the inability to discriminate the performance of genotypes under well-watered and drought stressed mega-environments as shown by the GGE biplots. Although GGE analysis was able to identify genotypes that have the similar sensitivity to the same environment, it was not able to explain why some of the winning genotypes in a particular environment were not significantly represented in another environment (Yan et al., 2007).

4.3 Genetic Relatedness and Implication for Stability for GY

In terms of genetic diversity and distance, wild accessions showed the highest level of relatedness with most clustering together in one subclade with resistant donor source, N-13. The only wild genotypes that clustered away from the rest were GEN048917 and GEN0444448 in Cluster 2, as well as GEN047293 in Cluster 3 (Figure 4). Landrace accessions were also distributed in different subclades within Cluster 1 with LODOKA and GEN044054 (Cluster 3) being the only landrace genotypes that clustered away from the rest. Improved varieties as well as the F4 generation of crosses were distributed within the population, an indication of low genetic relatedness but of high diversity. Most F_4 crosses clustered together with each other or with either of the parents used in the cross. However, some crosses grouped away from their progenies and parents, and this suggest the possibility that they were off types (Figure 4). These included, B35 × AKUOR ACHOT_ and B35 × LAND WHITE which clustered in completely different clusters from their parents and siblings (Figure 1). The wild relatives and landraces by clustering into distinct categories, would seem to suggest the existence of unique drought tolerance or striga resistance QTLs essential for GY stability and therefore the need to map and identify them for introgression into improved varieties (Cowan et al., 2020).

4.4 GY Correlated Traits Contributing to Stability of Performance

In sorghum, the stay-green trait is associated with more chlorophyll content and higher photosynthetic capacity ultimately leading to higher grain yields under drought stressed environments. Indeed, QTLs for stay-green have been mapped (Hausmann et al., 2004; Kebede et al., 2001) which correspond to QTLs for chlorophyll content (Crasta et al., 1999). In an earlier study, Ochieng et al., (2020), reported that though water stress reduced grain yield and yield related traits, RCC and GLAM, two prominent stay-green traits were positively correlated with grain yield under drought stress. The results reported here showed that landraces, such as LODOKA, OKABIR and AKUR-ACHOT scored the highest RCC, and GLAM and inevitably were among the highest grain yielders under water stress and outperformed known published stay-green drought tolerant sources, E-36-1 and B35. Equally, some wild relatives, such as accession, GBK 047293 had higher RCC, GLAM and GY than E-36-1 and B35 and performed as well as research bred cultivars such as IES 21400 DL. These findings underscore the fact that stay-green QTLs that are associated with drought tolerance in cultivated sorghum are also present in the landraces and wild relatives and help to determine the final GY and its stability under water stress. As would be expected, selection of these secondary traits, would reduce the effects of drought stress and lead to more stable grain yields in such environments (Sanchez et al., 2002).

On the other hand, F4 generation of F6YQ212 × B35 cross and GBK 045827 wild relative were the most consistently striga resistant genotypes across both field and potted artificially infested trials, though not necessarily high yielding as indicated by their HGW values in both trials (Muchira et al., 2021). F4 generation of B35 × E36-1 cross, two known sources of drought tolerance were also among the most striga resistant genotypes and as shown by the GGE data. Previous studies (Ochieng et al., 2020; Muchira et al., 2021) identified, the phenotypic traits that were associated with drought tolerance such as RCC, GLAM or with striga resistance such as ASNPC , NSFC as being strongly positively correlated with each other respectively, though this relationship was not so direct with grain yield, reaffirming the large environmental variance in the mega-environments that are due to microenvironmental effects that masked additive variance for yielding ability and stability. If adaptability can be classified as general or specific, then most genotypes performed well in only specific environments, but this would be expected since these two sets of trials were conducted in two different locations,

with only one but not both stresses being imposed in the same test plots. The most adapted genotypes, it would appear were the once that were ether highly drought tolerant, striga resistant or had one parent or both as drought tolerant or striga resistant in the generation of crosses. As previous argued by Hausmann et al., (2004) and Sah et al., (2016), drought tolerance and striga resistance mechanisms are expressed when there is increased photosynthates or repressed abscisic acid production. Whereas physiological proof is needed to ascertain this claim, it is apparent that drought tolerance or striga resistance QTLs or a combination of both confers stability and higher grain yields and are expressed by the genotypes that possess them, when grown in drought or striga prone environments.

5. Conclusion

The environment considered most ideal in this study was the striga infested field trial (Env3) where genotypes, E36-1 × MACIA, GEN 044120 and B35 × LANDWHITE showed the least interaction with the environment and whose productivity was determined by the properties of the genotype themselves rather than the environment. The drought stressed (Env1) and the well-watered (Env2) were the least stable, indiscriminative and had the lowest genotype × environment interactions. Genotypes, Macia, N13, ICSV111 IN, SRN39, GENO47293, IS9830, Framida, GENO 45827, F6YQ212 and generation of crosses of, B35 × ICSV III N, F6YQ212 × B35, ICSV 111 IN × B35, B35 × AKUOR ACHOT where one of the parents was either drought tolerant or striga resistant, were the most well adapted to all the four mega environments.

In order to gain full insight into the stability and adaptability of these genotypes for grain yield and minimize the effects of environmental variance, it is suggested that further trials should done in at least four seasons, in four locations where both drought stress and striga infestation are applied and artificially managed in the same field plots. Equally important especially in case of striga, it is recommended that striga free trials be setup to measure the extent to which striga affects particular traits. It is also recommended that the F4 generation of crosses with drought tolerance and striga resistance QTLs be selfed to fix the QTLs before any further testing. The germplasm identified here would be valuable to breeders aiming to develop sorghum varieties that combine both striga resistance and drought tolerance QTLs for subsistence and commercial production more specifically in the diverse agroecology of Eastern Africa.

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