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Novel sources of drought tolerance from landraces and wild sorghum relatives

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Abstract

Sorghum (*Sorghum bicolor* [L.] Moench) is the fifth most important cereal crop worldwide and second after maize (*Zea mays* L.) in Kenya. It is an important food security crop in arid and semi-arid lands, where its production potential is hampered by drought. Drought tolerance can be measured by a plant's ability to resist premature senescence, often described as stay-green. This study was carried out with the objective of identifying novel stay-green trait among wild and landrace genotypes of sorghum. Forty-four sorghum genotypes that included 16 improved, nine landraces, and 17 wild relatives of sorghum alongside known stay-green sources, B35 and E36-1, were evaluated under well-watered and water-stressed conditions in an alpha-lattice design of three replications. Data was collected on plant height (PHT), flag leaf area (FLA), panicle weight (PWT), 100-seed weight (HSW), relative chlorophyll content (RCC), number of green leaves at maturity (GLAM), days to 50% flowering (DFL), and grain yield (YLD). Genetic diversity was determined using diversity arrays technology (DArT) sequencing and quality control (QC) markers were generated using a java script. Lodoka, a landrace, was the most drought-tolerant genotype, recorded the highest numbers of RCC and GLAM, and outperformed B35 and E36-1 in yield under water-stress and well-watered conditions. The RCC was highly correlated with GLAM ($r = .71$) and with yield-related traits, HSW ($r = .85$), PWT ($r = .82$), and YLD ($r = .78$). All traits revealed high heritability (broad-sense) ranging from 60.14 to 98.4% for RCC and DFL, respectively. These results confirm earlier reports that wild relatives and landraces are a good source of drought tolerance alleles.

1 | INTRODUCTION

Sorghum ($2n = 2x = 20$) is considered a major staple food for a large portion of the world's population. Sorghum is the fifth most important cereal crop globally and is ranked second among staple food grains in semiarid tropics. The crop remains a critical component of food security for more

than 300 million inhabitants of Africa (Zhao, Che, Glassman, & Albertsen, 2019). Water stress is one of the major abiotic stresses affecting sorghum production in semi-arid areas, often experiencing long periods of drought and erratic rainfall (Rockström et al., 2010). Ordinarily, drought can occur at any stage of crop development, and the impact of moisture stress on yield is dependent on the stage of plant development. In sorghum, anthesis and grain-filling stages are the most vulnerable (Assefa, Staggenborg, & Prasad, 2010; de Camargo & Hubbard, 1999).

Several traits conferring drought tolerance have been reported in sorghum (Conley et al., 2001; Farré & Faci, 2006; Assefa et al., 2010; Schittenheim & Schroetter, 2014), including stay-green trait (Thomas & Ougham, 2014). Stay-green is a postflowering drought adaptation trait expressed by delayed leaf senescence as a result of improved water balance in the plant (Borrell et al., 2014; Subudhi, Rosenow, & Nguyen, 2000). The stay-green phenotype is considered functional when it is associated with greater biomass accumulation and enhanced crop productivity (Jordan, Hunt, Cruickshank, Borrell, & Henzell, 2012), or cosmetic/non-functional when chlorophyll is retained but the plant loses its ability to photosynthesize (Myers, Aljadi, & Brewer, 2018). Stay-green trait has been reported in several cereal crops including wheat (*Triticum aestivum* L.) (Christopher, Christopher, Borrell, Fletcher, & Chenu, 2016), maize (Belícuas, Aguiar, Bento, Camara, & Junior, 2014), and rice (*Oryza sativa* L.) (Hoang & Kobata, 2009). Only functional stay-green trait is considered important for crop improvement (Christopher et al., 2016).

Stay-green studies in sorghum have led to the identification of four major consistent quantitative trait loci (QTL) (*Stg1*, *Stg2*, *Stg3*, *Stg4*) (Crasta, Xu, Rosenow, Mullet, & Nguyen, 1999; Harris et al., 2007; Reddy, Ragimasalawada, Sabbavarapu, Nadoor, & Patil, 2014; Subudhi et al., 2000; Xu, Rosenow, & Nguyen, 2000). Some of these QTL are reportedly linked to enhanced grain yields (Borrell et al., 2014; Kassahun, Bidinger, Hash, & Kuruvinashetti, 2010; Reddy et al., 2014) and superior fodder quality (Blümmel, Deshpande, Kholova, & Vadez, 2017). However, most of these studies have been done using one major source of stay-green, B35/BTx642 (Rosenow, Quisenberry, Wendt, & Clark, 1983), with just a few studies using SC56 (Kebede, Subudhi, Rosenow, & Nguyen, 2001) and E36-1 (Haussmann et al., 2002). Stay-green sources B35 and E36-1 are from Ethiopia, while SC56 is from Sudan. Both Sudan and Ethiopia lie in the region where sorghum was first domesticated (Doggett, 1988), and the diverse germplasm that exists in this region would be a potential source of more novel stay-green alleles. A recent study of diverse sweet sorghum from Ethiopia reported an unexploited germplasm that can be included in breeding programs (Disasa et al., 2016).

Core Ideas

- New sources of stay-green from wild sorghum and landraces
- Three sorghum improved varieties show resilience under drought
- QC markers for hybridity testing developed

There is need to avoid the dependence on only a few sources of stay-green genes and alleles as is currently the case in global sorghum breeding programs. Crop wild relatives and landraces have been reported as reservoirs of useful genes for crop improvement (Brar & Khush, 2018; Nyamongo et al., 2015; Kyratzis, Nikoloudakis, & Katsiotis, 2019). The first step toward exploitation of this germplasm would involve screening and characterizing the novel alleles for their significance in crop improvement (Hokanson et al., 2010). In Kenya, most sorghum landraces grown by small-scale farmers freely exchange genes with their wild relatives growing in close proximity (Magomere, Ngugi, Obukosia, Mutitu, & Shibairo, 2015), although the potential of this genetic variation has not been fully exploited in breeding drought-tolerant sorghum genotypes. With the increasing effect of global warming, there is need to collect, screen, and identify novel sorghum germplasm harboring the stay-green trait that can be harnessed for adaptation to drought-prone agroecologies of eastern Africa.

The present study screened both wild and landrace sorghum alongside improved sorghum varieties and known stay-green sources, B35 and E36-1, under well-watered and water-stressed conditions in order to identify novel sources of stay-green. A unique set of molecular markers was also assessed for their potential in QC and marker-assisted backcrossing.

2 | MATERIALS AND METHODS

2.1 | Plant material and experimental layout

Sorghum genotypes comprising of 17 wild accessions, nine landraces, 16 improved varieties, and the two known stay-green sources, B35 and E36-1, were used in the study (Table 1). Fifty-two segregating F_2 populations were also part of the experimental design (data not presented). The entire wild and some landrace accessions were obtained from the Genetic Resources Research Institute of the Kenya Agricultural and Livestock Research Organization (KALRO). All the landraces and wild accessions

TABLE 1 Sorghum genotypes used in the study including the source of the germplasm and classification

Genotype	Source[a]	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. IESV23006 DL	ICRISAT	Improved variety	*Sorghum bicolor*
38. ICSV III IN	ICRISAT	Improved variety	*Sorghum bicolor*
39. HAKIKA	ICRISAT	Improved variety	*Sorghum bicolor*
40. CR35 ' 5	ICRISAT	Improved variety	*Sorghum bicolor*
41. IESV92043 DL	ICRISAT	Improved variety	*Sorghum bicolor*
42. WAHI	ICRISAT	Improved variety	*Sorghum bicolor*
43. IESV21400 DL	ICRISAT	Improved variety	*Sorghum bicolor*
44. IESV23010 DL	ICRISAT	Improved variety	*Sorghum bicolor*

[a]GeRRI, Genetic Resources Research Institute; ICRISAT, International Crops Research Institute for the Semi-Arid Tropics.

maintained in the gene bank have not been improved through any form of selection. The rest of the material was obtained from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Nairobi. The experiments were planted at KALRO Kiboko field station, Kenya (2.15° S, 37.75° E) in July 2017 in two blocks; the first block was irrigated throughout the plant growth cycle from sowing to physiological maturity stage, while the second block was water stressed to facilitate evaluation of genotypes for their response to drought as described elsewhere in the paper. Both experiments were laid out in a 12-by-8 alpha-lattice design replicated three times. The trial consisted of two-row plots of 2 m length) with an interrow spacing of 0.75 m and intrarow spacing of 0.25 m. Diammonium phosphate fertilizer was applied during planting at a rate of 100 kg ha^{-1} and the crop was top-dressed with urea 21 d after emergence at a rate of 40 kg ha^{-1} and then earthed up at 30 d after emergence. The crop was raised following the standard agronomic practices recommended in the area.

2.2 | Drought screening

The well-watered trial was irrigated three times per week, each time receiving 3 h of irrigation supplied at 25 mm per plot from sowing to soft-dough stage. Water was withdrawn in the water-stressed trial at 14 d post sowing; however, at 40 and 60 d after sowing, 25 mm of water per plot was applied twice per week with an interval of 2 d for each application period. Water-stress conditions were maintained until physiological maturity, which was 95 d after sowing.

2.3 | Data collection

Data on agronomic traits was collected on six randomly selected plants in the two rows from each of the three replications following the methodology described by IBPGR and ICRISAT (1993). Data was collected on the following traits: PHT (cm), FLA (m^2), DFL (counts), GLAM (count), RCC (soil plant analysis development readings) at maturity, PWT (kg), HSW (g), and YLD (t ha^{-1}). Grain yield data was determined on a plot basis as recommended by IBPGR and ICRISAT (1993).

2.4 | Genotyping, diversity estimation and quality control panel

Leaf tissues were sampled from the most representative plants per genotype at seedling stage and genomic DNA extracted using ISOLATE II Genomic DNA extraction kit

(Bioline Pty Ltd) according to manufacturer's instructions. Purity and quantity of the extracted DNA was determined using gel electrophoresis and a Qubit 2.0 Fluorometer (Life Technologies), respectively, with final dilution to 50 ng μl^{-1}. The DNA was sent to the Integrated Genotyping Service and Support at the Bioscience eastern and central Africa Lab at the International Livestock Research Institute hub for library construction and DArT sequencing (https://www.diversityarrays.com/products-and-services/applications/), as previously described (Wójcik-Jagła, Fiust, Kościelniak, & Rapacz, 2018). The resulting raw reads were processed using the GBS pipeline of the Trait Analysis by Association, Evolution and Linkage (TASSEL) 5.2.58 program (Bradbury et al., 2007). For drawing the neighbor-joining dendrogram, raw single nucleotide polymorphisms (SNPs) were filtered using a minor allele frequency of ≥0.05 and SNP minimum call rate of 100%. The dendrogram was drawn in Darwin 6.0.20 with 1,000 bootstraps (Perrier & Jacquemoud-Collet, 2006). For developing the QC panel, a set of 20 most informative SNPs was extracted from the SNP set used to draw the dendrogram using a java script (Ignacio, 2019).

2.5 | Statistical analysis

Analysis of variance, means, and variances for each quantitative trait was done in alpha-lattice design using GenStat v14.1 (VSN International, 2011). Treatment means were compared using Fisher's protected least significant differences at $p \leq .05$. The estimates of phenotypic and genotypic variance, genotypic and phenotypic coefficients of variation were done based on the formulas proposed by Syukur, Yunianti, and Kusumah (2011):

$$\text{Genotypic variance}: \ \sigma_g^2 = \frac{MS_g - MS_e}{r}$$

$$\text{Phenoypic variance}: \ \sigma_p^2 = \sigma_g^2 + \sigma_e^2$$

where σ_g^2 is genotypic variance; σ_p^2 is phenotypic variance; σ_e^2 is environmental variance (error mean square from the ANOVA); MS_g is the mean square of genotypes; MS_e is the error mean square; and r is the number of replications.

$$\text{Genotypic coefficient of variation}:$$

$$GCV = \left(\frac{\sqrt{\sigma_g^2}}{\bar{x}} \right) \times 100$$

FIGURE 1 A dendrogram showing two major clusters of the 38 genotypes analyzed. Cluster (a) contained wild and landrace accessions, while cluster (b) contained all of the improved varieties plus some of the landraces and wild accessions

Phenotypic coefficient of variation:

$$PCV = \left(\frac{\sqrt{\sigma_p^2}}{\bar{x}} \right) \times 100$$

where σ_g^2 is genotypic variance; σ_p^2 is phenotypic variance; and $\bar{x}$ is grand mean of a character.

Estimations of broad-sense heritability (H^2) of all traits were calculated according to the formula described by Allard (1960):

$$H^2 = \left(\frac{\sigma_g^2}{\sigma_p^2} \right) \times 100$$

where σ_g^2 is genotypic variance; σ_p^2 is phenotypic variance. Estimation of H^2 assuming selection intensity of 5% for individual and combined ANOVA were computed using the formula adopted from (Johnson, Robinson, & Comstock, 1955). The H^2 scores were classified according to (Robinson, Comstock, & Harvey, 1949) as follows: 0–30% = low; 30–60% = moderate; and >60% = high.

Simple linear correlation coefficients (Pearson, 1986) were calculated to understand the relationship among the studied agronomic traits as below:

$$PX, Y = \frac{\text{cov}(x, y)}{\sigma_x \sigma_Y}$$

where cov is the covariance, σ_x is the standard deviation of x, and σ_y is the standard deviation of y.

3 | RESULTS

3.1 | Genetic variation among sorghum accessions

A total of 26,291 raw SNPs were generated from 38 diverse genotypes (samples from six genotypes failed QC). The 38 genotypes included 11 improved varieties, eight landraces, 17 wild accessions, and two known stay-green sources, B35 and E36-1. After filtering for quality and ensuring no missing data, 803 SNPs were retained and used to assess genetic diversity between the 38 genotypes. Two major clusters were observed: one dominated by landraces and wild accessions, and another by improved varieties (Figure 1). Within the cultivated accessions cluster, there were three subclusters of improved varieties suggesting high similarities among the improved genotypes. Evidence of continuous gene flow between the wild and cultivated germplasm was revealed in the clustering of several wild accessions with cultivated ones. Genotype B35, the most widely used source of stay-green alleles, clustered with the wild and landrace accessions further confirming its mixed parentage. Genotype E36-1 clustered together with cultivated accessions. Apart from a few genotypes showing high similarities, the selected set of germplasm used in the study was diverse and likely to enhance the value of the improved varieties if integrated in breeding programs.

3.2 | Molecular markers for quality control and marker-assisted backcrossing

From the 803 SNP markers used for assessing genetic diversity (Figure 1), the 20 most informative markers for the 38 accessions (Table 2) were selected, which were well distributed across the genome. We confirmed the informativeness of the 20 markers by using them to draw a dendrogram for the 38 genotypes (Figure 2). The 20 markers differentiated the 38 genotypes (Figure 2) just as well as the 803 SNP markers did (Figure 1).

3.3 | Phenotypic variation of traits and heritability among diverse sorghum accessions

The mean performances of each genotype under well-watered and water-stress conditions are summarized in Supplemental Table S1. Seven genotypes (one landrace, two improved, and four wild accessions) were completely senescent under water-stress conditions. The rest of the analysis under drought conditions was therefore done with 37 accessions, which included 13 wild germplasm, eight

TABLE 2 The selected set of 20 most informative single nucleotide polymorphism markers selected from the 38 sorghum accessions that had been genotyped using DArT-sequencing

Chromosome	Position	Variant
1	21279335	C/G
1	45984426	C/T
2	7803138	C/T
2	77523709	G/A
3	13455829	A/G
3	57242431	A/G
4	1389787	T/A
4	2600536	C/T
4	3295616	C/G
4	56353491	C/G
4	59715414	T/C
4	65176114	C/G
5	53835336	T/C
5	56501094	G/A
5	61381207	A/G
6	49278472	G/T
6	50201989	C/G
6	57826860	G/A
8	54998738	A/C
9	786078	T/C

landraces, 14 improved varieties, and the two stay-green checks (B35 and E36-1). Analysis of variance performed using the 37 accessions that did not senesce revealed significant ($p \leq .05$; $p \leq .01$) differences across the genotypes for all the studied traits under both water-stress (Table 3) and well-watered conditions (Table 4). These differences were also revealed when the interaction between the two water regimes was compared except in the case of FLA (Table 5).

The highest genotypic and phenotypic variation was observed in PHT and DFL (Table 6). All traits revealed high heritability (broad-sense) that ranged from 60.14% for RCC to 98.40% for DFL (Table 6). Stay-green related traits, RCC and GLAM, both recorded high heritability at 60.14 and 63.71%, respectively.

When the effect of drought was determined using all the 44 genotypes and treating senescent genotypes under drought stress as missing data, we observed clear reductions in PHT, GLAM, RCC, DFL, FLA, and YLD (Figure 3). There was no observable difference in the means of HSW under stress and well-watered conditions, while an increase in PWT was observed under water stress.

We observed high correlations between RCC and yield components, with the highest correlations recorded between RCC and PWT ($r = .82$) and RCC and HSW ($r = .85$) (Table 7). Flag leaf area was negatively correlated with
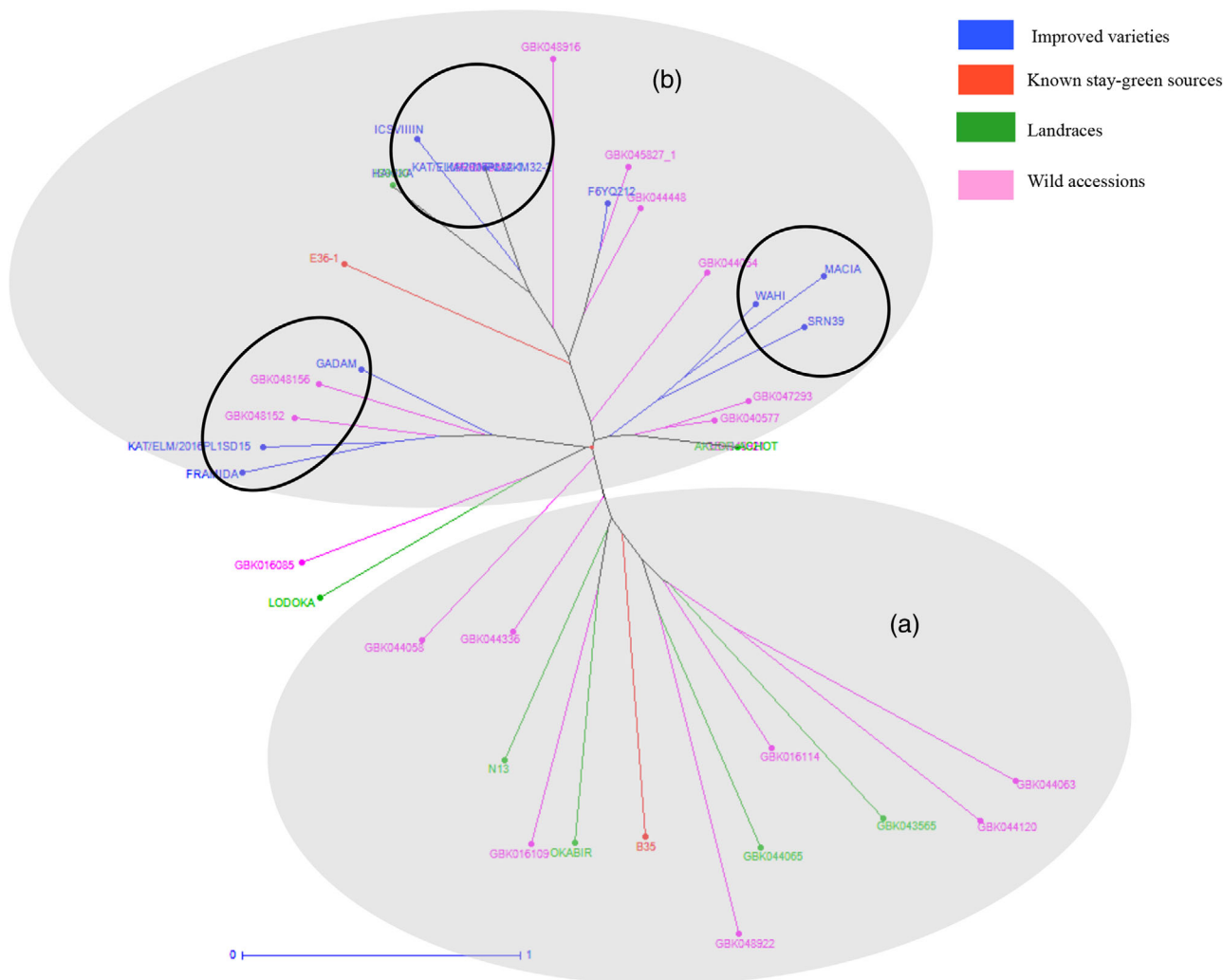
FIGURE 2 A dendrogram drawn using the 20 selected informative markers. Two clusters previously identified with 803 single nucleotide polymorphism markers (Figure 1) were still revealed

all traits except GLAM. Plant height was also highly negatively correlated ($r = 0.81$) with PWT.

3.4 | New sources of stay-green trait

Nine genotypes (LODOKA, IESV21400DL, IESV23006DL, IESV92043DL, IESV23010DL, OKABIR, GBK 016109, GBK 048156, and AKUOR-ACHOT) outperformed both E36-1 and B35 with respect to their RCC at maturity (Figure 4a), while seven genotypes (LODOKA, OKABIR, IBUSAR, F6YQ212, AKUOR-ACHOT, GBK 047293, and GBK 048917) had more GLAM than E36-1 and B35 (Figure 4b) under drought conditions. Ten (IESV23010DL, IESV23006DL, IESV92043DL, AKUOR-ACHOT, GBK 047293, LODOKA, WAHI, GBK 016114, GBK 045827, and OKABIR) of the 18 genotypes that had outperformed B35 when ranked using RCC measurement (Figure 4a) also yielded better

than both E36-1 and B35 (Figure 5). The landrace genotype LODOKA stood out as having the highest GLAM and RCC and was also among the top yielders, with a yield of 2.2 t ha^{-1} out of the highest recorded yield of 2.45 t ha^{-1}. All genotypes that yielded better than E36-1 (Figure 5) were considered as potential new sources of functional stay-green.

3.5 | Effect of drought on yield-related traits

Water stress revealed an overall negative effect on yield related traits (Supplemental Table S1; Figure 6). There was 5.30, 9.28, and 15.80% overall decrease in PWT, HSW, and YLD, respectively. Twenty-two, 18, and 15 out of the 37 accessions that did not senesce recorded higher PWT, HSW, and YLD, respectively, under water stress (Figure 6).

TABLE 3 ANOVA table showing the mean squares across 37 sorghum genotypes for each trait measured under water-stress conditions

SOV[a]	DF	PHT	RCC	GLAM	DFL	FLA	HSW	PWT	YLD
Rep	2	1929	46.77	1.413	34.48	0.002481	0.1641	0.001125	0.0005
Genotype	36	3555.8***	66.37***	6.133**	179.82***	0.0137***	0.675***	0.005708***	0.997***
Residual	71	730.1	26.66	2.796	2.888	0.003	0.1516	0.001071	0.1299

[a] SOV, source of variation; DF, degrees of freedom; PHT, plant height; RCC, relative chlorophyll content; GLAM, no. of green leaves at maturity; DFL, days to 50% flowering; FLA, flag leaf area; HSW, 100-seed weight; PWT, panicle weight; YLD, grain yield.
**Significant at $P < .01$.
***Significant at $P < .001$.

TABLE 4 ANOVA table showing mean squares across 37 sorghum genotypes for each trait measured under well-watered conditions

SOV[a]	DF	PHT	RCC	GLAM	DFL	FLA	HSW	PWT	YLD
Rep	2	3648.5	210.11	123.36	4.199	0.000484	0.0477	0.027492	0.027492
Genotype	36	7892.2***	50.86**	6.504**	94.66***	0.00066**	0.8205***	0.0026**	0.6139***
Residual	71	421.2	25	3.024	3.897	0.000307	0.2497	0.2497	0.001852

[a] SOV, source of variation; DF, degrees of freedom; PHT, plant height; RCC, relative chlorophyll content; GLAM, no. of green leaves at maturity; DFL, days to 50% flowering; FLA, flag leaf area; HSW, 100-seed weight; PWT, panicle weight; YLD, grain yield.
**Significant at $P < .01$.
***Significant at $P < .001$.

Only nine out of the 24 accessions that showed improvement in any one of the yield-related traits under drought were improved varieties, while the rest were either wild (11) or landraces (4). Neither B35 nor E36-1 showed an increase in their yield related traits under drought conditions.

4 | DISCUSSSION

This study highlighted the importance of including wild and landrace accessions for improving important traits in breeding programs. Molecular characterization of the germplasm revealed genetic relationship between wild, landrace, and improved genotypes that will be useful in future parental selection aiming to enhance the diversity within the breeding programs. Recent genetic analyses in sorghum have revealed similarities between commonly used breeding lines (Disasa et al., 2016) and unexploited landrace collections (Disasa et al., 2016; Mofokeng, Shimelis, Tongoona, & Laing, 2014; Upadhyaya, Dwivedi, Wang, & Vetriventhan, 2019). The clustering of wild and cultivated germplasm has been reported in other studies (Mutegi et al., 2010, 2011; Sagnard et al., 2011) and is indicative of gene flow between the two groups that could present both ecological and agronomic issues (Ohadi, Hodnett, Rooney, & Bagavathiannan, 2018). Some of the wild

TABLE 5 Combined ANOVA table showing the mean squares interaction of the two water regimes on the performance of the 37 sorghum genotypes

SOV[a]	DF	PHT	RCC	GLAM	DFL	FLA	HSW	PWT	YLD
Rep	2	2478.8	226.53	75.504	31.053	0.192	0.0544	0.19642	0.0184
Water regimes (W)	1	101724.5**	55.63***	199.485***	376.332***	0.2477***	0.1117ns[†]	0.3105ns	0.1802ns
Genotypes (G)	36	8394.5***	5.62**	9.817***	215.43***	0.1073***	0.9264***	0.5731***	1.2204***
W×G	36	3053.5**	162.81*	3.82*	59.049***	0.215ns	0.569***	0.2578*	0.3905***
Residual	143	611.2	2.24	3.563	3.452	0.0213	0.2	0.1567	0.1504

[a] SOV, source of variation; DF, degrees of freedom; PHT, plant height; RCC, relative chlorophyll content; GLAM, no. of green leaves at maturity; DFL, days to 50% flowering; FLA, flag leaf area; HSW, 100-seed weight; PWT, panicle weight; YLD, grain yield.
*Significant at $P < .05$.
**Significant at $P < .01$.
***Significant at $P < .001$. [†] ns, not significant.

TABLE 6 Heritability estimates of all traits measured across 37 sorghum genotypes using mean sum of squares from the combined water-stress and well-watered ANOVA

Trait[a]	Range	σ^2_g	σ^2_p	GCV[b]	PCV[c]	Broad-sense Hš
					—%—	
Plant height (cm)	65–235.7	2594.43	2798.17	33.63	34.92	92.72
Grain yield (t ha^{-1})	0.26–2.45	0.36	0.41	2.46	2.63	87.68
RCC (SPAD)	25.63–55.84	1.13	1.87	63.89	82.38	60.14
Hundred seed weight (g)	0.31–3.50	0.24	0.31	4.54	5.13	78.41
Panicle weight (kg)	6.08–11.50	0.14	0.19	3.38	1.98	72.66
Flag leaf area (m^2)	0.01–0.07	0.03	0.04	12.78	14.27	80.15
GLAM (counts)	6.08–11.50	2.08	3.27	3.45	4.33	63.71
Days to flowering (counts)	30–103	70.66	71.81	66.52	67.06	98.40

[a]RCC, relative chlorophyll content; SPAD, soil plant analysis development readings; GLAM, green leaves at maturity .
[b]GCV, genotypic coefficient of variation.
[c]PCV, phenotypic coefficient of variation.
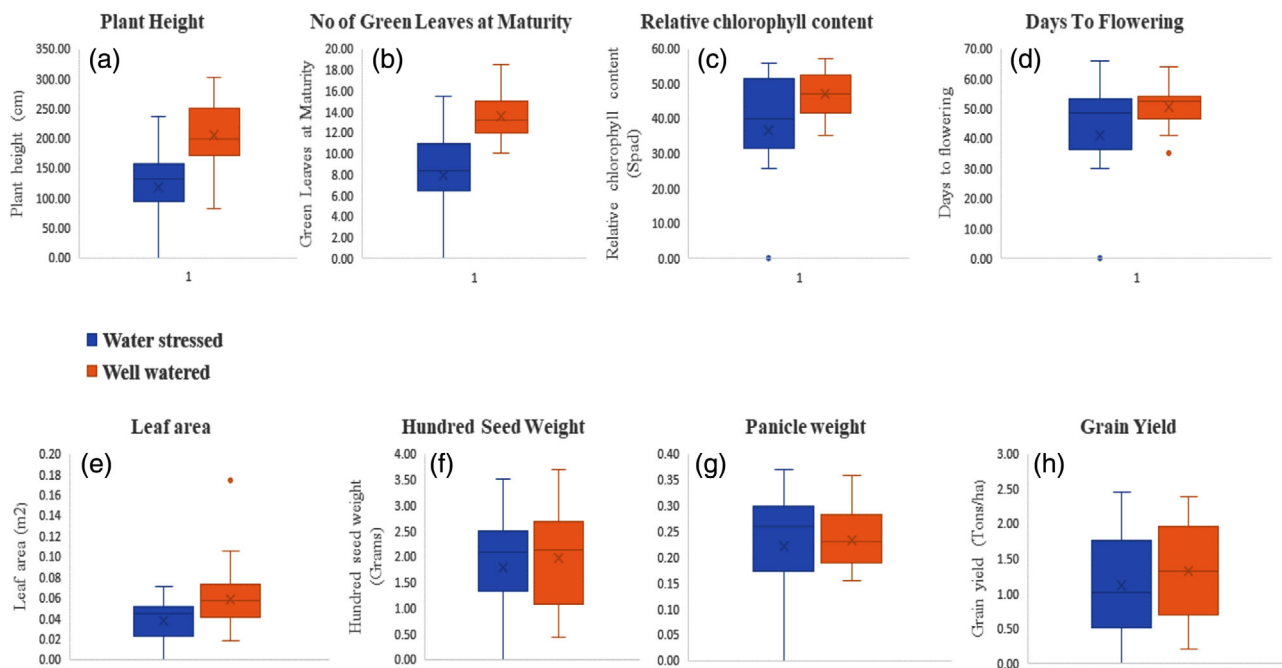
FIGURE 3 Box plots of the studied traits showing the mean performance of all the 44 sorghum genotypes under water-stressed (in Blue) and well-watered (Orange) conditions

sorghum relatives have been reported to be weedy (Okeno, Mutegi, de Villiers, Wolt, & Misra, 2012) and gene flow with improved germplasm could result in super weeds.

Wild sorghum is highly diverse (Billot et al., 2013; Mace et al., 2013; Sagnard et al., 2011) and has been used as a source of resistance to biotic (Wang et al., 2014; Mbuvi et al., 2017) and abiotic traits (Cowan et al., 2020) including stay-green. The oldest stay-green source, B35, is a BC1 derivative of IS12555, durra sorghum from Ethiopia

(Subudhi et al., 2000), while E36-1 was derived from the Ethiopian zera-zera germplasm collection (Thomas & Ougham, 2014). Cultivated sorghum is divided into five races (durra, caudatum, bicolor, guinea, and kafir) based on their morphology (Harlan & de Wet, 1972). Durra sorghum is very distinct with compact heads and is mainly grown along the edge of the Sahara and in India. Our results indicate the potential of discovering many more stay-green sources from wild accessions with

TABLE 7 Phenotypic correlations of traits measured under water stress

Traits[a]	PHT	FLA	GLAM	PWT	HSW	YLD	RCC
PHT	–	–	–	–	–	–	–
FLA	0.28[*]	–	–	–	–	–	–
GLAM	0.48[*]	0.44[*]	–	–	–	–	–
PWT	−0.81[***]	−0.38[*]	0.61[***]	–	–	–	–
HSW	0.63[***]	−0.19ns[†]	0.66[***]	0.74[***]	–	–	–
YLD	0.64[***]	−0.34[*]	0.55[***]	0.66[***]	0.73[***]	–	–
RCC	0.75[***]	−0.64[**]	0.71[***]	0.82[***]	0.85[***]	0.78[***]	–
DFL	0.55[**]	−0.54[**]	0.68[***]	0.36[*]	0.42[*]	0.35[*]	0.66[***]

[a] PHT, plant height; FLA, flag leaf area; GLAM, no. of green leaves at maturity; PWT, panicle weight; HSW, 100-seed weight; YLD, grain yield; RCC, relative chlorophyll content; DFL, days to 50% flowering.
[*] Significant at $P < .05$.
[**] Significant at $P < .01$.
[***] Significant at $P < .001$. [†] ns, not significant.

the five (GBK045827, GBK016114, GBK048922, GBK016109, and GBK047293) promising wild accessions clustering differently from B35 and E36-1. The genetic control of stay-green in these new sources will need to be studied and fully understood. These results further underscore the potential value of gene bank collections in contributing novel sources required for crop improvement and productivity enhancement. The nature of stay-green present in these new sources will need to be understood better through the development of relevant populations. Studies in B35 and E36-1 have reported that three of the major QTL identified in crosses involving B35 have also been reported in E36-1 (Haussmann et al., 2002; Subudhi et al., 2000; Thomas & Ougham, 2014). It will be necessary to establish the nature of QTL that will be mapped from the new sources and how they compare with B35 and E36-1 alleles.

Despite showing superior performance under drought for GLAM and RCC, the landrace LODOKA did not have the highest yields. The highest yielding genotypes were improved varieties: IESV203010 DL, IESV23006 DL, and IESV92043 DL. This underscores the need for enhancing the overall genetics of the identified drought-tolerant landraces in order to improve their yield. This will need to be done through introgression of the unique traits into farmer-preferred varieties or by selecting the respective landraces under intense cultivation and improving their agronomic traits. Most importantly, these three superior performing improved genotypes present extremely good material that should be promoted immediately under the arid and semiarid ecologies.

The high positive significant correlation coefficients observed between RCC and yield-related traits are consistent with previous reports in both sorghum (Borrell et al., 2014; Borrell, Hammer, & Henzell, 2000; Jordan et al., 2012; Kamal, Alnor Gorafi, Abdelrahman, Abdellatef, & Tsujimoto, 2019; Kassahun et al., 2010) and other cereals (Bekavac et al., 2007; Kamal et al., 2019), and confirms that the new stay-green genotypes identified in the current study are functional. It was surprising to see the positive effect of drought on yield-related traits in some genotypes. Drought has been reported to enhance heterosis in yield-related traits in pearl millet [*Pennisetum glaucum* (L.) R. Br.] (Yadav, Weltzien-Rattunde, Bidinger, & Mahalakshmi, 2000) and maize (Makumbi, Betrán, Bänziger, & Ribaut, 2011). In rice, QTL responsible for enhanced yield under drought have been mapped and used to improve the rice mega variety IR64 (Swamy et al., 2013). The fact that most of the genotypes that showed enhanced yield under drought were wild or landraces may suggest the presence of other traits in these uncharacterized germplasms that may have enhanced their performance. Such traits will need to be studied further and more supporting evidence generated before conclusions are made. Any additional factors contributing to yield losses under well-watered conditions will also need to be captured.

High heritability of stay-green related traits, RCC (60.14%) and GLAM (63.71%), is a positive outcome, as it indicates a high genetic control for the traits. Reports on heritability of stay-green have been variable in different crops including sorghum. Walulu, Rosenow, Wester, and Nguyen (1994) and Mkhabela (1995) reported high and low heritability values, respectively, while studying progenies generated using B35. These differences may result from the parameters measured and the inconsistencies in the environments used. Several studies reported four major QTL in B35 (Harris et al., 2007; Subudhi et al., 2000; Xu et al., 2000), three of which are shared with E36-1 (Haussmann et al., 2002). The high heritability recorded in the present study will need to be validated in the future using larger biparental populations created from the new sources identified.
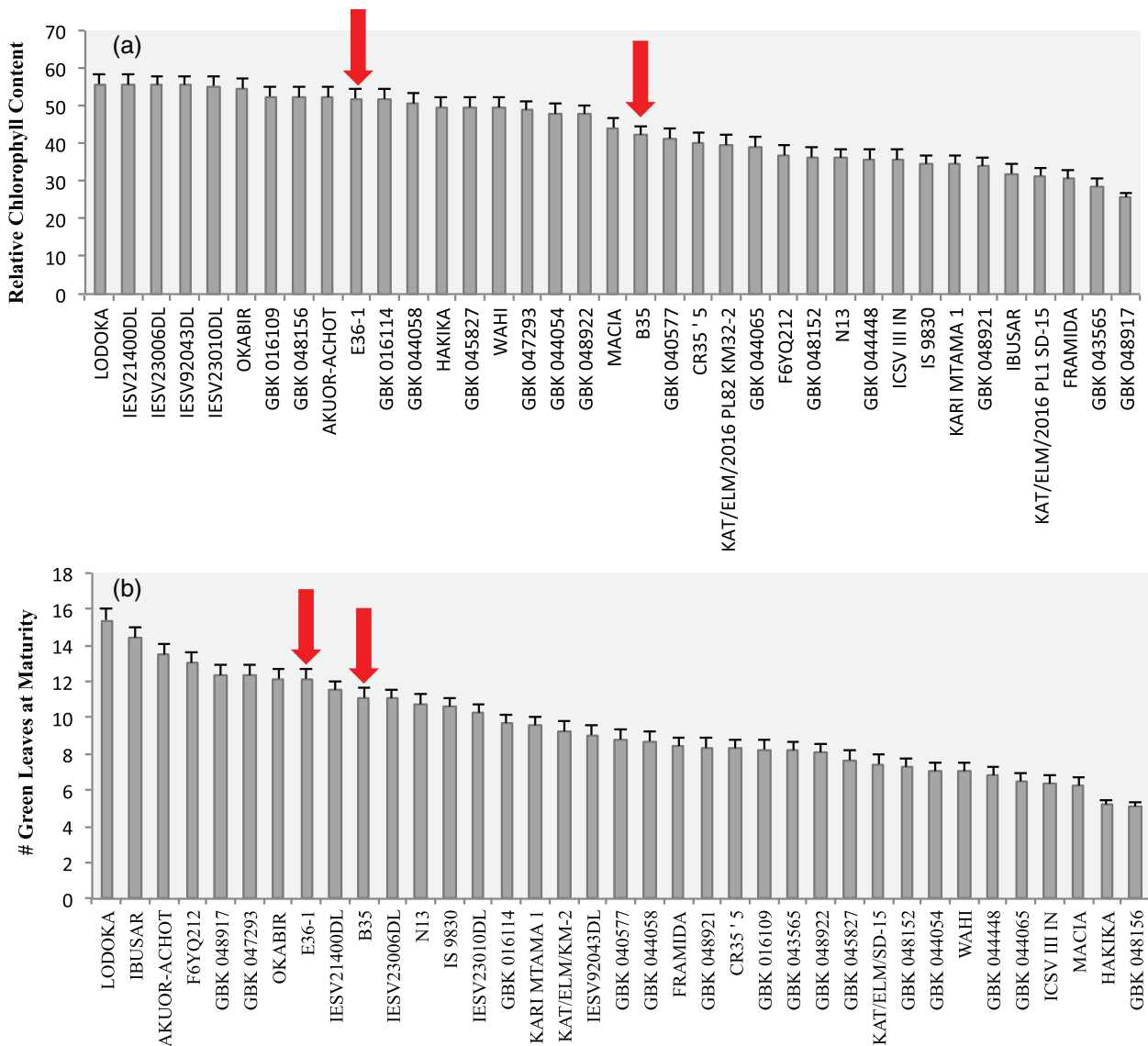
FIGURE 4 Performance of 37 out of the 44 sorghum genotypes that did not senesce under water-stress conditions in comparison with known stay-green sources, E36-1 and B35 as measured using (a) relative chlorophyll content and (b) green leaves at maturity

All the traits measured in the present study proved useful for the classification of the genotypes and showed significant variation across genotypes and environments. The lack of variation observed in FLA across environments could be due to the units used for measurement and the stage at which moisture stress was induced. Although there is a lack of information in cereals on the role of flag leaf during drought, it is known that flag leaf functionality is required for grain filling to occur (Biswal & Kohli, 2013). The reduced FLA among the drought-tolerant genotypes helps to reduce water loss, as these genotypes maximize water use efficiency at flowering (Farooq, Wahid, Kobayashi, Fujita, & Basra, 2009). A decrease in leaf expansion rate is an adaptive mechanism that usually precedes the reduction in stomatal conductance or
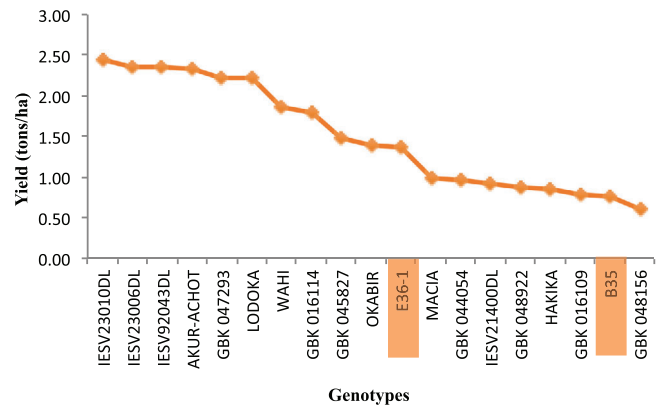
FIGURE 5 Yield of 19 top drought-tolerant sorghum genotypes in comparison with known stay-green sources, B35 and E36-1, under water stress
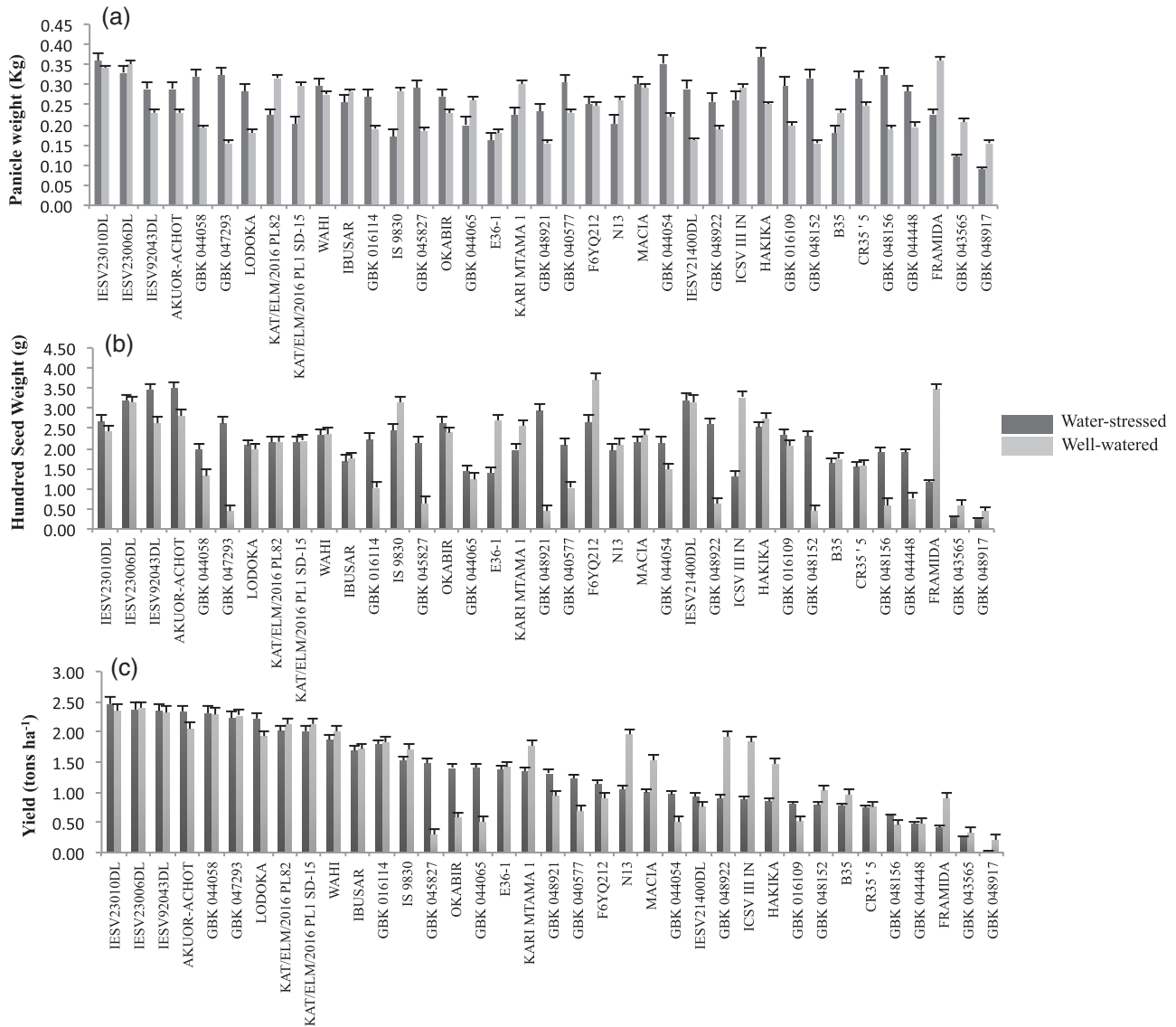
FIGURE 6 A comparison of (a) panicle weight, (b) 100-seed weight, and (c) yield traits observed for the 37 sorghum germplasm that survived under both well-watered and water-stressed conditions

photosynthesis (Farooq, Kobayashi, Ito, Wahid, & Serraj, 2010). Past studies in sorghum have reported high correlation between FLA and YLD (Munamava & Riddoch, 2001). Our results will still need to be validated to resolve the true relationship between FLA and yield-related traits.

A major concern for the introduction of wild accessions is the linkage drag (Zamir, 2001). The SNP marker set developed here for QC will enhance germplasm characterization, parentage verification, and confirmation of purity of genotypes. However, these markers will need to be developed into quick assays as has been done in other crops (Chen et al., 2016; Ertiro et al., 2015; Gemenet et al., 2020; Ndjiondjop et al., 2018) for more efficient application.

5 | CONCLUSION

Our results reveal the potential of discovering many more drought-tolerant sorghum genotypes in eastern Africa as the region currently holds a huge collection that is yet to be adequately characterized. Wild accessions will remain a viable source of novel alleles in the region, not only for stay-green, but other important traits as well. The developed SNP markers for QC will act as the first molecular toolkit for most of the breeders in the region who currently use mainly conventional breeding. The identified new sources of stay-green will need to be urgently characterized in order to ensure immediate deployment of these alleles into breeding programs. The observed positive effect of drought on yield of some accessions suggests that

the water regime used in the present study may need to be modified to ensure optimum conditions are used in future drought experiments.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.