



## Genetic Diversity Analysis of Stay Green and Some Drought Tolerant Traits in Sorghum (*Sorghum bicolor* (L.) Moench)

Saidu, G., Mahmood, M. S., Hussaini, I.  
Department of Agricultural Education,  
School of Vocational and Technical Education,  
Adamu Tafawa Balewa College of Education Kangere,

### ABSTRACT

*Sorghum bicolor* (L.) Moench is faced by several biotic and abiotic constraints that reduce its yield. Drought remains the main abiotic factor limiting the crop cultivation resulting in yield instability and food shortage. The objective of the study was to assess the genetic diversity among the sorghum genotypes for stay-green and drought tolerant traits. About 169 was obtained and screened from Bauchi and Zaria, Nigeria from their germplasm collection. Eighteen genotypes were selected for stay green traits were screened based on genetic diversity and stay green and other agro morphological traits under control and imposed drought condition in Bauchi and Zaria in 2023 raining season, randomized complete block designed was used on 18 sorghum genotypes. The genotypes were grouped into ABC major clusters elucidating a high level of genetic diversity which was useful for identifying suitable genotypes for stay green and drought tolerance breeding. The best characterized form of drought tolerance during crop growth is the non-senescence or “stay-green” trait. Genetic diversity analysis carried out with 18 out of 169 sorghum genotypes for drought tolerance using 18 stay-green specific polymorphic SSR markers revealed high level of polymorphism among the genotypes. The number of alleles produced by different primers ranged from 2 to 7 with an average of 4.0 alleles per primer. The polymorphic loci clearly discriminate all the genotypes. The similarity coefficients based on SSR markers ranged from 0.02 to 1.00. However, the alternative stay-green genetic sources were found to be distinct based on either all the SSRs employed or using only those associated with the stay-green trait in B35. Therefore, the physiological and biochemical basis of each stay-green source should be evaluated in order to enhance the understanding of the functioning of the trait in the various backgrounds. These genetic sources of stay-green could provide a valuable resource for improving this trait in sorghum breeding programmes.

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Stay Green (SG), Diversity, SSR Marker.

### INTRODUCTION

The stay-green trait has been used for years by breeder as a measure of post-flowering drought tolerance low soil moisture stress during grain-filling (Wang et al., 2011). The trait is characterized by green stems and green upper

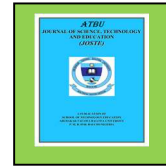
leaves when water is limiting during grain filling, which enables the stay-green genotypes to continue to fill their grain under drought conditions Thin et al.,(2015). Understanding the inheritance of traits, such as stay-green, is important for its successful application in plant

Corresponding author: Saidu, G. Mahmood

✉ [briefballagado@gmail.com](mailto:briefballagado@gmail.com)

Department of Agricultural Education, School of Vocational and Technical Education, Adamu Tafawa Balewa C. O. E., Bauchi.

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breeding programmes. Markers associated with regions of the genome controlling stay-green can be used in the breeding programme to assist in the precise introgression of those regions into novel varieties. Alleles of simple sequence repeats (SSR) associated with stay-green which might influence their tolerance on post-flowering drought stress. However, different genetic sources of stay-green may employ different genes and display different inheritance characteristics. Analysis of genetic diversity is important for crop improvement and provides essential information to enable more efficient use of available genetic resources and a platform for stratified sampling of breeding populations (Tomar 2012). Accurate assessment of the levels and patterns of diversity can be invaluable in the analysis of genetic variability in cultivars (Basavaraj, G. et al., 2013), identification of diverse parental combinations to create segregating progenies with maximum genetic variability for further selection (Ariyajaroenwong, P 2012) and introgressing desirable genes from diverse germplasm into the available genetic base (Thompson et al., 1998). Genetic relationships can be useful for the planning of crosses, assigning lines to specific heterotic groups and for precise identification with respect to plant varietal protection (Tomar 2012). Molecular markers have many advantages over phenotypic characters as they are unaffected by the environment. DNA-based markers, such as simple- sequence repeats (SSRs), have been or are being utilised in cultivar development,

Quality control of seed production, measurement of genetic diversity for conservation management, varietal identification and intellectual property protection (ABarik, S. et al., 2017) and are powerful tools in genetic similarity studies (Basavaraj, G.,2013). Furthermore, these markers can be used to obtain information on the genes that influence agriculturally important traits and to follow the introgression of these genes, thus facilitating the breeding process. SSRs are highly useful as genetic markers as they are codominant, occur in high frequency and appear to be distributed well throughout the genomes of higher plants and

animals (Appiah-Nkansah, et al., 2015) with simple Mendelian segregation (Basavaraj, G.,2013). SSRs have higher information content, as measured by expected heterozygosity and number of alleles and polymorphism, as compared to other markers. Indicating that SSRs are more discriminating. Their high information content and favourable characteristics of SSRs make them excellent genetic markers for many types of investigations, including marker-assisted selection (MAS) and finger-printing of germplasm collections (Appiah-Nkansah, et al., 2015). Comparative studies in crop plants have shown that they provide a powerful tool for discriminating between genotypes and for MAS (Zou 2014). In sorghum, SSRs are well distributed across the sorghum genome (Are et al., 2015). It is thus possible to avoid overrepresentation of certain regions of the genetic map that can produce inaccurate estimates of genetic similarities among individuals. SSRs have revealed high levels of polymorphism to allow the vast majority of Sorghum bicolor accessions, including those within working groups, to be distinguished by using a relatively small number of SSR loci (Adams et al., 2015)

## MATERIALS AND METHOD

Out of 164 sorghum genotypes eighteen sorghum genotypes were screened in Bauchi and IAR farm Samaru in 2023 wet season. The trial was laid out using randomise complete block design (RCBD) with three replications. Entries were grown on four row plots of 5m long with 75cm inter row and 25cm intra row spacing. Six to eight seeds were sown per hill and later thinned to 4 to 5 stands two weeks after sowing. Fertilizer (90kg N, 45kg P<sub>2</sub>O<sub>5</sub> and 45kg K<sub>2</sub>O) was applied in two split doses at 4 weeks and at about 6 weeks after planting using NPK 15:15:15 and urea. The experiments were kept weed-free by chemical weed control and manual weeding. Data were collected on days to 50% flowering, plant height (cm), number of leaves, number of internodes, stay green, brix degree at maturity, fresh stalk weight (Kg) and dry stalk weight (Kg). Brix degree was measured

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Corresponding author: Saidu, G. Mahmood

✉ [briefballagado@gmail.com](mailto:briefballagado@gmail.com)

Department of Agricultural Education, School of Vocational and Technical Education, Adamu Tafawa Balewa C. O. E., Bauchi.

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using a refractometer. Mean performance of each entry was calculated from the replicated plot means for all the measured traits. Analyses of variance (ANOVA) were performed on plot means for all the traits using the GLM procedure of the SAS program (SAS Institute., 2001). Eighteen Sorghum lines comprising sweet and non-sweet, were also collected in the field and characterised using SSR markers. In October the lines were characterised for genetic diversity using SSR markers for sorghum (A Barik, S.2017). Six lines were selected from the 4 clusters derived from the SSR based genotyping of the 18 sorghum lines (Figure 1). This was done by selecting lines with 3 high brix 3low brix content from each of the clusters. The six selected lines were used for population development for genetic studies.

## RESULTS AND DISCUSSION

The data collected in the raining seasons were subjected to ANOVA using GENSTAT 9.1 for assessment of the stay green genotypes. Genotypic differences were highly significant for all the traits observed (Table 1). Years differed significantly for stay-green score, time to 50% flowering, plant height and plant agronomic score. Genotype × year interactions also differed significantly for all observed traits except plant height. The overall stay-green score

of the genotypes ranged from 1.3 to 3.5 (SE±0.32). The stay green reported by (ABarik, S.et al. 2017, Haussmann et al. 2002) recorded an average stay-green score of 2.0 with grain yield 4.55 t ha<sup>-1</sup> (SE± 0.34) . The results of the analysis of variance for eight traits presented in Table 1. The result showed highly significant (p < 0.01) variations among the genotypes for all the traits days to 50% flowering, plant height (cm), number of leaves, number of internodes, stay green, brix content at maturity, fresh juice weight, dry biomass were indicated. The relative amount of variability is the best expressed in term of genotypic coefficient of variation (GCV), since this variable takes into consideration the mean values as well as the unit of measurement. The Analysis of variance revealed low estimate of GCV ranged from 1.13% - 28.70 for all studied except in grain yield which exhibited moderated GCV estimation of 58.04%. Similar trend was observed for phenotypic coefficient of variation that indicated highly significant different (p<0.01) for stay green with the values of 92.04%. Broad sense heritability (*H<sub>b</sub>*) which is an estimate of the total contribution of the genetic variance to the total phenotypic as reported by (Can 1999). Traits that had significant different(p<0.05) are number of leaves, days to 50% flowering, plant height, except brix content at maturity 85.38% and number of internodes 70.47%.

**Table 1:** Mean squares from Analysis of variance for Agro-morphological traits and Stay gtrrn levels of eighteen sorghum genotypes evaluated in Zaria in 2023 wet season

Source	DF	DFF	PHT	NL	NI	STG	BXM	FSW	DSW
Replication	2	12.67	16.67	2.57	0.57	0.43	2.02	9.16	2.25
Genotype	17	171.97**	25740.96**	13.17**	19.48**	0.77**	32.60**	44.73**	16.61**
Error	34	72.96	712.96	1.61	1.69	0.23	8.09	5.3	1.83

\* indicates significant at p<0.05; \*\*indicates significant at p<0.01. DFF= Days 50% Flowering, PHT= Plant height (cm), NL= Number of Leaves, NI= Number of Internodes, STG= Stay green, BXM= Brix degree at maturity (%), FSW= Fresh stalk weight (Kg), DSW=Dry stalk weight (Kg)

**Table 2:** Estimation of Genetic Parameters for Agromorphological Traits screened at Zaria in 2017

Trait	$\sigma_e^2$	$\sigma_g^2$	$\sigma_p^2$	GCV (%)	PCV (%)	<i>H<sub>b</sub></i>
Days 50% flowering	34.00	0.79	34.79	1.13	7.49	2.26
Plant height (cm)	72.96	12.03	84.99	1.26	3.35	14.16
Number of leaves	112.96	2.73	111.69	14.07	9.71	0.38
Number of internodes	1.61	3.84	5.45	16.13	19.22	70.47

Corresponding author: Saidu, G. Mahmood

✉ [briefballagado@gmail.com](mailto:briefballagado@gmail.com)

Department of Agricultural Education, School of Vocational and Technical Education, Adamu Tafawa Balewa C. O. E., Bauchi.

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Stay Green	1.69	1.12	2.81	58.04	92.04	39.77
Brix content at maturity	0.23	1.34	1.57	19.28	20.87	85.38
Fresh stalk weight (Kg)	8.09	2.81	10.90	18.25	35.93	25.80
Dry stalk weight (Kg)	5.30	3.03	8.33	28.70	47.61	36.34

$\sigma_e^2$ =Environmental variance.  $\sigma_g^2$ =Genotypic variance.  $\sigma_p^2$ =Phenotypic variance. GCV=Genotypic coefficient of variation. PCV=Phenotypic coefficient of variation.  $H_b$  =Broad sense heritability

### Mean Performance of the Stay green Genotypes Screened

The mean performance for the eight traits were highly significant influenced ( $p < 0.01$ ) by genotypes (Table 3) Comparing among eighteen genotypes in terms of days to flowering, KSV04, SAMSORG7, IS23525, CSR 01, SAMSORG7, SAMSORG 39, SAMSORG 03 and NRSS0003 were earliest ranged (71 – 74 days) while SAMSORG 41, CSR 02, SAMSORG 45, SAMSORG 46 75-78.78 were medium duration.. The remaining seven genotypes SAMSORG 44, SAMSORG 38, SWDANSADAU, SAMSORG 40, KSV 13, NRSS0005, KSV 12(81.33-94.67) were late duration types. In term of plant height KSV 04, IS23525, CSR 01, SAMSORG7, SAMSORG 39, SAMSORG 03, NRSS0005, are short types while SAMSORG 44, NRSS0005, CSR 02, SAMSORG 39 (411-292.05) are tall plant types. SAMSORG 46, SAMSORG 40, SAMSORG 45, KSV 15, CSR 01,

SAMSORG 41 SWDANSADAU, IS23525, SAMSORG 38, KSV04 ranged 275.61-181 are medium type, Brix content at maturity for SWDANSADAU, NRSS0005, IS23525 were high at maturity ranging 11.27 – 15.23. While SAMSORG 39, SAMSORG 38, KSV 12 and KSV 13 recorded the lowest Brix content ranging from 1.83 – 3.90. Fresh stalk weight ranged 5.87–16.07 SAMSORG 38, CSR02 and for dry fresh weight. CSR02, SAMSORG 39, were ranged 4.37–10.73. Number internodes were ranged from 9.00 –15.67 for KSV04, SAMSORG 44, and stay green ranged 0.8- 3.33 of SAMSORG 41, IS23525 respectively. Based on the International standard of classification of sweet sorghum (9.5 – 15.0 Brix contents), five genotypes; SWDANSADAU, NRSS0005, IS23525, CSR 01 and NRSS0003 were considered as Sweet sorghum with Brix content ranging from 11.87 to 15.23.

**Table 3:** Mean Performance for the Agro-morphological Traits and Stay green of 18 Sorghum Evaluated in 2023 Wet Season in Samaru.

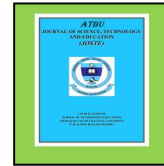
Genotypes	DFF	PHT	NL	NI	STG	BXM%	FSW	DSW
IS23525	71.67	252.67	11.33	11.33	3.33	13.47	11.10	5.23
SWDANSADAU	84.33	258.33	12.00	12.33	2.03	15.23	7.60	4.53
NRSS0005	93.67	409.67	14.33	14.67	1.93	14.07	13.00	6.33
NRSS0003	74.67	316.00	11.67	12.00	3.17	11.87	14.47	7.93
CSR 01	71.67	263.00	10.33	11.00	2.53	11.90	13.47	7.13
CSR 02	75.33	305.33	11.33	11.67	3.30	8.43	16.70	9.27
KSV 04	71.33	196.33	9.33	9.00	1.63	8.63	6.07	2.97
KSV 12	94.67	335.33	12.33	13.33	1.57	2.07	7.33	3.00
KSV 13	85.67	185.33	10.67	11.33	1.50	1.83	6.03	3.33
SAMSORG07	71.67	267.00	15.33	15.67	0.97	5.43	10.43	7.23
SAMSORG 40	84.33	273.33	13.00	12.67	1.63	4.23	6.03	5.30
SAMSORG 44	81.33	311.00	8.00	15.67	0.80	8.33	11.33	10.73
SAMSORG 41	75.00	261.33	10.33	11.67	1.00	6.23	9.57	8.07
SAMSORG 45	76.33	268.33	10.67	11.00	1.10	4.10	6.53	5.00

Corresponding author: Saidu, G. Mahmood

[briefballagado@gmail.com](mailto:briefballagado@gmail.com)

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SSAMSORG 46	77.00	275.33	9.33	10.33	1.63	5.77	5.87	7.57
SAMSORG 38	83.33	209.00	10.33	10.00	1.27	3.37	6.87	4.37
SAMSORG 39	72.67	292.67	15.33	14.67	1.50	3.90	5.87	4.77
SAMSORG 03	73.33	181.00	15.67	10.33	1.93	5.23	7.07	6.23
Means	78.78	275.61	11.74	12.15	1.82	6.01	9.19	6.06
CV (%)	7.65	7.07	6.73	11.68	4.67	4.00	5.22	4.92
SE	12.07	21.80	6.97	1.03	1.03	1.06	0.38	2.31

DF F= Days 50% Heading, PHT= Plant height (cm), NL= Number of Leaves, NI= Number of Internodes, SGY= Grain stay green, BXM= Brix degree at maturity (%), FSW= Fresh stalk weight (Kg), DSWT=Dry stalk weight (Kg)

#### Polymorphism and allelic diversity of SSR markers

The results of analysis of genetic diversity the number of alleles (A), gene diversity (D), heterozygosity (He) and polymorphic information content (PIC) of the SSR makers for the eighteen genotypes is presented in Table 3. The SSR markers generated a total of 71 putative alleles (different fragment sizes) with average of 2.29 alleles per locus among the 18 sorghum genotypes. Most of the markers generated 2 alleles, nevertheless nine markers generated 3 alleles per locus. The heterozygosity values (He) ranged from 0.00 to 0.78 with an average of 0.24. Markers gpsb067, mSbCIR223, mSbCIR246, sb5-206, Xcup11, Xcup14 and Xcup62 had the lowest *He* while gps148 and Xcup63 had the highest *He*. The PIC values of the markers ranged from 0.26 for the marker gpsb089 to 0.57 for sb5-206 with an average of 0.36. The average number of alleles in this study was 2.29 alleles per locus which was similar to 2.48 alleles per locus reported by Deesuth, O.(2016) and Pei *et al.* (2010) but lower than 6.19 reported by Reddy *et al.* (2010). In sorghum genetic diversity studies a greater number of alleles per locus (27) were reported by Smith *et al.* (2011) and 7.6 by Wang *et al.* (2009). Greater number of alleles generated by SSR markers suggests allelic richness, a useful indicator of genetic worthiness for subsequent selection and conservation strategies. The average heterozygosity observed in this study was higher to levels of heterozygosity (0.04) recorded among

Zambian sorghum genotypes (Ng'uni *et al.*, 2011) and lower than reported 0.60 by Deesuth, O.(2016) and Uptmoor *et al.*,(2003) who used 25 SSR markers and compared 46 sorghum accessions of diverse geographical collections. A comparatively high value (0.8) was also reported by Thudi and Fakrudin (2011) among rabi sorghum genotypes. The higher level of heterozygosity (allelic diversity) of SSR loci found in this study was probably associated with the wide range of genetic diversity represented in the germplasm of the sweet exotic sorghum and the local sorghum tested. Increased levels of heterozygosity indicate significantly greater proportion of genetic diversity, which will enhance selection response in breeding programs (Burks, P. S.,2015).

The Euclidean dissimilarity matrix was used to cluster genotypes using the UPGMA algorithm. The resulting dendrogram revealed two major groups A and B with distinct clusters and subclusters (Figure 1). Main group A consisted of clusters 1 and 2, group B consisted of clusters 3 and 4. Cluster 1 is divided into subcluster (a) and (b). Sub clusters a contained lines SAMSORG03 and a subclusters of SAMSORG39 and SWDANSADAU. Subclusters b contained NRSS0003, SAMSORG46, SAMSORG44, SAMSORG40, SAMSORG 7, CSR02 and SAMSORG45 in varying sub-clusters shown in Figure 2. Cluster 2 of Group 1 contained sub cluster c with SAMSORG41 and sub cluster d comprising KSV12 and KSV13. This shows a wide diversity among 18 sorghum genotypes evaluated.

Corresponding author: Saidu, G. Mahmood

✉ [briefballagado@gmail.com](mailto:briefballagado@gmail.com)

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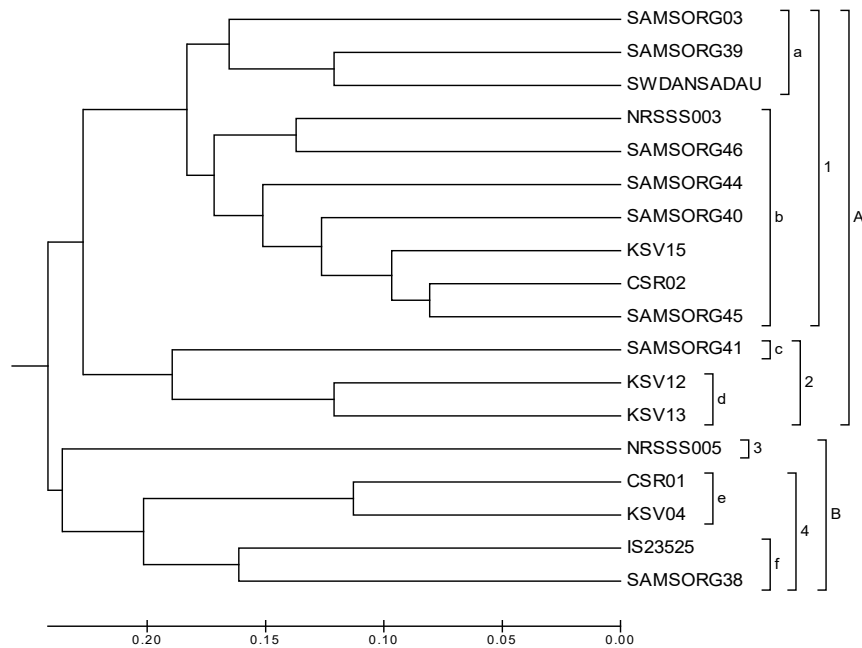


Fig 1. Dendrogram of 18 sorghum genotypes for stay green.

**Table 4:** Polymorphism and Allelic Diversity of the 31 SSR loci used to genotype the 18 sorghum lines

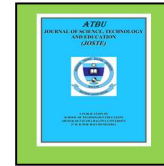
Marker	Major Allele Frequency	Number of Alleles (A)	Gene Diversity (D)	Heterozygosity (He)	Polymorphic Information Content (PIC)
gpsb067	0.56	2.00	0.49	0.00	0.37
gpsb089	0.81	2.00	0.31	0.28	0.26
gpsb123	0.58	3.00	0.53	0.11	0.43
gpsb148	0.61	2.00	0.48	0.78	0.36
gpsb151	0.53	3.00	0.58	0.50	0.50
mSbCIR223	0.78	2.00	0.35	0.00	0.29
mSbCIR238	0.64	3.00	0.50	0.50	0.42
mSbCIR240	0.83	3.00	0.29	0.17	0.27
mSbCIR246	0.78	2.00	0.35	0.00	0.29
mSbCIR248	0.67	2.00	0.44	0.11	0.35
mSbCIR262	0.67	2.00	0.44	0.11	0.35
mSbCIR276	0.61	2.00	0.48	0.11	0.36
mSbCIR283	0.72	2.00	0.40	0.22	0.32
mSbCIR286	0.58	2.00	0.49	0.28	0.37
mSbCIR300	0.64	3.00	0.50	0.06	0.42
mSbCIR329	0.61	2.00	0.48	0.33	0.36
sb4-72	0.69	3.00	0.44	0.33	0.36
sb5-206	0.44	3.00	0.64	0.00	0.57

Corresponding author: Saidu, G. Mahmood

✉ [briefballagado@gmail.com](mailto:briefballagado@gmail.com)

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SbAGB02	0.69	2.00	0.42	0.06	0.33
Xcup02	0.61	3.00	0.50	0.61	0.40
Xcup11	0.56	3.00	0.57	0.00	0.49
Xcup14	0.78	2.00	0.35	0.00	0.29
Xcup53	0.53	2.00	0.50	0.06	0.37
Xcup61	0.67	2.00	0.44	0.44	0.35
Xcup62	0.72	2.00	0.40	0.00	0.32
Xcup63	0.56	2.00	0.49	0.78	0.37
Xisep0107	0.53	2.00	0.50	0.50	0.37
Xisep0310	0.72	2.00	0.40	0.22	0.32
Xtxp10	0.58	2.00	0.49	0.39	0.37
Xtxp12	0.67	2.00	0.44	0.11	0.35
Xtxp15	0.75	2.00	0.38	0.28	0.30
Total	20.21	71.0	7.34	11.28	71.00
Mean	0.65	2.29	0.45	0.24	0.36

Group B is comprised of Cluster 3 having only NRSS05 and Cluster 4 containing sub cluster e having CSR01 and KSV04; and sub cluster f containing IS23525 and SAMSORG38. The groupings, though related, showed considerable levels of genetic diversity. This may suggest that the genotypes are genetically related as some of the released SAMSORG03 varieties were derived from the NRSS05, KSV12 and CSR02 series. However, the genotypes used in the study did not appear in the same cluster based on the brix content status or area of collection. This suggested wide genetic variations among the germplasm. This shows a wide diversity among 18 sorghum genotypes evaluated.

#### CONCLUSION AND RECOMMENDATION

There is wide genetic diversity among all 18 selected genotypes. However, the alternative stay-green genetic sources were found to be distinct based on either all the SSRs employed or using only those associated with the stay-green trait. Therefore, the physiological and biochemical basis of each stay-green source should be evaluated in order to enhance the understanding of the functioning of the trait in the various backgrounds. These genetic sources of stay-green could provide a valuable resource for improving this trait in sorghum breeding programmes. Research into molecular breeding for brix content on sorghum genotypes should be

encourage and pursued vigorously with view to shortening the time for development of improved and acceptable cultivars to farmers.

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Corresponding author: Saidu, G. Mahmood

✉ [briefballagado@gmail.com](mailto:briefballagado@gmail.com)

Department of Agricultural Education, School of Vocational and Technical Education, Adamu Tafawa Balewa C. O. E., Bauchi.

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Corresponding author: Saidu, G. Mahmood

✉ [briefballagado@gmail.com](mailto:briefballagado@gmail.com)

Department of Agricultural Education, School of Vocational and Technical Education, Adamu Tafawa Balewa C. O. E., Bauchi.

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Corresponding author: Saidu, G. Mahmood

✉ [briefballagado@gmail.com](mailto:briefballagado@gmail.com)

Department of Agricultural Education, School of Vocational and Technical Education, Adamu Tafawa Balewa C. O. E., Bauchi.

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