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Screening sorghum local landraces for potential drought tolerance stay-green quantitative trait loci (QTLs) sources in Burkina Faso

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Sorghum serves as a staple food for rural populations in Burkina Faso, but its cultivation faces limitations due to post-flowering drought stress. Consequently, there is a need for improvement to enhance resilience to moisture stress. The current investigation aims to identify drought-tolerant germplasm to enhance local susceptible elite varieties. One hundred and ten accessions, comprising improved and exotic materials, were evaluated under fully irrigated and terminal drought-stress conditions using an alpha lattice design. The accessions underwent PCR screening with 26 SSR markers to assess genetic diversity and identify alleles associated with stay-green traits. Seventeen local materials, including four improved varieties (Sariaso 04, ICSv 1049, Framida, and Sariaso 03), were identified as among the best genotypes with a high Stress Tolerance Index (STI). Except for the control (B35), twenty-nine top accessions, including one local variety (Ouedzoure), were recognized for their high spad value. Allelic diversity data indicated low genetic diversity (0.34) but identified important private alleles linked to stay-green quantitative traits loci (stg1 and stg4). These loci are situated in the genetic background of PSE146, PSE65, PSE69, PSE206, PSE226A and PSE288. Some local varieties have the potential to be utilized for production to mitigate the impact of drought in Burkina Faso and serve as valuable breeding material for developing new drought-tolerant varieties.

Key words: Local accessions, exotic, post-flowering, drought stress, tolerance, stay-green.

INTRODUCTION

Phytogenetic resources play a crucial role in agriculture, medicine, and industry, constituting a valuable asset for adaptation in dynamic environments (Barro-Kondombo, 2010). The evolution and genetic improvement of crop species rely on the useful genetic diversity present in the crop's germplasm (Charrier et al., 1997). Genetic diversity refers to the variation among alleles of genes within individuals of a species, influencing an organism's

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ability to acquire new traits through mutation or recombination. Throughout evolution, cultivated plants acquired traits that enabled them to survive and adapt to new environmental conditions.

According to FAO (2009), the biological foundation of global food security in agriculture depends on plant resources, with the future of world food production relying on domesticated species (CDB, 1996). However, despite over 250,000 available plant species, only about 3% (approximately 7,000) are utilized in agriculture (Vernooy, 2003). The cultivated species, grown for centuries and maintained using good agricultural practices, represent only a fraction of the available plant diversity. The underutilization of additional genetic resources is attributed to a lack of knowledge regarding their potential to contribute to feeding the world's population. While these species may contain undesirable genes, they are valuable primarily as sources of the few desirable genes they possess.

In Africa, especially in sub-Saharan countries, a significant portion of the population resides in rural areas and depends on crop and animal production for subsistence. Unfortunately, agriculture faces numerous constraints that hinder socio-economic development (Assogbadjo et al., 2009). In semi-arid areas, rural populations consistently contend with low and erratic rainfall distribution during cropping seasons, weakening their food security status.

In Burkina Faso, terminal drought is a frequent occurrence, significantly hindering sorghum production by disturbing the physiology of sorghum plants and causing issues such as barrenness, kernel abortion, or shriveled grain, ultimately reducing grain yield (Prasad et al., 2008). The adverse effects of terminal drought can be mitigated either by irrigation or by providing farmers with sorghum varieties capable of withstanding terminal drought. Unfortunately, smallholder farmers often cannot afford irrigation facilities (Derera, 2005). Therefore, breeding for drought tolerance represents the most sustainable alternative to address this major constraint. Developing sorghum varieties with post-flowering drought-tolerant traits should be a breeding priority in Burkina Faso, where terminal drought is prevalent.

In sorghum, stay-green is a well-characterized form of drought tolerance (Rosenow et al, 1983). Subudhi et al. (2000) reported that post-flowering drought adaptation in sorghum is linked to the stay-green phenotype, characterized by the maintenance of green stems and upper leaves under water limitation after flowering. Numerous research efforts have sought molecular markers associated with post-flowering (stay-green) drought resistance loci. Several stay-green QTLs have been mapped, such as those identified by Haussmann et al. (2002) and Sanchez et al. (2002), with molecular markers linked to these QTLs available (Kassahun et al., 2009).

Post-flowering studies have identified four major stay-green QTLs designated as Stg1, Stg2, Stg3, and Stg4, consistently recognized in various environments (Subudhi et al., 2000; Tao et al., 2000) and different genetic backgrounds (Subudhi et al., 2000). The same study also revealed two minor QTLs designated as stgA and stgB. Stg1 and Stg2 were mapped to sorghum chromosome 3, explaining approximately 20 and 30% of the phenotypic variance, respectively (Harris et al., 2007). At these loci, the stay-green alleles originate from BT x 642 (B35), the most common source of stay-green alleles. This information could be utilized to enhance local sorghum varieties for drought tolerance.

After a drought-induced food shortage in the early 1970s, sorghum breeding programs in the country were reinforced to provide farmers with high-yielding varieties, aiming to prevent another food crisis. The primary objectives focused on releasing exotic caudatum varieties, which are more productive than the Guinea race and respond well to intensified management in improved sorghum production systems (Barro-Kondombo, 2010). However, this initiative failed to have a measurable impact due to a lack of interest from stakeholders. Consequently, the introduction of exotic material was discouraged, prompting breeders to search for suitable breeding material within the available local sorghum germplasm to meet farmers' needs.

It has become essential to screen local germplasm and identify lines that may respond to climatic constraints, specifically drought stress, and are accepted by farmers. The probability of finding appropriate material is high due to the genetic diversification of Guinea sorghum. According to Harlan (1975), West Africa is a center of Guinea race diversification, and Burkina Faso may be at the heart of that center (Zongo, 1991). The present study aims to screen local landraces for potential drought-tolerant material and characterize Quantitative Trait Loci (QTLs) for their stay-green ability under drought conditions.

MATERIALS AND METHODS

Germplasm

One hundred and ten accessions were collected in 1999 from three districts located in two agro-ecological zones of the country. Ninety were collected in the West, forty-six in the southwest, and forty in the Eastern part of the country. The accessions were regularly self-pollinated for seed regeneration and conservation. Many accessions were found to be highly photosensitive, and the 50% flowering date of the majority of the collected accessions was too late for them to be grown during the post-rainy season.

Four accessions were obtained from ICRISAT, including two post-flowering drought-tolerant lines (B35, a durra line; ET36-1, a Guinea-caudatum from Ethiopia), and two pre-flowering drought-tolerant lines (RTx7000, RTx7078, from the USA). Additionally, three drought-tolerant lines (Grinkan, Tiandougou, and Tiandougou-coura) were obtained from Mali, and three drought-tolerant mutant lines (SAMURAI1&2 and Pahat) were obtained from Indonesia Details about genotypes are in annex 1.

Morphological characterization

The experiment field trial

Field trials were initiated during the post-rainy seasons (from November 2013 to March 2014) and were repeated from October 2014 to February 2015. Following a preliminary evaluation, certain early maturity accessions were identified and planted three weeks after the planting of late maturity accessions. An alpha lattice design (11*10) in three replicates was employed in two environments: fully irrigated conditions and terminal drought conditions.

In the fully irrigated trial, plants were watered every three days until physiological maturity. In contrast, under drought conditions, the trial was watered until genotypes reached 50% flowering, after which water was withheld for 21 days to induce terminal drought stress. All genotypes were subjected to each water regime to assess their responses across different conditions. The material was planted in single rows with spacing of 4 and 80 cm between plants. Three seeds were planted per hole at 40 cm intervals in each row.

The experiment took place at Valley du Kou (INERA station of Bobo) located at 11°22' N Latitude, 4°22' W Longitude, on ferruginous acid soil with a silty texture, where irrigation facilities are available. Terminal drought stress was achieved in water-stressed regimes by withholding water at 50% flowering for three weeks.

Data collection

Morphological data related to drought were collected from five plants in the middle of each row, including days to 50% flowering (Flo), plant height (PH), chlorophyll content (Spad), panicle size (PS), panicle weight (PWe), grain weight per panicle (GrWP), 100 grains weight (100GrWe), number of grains per panicle (NbGrP), yield under fully irrigated conditions (Yirr), yield under stress conditions (Ystr), leaf greenness (Stg), senescence or wilting (SNT), and grain filling rate (GFR). Additionally, two drought tolerance indexes were calculated: Stress Tolerance Index (STI) and the Geometric Mean Productivity (GMP).

Data analysis

The SAS computer program SAS 9.3 (SAS Institute, Cary, NC, USA) was utilized for data analysis. Analysis of variance was conducted to identify differences within genotypes (accessions), and Pearson correlations were calculated to identify correlations among parameters. Principal Component Analysis was employed to examine the structure of correlations between parameters. The eigenvalues and eigenvectors of the correlation matrix were derived and used to reduce the number of parameters in the statistical analyses (Daulfrey, 1976).

Cluster analyses were performed to group parameters together using the Euclidean distance metric. Data points with smaller distances between them were grouped together, and a dendrogram was plotted from these computed clusters to visualize the graphical relationship among accessions. The drought tolerance index (STI) and Geometric Mean productivity were used to rank the thirty top accessions.

Molecular characterization

Samples collection and DNA extraction

Leaf samples were collected from three-week-old plantlets and dried in a stove at 40°C for three days. After drying, leaf samples were ground using a Geno-grinder (RETSCH) at 500 strokes/min for 9 min (3 times, 3 min each), and then DNA was extracted following a Mixed Alkyl Trimethyl Ammonium Bromide (MATAB) method (Frost et al., 2007). A pre-heated (65°C) 750 µl of MATAB tampon was added to each sample, followed by incubating at 65°C in a shaking water bath for 20 min.

After incubation, 750 µl of chloroform-isoamylalcohol (24:1) was added to each sample and agitated manually 50 times before being centrifuged at 13,000 rpm for 20 min. 600 µl of the aqueous layer was transferred to a new tube of 1500 µl, then 600 µl of cold isopropanol was added, mixed, and kept at -20ºC for two hours. The samples were centrifuged at 13,000 rpm for 20 min, and the supernatant was decanted. Pellets were washed with 500 µl of 70% ethanol and centrifuged at 13,000 rpm for 20 min. The supernatant was decanted, and pellets were air-dried overnight or for 45 min in a stove at 40°C.

Finally, the samples were dissolved in 150 µl of TE (TE1X) and kept at ambient temperature for one night, then stored at -20°C. The working concentration of about 5 ng per µl was obtained from dilution of the initial DNA solution after checking DNA concentrations and quality on 0.8% agarose gels.

Polymerase chain reaction (PCR) and polyacrylamide gel electrophoresis

PCR was conducted in 10 µl reaction volumes containing 25 ng of genomic DNA template, 0.1 mM of dNTPs, 1x buffer, 200 µM of MgCl2, 0.1 µM of both forward and reverse primers, 0.1 µM of IRdye, 0.1 U of ampliTaq polymerase enzyme, and double-distilled water. The PCR was carried out in 35 cycles using a thermal cycler (MWG AG Biotech). The thermal cycler was programmed as follows: a denaturation at 94ºC for 4 min, followed by 10 cycles composed of 45 s of denaturation at 94ºC, annealing at TM-5°C with a reduction of 0.5ºC/cycle for 1 min, and an extension at 72ºC for 1 min 15 s. This was followed by 25 cycles, each consisting of denaturation (94ºC for 45 s), annealing (at TM-5ºC for 1 min), and extension (at 72ºC for 1 min 15 s). Finally, the reaction ended with a 5 min extension at 72ºC.

PCR products were subjected to electrophoresis in 6.5% polyacrylamide gels using a Licor 4300 DNA Analyzer system. Electrophoresis was carried out using 20 ml of 6.5% polyacrylamide (200 ml 10X TBE completed to 1L, 175 µl ammonium per sulfate (APS), and 25 µl TEMED). The gel was run at 1500V and 35mA constant power supply for 2 hours using a Licor DNA analyzer unit. For polymorphism assessment, the bands on the gel were coded as '1,' '2,' '3,' '4,' '5,' and '6' based on their allele position. Missing data were scored as 'x'.

Data analysis

Descriptive parameters for genetic diversity: The software Power Marker version 3.25 was utilized to determine descriptive parameters for genetic diversity, such as the average number of alleles per locus (N), the observed heterozygosity (Ho), and expected heterozygosity (He) across loci and populations (Belhkir et al., 2004). The software GenAlex version 6.41 (Peakall and Smouse, 2006) was employed to determine the number of different alleles (Na), loci with private (rare) alleles, and the number of common alleles. The same software was also used to assess the structure of genetic diversity through two fixation indexes (Fst and Fis) based on the heterozygosity rate defined by Wright (1965). Fst measures the genetic differentiation among sub-populations, while Fis measures the heterozygosity deficit in a sub-population, indicating intra-population genetic differentiation.

Table 1. F value for various traits recorded in the combined ANOVA.

Source	DF	Flo	Spad	Stg	PH	PS	GFR	GrWP	Nbgrpa	Yield
RH	1	3.37^{ns}	67.75***	139***	0.51^{ns}	189.63***	1315.49***	2889.99***	2997.75***	2894.73***
Geno	109	11.26***	4.10**	9.06**	68.77***	26.22**	20.35***	49.23***	57.07***	49.20***
RH*Geno	109	7.26***	4.471**	8.96**	6.14**	5.69**	10.74***	38.46***	39.25***	38.27**

***P<0.001; **P<0.01; *P<0.05; Flo= 50% flowering date; Spad=chlorophyll content value; PH= Plant height; GFR=grain filling rate; GrWP=grain weight/panicle; Nbgrpa=number of grain/panicles; Yield= grain yield.

Table 2. Pearson correlation coefficients, N = 110.

Correlation	Flo	Spad	Stg	PH	SNF	PS	GrFR	GrWP	Nbgrp	Ystr	STI
Spad	0.28*										
Stg	0.18^{ns}	0.479***									
PH	0.65***	0.44***	0.23*								
SNF	0.31**	0.36***	0.48***	0.22*							
PS	0.49**	0.45***	0.24*	0.66***	0.36***						
GrFR	-0.01^{ns}	-0.09^{ns}	0.20*	-0.15^{ns}	0.16^{ns}	-0.22***					
GrWP	0.045^{ns}	0.12^{ns}	0.31**	0.14^{ns}	-0.24***	-0.26***	0.59***				
Nbgrp	0.05^{ns}	0.11^{ns}	0.29**	0.11^{ns}	0.02^{ns}	0.18^{ns}	0.59***	0.95***			
Ystr	0.04^{ns}	0.12^{ns}	0.31**	0.14^{ns}	-0.25*	-0.26***	0.59***	0.99***	0.96***		
STI	-0.25**	-0.10^{ns}	0.13^{ns}	-0.27**	0.09^{ns}	-0.6***	0.46***	0.70***	0.67***	0.70***	
GMP	-0.29**	-0.12^{ns}	0.10^{ns}	-0.28**	0.05^{ns}	-0.11***	0.54***	0.73***	0.69***	0.73***	0.95***

*:Significant difference at 5%; **: highly significative difference at 5%; ***: very highly significative at 5%; ns: non significant.

RESULTS

Morphological characterization for drought trait, yield and yield components

Analysis of variance

Differences within genotypes were highly significant (P < 0.001) for all the morphological traits evaluated (Table 1). The environmental effect (different moisture stress) identified large differences for the same genotypes (P < 0.001) for chlorophyll value (Spad), panicle height, rate of grain filling, panicle weight, number of grain/panicle, and yield. The environmental effect did not affect the 50% flowering date and plant height. The genotype x water regime interaction was significant for all parameters (Table 1).

Pearson correlation

The chlorophyll content (Spad) was not positively correlated with the grain filling rate, yield, and its components (panicle size, grain weight/panicle, and number of grain/panicle) (Table 2). However, it was positively correlated with panicle size, leaf senescence, and plant height. The visual scoring of stay-green was positively and significantly correlated with all parameters except the stress tolerance index and the geometric mean productivity. Plant height was positively correlated with leaf senescence and panicle size but was not correlated with grain weight per panicle, grain number, and yield. In contrast, plant weight was negatively correlated with grain filling rate, STI, and GMP (Table 2).

The panicle size was negatively and significantly (P < 0.001) correlated with grain weight per panicle, yield under stress (Ystr), and grain filling rate, STI, and GMP (Table 2). The grain filling rate was highly positively correlated (P < 0.001) with the yield under stress and its components. The yield under stress was highly correlated with its components and STI and GMP.

Determination of principal variable through a Principal Components Analysis (PCA)

From the correlation analysis, the results revealed that phenotypic traits (Flo, Spad, stg, PH, SNF, GrFR) were mostly redundant, and the yield components (PWe, GrWP, Nbgrpa, Ystr, STI, GMP) also exhibited redundancy within them.

Principal components analysis was performed to determine the most important variables for the final discrimination of genotypes under terminal drought

Table 3. Eigen vectors used to determine principal variable form analysis of principal components.

Correlation	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
Flo	0.010	0.432	0.005	0.525	-0.163	0.562	-0.272	-0.311
Spad	0.054	0.366	0.267	-0.467	0.696	0.279	-0.028	-0.101
PH	0.035	0.493	-0.290	0.166	0.081	-0.001	0.075	0.783
SNF	0.127	0.268	0.791	-0.101	-0.427	-0.179	-0.121	0.178
PS	0.095	0.462	-0.238	-0.248	-0.315	-0.089	0.613	-0.383
GrFR	0.275	-0.148	0.348	0.548	0.325	-0.018	0.601	0.004
PWe	0.383	0.138	-0.092	-0.089	-0.031	-0.254	-0.096	0.021
GrWP	0.417	0.029	-0.099	0.043	0.048	-0.170	-0.134	-0.083
Nbgrpa	0.403	0.011	-0.105	0.099	0.130	-0.147	-0.317	-0.161
Ystr	0.417	0.031	-0.099	0.040	0.052	-0.172	-0.133	-0.084
STI	0.341	-0.221	-0.020	-0.234	-0.236	0.506	0.068	0.139
GMP	0.350	-0.243	-0.032	-0.173	-0.132	0.407	0.131	0.207
Eigen value	5.49	3.055	0.87	0.85	0.60	0.42	0.25	0.23
Variance (%)	45.76	25.46	7.27	7.12	5	3.52	2.10	1.99
Cumulative	45.76	71.23	78.5	85.5	90.62	94.14	96.24	98.23

conditions. The PC1 accounted for 45.76% of the variation, with yield, its components (Ystr, PWe, GrWP, and Nbgrpa), STI, and GMP as the major variables explaining variation among genotypes. The PC2 accounted for 25.46% variation and was associated with flo, Spad, PH, SNF, and PS. The third PC3 accounted for 7.27% of the variation and was associated with traits such as leaf senescence (SNF) and grain filling rate (Table 3).

Cluster analysis

From the principal components analysis, the variables associated with PC1 (yield, yield components, stress tolerance index, and geometric mean productivity) were used for hierarchical cluster analysis. The dendrogram was constructed to group accessions according to their root mean square distance (Figure 1). The minimum root mean square distance (0.038) was recorded between PSE58 and Sariaso09, and the maximum (3.55) was recorded between Grinkan and B35. The accessions were grouped into three major clusters.

Cluster I could be considered as an outlier as it contained only one accession (Grinkan, a drought-tolerant check). Cluster II was composed of three sub-clusters. The first sub-cluster contained one drought-tolerant check (Tiandougou-coura), one local variety (Ouedzoure), and 12 landraces. The second sub-cluster contained 45 accessions within which were six local varieties (Sariaso04, Sariaso07, Sariaso5, V1, V2, and Kapelga), three introduced materials (ICSV1049, Framida, SAMURAI: drought-tolerant check), and 36 landraces. The third sub-cluster contained 6 landraces and one improved variety (RTx7000). Cluster III comprised three sub-clusters. The first sub-cluster contained 13 landraces, the second 11 landraces and 3 improved varieties (Sariaso04, Sariaso09, and RTx7078). The third sub-cluster contained 19 accessions composed of 15 landraces and 3 local improved varieties and B35.

The accessions were classified according to their yield and yield components under terminal drought conditions. They were ranked from higher yielding to lower yielding. Accessions in cluster I exhibited high yielding capability, accessions in cluster II showed moderate yielding potential, whereas accessions in cluster III had low yielding potential under drought conditions. The classification was done according to the difference in the root mean square of accessions (Figure 1).

Performance of 30 top accessions according to their stress tolerance index

From the cluster analysis, accessions were ranked from higher to lower yield and yield components values. The highest root mean square was found for Grinkan, and the lowest value was found with B35, indicating that Grinkan was the highest yielding variety while B35 was the lowest. The 30 top accessions were selected based on their stress tolerance index, geometric mean productivity, and Spad value. Grinkan was the top accession with higher values of STI and GMP (4.4). Six landraces (Diankogo, S34, PSE184, CVS860, CVS427, and RSOEK71) and some drought-tolerant checks, Tiandougou, SAMURAI2, Tiandougou-coura, showed moderate values of STI and GMP, which were higher than that of B35. They rank among the fifteen top accessions. For the Spad value, B35 showed the highest chlorophyll content (55.7) in leaves two weeks after

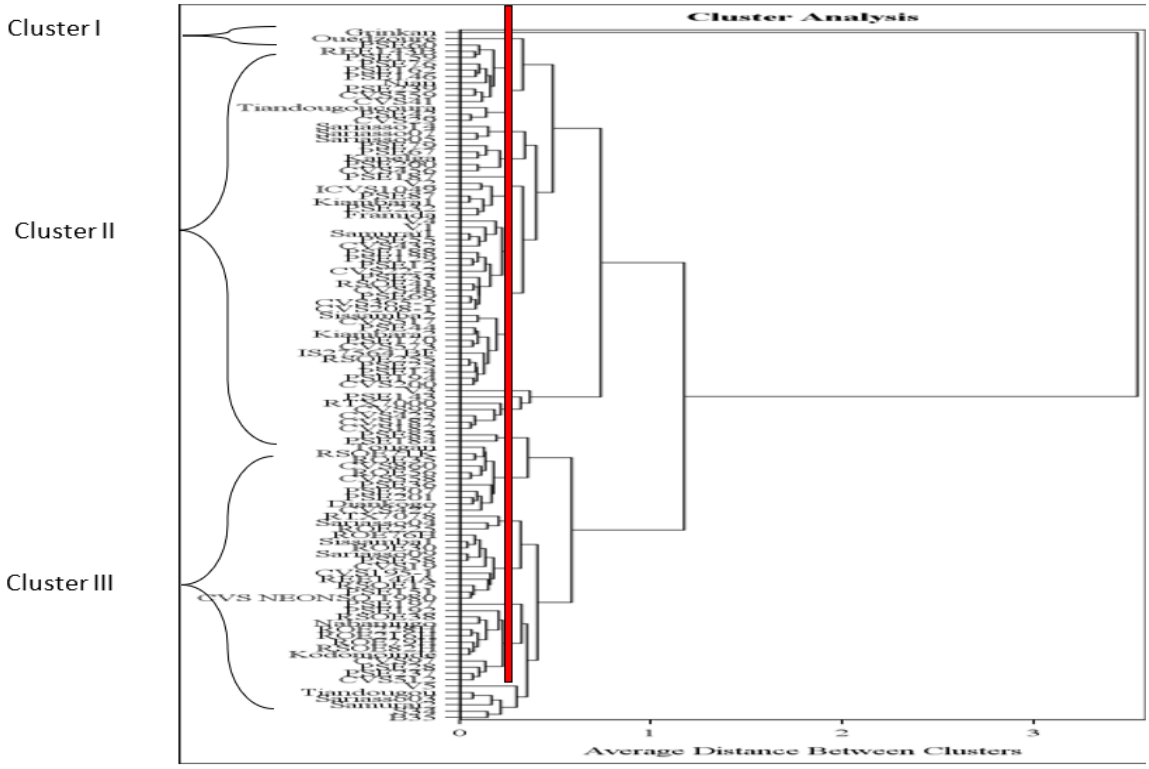

Figure 1. Dendrogram of the 110 sorghum accessions revealed by cluster analysis based on Root Mean Square (RMS) distance of their STI value.

flowering under terminal drought conditions. Grinkan was not among the 30 top accessions for this trait. The 29 accessions with Spad values ranking below B35 were all local landraces (Table 4).

Molecular characterization of accessions using SSRs markers

Twenty-six SSR markers were used in this study. The number of alleles ranged from 2 to 6, with an average of 3.5 alleles per locus (Table 5). A total of 93 alleles were identified. The unbiased expected heterozygosity ranged from 0.061 (Msbcir 225) to 0.613 (Gpsb032), with a mean of 0.367. The gene diversity (expected heterozygosity) ranged from 0.021 (Msbcir 225) to 0.553 (Gpsb032). The lower genetic differentiation was shown at Msbcir 225, with an Fst value of 0.026, and the higher distance was revealed by Xtxp225, with an Fst value of 0.601 (Table 5).

Allelic diversity patterns across the population

The allelic frequency was very low (<5%) and ranged from 2.23% (exotic accessions) to 2.69% (local accessions of Burkina). The rate of the number of common alleles was low in exotic material (AF=23.1%) and relatively high in accessions of local populations (from 69.2 to 80.8%). Twenty-two private alleles were observed in local accessions. Five private allelic forms were at locus Xtxp15. Seventeen private alleles were also observed in accessions, among which, five at the Gpsb014 locus, 6 at Xtxp123 locus, and 6 at Xtxp285 locus. In the background of improved varieties or exotic material such as B35, no private allele was observed. Within local accession PSE146, a private allele was observed at locus Xtxp15, which is responsible for stay-green 4. Within local accessions PSE65, PSE69, PSE206, PSE226A, and PSE288, some private alleles were found at locus Xtxp123, which is also responsible for the expression of stay-green 4.

Within local accession PSE234, a private allele at locus Xtxp285, which is responsible for the expression of stay-green 1. Another undetermined private allele was observed at locus Gpsb014 within accessions PSE235. Figure 2 shows an output of alleles as revealed by the marker Gpsb014.

DISCUSSION

Genetic improvement of species relies on the useful variability existing in available genetic resources (Charrier

Table 4. Performance of the 30 top genotypes according to their Spad value, STI and GMP.

Rank	Geno	STI	GMP	Rank	Geno	Spad
1	Grinkan	4.8	4.0	1	B35	55.7
2	Diankogo	3.7	3.5	2	Ouedzouré	55.3
3	S34	2.3	2.8	3	ROE225	53.4
4	Tiandougou	2.1	2.6	4	PSE143	51.1
5	Samuraï2	1.8	2.5	5	RSOE15	50.0
6	PSE187	1.6	2.3	6	PSE36	49.9
7	CVS860	1.6	2.3	7	CVS456	49.2
8	Tiandougou-coura	1.5	2.2	8	PSE60	48.3
9	CVS427	1.4	2.2	9	CVS22-2	48.3
10	V5	1.3	2.1	10	IS27564 BF	48.1
11	Sariasso04	1.2	2.0	11	PSE33	47.8
12	RSOE71K	1.2	2.0	12	CVS559	47.5
13	B35	1.2	2.0	13	CVS29	47.2
14	ROE79H	1.1	1.9	14	PSE159	47.2
15	PSE36	1.1	1.9	15	PSE197	47.1
16	REE143B	1.1	1.9	16	ROE76H	47.0
17	V2	1.0	1.9	17	ROE216H	46.1
18	ICVS1049	1.0	1.9	18	CVS432	46.0
19	ROE56	1.0	1.9	19	CVS558	45.5
20	ROE76H	1.0	1.9	20	PSE44	45.1
21	PSE232	1.0	1.8	21	PSE194	45.1
22	Framida	0.9	1.8	22	CVS208-1	44.9
23	PSE28	0.9	1.8	23	RSOE82H	44.9
24	CVS512	0.9	1.8	24	PSE188	44.7
25	PSE42	0.9	1.8	25	REE144A	44.4
26	CVS558	0.9	1.8	26	PSE58	44.4
27	RSOE15	0.9	1.7	27	RSOE38	44.3
28	Sariasso03	0.9	1.7	28	PSE201	44.3
29	RTx7078	0.9	1.7	29	ROE79H	44.2
30	Kiambara2	0.8	1.7	30	PSE76	44.2
	Means	1.4	2.1		Means	47.4

et al., 1997). In this study, the focus was on the genetic variation of agro-morphological traits related to drought, yield, and yield components to identify materials with high yielding capability under terminal drought conditions. Some accessions exhibited a significant difference for all agro-morphological traits. Zongo (1991) and Barro-Kondombo et al. (2008), using agro-morphological parameters of grain sorghum, reported that local sorghum accessions were highly diversified. Nebié et al. (2012) and Sawadogo et al. (2014) reported the existence of high variation among morphological characters that pointed out great variability among local accessions of Burkina Faso. The differences within materials showed that some of the accessions performed well under drought stress conditions, whereas some did not. This indicated a high probability of finding drought-tolerant genotypes within local accessions. The lack of correlation between chlorophyll content and grain filling rate, yield, and its components may be due to the fact that most of the local accessions did not have functional stay-green genes, such as some checks (B35 and ET36^{-1}). The positive correlation of visual scoring of the stay-green with the majority of the traits confirmed that greenness exhibited by local accessions did not have a positive impact on yield under stress conditions. This greenness was probably due to types C and D stay-green, which, according to Thomas and Smart (1993), are nonfunctional forms of stay-green, and plants of these types appear green but lack photosynthetic competence.

There was no correlation between plant height, grain weight per panicle, grain number, and the yield, and plant height was negatively correlated with the STI and GMP. Mutava et al. (2011) reported a negative correlation between plant height and grain weight, grain numbers, and yield in their investigation of sorghum genotypes for

Table 5. Descriptive parameters for genetic diversity.

Marker	Status	N	*Na*	*I*	*Ho*	*He*	*Fst*
Msbcir238	Stg	4	2.333	0.485	0.000	0.284	0.337
Gpsb014	NStg	5	3.000	0.722	0.002	0.401	0.242
Gpsb079	NStg	3	2.333	0.591	0.000	0.362	0.394
Gpsb098	NStg	4	3.000	0.790	0.006	0.459	0.181
Gpsb123	NStg	4	2.833	0.696	0.007	0.427	0.385
Gpsb133	NStg	2	1.500	0.239	0.000	0.165	0.327
Gpsb136	NStg	3	2.333	0.616	0.023	0.401	0.332
Gpsb158	NStg	3	2.167	0.478	0.004	0.286	0.456
Msbcir188	NStg	3	2.500	0.623	0.002	0.368	0.390
Msbcir222	Stg	3	1.833	0.152	0.000	0.071	0.034
Msbcir243	NStg	3	2.333	0.413	0.006	0.246	0.190
Sbagb02	NStg	3	2.000	0.460	0.000	0.313	0.264
Xcup11	NStg	2	2.000	0.510	0.000	0.339	0.243
Xcup43	NStg	3	2.333	0.590	0.010	0.365	0.331
Xtxp15	Stg	5	2.833	0.492	0.004	0.274	0.168
Xtx123	Stg	6	3.833	0.992	0.059	0.553	0.141
Gpsb032	Stg	3	2.667	0.873	0.016	0.553	0.056
Xtxp03	Stg	3	2.333	0.635	0.004	0.407	0.280
Msbcir225	Stg	4	2.333	0.145	0.017	0.060	0.026
Msbcir314	Stg	3	2.333	0.622	0.000	0.405	0.229
Sb5-236	Stg	4	2.667	0.500	0.005	0.279	0.092
Xtxp55	Stg	4	2.833	0.712	0.011	0.428	0.102
Xtxp72	Stg	3	2.000	0.448	0.004	0.278	0.328
Xtxp123	Stg	4	3.167	0.824	0.061	0.478	0.305
Xtxp225	Stg	3	2.167	0.285	0.028	0.157	0.601
Xtxp285	Stg	6	3.667	1.010	0.000	0.513	0.325
Mean		3.5	2.513	0.573	0.01	0.341	0.260
SE			0.095	0.01	0.003	0.02	0.027

traits related to drought tolerance. There was also a negative correlation between panicle size and grain filling rate, yield under stress conditions, STI, and GMP. This indicates that panicle sizes were more important than weak grain filling rate, low STI, and GMP indexes. Prasad et al. (2008) found that moisture stress from flowering to seed-set decreased percent seed-set and seed dry weight. The strong correlation between grain filling rates, yield under stress, grain numbers, STI, and GMP indicates that improvement can be made using these accessions. Craufurd and Peacock (1993) reported that sorghum grain yield was highly and positively correlated with seed numbers, and treatment effects affected during booting and flowering.

The principal components identified the main variables responsible for discrimination among genotypes. PC1 explained most of the variation observed in the yield (Ystr), yield components (Nbgrpa, GrWP, and PWe), and the indexes (STI and GMP) associated with yield, whereas PC2 was linked to morphological traits related to drought (PS, PH, Spad, and Flo). The PC3 was also important and was correlated with two morphological parameters (GrFR and SNF) related to drought stress. The remaining components explained less than 7% each of the variation observed among accessions. This indicates that major variables allowing differentiation among genotypes are associated with PC1, and variables associated with PC1, GrWP, and Ystr also contributed (0.417) more to the variation. Nbgrpa, PWe, GMP, and STI contributed, respectively, as follows (0.403, 0.383, 0.350, and 0.341) to the variation exhibited by PC1.

PC2 and PC3 explain the second-level variation among genotypes and are specifically correlated with morphological traits. The plant height, panicle size, 50% flowering date, and Spad value contributed, respectively (0.493, 0.462, 0.432, and 0.366), to the variation exhibited by PC2. Leaf senescence contributed highly to the variation revealed by PC3, while the grain filling rate contributed moderately. The analysis showed that yield and its components are more important sources of variation among genotypes than morphological parameters related to drought. The results suggest that

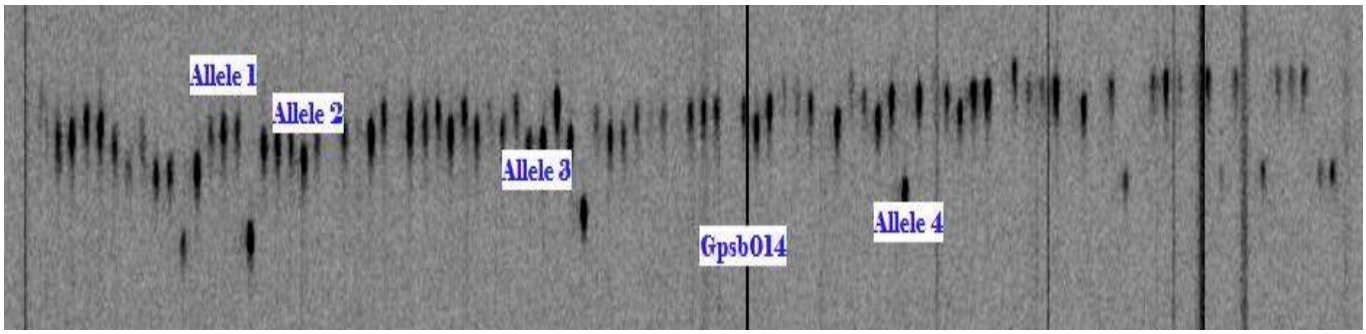

Figure 2. Picture illustrating the allelic diversity patern as revealed by the gel at the locus Gpsb014.

focus should be directed toward yield and its components while selecting directly for drought tolerance under moisture stress conditions. Ibrahim et al., 2014, found that grain yield exhibited a strong and positive phenotypic and genotypic correlation with its components. Vaisi and Golparvar (2013), in their investigation to improve grain yield and seed weight in oat, found that the selection of panicle number and grain number per panicle was relevant. However, morphological parameters such as chlorophyll content, leaf senescence, panicle size, and grain-filling rate could play important roles indirectly by allowing accessions to withstand moisture stress and somehow lessen yield loss. Tolk et al. (2012) found that during terminal moisture stress, panicle growth rate was linearly related to seed number for senescent and stay-green (non-senescent) hybrids, but they concluded that stay-green hybrids produced 10% more seed than senescent hybrids. They concluded that stay-green hybrids-maintained yield by retaining greater seed numbers.

The principal components analysis resulted in a hierarchical classification using variables associated with the major components (PC1). The accessions were grouped into different clusters based on the similarity index described by Vilain (1999) and Fernandez (1992). High-yielding and drought-tolerant accessions were grouped in cluster I. In cluster II, high-yielding and susceptible to drought accessions were grouped. In Cluster III, low-yielding and drought-tolerant accessions were grouped.

Cluster I contains only the drought-tolerant material (Grinkan) that performed well under both stress and non-stress conditions, exhibiting the highest STI and GMP values. Cluster II is primarily composed of local varieties (Sariaso04, Sariaso07, Sariaso5, V1, V2, Ouedzoure, and Kapelga) with high-yielding potential in research stations but susceptibility to terminal drought. Within the exotic material, some post-flowering drought-susceptible genotypes (RTx7000, SAMURAI1) were found in Cluster II, demonstrating that accessions in this cluster are susceptible to terminal drought.

Cluster III included 46 accessions, including six local improved varieties, one pre-flowering drought-tolerant line (RTx7078), and one post-flowering drought-tolerant line (B35). B35 is the most common source of stay-green used in breeding programs and is recognized as a low-yielding material. B35's presence in this group illustrates the low-yielding character of these accessions and their potential to withstand terminal drought. Sariaso04 and Sariaso09 are local high-yielding improved varieties, but they were found in the low-yielding group in this study. Although these materials did not yield well in this study, they are normally high-yielding and drought-susceptible. RTx7078 is known to be a pre-flowering drought-tolerant line. According to Hernandez (1992) and Vilain (1999), these materials should be classified within Cluster II accessions.

The ranking of the thirty top sorghum accessions was done according to the stress tolerance index, geometric mean productivity, and Spad value. Grinkan had the highest values of STI and GMP. Some local landraces (Diankogo, S34, PSE184, CVS860, CVS427, V5, and RSOEK71) also exhibited high STI and GMP. Except for Grinkan, which performed well under both conditions, the remaining accessions were grouped in Cluster III. This indicates that accessions with high STI and GMP were less productive but yielded more than B35. Most accessions with high STI and GMP were ranked below B35 for stress tolerance index and were classified into Cluster II, indicating that they yielded better under non-stress conditions than under moisture stress conditions. This suggests that high yield potential is not necessarily linked to drought tolerance ability. Mardeh et al. (2006) and Talebi et al. (2009) found similar results working on durum wheat. Belko et al. (2014) reported similar findings working on cowpea.

For the Spad value, B35 exhibited the highest chlorophyll content level (55.7) in leaves two weeks after flowering under terminal drought conditions. Ouedzoure, a local improved variety, as well as four landraces (CVS 558, PSE197, REE144, and RSOE38), displayed a high Spad value and yielded more under moisture stress conditions than non-stress conditions. The greenness exhibited by B35 was due to the expression of functional

stay-green genes. However, these landraces did not display premature leaf senescence and yielded more under stress conditions. This may be due to the impact of greenness on the yield under moisture stress conditions of these genotypes.

Borrell et al. (1999) found that, during grain filling, the decrease in the rate of leaf senescence from 3 to 1% leaf area per day resulted in doubling of grain size from about 15 to 30 mg. They suggested that sorghum grain size was correlated with the relative rate of leaf senescence; therefore, the stay-green trait has the potential to increase sorghum grain yield by improving both grain number and grain filling ability. Rosenow (1984), Henzell et al. (1992) and Tuinstra et al. (1998), reported a positive correlation between stay-green and grain yield. Kebede et al. (2001) reported that stay-green lines maintained photosynthetic capacity during grain filling, with the upper canopy leaves remaining actively photosynthetic even after physiological grain maturity. Rosenow et al. (1983), McBee (1984), Wolfe et al. (1988), and Borrell and Hammer (2000) found that sorghum hybrids containing the stay-green trait keep more photosynthetically active leaves than hybrids that do not contain it. These local varieties may need further investigation to determine whether they have functional stay-green genes or not.

For stay-green QTLs, the total allele number was 93, and the average allele per locus was 3.5, with allele numbers ranging from 2 to 6. Twenty-two private allelic forms were revealed by four markers. Two markers (Xtxp123 and Xtxp285) displayed six allelic forms, and two other markers (Gpsb014 and Xtxp15) exhibited five allelic forms. The importance of these private alleles included their presence at loci (Xtxp15, Xtxp023, Xtxp123, and Xtxp285), linking them to stay-green QTLs. According to Bhattramkki et al. (2000), markers Xtxp15, Xtxp023, and Xtxp123 are linked to stay-green 4, while marker Xtxp285 is linked to stay-green 1. This implies that other sources of stay-green exist within local material, and consequently, additional post-flowering drought-tolerant material may be available. Besides the common and known sources of stay-green genotypes such as BTx642 (B35), ET36-1, M35, SC56, and K19, other sources may be available within local Guinea lines. Gpsb123, Sbagb02, and Xcup11 exhibited four, four, and two allelic forms. Barro-Kondombo et al. (2010) found almost the same number of alleles per locus using Gpsb123 (4 alleles), Xcup11 (2 alleles), Sbagb02 (4 alleles), but Xtxp15 (6 alleles) exhibited one allele more. This study identified additional allelic forms at Sbagb02 and Xtxp15 and fewer at Gpsb123 than reported by Sawadogo (2015) on grain sweet sorghum. However, Nebie (2014) found in stem sweet sorghum an important number of allelic forms at Sbagb02 (20 alleles) with an average of 6.79 alleles per locus. Billot et al. (2013) found 19.2 alleles per locus with 39 allelic forms at Sbagb02.

The gene diversity (He) mean value was 0.34. Msbcir225 had the lowest gene diversity (He=0.021), while Gpsb032 had the highest gene diversity (He=0.553) within accessions. The genetic diversity values (2<N<6; Nm=3.5; Nt=93; P=86.54.64%; He=0.34) were relatively low in this study compared to previous studies. The gene diversity was close to that (He=0.37) reported by Barro-Kodombo et al. (2008) on grain sorghum and below the value (0.47 and 0.61) reported, respectively, by Nebié (2014) and Sawadgo (2015) on sweet stem and sweet grain sorghum. Ghebru et al. (2002), Deu et al. (2006), Barnaud et al. (2007), Sagnard et al. (2011) and Billot et al. (2013) found the following gene diversity: 0.77, 0.61, 0.57, and 0.674, working on grain sorghum accessions from Eritrea, Niger, Cameroon, Mali, and accessions representing the world collection.

Conclusion

Local sorghum accessions exhibited significant differences in agro-morphological traits related to drought tolerance. The days to 50% flowering (Flo), plant height (PH), panicle weight (PWe), grain weight/panicle (GrWP), and the number of grains per panicle (NbGrP) were morphological traits that discriminated genotypes. Traits such as yield under stress conditions (Ystr), stress tolerance index (STI), leaf senescence (SNF), Spad value (Spad), and Geometric Mean Productivity (GMP) were identified as indicators of drought tolerance. Additionally, morphological parameters like chlorophyll content, leaf senescence, panicle size, and grain filling played indirect roles in increasing the yield potential under moisture stress conditions. Notably, high-yielding and drought-tolerant genotypes (e.g., Grinkan) as well as high-yielding and drought-susceptible materials (Sariaso04, Sariaso07, Sariaso5, V1, V2, Ouedzoure, Kapelga, RTx7000, and SAMURAI1) were identified. Low-yielding and drought-tolerant material (B35) was also observed. Genotypes (Grinkan, Diankogo, S34, PSE184, CVS860, CVS427, V5, and RSOEK71) with high STI indexes were identified, suggesting their potential use by farmers for optimal production in drought-prone areas and their utility in breeding programs.

SSR markers revealed genetic diversity among local landraces, exotic materials, and within accessions. An important finding was the presence of private alleles in some local accession genomes, particularly in regions associated with stay-green traits. These alleles may play a crucial role in drought tolerance. The observed homozygosity level in local accessions indicates their stability, presenting opportunities for breeding purposes.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ABBREVIATIONS

ICRISAT, International Crops Research Institute for Semi-Arid Tropics; **INERA,** Institute of Environment and Agricultural Research; **FAO,** Food and Agricultural Organization; **QTL,** Quantitative Trait Loci; **PSE,** prospection sorghum ecotype; **STI,** stress tolerance index; **USA,** United States of America.

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Annexe 1. List of accessions used in the field evaluation.

N°	Accessions	Origin	N°	Accessions	Origin	N°	Accessions	Origin
01	B35	Ethi	38	PSE129	BF	75	ROE228H	BF
02	CVS NEONSO1980 BF		39	PSE14	BF	76	ROE30	BF
03	CVS182	BF	40	PSE143	BF	77	ROE35	BF
04	CVS187	BF	41	PSE146	BF	78	ROE56	BF
05	CVS19	BF	42	PSE151	BF	79	ROE76H	BF
06	CVS195-1	BF	43	PSE159	BF	80	ROE79H	BF
07	CVS200	BF	44	PSE162	BF	81	RSOE07	BF
08	CVS208-1	BF	45	PSE170	BF	82	RSOE15	BF
09	CVS22-2	BF	46	PSE187	BF	83	RSOE255	BF
10	CVS29	BF	47	PSE188	BF	84	RSOE38	BF
11	CVS365-2	BF	48	PSE192	BF	85	RSOE41	BF
12	CVS41	BF	49	PSE194	BF	86	RSOE71K	BF
13	CVS423	BF	50	PSE197	BF	87	RTX7000	USA
14	CVS427	BF	51	PSE200	BF	88	RTX7078	USA
15	CVS432	BF	52	PSE201	BF	89	S34	BF
16	CVS456	BF	53	PSE207	BF	90	Samouraï1	INDO
17	CVS48	BF	54	PSE232	BF	91	Samouraï2	INDO
18	CVS512	BF	55	PSE237	BF	92	Sariaso03	BF
19	CVS517	BF	56	PSE239	BF	93	Sariaso04	BF
20	CVS558	BF	57	PSE25	BF	94	Sariaso05	BF
21	CVS559	BF	58	PSE28	BF	95	Sariaso07	BF
22	CVS573	BF	59	PSE33	BF	96	Sariaso09	BF
23	CVS860	BF	60	PSE36	BF	97	Sariaso14	BF
24	CVS95	BF	61	PSE42	BF	98	Sepon82	ICRISAT
25	CVS97	BF	62	PSE44	BF	99	Sissamba1	BF
26	Diankogo	BF	63	PSE45	BF	100	Sissamba2	BF
27	Framida	ICRISAT	64	PSE55	BF	101	Tiandougou	Mali
28	Grinkan	Mali	65	PSE58	BF	102	Tiandougoucoura	Mali
29	ICSV1049	BF	66	PSE60	BF	103	Tougan	BF
30	IS27564 BF	BF	67	PSE67	BF	104	V1	BF
31	Kapelga	BF	68	PSE69	BF	105	V2	BF
32	Kiambara1	BF	69	PSE76	BF	106	V3	BF
33	Kiambara2	BF	70	PSE79	BF	107	V4	BF
34	kodomoindé	BF	71	PSE87	BF	108	V5	BF
35	Nabaningo	BF	72	REE143B	BF	109	PSE12	BF
36	Nian	BF	73	REE144A	BF	110	ROE225	BF
37	Ouedzouré	BF	74	ROE216H	BF			