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Evaluation and Association Mapping for Drought Tolerance in Sorghum [*Sorghum Bicolor* (L.) Moench]

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I. Introduction

Globally, sorghum is the fifth most important grain crop on the basis of production and grown across about 99 countries in the world. In Ethiopia 3.9 million tons with an average yield of 1.8 tons/ha is produced and it is one of the major food cereals after maize, teff and wheat [5]. More than 300 million people in developing countries consume sorghum as their principal food source especially for food-insecure peoples who are mainly living in the semiarid tropical regions [3].

Drought is one of the major limiting factors for yield stability in the semi-arid tropics, where rainfall is inadequate, non-uniform and erratic in distribution [6]. Worldwide, the yield loss each year due to drought was estimated to be around USD 10 billion [14]. Marker-assisted selection which involves the use of DNA markers is the most effective tool in a crop improvement through introgression of desirable genes of interest [17]. There is wide genetic variation for physiological and yield traits associated with tolerance to limited moisture stress within sorghum genotypes and these traits can be used for identifying drought tolerant genotypes of sorghum [13]. In plants, there are two approaches to identify genomic regions influencing expression of quantitative traits. The most common approach is to identify QTL in a segregating population through QTL mapping [18]. Alternatively, a relatively new approach being applied is association mapping, which is based on diverse populations being used to identify associations between allele frequencies and phenotypic variation [6]. Using 98 sorghum SSR markers and 107 accessions reported a total of 14 common SSR markers were associated with days to heading, days to flowering, culm length, number of tiller, number of panicle and panicle length [18].

Recently, [21] reported five markers associated with maturity date and plant height were identified on chromosomes 6, 9, and 10 using 242 sorghum accessions with 39 SSR markers which were evaluated at five environments. [25] reported two markers consistently associated with plant height at two environments using association mapping. In Ethiopia tremendous amount of variability exists in sorghum and a large number of accessions have been collected by the Institute of Biodiversity Conservation of Ethiopia [23].

II. Materials and Method

The field experiments were conducted in Ethiopia at Kobo Agricultural sub center site Amhara Regional State and Werer Agricultural Research Center Afar Regional State. Kobo is located 570 km East of Addis Ababa at altitude of 1513 masl and at latitude of $12^0$09'N and $39^0$38'E longitude. Werer Agricultural Research Center is located 278 km East of Addis Ababa at altitude of 740 masl and at latitude of $9^0$16'N and $40^0$9'E longitude.

The experimental materials consisted of 151 accessions of sorghum (*Sorghum bicolor*) having a chromosome number of 2n=2x=20. These accessions were collected by Institute of Biodiversity Conservation of Ethiopia from sorghum growing regions of Ethiopia. The field experiments in both locations were laid out in alpha lattice design, with three replications having eighteen blocks per replication. In both trials irrigation water was provided after sowing to ensure uniform germination. All the management practices were

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uniformly applied to all plots using the recommended practices. Phenotypic trait data were collected during plant growth and maturity for seven traits (days to 50% flowering, plant height, panicle exsertion, tiller number, panicle weight, grain weight per panicle and hundred grain weight) based on sorghum descriptor list [7].

a) DNA isolation and marker genotyping

Seedlings of 151 genotypes were raised in the green house and fresh leaves from 14 days old seedlings were harvested and dried with silica gel in zip locked plastic bag. Total genomic DNA was extracted following a modified CTAB (Cetyl Trimethyl Ammonium Bromide) extraction protocol [11].

The quality and quantity of the extracted DNA was determined by comparing the fluorescence of aliquots of DNA samples with a known concentration of λ-DNA after running them on 0.8% ((0.8g agarose dissolved in 100ml 1xTBE (Tris-Boric Acid-EDTA) buffer)) agarose gel that contained 0.3 μg/ml ethidium bromide solution. At the end of electrophoresis, the gel was visualized using UV light and photographed using a video capture (Flowgen IS 1000). All samples were normalized to the same concentration level (50ng) and used for PCR.

A total of 39 SSRs markers, including 22 di, 9 tri, and 4 tetra nucleotide or longer motifs and 4 compound repeats were used. These SSR markers were selected based on their uniform distribution in the sorghum genome. Four of them from chromosome 1, 3, 5 and 6, five of them were on chromosome 2, 7 and 8, two of them from chromosome 4 and three from chromosome 9 and 10. The PCR amplification was performed using Gene-Amp PCR system 9600(PE-Applied-Biosystems) in 96-well plates in a total reaction volume of 10μl reaction mixture containing 1μl DNA template (50ng), 1μl 10x PCR buffer, 2μl $MgCl_2$, 1μl of reverse primer, 1.0 μl forward primer directly labeled with 6-FAM (VIC, NED, PET fluoresce dyes), 0.5μl of each dNTP, 0.04 μl Taq DNA polymerase and 3.46 μl H_2O. The amplification profile consisted of initial denaturation of the template DNA at 95°C for 3 minutes, followed by 35 cycles, each for 30 sec at 95°C (denaturation), 1min at 56°C (annealing), and 1 min at 72°C (extension), and a final extension at 72°C for 3 mins.

III. Data Analysis

Statistical analyses were performed on phenotypic data for variances, genotype x environment interaction and heritability. The association analysis was done by using mixed linear model (MLM) with TASSEL software version 4.1.15 [1]. The population structure in 151 sorghum accessions was analyzed using 39 SSR markers. Each individual was assigned to sub-populations based on membership proportion in each sub-population using the software STRUCTURE ver.2.3.4 [14]. STRUCTURE was run with the admixture model, a burn-in period of 10,000 and 10,000 Markov Chain Monte Carlo repetitions [4]. The K matrix was generated in SPAGeDi for MLM analysis in the Q+K model.

$$y = X\alpha + Q + K + \varepsilon$$

y=observed vector, X = genotype, $X\alpha$ = fixed effects, Q= is population structure cofactor, K= kinship matrix cofactor and ε = random residual effects.

a) Marker Localization

To validate the association study between identified markers in this study and those mapped in previous studies were physically localized to chromosome accordingly [9]. The physical position of markers determined from map viewer searched against the sorghum genome database presented in www.phytozome.net /sorghum (03 January 2013). Alternatively, chromosome location of some SSR markers was provided by [12]. Molecular markers were located on chromosomes based on the physical distances in Mb using Map Draw 2.2 [11].

IV. Results and Discussion

The analysis of variance indicated highly significant difference (P<0.001) among accessions for all studied trait. Locations effects were highly significant (P< 0.01) for days to 50 % flowering, plant height and panicle weight. The location x genotype interaction was significant (P< 0.05) for plant height and highly significant (P< 0.001) for days to 50% flowering and panicle weight indicating that genotypes showed differential performance for these traits at the two locations. Heritability was for panicle weight (38.39-39.88%) and grain weight per panicle (36.72-41.39%) exhibited moderately high heritability estimates at both locations.

The population structure analysis result showed that posterior probability (Ln(P(D)) was stopped increasing and plateaued, as described by [4] in the range of 2-12 subpopulations (Figure 1).

The most likely K observed from K=4 explained by the highest value of(Ln (P (D))and this number was [21, 22].

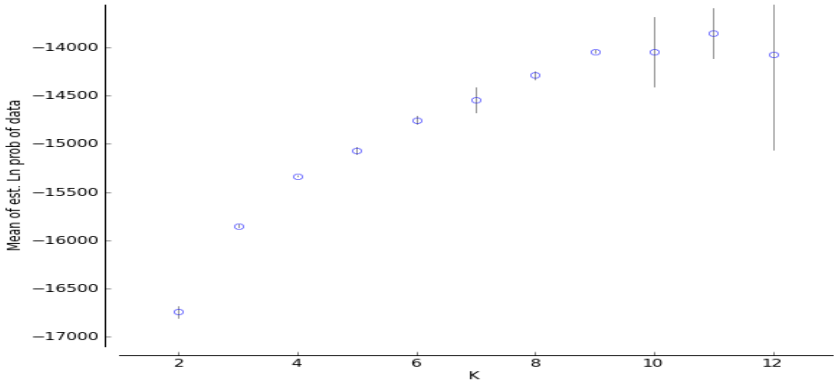

Figure 1 : Plot of mean likelihood L(K) and variance per K value from STRUCTURE on a dataset containing 151 individuals genotyped by 39 SSRs

The ad hoc statistics (ΔK) based on the second order rate of change of the likelihood showed a clear peak at the true value of K=4 (Figure 2). Therefore, k = 4 was selected and used for association analysis.

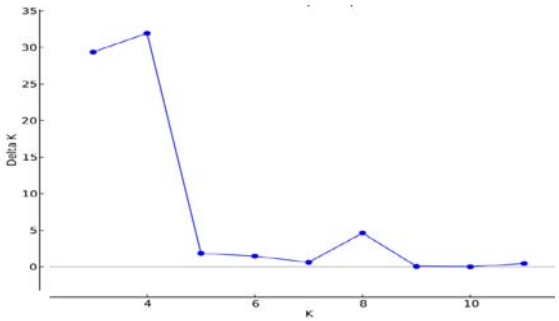

Figure 2 : Plots for detecting the number of K groups that best fit the data by delta K values

Plots of ancestry estimates provided the estimated membership coefficients for each individual in each cluster. Each individual is represented by a single vertical line, partitioned into K colored segments that represent individual's estimated membership fraction in each of the K inferred clusters (Figure 3).

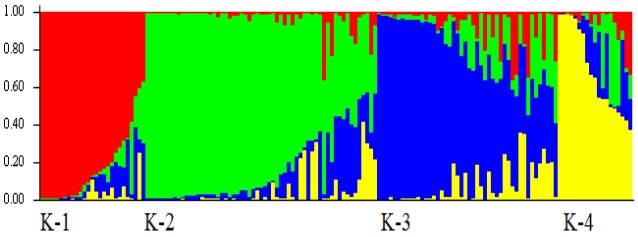

Figure 3 : Summary of plot estimates of population structure in the genotyped entries. The numbers (K-1, K-2, K-3 and K-4) were corresponding to the predefined population subgroups

To better examine patterns of association across different environments, associations with p-values between 0.05 and 0.01 were also shown. Association analysis based on two environments consistently four SSR markers Xtxp136, Xtxp015, mSbCIR300 and Xtxp278 associated with studied traits were identified (Table 1).

		Kobo					Werer				
Trait	**Marker**	**F**	**P**	**Chr**	**Pos.**	**R^2(%)**	**F**	**P**	**Chr.**	**Pos.**	**R^2(%)**
Days to 50 % flowering	Xtxp278	2.951	0.022	7	51.1	7.84	2.592	0.039	7	51.1	6.80
Panicle exsertion	mSbCIR300	2.754	0.03	7	58.3	7.28	2.896	0.024	7	58.3	6.72
Grain yield per panicle	Xtxp015	2.801	0.004	5	42.1	16	2.576	0.009	5	42.1	1.54
	Xtxp136	3.903	0.0225	5	57.6	5	3.478	0.017	5	57.6	6.93

p-values in bold are significant at $p < 0.05$

Only one marker (Xtxp015) was significantly associated with grain yield per panicle weight in Kobo and Werer. The reason that only one marker was associated with grain yield per panicle and for other traits was probably because inadequate coverage of the genome by the markers used in this study. Previous studies reported that one marker Xtxp145 was associated with sorghum grain yield on chromosome 6 [20].

[16] identified two SSR markers (Xtxp265 and Xtxp547) that were associated with days to flowering on chromosome 4 and 6 having phenotypic variance of 12 and 23 % respectively. In another study, [21] identified two markers (40-1897 and 44-2080) associated with days to 50 % flowering on chromosome 6 using 39 SSR markers and 242 sorghum accessions evaluated at five environments.

For days to 50 % flowering, alleles of 254bp and 248bp of marker Xtxp278 increased the days 50% flowering by approximately 1.01 day and 2.26 days each in 26 and 105 accessions respectively at Kobo. At Werer alleles of 254bp and 248bp of marker Xtxp278 reduced days to 50% flowering by approximately 0.49 days each in 26 accessions and increased 3.69 days each in 105 accessions.

For panicle exsertion, alleles 106bp and 112bp of marker mSbCIR300 increased panicle exsertion by 1.94 and 1.75 cm each in 130 and 16 accessions, respectively at Kobo. At Werer the same alleles 106bp and 112bp of marker mSbCIR300 increased panicle exsertion by 8.23 and 3.80 cm each in 130 and 16 accessions, respectively.

Two alleles 211bp and 215bp of marker Xtxp015 reduced grain yield approximately by 2 g and 2.19 g in 82 and 14 accessions, respectively. Allele 240bp and 237bp of marker Xtxp136 increased grain yield per panicle approximately by 2.16 g and 2.07 g in 78 and 68 accessions, respectively at Kobo. At Werer alleles 240bp and 243bp of marker Xtxp136 increased grain yield per panicle approximately by 0.53 g and reduced by 0.60 g in 128 and 12 accessions, respectively.

To validate the association between markers identified in this study and those mapped in previous studies, we physically localized our markers and previously mapped QTLs to the sorghum chromosomes.

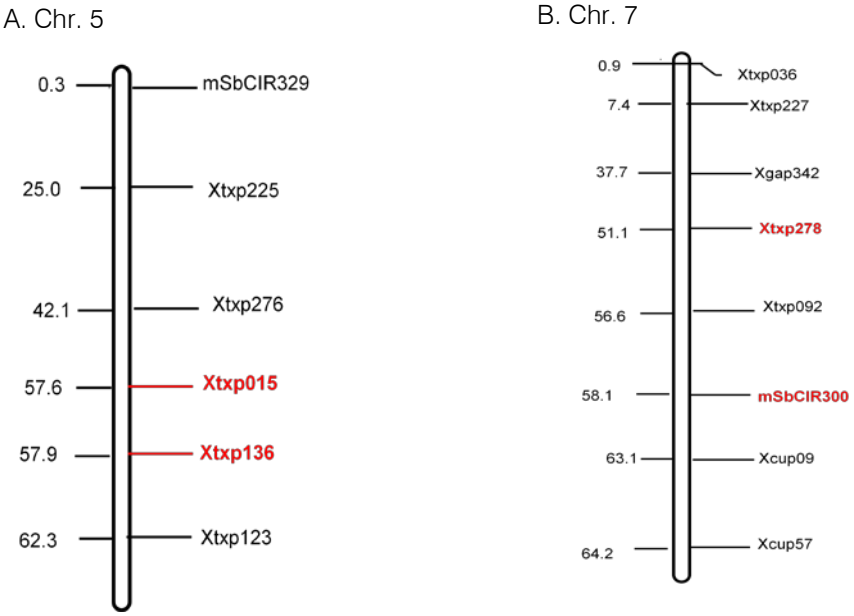

Figure 4 : Chromosomal locations of marker trait associations. The physical positions of markers in Mbp indicated on the left of the map and corresponding marker are indicated on the right. Identified markers in this study are showed in red color and markers previously mapped are in black color (A), shows SSR markers associated with grain weight per panicle (B), shows SSR markers associated with days to 50% flowering and panicle exsertion.

V. Conclusion

In this study, we identified two markers (Xtxp136 and Xtxp015) linked to grain yield per panicle, one (Xtxp278) to days to 50% flowering and one (mSbCIR300) to panicle exsertion consistently at two location. These SSR markers were co-localized with previously mapped QTLs for days to 50 % flowering, panicle exsertion and grain yield per panicle respectively. These markers could be used for marker-assisted selection of traits for drought tolerance improvement programmes and selection for drought tolerant genotypes. However, it should be validated further to improve the precision of study prior to apply in

marker-assisted selection. By increasing the number of markers genome wide association mapping must be conducted to find strong association with traits. Additionally, the vicinity of the indentified loci will need to be further investigated to search for gene homologs that are known to regulate each trait.

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