



Mapping of QTLs Associated with °Brix and Biomass-Related Traits in Sorghum Using SSR Markers

Tesfaye Disasa¹ · Tileye Feyissa² · Belayneh Admassu³ · Masresha Fetene⁴ · Venugopal Mendu⁵

Received: 18 July 2017 / Accepted: 7 January 2018 / Published online: 18 January 2018
© Society for Sugar Research & Promotion 2018

Abstract The non-renewability and greenhouse gas emission nature of crude oil led to the use of renewable plant-based biofuels as alternative energy. Sweet sorghum offers one of the best plant-based bioethanol production from its sugary stalk. Identification and mapping of quantitative trait loci associated with sugar-related traits in sorghum is a crucial step toward the improvement of the sugar content of the crop. The present study was carried out to identify and map QTLs associated with °Brix, stalk diameter and plant height using $F_{2,3}$ segregating mapping populations derived from a cross between grain sorghum (*Sorcoll 163*) and sweet sorghum (*Gambella*). Phenotypic evaluation was conducted in two different environments. A genetic map was constructed using 192 F_2 populations genotyped using 76 SSR markers. Estimated heritability for °Brix, stem diameter and plant height amounted to 0.88, 0.41 and 0.65, respectively. A total of seven QTLs distributed across five linkage groups that controls °Brix content were detected using Inclusive Composite Interval Mapping. Each QTL contributed 17.2–44.3% of the total phenotypic variation. The two QTLs located on linkage

group SBI-05 and SBI-06 repeated in both environments. These QTLs can be the target of the breeding programs in the future due to high heritability as well as stability in two different environments. The number of SSR markers used in present study is relatively fewer and fine mapping of the target regions should be considered in order to further dissecting the region. All the detected QTLs in this study are categorized as major QTLs, and it could be used further in sweet sorghum improvement program.

Keywords °Brix · Biomass · QTL mapping · SSR markers · Sweet sorghum

Introduction

The demand for the production of bioethanol from plant-based biomass materials is increasing globally. The demand is due to the fluctuating price of fossil fuels on world market combined with concern to protect the environment against the increase in greenhouse gas emissions resulted from the utilization of fossil fuels which contributed to the global warming. These necessitate gradually shifting from non-renewable energy source to plant-based biomass alternative energy source particularly in countries like Ethiopia to reduce its dependence on imported energy supplies and reduce the burden of foreign exchange.

Plant biomass-based biofuels can be derived from different sources like plant lignocellulosic material (bioethanol) or seed oil (biodiesel) or fermentable sugars (bioethanol) or starch (bioethanol). A number of crop plants are currently used as a source for biofuel production using their biomass. Sorghum (*Sorghum bicolor*) is among the preferred crops to be used for such purposes. Sweet sorghum is a type of sorghum which is among important

✉ Tesfaye Disasa
tesfayedisasa@yahoo.com

¹ National Agricultural Biotechnology Research Center, P.O. Box 2003, Addis Ababa, Ethiopia

² Institute of Biotechnology, Addis Ababa University, P.O. Box 1176, Addis Ababa, Ethiopia

³ USDA-ARS, 1691 S2700W, Aberdeen, ID 83210, USA

⁴ Ethiopian Academy of Science, P.O. Box 32228, Addis Ababa, Ethiopia

⁵ Department of Plant and Soil Science, Fiber and Biopolymer Research Institute (FBRI), Texas Tech University, Lubbock, TX, USA

biofuel feedstock for ethanol production globally (Murray et al. 2009). Moreover, fodder sorghum is promoted as a biomass crop for lignocellulosic-based ethanol production. The stalk of sweet sorghum is rich in sucrose which is used for the production of bioethanol via fermentation process. The sweet sorghum is characterized by reduced grain yield as compared to grain sorghum. The crop is categorized as the promising source of biofuel for the production of first (sucrose) and second (lignocellulosic material) generation of bioethanol. Sweet sorghum is among C4 crops which is characterized by significant level of directly fermentable stalk sugars. It has the potential to give high biomass under unfavorable conditions unlike many other C4 plants. The crop accumulates similar level of sugar to that of sugarcane (Murray et al. 2009). Under conducive environment, sweet sorghum crop has a significant potential of producing ethanol which is close to 8000 L of ethanol per hectare of land (Murray et al. 2009). Unlike sugarcane, sorghum is highly suitable for large-scale mechanization and seed-based production. Moreover, it is a relatively drought tolerance crop and requires low amount of water as compared to other types biofuel crops (Gnan-sounou et al. 2005). Besides, sweet sorghum has a huge adaptation potential ranging from extreme cold to high temperature environments. It is also highly suitable for both subsistence agriculture, semi-intensive and intensive agriculture which makes it an ideal crop to produce in various regions of the world. The utilization of sweet sorghum as bioenergy crop seems to be economically very visible as the cost of sugarcane production is three times more than the sweet sorghum cost of production (Reddy et al. 2005). Similarly, bioethanol production processing of stem sugar needs limited chemical reaction processes and less energy from the beginning to the final product than lignocellulosic-based technologies (Wang et al. 2009) hence presents a better alternative to lignocellulosic-based ethanol production.

Despite all these potentials, the global effort to improve the crop for bioenergy-related traits remains minimal. Little has been done in genetic diversity analysis of sweet sorghum genotypes using morphological and °Brix (Disasa et al. 2017) and molecular (Casa et al. 2005, 2008; Murray et al. 2009; Wang et al. 2009; Disasa et al. 2016) markers. Despite, Ethiopia/surrounding region is believed to be the center of diversity and origin for sorghum, not much attention has been given toward identification and mapping of QTLs conferring bioenergy traits using Ethiopian sweet sorghum germplasm. Hence, it is important to identify some novel genes/genomic regions that associated with various bioenergy-related traits.

Identification and mapping of economically important quantitative traits for the improvement of high stalk sugar-producing varieties is the major interest of many plant

breeders. Several approaches can be used in identification and mapping of the trait of interest in crop plants. Most of the studies have largely been focused on linkage or family mapping of quantitative trait loci (QTL) which involves the generation of mapping populations, screening of markers for polymorphism, construction of linkage map followed by QTL identification and mapping. This is an ideal approach especially for the identification of QTLs linked with the traits of interest using population derived from biparental crosses.

Identification and mapping of QTLs for several traits have been previously reported in grain sorghum. Among these, QTLs conferring stay-green (Tuinstra et al. 1996, 1997, 1998; Crasta et al. 1999; Xu et al. 2000; Tao et al. 2000; Subudhi et al. 2000; Kebede et al. 2001; Sanchez et al. 2002; Haussmann et al. 2002; Harris et al. 2007) and plant height traits (Lin et al. 1995; Pereira and Lee 1995; Rami et al. 1998; Hart et al. 2002; Klein et al. 2000; Natoli et al. 2002; Feltus et al. 2006; Ritter et al. 2008; Murray et al. 2008; Shiringani et al. 2010) are the most studied traits in sorghum. However, little attention has been given to identify and map QTLs conferring for bioenergy-related traits. Some of these works were based on selected traits derived from contrasting parents for stem sugar trait to identify and map stem sugar and other non-structural carbohydrates (Murray et al. 2008). Limited attempts on identification and mapping of QTLs linked to stem sugar and other agronomic traits were also previously reported (Shiringani et al. 2010; Guan et al. 2011). Similarly, QTL mapping for eleven sugar-related agronomic traits were also conducted using 184 recombinant inbred lines (RILs) derived from two different parents (grain and sweet sorghum lines) through crossing (Ritter et al. 2008). All of the identified QTLs so far were from exotic germplasm, and there was no study reported regarding QTL mapping of °Brix and related-sugar content traits using Ethiopian germplasm. Therefore, the current study aimed at identification and mapping of QTLs associated with °Brix and other biomass-related traits in sorghum using Ethiopian sorghum germplasms.

Materials and Methods

Development of Mapping Populations

Effort has been made to generate F₂ mapping populations by crossing one of the selected grain sorghum (*Sorcoll 163/07*) as a female parent and sweet sorghum (*Gambella*) as a pollen source. *Sorcoll 163/07* was inbred line characterized by its drought tolerance nature with an average plant height (224.5 cm), stalk diameter (15.5 mm) and °Brix (6.8). *Gembella* is farmers' preferred improved

genotypes characterized by its sugary stalk with an average plant height (192.3 cm), stalk diameter (14.0 mm), and °Brix (14.4).

Planting was undertaken at Melkassa Agricultural Research Center (39°21'E, 8°24'N) during main rainy season in 2012. The field was supplied with irrigation as the area experiences frequent rain failure. All the necessary agronomic management practices were implemented. Crossing was carried out using hand pollination technique with a great care. The heads were tightly covered with appropriate paper bag in order to avoid contamination from neighboring pollen. Enough seeds from the crosses were harvested and used to produce F₁ plants. Selfing of F₁ populations was undertaken at Water Agricultural Research Center (40°21'E, 9°33'N) during offseason in the same year using irrigation facilities. The seeds from F₁ populations were harvested and planted to produce F₂ mapping population for sugar-related traits and identify molecular markers that are strongly associated with the traits of interest. A total of 200 F₁ seeds were planted in green house of the National Agricultural Biotechnology Research Center based at Holetta (38°50'E, 9°05'N), Ethiopia in order generate F₂ plants from which genotyping was undertaken. In order to test in multiple environments, each F₂ plant was selfed to produce F₂ derived F₃ plants (F_{2:3}).

Experimental Design and Phenotypic Evaluation

Sufficient seeds derived from each F₂ population were planted across two locations; one at Melkassa Agricultural Research Center (39°21'E, 8°24'N) and the second one is at Dhera experimental station (39°19'E, 8°15'N) in Ethiopia. An α -lattice design was used with three replications for all the progenies along with the two parental lines in each trial. In both locations, a one-row plot of 5 m row length with the inter-row space of 0.75 m was used. Manual planting was undertaken followed by thinning to 0.2 m spacing after 3 weeks of emergence. Recommended amount of fertilizer was applied following the procedure used by Ayana (2001). Weed control and disease management was undertaken. The field was protected from bird damage.

Data were collected on three traits such as plant height, stalk diameter and °Brix. Plant height was measured as the length of the plant at physiological maturity stage ranging from the ground to the tip of the panicle. Stalk diameter was measured using appropriate digital caliper (World Precision Instruments, Shanghai Trading Co., Ltd). The measurement was taken at three locations along the height of the plant starting from 20 cm above ground. Stem juice was extracted from stalks harvested just before reaching full maturity stage. One to two drops of the extracted juice was applied on top of the hand-held refractometer

(TEC++ DR. VOLKER SCHMIDT GM BH, Deutschland) in order determine the percentage of soluble solids (°Brix).

Phenotypic Data Analysis

Minitab v.16 statistical software (Minitab Inc. 2010) was used to estimate the normality of the data for each trait based on the data collected from two locations. Similarly, other major statistics including standard deviation and percent coefficient of variation (CV) of the three traits were analyzed using SAS software v. 9.2 (SAS inst. 2008). METAR (Multi Environment Trial Analysis for R) Version 2.1 software was used to calculate the broad-sense heritability, while the PROC CORR procedure was used to compute the Pearson correlation coefficient.

DNA Extraction and Polymorphism Screening

Young leaf tissues were collected from each parent, *Sorcoll 163/07* and *Gambella* varieties in order to extract genomic DNA that will be used to screen polymorphic markers using the two selected parents. Promega Wizard[®] Genomic DNA Purification Kit (Madison, USA) was used to isolate the genomic DNA. Kit based DNA isolation was preferred in order to obtain good quality and quantity DNA molecules. The purified genomic DNA was stored in +4°C followed by quality checking using 0.8% agarose gel stained with GelRed[®] (Biotium, USA). The concentration of the genomic DNA was quantified using spectrophotometer. Based on the recorded concentration for each genomic DNA samples, the working DNA solution was further diluted to the concentration of 10 ng/μl (Disasa et al. 2016).

A number of literatures from previously published sorghum SSR markers mapping were used in order select the most polymorphic markers (Bhatramakki et al. 2000; Menz et al. 2002; Wu and Huang 2006; Li et al. 2009; Ramu et al. 2009). Nearly 300 selected sorghum SSR primers that are found to have full coverage of the ten linkage groups were identified. A total of 10 μl PCR volume was used with a combination of 30 ng/μl template DNA, 0.04 pmol forward primer, 0.2 pmol reverse primer, 1 × taq buffer, 2 mM MgCl₂, 0.16 mM each of the four deoxynucleotide triphosphates (dNTPs), 0.16 pmol for each four different fluorescent labels and 0.2 u Amplitaq (SibEnzyme Ltd, Russia). The entire forward primers were supplemented with fluorescently labeled detectors such as FAM, PET, NED or VIC (Applied Biosystems, USA) on the 5' end (Schuelke 2000). These will enable to detect the amplified products independently.

Polymerase chain reaction was performed using a GeneAmp[®] PCR System 9700 thermal cycler (Applied

Biosystems) adjusted for initial denaturation at 94 °C for 15 min, the second denaturation was programmed at 94 °C for 30 s, annealing temperature set at 50 °C for 1 min while extension of the product was adjusted at 72 °C for 2 min and final elongation at 72 °C for 20 min (Disasa et al. 2016).

To confirm whether the PCR product was successfully amplified or not, a 2.0 µl volume of the PCR products was obtained and checked using a 2% (w/v) agarose gel stained with GelRed® (Biotium). The result was visualized under UV. Amplified products were selected followed by co-loading of four PCR products together. The volume of each PCR products is depending on the strength and nature of fluorescent label used. The products were supplemented with Hi-Di™ Formamide (Applied Biosystems, USA) and internal size standard, GeneScan™ 500 LIZ® (Applied Biosystems, USA). Fragments with different sizes were separated using capillary electrophoresis techniques using an ABI Prism® 3730 Genetic analyzer (Applied Biosystems, USA), and fragment sizes scoring was done using GeneMapper 4.0 software (Applied Biosystems, USA).

Genotyping F₂ Mapping Populations

A total of 192 F₂ progenies and their parents were genotyped in these experiments. Fresh leaf tissues were collected from young seedlings, and genomic DNA was isolated using Promega Wizard® Genomic DNA Purification Kit (Madison, USA). The same PCR protocol and thermal cycler used to screen polymorphism among the parents was also applied here. A total of 81 SSR primers that show polymorphism between the parents, *Sorcoll 163/07* and *Gambella*, were used to genotype the entire mapping populations. SSRs were amplified and separated based on fluorescent labeled forward primers. The entire polymorphic markers were analyzed in all mapping populations including parents using the ABI PRISM_3730 genetic analyzer (Applied Biosystems, USA). GeneMapper software, v4.0 was used to collect the data. The scored markers and F_{2:3} mapping populations were used to analyze QTL associated °Brix traits. Fragment scores were recorded as 2, 0 and 1 for *Sorcoll 163/07*, *Gambella* and *heterozygous*, respectively. Missing values were recorded as – 1 for individual marker across the entire populations.

Linkage Map Construction

Based on 76 successfully scored markers across 192 progenies and parents, the linkage map was constructed using IciMapping software v 4.0 (Li et al. 2007). Based on the logarithm of odds (LOD) threshold of 3.0, linkage groups were identified and map distances (in Centi-Morgan units) was computed based on the Kosambi mapping function

(Kosambi 1944). The relationship between genetic distance in (*m*) and recombination frequency (*r*) is given by

$$m = 25 \ln \left(\frac{1 + 2r}{1 - 2r} \right) \quad \text{Or} \quad r = \frac{1 e^{4m} - 1}{2 e^{4m} + 1}$$

The linkage map was compared and assigned to chromosomes according to sorghum censuses map (Mace et al. 2009).

The whole polymorphism screening of parents and F₂ genotyping activities were undertaken at International Crop Research Institute for Semi Arid Tropics (ICRISAT-Nairobi), regional program, whereas DNA extraction was carried out at the National Agricultural Biotechnology Research Center of the Ethiopian Institute of Agricultural Research (EIAR), Ethiopia.

QTL Analysis

Identification and mapping of the QTLs were based on three different approaches such as single-marker analysis (SMA), interval mapping (IM) and inclusive composite interval mapping (ICIM) using IciMapping software v 4.0 (Li et al. 2007). The results from IM and ICIM were compared. Finally, ICIM was used for the analysis. LS means of each of the traits were used for QTL mapping. Missing phenotypic data was scored as – 100.00.

Results and Discussion

Phenotypic Evaluation

For traits such as plant height and stem diameter, there was no significant difference between the two parents as well as the progenies in both environments (Table 1). However, °Brix value measured for *Gambella* was significantly ($P < 0.01$) higher than that of *Sorcoll 163/07*. The phenotypic value of °Brix showed continuous variation across the progenies in two locations and normally distributed with a moderate value of skewness and kurtosis (Table 1). Environmental means for °Brix were 8.9 and 9.1 in the two locations. The highest (15.8) and lowest (4.5) °Brix values were recorded among the progenies at Dhera and Melkassa stations, respectively.

The heritability of the traits such as °Brix, stalk diameter and plant height were 0.88, 0.41 and 0.65, respectively. °Brix showed a high heritability, whereas the stalk diameter and plant height showed moderate to high heritability. Pearson's correlation coefficients analysis showed that there was no significant correlation between °Brix and plant height ($r = 0.089$, $P > 0.05$), but a slight negative correlation was observed between °Brix and stem diameter

Table 1 Phenotypic values and statistical analysis of °Brix, stalk diameter and plant height values in different environments

| Locations | Traits | Mean | Variance | Std error | Skewness | Kurtosis | Min | Max | Range | <i>P</i> value |
|-------------------|---------------------|--------|----------|-----------|----------|----------|--------|--------|--------|----------------|
| MARC | °Brix | 8.88 | 8.56 | 2.93 | 0.46 | − 1.06 | 4.50 | 14.90 | 10.40 | 0.00 |
| | Stalk diameter (mm) | 16.19 | 5.23 | 2.29 | 0.11 | − 0.35 | 11.40 | 23.30 | 11.90 | 0.36 |
| | Plant height (cm) | 199.34 | 1811.43 | 42.56 | − 0.06 | − 0.48 | 104.60 | 306.30 | 201.70 | 0.13 |
| Dhera sub station | °Brix | 9.10 | 6.89 | 2.62 | 0.76 | − 0.54 | 5.70 | 15.80 | 10.10 | 0.00 |
| | Stalk diameter (mm) | 13.12 | 2.76 | 1.66 | 0.14 | − 0.21 | 9.10 | 17.30 | 8.20 | 0.44 |
| | Plant height (cm) | 203.67 | 1414.54 | 37.61 | − 0.24 | − 0.55 | 90.30 | 287.60 | 197.30 | 0.08 |
| Combined location | °Brix | 8.99 | 6.84 | 2.61 | 0.61 | − 0.96 | 5.50 | 14.90 | 9.40 | 0.00 |
| | Stalk diameter (mm) | 14.66 | 2.52 | 1.59 | 0.10 | − 0.36 | 11.00 | 19.10 | 8.10 | 0.59 |
| | Plant height (cm) | 201.50 | 1156.04 | 34.00 | − 0.13 | − 0.29 | 108.20 | 285.40 | 177.20 | 0.56 |

($r = -0.320$, $P < 0.05$). The result also showed that there was no significant correlation between plant height and stem diameter traits ($r = 0.145$, $P > 0.05$).

°Brix combined with other sugar-related traits are the most important traits for the utilization of sorghum crops as a bioethanol feedstock. Biomass-related traits such as plant height and stalk diameter are also important agronomic traits for the production of ethanol from sweet sorghum crop as they determine the overall sugar yield. Any positive correlation between these traits helps to improve the stalk juice yield of the crop. According to Murray et al. (2008), the total stem sugar yield per hectare is dependent on two traits, sugar concentration in the stem and stem juice yield per hectare. Increasing sugar concentration would be an important step to increase energy density and reduce processing and transportation costs. Identification and introgression of QTLs that are associated with both high sugar concentration and stem juice yield into elite varieties will be an important step. Therefore, QTLs that influence °Brix content would be good breeding target for biofuel improvement program in the future since °Brix had strong positive correlation with the sugar concentration and stem juice yield traits (Ritter et al. 2008).

Our result showed that no significant correlation between °Brix and plant height, which deviates from previously reported study whereby most of sugar-related traits showed significantly positive correlation with plant height (Ritter et al. 2008; Murray et al. 2009). The deviation may be due to non-contrasting parent (no significant variation between the two parents) for plant height trait and stem diameter used to develop mapping populations. The high heritability ($H = 0.88$) estimate for °Brix trait was in agreement with previous study conducted by Ritter et al. (2008) using the same trait ($H = 0.86$). However, the result is higher than the study reported by Murray et al. (2008), Shiringani et al. (2010) and Guan et al. (2011) which are reported to be $H = 0.65$, $H = 0.59$ and $H = 0.76$, respectively. This high heritability estimate nature of the

trait has an implication that the trait is suitable for QTL mapping studies as compared to traits with low heritability potential.

Linkage Map Construction

Data from fragment analysis showed that among 304 polymorphic SSR markers tested, 81 (26.6%) found to be polymorphic between the two mapping parents. In contrast, 97 (31.9%) were unable to discriminate the two parents and considered as monomorphic markers and the remaining 68 (22.4%) markers did not produce distinct and reproducible patterns of polymorphism among the two parents. The polymorphic markers were fairly distributed across the ten linkage groups.

Out of the 81 polymorphic marker loci used for genotyping F_2 mapping populations, five of them (6%) were failed to discriminate the F_2 progenies and excluded from the analysis. Most of the 76 SSR markers used here showed good segregation pattern, which did not significantly deviate from the expected 1:2:1 segregation ratio. The distributions of markers more or less appear to be evenly distributed across the ten linkage groups except few numbers of markers on some of the linkage groups due to the difference in length of chromosomes (Fig. 1). Out of the 76 polymorphic marker loci, six of them could not assign to any specific linkage group and excluded from the final analysis.

QTL Detection and Analysis

Two major QTLs influencing the °Brix were detected on linkage group SBI-05 and SBI-06 at a LOD threshold of 3.5 using the data collected from Melkassa Agricultural Research Center. In contrary, QTLs influencing plant height and stalk diameter were not detected at similar LOD cut value (Fig. 2). The two QTLs associated with °Brix were found to be major QTLs and each contributed

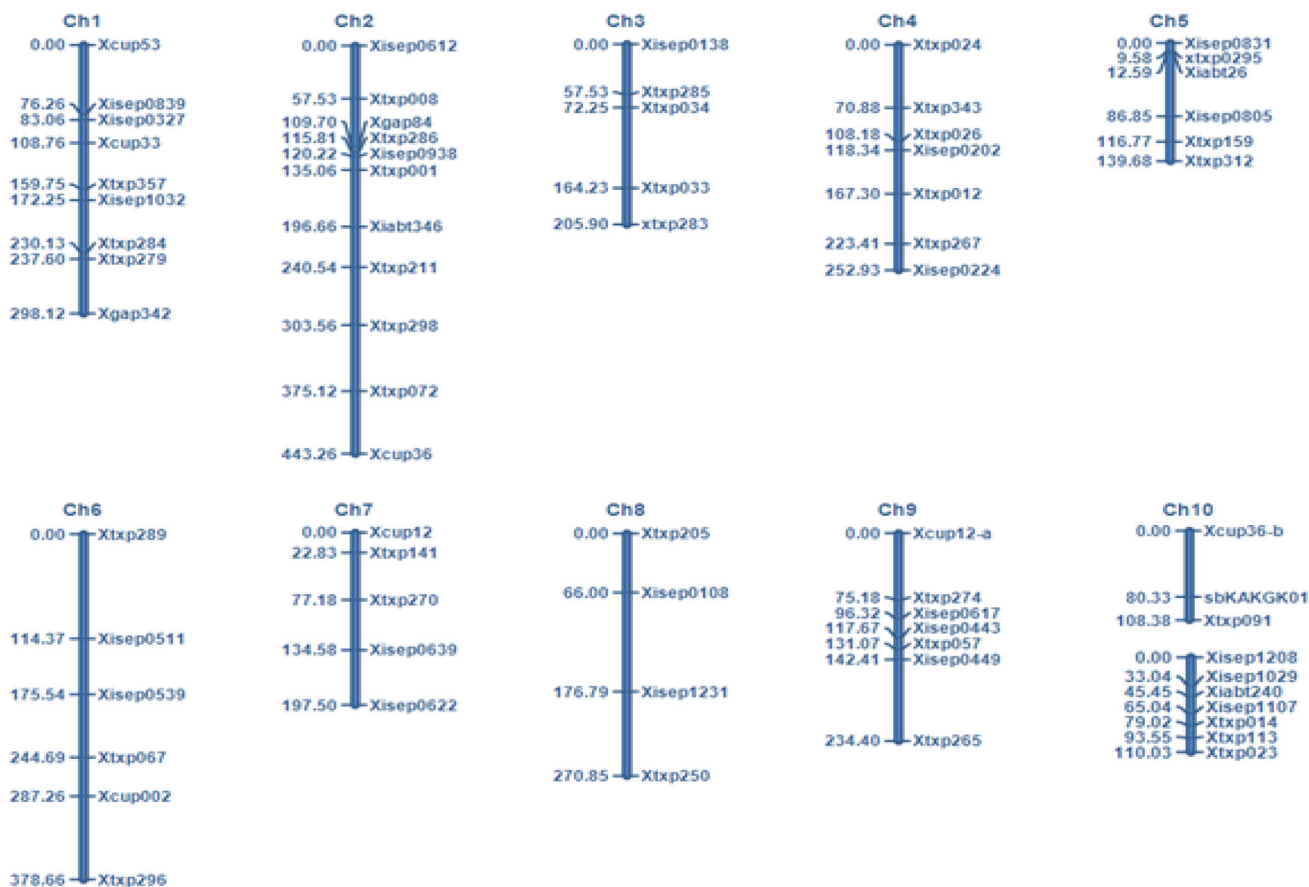
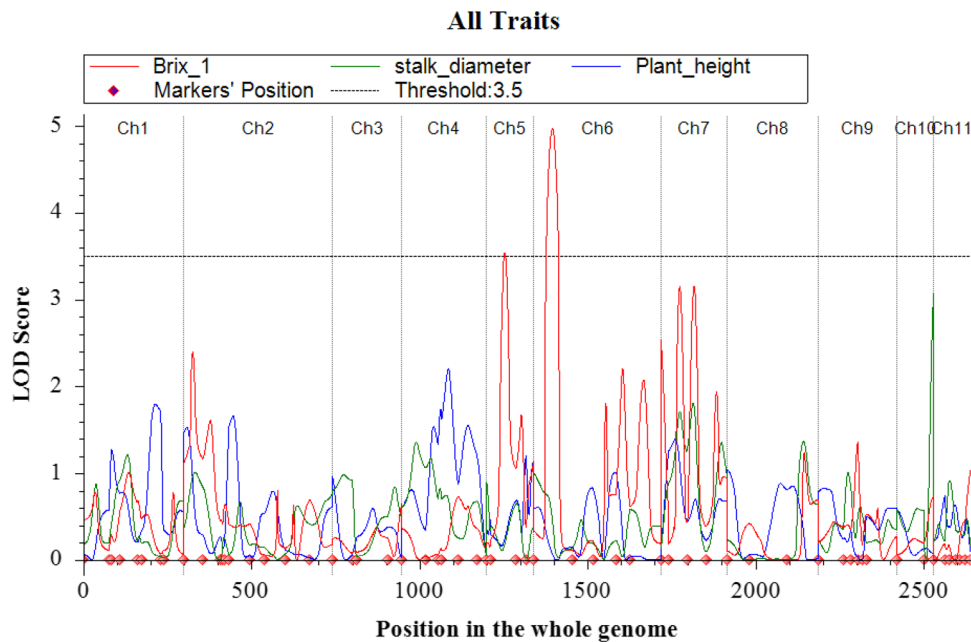


Fig. 1 Constructed genetic linkage map using 192 F₂ mapping populations genotyped using polymorphic SSR markers

Fig. 2 Detected quantitative trait loci for °Brix trait in sorghum at LOD threshold 3.5 (at MARC environment)



40.0–44.7% of the total phenotypic variation (Table 2). The first QTL on the SBI-05 linkage group lies between *Xiabt26* and *Xisep0805* with a LOD value of 3.5, whereas

the second QTL on the linkage group SBI-06, located between *Xtxp289* and *Xisep0511* with a LOD value of 5.0. The additive effect of each QTL on linkage group SBI-05

Table 2 Linkage groups, position and flanking markers of detected quantitative trait loci associated with °Brix trait evaluated at MARC and Dhera environments

| Locations | Linkage groups | Position | Left marker | Right marker | LOD | PVE (%) | Add | Dom |
|-----------|----------------|----------|-------------|--------------|------|---------|--------|--------|
| MARC | SBI-05 | 54 | Xiabt26 | Xisep0805 | 3.54 | 39.97 | − 1.59 | − 2.97 |
| | SBI-06 | 55 | Xtxp289 | Xisep0511 | 4.97 | 44.68 | − 1.93 | − 2.67 |
| Dhera | SBI-02 | 80 | Xtxp008 | Xgap84 | 3.52 | 25.69 | − 1.32 | − 2.21 |
| | SBI-02 | 165 | Xtxp001 | Xiabt346 | 3.57 | 39.94 | − 1.77 | − 2.38 |
| | SBI-05 | 121 | Xtxp159 | Xtxp312 | 4.33 | 17.21 | − 2.50 | − 2.14 |
| | SBI-06 | 58 | Xtxp289 | Xisep0511 | 4.40 | 44.27 | 2.34 | − 1.68 |
| | SBI-06 | 329 | Xcup002 | Xtxp296 | 3.87 | 38.39 | − 1.74 | − 2.47 |
| | SBI-07 | 98 | Xtxp270 | Xisep0639 | 5.45 | 39.61 | − 2.36 | − 2.13 |
| | SBI-10 | 40 | Xcup36 | sbKAKGK01 | 3.51 | 41.11 | 1.69 | − 2.53 |

and SBI-06 was − 1.59 and − 1.93%, respectively. Similarly, the dominance effect was − 2.97 and − 2.67%.

Seven significant QTLs that are associated with the °Brix content (LOD threshold ≥ 3.5) were detected from the experiment evaluated at Dhera station (Fig. 3). Similar to Melkassa environment, no QTL was detected that is linked with plant height and stalk diameter traits. The QTLs were distributed across five linkage groups such as SBI-02, SBI-05, SBI-06, SBI-07, and SBI-10 (Table 2). The LOD values of the QTLs were ranging 3.5–5.5, while each QTL was explaining a phenotypic variation ranging from 17.2 to 44.3%. The additive effect of each QTL ranged from − 2.5 to 2.3%, where as dominant effect ranged from − 3.0 to − 1.7% of the °Brix content.

A total of seven QTLs were detected using the combined data collected from two locations using LOD

threshold ≥ 3.5 , whereas no QTLs influencing plant height and stalk diameter were identified (Fig. 4). Two of the QTLs conferring for total soluble solids were consistent across the two environments and located on the same linkage groups (SBI-05, SBI-06). The remaining QTLs were located on linkage group SBI-02, SBI-07 and SBI-09 with a LOD value ranging from of 3.5 to 5.4 (Table 3). Three QTLs were detected and mapped on linkage group SBI-07 with pairs of flanking markers *Xtxp141–Xtxp270*, *Xisep0639–Xisep0639* and *Xisep0639–Xisep0622* (Fig. 5). The three QTLs are considered as independent because the distance between them is greater than 20 *Centi-Morgan* (Shiringani et al. 2010). The QTL on the SBI-09 linkage group was detected between markers *Xisep0449* and *Xtxp265*. Almost all the identified QTLs are considered as major QTLs contributed high percentage of phenotypic

Fig. 3 Detected quantitative trait loci for °Brix trait in sorghum at LOD threshold 3.5 (at Dhera environment)

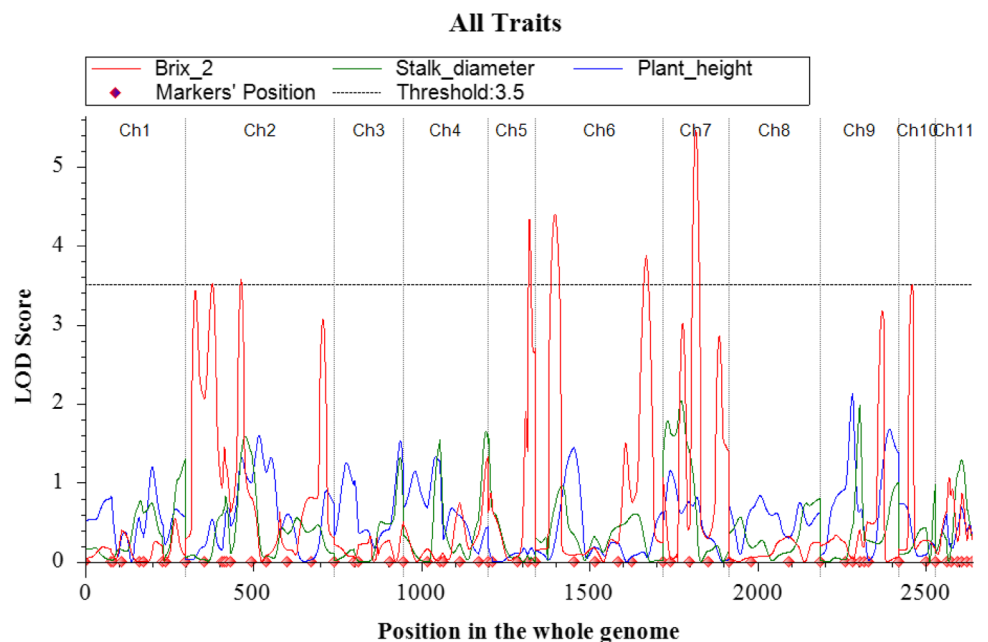


Fig. 4 Detected quantitative trait loci for °Brix trait in sorghum at LOD threshold 3.5 (combined locations)

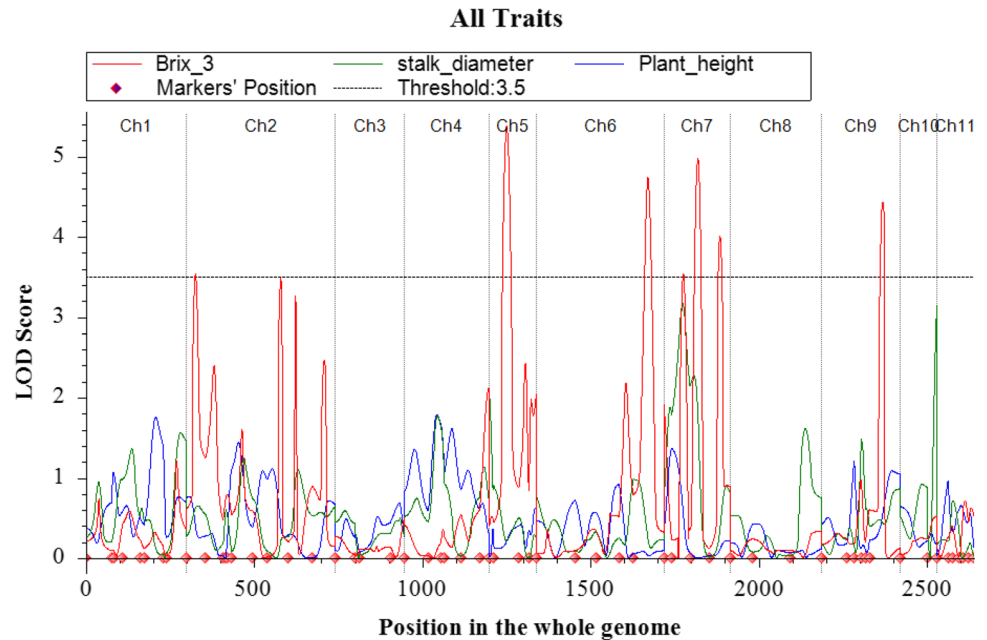


Table 3 Linkage groups, position and flanking markers of quantitative trait loci associated with ° Brix trait evaluated at MARC and Dhera environments (combined locations)

| Linkage groups | Position | Left marker | Right marker | LOD | PVE (%) | Add | Dom |
|----------------|----------|-------------|--------------|------|---------|--------|--------|
| SBI-02 | 27 | Xisep0612 | Xtxp008 | 3.54 | 40.13 | − 1.92 | − 2.48 |
| SBI-05 | 51 | Xiabt26 | Xisep0805 | 5.38 | 46.76 | − 1.87 | − 2.54 |
| SBI-06 | 331 | Xcup002 | Xtxp296 | 4.74 | 49.14 | − 2.10 | − 2.25 |
| SBI-07 | 58 | Xtxp141 | Xtxp270 | 3.54 | 38.51 | − 2.25 | − 2.24 |
| SBI-07 | 101 | Xtxp270 | Xisep0639 | 4.97 | 45.35 | − 1.99 | − 2.75 |
| SBI-07 | 167 | Xisep0639 | Xisep0622 | 4.01 | 43.59 | − 1.83 | − 3.00 |
| SBI-09 | 182 | Xisep0449 | Xtxp265 | 4.43 | 49.77 | 2.12 | − 2.26 |

variance which ranged from 38.5 to 49.8% of the phenotypic variation with the additive effect ranging from − 2.3 to 2.1%. Besides, the dominance effect ranged from − 3.0 to − 2.3%.

All of the detected QTLs were found to be major QTLs explaining phenotypic variation ranging from 38.5 to 49.8% and greater than most of the studies conducted in the past using grain and sweet sorghum genotypes (Ritter et al. 2008; Shiringani et al. 2010; Guan et al. 2011). However, direct comparison is difficult due to the difference in type of mapping populations and number of markers used in the study.

Two similar QTL located on SBI-05 and SBI-06 that are influencing °Brix traits were detected in both MARC and Dhera environments. Similar studies were reported by Ritter et al. (2008) who detected similar QTLs across two environments for many agronomic traits. This is a good indication of the high heritability nature of the trait. The

repeatability of these QTLs has also an implication that they are useful for the introgression of these QTLs into elite breeding lines due to their stability in different environments. Similar result was reported by Ritter et al. (2008) on two linkage groups such as SBI-05 and SBI-06 with major QTLs. Our findings are also partly inconsistent with the previously reported result by Guan et al. (2011) who detected four QTLs out of which two of them located on linkage group SBI-02 and SBI-07, whereas the remaining two QTLs were detected on linkage group SBI-02 and SBI-07 across two environments.

Our findings also showed the repeatability of those previously reported QTLs at different times on various linkage groups. These major QTLs were located on SBI-02 (Shiringani et al. 2010; Guan et al. 2011), SBI-05 (Ritter et al. 2008), SBI-06 (Murray et al. 2008; Ritter et al. 2008), SBI-07 (Murray et al. 2008; Guan et al. 2011). This is a good indication that these QTLs are reproducible and

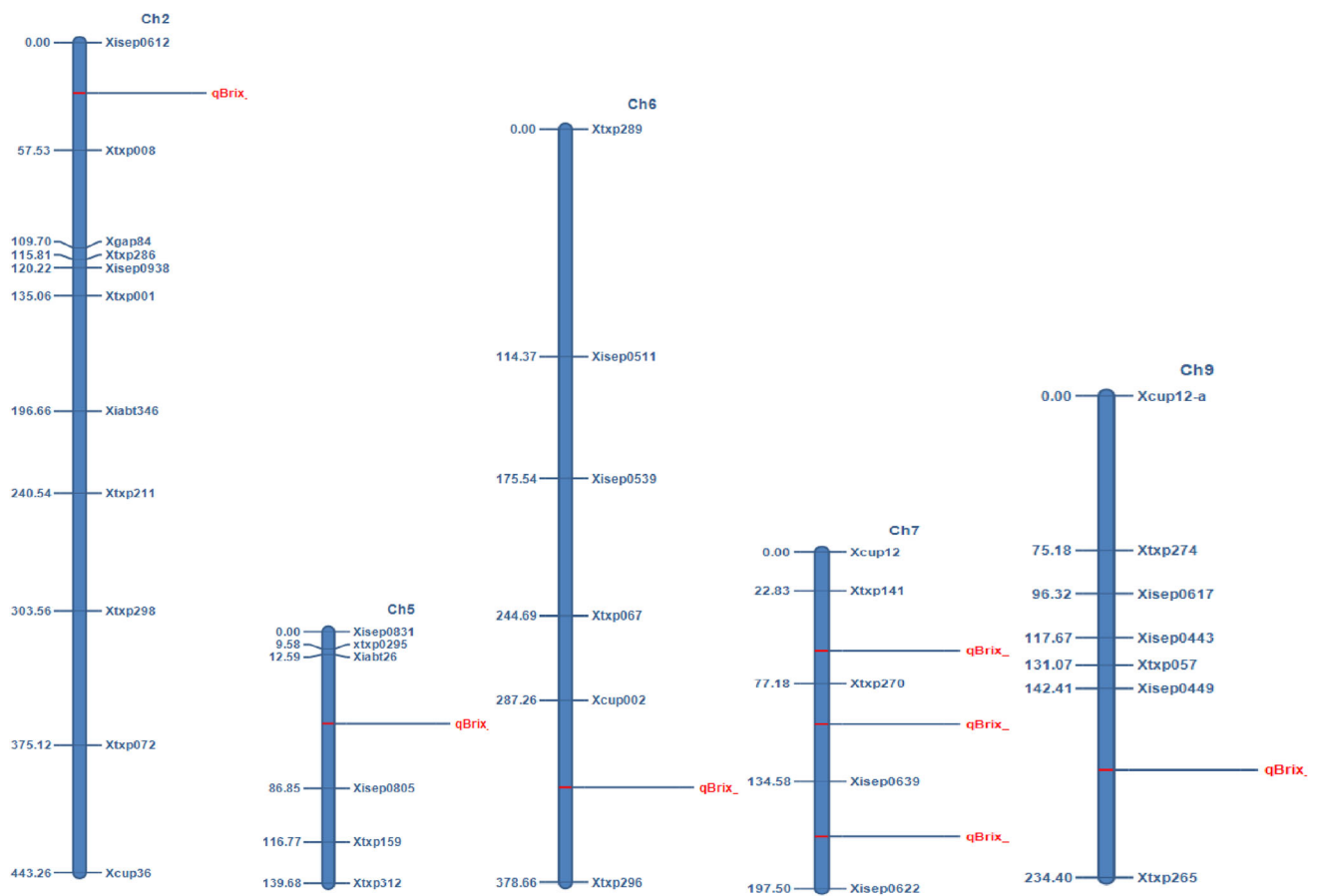


Fig. 5 Map of QTLs controlling °Brix using F_2 genotypic data and combined phenotypic data of $F_{2:3}$ mapping populations using a LOD cutoff of 3.5

important to be utilized in breeding program. Besides, we have identified a new major QTL on linkage group SBI-09. This QTL has never been reported previously and may be unique to Ethiopian sorghum germplasm. In contrary, our study failed to detect previously reported significant QTLs distributed on various linkage groups such as SBI-01 (Guan et al. 2011), SBI-03 (Murray et al. 2008; Guan et al. 2011), SBI-04 (Bian et al. 2006; Shiringani et al. 2010) and SBI-10 (Bian et al. 2006).

The major goal of many sweet sorghum breeders is to develop cultivars with improved stem sugars both in terms of sugar concentration and stem juice yield. QTLs that control these important traits are usually controlled by multiple genes and require dissection of the chromosomal region(s) using selected molecular markers. Successful identification and mapping of these novel QTLs plays a significant role to introgress the QTLs into locally adapted sweet sorghum genotypes. Major QTLs with high repeatability across locations were identified and mapped in this study. The high °Brix heritability observed in this experiment has implication that successful utilization of these novel QTLs in breeding program in the future to

improve the sweet sorghum genotypes. Therefore, the current study will serve as a foundation to start advanced sweet sorghum breeding program in Ethiopia through marker-assisted breeding approaches to improve the quality of the sorghum lines for bioethanol industry.

Acknowledgments The authors are very grateful for the financial support provided by the Swedish International Development Agency (SIDA) through Bio-Innovate project. The authors also acknowledge the Lab facilities support provided by the International Crop Research for Semi-Arid Tropics (ICRISAT-Nairobi).

Compliance with Ethical Standards

Conflict of interest We verify that we have no potential conflict of interest.

References

- Ayana, A. 2001. Genetic diversity in sorghum (*Sorghum bicolor* (L.) Moench) germplasm from Ethiopia and Eritrea. Ph.D. dissertation, Addis Ababa University, Addis Ababa, 229.
- Bhatramakki, D., J. Dong, A.K. Chhabra, and G.E. Hart. 2000. An integrated SSR and RFLP linkage map of *Sorghum bicolor* (L.) Moench. *Genome* 43: 988–1002.

- Bian, Y.L., S.J. Yazaki, M. Inoue, and H.W. Cai. 2006. QTLs for sugar content of stalk in sweet Sorghum (*Sorghum bicolor* L. Moench). *Agricultural Science of China* 5: 736–744.
- Casa, A.M., S.E. Mitchell, M.T. Hamblin, H. Sun, J.E. Bowers, A.H. Paterson, C.F. Aquadro, and S. Kresovich. 2005. Diversity and selection in sorghum: simultaneous analyses using simple sequence repeats. *Theoretical and Applied Genetics* 111: 23–30.
- Casa, A.M., G. Pressoir, P.J. Brown, S.E. Mitchell, W.L. Rooney, M.R. Tuinstra, C.D. Franks, and S. Kresovich. 2008. Community resources and strategies for association mapping in sorghum. *Crop Science* 48: 30–40.
- Crasta, O.R., W.W. Xu, D.T. Rosenow, J. Mullet, and H.T. Nguyen. 1999. Mapping of post flowering drought resistance traits in grain sorghum: association between QTLs influencing premature senescence and maturity. *Molecule, Gene, Genetics* 262: 579–588.
- Disasa, T., T. Feyissa, A. Admassu, R. Paliwal, S.M. De Villers, and D.A. Odeny. 2016. Molecular evaluation of Ethiopian sweet sorghum germplasm and their contribution to regional breeding programs. *Australian Journal of Crop Science* 10: 520–527.
- Disasa, T., T. Feyissa, and A. Admassu. 2017. Characterization of Ethiopian sweet sorghum accessions for °Brix, morphological and grain yield traits. *Sugar Tech* 19: 72–82.
- Feltus, F.A., G.E. Hart, K.F. Schertz, A.M. Casa, S. Kresovich, S. Abraham, P.E. Klein, P.J. Brown, and A.H. Paterson. 2006. Alignment of genetic maps and QTLs between inter- and intra-specific sorghum populations. *Theoretical and Applied Genetics* 112: 1295–1305.
- Gnansounou, E., A. Dauriat, and C.E. Wyman. 2005. Refining sweet sorghum to ethanol and sugar: economic trade-offs in the context of North China. *Bioresource Technology* 96: 985–1002.
- Guan, Y., H. Wang, L. Qin, H. Zhang, Y. Yang, F. Gao, R. Li, and H. Wang. 2011. QTL mapping of bio-energy related traits in Sorghum. *Euphytica* 182: 431–440.
- Harris, K., P.K. Subudhi, A. Borrell, D. Jordan, D. Rosenow, H. Nguyen, P. Klein, R. Klein, and J. Mullet. 2007. Sorghumstay-green QTL individually reduce post-flowering drought induced leaf senescence. *Journal of Experimental Botany* 58: 327–338.
- Hart, G.E., K.F. Schertz, Y. Peng, and N.Y. Syed. 2002. Genetic mapping of *Sorghum bicolor*(L.) Moench QTLs that control variation in tillering and other morphological characters. *Theoretical and Applied Genetics* 103: 1232–1242.
- Hausmann, B.I., V. Mahalakshmi, B.V. Reddy, N. Seetharama, C.T. Hash, and H.H. Geiger. 2002. QTL mapping of stay-green in two sorghum recombinant inbred populations. *Theoretical and Applied Genetics* 106: 133–142.
- Kebede, H., P.K. Subudhi, D.T. Rosenow, and H.T. Hguyen. 2001. Quantitative trait loci influencing drought tolerance in grain sorghum (*Sorghum bicolor* L. Moench). *Theoretical and Applied Genetics* 103: 266–276.
- Klein, P.E., R.R. Klein, S.W. Cartinhour, P.E. Ulanich, J. Dong, J.A. Obert, D.T. Morishige, S.D. Schlueter, K.L. Childs, M. Ale, and J.E. Mullet. 2000. A high throughput AFLP-based method for constructing integrated genetic and physical maps: Progress toward a sorghum genome map. *Genome Research* 10: 789–807.
- Kosambi, D.D. 1944. The estimation of map distances from recombination values. *Annals Eugene* 12: 172–175.
- Li, H., G. Ye, and J. Wang. 2007. A modified algorithm for the improvement of composite interval mapping. *Genetics* 175: 361–374.
- Li, M., N. Yuyama, L. Luo, M. Hirata, and H. Cai. 2009. In silico mapping of 1758 new SSR markers developed from public genomic sequences for sorghum. *Molecular Breeding* 24: 41–47.
- Lin, Y., K.F. Schertz, and A.H. Paterson. 1995. Comparative analysis of QTL affecting plant height and maturity across the Poaceae, in reference to an interspecific sorghum population. *Genetics* 141: 391–411.
- Mace, E.S., J.F. Rami, S. Bouchet, P.E. Klein, R.R. Klein, A. Kilian, P. Wenzl, L. Xia, K. Halloran, and D.R. Jordan. 2009. A consensus genetic map of sorghum that integrates multiple component maps and high-throughput Diversity Array Technology (DArT) markers. *BMC Plant Biology* 9: 1–13.
- Menz, M.A., R.R. Klein, J.E. Mullet, J.A. Obert, N.C. Unruh, and P.E. Klein. 2002. A high-density genetic map of *Sorghum bicolor* (L.) Moench based on 2926 AFLP, RFLP and SSR markers. *Plant Molecular Biology* 48: 483–499.
- Murray, S.C., W.L. Rooney, S.E. Mitchell, A. Sharma, P.E. Klein, J.E. Mullet, and S. Kresovich. 2008. Genetic improvement of sorghum as a biofuel feedstock: I. QTL for stem sugar and grain nonstructural carbohydrates. *Crop Science* 48: 2165–2179.
- Murray, S.C., W.L. Rooney, S.E. Mitchell, and S. Kresovich. 2009. Sweet sorghum genetic diversity and association mapping for °Brix and height. *The Plant Genome* 2: 48–62.
- Natoli, A., C. Gorni, F. Chegdani, P. Ajmone Marsan, C. Colombi, C. Lorenzoni, and A. Marocco. 2002. Identification of QTLs associated with sweet sorghum quality. *Maydica* 47: 311–322.
- Pereira, M.G., and M. Lee. 1995. Identification of genomic regions affecting plant height in sorghum and maize. *Genetic Resources and Crop Evolution* 90: 380–388.
- Rami, J.F., P. Dufour, G. Trouche, G. Fliedel, C. Mestres, F. Davrieux, P. Blanchard, and P. Hamon. 1998. Quantitative trait loci for grain quality, productivity, morphological and agronomical traits in sorghum (*Sorghum bicolor* L. Moench). *Genetic Resources and Crop Evolution* 97: 605–616.
- Ramu, P., K. Bantie, S. Senthilvel, C.A. Kumar, B. Jayashree, R.T. Folkertsma, L.A. Reddy, M.S. Kuruvinashetti, B.I.G. Haussmann, and C.T. Hash. 2009. Exploiting rice–sorghum synteny for targeted development of EST-SSRs to enrich the sorghum genetic linkage map. *Genetic Resources and Crop Evolution* 119: 1193–1204.
- Reddy, B.V.S., S. Ramesh, P.S. Sanjana Reddy, B. Ramaiah, P.M. Salimath, and R. Kachapur. 2005. Sweet sorghum: A potential alternate raw material for bio-ethanol and bio-energy. *Journal of SAT Agricultural Research* 1: 1–8.
- Ritter, K.B., D.R. Jordan, S.C. Chapman, I.D. Godwin, E.S. Mace, and C.L. McIntyre. 2008. Identification of QTL for sugar-related traits in sweet and grain sorghum (*Sorghum bicolor* L. Moench) recombinant inbred population. *Molecular Breeding* 22: 367–384.
- Sanchez, A.C., P.K. Subudhi, D.T. Rosenow, and H.T. Nguyen. 2002. Mapping QTLs associated with drought resistance in sorghum (*Sorghum bicolor* (L.) Moench). *Plant Molecular Biology* 48: 713–726.
- Schuelke, M. 2000. An economic method for the fluorescent labelling of PCR fragments. *Nature Biotechnology* 18: 233–234.
- Shiringani, A.L., M. Frisch, and W. Friedt. 2010. Genetic mapping of QTLs for sugar-related traits in a RIL population of *Sorghum bicolor* L. Moench. *Genetic Resources and Crop Evolution* 121: 323–336.
- Subudhi, P.K., O.R. Crasta, D.T. Rosenow, J.E. Mullet, and H.T. Nguyen. 2000. Molecular mapping of QTLs conferring stay-green in grain sorghum (*Sorghum bicolor* L. Moench). *Genome* 43: 461–469.
- Tao, Y.Z., R.G. Henzell, D.R. Jordan, D.G. Butler, A.M. Kelly, and C.L. McIntyre. 2000. Identification of genomic regions associated with stay-green in sorghum by testing RILs in multiple environments. *Genetic Resources and Crop Evolution* 100: 1225–1232.
- Tuinstra, M.R., E.M. Grote, P.B. Goldsbrough, and G. Ejeta. 1996. Identification of quantitative trait loci associated with pre-

- flowering drought tolerance in sorghum. *Crop Science* 36: 1337–1344.
- Tuinstra, M.R., E.M. Grote, P.B. Goldsbrough, and G. Ejeta. 1997. Genetic analysis of postflowering drought tolerance and components of grain development in *Sorghum bicolor* (L.) Moench. *Molecular Breeding* 3: 439–448.
- Tuinstra, M.R., G. Ejeta, and P.B. Goldsbrough. 1998. Evaluation of near isogenic sorghum lines contrasting for QTL markers associated with drought tolerance. *Crop Science* 38: 835–842.
- Wang, M.L., C. Zhu, N.A. Barkley, Z. Chen, J.E. Erpelding, S.C. Murray, M.R. Tuinstra, T. Tesso, G.A. Pederson, and J. Yu. 2009. Genetic diversity and population structure analysis of accessions in the US historic sweet sorghum collection. *Theoretical and Applied Genetics* 120: 13–23.
- Wu, Y.Q., and Y. Huang. 2006. An SSR genetic map of *Sorghum bicolor* (L.) Moench and its comparison to published genetic map. *Genome* 50: 84–89.
- Xu, W., P.K. Subudhi, O.R. Crasta, D.T. Rosenow, J.E. Mullet, and N.T. Nguyen. 2000. Molecular mapping of QTLs conferring stay-green in grain sorghum (*Sorghum bicolor* L. Moench). *Genome* 43: 461–469.