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Review article

Diversity assessment of sorghum germplasm and its utilization in genetic analysis of quantitative traits-A review

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Abstract

Utilization of plant genetic resources can play a key role in sustainable agriculture and food supply, especially after shortages due to the increasing number of people and global climate change. The use of plant genetic resources (PGR) is believed to be one of the important tools in sustainable methods of crop improvement. Conservation and utilization of plant genetic resources are valuable to meet future needs and rising issue of food security. Application of advanced molecular techniques has gained significant attention for assessment of plant genetic resources and their conservation. Mapping and QTL analysis of important morphological and agronomic traits are primary and significant approaches for germplasm enhancement in crops. In particular, employing the improved crop varieties contribute to crop adaptation and production, and in turn, answers the increased food demand due to global climate change. Identification of QTLs using DNA markers is the first strategy in marker-assisted selection (MAS) for phenotypic traits, including stress tolerance in plant breeding. In this review, we describe the importance of genetic diversity in sorghum germplasm and its utilization in crop improvement through genetic analysis of important traits. The use of two different approaches of QTL mapping, namely family based linkage mapping and linkage disequilibrium based association mapping are discussed. This article surveys how QTL-based methods contribute to a better understanding of genetics mechanism involved in controlling phenotypic traits of interest. Some of the case studies of QTL mapping, controlling important traits in sorghum, are also reported in this review.

Keywords: Genetic diversity; molecular markers; QTLs; association genetics; linkage disequilibrium; morphological traits; abiotic stresses.
Abbreviations: QTL_quantitative trait loci; PGR_plant genetic resources; LD_linkage disequilibrium; SSR_simple sequence repeats; MAS_marker assisted selection.

Introduction

Sorghum [*Sorghum bicolor* (L.) Moench], is a C_4 grass that diverged from maize about 15 million years ago, and is the fifth most important cereal grown worldwide (Doggett 2008). This grain and forage crop is especially important in the semi-arid tropics because of its unusual tolerance to hot and dry environments. Recently, sorghum has been identified as a key plant species for the comparative analysis of grass genomes (Dillon et al., 2007), and as a source of beneficial genes for agriculture. The sorghum genome is relatively small (750 million base pairs (Mb), which has been completely sequenced (Paterson et al., 2009). Its germplasm has extraordinary genetic diversity (Kong et al., 2000; Djè et al., 2010). The sorghum's incremental divergence from maize and rice (Doebley et al., 1990) makes it ideally suited for discovery and analysis of grass genes through comparative genomics.

The improvement of crop genetic resources is dependent on continuous infusions of wild relatives, traditional varieties and the use of modern breeding techniques. Diversity in germplasm is important for any breeding program, since it directly affects the potential for genetic gain through selection (Kotal et al., 2010). It also allows the plant breeders to make a classification of germplasm into heterotic groups to maximize heterosis (Menz et al., 2004).

Genetic diversity within and between populations is routinely assessed using morphological, biochemical and molecular techniques. Though morphological characterization has been traditionally used to assess genetic variation, the genetic information provided by morphological characters is often limited and expression of quantitative traits is subjected to strong environmental influence (De Vicente and Fulton, 2003). Similarly, biochemical methods have been useful in analysis of genetic diversity as they reveal differences between seed storage proteins or enzymes encoded by different alleles at one (allozymes) or more gene loci (isozymes). Even though these methods reduce the environmental influence; however, they have less power to detect low levels of variation (De Vicente and Fulton, 2003).
Molecular markers were developed soon after the plant biotechnology era to create high-resolution genetic maps and exploit genetic linkage between markers and important traits in crop plants (Edwards et al., 1987; Paterson et al., 1988). Over the last few years, molecular marker techniques have been increasingly employed in analysis of variation (Agarwal

et al., 2008; Mondino et al., 2009). Molecular markers offer numerous advantages over conventional phenotypic based alternatives as they are not affected by environmental factors, applicable to any part of the genome, easy and economical in use, and are able to distinguish polymorphisms, which do not produce phenotypic variation.

Many DNA based marker techniques such as Restriction Fragment Length Polymorphisms (RFLP), Random Amplified Polymorphic DNA (RAPD), Simple Sequence Repeats (SSR) and Amplified Fragment Length Polymorphism (AFLP) have been commonly used in different studies of plant sciences (De Vicente and Fulton, 2003; Shehzad et al., 2009a). Among them, microsatellites or Simple Sequence Repeats (SSRs) are highly versatile genetic markers due to their codominant inheritance, high polymorphism and reproducibility. Similarly, ESTs are a potentially rich source of SSRs that reveal polymorphisms not only within the source taxon, but in related taxa, as well. The most recent and modern unit of genetic variation is the single nucleotide polymorphism or SNP.

The small and completely sequenced genome of sorghum makes it an ideal crop for the application of genomics-based breeding methods. Using high-density SNP array technologies (Batley and Edwards, 2007) or genotyping-by-sequencing (Chia and Ware, 2011; Davey et al., 2011; Elshire et al., 2011; Morris et al., 2013) and next-generation sequencing platforms (Metzker, 2010), has considerably accelerated the selection gain and improved the effectiveness of breeding.

QTL mapping is an efficient approach for identifying genomic regions linked to complex growth traits and pyramiding the desirable alleles to improve drought tolerance in crops. Two main approaches are used for genetic mapping: family-based linkage (FBL) mapping and linkage disequilibrium (LD)-based association mapping (Mackay et al., 2007). FBL is a classical approach, in which the LD is created by developing a mapping population (e.g., F_2, doubled haploid, backcross, RILs, near-isogenic lines). Then the population is genotyped and examined for the trait(s) of interest in different environments. In FBL, the accuracy of gene mapping depends on the size of mapping population, genetic variation covered by the population, and number of molecular markers applied. Although, FBL is being used for gene mapping in crop plants, it is costly and has low resolution due to lower number of meiotic events and evaluates fewer alleles in a relatively longer time (Stich et al., 2008; Ross-Ibarra et al., 2007).

In contrast, LD-based association mapping have several advantages over conventional FBL mapping such as (1) requires a collection of diverse materials that is used to associate genetic markers with phenotypes of interest (i.e., many allele evaluated simultaneously), (2) offers higher resolution due to the exploitation of relatively higher number of meiotic events throughout the history of germplasm development, (3) takes population structure into account, which reduces the frequency of false positive associations (Ross-Ibarra et al., 2007), (4) the possibility of using historically measured phenotypic data and (5) no need for the development of expensive and tedious biparental population that makes this approach time saving and cost effective (Kang et al., 2008; Kraakman et al., 2004).

Sorghum is an ideal crop for association-mapping methodologies because of its moderate level of LD and its self-pollinating mating system (Hamblin et al., 2005). A panel of diverse grain sorghum germplasm for association mapping was previously reported by (Casa et al., 2008). Similarly, several QTLs have been reported by association mapping using a sorghum diverse set (Shehzad et al., 2009b; El Mannai et al., 2012). Recently, several classical loci for plant height and inflorescence architecture were mapped in sorghum using genome-wide association mapping, (Morris et al., 2012). This review will highlight the importance of sorghum genetic resources and their utilization in genetic analysis of important agronomic traits.

Diversity in Sorghum

Sorghum is believed to have a wide range of diverse germplasm. Plant genetic resources play an important role in generating new high yielding crop varieties with desired traits. Various research methodologies are employed in *in situ* and *ex situ* conservation of genetic diversity in plants. The amount of genetic diversity and rate of genetic erosion is quantified in various crops using different methods and models (Mekbib, 2012). Success of any crop breeding program is based on the knowledge and availability of genetic variability for efficient selection. Morphological, biochemical and molecular procedures have been exploited for evaluating these resources. Use of molecular techniques is becoming much more important in assessment of genetic diversity than phenotypic evaluation. Sorghum is rich in its genetic resources and conserved at many centers around the world, including International Crops and Research Institute for the Semi-Arid Tropics (ICRISAT) in India, the National Plant Germplasm System (NPGS) in USA, Chinese Crop Germplasm Information System (CGRIS) in China, etc.

Core collection of plant genetic resources

Genebanks around the world hold collections of the genetic resources of crop plants for long-term conservation and for ease of access by plant breeders, researchers and other users. Usually these collections are so large conservation and utilization of these genetic resources becomes impossible. For this reason, a limited or "core collection" could be established from an existing large collection (Frankel et al., 1984). With minimum similarity between its entries, the core collection is of limited size and chosen to represent the genetic diversity of a large collection, a crop, a wild species or group of species. It does not replace the existing collection or material, from which it is obtained. The basic scheme of core collection development and its proper utilization is given in Fig 1. Development of a core collection depends on the objectives for making the core set. It could be a world core collection, germplasm specific core collection or trait specific core collection. Mini core collections are also developed from large core collections for efficient utilization based on specific objectives. Due to the availability of large germplasm and wide range of diversity, several studies have been reported on the development of sorghum core collections and their utilization in genetic improvement (Grenier et al., 2001a, b; Upadhyaya et al., 2009). Recently, Billot et al. (2013) screened composite germplasm collection of 3367 samples and developed a community resource of reference set of 384 lines. The reference set was further evaluated with EST-SSR by Ramu et al. (2013). We also developed a SSR based sorghum diversity research set (SDRS) of 107 accessions (landraces) (Shehzad et al.,. 2009a) preserved at National Institute of Agrobiological Sciences (NIAS), Genebank, Japan. The list of accessions with other details is listed in Supplementary Table 1. The SDRS was developed from a geographically diverse base

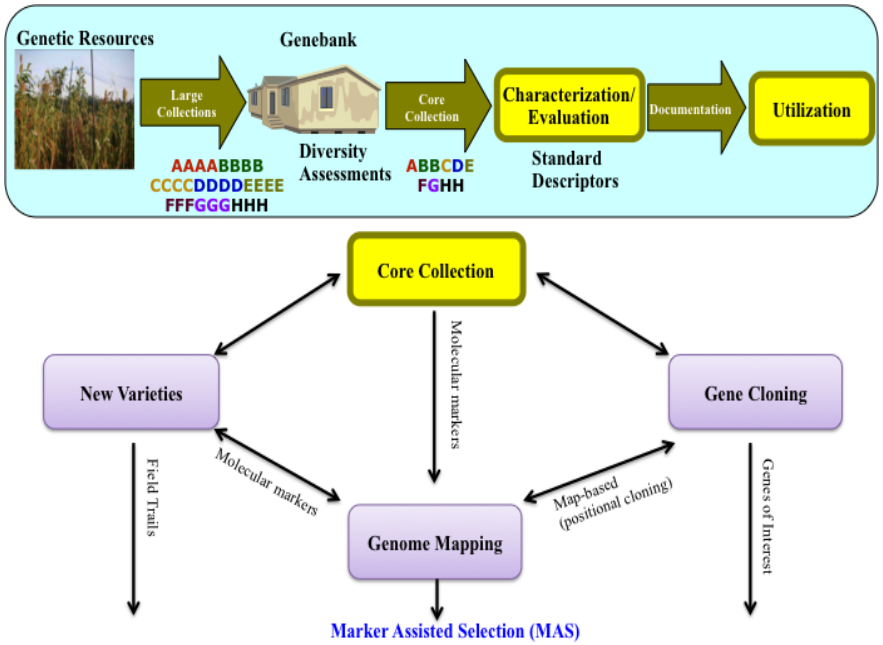

Fig 1. A General sketch showing the function of Genebanks in utilization of genetic resources through development of core collections by molecular techniques.

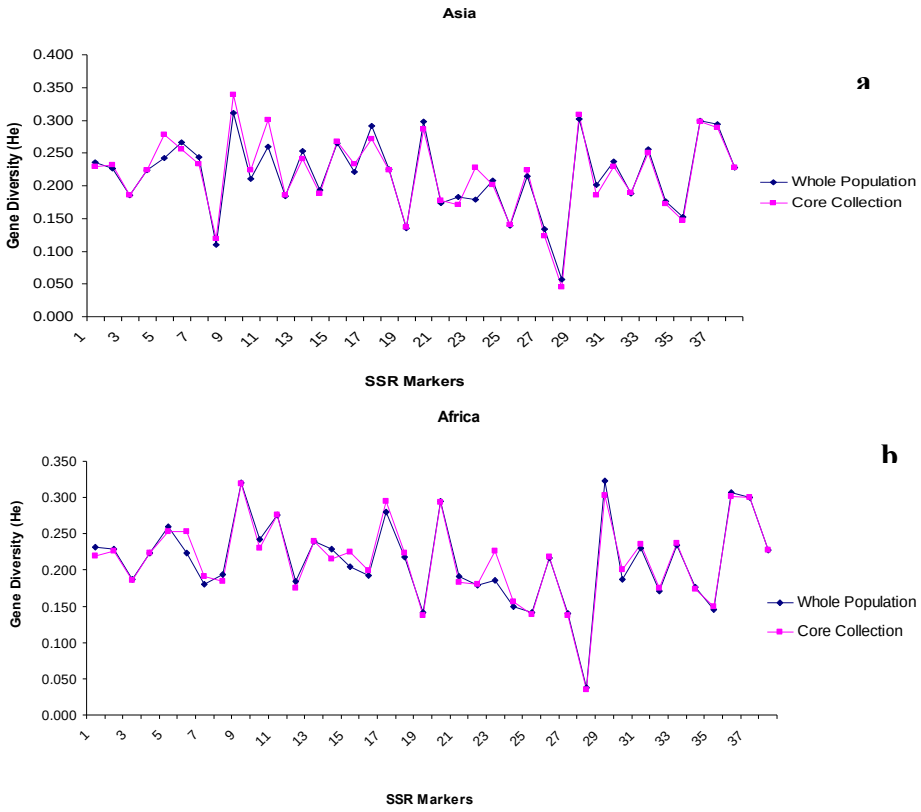

Fig 2. Comparing gene diversity values (*He*) of 38 SSR markers in whole population and core collection: (a) 206 Asian accessions, (b) 114 African accessions.

population of 320 sorghum landraces by the assessment of SSR markers, which were randomly selected from all linkage groups of sorghum. The representativeness of the collection can be judged by comparing with original population. The core set developed in this study retained 99.93 % of total alleles present in whole population. The comparison between gene diversity (*He*) estimates of SSR loci in whole population and core collection showed a negligible change as given in Fig 2 (a: Asia, b: Africa). These results validated the accuracy of our method for the development of core collection. A wide range of variation can be observed for different important agronomical and physiological traits in the germplasm, including panicle shape, ranging from loose to very compact type (Fig 3).

Genome-wide association analysis in sorghum

Recently, the idea of utilization of association mapping in crop plants is attracting more attention than conventional linkage mapping. Association mapping can be classified into two main categories (Chengsong et al., 2008). First one is candidate-gene association mapping. Here, candidate genes are selected based on prior information from different ways e.g. mutational analysis, biochemical pathway or linkage analysis. It is trait-specific and low cost, but there is a chance to miss other unknown loci. Another is the genome-wide association mapping, where genome-wide marker polymorphisms are used to study casual genetic variations. Although a large number of markers are necessary for detecting association with complex morphological traits in general, it does not require any prior information on candidate genes and there are chances to detect unknown loci (Joosen et al., 2009, Shehzad et al., 2009b).

Agronomic and morphological traits

We have used our representative set of 107 sorghum accessions (SDRS), which has retained maximum genetic diversity as well as a wide range of phenotypic variations in QTL mapping and genetic studies using association genetics. The core collection was effectively utilized in association mapping of morphological traits and several loci have been identified to be associated with morphological traits.

The SDRS was sown during sorghum sowing season in two replications. Data were recorded on 26 important morphological traits involving culm length, number of tillers per plant, panicle length, grain weight per panicle, days to heading, days to flowering, leaf length, leaf width and other qualitative traits according to the standard sorghum descriptors at Genebank. Analysis of variance showed a highly significance difference among all accessions for most of the traits. Morpho-agronomic traits could not effectively classify the accessions according to geographic origin.

For association mapping purpose, 98 sorghum SSR markers (selected from Bhattramakki et al., 2000, Kong et al., 2000 and Taramino et al., 1997) were used and all accessions in SDRS were genotyped (Shehzad et al., 2009b). The population structure was inferred with Bayesian clustering analyses with the admixture models, in which the number of populations (*J*) ranged from 2 to 9. Markov chain Monte Carlo (MCMC) sampling was repeated 1 x 10^5 times after 1 x 10^4 cycles of a burn-in period. The optimal number of populations was determined as $J = 3$ on the basis of estimated logarithmic posterior probability of the Bayesian clustering. Three different models were applied for association analysis to identify QTLs controlling major agronomic traits, which were (i) General linear model (GLM), (ii) Mixed linear model (MLM) and (iii) Bayesian based on Markov chain Monte Carlo (MCMC) algorithm. A total of 14 common significant SSR loci were identified by three different models of association analysis namely, single-QTL models with the effects of population structure, single-QTL models with the effects of population structure and familial relatedness, and multiple-QTL model with the effects of population structure. These loci were associated with 12 different morphological traits, including days to heading, days to flowering, culm length, number of tillers, number of panicles and panicle length. In this study, some common QTLs were identified for more than a single trait, for example, a single locus *Xtxp100* (Kaf) in linkage group B (LG-B) was found to be highly significant for two similar traits such as number of panicles and number of tillers. Similarly, the locus *Xtxp23* (LG-J) had association with three correlated traits i.e., days to heading, days to flowering and days to maturity. These findings could be further utilized in sorghum whole genome association mapping and will serve as landmark in mapping QTLs by association analysis.

Flowering time

Flowering time is one of the essential traits determining adaptation during crop domestication. In sorghum, flowering is considered as a crucial event as it plays a key role in adaptation and geographical distribution of this crop. The effects of photoperiod on flowering time in sorghum are essential for the crop domestication. These effects are not well understood yet (Mauro-Herrera et al., 2013; Michael et al., 2008; El Mannai et al., 2011).

El Mannai et al. (2011), performed association analysis to identify the QTLs controlling flowering time and photoperiod sensitivity using 107 accessions of sorghum grown under natural condition. A wide range of variation in flowering time was observed within a diversity research set of 107 sorghum accessions ranging from 56 to 133 days. The whole collection was classified into early, medium and late flowering groups. After selection, 45 accessions were grown under three different environments of photoperiod (11hr, 12hr and 15hr). Four QTLs controlling flowering time were detected under natural condition of day-length at threshold 2.5, using the K model. A total of 7 flowering time loci were detected under controlled conditions of day-length. In this study, one photoperiod dependednt QTL was detected on chromosome 1, while another photperiod independent QTL deteted on chromosome 4. The results showed that sorghum gradually responded to the flowering time during the process of domestication by two mechansims; firstly the sensitivity or insensitivity of genotypes to photoperiod and secondly, the inheritance of ealry or late flowering characteristic. We suggested that the exact photoperiod requirement for sorghum flowering lies in the interval of 11–12 h

Inflorescence architecture

Sorghum panicle (inflorescence) has a large diversity in type, ranging from very open and loose to a very compact type. In this study, we used a common set of quantitative measures of panicle dimensions and structure to capture the different panicle architectures such as compact and loose panicle types (Witt Hmon et al., 2013). We analyzed the variations in panicle related traits of sorghum core set of 107 accessions. We found that the patterns of frequency distribution of panicle

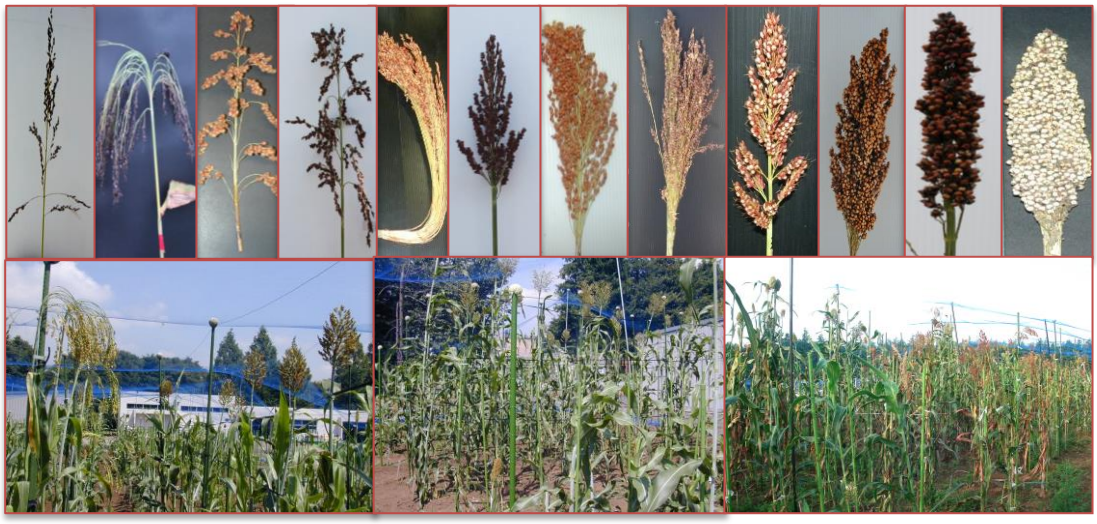

Fig 3. Phenotypic diversity among accessions in sorghum diversity research set (SDRS) for different agronomic traits.

Fig 4. Effect of Abiotic Stresses on the growth of sorghum in comparison with control conditions. (a) Control Vs. Salt Treated (b) Control Vs. Drought Treated

traits reflect the distribution of different origins. Similarly, data was recorded on yield components and its association with panicle architecture was assessed (unpublished data). A total of 41 loci have been found associated with 14 panicle traits on using the statistical model conferring both population structure as well as kinship. Among them ten QTLs were responsible for elongation traits, two branching traits and eight other panicle related yield component traits, including panicle number, panicle shape, panicle type and grain weight per panicle. The distribution of different panicle compactness and shapes showed clear trends between Asian and African accessions.

Abiotic stress tolerance (salinity/drought)

Abiotic stresses are considered to be the major limiting factors of plant growth and yield production. Climate change is thought to be a major source of abiotic stress for crops (Mittler et al., 2010). Changing patterns of temperature and rainfall are having an impact on the cultivation of food and other crops, with some formerly productive areas suffering from drought, flooding or extremes of temperature. In order to alleviate the economic impact of crop failures, research is being carried out for development, through breeding or genetic engineering of crop plants that are more resistant to these forms of stress.

Abiotic stress factors remain a major constraint to the growth and productivity of crops, especially when it occurs during developmental stages. Due to high interaction of environment and genotype, evaluation of germplasm for tolerance to abiotic stresses in a target environment is important. Breeding for abiotic stress tolerance strives to contribute to our knowledge on the genetics underlying tolerance to abiotic stresses, in particular to drought and salinity. Increased understanding of the genetics of the plant's response to drought and salinity will enable focused breeding for increased tolerance to these abiotic stresses, which will ultimately lead to crops that consistently give high yields even on marginal land and when experiencing drought or salinity stress (Henry et al., 2004; Shehzad and Okuno 2013).

Sorghum is an excellent crop for the analysis of genes contributing to environmental stress tolerance and other traits (Doggett, 2008). It has a diverse germplasm collections and a small genome size, which prove valuable for comparing with genomes of other grass species including maize and rice.

We used a diverse germplasm collection of 107 sorghum accessions (Shehzad et al., 2009a) as a mapping population to assess the natural variations for tolerance to abiotic stresses. Association analysis approach was applied to identify chromosomal regions controlling the complex mechanism of tolerance to salinity and drought stresses.

To establish an appropriate screening method, we have conducted experiments under controlled and stressed conditions for drought and salinity. Phenotypic data was recorded for different important morphological traits, which were affected either directly or indirectly by these stresses. The response of genotypes for abiotic stresses varied with level of tolerance and susceptibility as shown in Fig 4. For association analysis, different models were applied to identify the most reliable method of QTL identification. In case of salt tolerance experiment, we used 0, 150, 200 and 250 mM concentrations of salt and 250 mM was found to be the most limiting with low coefficient of variation (CV%) within genotypes (unpublished data). In our preliminary studies, we used association mapping approach to identify QTLs responsible for salt tolerance in sorghum. We have found four major QTLs for traits related to salt tolerance in sorghum. Among them, one QTL was located on chromosome 1 at the position where a gene for leaf senescence is located in sorghum.

Similarly, in cereals, the plants are more sensitive to drought stress at any stage of their development. Several stay-green QTLs are co-localized with QTLs for grain yield, flowering time, and plant height (Borrel et al., 2000; Jordan et al., 2003; Kassahun et al., 2009; Sabadin et al., 2012). We conducted an experiment under controlled (well-watered) and drought stress conditions, and the phenotypic values of 23 morphological traits were recorded (Sakhi et al., 2013). The mean values for reproductive growth traits such as panicle exertion, panicle length, panicle width, panicle weight and grain weight per plant showed significant reduction under the drought stress conditions, compared with control conditions. Under the control conditions, association analysis found 17 QTLs related with 12 traits on chromosomes 1, 2, 4, 8, 9, and 10, with $-\text{Log}_{10}$ (*P*) ranging from 2.5 to 7.6 and explaining 9.5% to 57.5% of the total phenotypic variance for the traits. Under the drought stress conditions, 9 QTLs associated with 8 traits were identified on chromosomes 1, 2, 3, and 10 that explained 9% to 61.2% of the total phenotypic variance for the traits, with $-\text{Log}_{10}$ (*P*) ranging from 2.5 to 3.5. QTLs for some traits were detected only under the drought stress condition, suggesting that these traits are important in drought tolerance. Leaf drying score (LDS), defined as the percentage of fully dry leaves on each plant. It was used as a direct parameter for the assessment of drought tolerance/sensitivity. Under the drought stress conditions, LDS ranged from 5% to 88% (average of 49%), while in the control treatment, no dry leaves were observed. A locus *Xtxp149* on chromosome 1 had strong association with LDS. These results will serve as landmark for cloning of genes controlling abiotic stress tolerance in sorghum through map-based cloning strategies.

Family based linkage analysis in sorghum

In sorghum, different kinds of molecular markers (e.g., restriction fragment length polymorphism [RFLP] markers, random amplified polymorphic DNA [RAPD] markers, amplified fragment length polymorphism [AFLP] markers, and simple sequence repeat [SSR] markers) have been used to develop linkage maps. Recently, QTL mapping has been widely used to locate chromosome regions harboring genes for important agronomic traits. Due to the presence of large diversity in our core collection, we have been using it for the selection of parents for the development of segregating population. Such populations are then used in family based linkage analysis studies for various important traits. These populations also helped us in validation of QTLs identified in association mapping.

Linkage mapping for yield and yield component

Yield is generally believed to be controlled by many genes and is very difficult to manipulate in breeding programs (Betrand et al., 2008). We investigated quantitative trait loci (QTLs) associated with yield and yield-related traits in sorghum. We measured eight morphological traits related to yield potential in a F_2 population derived from a cross between African and Japanese sorghum landraces. We also developed a genetic linkage map of 137 sorghum genome-based simple sequence repeat markers. The F_2 population showed a significantly greater range of variation than the parents (i.e., transgressive segregation) for all eight traits studied. In this study, we used two different approaches of linkage mapping as (i) single-QTL based (composite interval mapping "CIM") and (ii) multiple-QTL based (Bayesian interval mapping "BIM") (Shehzad and Okuno unpublished). We identified a total of 52 QTLs associated with the eight traits (culm length, number of tillers, panicle length, culm diameter, leaf length, leaf width, grain weight/panicle, and 100-grain weight). The percentage of phenotypic variation explained by these QTLs ranged from 3.1% to 36.3%. In this experiment, we identified a major QTL for culm length (*qtl7*CL) on chromosome 7 between SSR markers *SB4096* (93.6 cM) and *SB4024* (109.6 cM), which is the same location as one of the major dwarfing genes in sorghum *dwarf3 (dw3)*. Similarly, QTLs identified for leaf length and leaf width were in similar genomic regions as previously identified as stay-green QTL regions.

Linkage analysis of flowering time

In this study, we identified QTLs for flowering time using an F_2 population derived from a cross between Kikuchi Zairai, a late-flowering cultivar originating from Japan and SC112, an early-flowering cultivar originating from Ethiopia (El Mannai et al., 2012). The F_2 plants were grown with their parents under a natural day length conditions in early May 2008 in the experimental field of Tsukuba University. Similarly, the F_2 lines were also grown in 12 h day length in controlled condition. Linkage analyses were performed in both populations and two linkage maps were constructed using 213 simple sequence repeat markers. Under natural day length conditions, nine QTLs controlling flowering time were identified in F_2 population namely, *qFT1-1* and *qFT1-2* on chromosome 1, *qFT2* on chromosome 2, *qFT3* on chromosome 3, *qFT5b* on chromosome 5b, *qFT7* on chromosome 7, *qFT8* on chromosome 8, *qFT8b* on chromosome 8b and *qFT10* on chromosome 10. Furthermore, seven QTLs were identified under 12 h controlled day length. Among them, *qFT1-2* on chromosome 1, *qFT2* on chromosome 2, *qFT3* on chromosome 3, *qFT5b* on chromosome 5b and *qFT10* on chromosome 10 were similarly identified under natural day length. The *qFT5* on chromosome 5 and *qFT6b* on chromosome 6b were mapped only under 12 h day length. The flowering time in sorghum is controlled by a large number of QTLs with small effects, suggesting the genetic architecture of the flowering time in sorghum. We also investigated the interaction of the QTLs,

controlling flowering time in sorghum with the photoperiod that appears to be fundamental to the improvement of this crop and to feed the world's expanding populations.

Conclusions

Use of advanced molecular and biotechnological techniques will enhance the conservation and utilization of genetic resources. There is a need to develop highly advanced throughput phenotyping system for assessment of complex agro-morphological traits. Several areas of research need to be elaborated more such as development of molecular markers and linkage maps, development of EST libraries, comparative genomic studies including model plants such as Arabidopsis, rice and sorghum, and development of TILLING (Targeting Induced Local Lesions IN Genomes) techniques for the identification of genes and their function.

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