Chapter 2

Genetic Improvement of Sorghum for Biomass Traits Using Genomics Approaches

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Abstract

Nonrenewable energy resources deplete with the passage of time due to rapid increase in industrialization and population. Hence, countries worldwide are investing dearly in substitute energy resources like biofuel from miscellaneous set of feedstocks. Among the energy crops, sorghum serves as a model crop due to its drought tolerance, small genome size (730 Mb), high biomass, dry matter contents, quick growth, wide adaptability to diverse climatic and soil conditions and C4 photosynthesis. Sweet sorghum with high sugar content in stalk is an efficient feedstock for advanced biofuels and other bio-based products from sugars. However, high biomass sorghum has the utility as a feedstock for cellulosic biofuels. The enhanced yield of monomeric carbohydrates is a key to cheap and efficient biofuel production. The efficiency of lignocellulosic biofuels is compromised by recalcitrance to cell wall digestion, a trait that cannot be efficiently improved by traditional breeding. Therefore, scientists are looking for solutions to such problems in biomass crop genomes. Sorghum genome has been completely sequenced and hence this crop qualifies for functional genomics analysis by fast forward genetic approaches. This chapter documents the latest efforts on advancement of sorghum for biomass potential at morphological and molecular level by exploiting genomics approaches.

Keywords: biofuel, sorghum, association mapping, lignocellulosic feedstock, genomics, microRNAs, marker-assisted selection

1. Introduction

Though biofuels coevolved with cars, they received less importance in the past, due to abundant availability of fossil fuels at an economical price. Today, the world is experiencing

higher risks to energy security as well as its efficient utilization. These include disruptions to the supply of imported fossil fuels, limited availability of fossil fuels, and energy price spikes. Carbon dioxide emissions leading to global warming have further worsened the situation. In this context, biofuels are regaining interest, being able to present an attractive alternative to petroleum products since these are known to be biodegradable as well as renewable resources.

While primary biofuels have utility in electricity generation, secondary biofuels generated by biomass processing are used in motorization and several industries. There are first-, second-, and third-generation categories of secondary biofuels depending on the type of raw material used and the processing technology applied. Feedstocks of starch and sugar are being utilized in the production of ethanol. Corn, wheat, and milo are starch-based feedstocks, whereas sugarcane and sugar beet are sugar-based feedstocks. Sugar-based feedstocks contain simple sugars, which can be readily extracted and fermented. Sorghum (Poaceae) serves as both sugar- and biomass-based feedstocks. The crop grows well with minimal input requirements on marginal areas. Sorghum varieties possess diverse phenotypic traits to suit their usage as food, feed, energy, and sugar production. Energy sorghum has high lignocellulosic biomass that can be converted into biofuels. An economic comparison shows that cost per ton of sorghum biomass is lesser than other potential biomass crops including switchgrass [1].

High yield and stress tolerance are two main characteristics attracting the scientists and researchers to sorghum as a promising source of biomass. Efforts for genetic improvement of biomass sorghum directly reduce the overall cost of biomass-to-ethanol conversion, mainly affected by lignin content and its composition. Plant cell walls constituting most of the biomass are mainly composed of cellulose, hemicelluloses, and lignin. Lignocellulosic biofuels are produced via three processes: pretreatment, hydrolysis, and fermentation. The enhanced yield of monomeric carbohydrates leads to cheap and efficient biofuel production. Progress in sorghum feedstock genomics research is a key to enhanced bioenergy production. It relies on the integrated use of breeding and biotechnology. The tremendous source of genetic variability in sorghum world collections has made a significant contribution to sorghum improvement in many countries. Sorghum has a diverse germplasm and a relatively small diploid genome of 760–810 Mbp [2] making it well suited for genomics approaches.

2. Genetics of sorghum biomass traits

The processes like crop phenology, vegetative and productive growth exert a strong influence on biomass yield of energy sorghum. These processes are portrayed by the morphological and physiological characteristics of sorghum [3]. Improvement in sorghum yield depends on the nature and extent of genetic variability, heritability, and genetic advance in the base population. Energy sorghum has characteristic features that are associated with high biomass. These include plant height, flowering time, number of leaves and flag leaves per plant,

leaf length-width area, flag leaf area index, fresh and dry biomass index, brix value, and days to maturity [4].

Significant variability for genotype, general combining ability (GCA), and specific combining ability (SCA) for different components has been observed in sorghum [5, 6]. Genetic variability for biomass-related traits in sorghum has been reported by many scientists [7–9]. Hawkins [10] proposed an ideotype of high yielding and high biomass sorghum being tall, lodging resistant and moderately photoperiod sensitive for maximum vegetation.

Plant height in sorghum is controlled by four independently inherited *Dw* (Dwarf) genes, viz., *Dw1*, *Dw2*, *Dw3*, and *Dw4* [11]. It is determined by the interplay of the internode length and the number of nodes it produces before flowering. The *Dw* genes have partial dominance for tallness, and their effects are additive in nature. Dwarfing genes have been isolated, and dwarf forage hybrids have been developed by incorporating *Dw2* gene into forage seed and pollen parents, leading to 11% increase in leafiness but 30% decrease in forage yield [12]. High heritability has also been reported for plant height [13], number of leaves/plant [14], brix value [15], and leaf area [16] in sorghum. All these traits are under genetic control and improved in early generations.

Lignin is present within plant secondary cell wall. It not only gives rigidity and support to plant cell wall but also enhances water conductance and acts as a protective barrier against microbes [17]. Since lignin lowers the yield of fermentable sugars from cell walls, its higher concentration will negatively affect the morphogenic and industrial potential of lignocellulosic biomass [18]. Higher lignin is also associated with poor forage quality of sorghum owing to reduced access to proteins and other nutrients in the cell wall matrix. Hence, pretreatment of lignocellulosic biomass is essential to degrade lignin material in the cell wall. Researchers are trying to modify biomass composition of sorghum by targeting the genes that encode enzymes of the monolignol biosynthetic pathway [19].

Brown midrib (*bmr*) mutants arose from novel mutations in phenylpropanoid pathway leading to low lignin concentrations. Sorghum *bmr* mutants developed by chemical mutagenesis were characterized by low lignin [20]. Several allelic *bmr* genes, namely, *bmr 12*, *18*, *26*, and *6*, have been introgressed and characterized in sorghum. Most of the sorghum *bmr* mutants exhibited higher yield of fermentable sugars. A decreased caffeic acid O-methyltransferase activity was reported during evaluation of allelic genes *bmr12* and *bmr18*. Similarly, a low cinnamyl alcohol dehydrogenase activity was linked to *bmr6* [21].

In sorghum, the stay-green is a recessive trait causing retention of green leaf area at maturity (GLAM). This character may be functional or cosmetic, indicating continued leaf photosynthesis capacity during grain filling or discontinued photosynthesis from leaf greenness, respectively. High yield potential of sorghum in water scarce environment is governed by the functional stay-green. The pleotropic stay-green leads to arrest protein decline in aging leaves [22]. Sorghum stay-green types have been developed worldwide by conventional breeding. Exploiting stay-green trait in breeding programs may result in genetic enhancement of sorghum yield, industrial value, and biotic and abiotic resistance. Stay-green alleles in sorghum individually enhance grain yield under limited water availability via modification in plant architecture and water

uptake patterns. This is a quantitative trait governed by nuclear genes [23]. Various sorghums have been reported exhibiting different types of stay-green phenotypes [24].

Sorghum is a short-day plant belonging to semiarid region. The photoperiod sensitivity of sorghum is a recessive trait that causes longer vegetative period, which supports plant growth and in turn the green mass production. Sorghum maturity trait is controlled by six genes: *Ma1*, *Ma2*, *Ma3*, *Ma4*, *Ma5*, and *Ma6*. *Ma1* gene is known to be regulated by photoperiod in order to effect height and flowering time. *Ma1* has the largest impact on flowering date of all the maturity genes [25]. The *Ma5* gene, when present in the dominant form together with *Ma6*, very strongly inhibits floral initiation regardless of day length [26, 27].

Sorghum is referred as perennial grass due to its tillering capacity. The number and types of branching in sorghum are genetically controlled. Secondary and tertiary branches termed as vegetative branching are thought to be controlled by the same genetic factors [28], while distinct genetic elements might control tillering or mature branching in sorghum. Tillering enhances accumulation of sugars in sorghum stem for biofuel production [29, 30]. It imparts greater leaf area leading to higher intercepted radiations and thereby also affects biomass accumulation in sorghum. High-tillering sorghum thrives best in appropriate growth conditions where all the resources are best utilized [31]. Sorghum plants with excessive tillers and limited water availability exhibit low biomass and grain yield potential [32].

Sorghum is a C4 plant with characteristic drought tolerance owing to its efficient root system. It is a single-stemmed grass anchored by spreading and fibrous root system having primary, secondary, and supporting roots. The roots may extend from 1.5 to 2.5 m up to 1 m below the soil in all directions. Higher biomass yield is associated with the cloning of genes linked with root loci and improvement in root structure genetic design.

Significant variability in leaf size has been noticed in sorghum. This affects energy-capturing potential, conversion into biomass, and physiological activity. Smaller leaves are adapted to dry and hot regions, whereas in cooler and humid climates, plants with larger leaves are found having insufficient energy conversion capacity. Several other traits are also desirable for energy sorghum, like low grain yield, resistance to lodging, low water content, and biomass quality [32].

3. Genetic diversity evaluation using quantitative biomass traits of sorghum

Sorghum genetic resource characterization is based on morphological, biochemical, and molecular marker approaches. The most economical approach is morphological characterization for diversity evaluation and identifying promising genotypes. The agro-morphological characterization of sorghum is well reported [33]. The genetic diversity of sorghum germplasm comprising of different sorghum types was evaluated [34] by exploiting the quantitative characters. Nine of 14 quantitative traits were selected on the basis of their diverse nature. Panicle width, stem girth, and leaf breadth proved more diverse traits as indicated by principal component analysis. The hierarchical cluster analysis grouped sorghum germplasm into six classes. The clusters 1 and VI contained the maximum and minimum numbers of accessions, respectively,

while the clusters VI and IV were the most distantly related among all the clusters. The accessions grouped in cluster III had the highest average yield and hundred seed weight. The present study indicated that high yielding and diverse accessions can serve as better parents for sorghum variety development.

Disasa et al. [35] conducted a study to characterize the brix degree, grain yield, and some morphological traits of 180 sorghum accessions from Ethiopia and analyzed them under different environments. Greater variability was observed among genotypes collected from different areas. Genotypes collected from northern areas of the country showed high brix value, while the rest of the collection contained high biomass for sugar stalk yield. Cultivars with high-biomass traits and brix value were recommended for utilization in breeding programs to develop sugar-rich sorghum genotypes.

In a recent study, the genetic divergence among 208 Pakistani sorghum genotypes was estimated [36] by evaluating the 14 different quantitative traits for two consecutive years. Multivariate tools like principal component analysis (PCA) and unweighted pair-group method with arithmetic mean (UPGMA) analysis were employed. Study revealed broader variability in fresh biomass, dry biomass, flag leaf area index, leaf area index, and plant height. Broad-sense heritability was reported to be >80% for all traits in both years. The PCA showed that all the biomass-related traits with eigenvalue >1 were contributing significantly in the first three PC axes (75.39 and 71.21% for both years). This indicated the presence of maximum variability among these genotypes. Such a diverse germplasm might be a good candidate for varietal development. Pearson correlation analysis indicated that fresh and dry biomass had a significant positive correlation with leaf area index, number of leaves per plant, flag leaf area index, days to maturity, and 50% days to flowering for 2 years. UPGMA analysis classified the germplasms into 141 morphotypes and 7 classes in the first year and 136 morphotypes and 5 classes in the second year. The genotype P-13-2013 was found to be the best performer with relevance to traits such as the number of leaves per plant, stem thickness, leaf length, fresh biomass, dry biomass, and flag leaf area index. The genotypes Indian-6, BM-726, P-10-2013, and Johar-2013 showed good performance in terms of fresh biomass and days to 50% flowering.

Further, Shokat [37] performed assessment of 1300 diverse USDA sorghum collections on the basis of morphological traits for two consecutive years (2015–2017). The 24 high-biomass sorghum lines selected from the first year trials were further investigated for biomass-related traits in the second year. Out of nine traits evaluated, viz., germination percentage, number of leaves per plant, number of nodes per plant, days to 50% flowering, stem girth, fresh biomass, dry biomass, days to maturity, and plant height, three proved statistically significant, including number of leaves, number of nodes, and stem girth. Sorghum accession number NSL-54978 gave highly significant value for the number of leaves and number of nodes, while sorghum accession number PI-525981-01-SD exhibited significantly higher value for stem thickness. Correlation analysis indicated a significant relationship among stem girth and fresh biomass, days to maturity, and fresh biomass. PCA exhibited 48.9% expression for the number of leaves and number of nodes, while 46.9% expression was recorded for fresh biomass. The biplot analysis showed maximum diversity in fresh biomass, stem girth, days to maturity, and plant height characters (**Figures 1** and **2**).

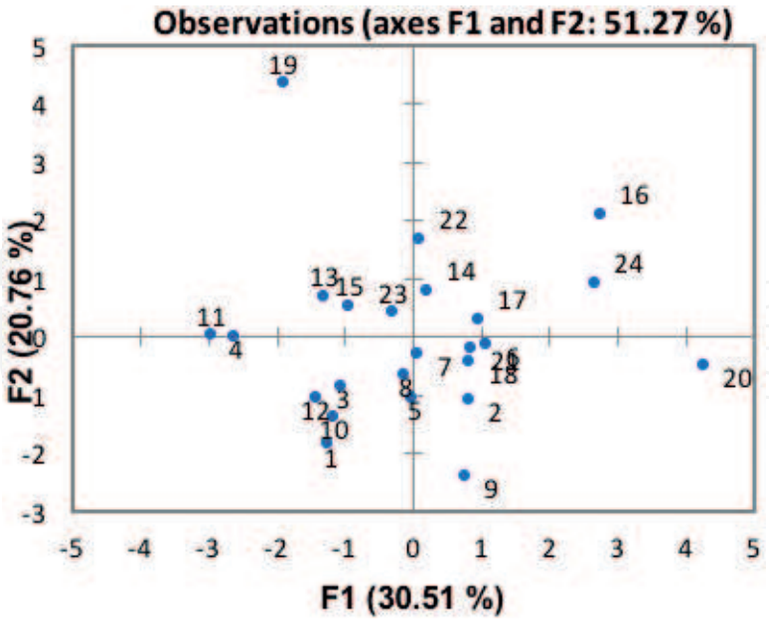

Figure 1. Biplot with sorghum genotypes.

UPGMA analysis generated 9 morphotypes of 24 sorghum genotypes (**Figure 3**). Total of 24 genotypes were divided into five different classes. Cluster analysis revealed that the main cluster was divided into two major clusters. The first subcluster comprised four genotypes (6, 24, 16, and 20), while the second subcluster was further subdivided into four different small clusters. Two genotypes were present in the cluster with blue-colored cluster, and 11 genotypes were placed in light blue-colored cluster. The class represented by red color consists of two genotypes, whereas the class represented by green color includes three genotypes.

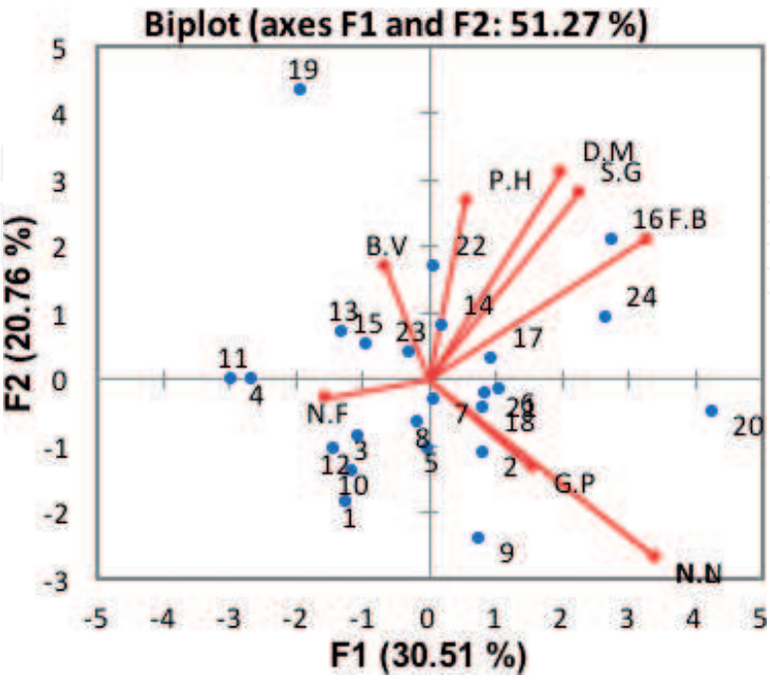

Figure 2. Biplot with cumulative sorghum variables and genotypes.

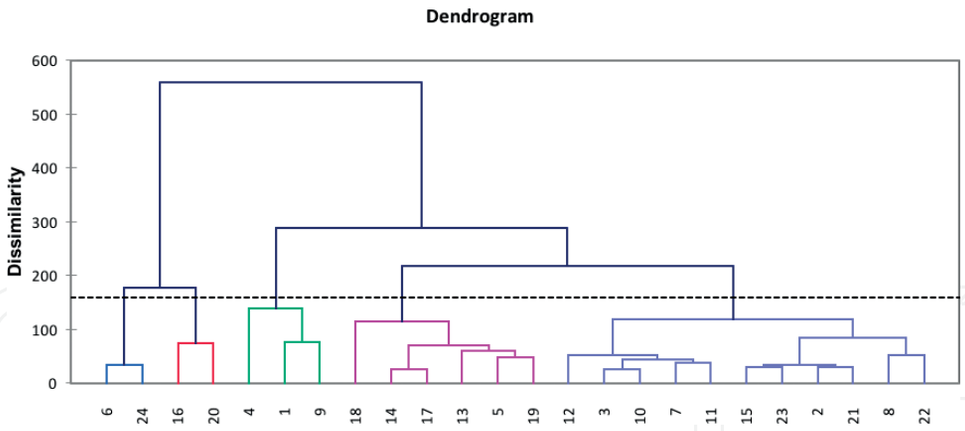

Figure 3. Cluster analysis of 24 sorghum genotypes on the basis of morphological characters.

4. QTL mapping of quantitative traits in sorghum

Germplasm characterization using morphological traits has some limitations. The expression of a phenotype is mostly influenced by the environment and depends upon plant organ as well as plant developmental stages. Owing to these shortcomings, it is the least preferred means of characterizing crop germplasms. Hence, investigating DNA polymorphism is a reliable means of genetic diversity assessment. Molecular markers are extensively used in molecular breeding being reliable, abundant, phenotypically unbiased, and time and stage independent. These markers are helpful in improving breeding programs through different ways. The marker-assisted selection (MAS) technology makes use of an association between the expression of desired characters and markers present in the DNA. Quantitative trait loci (QTL) for many traits can be evaluated by using molecular markers [38]. For a given trait in a particular population, increasing marker density can increase the resolution of the genetic map, thus enhancing the precision of QTL mapping. Genetic mapping studies are based mainly on BTx623 and other grain sorghum types. Widely used polymerase chain reaction (PCR)-based markers are RAPD (random amplified polymorphic DNA), AFLP (amplified fragment length polymorphism), SSRs (simple sequence repeats), STS (sequence-tagged sites), and DArTs (diversity arrays technology) [39–41].

Recently, there has been a growing interest in exploiting QTL mapping for different traits. About >700 QTLs have been identified for several traits in sorghum (http://www.gramene.org). However, fewer studies have been carried out to find out the molecular basis of these traits.

The biomass trait of sorghum depends on stem height and thickness, which are vital for bioethanol production. Taller varieties produce higher biomass with thicker stem and higher sugar contents. Height is positively correlated with biomass production and independent of stem structural composition like cellulose, hemicellulose, and lignin content. The QTL for total dry biomass has been found to localize with height QTLs [42, 43]. In sorghum, height is controlled by few QTLs. Genetic study has identified four loci controlling stem height: *Dw1*, *Dw2*, *Dw3*, and *Dw4* [44]. *Dw3*, which encodes a P-glycoprotein that controls polar auxin transport, has been cloned [45]. This gene is also co-localized with a height QTL on chromosome 7 [42]

and *Dw2* with QTL on chromosome 6 [46]. Another QTL on chromosome 9 was also found for height [42]. Using 377 sorghum accessions and 49 SSR markers, a height QTL (Sb-HT9.1) was mapped. Likewise, Murray et al. [43] used 47 SSR and 322 SNP markers on 125 genotypes of sorghum and identified two associations for height on chromosomes 6 and 9.

Maturity (days to 50% flowering) is also positively correlated with the biomass production [47]. The photoperiod sensitivity in sorghum was initially reported to be controlled by single maturity locus Ma1 [48]. Any genotype with a dominant Ma1 allele will show a photoperiod response, while the homozygous recessive (Ma1) will flower early. Ma1 was cloned and reported as pseudo-response regulator protein 37 [49]. The first maturity cloned locus in sorghum was Ma3 that encoded a phytochrome B [50]. Genotypes with total loss of functional ma3R allele of Ma3 are insensitive to photoperiod and flower early regardless of Ma1 allele and day length. There is an epistatic interaction between Ma1 and Ma3. Few more maturity loci have also been reported in sorghum, e.g., Ma2, Ma4, Ma5, and Ma6, with very little information about their functions. Ma2 is unmapped and shows interaction with Ma1 [51], while Ma4 is thought to be on chromosome 10 [26]. For the production of photosensitive hybrids from two plants, the Ma5-Ma6 interaction has been extensively used by the biomass sorghum seed industry.

Murray et al. [52] identified one QTL for brix (located on chromosome 1) by using 47 SSRs and 322 SNPs for a diverse panel of 125 sweet sorghums. Six marker loci related to plant height and 10 loci to plant maturity were identified [53] by using 14,730 SNPs for sorghum mini core collection. Once identified, QTLs need validation/confirmation in varying experimental conditions prior to exploitation for MAS. Wang et al. [54] used 181 recombinant inbred lines (Shihong137, a dwarf grain sorghum, x L-Tian, a tall sweet sorghum) to validate QTLs controlling plant height, biomass, juice weight, and brix value.

The study identified seven QTLs for biomass-related traits including plant height, juice, and stem fresh weight under four different environmental conditions, while three of these seven QTLs were under strong epistasis. Co-localization of many biomass-related QTLs with previously reported height QTLs confirmed that plant height regulates biomass in sorghum. On the other hand, few QTLs, namely, qSFW1–qSFW2, qSLFW6–qSLFW1, and qSLFW6–qSLFW2, were mapped to chromosomal positions where no height QTLs were located.

5. Association mapping for biomass traits in sorghum

Linkage mapping and association mapping (AM) can both be used to identify QTLs by genotyping and phenotyping the segregating populations. For association mapping (AM), the population screened on the basis of phenotypic performance is subjected to molecular marker analysis, followed by the assessment of population structure and linkage disequilibrium (LD) (**Figure 4**). Linkage mapping requires few markers, due to high linkage disequilibrium (LD), but has low resolution, while association mapping needs a large number of markers to conduct a genome-wide scan of a large number of diverse lines with low levels of LD. Association mapping has

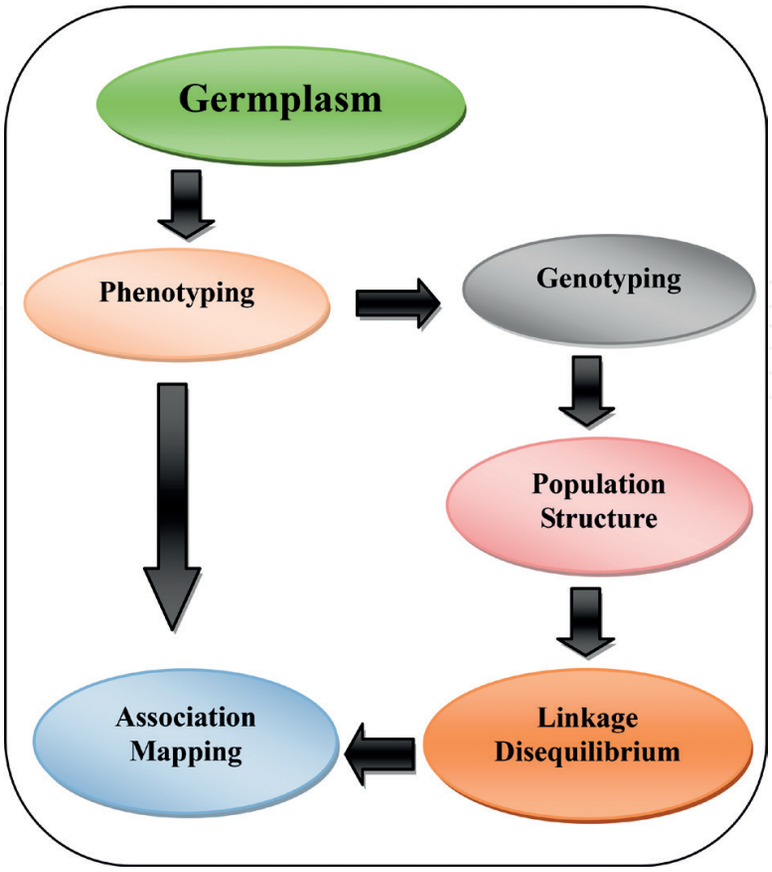

Figure 4. A simplified flow chart showing different stages of association mapping for tagging a gene of interest using germplasm accessions [36].

the ability to evaluate multiple haplotypes. Moreover, association analysis is an efficient strategy to genetically dissect the complex traits that deviate from classical Mendelian pattern of segregation.

Though originally designed for human genetics, exploitation of association mapping is picking momentum in plant improvement. In sorghum, association mapping is being applied for its genetic enhancement by phenotypic evaluation of sorghum germplasm, identifying and mapping QTLs associated with desired traits and selecting the genotypes (parents) that carry favorable alleles for gene introgression through MAS. Using 107 representative sorghum accessions and 98 SSR markers, Shehzad et al. [55] reported the association of 14 SSR loci with four traits including days to heading, days to flowering, number of panicles, and panicle length in sorghum. Another report identified two SSR markers consistently associated with plant height under two different environments [56]. Plant height and maturity date were also reported to be associated with 5 out of 39 SSR markers on chromosomes 6, 9, and 10 in 242 sorghum accessions [57].

About 300 diverse accessions of sorghum were evaluated [58] to conduct association analysis of seedling phenotypic variation during cold and heat stress treatments. They identified and validated 30 and 12 SNPs associated with cold and thermal tolerance, respectively, to determine the haplotypes in sorghum.

Recently, association mapping is performed [36] for biomass-related traits in 208 sorghum accessions of Pakistan. Diversity and structure analysis as well as association mapping analysis were performed on 94 diverse accessions, which were selected through PCA of 208 sorghum accessions. About 215 alleles were detected with an average of 3.47 alleles per locus. The range of alleles varied from 2 to 5. The polymorphic SSR markers were used to identify molecular diversity, population structure, linkage disequilibrium, and marker trait associations (MTAs). Major allele frequency was ranged from 0.13 to 0.74. The average PIC value of primers was 0.51 that ranged from 0.25 to 0.62. The admixture model-based structure analysis revealed four admixture subpopulations, which indicated that all domesticated cultivars had common ancestor with continuous gene flow. The haplotype LD block analysis showed strong linkage between xtxp219 (located at 66.13 Mb) and Xcup37 (located at 61.90 Mb) with the R2 value of 1.00, which depicted that no recombinational event occurred between these two loci on chromosome 6. The markers, Xcup12 (54.22 Mb) and sb4–sb72 (41.44 Mb) were strongly linked with R2 value of 0.90. The markers within the range of R2 value 0.60–0.70 were Xcup36 (47.11 Mb), xtxp127 (44.97 Mb), xtxp045 (49.28 Mb), sb4–sb72 (41.44 Mb), SB3630 (52.81 Mb), and xtxp127 (44.97) on chromosome 6. The LD decay was estimated to be up to 10 Mb in case of chromosome 6 with R2 value of 0.440 by using 23 polymorphic SSRs. The haplotype LD block analysis of chromosome 9 showed strong linkage between SB5111 and Xcup18 with R2 value of 1.00. The pair-wise LD decay analysis revealed LD decay at 50 kb at R2 value of 0.023.

Seven marker trait associations (MTAs) were detected by mixed linear model (MLM) approach with phenotypic variability ranging from 9.13 to 13.9% for the first year and from 6.25 to 23.05% for the second year. Four MTAs were associated with plant height, days to 50% flowering, and leaf length on chromosome 6 and three on chromosome 9 with the same traits. A total of five SSR markers expressed significant MTAs; three of these (Xgap072, Xtxp265, and SB3789) were associated with plant height, days to 50% flowering, and leaf length traits on chromosome 6. Two markers Xtxp283 and SB5040 were associated with plant height, leaf length, and days to 50% flowering on chromosome 9. Hence, chromosomes 6 and 9 appeared to carry important QTLs for biomass-related traits in sorghum.

6. Conclusion

Considering the global drive to explore alternate sources of renewable energy, biomass sorghum stands out for meeting economic demands like greater variability, short development cycle, and high calorific value in boilers. Moreover, sorghum is a good genetic model having integrated genetics-genomics-breeding platform, wide adaptability across varying environments, and diverse germplasm across the globe exploited for food, feed, fiber, and biofuel. Our chapter describes the efforts being carried out to identify and improve biomass in sorghum genotypes with great agronomic and energetic potential, dissecting sorghum genome by using omics approaches to explain the genetics of these traits and documenting the association of these traits with the genetic fingerprints of sorghum crop. The scientific record suggests that there is abundant genetic variation within existing sorghum germplasm to play around for developing high-biomass sorghum.

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