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Chapter

Sorghum an Important Annual Feedstock for Bioenergy

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Abstract

Plant-based renewable biofuels guarantee sustainable solutions to food and energy demands. High-biomass C4 grasses including sugarcane, corn, and sorghum are potential candidates for bioenergy. Among these, sorghum enjoys the status of a highly diverse food, feed, and biofuel source worldwide. The natural attributes like abiotic stress tolerance, diverse genetic base, viable seed industry, and sound breeding system make sorghum a perfect candidate for establishing an efficient and low-cost biofuel industry. Scientists are exploring ways to exploit forage, sweet, and biomass sorghums as climate-smart energy crops. In this context, conventional breeding has played a significant role in developing high-yielding sorghum varieties. For biomass sorghum, stem compositional analysis helps screen low lignin and high polysaccharide types as feedstocks for biofuels. Recent tools of phenomics, genomics, proteomics, and genome editing are key players of designing eco-friendly bioenergy sorghum. Here, we report stem compositional analysis and proteomics-based evaluation of USDA sorghum germplasm as a baseline to develop sorghum as a biofuel feedstock.

Keywords: fossil fuels, feedstock, lignocellulosic biomass, C4 crops, genome, proteomics, hemicellulose, SSR

1. Introduction

In the present-day-global-warming era and with ever-increasing number of automobiles on the roads, fossil fuel reserves are going to be scarce and depleted over next few decades. In order to provide a safer environment to our future generations, we need to use energy wisely and economically and look for alternative fuel sources like biofuels, derived from crops and their waste products [1].

Biofuels are considered zero net emitters as they use atmospheric carbon dioxide for their growth and afterward release the same when burnt in the vehicles. The biofuels are generally classified as "conventional" (the first generation) and "advanced" biofuels (the second-, third-, and fourth-generation biofuels). Biodiesel and bioethanol are categorized as first-generation biofuels. These are produced from food crops rich in higher fermentable carbohydrate level. The second-generation biofuels are most commonly extracted from switchgrass, jatropha, miscanthus, and the residues of food crops. Often, industrial wastes are also used for the production of second-generation biofuel. The biofuels extracted from algae

are classed as third generation. Major crops used for the production of biofuel are sugarcane, corn, wheat, sorghum, sugar beet, and cassava [2].

The choice of the most efficient biofuel depends upon its life cycle analysis, climatic, and economic factors. Moreover, its transportation cost to refinery, price of biofuel, and greenhouse gases also matter. Plant-based feedstocks for biofuels include crops like corn, sugarcane, soybean, poplar, sorghum, switch grass, etc. The cost-effective biofuel production depends upon the exploitation of high-yielding energy crops. Designing climate-smart energy crop with optimized composition to suit the growers, consumers, and industry needs is the backbone of cost-competitive biofuel industry. C4 grasses provide a perfect fit to this definition owing to higher photosynthetic rate, productivity, and broader genetic base of germplasm. Sorghum is a short duration crop of about 3–4 months and produces higher biomass yield with less inputs. These characteristics make sorghum a popular biofuel feedstock [3]. Sorghum has different end-use types including biomass, forage, sweet, and grain sorghums. Energy sorghum including biomass and sweet type varieties is the most efficient and climate-smart feedstock being able to grow with less inputs on marginal lands under harsh climatic conditions and having ability to utilize more sunlight [4–6].

It has diverse germplasm owing to extensive breeding and natural selection [7]. Sorghum is a crop of subsistence worldwide, the fifth most important cereal crop and an important component of poultry industry [8]. It is very responsive to biotechniques ranging from simple in vitro culture to transgenics, cisgenics, and genome-editing technologies. However, the outcrossing of sorghum with its weedy relatives has prevented regulation of GM technology in this crop. All above-ground parts of sorghum, starch, sugar, or stem biomass are utilized for the first- and second-generation biofuel production [9]. Though sweet sorghum has been widely used as a biofuel source, biomass sorghum has also been recently recognized as a promising feedstock for cellulosic ethanol production. This sorghum type usually has stem higher than 5 m, more number of leaves, fibrous roots, greater potential for vegetative growth, and is suitable for mechanization [10]. Besides producing second-generation ethanol, biomass sorghum also releases energy during biomass combustion [11]. It is a good substitute to corn and sugarcane with additional benefit of less water consumption. It is an annual grass having higher dry matter yield like perennial crops but in less duration, thus facilitating cheaper crop rotation. Recent wide scale applications of omics approaches like phenomics, genomics, proteomics, and metabolomics are enhancing the efficiency of sorghum breeding processes. Being an important element of system biology approach, omics analysis dissects the association between genes and proteins within diverse phenotypes. Genome analysis further refines this integration. Sorghum yields fuel and chemicals form sugars and cell wall biopolymers. Sorghum is a widely grown summer forage of Pakistan, while its biofuel potential is yet to be explored in the country. Information on sorghum stem quality traits is vital for designing eco-friendly biofuel source. Present study intended to demonstrate the basis of morphological characterization of 24 USDA sorghum genotypes selected under Pakistan conditions. These genotypes were subjected to proximate analysis to measure stem quality traits like crude protein, ash contents, neutral detergent fiber, acid detergent fiber, hemicellulose, cellulose, and acid detergent lignin. Translational analysis indicated a unique band of 56.1 kDa in 12 out of 24 genotypes. This uncharacterized protein is supposed to be translated by Dw1 gene (Sobic.009G229800) comprising of 510 amino acids and controls the internodal length in sorghum. In this chapter, stem composition evaluation and proteomics-based recent research involving USDA sorghum germplasm is reported in order to screen promising energy-type sorghum.

2. Analyzing sorghum biomass potential

2.1 Phenotyping biomass sorghums

Sorghum biomass is influenced by genetic and environmental factors [12]. The identification of variation in phenotypic, genetic, structural, and physiological characters of energy sorghum is vital to its improvement. Sorghum biomass improvement model relies on integrating several genomic-assisted techniques with phenomics approaches. Common field-based selection of high biomass sorghum depends upon characterizing biomass-related morphological traits like days to flowering (days after sowing), plant height, fresh biomass yield, dry matter, and dry matter yield, plant height, stalk diameter, leaf number, leaf width, leaf length, leaf angle and leaf area index, etc. [13]. Several studies report on morphological diversity assessment of sorghum for biomass traits in the field environment [14, 15].

Accurate and comprehensive phenotypic data are the baseline to elucidate genetic mechanisms underlying complex quantitative biomass traits. Since biomass-related traits are measured via destructive sampling, recording morphological data during the entire growing period of energy sorghum is possible only once. Manual, nondestructive sampling for these traits over complete development of sorghum is impossible. As compared to relatively cheaper technologies of genomic selection, association mapping and GWAS, reliable phenotyping is laborious and expensive. About 20 years back when genotyping techniques were fast advancing, improving phenotyping approaches was completely ignored. Recently, there has been a growing interest in developing effective sorghum phenotyping methods. The work started with optimizing high-throughput phenotyping systems for model plants under controlled environments. Later on, field-based phenotyping platforms were devised for short stature crops [16]. In the last 5 years, different approaches have been excogitated with promising capabilities of recording sorghum phenology in field environments. Some of these include various UAS platforms [17, 18], field-based robotic phenotyping system [19], unmanned aerial system [20], ultrasonic sensors [21], the light detection and ranging (LiDAR) [22], the time of flight cameras [23], tomography imaging [24], Kinect v2 camera [21], RGB and NIR imaging [25], and Phenobot 1.0 [26]. The next-generation phenomics tools generate enormous amount of data that are being translated via machine- learning statistical approaches into trait descriptions, relevant to sorghum breeders [27].

2.2 Analyzing biomass stem composition

The composition of biomass derived from forage, grain, and sweet sorghums has been well characterized [28]. The research on exploiting forage sorghum as biofuel was initiated in 1980s, which led to the development of photoperiod-sensitive-energy sorghum hybrids [29]. These are high biomass yielders [30]. Being relatively a recent introduction, the stem composition knowledge of energy sorghum is still limited. Up till now, a majority of research on sorghum biomass feedstock has focused more on improving yield than the quality components. So, there is a need to accurately conduct the biochemical analysis, since stem composition is the basic element influencing biofuel yield.

Plant cell walls are the main constituents of biomass that provide strength and limited plasticity to cell. The cell wall serves as a tough physical barrier, protecting interior of the cell against biotic and abiotic stresses. It is a multilayered structure composed of polysaccharides and proteins, which are important contributors of

biofuel quality and energy conversion processes. The polysaccharides are cellulose (a polymer of glucose), pectic compounds (polymers of galacturonic acid), and hemicellulose (a polymer of a variety of sugars including xylose, arabinose, and mannose). Cellulose is the largest source of glucose for biofuels. Glucuronoarabinoxylan (GAX) hemicellulose complex is linked to lignin. Since lignin component of plant cell wall provides structure, it cannot be converted to carbohydrates and hence is recalcitrant to conversion protocols. Likewise, ash content also reduces biomass to biofuel conversion reaction. Certain constituents of cell wall are water soluble like sugars, proteins, amino acids, mixed-linkage glucans, and phenolic glycosides, whereas chlorophyll, lipids, and waxes are water-insoluble ingredients that need ethanol extraction.

Different studies have reported various approaches for compositional analysis of energy sorghum leaves and stem. In some sorghum genotypes, proportion of cellulose can vary between 27 and 52%, while the range of hemicellulose content is 17–23% and lignin content is 6.2–8.1% [31, 32]. Along with the biomass yield, low lignin, high cellulose, and hemicellulose contents are also the desirable selection attributes for energy sorghum genotypes [33]. Such sorghums exhibit wide variations in biomass composition [34]. Now a days, near-infrared spectroscopic (NIR) analysis is routinely used for high-throughput computation of biomass composition [28].

Cellulosic bioethanol production requires three main steps: pretreatment, hydrolysis, and fermentation [35] (**Figure 1**). Pretreatment is performed to fractionate lignocellulose into different components via physical (boiling, steaming, and ultrasonication), chemical (acid, alkali, salts, etc.), physiochemical (ammonium fiber explosion or AFEX), and biological methods (bacteria and fungi). It increases porosity and surface area of the substrate. During hydrolysis, nonstructural carbohydrates are degraded in to sugars. Enzyme-based hydrolysis is preferred over acid hydrolysis being a mild and cost-effective process.
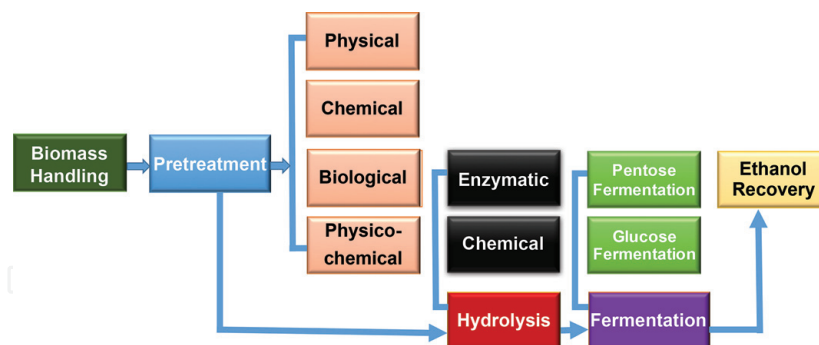
Figure 1.
Flow chart of sorghum cellulosic ethanol production process.

The process of fermentation proceeds under liquid or solid state in the presence of bacteria or yeast [36]. In a recent study, 24 sorghum genotypes (**Table 1**) were subjected to stem compositional analysis [37]. These genotypes had previously been selected on the basis of morphological traits [38].

The dried stem samples of these genotypes were grinded and used for measuring crude protein (%), ash contents (%), neutral detergent fiber (NDF %), acid detergent fiber (ADF %), hemicellulose (%), cellulose (%), and acid detergent lignin (ADL %), using the respective formulae:

$$\text{Crude protein } \% = \frac{0.1 \text{ N } H_2SO_4 \times 100 \times 6.25 \times (0.0014 \times \text{total diluted volume})}{\text{Weight of sample} \times \text{diluted digested material (ml)}}$$

(1)

Sr. #	Genotype #	Sr. #	Genotype #
1.	PI-609239-01-SD	13.	PI-329875-03-SD
2.	PI-620625-01-SD	14.	PI-330039-02-SD
3.	PI-648173-01-SD	15.	PI-330022-01-SD
4.	PI-648187-01-SD	16.	PI-456415-03-SD
5.	PI-454464-03-SD	17.	PI-329488-02-SD
6.	PI-570039-02-SD	18.	PI-155871-02-SD
7.	PI-525981-01-SD	19.	PI-457393-02-SD
8.	PI-329569-01-SD	20.	PI-329480-02-SD
9.	PI-583832-02-SD	21.	PI-303658-02-SD
10.	PI-329733-01-SD	22.	PI-303656-01-SD
11.	PI-456441-03-SD	23.	NSL-54978
12.	PI-329471-02-SD	24.	PI-257595-01-SD

Table 1.
Sorghum genotypes used for stem compositional analysis.

$$\text{Ash\%} = \frac{\text{Weight of ash} \times 100}{\text{Weight of sample}} \qquad (2)$$

$$\text{NDF\%} = \frac{(\text{Weight of crucible residue}) - \text{Weight of crucible} \times 100}{\text{Weight of sample}} \qquad (3)$$

$$\text{ADF\%} = \frac{(\text{Weight of crucible} + \text{ADF residue}) - \text{Weight of crucible} \times 100}{\text{Weight of sample}} \qquad (4)$$

$$\text{Hemicellulose \%} = \frac{(\text{NDF} - \text{ADF}) - \text{Weight of crucible} \times 100}{\text{Weight of sample}} \qquad (5)$$

$$\text{Cellulose\%} =$$
$$\frac{(\text{Weight of crucible} + \text{ADF residue}) - \text{Weight.of crucible} + \text{residue after 24 NH}_2\text{SO}_4 \times 100}{\text{Weight of sample}}$$
$$(6)$$

$$\text{Lignin/ADL (\%)} =$$
$$\frac{(\text{Weight of crucible} + \text{residue of cellulose}) - \text{Weight of crucible} + \text{residue after combustion} \times 100}{\text{Weight of sample}}$$
$$(7)$$

Statistical analysis indicated highly significant variations among all sorghum genotypes for crude protein, ash contents, NDF, ADF, ADL, hemicellulose, and cellulose contents (**Table 2**).

PCA analysis of different biochemical traits indicated three principle components (PC1, PC2, and PC3) having Eigen values greater than 1 (**Table 3**). The cumulative variability of three PCs was 82.94% for the studied genotypes. The total variability in traits shared by three PCs was 37.48, 27.37, and 18.096%, respectively. Different biomass-related traits added more than 34% of variation factor in PC1 such as: ash contents (43.7%), ADL (47.6%), cellulose (45.5%), hemicelluloses (37.4%), and NDF (48.5%). PC1 showed weak and positive correlation with crude protein (0.000%) and ADF (0.012%). The PC2 contributed for 27.37% of total variability. PC2 showed positive and strong correlation with the traits such as ADL (38.4%), ADF (50.1%), and cellulose (46.8%). Weak and negative correlation was

Eigen vectors	PC1	PC2	PC3
AC	0.437	−0.218	0.373
ADL	0.476	0.384	0.134
ADF	0.012	0.501	−0.191
C	0.455	0.468	0.097
CP	0.000	−0.188	0.777
HC	0.374	−0.481	−0.293
NDF	0.485	−0.261	−0.328
Eigen value	2.623	1.916	1.267
Variability %	37.476	27.371	18.096
Cumulative %	37.476	64.847	82.943

PC, principle component; SD, standard deviation; CV, coefficient of variation; AC, ash contents; ADL, acid detergent lignin; ADF, acid detergent fiber; C, cellulose; CP, crude protein; HC, hemicellulose; NDF, neutral detergent fiber.

Table 2.
*Principle component analysis (PCA) related to biomass traits in sorghum.*

Variables	Minimum	Maximum	Mean	SD	CV (%)
CP	4.927	10.927	7.808	1.414	1.37
AC	5.217	19.470	12.418	3.877	2.39
NDF	54.633	81.500	63.947	6.411	2.29
ADF	26.167	54.500	34.410	6.994	4.34
ADL	1.500	8.000	3.160	1.316	14.17
HC	22.087	44.150	31.419	5.981	1.64
C	29.000	57.167	39.250	7.331	2.03

SD, standard deviation; CV, coefficient of variation; AC, ash contents; ADL, acid detergent lignin; ADF, acid detergent fiber; C, cellulose; CP, crude protein; HC, hemicellulose; NDF, neutral detergent fiber.

Table 3.
Descriptive statistics for quantitative traits of sorghum germplasm.

observed for ash contents (21.8%), crude protein (18.8%), hemicellulose (48.1%), and NDF (26.1%). Crude protein and ash contents showed 77.7 and 37.3% of the factor variations in PC3, respectively.

Biplot analysis described that variables were greatly obliged as vectors; comparative length of the vector was distinguished as the relative proportion of the variability in each variable. The traits like ADL and CP, which were plotted near the central point, showed more similarities, while cellulose, ADF, NDF, and HC displayed more variability (**Figure 2**). Significant characters such as ADL, ADF, and cellulose were located at positive and positive coordinate region in biplot. Traits like AC, NDF, and HC were allocated at negative coordinate (**Figure 2**). Variability in the traits explains the variations among genotypes, which can be used in sorghum breeding plan effectively. Correlation analysis among biofuel-related stem compositional traits indicated that concentration of protein and lignin contents showed negative interaction with cellulose and hemicelluloses (**Table 4**). It showed that significant genetic variability is present among 24 sorghum genotypes. In sorghum, cellulose and hemicellulose contents play significant role in biofuel quality. For fiber

Biplot (axes F1 and F2: 64.85 %)
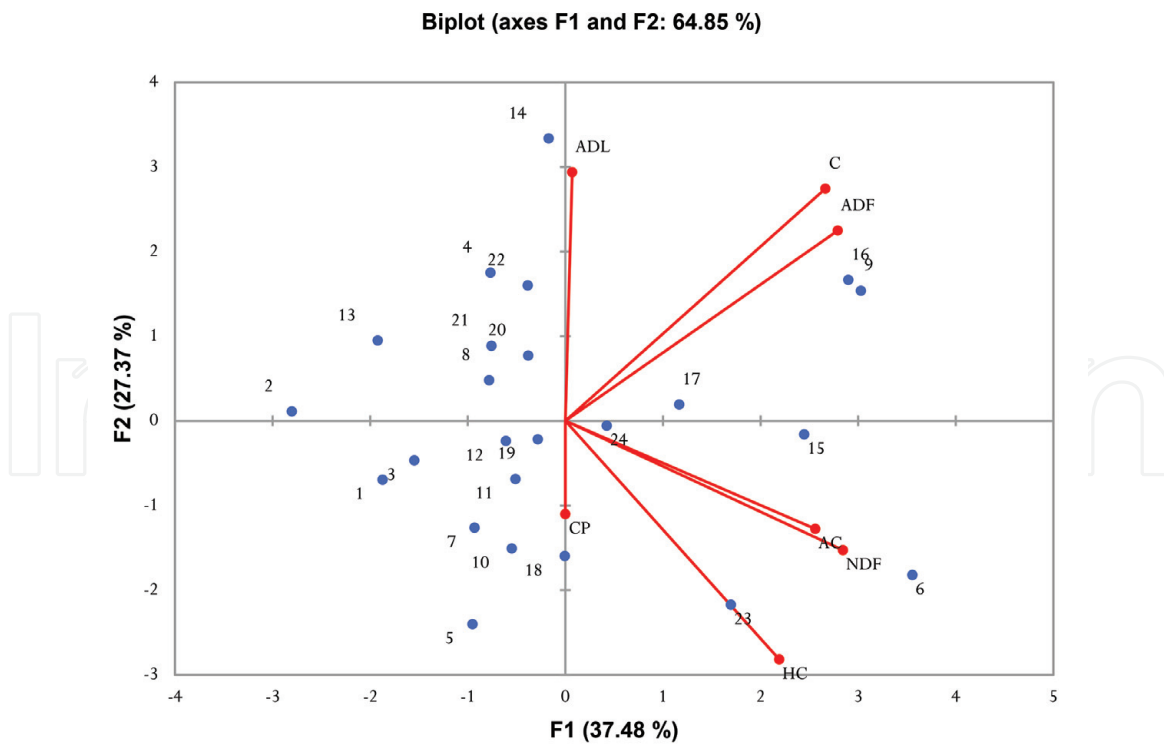
Figure 2.
PCA grouping of 24 USDA sorghum genotypes using quantitative traits.

| Traits	CP	AC	NDF	ADF	ADL	HC	C
CP	1	0.347[*]	−0.128	−0.066	−0.182	−0.051	−0.080
AC	0.347	1	0.473[*]	0.362[*]	−0.173	0.431[*]	0.311[*]
NDF	−0.128	0.473	1	0.293[*]	−0.033	0.802[**]	0.289[*]
ADF	−0.066	0.362	0.293	1	−0.153	0.067	0.955[**]
ADL	−0.182	−0.173	−0.033	0.153	1	−0.313	0.335[*]
HC	−0.051	0.431	0.802	0.067	−0.313	1	−0.016
C	−0.080	0.311	0.289	0.955	0.335	−0.016	1

[*]*Normal correlation.*
[**]*Strong correlation.*

Table 4.
*Correlation coefficients of various traits of sorghum genotypes.*

analysis, NDF, ADL, and ADF are generally used as standard quality testing techniques [39], while lignin concentration markedly affects the efficiency of hydrolysis [40].

Study reports that by increasing the level of lignin, cellulose and hemicellulose concentrations decreased. The genetic relationships among 24 genotypes were identified through construction of dendrogram on the basis of similarity matrix utilizing the UPGMA algorithm (**Figure 3**). The genotypes were grouped into two main clusters: only two genotypes (PI-583832-02-SD and PI-456415-03-SD) were present in subcluster-1, while the subcluster-2 was divided into smaller groups. The genotypes PI-570039-02-SD, PI-330022-01-SD, and NSL-54978 were grouped together and showed some distinctness from rest of the members of the group, whereas the maximum genetic relatedness was found among genotypes PI-329569-01-SD and PI-303658-02-SD followed by genotypes PI-329733-01-SD, PI-525981-01-SD, PI-303656-01-SD, and PI-648187-01-SD. The genotypes
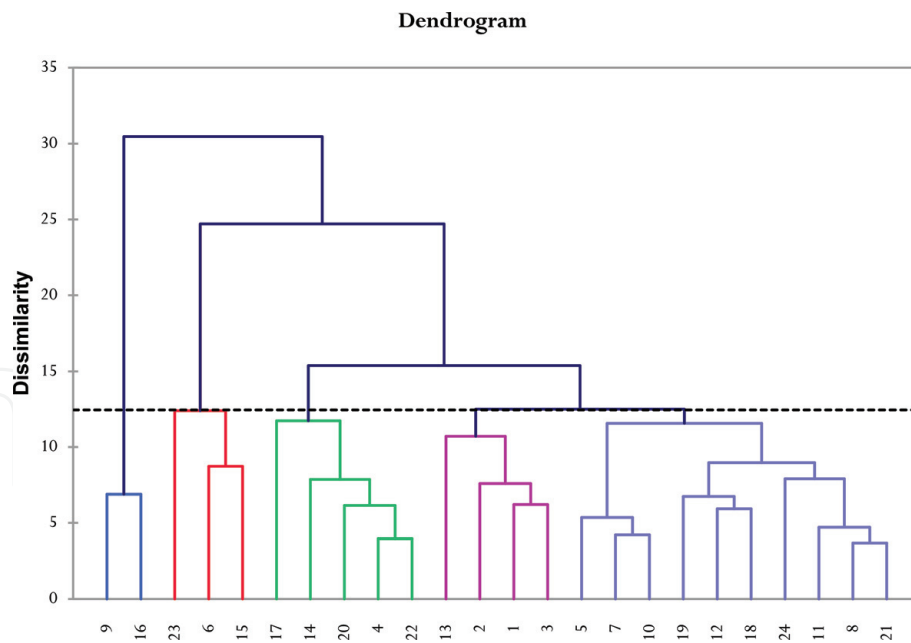
Figure 3.
Classification of 24 sorghum genotypes using UPGMA cluster analysis.
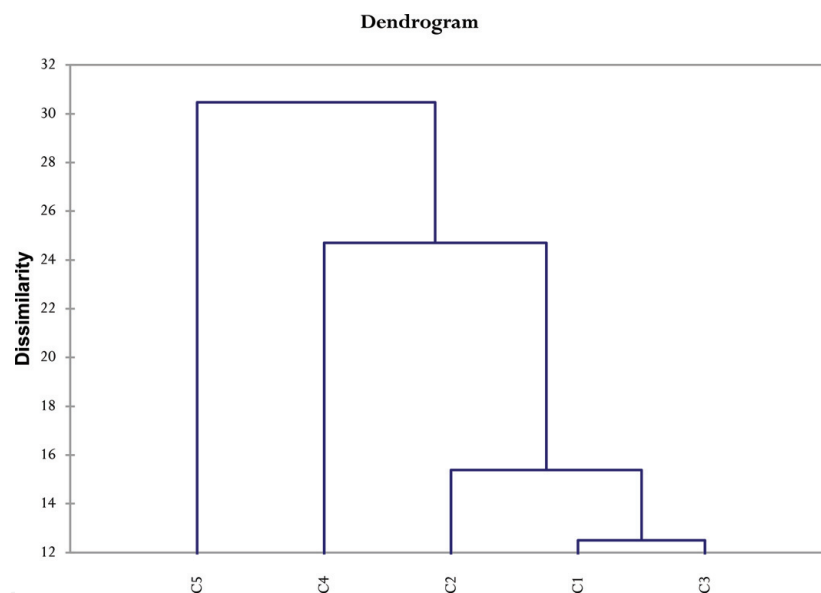
Figure 4.
Cladogenesis studies using homology-based classification of 24 sorghum genotypes.

PI-583832-02-SD and PI-329733-01-SD were also found genetically distinct from rest of the genotypes used in the study (**Figure 4**). Variance decomposition for optimal classification showed that there were 23.41 and 76.59% variances present within and between classes, respectively.

The sorghum germplasm with less lignin and protein contents is desirable for biofuel production. Sorghum genotype PI-609239-01-SD had maximum value of NDF (83.5%) and ash contents (19.5%), while genotype PI-303658-02-SD exhibited the maximum value (57.5%) of cellulose content.

Though sorghum is viewed as a cheap source of biofuel being able to grow on marginal lands, few studies have indicated the lower biofuel potential of energy sorghums grown on marginal lands than the crop land [41]. Hence, screening of energy sorghum having stress tolerance, with efficient production technology and conservation tillage practices, is the key element of sustainable commercial production of energy sorghum [5].

2.3 Transcriptional and translational analyses of sorghum biomass

The mysterious relationship between phenotype and genotype can be revealed by applying various biotechnological approaches such as proteomics, transcriptomics, and metabolomics [42]. In transcriptomics, a huge set of gene libraries can be established by employing different techniques of bioinformatics and next-generation sequencing [43]. Over the last decade, expression profiling experiments for genome-wide investigation in sorghum have been carried out to analyze responses to numerous abiotic and biotic stresses, to determine tissue-specific and genotype-specific gene expression motifs, and to disclose the genetic modification and expression divergence between different sorghum varieties.

RNA-seq technology for expression profiling has been applied in sorghum to study different gene functions [44]. This technique gives a precise assessment of gene expression at different stages of sorghum plant development [45].

Proteomics offers the set of the most efficient tools for recognition, assessment, and quantification of unique proteins. Our recent study [44] merged transcriptomic and proteomic approaches for screening sorghum germplasm best suited for bioenergy and for comparative analysis of protein expression of elite sorghum germplasm. The study was based on 24 USDA sorghum genotypes selected for biomass potential in the field experiments, which is already reported in this chapter [37]. For translational analysis, 12 out of 24 selected genotypes were divided into three groups based on stem height, since height is directly correlated with biomass in sorghum. Four short stature genotypes were chosen as negative control (**Table 5**).

Sr. #	Genotypes	Height-based groups
1.	NSL-54978	Tall
2.	PI-456441-03-SD	
3.	PI-525981-01-SD	
4.	PI-303656-01-SD	
5.	PI-457393-02-SD	Medium
6.	PI-583832-02-SD	
7.	PI-620625-01-SD	
8.	PI-456415-03-SD	
9.	PI-648187-01-SD	Small
10.	PI-609239-01-SD	
11.	PI-330039-02-SD	
12.	PI-329733-01-SD	
13.	PI-643630-01-SD	Negative control
14.	PI-643735-03-SD	
15.	PI-643581-01-SD	
16.	PI-642993-01-SD	

Table 5.
Sorghum genotypes and their respective groups based on height.

The *in vitro*-germinated, 15-day-old sorghum seedlings were used for protein extraction. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) revealed diverse banding pattern of proteins ranging in size from 14.9 to 124 kDa with different expression levels in all studied genotypes (**Table 6**).

Genotype	Protein weight (kDa)							
NSL-54978	124	97.6		64	56.1	40.5	38.7	32	14.9
PI-456441-03-SD	124	97.6		64	56.1	40.5	38.8	32	14.9
PI-525981-01-SD	124	97.6	71	64	56.1	40.5	38.8	32	14.9
PI-303656-01-SD	124	97.6		64	56.1	40.5	38.8	32	14.9
PI-457393-02-SD			71	64	56.1	40.5	38.8	32	
PI-583832-02-SD		97.6	71	64	56.1	40.5	38.8	32	
PI-620625-01-SD			71	64	56.1	40.5	38.8	32	
PI-456415-03-SD			71	64	56.1	40.5	38.8	32	
PI-648187-01-SD		97.6	71	64	56.1	40.5	38.8	32	
PI-609239-01-SD			71	64	56.1	40.5	38.8	32	
PI-330039-02-SD		97.6		64	56.1	40.5	38.8	32	
PI-329733-01-SD		97.6		64	56.1	40.5	38.8	32	
PI-643630-01-SD		97.6		64		40.5		32	
PI-643735-03-SD		97.6		64		40.5		32	
PI-643581-01-SD				64		40.5		32	
PI-642993-01-SD				64		40.5		32	

Table 6.
SDS-PAGE-based banding pattern of various proteins in sorghum genotypes.
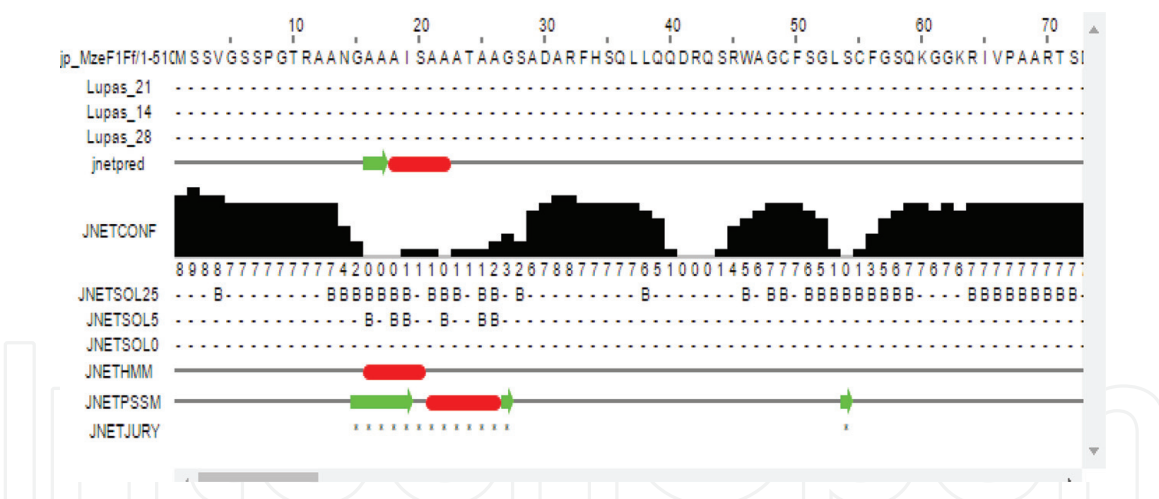
Figure 5.
Secondary structure prediction of SORB1_3009G229800 protein responsible for stem internodal length.

SDS-PAGE showed nine different bands in 12 selected sorghum genotypes. The banding pattern of four negative controls was different from the selected ones, which revealed low expression of proteins. The study showed a unique band of 56.1 kDa present only in all selected genotypes. This band represents a hypothetical protein Sobic.009G229800, which has 510 amino acids (**Figures 5** and **6**) and controls the internodal length of stem in sorghum, which is why short-stature sorghum genotypes were devoid of this protein.

Height is positively correlated with biomass production [46] and is reported to be independent of stem structural composition like cellulose, hemicellulose, and lignin contents [47]. The Quantitative trait loci (QTL) for total dry biomass has been found to be localized with height QTLs [48]. Hence, breeders aim for taller

hypothetical protein SORBI_3009G229800 [Sorghum bicolor]
Sequence ID: KXG22524.1 Length: 510 Number of Matches: 1

Range 1: 1 to 510 GenPept Graphics

Score	Expect	Method	Identities	Positives	Gaps
1041 bits(2692)	0.0	Compositional matrix adjust.	510/510(100%)	510/510(100%)	0/510(0%)

```
Query  1    MSSVGSSPGTRAANGAAAISAAATAAGSADARFHSQLLQQDRQSRWAGCFSGLSCFGSQK   60
            MSSVGSSPGTRAANGAAAISAAATAAGSADARFHSQLLQQDRQSRWAGCFSGLSCFGSQK
Sbjct  1    MSSVGSSPGTRAANGAAAISAAATAAGSADARFHSQLLQQDRQSRWAGCFSGLSCFGSQK   60

Query  61   GGKRIVPAARTSDGNGSNARGNGQSGANSNQNMPMNLSLLAPPSSPASFSNSALPSTAQS   120
            GGKRIVPAARTSDGNGSNARGNGQSGANSNQNMPMNLSLLAPPSSPASFSNSALPSTAQS
Sbjct  61   GGKRIVPAARTSDGNGSNARGNGQSGANSNQNMPMNLSLLAPPSSPASFSNSALPSTAQS   120

Query  121  PNCFLSVSANSPGGPTSNMFAVGPYANEPQLVSPPVFSTYTTEPSTAPLTPPPELAHATT   180
            PNCFLSVSANSPGGPTSNMFAVGPYANEPQLVSPPVFSTYTTEPSTAPLTPPPELAHATT
Sbjct  121  PNCFLSVSANSPGGPTSNMFAVGPYANEPQLVSPPVFSTYTTEPSTAPLTPPPELAHATT   180

Query  181  PSSPDEHNMPFLSTAYSGGSGLQASYPLYPESPCSSLISPASVTPRTGLSSPIPEQEVPP   240
            PSSPDEHNMPFLSTAYSGGSGLQASYPLYPESPCSSLISPASVTPRTGLSSPIPEQEVPP
Sbjct  181  PSSPDEHNMPFLSTAYSGGSGLQASYPLYPESPCSSLISPASVTPRTGLSSPIPEQEVPP   240

Query  241  AHWKTSRSACDTPYFRASPIPEQETTAQWKTSRSACDTPYARNSPTNIFGLDSSTPRNYM   300
            AHWKTSRSACDTPYFRASPIPEQETTAQWKTSRSACDTPYARNSPTNIFGLDSSTPRNYM
Sbjct  241  AHWKTSRSACDTPYFRASPIPEQETTAQWKTSRSACDTPYARNSPTNIFGLDSSTPRNYM   300

Query  301  LDSNFFRPAASAQFYLDQAQQTFPHNGGRVSVSREKQDADEIEAYRASFGFSADEIVQSQ   360
            LDSNFFRPAASAQFYLDQAQQTFPHNGGRVSVSREKQDADEIEAYRASFGFSADEIVQSQ
Sbjct  301  LDSNFFRPAASAQFYLDQAQQTFPHNGGRVSVSREKQDADEIEAYRASFGFSADEIVQSQ   360

Query  361  SYVGIPDAVDESFSISPFGNNAPATEICPFSDLPNEVQKVDKSCAYAKDGTSPKKSANQL   420
            SYVGIPDAVDESFSISPFGNNAPATEICPFSDLPNEVQKVDKSCAYAKDGTSPKKSANQL
Sbjct  361  SYVGIPDAVDESFSISPFGNNAPATEICPFSDLPNEVQKVDKSCAYAKDGTSPKKSANQL   420

Query  421  SIDSPNKVLRLDVFKGTKGGHQSEDEGIVKDGHPFRRTTDEISLKPIEVRKKSLPGHSCS   480
            SIDSPNKVLRLDVFKGTKGGHQSEDEGIVKDGHPFRRTTDEISLKPIEVRKKSLPGHSCS
Sbjct  421  SIDSPNKVLRLDVFKGTKGGHQSEDEGIVKDGHPFRRTTDEISLKPIEVRKKSLPGHSCS   480

Query  481  DAEIEYRRTRSLRDANGVLSRRSALARQLH    510
            DAEIEYRRTRSLRDANGVLSRRSALARQLH
Sbjct  481  DAEIEYRRTRSLRDANGVLSRRSALARQLH    510
```

Figure 6.
Blast result for confirming the SORB1_3009G229800 protein against NCBI database.

Names and taxonomy	
Protein	Uncharacterized protein
Gene	SORBI_009G229800
Organism	*Sorghum bicolor*
Taxonomic identifier	4558 [NCBI]
Proteomes	UP000000768
Chromosome	9
Sequence databases	CM000768 Genomic DNA Translation KXG22524.1
Last sequence update	November 2, 2016

Table 7.
Profile of SORBI_3009G229800 protein translated from Dw1 *gene and upregulated in top sorghum genotypes.*

genotypes in sorghum biomass improvement plans. Chromosomes six, seven, and nine carry QTLs for height in sorghum. This protein (Sobic.0 09G229800) is considered to be translated from Dw1, a gene greatly conserved in plants (**Table 7**). Earlier reports showed that *Dw1* enhances the internodal length and weight of sorghum plant [49] and is in turn important for plant biomass production.

3. Conclusion

Energy sorghum is considered to be a promising biofuel feedstock to counteract the depleted fossil fuel reserves. To keep pace with fast progressing sorghum genomics, recent phenomics tools have been evolved that are more efficient than traditional laborious field-based manual phenotyping methods. This chapter describes the results of recent studies involving 24 selected biomass sorghums. The

genotypes with low lignin, high cellulose, and hemicellulose components have been identified. Furthermore, with the help of translational analysis, an uncharacterized protein (Sobic.009G229800) is identified in tall sorghum genotypes. It regulates plant height by altering the length of internodes. Sorghum feedstock's stem compositional analysis, genomics, phenomics, and proteomics are enabling technologies extensively used by sorghum researchers for selection of elite sorghum germplasm with biofuel potential.

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