

Review Article

A Review on the Sorghum for biofuel and microRNAs

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Abstract

Energy is lifeline in development and progress of a country, with pivotal use in industrial and agricultural sectors. While fossil fuels are primary source of energy, its rapid depletion is major concerns of the world today. Developed countries are finding alternates to strengthen its economic backbone by resolving limited supply of energy issues and meeting their enormous demands. Plants can offer better alternative as biofuels to address the energy crisis and meet projected energy demand. Recently, sorghum based research remained center of attention due to its exceptional properties to grow under dry, and hot environmental conditions with limited water requirement. All parts of sorghum have economic values with usage in syrup, sugar, fuel, alcohol, bedding and paper production. Whereas, Sorghum stalks are enriched source of carbohydrates with 16-18% of fermentable sugar that makes it potential candidate for bioethanol production. Bioethanol is considered environmental friendly as it reduces greenhouse gases and replaces MTBE (Methyl tert-butyl ether) pollutants in air. Yet, the benefits of sorghum as biofuels comes with a challenge of rapid degradation of sugar, therefore immediate harvest after maturity can ensure high sugar content. This review covers scope and recent research on sorghum in Pakistan and indicates its usage as an ideal feedstock to meet present energy crisis. Currently, developed countries are exploiting sorghum in bioethanol production due to its high tolerance to drought and salt, and improved sugar content in lieu of using sugarcane and maize. Moreover, studies involving high-tech research and role of microRNA in high yield and improved sugar content in biofuel production of sorghum are also addressed in this review.

Keywords: Sorghum; Biofuels; miRNA; Bioethanol; Economic and environmental concerns

Introduction

Sorghum based research in Pakistan

Pakistan is an agriculture based country with variety of important cereal crops, including sorghum. Sorghum ranks fifth among important cereal crops of the world, and is top summer grass of Pakistan and commonly

called as 'Jawar.' It is widely used as staple food for humans besides its usage as fodder for animals. Sorghum is warm-weather crop and adapted to wide range of soil and climate conditions [1]. Its grain comprises of 70% carbohydrates, 10-12% protein and 3% fats, while fodder is highly enriched with

prussic acid and oxalic acid, therefore grains can be introduced in feeding programs for poultry and cattle [2]. Around 2.35 million hectares of land, contributing in 12% of the cultivated land is cropped area for fodder production in Pakistan (Agricultural Statistics of Pakistan, 2005). Though production of good quality forage requires well organized livestock industry, food crop land cannot be compensated for the fodder cultivation. Therefore, legumes are inter-cultured with cereal and forage crops to increase fodder supply. Although cereal crops give high yield, their protein content is lower than legumes [3]. Yield of sorghum is higher even from small farm area as compared to other plants however, the forecasted values of sorghum production revealed decrease in cultivation trend in near future. This decrease in cultivation trend is due to improper use of sorghum for industrial purpose and use of low yielding varieties [4]. Nevertheless, the research on sorghum has been increased in recent years while advancing from physio-morphological characteristics. Previously, germplasm characterization was performed using physiological, morphological or isozyme markers. Recently, DNA finger printing has removed the barriers in germplasm characterization by providing enough genetic information that was unavailable at phenotypic level. Tabbasum and her coworkers [5] distinguished two sorghum hybrids while identifying 78.98% genetic resemblance among them using random amplified polymorphic DNA (RAPD) [5]. Advancement in molecular techniques has helped identifying genetic diversity among crops and thus use of agronomic, physiological and morphological traits have been improvised to the level of biochemical and molecular markers. A study conducted by Mehmood et al. [6] approached phylogenetic relationship and genetic diversity of 10 sorghum varieties through

RAPD analysis, and found 78.94% polymorphism. Through their work sorghum varieties, YSS-9 and 84G01 showed distant relationship, while RARI-S3 and RARI-S-4 were found to be closely related [6]. Sorghum has diverse genome, therefore, genetic fingerprinting provides an efficient marker system. In 2010, another genetic diversity study on 29 sorghum varieties was conducted among exotic, and approved local lines using RAPD, revealing 95% polymorphism among the varieties. They found that the genotypes of exotic lines K-A-113 and Indian III exhibited maximum similarity, whereas F-606 and F-601 showed distinctive features [7].

Till date genotypes of many crops are evaluated using physiological markers, as recent study [8] evaluated drought tolerance in sorghum (80 accession) using physiological parameters revealing significant differences in all accessions under stress. Osmotic pressure was considered potential trait for drought tolerance, while five accessions (80265, 80114, SS-95-4, SS-97-7 and 80377) were found widely tolerant to water stress [8]. Furthermore, a study conducted on role of exogenous salicylic acid in salinity tolerance on post germination seedlings (PARI-S-4 and YSS-9) revealed that low level of salicylic acid reverse the impact of salinity in seedlings but at high salt concentration (50 mg L⁻¹): beyond threshold causing high level of Na⁺/ K⁺ ratio, salicylic acid was found ineffective in reducing salinity [9]. Another study on determining effect of exogenous proline in reducing impact of salinity in sorghum cultivars revealed that low concentration (50 mM) of proline boost physiological characteristics of sorghum, whereas as high concentration (100 mM) together with high salt concentration alleviated the adverse effect caused by salt stress [10]. Akram et al. [11] determined genetic variability for drought tolerance in

20 genotypes of sorghum cultivars using 10 RAPD primers. Maximum similarity of 95.5% was noted between YSS-17 and PARC-SS-1 genotypes, while minimum similarity of 51.5% was observed for DS-97-1 genotype. Furthermore, maximum similarity was observed between YSS-10 (C) and YSS-18, YSS-98 and SV-10, CSV-15 and Rasili, VI-1 and RS-29 [11]. In another study, 17 of sorghum landraces were evaluated for grain yield and drought tolerance using physio-morphological markers at seedling and post flowering stages, revealing high genetic difference in water stress tolerance for higher grain yield [12]. Hussain et al. [13] evaluated eight genotypes of sorghum of Potohar region for various grain and associated traits, showing significant difference in grain yield, plant height, stalk yield, days to 50% flowering and maturity. Among tested varieties of sorghum, SPV-462 was stalk producing variety with higher grain yield, whereas Johar and CSP-15 SPV were high grain producing varieties, while SPV and YSS were high stalk producing varieties. Among the sorghum varieties tested, PARC-SS-2 took minimum days to 50% flowering, whereas, CSV-15 and YSS-9 took longest time to 50% flowering [13]. Sher et al. [14] investigated the effect of harvest time associated with P and S fertilization on yield and quality of forage sorghum (*Sorghum bicolor* (L.) Moench). They concluded that harvesting at maturity stage along with P and S fertilization increases forage yield and other quality related traits as: 30% decrease in leaf hydrocyanic acid content, and 50% increase in stalk soluble-solid content widely used as an indicator of forage juiciness and palatability [14]. Furthermore, a study [15] on effect of potash fertilizer dose on sorghum hybrid (Pioneer MR-Buster) and maize hybrid (Pioneer 3062) showed high grain yield with increase in potash level and number of split

applications. Potash fertilizer caused significant differences in all parameters except for plant height, thus, highest grain yield was recorded in maize (8014 kgha-1) with three-split dose of 120 kgha-1, as compared to control with minimum grain yield. Therefore, recommended application of 120 kgha-1 of potash fertilizer with 3 splits can help improve grain yield for both crops [15].

In Pakistan, salinity is among major challenges that adversely affect the yield and growth of crops to various extents. Therefore, cultivation of salinity tolerant lines can bring solution to obtain economical yield. Kausar et al. [16] screened physiological parameters of sorghum lines to identify salinity tolerance. Among all sorghum lines, Sandalbar and JS-2002 were considered tolerant, while JS-263 and Hegari had medium tolerance. Sorghum line Noor was conceived as medium sensitive, whereas, PSV-4 and FJ-115 were found sensitive to salinity [16]. Another study for effects of heavy metal (NiCl) on morphological characteristics of sorghum demonstrated that increasing concentration up to 90 ppm (of NiCl) can adversely reduce dry weight of sorghum while 30ppm was considered minimum threshold level [17]. Seed priming technique widely used to stimulate the seed emergence and seedling growth has been studied by Shehzad et al. [18] to evaluate effect of these different techniques such as: un-soaked seed (control), Halopriming with KNO₃ and CaCl₂ (1% solution), Hydro-priming (soaked with distill water) etc. On germination and seedling growth of three sorghum varieties (JS-2002, Hegari, and JS-263). All priming techniques accelerated germination rate by 50%, while Halopriming with KNO₃ and CaCl₂ helped improving seedling growth and sorghum emergence [18]. Genetic potential of 20 sorghum genotypes was evaluated for drought

tolerant traits (heritable and measurable) after artificially creating water stress via PEG treatment and five (80353, 80365, 80199, 80204 and 80319) were found superior to be used in drought tolerant breeding programs. Therefore, crops at early growth stages can be screened using different heritable morphological parameters for drought stress [19]. Seven advanced lines of sorghum, i.e., Noor, Hegari, F-9917, F-207, F-214, JS-2002 and PC-1 were evaluated for nutritive profile as well as dry matter and forage material, and Hegari genotype was found superior in terms of forage material as well as nutritive dry matter due to high leaf area [20]. F-9917 and F-214 displayed poor performance in terms of ash content, crude protein and fiber needed to be served for forage purpose. These findings on new genotypes revealed that genetic variation among sorghum genotypes can contribute in potential traits for forage purpose and therefore, can be exploited [20]. Although hydrogen cyanide (HCN) is associated with it, sorghum is considered main forage crop for livestock. Zahid et al. [21] determined the effects of HCN content at different growth stages (of plant, i.e., 3rd leaf (GS1), pre-booting (GS2) and 50% heading stage (GS3) and post cutting) for local sorghum cultivars: including JS-2002, and Chakwal sorghum. Sorghum JS-2002 was considered safe for forage purpose due to high crude protein percentage, lowest HCN content and, higher crude fiber at pre-booting stage. Moreover, though the content of HCN was found high in young and early growth stages, yet it decreased and crude fiber increased in mature and advanced growth stages. Furthermore JS-2002 cultivar, at GS2 stage, presented lowest content of HCN after 18 hrs of post cutting [21]. Individual and combined allelopathic effects of root/shoot powder and water extract of sunflower and sorghum applied on soil were evaluated for seed emergence and

growth of rice and weeds: (*Dactyloctenium aegyptium*, *Trianthema portulacastrum* and *Eleusine indica*. Sorghum) and it was found that sunflower water extract caused high inhibitory effects by reducing 50% time required for seed emergence compared to the powder treatments [22].

During 2011, a new sorghum hybrid variety was introduced for general cultivation in Pakistan. This dual purpose sorghum hybrid Sorghum 2011 was developed by crossing Australian No.7 (exotic) with Sugrorib (local) through pedigree breeding method in year 1998-99. During 2002-03 to 2009-10 homozygous progenies no. F6-6019 were bulked to evaluate various traits for high yield and uniform fodder, and Sorghum-2011 hybrid showed exceptional performance compared to Hegari, JS-263 and JS-2002 existing cultivars, in terms of high nutritional value, low fertilizer requirement, improved water efficiency, high grain yield, robust growth, wide adaptability and green fodder with minimum HCN content [23]. Akhtar et al. [24] demonstrated impact on quality of forage sorghum when these were sown individually or together with forage legumes. Quality and quantity of forage sorghum intercropped with forage legumes, was significantly higher as there was better mixed green forage yield obtained when forage sorghum was grown in 30 cm apart rows and cluster bean was in between the rows [24].

Another study was done on response of sorghum varieties JS-263 (old cultivar), JS-2002 and Chakwal Sorghum (recent cultivars) on different levels of soil moisture using physiological and morphological traits. At high soil moisture JS-2002 showed higher potential than Chakwal Sorghum while JS-263—old genotype showed insufficiency to face drought. Therefore, JS-2002 and Chakwal Sorghum holds promising future in areas subjected to dry spells [25]. Hayyat et al. [26] demonstrated

the effects of viable textile effluent concentrations, on physiology of Sorghum vulgare Pers CV-5000. They concluded, that the plant growth was adversely affected by maximum concentration of textile effluents which was found injurious in comparison to control [26]. Six sorghum parent varieties (V-1, SV-6, CVS-13, SPV-462, RARI-S-10, TSS-9) and nine crosses were evaluated for fodder yield by heterosis and combining ability, revealing significant difference for all the traits under assessment. This study [27] indicated that a significant improvement in fodder yield of crosses is possible due to better and robust performance of these genotypes than parent heterosis along with good general combiner parents (V1, CVS-13). One of such sorghum line TSS-9 was selected for number of tillers per plant, while V-1 presented highest GCA for plant height, and CVS-13 was noted for stem thickness, fresh weight per plant, and dry weight per plant [27]. In a study eight sorghum varieties (JS-2002, JS-263, MR-Sorghum-2011, Hegari, Pak-China-1, Sandal Bar, F-7017 and F-114) compared for yield and other quality related attributes, showed significant difference in dry matter yield, morphological traits, forage yield, and quality parameters (Table 1). Pak-China 1 had difference in maximum number of traits, while F-114 differed in minimum traits. F-7017 and Sandal bar showed maximum crude protein and crude fiber compared to other varieties [28]. Raza and Naheed [29] determined correlation of high yield and its related biometric characteristics among sorghum hybrids improving yield. Their results concluded that biometric characteristics: such as yield and plant population; number of green leaves and plant population; plant height and yield were positively correlated [29]. In another study sorghum accessions (10) were evaluated for drought tolerance and showed great variability for water stress

tolerance at seedling stage. Among all traits studied in the young seedlings; shoot related traits were found highly sensitive to water stress. Thus accession No.1749 was considered drought tolerant, while F-2007 and F-2008 were found drought susceptible [30]. Kausar et al. [31] explored variation in physiological parameters due to salinity stress related to nitrogen metabolism of sorghum (Table 1). The sorghum genotypes having efficient N-metabolism were considered tolerant due to high biomass production under saline stress. Salinity influence was less in sorghum lines JS- 2002 and Sandalbar and thus were categorized as tolerant, while Noor was found to be medium sensitive and FJ-115 was labeled as sensitive one [31].

Recently, a new pathogen *Curvularia lunata* (Wakk.) was identified from the leaves of sorghum in Punjab Province, Pakistan. Early symptom indicat small reddish brown lesion that further increases in size and merges to form oblong lesion with chlorotic centers and affects the entire field. This pathogenic strain showed 99% of 584-bp sequence resembles with *C. lunata* strain pingxiang a causative agent of leaf spots of lotus particularly in China. *Curvularia* species are known for causing leaf spot disease in various grass species, yet identification *C. lunata* on *S. bicolor* in Pakistan is among primitive reports [32]. Kandhro et al. [33] demonstrated economical, effective and eco-friendly weed management strategy by intercropping sorghum dwarf variety MR-Buster, sunflower hybrid Hysun-39 and cotton seeds variety Sindh-1. Their results showed that intercropping of these three seeds, i.e cotton, sorghum and sunflower can significantly enhance yield and reduce 59.6% of weeds. Moreover, twice inter-culturing can decrease 67.5 % of weeds whereas application of Dual Gold 960 EC controlled 53.4 % of weed, while significant strengthening the

cottonseed yield. The analysis of variance showed that intercropping of sorghum or sunflower with cotton suppressed weeds significantly by 59.6 %, respectively and significantly enhance growth, while inter-culturing twice reduced further weeds and gave significantly higher cottonseed yield. Among all the strategies, total crop productivity was highest through intercropping of sunflower and sorghum with cotton as compared to Dual Gold 960 usage in term of cottonseed yield [33].

A study conducted by Mehmood et al. [34] evaluated impact of mulching (no mulch application, poultry manure mulch, and wheat straw mulch) and tillage application (zero tillage, reduced tillage and conventional tillage) on soil fertility and sorghum grain productivity. Poultry mulching enhanced soil fertility and sorghum grain yield significantly higher than other mulching practices, while tillage affected all soil properties except organic matter, and soil pH, whereas conventional tillage was found superior for grain yield. Their study concluded that limited poultry manure mulching and tillage can improve soil properties and sorghum grain yield [34]. A field experiment [35] determined growth and efficient uptake of nitrogen (N), potassium (K) and phosphorus (P) in sorghum from different organic components [Humic acid (HA), Farmyard manure (FYM), and Press mud (PM) and its compost from rock phosphate] in sorghum. The grain and dry mass yield, as well as plant height and uptake of nutrients significantly increased due to organic composts in saline effected soil. Therefore, different types of organic compounds have potential to improve growth by increasing nutrient uptake in saline effected soils [35]. Crop breeding is an excellent strategy to increase plant yield and producing stress resistant varieties. Ten sorghum genotypes were tested through physio-morphological

traits for their resistance toward drought stress, together with impact of drought on fodder yield and quality. Three different levels of water stresses (100%, 75%, and 50%) provided to sorghum genotypes revealed variable results. Sorghum-11 and NARC-11 were reported to be drought tolerant, while F-114 was found drought sensitive [36]. Sorghum genotypes differed from each other on the basis of morphological traits, through a study conducted by Ghani et al. [37] that evaluated grain and other quality associated traits in sorghum. Their study reported following: YSS-17, YSS-10 (Cream), and YSS-9 were high grain producing, while YSS-10 (Cream) and YSS-9 also showed high fodder yield. YSS-98 (control) was reported for 50% flowering in minimum 79 days, whereas, YSS-10 (Cream) took maximum 86 days to 50% flowering. Among the tested genotypes, the study showed YSS-9 as dual-purpose variety with grain having no tannin contents and high grain and fodder yields [37]. Twenty four genotypes of sorghum including SS 97-2 (S1), PARC-SS-1, No. 1500, No. 1692, T-3-DADU, 1572-T, S-98-6, SS-95-4, No. 1761, No. 1542, No. 1620, No. 1728, SP-1832, SS-97-10, SS 98-3, No.1828 (2001), F-9806, R-19, No. 1623, BR-123, PARC-SV-2, SS-95-RG, SS-95-RG and BMR-RED UNI) were tested for morphological traits and yield to determine heritability, genetic variability, and genetic advance. The results revealed high variability for all the studied characteristics, whereas phenotypic co-efficient of variation was slightly higher than genotypic co-efficient of variation. This study indicated that expression of physiological and morphological traits were highly heritable in sorghum population and can be exploited to improve crop productivity in breeding program [38]. Another study on fodder yield potential of nine sorghum varieties through evaluating morphological characteristics

showed, significant differences in fodder yield, number of leaves and leaf area, yet plant height was found insignificant trait. Number of leaves was highly variable among all varieties, yet variety-Local Tandojam gave highest yield, whereas variety Giza-3 had the lowest yield [39].

A study was conducted on phyto-extraction of heavy metals: like lead, chromium, and cadmium (along with EDTA as chelating agent) having different concentrations at various physiological levels in sorghum. The study revealed that the heavy metal accumulation of cadmium, chromium, lead, and EDTA adversely affected shoot length, fresh weight and dry weight of *S. bicolor*. The metal uptake and accumulation also increased by increasing metal concentration, whereas 5mM EDTA enhanced metal uptake [40]. Sorghum and sunflower contains several allelochemicals inhibitory metabolites now used as natural and cost effective tool for weed suppression. A study tested impact of inter-culturing:, Dual Gold 960, sorghum and sunflower water extracts different concentrations and intervals, as well as sorghum and sunflower water extracts in combination with Dual Gold 960, for controlling weeds (Table 1). The results

suggested that application of sorghum water extract in combination with Dual Gold reduced 66.6% weeds with improved yield of cottonseed allowing maximum income benefits, followed by sunflower combined with Dual Gold that lowered 65.5% weeds, while lastly sole application of Dual Gold reduced weeds by 55.9% with low yield of cottonseed. Nevertheless, inter-culturing twice exponentially suppressed 67.7% of weeds, yet had low income benefits as compared to sorghum and sunflower water extracts in combination with Dual Gold. Therefore, sorghum water extract with Dual Gold showed maximum benefits in terms of net income, yield and low weed [41]. Another study conducted on allelopathic effect of sunflower and sorghum powder via soil incorporation and water extracts, showed substantial reduction of germination and morphological traits of cotton seedlings. Allelopathic effects of sorghum powder on growth and morphological traits of cotton seedlings were found superior compared to water extracts. Therefore, utilizing the allelopathic potential of sorghum and sunflower can be effective strategy for weed management [42].

Table 1. Sorghum based research in Pakistan

S. No.	Sorghum variety	Purpose	Result	Refs.
1.	Two hybrids of Sorghum	RAPD analysis to find DNA polymorphisms	8 (40%) primers detected polymorphism between the hybrids, 33% were polymorphic	[5]
2.	10 Sorghum varieties: DS-97-1, 84-Y-00, RARI-S-3, RARI-S-4, Mr. Buster, 86-G-87, 84-Y-01, 85-G-83, PARC-SS-1, YSS-9	Molecular characterization	(RARI-S3 and RARI-S-4 are closest; YSS-9 and 84G01 show distant relationship)	[6]
3.	29 Sorghum: Hegari Noor, JS-2002 F-507 F-603 F-505 F-215 FJ-9601 F-9809 F-502 F-9917 F-2012 F-9606 F-2022 F-214 F-2020 F-2018 F-9706 F-9603 F-604 F-601 F-508 F-606 Indian III, Australian-7 K-A-113 Australian-6 China small A82L-36	Genetic divergence among genotypes, using RAPD markers	Highest similarity between Indian III and K-A-113 (both exotic lines), while the F-601 and F-606 were most diverse genotypes	[7]

4.	Sorghum Seed of 80 Sorghum accessions	Drought tolerance through physiological markers	Five accessions (80265, 80114, SS-95-4, SS-97-7 & 80377) were tolerant	[8]
5.	Two Sorghum lines, viz., PARI-S-4 and YSS-9	Role of exogenous salicylic acid in salinity tolerance	Application of 25 ppm SA was effective in salt tolerance	[9]
6.	Two cultivars of Sorghum	Salt tolerance by exogenous application of proline	Proline alleviated the adverse effects of salt stress	[10]
7.	Rari-S-4, SPV-462, CSV-15, RS-29, PARC-SV-10, YSS-9, PARC-SS-2, Johar	Evaluation of physiological parameters	SPV-462, CSP-15 and Johar produced higher grain yield, YSS-9 and SPV-462 produced higher stalk yield, YSS-9 and CSV-15 took maximum days to 50% flowering while variety PARC-SS-2 took minimum days to 50% flowering. PARC-SS-2 and Johar showed earliness to mature, while YSS-9 matured late	[13]
8.	Seeds of 20 Sorghum (RASILI, VI-1, SPV-462, CSV-13, DS-97-1, PARC-SS-1, RARI-S-3, RARI-S-4, YSS-9, RS-29, PARC-SS-2, YSS-98, YSS-10 (Red), YSS-10 (Cream), YSS-17, YSS-18, YSS-19, SV-10, JV-2002, CSV-15)	Drought tolerance (using 10 RAPD primers)	DS-97-1, SPV- 462, YSS-10(R), YSS-19, JV-2002, PARC-SS-2 and CSV-13 were found genetically diverse (genotypes are an important source for drought tolerance)	[11]
9.	Chakwal Sorghum (var Moench)	effect of harvest time associated with P and S fertilization on yield and quality of forage	Quality traits are improved when plants were harvested at a more advanced maturity	[14]
10.	Seventeen Sorghum landraces	Exploited to establish morpho-physiological criteria for drought tolerance and higher grain yield in sorghum at seedling and post flowering stages	Morpho-physiological markers can be exploited for drought tolerance in breeding programme	[12]
11.	Maize hybrid (Pioneer 3062) and Sorghum hybrid (Pioneer MR-Buster)	Effect of split doses of potash fertilizer on maize and Sorghum	In sorghum and maize maximum grain yield was recorded with the application of 120 kg/ha-1 of Potash with three splits.	[15]
12.	JS-2002, JS-263, Hegari-Sorghum, PSV- 4, Sandalbar, Noor, FJ-115	Salinity tolerance	JS-2002 and Sandalbar (tolerant), Hegari- sorghum	[16]

			and JS-263 (medium tolerant), Noor (medium sensitive), FJ-115 and PSV-4 (sensitive)	
13.	Twenty accessions of Sorghum (var Moench) 80353, 80365, 80199, 80204, 80319, SS-97-7, 80174, SS-95-4, 80077, 80265, 80369, 80374, 80381, 80236, 80203, 80376, 80158, 80364, 80114, 80214	Drought tolerance	promising drought tolerant accessions (80353, 80365, 80199, 80204 and 80319)	[19]
14.	Three Sorghum genotypes: Hegari, JS-263 and JS-2002	Effect of Seed priming techniques on seed emergence and seedling growth	seed priming accelerates emergence of sorghum	[18]
15.	Seven lines of Sorghum: Hegari, Noor, F-214, JS-2002, F-207, PC-1 and F- 9917	Performance of forage material and nutritional profile of dry matter	Hegari was preferred for forage purpose	[20]
16.	JS-2002, Chakwal Sorghum and local cultivar	Identify the suitable cultivars having low HCN content Stress disrupts normal growth and contribute toward increased HCN toxicity. Common cause of HCN in sorghums is drought	JS-2002 has the lowest HCN content	[21]
17.	Sorghum (<i>Sorghum bicolor L.</i>)	Effect of NiCl (salt)	Morphological parameters effected at high concentration	[17]
18.	Sorghum and Sunflower	Allelopathic effects of water extracts of sorghum and sunflower alone and in combination on the germination and seedling growth of rice and weeds	sorghum with sunflower water extracts showed more inhibitory effects on germination	[22]
19.	Sorghum-2011: a cross Sugrorib (local) × Australian No.7 (exotic), Sorghum-2011, JS-2002, JS-263, Hegari	Develop dual purpose sorghum cultivar	Sorghum-2011 superior than other cultivars	[23]
20.	<i>Sorghum bicolor L.</i>	Agro-qualitative response of forage sorghum (<i>Sorghum bicolor L.</i>) sown alone and in mixture with	Sorghum intercropped with cluster bean produced significantly higher mixed green forage yield	[24]

		forage legumes		
21.	JS-2002, Chakwal Sorghum and JS-263	Growth response in drought	(JS-2002 and Chakwal Sorghum)	[25]
22.	<i>Sorghum vulgare</i> Pers CV-5000	Effects of textile effluent	Textile effluents are injurious at high concentrations.	[26]
23.	Nine Sorghum crosses and their six parents (V-1, SV-6, CVS-13, SPV-462, RARI-S-10, TSS-9)	Estimation of heterosis for fodder yield	Heterosis in fodder yield is feasible Better combiner parents (V1, CVS-13)	[27]
24.	Ten different accessions of Sorghum: F.C-26-I, NO.1749, F.S.9902, F-2007, AK-113, NOOR, F-2-2007, F-2008, M.R-SORG- 2011, F-114.	Drought tolerance at seedling stage	Promising drought tolerant accession (NO.1749) and drought susceptible (F-2007 and F-2008)	[30]
25.	Eight forage Sorghum cultivars: JS-2002, JS-263, MR- Sorghum-2011, Hegari, Pak-China-1, Sandal Bar, F-7017 and F-114	Comparative performance of different sorghum forage cultivars regarding yield and quality attributes	Maximum physiological value recorded in Pak-China-1 followed by F-114, and minimum in Sandal-Bar Crude protein and crude fiber were more in Sandal bar and F-7017	[28]
26.	Two tolerant (JS-2002 and Sandalbar) and two sensitive (Noor and FJ- 115) Sorghum genotypes	Salinity induced changes in nitrogen metabolism of sorghum	Salinity influenced Noor as medium sensitive and FJ-115 sensitive. Sorghum lines JS-2002 and Sandalbar were tolerant, Noor medium sensitive and FJ-115 as sensitive	[31]
27.	Seeds of cotton: variety Sindh-1, Sorghum dwarf variety: MR-Buster, Sunflower hybrid: Hysun-39	Weed management through intercropping	Intercropping of both sorghum and sunflower in cotton was effective in weed management	[33]
28.	Sorghum hybrid (46 genotypes)	Find correlation among yield of sorghum hybrid and its isometrical characters	Yield and plant population; number of green leaves and plant population; plant height and yield were correlated	[29]
29.	Leaves of Sorghum	Leaf spot disease	Pathogen identified as <i>Curvularia lunata</i>	[32]
30.	Sorghum variety JS-2002	Effect of tillage and mulching practices on grain yield and soil fertility	Reduced tillage operations and poultry manure mulching is suitable for improving soil properties and grain yield of sorghum	[34]
31.	Sorghum	Influence if organic materials on growth and phosphorus uptake	use of different organic materials improves sorghum growth	[35]
32.	Eight Sorghum genotypes: YSS-9, YSS-10, YSS-17, YSS-10, YSS-98, YSS-18, YSS-19, YSS-32	Grain yield and related traits	YSS-9 proved as a dual-purpose variety with reasonable grain and fodder yields	[37]
33.	Ten genotypes of Sorghum: FS-08 FSD-11 NOOR F-114 F-113 NARC-11 AARI-10 AARI-08 FA-08	Drought tolerance	NARC-11 and Sorgh-11 were drought tolerant F-114 was drought sensitive	[36]

	Sorgh-11			
34.	SS 97-2 (S1), PARC-SS-1, No. 1500, No. 1692, T-3-DADU, 1572-T, S-98-6, SS-95-4, No. 1761, No. 1542, No. 1620, No. 1728, SP-1832, SS-97-10, SS 98-3, No.1828 (2001), F-9806, R-19, No. 1623, BR-123, PARC-SV-2, SS-95-RG, SS-95-RG and BMR-RED UNI	Yield and morphological traits	No. 1623 showed higher stalk yield while SS 97-2 (S1) give highest grain yield	[38]
35.	Nine sorghum varieties: Local Tandojam, F-9902, F-9917, No. 1863, JS-263, F-9909, Js-2002, Local Quetta, Local D. I. Khan, Giza-3	Green fodder yield potential	Local Tandojam produced highest green fodder yield while Giza-3 produced lowest yield	[39]
36.	Sorghum	Phyto-remediation of Lead, Chromium and Cadmium	Application of 5mM EDTA enhanced the uptake of heavy metal	[40]
37.	Sorghum or sunflower water extracts	Weed management	Dual Gold (herbicide) in combination with sorghum or sunflower water extracts is effective weedicide	[41]
38.	Sorghum and sunflower	Phytotoxic potential on cotton seedlings	Sorghum showed superiority over sunflower in allelopathic efficiency	[42]

Research on biofuel production from Sorghum worldwide

Sweet sorghum is an emerging, potential candidate to serve for biofuel production due to its vast adaptability, easy cultivation, and high yield potential. Intensive exploitation of its diverse germplasm in various breeding programs has led to improved syrup, grain and forage yield. Monk et al. [43] performed study on increasing yield of sorghum hybrid in USA. The study demonstrated that both stalk and grain of this crops can contribute in energy sector such as 60 Mg ha⁻¹ of fresh biomass can produce 5000 liters ha⁻¹ of ethanol [43]. Another study explored potential of sorghum as a renewable energy resource, as six sorghum cultivars were assessed for fermentable sugar production by determining agronomic traits, physiological characteristics and yields in nine different locations of USA [44]. Total sugar yield was variable for different locations, ranging from 4 Mg ha⁻¹ to 10.7

Mg ha⁻¹, whereas Hawaiian island contributed highest yield of sugar with 12 Mg ha⁻¹. Furthermore, per these sugar yields theoretical ethanol production was ranged between 2129 L ha⁻¹ to 5696 L ha⁻¹. Sweet sorghum are high adaptable as compared to other tropical plants that makes them superior and exceptional source of fermentable carbohydrates in diverse regions [44]. Smith and Buxton [45] determined impact of various factors such as: nitrogen fertilizers, as well as irrigated and non-irrigated conditions of USA on sugar yield of sweet sorghum. Addition of Nitrogen fertilizers had minute effect on improving fermentable sugar production. Their finding showed average of 3100 to 5235 liters ha⁻¹ of ethanol yield for 2 year, suggesting potential use of sweet sorghum as an energy crop [45].

Biofuels hold promising future in energy and transport sector to supply electricity and liquid fuels without any modifications in

current infrastructure. Woods [46] studied the potential of sweet sorghum under available condition and resources of Zimbabwe. The finding suggested 60 tons of total fresh weight of sorghum yielding 45 tons of fresh stems contributed in 3000l/h of anhydrous ethanol and 12.6 GJ of electricity. Furthermore, he suggested that sweet sorghum can contribute in region's electricity and liquid fuel by utilizing only 1% of the land for growth and processing of crop [46]. Monti and Venturi [47] evaluated performance of four monocultures of sorghum and wheat cultivars at variable doses of nitrogen fertilizers to determine net energy, and energy use efficiency. Sweet sorghum could contribute maximum 50% of net energy than wheat and fiber sorghum when grown in climatic conditions of Italy (Figure 1). These finding suggested that ethanol production can be maximized to 90% if dry matter and bagasse of crop is also used; otherwise contributed net energy ratio was low. Moreover, highest ethanol production is obtained from straw, and resulting in low percentage of ethanol if straw is not processed [47]. However, structural carbohydrates makes up lignocellulosic biomass on digestion and thus fermentation has high potential to produce energy per hector as compared to nonstructural carbohydrates that include sugar and starch. Murray et al. [48] demonstrated that energy yields can be maximized in sweet sorghum if grain and biomass related traits are targeted for improvement. They evaluated genetic basis of 31 traits involved in biomass yield in F1 generation of grain sorghum 'BTx623' and 'Rio' sorghum line, and identified 110 quantitative trait loci. Many structural and non-structural carbohydrate related QTLs were found co-localized with flowering time, height, and stand density-tillering. They identified separate genetic control for structural carbohydrates and for protein

accumulation in stem, grain and leave that contribute in maximizing energy yields [48]. They further identified QTLs responsible for high yield, with altered composition of stem sugar and grain with no pleiotropic effects. For example, a QTL on chromosome 3 was responsible for 25% genetics variance for stem sugar concentration while remained disassociated with any grain QTL. This finding suggested that non-structural yield can be improvised by altering genetic potential of grain and stalk by opting for QTLs responsible for sweet and high grain traits. This strategy can develop feedstock with qualitative traits and high yield to counter energy and food crisis [49].

Zhao et al. [50] evaluated carbohydrates, biomass and calculated ethanol yield (CEY) of five sorghum cultivars in Beijing, China from the day of anthesis to 40 days after anthesis (DAA). Although, all cultivars differed significantly in dry biomass, and carbohydrate content; yet, hybrid as feedstock for bioethanol conversion was comparatively higher performer than other cultivars. The environmental conditions and harvest time also influenced carbohydrate content and biomass production that ultimately affects the ethanol yield. However, the CEY increased with increase in crop cultivation duration and after anthesis period due to elevated accumulation of carbohydrates [50]. Miller and Ottman [51] determined the effect of frequent irrigation on sorghum growth and ethanol yield. They depleted plant available soil water during pre-anthesis and post-anthesis stage of sorghum by 65% in Tucson, AZ, USA (Figure 1). The result suggested that frequent irrigation showed negligible impact on biomass and ethanol yield, whereas, water stress with 50% depletion reduced biomass per unit of water with no increase in sugar concentration or accumulation at harvest of sweet sorghum [51].

Wortmann and workfellows [52] evaluated sweet sorghum, corn, and grain sorghum for energy use efficacy, along with greenhouse gases (GHG) emissions and ethanol yield at seven different locations in Nebraska, USA. Grain crops showed 21% to 33% more calculated ethanol yield and net energy yield than sweet sorghum, although mean net energy yield of an earlier-maturing sweet sorghum cultivar was equivalent to grain crops. The total energy utilized to convert grain crops to ethanol was 23% lower than that utilized on sweet sorghum. However, sweet sorghum and grain crops reduced GHG emissions by 69% than gasoline. The byproducts of grain crops during ethanol production were utilized efficiently, while sweet sorghum bagasse were returned to field as soil fertilizers. Sweet sorghum cultivars are competitive candidates for producing biofuel, but not as liquid transportation fuel as grain crops performed better [52]. Economou et al. [53] demonstrated conversion of sweet sorghum into biodiesel via oleaginous fungus *M. isabellina* in Greece. The fungus effectively converted sugar and nitrogen in sweet sorghum directly into storage lipid. The process was performed on semi-solid fermentation that had certain advantages like high quality oil—compared to liquid and solid state fermentation. Their findings concluded that lipid accumulation is possible due to nitrogen limitation allowing biomass growth and oil synthesis, whereas semi-solid state fermentation effectively extracted sugar from stem of sweet sorghum and lead to lipid accumulation [53].

Audilakshmi et al. [54] facilitated breeding of sweet sorghum for bioethanol purpose in relation to presence of high sugar content. They crossed 27 B × BJ 204 (a Chinese line) and 27 B × kellar (a US sweet sorghum line) to find generation mean and frequency distribution of useful traits during 2006 and 2007 in India. They reported that mean

performance of F1 generation comprised of high brix and sucrose content (due to Parent 2 having high sugar percentage). In F1 generation: stalk, juice yield and plant height were marked as over dominant traits. In F2 generation sucrose percentage and Brix were found polygenic traits, although, juice and stalk were marked as oligogenic. These dominant and over-dominant traits were utilized in hybrid breeding programs to enhance production of bioethanol [54]. Sweet sorghum hybrids vary with respect to biomass yield and variable level of nitrogen that ultimately affect quality of extracted juice. Mosalia et al. [55] determined the impact of varying levels of nitrogen fertilizers on biomass, juice yield, and forage and bagasse quality in USA. Sorghum varieties harvested and evaluated at soft dough stage for required traits, showed increase in forage and juice yield due to fertilizers [55]. Guigou, et al. [56] studied effects of post-harvest conditions on three sweet sorghum varieties (M81, Topper and Theis) used for bioethanol production. Juices were obtained by milling whole plant, stalk and plant without panicle. Linear relationship was found between Brix degree and fermentable sugar concentration. Fermentable juice of sweet sorghum differed with respect to extraction treatments, yet showed similarity in fermentation efficiency and sugar concentrations. Theis and Topper varieties were superior in terms of ethanol yield, concentration and fermentation efficiency. Furthermore, post-harvest techniques and selection of desirable variety played critical role in bioethanol production from sorghum [56].

Sweet sorghum is widely cultivated as summer crop due to high resistance to salinity, while being productive through drought and during limited water resources. Vasilakoglou et al. [57] conducted a research in Greece to access the productivity of grass sorghum Susu as well as, sweet

sorghum varieties, Sugar graze, M-81E, Urja and Topper-76-6, along with grain sorghum cultivar KN-30 in terms of biomass, total fermentable sugar, juice and ethanol yield. The cultivars were grown in high saline conditions with low to intermediate water for irrigation. Sugar graze and Urja cultivars were high ethanol producing varieties with high juice content, for ethanol production when grown in saline soil and 50-75% irrigation [57]. Xu et al. [58] demonstrated photosensitive sorghum (PS) grown in fields of USA, has great potential for bioethanol production due to high yield, drought tolerance, better sugar content and high biomass. Variable concentrations (0.5 to 1.5%) of diluted sulfuric acid were used as pretreatment to hydrolyze cellulose for ethanol yield. and highest glucose yield of 80.3% was obtained with 1.0% of acid. The efficiency of hydrolysis increased up to 94.4% as the acid concentration rose up to 1.5% due to high degradation of available cellulose. Their findings reported 74.5% of ethanol yield due to above mentioned processing conditions, where 1g of sorghum produced 0.2g of ethanol [58]. Similar study conducted by Zhang et al. [59] produced butanol from sweet sorghum bagasse via acetic acid treatment to hydrolyze hemicellulose-rich biomass using *Clostridium acetobutylicum* ABE080—an acetic acid resistant strain. Hemicellulose hydrolyzed by 2g/l of acetic acid at 121°C for 10 minutes produced various compounds such as: carbohydrates, furfural, organic acid, hydroxymethyl furfural (HMF) and phenolics. Detoxification process with laccase eliminated phenolic compounds that were generated as by-products and served as primary inhibitors in hydrolysate step resulted in 8.5 g/l of butanol [59]. Ananda et al. [60] investigated the effect of drought and heat stress on glucose of sorghum grain at different phenological stages and also its subsequent impact on bioethanol production

in USA. The abiotic stress had negligible impact on glucose levels from grains as compared to control, yet an increase in ethanol production, up to 4.5%, was observed in flowering and seed-setting stage. However, the heat stress significantly reduced glucose content and bioethanol production by 9% in different seed filling stages. Thus, abiotic stresses adversely affected phenological stages of sorghum; ultimately reducing ethanol yield [60].

Sorghum is widely adapted around the world and serves as the most attractive feedstocks for liquid fuels, due to efficient conversion of CO₂ into sugar units. A study conducted in USA evaluated use of sweet sorghum in biofuel production [61]. Their research reported that sweet sorghum comprised of 37% juice, 36% bagasse, 19% leaf and 8% seed head, all of which can be utilized in producing bioethanol via *Saccharomyces cerevisiae*. Around 229.8 Kg of carbohydrates from 1ton of sorghum can produce 59 kg of ethanol [61]. Qureshi et al. [62] evaluated production of ethanol from sweet sorghum grains and thereby sugar tolerance in *Saccharomyces cerevisiae* in Pakistan. Sorghum varieties were simultaneous saccharificated and fermented to produce ethanol from starch grains. The findings revealed that *S. cerevisiae* can tolerate 25% glucose and 11% ethanol. Separate hydrolysis and fermentation showed 95% fermentation efficiency in ethanol production, whereas, simultaneous saccharification and fermentation showed high concentration of ethanol [62].

Erickson et al. [63] focused on optimizing production of sweet sorghum using various management practices for bioenergy purpose. The effect of N-fertilizers' rate on biomass, sugar yield, brix as well as N and P recovery were investigated for sweet sorghum in Florida, USA. N-fertilizers had no impact on biomass of sorghum, yet led to greater N-recovery and minor gain in sugar

yield. The results suggested that proper management of nutrients can contribute in optimizing sugar yield in sweet sorghum for bio-based products [63]. Another study conducted by Holou and Stevens [64] determined optimum rate of N-fertilizers in different soil materials (on silt loam, sandy loam, and clay soils) to produce high yield sweet sorghum at Missouri, USA. N-rate and soil material significantly affected juice yield by increasing sugar content (particularly due to N-fertilizers). However, negative correlation existed between juice yield and sugar content of sweet sorghum. Sorghum displayed high sugar content when grown in silt loam compared to that in clay. Addition of N-fertilizers in clay soil increased sugar yield of sweet sorghum, while in silt loam it displayed high sugar content only if cultured after corn. Significant difference existed in juice yield ranging 2-9.9 Mg/ha due to N-rates, soil and other factors. Whereas, sorghum required low quantity of N-fertilizers if planted after cotton or soybean. In general, 67 kg of N-fertilizers were enough to optimize sugar, juice and bagasse yield [64].

Sambusiti et al. [65] demonstrated production of methane from five sorghum varieties via alkaline pretreatment in France. The samples were pretreated with NaOH in closed bottles for 12h at 55°C. The pretreatment reduced sorghum into lignin, cellulose, hemicellulose and galacturonic acids; thus further accelerating hydrolysis of cellulose and hemicelluloses. However, NaOH pretreatment was unsuccessful for increasing methane yields [65]. Rutto et al. [66] conducted a research on five sorghum varieties (Dale, M81E, Sugar Drip, Della and Keller) to evaluate quality of grain and juice as potential source of bioethanol in USA (see Figure no.1). Thus, stem weight and juice volumes were correlated ranging from 10 to 24 m³·ha⁻¹, though brix value ranged between 14-19%. Yet, production of

bioethanol from Keller and M81E was greater than theoretically estimated ethanol and sugar yields. The findings reported low grain, but high starch content in Keller than other cultivars being potential source for fermentable grain and sugar [66]. Martin et al. [67] demonstrated effective, high throughput method to screen feedstock toward biomass, cell wall digestibility, and fermentable sugar at 10 days post-anthesis for biofuel production in Australia. They designed a mathematical method along with high throughput strategy using fourth internode of plant to assess soluble sugar, stalk biomass, stalk fermentable sugar, and cell wall composition. The high throughput method using partial least squares (PLS) modeling measured soluble glucose, sucrose, and fructose in juice via Fourier transform infrared (FTIR) spectra. The findings showed sugar PLS correlated with brix and gas chromatography–mass spectrometry (GC-MS) [67].

Forage sorghum, Brown Mid-Rib (SBMR) and non-BMR (SNBMR) types, and sunn hemp (*Crotalaria juncea* L.) are widely used as fiber and forage crop in USA. Kamireddy et al. [68] evaluated these crops for biofuel purpose. The feedstock were treated with dilute acid and further by enzymatic hydrolysis cellulase. SNBMR showed highest xylose yield with 95% wt of xylose, than that of SBMR with 91 at combined severity factor (CSF). while sunn hemp showed maximum of 75% wt of xylose at CSF. On harsh pretreatment conditions, the amount of xylose decreases due to degradation. Similarly, after enzymatic hydrolysis glucan saccharification yield was highest in SNBMR with 90% wt, while SBMR showed 84% wt, and Sunn hemp showed 68% wt at CSF. They concluded that SBMR and SNBMR can serve as potential candidate for biofuel production due to less crystallinity than Sunn hemp [68].

Recently, biofuel research has been accelerated in Pakistan due to scarcity of resources (Figure 1). Lignocellulosic biomass is pretreated to convert complex it into simpler substances for hydrolysis and fermentation. *Sorghum bicolor* straw potential lignocellulosic feedstock was evaluated for various pretreatment conditions (Acid concentration, Time and Temperature) in Pakistan. Samples were analyzed using HPLC, where maximum sugar value was observed with 2% H₂SO₄ at 121°C for 10 minutes, suggesting that optimized pretreatment conditions were effective prerequisite for production of bioethanol [69]. In India, Rao et al. [70] compared sorghum hybrids with open pollinated varieties (OPVs) for various quality related traits to produce potential bioethanol and high yielding grains. Sixteen genotypes were evaluated for sugar and stalk related traits. Stalk yield from sorghum hybrids and OPV ranged between 29.4 to 46.5 t ha⁻¹, while grain yield was recorded higher for hybrids than OPVs. SPSSV30 showed highest total soluble sugar and juice Brix. Among hybrid group genotypes: SPSSH 24, SPSSH 27, PAC52093, whereas among OPV genotypes: SPSSV 20, SPSSV 27 and SPSSV 15, showed superior bioethanol and total sugar yields. Hybrids can support energy sector due to 10-18% high production of stalk biomass and bioethanol yields compared to OPV [70].

Sugarcane has served as renewable alternative source to solve energy crisis in developed countries, though, after paying heavy price of using agricultural land, nutrients and water. Recently, the research has been shifted toward use of sorghum cultivars as an alternative source of energy due to its high adaptability, resistance and limited use of water making it an excellent candidate. Dutra et al. [71] evaluated bioethanol production from eight sorghum cultivars developed in state of Pernambuco,

Brazil. The cultivars were pretreated, then fermented by *S. cerevisiae*, at 33°C for 6 hours. Sorghum cultivars SF 15 showed highest bioethanol potential, followed by BR 506 [71].

Theuretzbacher et al. [72] evaluated three sorghum varieties for bioethanol and biogas synthesis and compared the outcomes with a maize variety. One grain variety “C” (Chopper), and two sweet sorghum varieties “SG1” (Sugargraze I) and “SG2” (Sugargraze II) were grown in eastern Austria. The varieties were analyzed for sugar content and composition to determine sorghum potential to produce biofuel. SG1 and C varieties showed highest and improved energy output than maize. Furthermore, biogas and bioethanol production was variable in among sorghum varieties due to difference in sugar and starch. Variety C provided 54% bioethanol and 46% methane, while SG1 contributed in 30% bioethanol and 70% methane [72]. Lignocellulosic biomass are converted into bioenergy based products via various pretreatment and fermentation strategies. A study conducted by Vandenbrink et al. [73] investigated crystallinity index trait, and feedstock composition of twenty sorghum varieties grown in USA. They performed enzymatic hydrolysis (using *Aspergillus niger* cellulases and *Trichoderma viride*) on sorghum to obtain high sugar yield. The study illustrated positive correlation of hydrolysis yield potential with stem (due to presence of lignin content) as well as negative correlation due to crystallinity index. Thus, sorghum leaf showed negative correlation for hydrolysis due to absence of lignin. However, each genotype efficacy was variable with respect to pretreatments (ammonium hydroxide and *T. viride* cellulose). These pretreatment factors can be optimized to obtained bioethanol in large-scale. Similarly, butanol synthesis were positively correlated with stem tissues, while

suggesting that selection of genotype with high ratio of stem to leaves can improve end product yield [73].

Cotton et al. [74] studied biomass and cellulosic properties of forage sorghum differed on basis of brown midrib trait (i.e., bmr12) to produce ethanol under limited irrigation, while comparing to dry land (no irrigation) conditions of Southern High Plains of the USA (see Figure no.1). The Sorghum Partners 1990 produced highest biomass under irrigated and dry land conditions, yet, PaceSetter bmr showed highest cellulosic efficiency on simultaneous saccharification and fermentation to produce bioethanol. The study demonstrated that irrigation can increase up to 49% of biomass and 72% of cellulose for bioethanol production compared to limited irrigation (had no effect on biomass and biofuel production). The findings concluded that low precipitation did not affect the cultivars, while high precipitation increased cellulose by 28% in sorghum Partners 1990 that further amplified bioethanol production. The results showed that precipitation and irrigation were crucial factors that directly impacted biomass and biofuel production [74]. Mehmood et al. [34] evaluated four varieties of sorghum (84-Y-01, 85-G-86, Mr. Buster and RARI S-3) to unleash their use as ideal feedstock for bioethanol production in Pakistan. The straw of sorghum varieties were pretreated with diluted sulfuric acid and enzymatically hydrolyzed to ensure complete sugar extraction and conversion to bioethanol. Their study assigned following sequence to sorghum varieties with respect to sugar yield: 85-G-86>Mr. Buster> 84-Y-01 > RARI S-3. Nevertheless, variety (85- G-86) was considered ideal feedstock due to low by-products, furans and high sugar yield [34]. Similar study was conducted by Dalla Marta et al. [75] to examine sorghum growth at different developmental stages under

constant water stress in Italy. They determine its potential use as first and second generation biofuel. The study concluded that duration of growth and cultivation period should depend on objective of use. Such as, if the target is bioethanol production, then longer duration for cultivation period was suitable, on the contrary, flowering stage was considered suitable harvest time to address optimal use of water [75]. Another study was performed in Guatemalan Pacific coastal plains on six varieties of sorghum to evaluate sugar potential in stem for bioethanol and nutritional value from grains. Of all varieties Top 76-6 variety was found exceptional in both terms of grain nutrition and high sugar value in stem with capacity to produce 2465 l ha⁻¹ of ethanol. Grains had 83.2% of digestible proteins and low level of polyphenols [76]. Sweet sorghum was investigated as an alternate feedstock for bioethanol by Embrapa (Brazilian Agricultural Research Corporation of Maize and Sorghum). Fernandes et al. [77] used juices of 4 four sorghum varieties (BRS 506, BRS 508, BRS 509, BRS 511 and BRS) and evaluated their nutritional contents including, sugar, starch and respective theoretical ethanol yield. The study concluded that three varieties (BRS 508, BRS 509 and BRS 511) were potential candidates for bioethanol as their maturity exceeds 30 days. The nutritional value in sorghum varieties decreases with increase in cultivation duration. BRS 511 showed highest sugar production, whereas BRS 508 yielded 90.5% theoretical bioethanol within 8 hours of fermentation, which is similar to bioethanol produced from sugarcane juice [77].

Cuevas et al. [78] agronomically evaluated 925 accessions (originally belonged to different countries and compared them with accessions of USA) obtained from U.S. National Plant Germplasm System (NPGS).

They evaluated physiological parameters, morphological parameters, fermentable sugar, juice volume, brix values, and disease responses toward rust (*Puccinia purpurea*) and anthracnose (*Colletotricum sublineolum*). The findings concluded that nine accessions belonging to Ethiopia, South Africa, Sudan, Zimbabwe showed high brix values than accessions belonging to USA. Similarly, dry mass of accessions belonging to Ethiopia and USA were higher than reference number of other countries. New sources of rust and anthracnose disease resistance were identified, while accession from Tanzania was found resistant to both diseases. The findings showed that NPGS sorghum collection consisted of valuable germplasm that can be exploited in breeding programs to fulfill biofuel purpose paving integration of such germplasm in genetic diversity and new varieties for bio-based products [78]. Mocoer et al. [79] crossed sweet and grain kaoliang sorghum to obtain recombinant inbred lines to find genetically stable biomass, as well as morphological and biofuel related traits in field trials conducted in Denmark and China. They determined 53 QTLs using PAV markers (presence and absence variant) from which 18 QTLs were common for both countries. However, out of 15 traits only eight were genetically stable and heritable. Plant height was positively correlated with biomass, whereas juice and brix content were negatively correlated. In Denmark trials sheading stage was correlated with biomass and morphological traits. QTLs for maturity identified on two chromosome of SBI01 and SBI02 were associated with early chilling tolerance; suggesting that accelerating maturity built the tolerance to low temperature. The finding also suggested that selection of sorghum on basis of early maturity, plant height, and high Brix content can contribute in bioenergy and biofuel alternative for Northern Europe [79].

Sweet sorghum is considered analogous to sugarcane due to sugar accumulation, Brix content, and lignocellulosic residues (pretreated to obtain sugar for ethanol). A study was conducted in Kenya on 16 sweet sorghum bagasse, which could be sustainable source of biofuel [80]. Sweet sorghum bagasses were dried and pretreated with alkaline hydrogen peroxide and phosphoric acid to yield 63.40% and 49.12% of glucose. Further hydrolysis and fermentation of bagasse by *Trichoderma reesei* via cellulose enzyme produced 88% (treatment with sodium hydroxide) and 77% (treated with phosphoric acid) of sugar yield. The study suggested that bioethanol can be produced from sorghum bagasse to widen the scope and strength of bioenergy sector [80]. Similarly, Khalil and his colleagues [81] analyzed productivity and biofuel related traits from bagasse of five sweet sorghum varieties in Egypt. Tested varieties differed in juice content, bagasse and stalk yield. Extracted juice was fermented by *Zymomonas mobilis* ATCC 29191 and *Saccharomyces cerevisiae* ATCC 7754 to produce bioethanol. Three sorghum varieties were squeezed directly to obtain juice while Ramada, Mn-1054, and SS-301 rich in fiber had to be pretreated with 2% H₂SO₄ (98%) at 120°C to extract juice from sugar rich filtrate for bioethanol production. The finding showed that mixed-cultured treatment can help to obtain highest juice such as: 1 Kg of SS-301 variety produced 160mL of bioethanol from juice and bagasse [81].

Biofuel from crops not only reduces our dependency on depleting fossil fuel but also lowers CO₂ emission in environment. Currently, bioethanols are commercially available and used in certain percentages with gasoline in developed countries like USA, and Brazil. Therefore, production of bioethanol depends on agricultural land, crop production, resources, as well as

governmental and social policies. Xuan et al. [82] evaluated optimum time required for sowing sorghum seeds and determined suitable cultivation condition required to obtain maximum yield for biofuel production in Vietnam. Suitable cultivation conditions to obtain >5000 L ha⁻¹ yield of ethanol were 5.5 pH, along with Al and Zn content ranging between 39.4 and 0.6 g kg⁻¹. pH higher than 6.0 can reduce biomass and bioethanol yield through altering Zn content, however, other minerals like P, N, K, Organic C, and Fe are less likely to contribute in biomass. Their finding recommended that cultivar 4A can highly contribute in ethanol production due to its high resistance toward pest diseases especially cut worm (*Agrotis spp*) [82]. Maw et al. [83] tested the effect of N-fertilizers on dry mass, stem juice yield, sugar yield, theoretical ethanol juice, lignocellulosic ethanol yield, and total ethanol yield from two sorghum varieties (Dale and Top 76-6) grown in Missouri,

USA (see Figure no.1). N-fertilizers failed to show significant impact on brix content; yet, other parameters were highly influential in both varieties, though. highest ethanol yields was obtained with 168 kg ha⁻¹ of N-fertilizers. Whereas low temperature and high precipitation levels were also important factors to contribute in ethanol yields [83]. Mengistu et al. [84] determined water use efficiency for sweet sorghum and its impact on biofuel production under two different climatic conditions of South Africa (Hatfield and Ukulinga research farm). Theoretical ethanol production was calculated through Brix % produced as well as stalk yield. The findings concluded that sweet sorghum has variable water use efficiency (WUE) under different climatic conditions, such as Hatfield produced maximum of 0.70 L-m⁻³ of ethanol, which was comparatively higher than that from Ukulinga research farm. Therefore, WUE of sweet sorghum was considered sensitive to plant density [84].

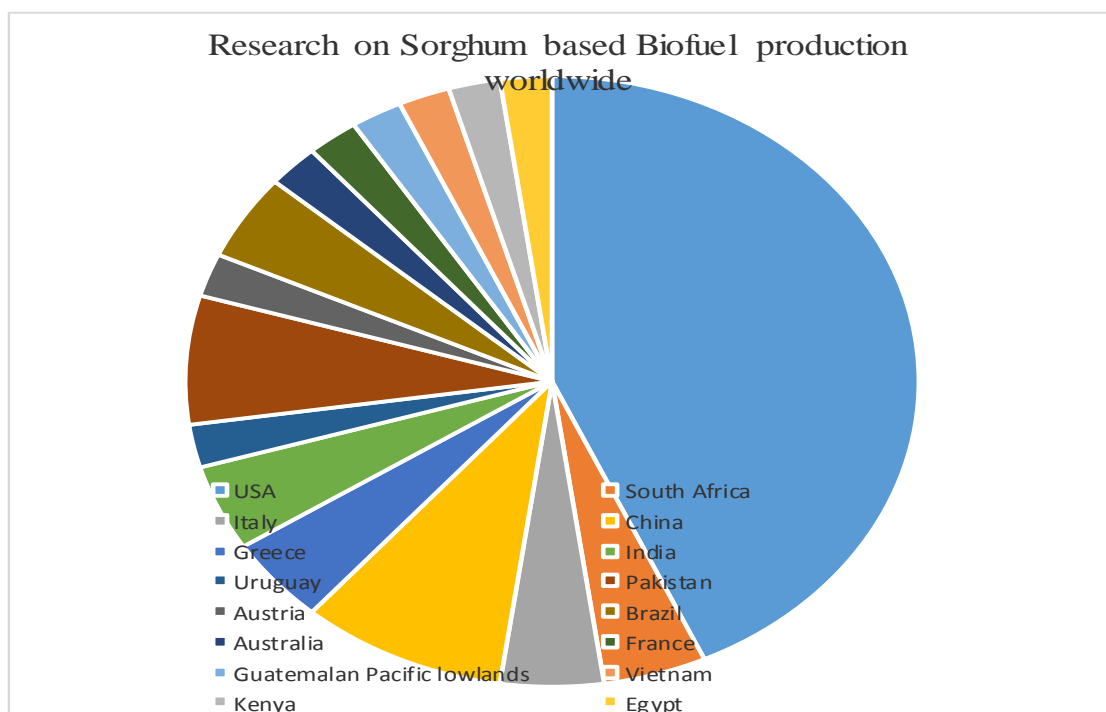


Figure 1. Biofuel production from sorghum worldwide

Role of microRNA in biofuel production in different crops

MicroRNA (miRNA) plays important role in development and stress resistance of plants via post transcriptional gene regulation. miRNA are small RNAs that are usually 21-22 nt long, and produced in series from splicing action of RNase-III like enzyme Dicer on its precursors with short hairpin-loop structures [85]. miRNA are negative regulators of gene expression and act by splicing or inhibiting the target mRNA for protein translation [86]. Keeping function of miRNA in view, many novel miRNAs has been identified and further identification is a key to unravel the mechanism of stress tolerance.

Cultivation of Brachypodium, Miscanthus and switchgrass are emerging with broad scope in bioenergy field. Switchgrass is center of attention worldwide due to its wide adaptability to grow, and tolerate drought spells. However, the basis of genome responsible for such traits is still unexplored. Recent research shows that microRNA are responsible for gene expression in growth, development and adaptation to wide range of circumstances (Table 2). Matts et al. [87] identified miRNA families in switchgrass responsible for growth and development with tissue-specific expression, and ubiquitous expression in some species. The findings showed expression of miR395 and miR399 under optimal level of sulfate and phosphate, and slightly altered under phosphate and sulfate deficit conditions. They suggested 4 target mRNA like NAC domain containing transcription factor, apetala 2-like, Squamosa promoter binding-like factor, and HD-Zip homologs and 37 genes as targets for miRNA [87]. Similar study performed by Xie et al. [88] identified 121 miRNA in switchgrass belonging to 44 families using comparative genomics approach. These miRNA targeted 839 proteins coding genes and played important

role in biological, metabolic processes and plant development. Whereas gene ontology showed that miRNA has crucial role in 527 biological processes on the basis of these targets. Among these processes, 23 processes are involved in metabolic activities of carbohydrates, lipids and xylem formation. The findings analyzed 118 metabolic pathway networks involved in oxidative stress response, hormone regulation, carbon fixation, metabolism of fat and sucrose and other secondary metabolites [88].

Zanca et al. [89] reported 19 sugarcane miRNA precursors responsible for high bioethanol yield in sugarcane and found similar to sorghum at nucleotide and secondary structure levels (Table 2). Mature miRNA can be accumulated in specific tissues and organs of sugarcane. The findings determined 46 potential targets for 19 miRNA of sugarcane several targets of conserved miRNA were involved in plant development as transcription factors. The findings classified 19 miRNA precursors in sugarcane and one miRNA precursor in sorghum into 14 families. Comparative analysis of sugarcane with sorghum showed common homologous miRNA and their targets in genome of these two species. Hence, such conservation may help to clarify specific aspects of miRNA regulation and evolution in the polyploid sugarcane [89].

Foxtail millet (*Setaria italica*) belongs to poaceae family and is widely used as food, fodder and as model crop for biofuel grasses. Zhang et al. [90] drafted genome onto nine chromosomes and annotated 38,801 genes. The findings demonstrated that reshuffling events of foxtail, sorghum and rice contributed in divergence of the crops. Two reshuffling events of key chromosomes were identified via collinearity between 2 and 9 chromosome of foxtail millet with 3, 7, 9, and 10

chromosomes of rice that occurred after divergence of foxtail and rice. Single reshuffling event occurred between foxtail millet chromosome 3 with rice chromosome 5 and 12, after divergence of sorghum and millet [90]. Recent study showed genetic improvement of switchgrass biomass to develop emerging bioenergy crops. miR156 precursor was overexpressed in switchgrass and its effect on squamosa promoter binding protein like (SPL) genes were determined via microarray and RT-PCR. The findings characterized biomass yield, forage digestibility, saccharification efficiency and morphological alterations. miR156 overexpression suppresses SPL gene and is associated with apical dominance and transition in flowering time; while, low expression of miR156 was sufficient to increase biomass with normal flowering time, and disruption of apical dominance. Moderate expression of miR156 improved biomass, yet inhibits flowering. Thus, low and moderate expressions contribute in 58%-101% high biomass yield, though high miRNA expression result causes stunted growth (Table 2). Consequently, the degree of morphological alterations depends on level miR156 expression. High expression enhances solubilized sugar yield, forage digestibility and biomass yield by increasing tiller number [91].

Switchgrass dedicated biofuel crop is broadly cultured for its high adaptability for marginal lands and high biomass yield. However, limited knowledge is available on basic mechanism of its gene expression under stressful conditions. Study conducted by Sun et al. [92] demonstrated expression of regulatory miRNA on physiological parameters under salt and drought stress. The finding indicated that 1% salt stress adversely effected germination rate and growth, whereas, drought stress showed slight impact on germination. The miRNA expression of switchgrass under salt and

drought stress was dose-dependent, and upregulated the gene expression by 0.9 folds and downregulated by 0.7 folds. Though, miRNA under both stresses showed similar range of expression yet, miRNA were more sensitive in drought stress, as miR156 and miR162 displayed significant expression, suggesting role of miRNA to cope up with drought stress (Table 2). Therefore, transgenic lines can improve bioenergy crops for biofuel production [92]. Drought spells are widely known to reduce the yield of majority crops. Certain genes are expressed to plant cope up with drought stress and manage water, yet this mechanism remains unexplored in majority of crops. Ferreira et al. [93] conducted a study to explore expression of miRNA in two cultivars of sugarcane under drought stress. Sugarcane cultivar RB855536 known for low drought tolerance and RB867515 cultivar with high tolerance were grown for 3 months and subjected to drought stress for 2-8 days. The results revealed identification of 18 miRNA families via deep sequencing, out of which 7 miRNA were differentially expressed in drought stress. Furthermore, differential expression of miRNAs depends on duration of stress, such as 6 miRNAs were differentially expressed after 2 days and 5 miRNA were expressed at 4 days of stress [93].

Brachypodium distachyon belongs to Poaceae family and known to produce biofuel. Jeong et al. [94] sequenced 17 small RNA libraries and identified miRNA populations representing diverse stress conditions and tissues. The study identified 115 miRNAs using computational tools, which included conserved, non-conserved and novel miRNA. PARE (Parallel Analysis of RNA Ends) based identification of miRNA cleavage function were used to construct PARE libraries. The PARE libraries constructed from key tissues resulted in ~70 million raw sequences and

~5 million genome matched sequences. PARE data analysis of miRNA mediated cleavage represented 250 sites involved in miRNA targets. Furthermore, characterization of target sequences and miRNA in *Brachypodium* were involved in tissue-preferential control of miRNA family members, responsible for differential target cleavage. The PARA data from other crops and *Brachypodium* provided insight on role of miRNA and its target regulation to exploit various features to fulfill biofuel purpose [94]. Another study performed by Green [95] identified genome wide targets of miRNA in *Arabidopsis* using PARE analysis and applied PARE further to *Brachypodium distachyon* genome. The findings suggested identification of new miRNA and regulations with respect to environmental stresses and type of tissue. 260 targets of miRNA were identified using PARE sequences were responsible for precise miRNA guided cleavage. PARA analysis revealed that differentially expressed miRNA guide is responsible for specific target RNA cleavage in tissue-preferential manner miRNA-target RNA regulation. Therefore, specific miRNA targets RNA association with known physiological functions, providing insight on gene regulation and response under environmental stresses in different tissues [96]. Recently, genomic tools are used to exploit lignocellulosic synthesis genes to harness high yield of biomass, however, the lack of complete genome in majority of bioenergy grasses hinders the study on crop

improvement. Muthamilarasan et al. [97] studied foxtail millet and identified gene families responsible for monolignol biosynthesis (PAL, C4H, 4CL, HCT, C3H, CCoAOMT, F5H, COMT, CCR, CAD), callose (Gsl) and cellulose (CesA/Csl). Comparative analysis of lignocellulose biosynthesis genes of foxtail millet with genome of C4 crops showed high resemblance with switchgrass followed by sorghum and maize. Whereas, expression profiling revealed that lignocellulosic gene was differentially expressed under abiotic stresses and hormonal treatments. The results suggested that monolignol biosynthesis proteins were highly diverse, while Gsl and CesA/Csl proteins were homologous to *Oryza sativa* and *Arabidopsis thaliana* [97]. Furthermore, lignocellulosic crops faces economic barrier during biofuel production due to cell wall recalcitrance. Many researchers are actively working to explore genes that can offer solution to fix this problem via genome wide study. miRNAs are known to be involved in all biological, developmental and metabolic processes due to broad functions of their targets. Alteration in miRNA expression can lead to pleiotropic effects. Such as miRNA regulates physiological and biological traits such as low expression of miR156 increases biomass and reduces recalcitrance, while high expression of miRNA in bioenergy switchgrass and poplar reduces lignin content, increase biomass and flowering time with improved responses toward harsh environment [98].

Table 2. Role of miRNA in crops for biofuel production

S No.	Crop	No. of miRNA	miRNA	Refs.
1.	Switchgrass (<i>Panicum virgatum</i> L.)	20 conserved miRNA families. 37 genes were predicted as targets for miRNAs and 4 target mRNAs	miR156a,b, miR156e, miR156f, miR156k, miR159b, miR160, miR164a, miR164c, miR166, miR167b, miR168, miR169a, miR169c, miR169d, miR169b, miR169k, miR171g, miR172a, miR172c, miR172b, miR172d, miR319, miR393, miR394, miR395n, miR396n, miR397, miR398n, miR399, miR408, miR437, miR444, miR528	[87]
2.	Switchgrass	121 potential miRNAs, belonging to 44 families		[88]
3.	Sugarcane (<i>Saccharum spp.</i>)	19 miRNA precursors a total of 46 potential targets for 19 sugarcane miRNAs	miR156a, miR159a, miR167a, miR168a, miR169, miR396a, miR827, miR408a, miR437, miR444, miR528, miR1128, miR1432, miR319b	[89]
4.	Foxtail millet (<i>Setaria italica</i>)	Seven homologues of miRNA	miR169g	[90]
5.	Switchgrass	miR156b precursor overexpression improved biomass, solubilized sugar yield and forage digestibility	miR156b	[91]
6.	Switchgrass	miR156 and miR162, showed significant change in expression	miR156, miR157, miR159, miR162, miR167, miR169, miR172, miR395, miR396, miR397, miR398, miR399	[92]
7.	Sugarcane (<i>Saccharum spp.</i>)	miRNAs differentially expressed under drought stress	miR156, miR160, miR164, miR166, miR167, miR169, miR171, miR172, miR319, miR390, miR393, miR394, miR396, miR397, miR399, miR528, miR529, miR1432	[93]
8.	Wild grass <i>Brachypodium distachyon</i>	116 miRNAs conserved and non-conserved miRNAs	miR5163b-3p, miR5181e, miR51851-3p.2, miR7731, miR7738-3p, miR7754-3p, miR9480ab, miR9481a, miR9481b, miR9482, miR9483ab, miR9484, miR9485, miR9486a, miR9486a, miR9486b, miR9487, miR9488, miR9489, miR9490, miR9491, miR9492, miR9493, miR9494, miR9495, miR9496, miR9497, miR9498, miR9499	[94]
9.	Wild grass	80 new miRNA precursors (conserved and non-conserved) 260 targets of new and known miRNAs with PARE sequences at the precise cleavage site were identified and characterized	miR166, miR156, miR529, miR5163b-3p	[96]
10.	Foxtail millet (<i>Setaria italica</i> L.)	Potential miRNAs target few genes for post-transcriptional gene silencing	miR156d-1, miR156d-2, miR395b, miR114-npr, miRn29	[97]
11.	Wild grass	Metabolism, development, stress response	miR156, miR160, miR167, miR169, miR172, miR397, miR398, miR414, miR5200	[98]

12.	Cassava (<i>Manihot esculenta</i>)	Metabolism, development, stress response	miR156, miR160, miR164, miR172, miR395, miR482	[98]
13.	<i>Jatropha curcas</i>	Metabolism, development, stress response	miR004, miR156, miR172, miR395, miR398, miR5201,	[98]
14.	Maize (<i>Zea spp.</i>)	development, stress response	miR156, miR160, miR164, miR166, miR167, miR169, miR172, miR319	[98]
15.	Poplar (<i>Populus spp</i>)	Metabolism, development, stress response, Pathogen response, Cold response	miR156, miR159, miR160, miR164, miR166, miR169, miR172, miR319, miR472, miR1445, miR1446	[98]
16.	Sugarcane (<i>Saccharum Spp.</i>)	development, abiotic stress response	miR156, miR159, miR164, miR397, miR399, miR528	[98]
17.	Switchgrass	Metabolism, development, biofuel yield, abiotic stress response	miR156, miR164, miR166, miR167, miR172, miR398, miR414, miR444, miR477, miR528, miR531, miR854, miR1535, miR1848, miR2102, miR2118	[98]

Role of identified miRNAs in Sorghum

Sorghum bicolor is well known staple crop in developing world and closely related to maize. It is desired crop due to high adaptability to wide range of climatic conditions, short production time, salinity and drought tolerance making it potential candidate for cultivation for biofuel production. Bedell et al. [99] suggested that 95% of sorghum genes are sequenced tagged with 65% coverage across its length, generated sequence through hypomethylated portion of genome using methylation filtration (MF) technology. They studied functional parts of sorghum genome using MF technology to obtain information on miRNA, promoters, single sequence repeats, exons and introns, while minimizing interspersed repeats. MF based sequence is powerful source for comparative genomics with grasses and other important crops [99]. Previously, 286 miRNA genes clustered into 43 family has been identified in Arabidopsis, maize, and rice. Furthermore, Dezulian et al. [100] reported identification of 200 miRNA genes belonging to 43 families in genome of sorghum, maize, medick and poplar and expression of 37 miRNA precursors of sugarcane and soybean via computation tools. The study analyzed plant precursors such as: conserved

precursors, secondary structure of miRNA and stem length. The findings suggested two classes of miRNA plant precursors. The abundant precursor class has strong conserved blocks that are corresponding to mature miRNA and its complementary sequence, while less frequent precursor class has two additional conserved blocks with long stem region and extensive secondary structure that includes miR159/319 and miR394 families [100]. miRNA are wide spread in all organism. Some miRNAs are highly conserved in plant species and linked to common ancestors in early evolution, with minute difference or absence of nucleotide substitution among plant species. Whereas, others potential miRNA are expressed and regulated under biotic and abiotic environmental stresses. Some miRNA are tissue specific and expressed under developmental switching. Previously, 75 miRNA belonging to 14 families were identified. Zhang et al. [101] suggested that EST analysis can identify new miRNA, and its targets (Table 3). They predicted RNA secondary structure of 812 EST sequence and identified 338 new miRNA in 60 plant species. miRNA are present among plant species in abundance and involved in variety of developmental, signal transduction and

environmental stresses that stimulate miRNA synthesis and regulation [101].

Zhang et al. [102] identified 481 miRNA of 37 families from 71 different plants via EST analysis. The research revealed that miRNA in some plants were clustered together polycistron suggesting similar expression and transcription. Number of miRNA identified were related to available EST instead of evolutionary relatedness to *A. thaliana*, indicating that conserved and phylogenetic relation are linked to presence and absence of miRNA. However, miRNA expression existed in early evolutionary stages of plant and remains functional for 425 million years. Many miRNA families are conserved among major plant lineages like: monocots, gymnosperms, mosses, and eudicots [102] (Table 3). Zhang et al. [103] performed genome wide analysis of miRNA genes in maize, and characterized their structure, evolutionary relatedness and expression. Using computational homology and secondary structure modeling approach about 150 genes of 26 miRNA families were identified and validated. Moreover, 68 miRNA precursors belonging to 18 families were highly conserved among several species of plants, depicting this class of genes have splice variation and similar sites for alternative transcription. Analysis of sequence variation in diverse maize inbred lines teosinte accessions suggested that evolution of flanking sequences are similar to other genes. Additionally, the research suggested that, duplicated miRNA genes undergo gene loss like protein coding genes with only 35% ancestral sites retained; though the retained number is higher than protein coding genes [103].

Paterson et al. [104] determined genome-wide sequence of sorghum via shotgun sequencing. About 98% genes in chromosomal context were validated by physical genetic, and syntenic information. The study revealed that one-third of

sorghum genome is genetically recombined having gene density and gene order similar to rice. About 75% of sorghum genome is larger than rice genome because of retro-transposon accumulation in recombinationally recalcitrant heterochromatin. However, repetitive DNA and genes were preserved since 70 million year ago, yet prior to sorghum rice divergence most of the duplicated genes had lost one member. Sorghum genome comprises 7% sorghum-specific genes and 24% grass-related genes. Therefore, duplicated miRNA genes contribute in drought tolerance of sorghum [104]. Zhang and coworkers [58] sequenced small RNA library by identifying miRNAs in Sorghum. The findings revealed 13 novel miRNA, 7 conserved miRNA and 29 miRNA families similar to monocots. Differential expression of conserved and novel miRNA indicated diverse role of 125 genes. These miRNAs were involved in diverse processes, and provides insights on miRNA controlled process that can be manipulated to improve biomass and stress tolerance in sorghum [58].

Majority of biofuel producing crops like: sorghum, Miscanthus, switchgrass, sugarcane and corn belong to tribe of Andropogoneae. Sorghum has simple genome as compared to other species due to lack of additional rounds of whole genome duplication events, and contribute in possibility to generate high quality genome sequence of sorghum. The research characterized small RNA from grains, stems of sweet sorghum and F2 generation (derived from cross segregated for flowering time and sugar contents). Variation in expression of miR395 and miR172 were correlated with flowering time, whereas variation in expression of miR169 was correlated with sugar content of stems. Furthermore, due to genotypic differences in miR395* were expressed equivalently as

abundant as miR395 in sweet sorghum though lacked expression in grain sorghum. The research predicted 7 new miRNA and 27 known miRNA from expression of 25 miRNA families in sorghum genome. The study validated small RNA sequences from the stem of sweet sorghum for previously predicted miRNA, depicting its role in flowering time and stem sugar accumulation [105]. Katiyar et al. [106] identified miRNA and its target genes from 1379 unique and known miRNA of 33 different crops via Genomic Survey Sequence (GSS) and EST using computational tools. They determined 37 miRNA of 10 miRNA families with 72 potential target genes. These predicted targets lay foundation to understand miRNA function and involved in plant development and growth [106] (Table 3).

MicroRNA are involved in gene expression and plays crucial role in environmental stresses and development. Thiebaut et al. [107] predicted small RNA via deep sequencing and discovered novel miRNA using bioinformatics tools that regulated, pathogen infections, drought and salt stresses in sugarcane. Using miRNA precursors in sorghum genome 623 new mature miRNAs were identified in sugarcane, from which 44 miRNA highly unique in their biological function. Sets of miRNA in sugarcane were known to target zinc finger protein, Myb, and serine/threonine kinases. RPP2B protein and MADS-box transcription factor were involved in disease resistant protein and plant development, regulated by miRNA cleavage and DNA methylation. Comparative analysis of miRNA between monocots provides valuable insight on conservative miRNA and their targets in unidentified genome sequence of plants [107].

Sorghum is C4 plant with high water efficacy and high adaptability to semi-arid regions. Pasini et al. [108] evaluated

physiological and molecular basis of sorghum genotype during drought response in “dry-down” experiment, where stress was determined by water potential in 4-leaf-old plants. 1205 genes were up-regulated due to drought stress and involved in carbon metabolism (NADP-ME), detoxification (CYPs, GST, AKRs), signal transduction (phosphoesterases, kinases, phosphatases), osmoprotection mechanisms (P5CS), regulation of transcription (bZIPs, MYBs, HOXs), and stability of protein membranes (DHN1, LEA, HSPs). The findings suggested differentially expressed genes responsible for upregulation of 4 miRNA families, whereas one family was downregulated during early phase of drought stress [108]. In another similar study, Katiyar et al. [109] constructed small RNA libraries to identify tasi-RNAs and drought-responsive miRNAs via next generation sequencing on drought susceptible (C43) and drought tolerant (M35-1) sorghum lines. Research showed that around 241 miRNA has been identified in sorghum and deposited in the miRBase. The research identified 526 novel miRNA and 97 conserved miRNA belonging to unique miRNA families from sorghum, out of which 97 were regulated by drought stress (49 down regulated, 32 up-regulated, and 14 miRNA depicted contrasting expression between genotypes). A maximum of 18 miRNA were differentially regulated in tolerant and sensitive genotypes under drought stress condition. Genotype dependent miRNA regulation under drought stress contributed in differential drought tolerance of sorghum genotypes. Around 1300 target genes related to conserved and novel miRNA were identified with specific role related to biological, cellular, metabolic, and development processes. Stress responsive miRNA, tasi-RNA and their respective targets identified via genome-wide search can unravel genetic and

molecular mechanism responsible to cope up with drought stress, thus providing a key role to improve stress response and biomass production in sorghum [109].

Recently, Sanousi et al. [110] studied regulatory mechanism of miRNA related to drought and salinity in sorghum. They studied expression profiling of small regulatory RNAs in pre- and post-flowering stages of six sorghum genotypes under drought and salinity stress using RT-qPCR. The findings suggested that miRNA expression profile was dependent on dosage, whereas each miRNA exhibited different expression in response to stress in all genotypes. Furthermore, the expression of following miRNAs: miR156, miR167, miR168 and miR399 were variable as compared to previous studies, which suggested adaptation of sorghum toward stresses. Therefore, using transgenic technology sorghum can be exploited to produce improved varieties [110]. Alternative polyadenylation (APA) and alternative splicing of pre-miRNA contribute in diversity of transcriptome, expression regulation and coding capacity of genome in eukaryotes. These transcriptomes are analyzed by second-generation sequencing technologies. Abdel-Ghany et al. [111] sequenced sorghum transcriptome and developed pipeline—Transcriptome

Analysis Pipeline for Isoform Sequencing (TAPIS) (tool used for iso-sequencing data analysis) to identify APA sites and full-length splice isoforms. The findings suggested transcriptome-wide full-length isoforms over 11,000 novel splice isoforms and discovered more than 2,100 novel genes and ~ 11,000 expressed genes of APA [111] (Table 3).

Small RNAs are responsible for various developmental processes, whereas miRNA expression is regulated by environmental stresses in plant species. Hamza et al. [112] determined the expression profile of 8 down-regulated miRNA in 11 elite sorghum genotypes under drought stress and watered conditions. Expression level of miR396 and miR398 was highest in all sorghum genotypes, yet high deregulation was observed in, miR166, miR167, miR168, miR393, miR396, miR397-5p. The study suggested that high gene expression profile in grain sorghum HSD3226 in well-watered conditions; shifted the expression profile under drought stress. N98 and Atlas—forage accession showed contrasting expression profile of miR397-5p under drought stress depicting active mechanism to tolerate drought stress. Moreover, the research provides knowledge on miRNA with potential to improve drought stress in other cereal crops [112].

Table 3. Role of miRNA in Sorghum

S. No	Identified miRNAs' families	miRNA associated (biotic/abiotic) traits	NGS/Bioinformatics approach used	Refs.
1.	44 (85%) miRNAs identified in sorghum	DREB1-like proteins	Methylation Filtration (MF) Technology, bioinformatics	[99]
2.	sbi-miR156a,b,c,d, miR157, miR159a,b, miR165, miR166, miR168, miR169, miR170, miR171, miR172	Biotic or abiotic environmental stresses and hormone signaling	Bioinformatics tool (EST analysis)	[101]
3.	sbi-miR156a,b,c,d, miR159, miR160a,b,c,d,e, miR164, miR164b, miR166a,b,c,d,e,f, miR167a,b,c,d,e,f,g, miR168, miR169a,b,c,d,e,f,g,h,i, miR171a,b,c,d,, miR172a,b,c,d,e,	conserved sequence blocks	Bioinformatics tools	[100]

	miR319, miR393, miR394a,b, miR395a,b,c,d,e, miR396a,b,c, miR399a,b,c,d,e,f			
4.	A total of 481 miRNAs, miR156, miR172, miR319, miR396	evolutionarily conserved miRNA families	Bioinformatics	[102]
5.	miR156, miR159, miR160, miR162, miR164, miR166, miR167, miR168, miR169, miR171, miR172, miR319, miR390, miR393, miR394, miR395, miR396, miR397, miR398, miR399, miR408, miR482, miR528, miR529, miR827, miR1432	Involved in secondary metabolic processes, biological processes including gene expression and response to stimuli	Next generation sequencing Bioinformatics	[103]
6.	miR156a,b,c,d,e, miR159,b, miR160a,b,c,d,e, miR162, miR164b,c, miR166a,b,c,d,e,f,g, miR167a,b,c,d,e,f,g, miR168, miR169a,b,c,d,f,g,i, miR171a,b,c,d,e,f, miR172a,b,c,e, miR319, miR390, miR393, miR394, miR395a,b,d,e,f, miR396a,b,c, miR397, miR399a, b,c,d,e,f,g,h,i, miR408, miR437, miR444, miR528, miR529, miR821, miR1432, miR1435, miR1436, miR1439	Rice miRNA 169g, upregulated during drought stress Cytochrome P450 domain-containing genes, often involved in scavenging toxins such as those accumulated in response to stress	Genome sequencing Integration of shotgun assembly with genetic and physical maps -Bioinformatics (BLAST)	[104]
7.	25 miRNA families:miR395, miR169d, miR172c, miR437g, miR169, miR172, miR169a,b,c,d,e,f,g,h,i, miR172a, b,c,d,e, miR395a,b,c,d,e,f,g,h, miR5381, miR5382, miR5383, miR5384, miR5385, miR5386, miR5387, miR5388, miR5389	Regulation biological processes other than the sulfur metabolism	SOLiD next generation sequencing system, Bioinformatics tools	[105]
8.	miR156, miR159 miR160, miR164, miR165, 166, miR167, miR168, miR169, miR170/171, miR172, miR319, miR390, miR393, miR394, miR395, miR396, miR397, miR398, miR408, miR437, miR444, miR528, miR529, miR530, miR827, miR894, miR1126, miR1318, miR1436, miR2118, miR2910, tasiRNA3a, tasiRNA3b, miR5564a, miR5566, miR5568, miR5387b, miR5569, miR5570, miR5565, c, d, e, f, miR5564b, miR5567	Drought tolerance, physiological processes and biotic and abiotic stress responses The predicted targets are pre- dominantly transcription factors: miR156 is predicted to target 7 Squamosa promoter binding transcription factors; miR159, 4 MYB transcription factors; miR160, 6 auxin response factors	Bioinformatics tools (prediction of miRNA targets, BLAST) (5'RACE) assay	[59]
9.	sbi-miR156j,k,l,m, miR166l, miR166m,n,o,p,q,r,s,t, miR167j,k, miR168b,c, miR171l,m, miR390b, miR396f,g,h,i,j,k, miR398b, miR399l, miR444a,b,c	In leaf development, and evolutionarily conserved in all land plants. Regulates expression of the HD-ZIP III (class-III homeodomain-leucine zipper) miR444 family members are conserved only in monocot species	Bioinformatics tools (used ESTs and GSS to predict miRNAs)	[106]

10.	sbi-miR164,b,c, miR395a,f,k,i, miR399a,b,	Drought affected gene expression. Regulation of transcription (bZIPs, MYBs, HOXs), signal transduction (phosphoesterases, kinases, phosphatases), carbon metabolism (NADP-ME), detoxification (CYPs, GST, AKRs), osmoprotection mechanisms (P5CS) and stability of protein membranes (DHN1, LEA, HSPs)	Transcriptome analysis using a high density microarray Bioinformatics tools (analysis of drought-related genes and stay green QTLs)	[108]
11.	sbi-miR44, miR94, miR105a,b, miR108, miR114, miR131, miR141, miR149, miR182a,b, miR191, miR192, miR205, miR221a-h, miR224, miR229, miR255, miR263a-c, miR269, miR313, miR322, miR324, miR337, miR383 down-regulated under drought miR169d-l, miR529, miR57, miR111, miR211, miR245, miR266, miR339, miR387 up-regulated in M35but down regulated in C43 miR160a, miR396b-c, miR396d-e, miR5385, miR4, miR6, miR19, miR26, miR41, miR46, miR48, miR76a-d, miR82, miR87, miR119, miR138, miR144, miR151, miR164, miR259, miR176, miR178a-b, miR180a-c, miR212, miR240, miR285, miR287, miR292, miR304, miR310a-e, miR314a-c, miR316, miR335, miR336, miR340, miR351, miR368, miR373, miR385, miR390, miR391, miR392a-c, miR412, miR413, miR416 down-regulated in M35 but up regulated in C43 miR2118e, miR2275, miR36, miR59a-c, miR64, miR120a-b, miR200, miR215, miR223, miR227a-c, miR256, miR268, miR295, miR297a-c, miR301, miR344, miR359, miR360a-c, miR376	Drought stress expression miRNAs were found to be involved in cellular, metabolic, response to stimulus, biological regulation, and developmental processes	Next-generation sequencing, Small RNA Library Construction and Sequencing, Bioinformatics Analysis of sRNA Sequences, Differential Expression Analysis of miRNAs	[109]
12.	miR156d, miR156, miR159a, miR167c,g,f, miR168, miR393a,b, miR160f, miR166a-d, miR166f, miR168, miR171, miR399b, miR1435b	Regulation of salinity stress and drought stress	RT-qPCR	[110]
13.	sbi-miR171h, miR5567, miR171d, miR528, miR166j, miR167d, miR156d,	Sequenced the sorghum transcriptome o identify	Bioinformatics tool (BLAST), RT-PCR, RNA-	[111]

	miR5568d, miR1432, miR5568f, miR319a, miR5567, miR159a, miR408, miR168, miR397, miR166d, miR396c, miR6225, miR169k, miR169l, miR5565g, miR5567, miR399h, miR156e, miR167c, miR166h	full-length splice isoforms and APA sites.	sequencing, Differential gene expression analysis	
14.	miR396, miR393, miR397-5p, miR166, miR167, miR168	Regulate drought and other abiotic stresses	expression profiling	[112]

Conclusion

Under hot and dry weather conditions, sweet sorghum is best choice as bioethanol to deal economic and environmental concerns. In transport sector biofuels are highly promoted. Recently, majority of research focuses on development of renewable resources, sustainable development, and environmentally friendly process. Use of sorghum biomass for energy purposes is of particular importance today because it allows reduction of greenhouse gases. Bioethanol is by far the most used biofuel for transport around the world. Although identification of potential miRNAs feedstocks have improvised and enhanced bioethanol production, one area of research possible in the future could explore the role of miRNAs in the factors of digestion, including the possible manipulation of miRNAs in microbial communities to improve biodegradability. In future miRNAs could be another way to enhance selective characteristics to biofuel production mechanisms.

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Authors' contributions

Data collected by: SH Ali & M Din, Wrote Paper: SH Ali, MYK Barozai & AN Azziz.

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