

BREEDING CLIMATE SMART FORAGE SORGHUM – A REVIEW

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SUMMARY

Unfavourable weather conditions like extreme of temperature heat or cold, drought, salinity etc. hinder the growth and development of the plants. These abiotic stresses cause huge productivity losses and are of main concern for the agricultural scientists to meet the challenges of global food security and nutritional requirements. Since the time of the green revolution, the scientists have been working on engineering plant mechanisms to overcome the abiotic stresses in cereals, pulses and oil seed crops to increase productivity but now it is high time to think about improvement of fodder crops. If we want to improve animal health and in turn human nutrition and health, we must use advanced genomic tools for forage crop improvement. Forage sorghum is a leading forage crop in India and improving forage sorghum for stress tolerance, forage yield and quality has great implications for farmers with small land holding. In this review paper, main focus is on forage sorghum biodiversity, its importance as forage crop, effect of climate on its morphological and quality attributes and use of advance techniques like genomics, transcript-omics, proteomics, metabolomics, and phenomics for development of better cultivars of forage sorghum. There is ample scope for improvement of forage yield and quality of sorghum.

Key words: Forage sorghum, quality, climate resilience, molecular

Sorghum is the world's fifth most important cereal crop cultivated in semi-arid tropics of the world. Globally an estimated 500 million people rely on sorghum as their primary staple food (Morris *et al.*, 2013). It is a cereal crop with multiple uses and has great demand not only as food but as animal feed, fodder and biofuel production also. It is a C₄ diploid crop having 2n = 20 with some tetraploid and hexaploid wild and weedy relatives. Sorghum is an annual plant similar to corn with white, green and brown colored midrib, waxy leaves with sweet and juicy stem. It is tolerant to drought, salinity, and water stagnation and highly responsive to fertilizer application due to which it is easily grown in Arid and Semi-Arid Tropics (Tari *et al.*, 2013). USA being the major exporter of sorghum grain while China being the major importer. In North-India it is mainly cultivated as fodder crop during summer and *kharif* seasons and in South India it is mainly grown for grain round the year. Due to its high green biomass yield, palatability and digestibility it is relished by animals. *S. bicolor* is widely cultivated species spread over 120 countries of Africa, Asia, Australia, and Europe including tropical, semi-tropical, arid and semi-arid environments (FAOSTAT, 2015).

The genus *Sorghum* belongs to family Poaceae and is indigenous to Africa, and the present day varieties

of grain and forage sorghum were evolved in this continent. Sorghum reached China and in Western Hemisphere much later. It spread from Africa to America (early 17th century) and Europe with the slave traders and colonists, respectively. In US it was grown primarily as a source of sugar for syrup. Demand for drought resistant forage sorghum varieties was created in West. Extensive forage cultivation in United States started only after the introduction of Black Amber (also called "Chinese sugarcane") by way of France after 1850s. Johnson grass was imported from South Carolina for forage in 1830. After that various forage sorghum single cut, multicut, dual type varieties/hybrids were introduced from other countries and developed domestically (Bhat 2020). Genus *Sorghum* comprises of 25 species divided into five subgeneras: *Chaetosorghum*, *Heterosorghum*, *Parasorghum*, *Stiposorghum* and *Eusorghum* (USDA-ARS 2012). The *Eusorghum* contains the agronomically important species *viz.* *Sorghum propinquum* and *Sorghum halepense* L. (Paterson *et al.*, 1995). Due to huge variability within species classification of *S. bicolor* has been controversial and challenging. Currently, *S. bicolor* has three recognized subspecies namely subsp. *bicolor*, subsp. *verticilliflorum* (Steud.) and subsp. *drummondii* (Steud.). The subspecies *bicolor*

includes the domesticated sorghum used for grain. Based on floral morphology it is divided into five interfertile races (*Bicolor*, *Kafir*, *Caudatum*, *Durra* and *Guinea*) and 10 intermediate races viz., guinea-bicolor, caudatum-bicolor, kafir-bicolor, guinea-caudatum, durra-bicolor, guinea-kafir, guinea-durra, kafir-caudatum, durra-caudatum and kafir-durra (Harlan and deWet 1972; Morris *et al.*, 2013) having different morphological features and diverse uses. Cultivated sorghum is close relative of perennial sorghum species *Sorghum halepense* (Johnson grass) (Arriola *et al.*, 1996). Natural hybrid segregant between *S. bicolor* (L.) Moench and *Sorghum arundi-naceum* (Desv.) Stapf cross called *sudangrass* has originated in region from Southern Egypt to Sudan. It is mainly used as fodder for animals as it can be grazed or utilized in cut-and-carry forages, as silage or hay.

Suitability of sorghum as green fodder

Sorghum, sudangrass and sorghum x sudangrass hybrids due to their high tillering and fast regeneration potential produce nutritious fodder and fits very well in different cropping patterns (Marsalis, 2010). Forage sorghum is major forage crop of summer and *kharif* season. It is one of the main summer forages having moderate tolerance to saline and sodic soils and has ability to tolerate soil toxicities much better than maize, bajra etc. Less irrigation and fertilizer requirements make it more economical. Its stem is juicier (brix value upto 20%), has high crude protein content and *in-vitro* dry matter digestibility as compared to other cereal forages (Aruna *et al.*, 2018). Multicut varieties harvested only after 55-60 days of sowing and single cut will be harvested at 50% flowering stage or at heading stage to get maximum yield with quality fodder. Dry matter yield potential of forage sorghum is good even in the rainfed low-fertility farming situations of the semi-arid tropics (Reddy and Blümmel, 2020). Sorghum, sudangrass and sorghum x sudangrass yield multiple cuts unlike fodder maize (Mut *et al.*, 2017). As a silage crop, sorghum can withstand more heat. Forage sorghum has higher dry-matter accumulation potential as compared to other annual cereals when equal amount of water supplied (Teutsch, 2002). Therefore, sorghum is an ideal forage crop due to its high tillering, quick growth habit, good regeneration potential, leafiness, high green and dry fodder yielding ability, wide adaptability and tolerance to salinity, drought and water stagnation.

Target traits for forage sorghum breeding

To combat global fodder scarcity and to improve animal health development of climate-resilient forage crops is critical to safeguard. Sorghum has ability to grow in harsh conditions. Success of any crop improvement program depends on the extent of available genetic variability (Begna, 2021). Various researchers have worked to find the traits associated with fodder yield and quality in forage sorghum. Out of these some morphological and biochemical traits have been genetically analysed and used successfully to develop varieties having wide adaptability, higher fodder yield, superior fodder quality and resistance to biotic and abiotic stresses (Biswas *et al.*, 2020). In sorghum, there is enough variability for various traits like days to 50% flowering, early vigor, plant height, leaf length, number of leaves, leaf width, stem thickness, green and dry biomass, regeneration potential in case of multicut, etc. Good forage quality is correlated with high leaf: stem ratio, absence of wax on the stem, tan plant type, stalk sweetness, high digestibility, high protein and low HCN. Tan plant type is known to be highly correlated to resistance to various foliar diseases. Forage sorghum germplasm was studied for various important morphological and quality traits Grewal *et al.* (1996), Pahuja *et al.* (2005), Reddy *et al.* (2006) and Vijaylaxmi *et al.* (2019) using germplasm collection of ICRISAT. High heritability coupled with high genetic advance for various traits in diverse material indicates that selection based on total soluble solids, leaf: stem ratio and stem girth may be useful for further improvement of forage sorghum cultivars for good quality fodder (Singh *et al.*, 2016 and Thant *et al.*, 2020).

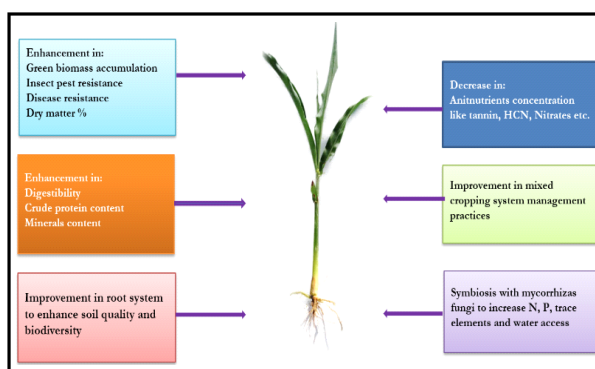


Fig. 1. Target traits for forage sorghum improvement.

Target traits that are crucial for adaptation and enhancing production in forage sorghum under changing climatic scenario are as follows:

1. High green and dry fodder yield
2. Profuse tillering and good regeneration potential
3. High grain yield potential in multicut types
4. High TSS% (Total soluble solids)
5. Responsive to fertilizers
6. Biotic resistance (like foliar disease resistance and insect pest tolerance)
7. Resistance to lodging
8. High palatability
9. Low hydrocyanic acid and lignin content
10. High crude protein and *in-vitro* dry matter digestibility.
11. Heat tolerance
12. Cold tolerance

Sorghum is an often cross pollinated crop and various breeding techniques are followed for its improvement like mass selection (simplest and least expensive of plant-breeding procedures) or we can say phenotypic selection is used widely since ages to improve landraces that have been passed down from one generation to the next over long periods at farmer's field (Witcombe *et al.*, 1996). Using mass selection, we can improve traits namely green biomass yield, dry biomass yield but for quantitative traits such as regeneration potential and tillering ability which are having low heritability but amenable for improvement through progeny selection more effectively as compared to phenotypic selection (Dalid *et al.*, 2018).

Pure line selection technique is widely used in the breeding of forage crops. In pure line selection any progeny superior to the existing variety is released as a new "pure-line" variety when large numbers of genetically variable landraces are available. In India, forage sorghum breeding started during 1930s and upto 1980s numerous varieties have been released using pedigree selection like SSG 59-3 which was 1st multicut forage sorghum variety in India after that various single cut and dual type varieties like Pusa Chari (1st single cut variety), HC 136, HC 260, HC 308, PC 5, PC 7 etc. were released at state and national level. In recent years, the importance of this breeding method for major cultivated species has declined but is still in use for improvement of forage crops. Later on during 20s hybridization was used in breeding of forage crops to combine desirable genes present in two or more varieties and to produce pure-breeding progeny superior in many aspects to the parental types. CMS based hybrids *viz.*, CSH20MF and CSH24MF were released from G.B. Pant University of Agriculture Science and Technology under AICRP on Sorghum

programme for cultivation at national level. In 2017, a new multicut variety CSV33MF was released using mutation breeding from Co(FS)-29, which has annual forage yield potential of more than 100 tons/ha from 3 cuts and has potential for 7-8 cuts under favourable conditions and a new multicut hybrid CSH43MF has also been released in 2020 at national level possessing high fodder yield and quality.

Genetics of important morphological and quality traits

Forage sorghum improvement started after its domestication but its genetics was mainly exploited during 19th century for major traits like days to 50% flowering, number of tillers per plant, regeneration potential, plant height, leaf length, leaf breadth, no. of leaves, stem girth, crude protein, *in-vitro* dry matter digestibility etc. Study of genetic architecture of these traits is imperative before planning any forage sorghum improvement program.

Days to Flowering

It is an important trait for all the forage crops. There is tremendous amount of diversity for days to 50% flowering in sorghum. Early types are preferred in grain sorghum whereas late flowering is desirable for forage sorghum. Flowering in sorghum is highly influenced by the genotype, environment, temperature and photoperiod. Quinby in 1967 identified 4 loci Ma₁, Ma₂, Ma₃ and Ma₄ in sorghum related to maturity. Days to 50% flowering varies from 38-150 days in sorghum but for fodder purpose genotypes having late flowering (>80 days) are preferred. Days to 50% flowering is also highly correlated with good quality like high concentration of crude protein% and IVDMD % in forage sorghum. As far as its genetics of days to 50% flowering is concerned various workers have studied it some reported that it is governed by additive/non-additive gene action. It is also highly heritable trait and have high to moderate genetic advance (Thant *et al.*, 2021 and Akshitha, 2022). But its genetics and GXE interaction needs to be studied at molecular level.

Plant height, stem girth, internode number and length

There is huge diversity for plant height, stem girth, number of internodes and internode length in sorghum. In most of the sorghum growing areas of the world tall stature is preferred for fodder purpose.

TABLE 1
Released varieties/hybrids of forage sorghum popular in India

Released varieties/hybrids of forage sorghum	Main characteristics (GFY and DFY q/ha)
SSG 59-3	GFY: 750; DFY: 200; first multicut variety released at national level, is tolerant to water logging and drought. It is sweet stalked, and have high early vigor
CSV 33MF	GFY: 1040; DFY: 280; multicut variety, tall, pyramidal and very loose panicle, resistant to all major diseases and tolerant to pest infestation, resistant to lodging
Pant Chari 4	GFY: 425-475; DFY: 130; single cut variety, tolerant to bird damage, tolerant to stem borer and shootfly with good fodder quality
Pant Chari 5	GFY: 482; DFY: 134; dual purpose variety, juicy, sweet, resistant to major foliar diseases
HC 308	GFY: 530; DFY: 175; is a single cut variety, juicy, sweet, resistant to major foliar diseases and resistant to lodging
CSH 20MF	GFY: 872; DFY: 243; multicut hybrid, tall, juicy, sweet, resistant to foliar disease
CSH 24MF	GFY: 914; DFY: 232; multicut hybrid, having tan plant color, resistant to major foliar diseases and insects, lodging resistant, good hybrid seed yield
CSV 21F	GFY: 410; DFY: 125; single cut variety, tall, tolerant to shoot fly and stem borer
CSV30F	GFY: 420-450; DFY: 120-130; single cut variety having non-tan plant type, tall, symmetric panicle shape, semi-loose ear head compactness.
CSV 32F	GFY: 463; DFY: 178 ; single cut variety, tall, sweet, juicy, resistant to foliar diseases
CSV 43BMR	GFY: 150-200; first brown midrib fodder purpose variety released in India. It contains less lignin (4.12% ADL), high brix, juicy and good for silage.
CSH 43MF	GFY: 900-950; DFY: 210-230; multicut forage sorghum hybrid, tall, having low HCN content, high protein content, high digestibility and resistant to foliar disease.
CSV 44F (Single cut)	GFY: 407; DFY: 112; single cut forage sorghum variety, tall, sweet, juicy, high TSS%, tolerant to shoot Southern region fly and stem borer
CSH 40F	GFY: 560; DFY: 155; single cut forage sorghum hybrid, tall, sweet, juicy, low HCN, high TSS%, high crude protein percent (8.4%) and in-vitro dry matter digestibility (53%)
HJ 541 (Single cut, Haryana)	GFY: 525-550; DFY: 160-180; single cut forage sorghum variety, tall, sweet, juicy, low HCN, high digestibility, tolerant to shoot fly and stem borer
CSV 53F	GFY: 482; DFY: 152; single cut forage sorghum variety, tall, sweet, juicy, low HCN, high TSS%, tolerant to shoot fly and stem borer, resistant to foliar diseases.

There are 4 important loci in sorghum involved in controlling plant height Dw_1 , Dw_2 , Dw_3 and Dw_4 (Quinby & Karper, 1957). Tallness is dominant over dwarfness and effect of these four genes is additive in nature. Generally, hybrids are found taller than their parents and for plant height 59 % heterosis was reported by various workers (Patel *et al.*, 2018). The hybrids between sorghum x *sudan* grass are taller and have high heterosis over standard check and superior parent. But expression of heterosis for plant height is greatly influenced by the growing conditions like rainfall, soil fertility etc. Both additive and additive x additive gene interactions have been reported for plant height. High heritability in association with high genetic advance was observed for plant height which indicates that these characters are associated with additive gene action (Patel *et al.* 2018). Similar results were also observed by Jain *et al.* (2010) and Jadhav *et al.* (2011).

Tillering

Effectiveness of apical dominance determines the tillering ability of a genotype. Inhibiting influence of apical dominance on auxiliary bud development is under hormonal control. Number of tillers vary from 1-15 in various plant types. Single cut types are mostly single stemmed but multicut types have tillers along with main stem and number of tillers may vary from 2-15. Multicut varieties like SSG 59-3 and CSV33MF have more number of tillers as compared to multicut hybrids like CSH20MF, CSH24MF and CSH43MF. High heritability and genetic advance was reported by various workers for number of tillers per plant (Subramanian *et al.*, 2019). Kebrom *et al.* (2006; 2010) identified a gene *tb1* controlling tillering transcription factor in forage sorghum but more detailed study on genetics of tillering is required to develop multicut types in forage sorghum.

Leaf traits

There is considerable amount of variation in number of leaves, leaf length and leaf breadth in forage sorghum but no consistent difference in number of leaves in forage sorghum varieties and hybrids was reported. Vinoth *et al.* (2021) reported 20%, 29% and 5 % heterosis over better parent for number of leaves, leaf length and leaf breadth, respectively. Several workers have reported involvement of additive and non-additive gene actions in the inheritance of leaf traits (Akbari *et al.*, 2012) but some workers also reported involvement of epistatic gene interaction in their inheritance. Vijaylaxmi *et al.* (2019) reported high heritability for number of leaves. Till date no molecular study was done on leaf characters as these are important characters in both grain and forage types and use of molecular breeding tools is very important for their improvement. Akshitha, 2022 reported that crude protein percent was positively correlated with number of leaves per plant.

Yield characters

Leaf weight, stem weight, green fodder yield and dry matter yield are major characters of fodder yield in sorghum. Extensive studies have been carried out to estimate heterosis for green and dry fodder yield. In case of forage sorghum upto 66% heterosis was reported by (Prakash *et al.*, 2010) for green and dry fodder yield. However, hybrids have shown high heterotic potential for green and dry fodder yield over their parents. High heterosis was reported in summer season for both green and dry fodder yield as compared to monsoon season which may be due to favourable conditions for gene expression during summers as compared to monsoon (Sheoran *et al.* 2005). But study of genes/QTLs affecting yield needs to be investigated in detail using molecular breeding techniques. High heritability accompanied with high genetic advance as per cent of mean for leaf: stem ratio, green fodder yield per plant and dry matter yield was reported by Ranjith *et al.* (2017) and Kour and Pradhan (2016).

Quality

In any forage crop quality of fodder is an important trait. The major quality attributes in forage sorghum are crude protein, IVDMD%, ADF (Acid detergent fibre), NDF (Neutral detergent fibre), and

toxic constituents like HCN and tannin. The stage of crop growth is also an important factor influencing fodder quality and quantity. Maximum forage yield and quality is reported at the stage of days to 50% flowering.

Crude protein and digestibility are important parameters which determine nutritive value of the fodder. In some studies, positive correlation has been reported between crude protein and digestibility (Vijaylaxmi, 2015). Concentration of crude protein and IVDMD vary from 3.0 - 8.5 % and 40.4 - 66.2%, respectively in forage sorghum. Both these traits are mainly governed by additive gene action. High heterosis has been reported for quality traits by various workers in forage sorghum. High heritability and high genetic advance was reported for crude protein and IVDMD% (Deep *et al.*, 2019). Some workers have reported that IVDMD% was highly heritable as compared to crude protein (Vijaylaxmi *et al.*, 2019). High positive heterosis for protein content has been observed by Akabari and Parmar (2014), Akabari *et al.* (2014).

Total soluble sugar is also an important quality parameter. It affects the palatability and acceptability of sorghum fodder by animals. Its concentration varies from 4-17% in forage sorghum. Varieties/hybrids with high TSS% are preferred by animals. There are some improved varieties which are having more than 10% TSS for e.g. CSV 44F, CSV 53F etc. Vijaylaxmi *et al.* (2019) reported high heritability and high genetic advance for TSS%. ADF%, NDF% and hemicellulose concentration vary from 65-72%, 40-45% and 26.3%, respectively in forage sorghum (Singh *et al.*, 1997). High heritability and high genetic advance has been recorded for these parameters and they are governed by additive gene action (Kumar *et al.*, 2011).

In addition to these quality parameters sorghum also possesses some anti-nutritional factors which adversely affect the forage quality and make it unsuitable for fodder purpose for e.g. HCN and tannin. Range of HCN in sorghum vary from 0-1000 and above ($\mu\text{g/g}$ on fresh weight basis) which is toxic for animals (Aruna *et al.*, 2011). The permissible limit of HCN for forage types is from 0-200 $\mu\text{g/g}$. Its concentration is high at the early stage of crop growth *i.e.* from 30-35 days after sowing and after this stage its concentration starts decreasing. There are various factors which affect the HCN concentration in sorghum like plant age, plant morphology, genotype, environmental stress like drought, soil fertility etc. (Fjell *et al.*, 1991). HCN concentration increase in case of water stress. Chakraborty *et al.* (2020) reported that HCN was

negatively correlated with TSS content in forage sorghum. High genetic advance and high heritability was reported by (Akshitha, 2022) for HCN concentration. Significant negative heterosis was reported by various workers for HCN concentration in forage sorghum (Kumar *et al.*, 2013). Similarly, tannin is also anti-nutritional factor present in sorghum. It causes bitterness of fodder and affects its palatability and digestibility. Its concentration varies from 1- 6.2% on dry matter basis. Its concentration in plant decreases 20 days after sowing but it again starts increasing after 50-55 days of sowing with simultaneous increase in TSS% (Aruna *et al.*, 2011). Leaves contain high tannins as compared to stem. Varieties/hybrids which are susceptible to leaf spots diseases possess high tannin concentration as compared to resistant varieties as it reduces the digestibility of sorghum leaves. Negative heterosis for tannin content was reported by Bibi *et al.* (2012), Kamatar *et al.* (2003), Jadhav and Deshmukh (2017).

Traits governed by additive and non-additive gene actions are generally improved by recurrent selection method of breeding (Akabari *et al.*, 2012). But use of molecular breeding tools would certainly speed up the process of improvement for these traits. Even after remarkable development in the field of molecular biology very little work has been done on molecular breeding in forage sorghum using advanced genomic tools. Till now genes/gene location for few traits has been studied by using molecular breeding techniques for e.g. *plant height* (tall:short; located on chromosome no.SBI-06, SBI-07, SBI-09); *leaf color* (normal:brown midrib; located on chromosome no.SBI-07 & SBI-04); *stem type* (pithy:juicy; located

on chromosome no.SBI-06); *plant color* (purple:brown; located on chromosome no.SBI-06); *leaf sheath* (waxy:bloom; located on chromosome no. SBI-10) and *tillering* (non-tiller: tiller; located on chromosome no.SBI-01) were studied by Klein *et al.* 2008; Saballos *et al.* 2008; Xu *et al.* 2000; Srinivasan *et al.* 2009; Burrow *et al.* 2009; Kebrom *et al.* 2006, respectively. Choi *et al.*, 2020 identified 14 candidate genes of dhurrin metabolism. The expression of these candidate genes was highest at the seedling stage and decreased at the adult stage. The expression of these genes will be helpful in prevention of HCN toxicity, drought tolerance and pathogen resistance.

Molecular Breeding for forage sorghum improvement

Fodder crops are generally raised on marginal and degraded lands. There is little scope of increase in area under fodder crops due to rise in demand of food for human population. So, the need of the time is that some climate resilient high yielding varieties of major fodder crops must be developed to feed the growing livestock population with quality fodder (Ghosh *et al.*, 2016). Some plant species have in built molecular regulatory mechanisms to develop resilience to biotic and abiotic stresses *via* release of some anti-nutritional factors like sorghum have high hydrocyanic acid concentration in drought conditions and release of phenolic compounds against insect pest attack. Thousands of genes are involved in adaptation of particular genotypes to specific environment which in turn also determine plant plasticity.

Plasticity is the ability of a plant to respond to

TABLE 2
QTLs/genes identified for various traits in forage sorghum

Trait Studied	Gene/QTL	Gene ID	Protein encoded	References
Tillering	tb1	Sobic.001G121600	Tillering Transcription factor	Kebrom <i>et al.</i> (2006; 2010)
Cytoplasmic male sterility	Rf1/SbPPR13	Sb08g019750	Fertility Pentatrigo peptide repeat protein	Klein <i>et al.</i> (2005)
	Rf2	Sobic.002G057050	Fertility Pentatrigo peptide repeat protein	Madugula <i>et al.</i> (2018)
	Rf6	Sobic.004G004100	Fertility Pentatrigo peptide repeat protein	Praveen <i>et al.</i> (2015)
Brown midrib	bmr2	Sb04g005210	Brown midrib 4-Coumarate: coenzyme A ligase	Saballos <i>et al.</i> (2012)
	bmr6	Sobic.004G071000	Brown midrib Cinnamyl alcohol dehydrogenase	Saballos <i>et al.</i> (2009)
	bmr12	Sobic.007G047300	Brown midrib Cafeic O-methyl tranferase	Sattler <i>et al.</i> (2012)

specific environmental conditions and these days' environments have become more unpredictable so it plays a pivotal role to ensure global food security in changing climate scenario (Muluneh, 2021). The available traditional conventional breeding approaches are under exploitation for fodder crop improvement since the inception of forage crop breeding. Due to rapidly changing climatic scenario there is an unpredictable adverse effect on agricultural productivity including fodder crops (Lin et al., 2011). Therefore, the biomolecule triggered defence systems identification and their activation/deactivation are the major focus points to increase crop productivity. In the present chapter, we tried to discuss the impact and future prospects of the advanced molecular breeding approaches for development of climate smart forage sorghum genotypes resistant to biotic and abiotic stresses (Fig. 2)

Molecular markers approaches which were developed in 20th century like RFLPs, RAPDs, SSRs etc, were less accurate, costly and laborious techniques but discovery of SNPs and InDels high-density marker arrays from next generation sequencing method facilitated the GBS (genotyping-by-sequencing); GWAS (genome wide association studies) and GS (genomic selection) approaches, which are fast and accurate as compared to conventional phenotypic selection approaches for discovery of novel genes for abiotic stress tolerance in forage sorghum and their further use in varietal development. Li et al., 2018 identified 5, 3, 3, 7, and 24 QTNs related to crude protein, cellulose, hemi cellulose, acid detergent fibre and neutral detergent fibre, respectively using GWAS. Yamazaki *et al.* (2020) using genome-wide association study identified two single nucleotide polymorphism in sorghum that were significantly associated with biomass production. Similarly, Choi *et al.*, (2020) identified 14 candidate genes of dhurrin metabolism. The expression of these candidate genes was highest at the seedling stage and decreased at the adult stage. This expression of these genes will help in selection of accession for fodder breeding programs to prevent HCN toxicity in livestock or promote drought tolerance or pathogen resistance. Lignin is a phenylpropanoid polymer which improves strength and rigidity in cell walls, as well as mechanical support for leaf blades and stems. But it adversely affects the digestibility of forage in animals (Frei, 2013). Lignin reduction while increasing sugar and cellulose content are current priorities in breeding, in order to improve digestibility for livestock (Jung *et al.*, 2012). Niu *et al.*, 2020 identified nine quantitative trait loci (QTLs) for lignin

content using GWAS technique. However, the complex genetics of quality traits in forage sorghum is not well elucidated till date.

Gene introgression, gene pyramiding with the help of high-throughput phenotyping techniques and marker-assisted breeding provided a new avenue for enhancing the selection efficiency and development of abiotic stress resistant genotypes for future crop production. In forage improvement programmes, where cultivars and breeding populations are characterized by high genetic diversity and substantial G × E interactions, precise and efficient phenotyping is very essential to meet future challenges imposed by climate change, growing demand of fodder and declining resources (Walter 2012). But some new issues are there which need to be addressed like development of forage sorghum genotypes adapted to post rainy (*rabi*) season which can withstand cold stress suitable for tropical countries for availability of forage sorghum in spring/winter season.

High reliability and fast analysis approach of next generation sequencing techniques had transformed dramatically the genomics-assisted breeding approaches. Forty-four sorghum genotypes including 16 improved, nine landraces and 17 wild relatives of sorghum were studied by using SNP markers and Ochieng *et al.* (2021) reported that wild relatives and landraces are a good source of drought tolerance alleles. Functional genomics approaches are major genomic tools which helps in identification of genes/QTLs responsible for the major abiotic stresses from wild relatives in forage sorghum. Which in turn are helpful for development of forage sorghum genotypes tolerant to abiotic stresses mainly cold and drought.

As sorghum is a C₄ crop having versatile end uses for forage, biofuel and food. Genome references of BTx623 and Rio were major breakthroughs for deciphering the underlying causes for grain and sweetness accumulation in plant (Cooper *et al.* 2019). But another aspect is that feed and fodder are equally important in developing countries like India. Now a step ahead Joint Genome Institute and HUDsonAlpha (Boyles et al., 2019) jointly announced an additional sequencing of 12 sorghum genotypes using whole genome sequencing for pan-genome for sorghum. It will be helpful in identification of target genes and their expression pattern study. Forty-four sorghum accessions were sequenced using WGS at a minimum coverage of 16x (Mace *et al.* 2013). Use of advance technique to study genetic architecture of forage sorghum is urgently required.

The grain sorghum genome was sequenced by Paterson *et al.* 2009 and later on many genes/QTLs were identified and introgressed in different genetic backgrounds to enhance crop yield under biotic and abiotic stresses. But major QTLs have been identified related to drought and salinity in grain sorghum (Sanchez *et al.* 2002), but cold tolerance is also a major problem which needs to be addressed that can provide novel opportunities for abiotic stress tolerance. Upadhyaya and co-workers in 2013 identified few SNPs linked to biomass and height in sorghum under salinity through linkage disequilibrium. Recombinant inbred line was studied by Wang *et al.* (2020) and they identified QTL clusters which control the plant height, total biomass, and fresh weight in forage sorghum. They also reported some SNPs that were significantly associated with the salt tolerance index. These SNPs might serve as target sites for marker-assisted selection in improving the salt tolerance of forage sorghum. To be precise a focused research for novel allele's identification in wild germplasm for development of cold tolerant forage sorghum genotypes is required for cultivation in tropical countries especially in Germany, Poland, and Denmark etc.

Sorghum genus is having huge and diverse germplasm but in the recent past, a handful of studies have been conducted in forage sorghum using molecular biology approaches for incorporation of newly identified major variation from landraces, ancestral or wild crop relatives into elite genetic backgrounds. But if it is explored precisely with new advanced tools like multi-omics will surely help in development and commercialization of climate-smart genotypes with yield increment with biotic and abiotic stress tolerance/resistance (Kumar *et al.*, 2022).

Use of the available CMS system is also a big advantage for sorghum improvement. Various hybrids have been released in India and worldwide in sorghum for grain, fodder and sweet sorghum types. Three fertility restorer genes viz., rf_1 , rf_2 , rf_4 and rf_5 belonging to pentatricopeptide repeat (PPR) gene family have been identified in sorghum (Praveen *et al.*, 2015). Use of molecular approaches is required for development of better restorers in forage sorghum having ability to develop cold and salinity tolerant forage sorghum hybrids because in rapidly changing climate scenario these two traits are of prime importance in breeding programme.

Nested Association Mapping (NAM) populations architecture dissection provides incredibly powerful resources for genomic dissection. Its major advantage is that it delimits the detection of false

positive and allows detection of small effect loci specially using GWAS (genome wide association studies). Bouchet *et al.*, (2017) developed a nested association mapping (NAM) population using 10 diverse global lines crossed with an elite reference line RTx430 in sorghum for adaptive traits like flowering time and plant height dissection. Similarly, we can develop NAM populations in forage sorghum for morphological and quality traits.

Targeted genome-editing using CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats/ CRISPR associated protein 9 nuclease) is the latest technology for genome improvement via knocking out or silencing target genes or genomic regions, point mutation and their manipulation for development of plant system suitable for changing climatic conditions. In major cereal crops numerous reports of CRISPR/Cas9 are available but there is not a single report on use of CRISPR/Cas9 for fodder crop improvement, but efforts are underway. Lui *et al.*, 2019 used CRISPR/Cas9 system for investigating sorghum genome editing through biolistic bombardment for two genes CAD (cinnamyl alcohol dehydrogenase) and PDS (phytoene desaturase). Successful genome editing has been achieved in sorghum genotype Tx430. Sequencing also confirmed gene editing and their heredity. But now focus is on crucial elements to establish a well CRISPR/Cas9 system for forage sorghum. Targeted gene editing in sorghum helps to generate elite cultivars for high grain yield (Grain sorghum), sugar accumulation (sweet sorghum), tillering and regeneration (forage sorghum), lignocellulosic biomass (bmr types) etc. having abiotic and biotic stress tolerance.

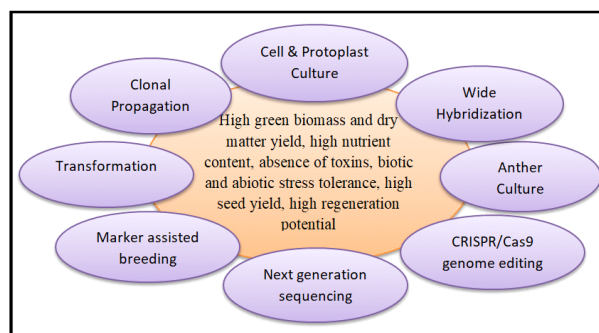


Fig. 2. Biotechnological tools for forage crop improvement.

Future Prospects

Use of advanced tools and techniques for agricultural progress in 21st century are playing a major role in developing new crop varieties with market preferred traits which can withstand adverse abiotic

stresses would further help in fighting vagaries of weather. Improved weather forecasting methods coupled with these improved cultivars and better agronomy will greatly help in increasing forage productivity. Advances in climate science are helpful in issuing of advisory to the farmer's from time to time regarding sowing time, selection of right variety and maintenance practices for various crops. Simultaneously the use of genomic tools in crop improvement would help in development of efficient products, easy evaluation, and selection of most adapted genotypes with market desired attributes. But more rigorous, multidisciplinary and integrated approaches/multi-omics can speedup crop improvement programs manifold for accumulation of efficient and favourable alleles like stay-green, number of tillers per plant, regeneration potential, total biomass and tolerance to biotic and/or abiotic stresses definitely in genetic background of forage sorghum *vis-a-vis* from different backgrounds. High throughput phenotyping and genotyping techniques certainly offer troundays potential for identification and selection of salt-tolerant sorghum varieites with high forage productivity. Thus knowledge trait interactions is very important for success of any breeding programs. Further it will also help to develop genotypes that are more resilient to climate change for fodder security in future.

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