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REVIEW ARTICLE

Improving Water Stress Resilient Crop Breeding Using Phenomics and Genomics Information Derived from Sorghum (*Sorghum bicolor* L.)

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ABSTRACT

In this unpredictable climatic scenario, increasing crop productivity under low water availability is the foremost challenge. The crops are further seriously affected, and the yields are drastically reduced due to elevated temperature, greenhouse gases, and humidity during the water stress period. To ensure food security in the coming decades, scientists have summoned to increase the high-quality food with these climatic vagaries. Though several agronomic and management strategies were proposed to mitigate the water stress, genetic improvement of crops with improved drought tolerance is the simple, sustainable and affordable option. Nevertheless, identification and molecular understanding of the appropriate breeding traits that can alleviate the impact of water stress on crop plants are the trickiest part of this strategy. Sorghum (*Sorghum bicolor* L.) is gaining its importance in water stress tolerance plant breeding, as it has several clearly defined drought-tolerant component traits that promote productivity under low water environments. The genomics and phenomics information generated in *S. bicolor* would immensely help breeding plants resilient to the challenges of a water scarcity. This paper describes the molecular mechanisms of drought tolerance using *sorghum bicolor* as a model and how this information can be extrapolated to breed better cultivars in other crops.

Keywords: *Resilient Plant Breeding; Drought Stress; Phenomics; Drought Tolerant Component Traits; Genomics Assisted Breeding.*

INTRODUCTION

Water Stress and its Impact on Crop Production

The percentage of the planet affected by drought has more than doubled during the last 40 years (Vicente-Serrano *et al*., 2020). Although water stress has various impacts on living organisms, including human beings, the primary victim would be crop plants as they cannot move away from stress. Among the several stresses that havoc crop productivity, drought is considered the primary cause for poor plant growth and development, thereby leading to huge loss and frequently threatening food security.

Usually, water stress or drought arises due to poor availability of soil water and constant loss of water from plant through transpiration that support average crop productivity. It is speculated that the harmful effects of water stress are going to increase rapidly in the coming years due to changing climatic conditions and global warming. Further, incrementing water use byever-growing urbanized human population will also lead to a severe shortage of water in the future. Although the rainfall is adequate, poor distribution of the rainfall in a calendar year or alterations in the rainfall pattern also leads to water stress in plants.

Sometimes water stress also occurs strictly not because of a water deficit in the environment: there is enough water in the soil, but several factors (such as salinity, low soil temperatures and flooding, fertilizer misapplication, high temperature, high intensity of light, dry wind *etc*.,) prevent or decrease water uptake by roots and subsequently lead to water stress in plants. This type of drought is called pseudo-drought or physiological drought (Arbona *et al.* 2013) and in this case, atmospheric conditions are not determining factors for drought. Essentially, when the water potential of the soil is lower than the water potential of plants, drought stress occurs.

Water stress leads to several adverse effects in growth and physiology of the crop plants (Bakht *et al.* 2020 and references therein) *viz*., i) it impedes germination, ii) reduces leaf water potential and relative water content, diminishes turgor pressure and stomatal activity that lead to stomatal closure and limitation of gas exchange and transpiration

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(subsequently disordered enzyme activities (most of the enzymes are not produced, but some of them are induced at high rate (examples: late embryogenesis abundant protein, heat shock proteins, peroxidases) and decreased energy supply from photosynthesis due to increase in leaf and canopy temperature besides production of reactive oxygen species (ROS), iii) resulting in oxidative damage in chloroplasts through alterations in Photosystem I and II, iv) affects plant mineral nutrition and disrupts ion homeostasis, v) messing up the metabolic processes, vi) decreases the cell membrane stability and damages cell division, expansion and elongation, vii) reduces stem length viii) decreases the number and size of leaves (due to decrease in the number of stomata, cell wall thickening, cutinization of the leaf surface and developed conductive system (thereby increase in the number of large vessels), submersion of stomata in succulent and xerophyte plants, the formation of tube leaves in cereals and premature leaf senescence) which lead to lessening in biomass, ix) increase in the different system development but at the cost of decreasing the shoot development, affecting the normal growth and development of reproductive organs and x) ultimately affecting the economic yield.

The response and acclimation of plants to drought stress, as well as other stresses that cause soil water deficit, may be governed by the action of wide array of molecules and metabolites, including protective compounds and hormones. Recently Wojtyla *et al.*, 2020 have reviewed a snapshot of wide array of such molecules and metabolites in crop plants that are involved in drought sensitizing, drought-responsive, water stress signal transduction, memorizing and responding later stages, regulating and modifying the gene expression and epigenetic control of selected genes under water stress (such as DNA methylation, histone modifications and gene silencing by small RNA (sRNA), including micro RNAs).

It should also be noted that there are varying intensities and duration of water stress that differently affect the plant depending on the phenological stage of the crop plants. Drought stress varies in levels of intensity, duration, spatial extent and impact. For drought stress, the severity and duration of the stress and responses of plants after stress removal and interaction between stress and other factors are extremely important (Boopathi, 2020). Severe drought occurrences have drastic impacts on plants and sometimes, it may lead to complete yield loss. For instance, drought stress occurs at the germination or reproductive phase has more impact on yield than it occurs during the vegetative phase for a short period of time. Further, response to the drought stress greatly varies from crop to crop and even within the crop species (which clearly relies on crop stage, soil heterogeneity, micro-climatic conditions and so on).

Additionally, it is prominent that mild soil water deficit (for short periods) may enhance stress tolerance by inducing stress memory, also referred to as imprint, training, priming, hardening, conditioning and acclimation. Information about stress memory expressed as behavior of plants subjected to recurrent drought, is still limited. The molecular reaction of plants to a subsequent drought exposure can be specified in an enhanced response, a more efficient response and a more rapid response. These mechanisms of drought stress imprint are based on plant response on metabolomics, proteomics and transcriptomics levels. Drought stress memory has been observed in several species such as *Arabidopsis*, maize and switch grass (Wojtyla *et al.* 2020), but a lot has to be done to design a fruitful strategy to mitigate the drought stress in plants.

Water Stress-Related Biotic and Abiotic Stresses: Interaction and Impact on Crop Productivity

It is not uncommon to realize the fact that whenever the water stress occurs in the field, crop also experiences several other biotic (herbivores, insects, pathogens (bacteria, viruses, fungi and nematodes) and abiotic stresses (adverse conditions such as increased soil and canopy temperature, light intensity, dry wind speed *etc.*). Simultaneous occurrences of several stresses actually speed up the process of complete loss of crop productivity. On the other hand, it has also been reported that trade-off is often seen in plant adaptation between different biotic and abiotic stresses. However, initial exposure to one stress often leads to an enhanced state of tolerance to different stresses, designated as cross-tolerance. Plants often prioritize their response to one stress over that to another (examples are shown in Khan *et al.* 2020; Tajima *et al.* 2020)

Recent researchers have started understanding the molecular basis of combined biotic and abiotic stress interactions. Simultaneous occurrence of several biotic and abiotic stresses during plant growth provokes complex pathways controlled by different signaling events and finally lead to positive or negative impact of one stress over the other (Pandey *et al*. 2015). Evidence suggest that under combined stress plants exhibit tailored physiological and molecular responses, in addition to several shared responses as part of their stress tolerance strategy. Besides, the existence of crosstalk between plants independently exposed to biotic and abiotic stresses and their positive (cross-tolerance) or

negative influence on plants have also been shown (Ramegowda and Senthil-Kumar 2015)

However, our knowledge on molecular and physiological responses under stress combination is limited; therefore, further studies are required to understand these mechanisms. A high throughput functional genomic approach in association with high throughput stress effect quantification methods in model plant species would also hasten the process to identify key strategies that can be used to develop crop plants tolerant to simultaneous stresses.

Drought Tolerance Mechanisms

As the plants are sessile, they cannot move into an environment where it is conducive for their normal growth and development; instead, they have a myriad of mechanisms to overcome the problem of shortage of water. It should be noted that these mechanisms are not common in all water stress conditions since it is varied from plant to plant and situation to situation (mild to severe water stress, duration and intensity of the drought). As soon as the root signals the water scarcity in the soil, the plant respond physiologically in several ways: turgor loss (by reduced activity of RuBisco, PEP carboxylase, NADP malic enzyme, pyruvate phosphate dikinase and increased expression of ABA biosynthetic genes and ABA-responsive genes), impairment of osmotic adjustment (by the accumulation of compatible solutes such as proline, trehalose, polyamines), decreased transpiration rate due to closure of stomata (low stomatal conductance), increase in antioxidative enzymes such as SOD, CAT and APX, synthesis of specific proteins such as LEA, *etc*., decreased ratio of CO_2/O_2, decrease in accumulation of ROS, reduced photosynthetic rate and reduced growth (Tajima *et al.* 2020)

In response to water stress, a number of genes are expressed. The list of drought-responsive genes is ever-growing owing to the constant emergence of novel tools and methods that efficiently characterize such genes. In general, the drought-responsive genes are distinguished into three types: (1) genes encoding products that straightaway protect plant cells against water stress such as heat stress proteins (HSPs) or chaperones, aquaporins (water channel proteins), LEA protein, osmolytes, anti-freezing proteins, detoxification enzymes and free-radical scavengers (2) those that are involved in the regulation of gene expressions such as protein kinases MAPK, MAPKKK, CDPK, SOS kinase and phospholipases and transcriptional factors such as MYB, MYC, NAC and bZIP and (3) those that are involved in water and ion uptake and transport (Bakht *et al.* 2020).

At the morphological level, the plants respond to water stress by fastening the life cycle, producing more root system, reduced leaf number, area and size (by way of rolling in case of rice), drooping of flowers *etc*.,

In general, water stress is mitigated by the plants by three ways: Escape, Avoidance and Tolerance. Escape mechanisms involve ceasing the life cycle early and thereby escape from the severe water stress before it affects the plant system. Avoidance includes a variety of protective mechanisms (includes decreased stomatal conductance, rolling of leaf and senescence and impairment of growth) that delay or prevent the negative impact of drought on plant. In contrast, tolerance mechanism is the potential of a plant to acclimatize a stressful situation which is characterized by a higher content of chlorophyll, higher stomatal conductance, photosynthesis and maintenance of growth and osmotic adjustment (Ludlow 1993).

Incorporating the above knowledge in improving drought tolerance in crop plants through various breeding and agronomic strategies were not producing sustaining results since each strategy has some problems and limitations because of the complexity of drought effects on plants and the plants' responses to the drought. For example, the role of roots in extracting the water from deep soil during the drought period is considered as the priority trait in drought resistance crop breeding programs. On the other hand, root development is the function of genotype and soil and climatic environments. Of late, it is recognized that root system improvement alone will not be sufficient for this purpose since it is difficult to decide what do we need from a root system to increase drought resistance? deep or shallow roots, more or less roots, a prolific or limited branching root system, high or low sensitivity to soil water shortage and/or high or low root hydraulic conductivity?

It was concluded by Palta and Turner (2019) that root system that will lead to an increase in drought resistance depends on the amount and distribution of rainfall, the soil characteristics, the nature and timing of drought and several other plant characteristics. Therefore, incorporation of appropriate drought tolerance component traits in the breeding program often depends on the ultimate aim of the breeding program (such as understanding the genetics of drought tolerance mechanisms, increasing the drought tolerance of the existing cultivars or producing sustainable yield under water stress) and it is realized that the decision making is often a complicated process and often end up to look for other alternative approaches.

Approaches for Mitigating Drought Stress

In order to overcome the effect of water stress

on crop productivity, various strategies are being suggested. They may include one or combination of the following: i) seed priming ii) foliar application of exogenous water stress moderating chemicals and/or minerals (such as calcium, silicon), iii) application of microbial consortium and iv) soil water conservation methods (Sabagh *et al.* 2020). For example, seed priming and exogenous application of growth regulators and osmolytes such as glycine betaine and proline at various crop growth stages plays a significant role in inducing resistance against abiotic stresses, including drought (Farooq *et al.* 2006)

Seed priming (which is pre-sowing hydration of seeds or treating the seeds with ascorbic acid and potassium chloride) is an important and short-term approach that helps to initiate the germination metabolism under drought condition and Ajouri *et al.* (2004) recorded a 44% increase in the germination of wheat seeds through seed priming under drought conditions. Although osmolytes have generated positive results, their actual roles in plant osmotolerance still remain debatable. Similar to organic osmoprotectants, the application of abscisic acid and yeast significantly improved maize under water deficit conditions (Abdelaal *et al.* 2017) Thus, it has been shown in several studies that the above four strategies ameliorate the adverse effects of drought stress on the crop productivity.

On the other hand, these strategies require additional cost, effort, time and labour to mitigate the drought stress in the field. Further, such strategies would serve better when there is early drought, or the duration of the water stress is for minimum period of ten days. Therefore, scientists are looking for an alternative, simple and affordable strategy that can overcome the above limitations. Plant breeding approaches that focus on evolving novel cultivars with improved drought tolerance have been found as viable and sustainable approach to mitigate the stress, despite the fact that it possesses several hurdles, as discussed above and below sections.

Resilient Crop Breeding for Drought: Understanding the Reasons for Slow Progress and What is really needed?

Plant breeding for drought tolerance enhancement in crop plants strongly requires the insights on the genetics of drought tolerance mechanisms and exploration and incorporation of genetic variation in drought tolerance traits in breeding program. Several pieces of evidences suggest that plant response to water stress is controlled by more than one gene and is highly influenced by environmental variation (Foolad *et al.* 2003).

The quantification of drought tolerance has several serious difficulties since it is a developmentally regulated and stage-specific phenomenon. Direct selection in the field is unlikely because of uncontrollable environmental factors (such as variations in rainfall, interactions with extreme temperatures and variations in salinity and nutrient availability) that adversely affect the precision and repeatability of such trials. There is no trustworthy field screening technique that can be used across the years, plant generations and phenological stages. This is because drought tolerance at one plant developmental stage may be poorly correlated with the tolerance at other developmental stages.

Therefore, it is essential to identify specific stages from the phenology of the plant (including seed germination and vigorous emergence, seedling survival and growth, proper vegetative and reproductive growth) and should be evaluated separately for the assessment of drought tolerance and identification of its genetic components. Each developmental stage, which should be considered as a separate trait, may require a different screening procedure and simultaneous or sequential screening may be impractical or impossible. It requires specialized personnel and extensive investments in field nurseries or greenhouse facilities. These complexities have led to limited success in developing drought-tolerant plants or improving crop yields in dry environments (Boopathi 2020). In conclusion, regardless of many decades of research, drought tolerance continues to be a major defy to plant breeders, partly because of the apparent complexity of this trait.

At this point, a promising approach is proposed which can facilitate selection and breeding for complex drought tolerance traits: identify simply inherited genetic markers that are linked with the drought tolerance trait(s) of interest and use them as indirect selection criteria. The trickiest part of this approach is the identification of causal or functional genetic marker linked to the drought resistance component trait that has definite impact on economic yield under water stress conditions (Paterson 2008). During the last three decades, a large numbers of marker-trait associations (referred as quantitative trait loci, QTL) have been reported in several crops and it has been proposed that such association not only allows genetic dissection of physiological and molecular mechanisms underlying complex drought tolerance traits, but also accelerates transfer of QTLs through a process known as marker-assisted selection (MAS).

This special kind of selection facilitates the transfer of desirable genes without having to phenotypically evaluate plants for the trait(s) of interest in every generation under drought stress

besides reducing both the number of generations required to transfer a trait and the extent of "linkage drag", which is often a recognized as a difficult part when transferring genes from exotic sources (Boopathi 2020)

Sorghum: An Ideal Crop for Climate Change

Sorghum [*Sorghum bicolor* (L.) Moench], also known as jowar, is an annual crop belonging to family Poaceae, subfamily Panicoidae, tribe Andropoganae and subtribe Sorghastrae (Price *et al.* 2005). The primary center of origin is Abyssynia. It was domesticated in Northeast Africa. Sorghum is an often cross - pollinated crop and because of that it holds significant diversity in morphology as well as in agronomic traits (Rani *et al.* 2013). It is one of the major food crops of the world, particularly Africa and Asia. It has a solid cylindrical rod like stem of about 1 to 3 meters high, with terminal inflorescence, which includes one or two spikelets with bisexual flower. It produces caryopsis type of seeds that possesses ~ 4 mm diameter (Ramatoulaye *et al.* 2016)

After rice, wheat, corn and barley, it is the fifth most important crop in the world and considered as the main cereal for over 750 million people living in semi-arid tropics of Africa, Asia and Latin America and cultivated in about 98 countries (CCCF, 2011). In the world level, sorghum is cultivated in an area of about 39.93 million ha (Mha) with production of 59.35 million metric tons (MMT). More than 90% of sorghum production areas are in Africa and Asia. Globally, Asia accounts for 22% of area with 18% of production. India accounts for 70% of sorghum production of the Asian continent. In India, it is cultivated in an area of 4.01 Mha with production and productivity of 3.70 MMT and 920 kg/ha, respectively (USDA 2019). India globally ranks third in area after Sudan (7.0 Mha) and Nigeria (5.8 Mha) and stands fifth in production after Nigeria (6.8 MMT), Ethiopia (5.0 MMT), Sudan (4.5 MMT) and Mexico (4.7 MMT) (www.icrisat.org).

Genus "Sorghum" was classified into five sub genera *viz*., *Heterosorghum* (n = 20), *Parasorghum* (n = 5, 10, 15, 20), *Eusorghum* (n = 10, 20), *Chaetosorghum* (n = 20) and *Stiposorghum* (n = 5, 10, 15, 20) (Ejeta and Grenier 2005). Subgenus *Eusorghum* contains all the cultivated sorghum within it. It contains three species *viz*., *S. halepense, S. propinquum* and *S. bicolor* (De Wet 1978). *S. bicolor* contains three sub species *viz*., *S. bicolor drummondii, S. bicolor bicolor, S. bicolor arundinaecium* (Harlan and De Wet 1972; Wiersema and Dahlberg 2007). All the cultivated species of sorghum is under *S. bicolor* subsp. *bicolor* and there are five cultivated races *viz*., *bicolor, guinea, cudatum, kafir* and *durra*. There are also 10 intermediate races (Harlan and De Wet 1972). The cultivated sorghum evolved from wild sorghum *i.e*., *S. arundinaecea, S. verticelliflorum, S. sudanense and S. aethiopicum*. Dura is the oldest and primitive of five races that originated from Ethiopia and evolved in West Asia. Among the cultivated races, *guinea, caudatum* and *kafir* have opted for various parts of Africa and South Africa (Mundia *et al.* 2019).

Cultivated sorghum is a diploid species with ten pairs of chromosomes (2n = 20). The genome size was estimated between ~ 700 Mbp (from *Cot* analysis) to 772 Mbp (from flow cytometry) (Paterson 2008). As that of rice, which acts as a model plant for C3 photosynthesis, sorghum acts as a model crop for C4 photosynthesis in tropical grasses. A low level of gene duplication made it an attractive genome model. Genome size of sorghum is 60 % larger than rice and it is one fourth of maize or human. In angiosperm, the first BAC library was established for sorghum. Whole-genome sequence (~730 Mbp) was made using sorghum cultivar BTX623 by shotgun method (Paterson *et al.* 2009)

Besides as food crop, it is also used as an important dry fodder crop and used in production of sugar/syrup, wax, alcohol, starch, edible oil and in brewing. It has an immense potential for production of lingo – cellulosic ethanol and energy by means of biomass combustion (Mullet *et al.* 2014). Nutritionally, sorghum is superior to other cereals since it has higher fibre content, mineral and slower digestibility (Rao *et al.* 2010; Gorthy *et al.* 2017). It contains about 72.6% carbohydrate, 10 to 12% protein, 3% fat, 1.6% mineral. It is a rich source of amino acids (mainly lysine), riboflavin and folic acid along with Vitamin-B complex especially niacin (vitamin B_6). It contains nitrogen (212 mg / 100 g) and starch (5.6 % to 7.3 %) in high quantity along with copper, zinc and molybdenum. Bran protein contains four times lysine, two times arginine and glycine than endosperm protein (Rana *et al.* 1978). Phytic acid form complex with protein because of its binding capacity and these insoluble forms are readily available for animals and humans. From the analysis of several sorghum lines, it was evident that seed phytic phosphorus varies between 170 and 380 mg per 100 g and it's about 85% of total phosphorus (Ramatoulaye *et al.* 2016)

Cultivated and Conserved Accessions of Sorghum: Importance and Utilization in Drought Tolerance Breeding

Land races and wild relatives of sorghum are resistant to biotic stresses (such as diseases, pathogens and insects) and abiotic stresses (such as drought, salinity and high temperature). However, continuous use of varieties and hybrids during the past several decades steered landraces and wild relatives vulnerable for extinction. Hence, collection

followed by conservation of the germplasm by different approaches are very important to prevent the extinction of the landraces of the sorghum to preserve their desirable agronomically, economically and nutritionally important traits (Gorthy *et al.* 2017).

Sorghum germplasm had been collected from Africa and Asia and maintained by various international institutes such as International Crops Research Institute for Semi-Arid Tropics (ICRISAT), Institut de Recherches Agronomiques Tropicales et des Cultures Vivrieres (IRAT), Office de la Recherche Scientifique et Technique d'Outre-Mer (ORSTOM), International Board for Plant Genetic Resources (IBPGR), United States Department of Agriculture (USDA) and national institutes such as Indian Institute of Millets Research (IIMR), National Bureau of Plant Genetic Resource (NBPGR) and National Research Center for Sorghum (NRCS). A sum of 26,093 and 5,287 sorghum accessions are being conserved in NBPGR and IIMR alone, respectively.

Sorghum germplasm can also be obtained from National Germplasm Resource Information Network (GRIN), European Plant Genetic Resources Online Catalogue (EURISCO), Plant Genetic Resource Gateway (PGRG), International Crop Information System (ICIS) and Chinese Crop Germplasm Information System (CGRIS).

From the large set of available germplasm, core and mini core collections for specific purpose or traits can be framed for further study which will help to identify the accession(s) with trait of interest such as drought tolerance and also helps in recognition of new source of variation. For example, form 242 mini core accessions, several accessions were identified as useful: 70 accessions for biotic stress resistance, 12 accessions for abiotic stress resistance, 13 accessions related to bioenergy traits and 27 accessions related to nutritional traits (Upadhyaya *et al.* 2019).

From ICRISAT, 242 cultivars (including 35 hybrids/varieties for India and SAMSORG 47, 48 and 49 for Nigeria) have been released for commercial cultivation from the available germplasm lines and they are widely cultivated by the farmers. Maldandi (M 35-1) is one of the popular landraces for its high yielding capacity (www.icrisat.org). Similarly, sorghum germplasm improvement program has led to the release of several cultivars and hybrids specific to the particular ecosystem in Indian states (examples include sorghum Hybrid CO5, K tall, CO30, K11 and Paiyur1 for Tamil Nadu, (www.agritechportal.tnau.ac.in), Nandyal, Guntur and Anakpalle series for Andhra Pradesh, Fulgar white, Fulgar yellow, Kanvi, Hagari and Vanigar for Karnataka, Budh, Perio, Sundhia and Chasatio for Gujarat, NJ 156, NJ 164, PS 13, Saonar, Ramkel, Aispuri, Dagdi, Maldani 35-1 and Ganeri 2 for Maharastra (Tonapi *et al.* 2011). Despite these significant genetic improvement programs, the improvement of sorghum production, particularly in India, has met with limited progress.

Sorghum cultivation is subjected to various abiotic and biotic stresses from the time of sowing to the harvest. Main biotic stresses, especially pests, which limit sorghum production are shoot fly, shoot bug, stem borer, head bug, aphid and grain mold. Shoot fly is a serious pest which results in a loss of about 80-90% of grain yield and 68% of fodder yield (Kahate *et al.* 2014). Since sorghum is cultivated in rainfed production systems, drought is another primary key constraint for productivity. In addition, cold (where sorghum is grown in post rainy season), soil acidity (associated Al^{3+} toxicity mostly in Latin America) and salinity (in some parts of India and Middle East countries) are also emerging as new challenges (Reddy 2019) to sorghum production.

Among all these stresses, drought in combination with high temperatures causes major grain yield reduction in sorghum attributed mainly to variation in total biomass accumulation (Craufurd and Peacock 1993). Drought stress reduced grain yield significantly when it occurred at the flag leaf stage and at flowering. The reduction of grain yield at the flowering stage was due to a significant reduction of grain number (Castro-Nava *et al.* 2012). Certain micronutrients such as grain K and Fe contents were associated with dehydration/drought tolerance in sorghum. However, the usefulness of dehydration tolerance can be realized only if it is placed in a genetic background that has other mechanisms related to maintenance of production under moisture-deficit environments (Reddy, 2019).

Sorghum Genetic Improvement under Water Stress: Contributions of Conventional Breeding Strategies

All the mechanisms of drought tolerance have been described in sorghum: drought escape, avoidance and tolerance (Ludlow 1993). Early maturity or short duration is a well-known "drought escape" mechanism through which the crop completes its life cycle before the onset of severe moisture deficits and is often associated with a reduction in yields. Short-duration sorghums have lower evapotranspiration rates because of smaller leaf area and smaller root densities compared with long-duration ones and in some measure, yield loss can be overcome by increasing the plant density (Blum 1979).

Drought avoidance in sorghum is a mechanism for circumventing lower water status or to conserve a relatively higher level of hydration in tissues during water stress by maintaining cell turgor and

cell volume either through forceful water uptake by an improved root system (such as deep penetration of roots, adequate root density through the soil profile and adequate longitudinal conductance in the main roots) or through decrease in water loss from transpiration and other nonstomatal pathways (such as through the development, structure and surface properties of the canopy, ability to adjust the leaf area and cuticle according to moisture availability and functional attributes such as osmotic adjustment) (Ludlow and Muchow 1990).

Drought or dehydration tolerance is a mechanism by which plant maintains metabolism even at low water potential. There are multiple ways for a plant to remain green and productive. Stay-green is an important post-flowering drought tolerance trait in sorghum. Stay-green cultivars/hybrids produce significantly greater total biomass after anthesis, retain greater stem carbohydrate reserves, maintain greater grain growth rates and have significantly greater yields under terminal drought stress than related but senescent cultivars/hybrids. Stay-green genotypes are less susceptible to lodging and more resistant to charcoal rot (Mahalakshmi and Bidinger 2002). A stay-green phenotype may arise if the onset of senescence is delayed (type A), the rate of senescence is reduced (type B), chlorophyll is retained but photosynthesis declines (type C), greenness is retained due to rapid death at harvest (type D), or the phenotype is greener to begin with (type E). The maintenance of leaf photosynthesis characterizes functional stay-green during grain filling (types A, B and E). Also selection for both stay-green and grain yield should be undertaken simultaneously in plant breeding programs to ensure that delayed senescence is not due to low sink demand (Borrell *et al.* 2014).

Several breeding approaches have been used for transferring the stress resistance from wild varieties to susceptible sorghum elite cultivars by utilizing the variation existing between them which are the basic for plant breeding. Steps involved in such breeding methods are creation of variation, selection, evaluation and multiplication of breeding materials. Genetic variations are mainly created by traditional methods such as germplasm collection, hybridization, mutation and polyploidy. Back crossing is another important technique used by breeders to transfer the particular trait/ gene from the donor into the desirable sorghum line.

Sorghum production environments have been grouped into different target populations of environments (TPE) using long-term climatic data and used for multi-locational testing of genotypes developed for each drought pattern of TPE. Different methods have been proposed for screening (such as line source irrigation and managed water stress conditions) and selection of breeding materials (by employing either indirect selection methods such as the use of polyethylene glycol or potassium iodide to artificially induce water stress or direct selection methods in which the plants are grown directly in drought-prone areas during dry season that impose water stress when the plants are in germination and seedling emergence stage, post-emergence or early seedling stage, midseason or pre-flowering stage, post-flowering or terminal stage and total biomass, yield and its components are used as criteria for selection) (Boopathi 2020).

For example, in regions where terminal (otherwise called as end-of-season) drought stress is common, such as those in the Indian peninsula, evolving early maturing genotypes enables them to escape terminal drought. Under terminal drought, typically experienced by post-rainy season sorghums in India, early maturing improved sorghum cultivars such as CSH 1 (100 days and 4 t/ha), CSH 6 (95 days and 3.2 t/ha) and NK 300 (88 days and 4 t/ha) produced better grain yields than long-duration cultivars such as M 35-1 (105 days 1.9 t/ha) and SPV 86 (108 days and 3 t/ha) (Seetharama *et al.* 1982). However, under this terminal water stress, short-duration sorghum genotypes produce equal grain but less dry matter than long-duration cultivars. The lowering of yield due to early maturity can be reduced by increasing planting density as described earlier (Blum 1979).

Genetics of drought tolerance in sorghum has been reviewed by Reddy (2019) and concluded that characters such as stay-green, root volume, leaf area index, plant height and harvest index showed high value for phenotypic- and genotypic coefficient of variation, higher estimates of heritability and genetic gain and thus indicated the presence of additive gene effect. This suggested that there is considerable scope for the selection of these traits, which can increase drought tolerance potential in sorghum. Based on these facts, four basic approaches to breeding for drought tolerance/ resistance have been proposed.

The first is to breed for high yields under optimal conditions, *i.e.*, to breed for yield potential and then to assume that this will provide a yield advantage under suboptimal conditions. The second is to breed for maximum yield by empirical selection in the TPE. The third approach is to incorporate the selected physiological and/or morphological mechanisms conferring drought tolerance into traditional breeding programs. The fourth breeding approach involves identifying key traits that confer drought tolerance at specific phenology and its introgression into the high-yielding background (Reddy 2019).

Although difficult to combine, some sorghum

hybrids containing both pre-and post-flowering drought resistance have been developed (Rosenow *et al.* 1997). Rao *et al.* 1999 reported the superiority of sorghum hybrids over their parents for leaf area and dry matter production under both pre-and post-flowering drought stress. The increased performance of hybrids than their parents is due to greater growth rates and greater total biomass production, larger grains and higher harvest index with or without an apparent increase in leaf photosynthetic rates (Menezes *et al.* 2015).

In contrast, due to the complexity of drought resistance and low genotypic variance and genotype (G) x environment (E) variance, genetic gains made in one season may be lost in consequent seasons. Yield-based indices may lead to the development of genetic materials with specific adaptation but have a limited role in developing genetic stocks or varieties suitable for other similar water stress locations *i.e*., TPE. Progress can be made by selecting for resistance components specific to the particular TPE rather than the entire set of phenotypes that favor drought tolerance. To this end, it would be highly desirable to design a breeding program that focuses on incorporating a specific set of component traits of drought tolerance. Recently, Birhan *et al.,* (2020) have identified sorghum genotypes that were well suited for specific drought stress adaptation through traditional breeding and research. On the other hand, in the conventional method of breeding, there exist, always, a problem of linkage drag besides their laborious and time taking techniques (Collard and Mackill 2008).

In summary, the main problems associated with conventional breeding approaches that limit the rapid progress are: i) problems associated with breeding techniques that involve more complex drought resistance traits that are controlled by several other genes, ii) break down of tolerance mechanisms by several factors and iii) lack of complete information on genetic control of resistance, combining ability, gene action, gene effect and linkage drag on crop improvement under water stress environment. Here comes the use of molecular markers to overcome these problems.

Molecular Markers and Its Applications in Sorghum Breeding

Molecular marker is a DNA fragment that has a definite location on the genome. Molecular markers are being used for a variety of purposes in plant sciences, including to tag and trace the segregation of complex phenological traits such as drought tolerance and use them to increase the breeding efficiency within a short time, provided the genetics of these traits were well established with these markers (Boopathi 2020). Different types of molecular markers (such as Restricted Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeat (SSR), Expressed Sequence Tags (EST), Inter- Simple Sequence Repeat (ISSR) and Single Nucleotide Polymorphism (SNP)) are available for this purpose (Gupta and Varshney 2000). Among these, SNPs are considered to be more useful in the case of dissecting out the genetic basis of more complex phenotypes such as drought tolerance and further genetic improvement of drought tolerance using marker-assisted selection (MAS) in sorghum and other crops as described below.

SNPs: The Ultimate Marker that can revolutionize the Sorghum Breeding

In 1996, Lander proposed the use of SNP as a potential marker and it was first demonstrated by Wang *et al*., (1998). SNP represents a single nucleotide change in specific loci due to single base transition, transversion, insertion, or deletion. Transition is found to be common and almost 2/3 of all SNPs are transition and the majority of the time, they are silent mutation (*i.e*., less likely to have amino acid substitutions) due to the wobble hypothesis (Zhao and Boerwinkle 2002).

SNP genotyping helps to measure the variation of SNP between the members of species. The main advantages of SNP markers include, they are abundant, genetically stable and can be used for automated high throughput analysis (Al-Samarai and Al-Kazaz 2015). As they are conserved during evolution, SNPs are assayed by various methods (such as i) hybridization-based methods that employ Microarrays, ii) analytical methods that use post PCR amplification products and iii) recently by employing methods that use next-generation sequencing). Such methods are briefly described hereunder since it is imperative to know the principle of SNP detection assays which is essential to select appropriate assay for a proficient genetic dissection of drought resistance component traits (which are complex and difficult to dissect using conventional tools) in sorghum and other crops.

Dynamic allele-specific hybridization (DASH)

It is a hybridization-based method where the genomic segment is amplified using biotinylated primers attached to beads (Jobs 1999). Amplified products are added to the streptavidin column to remove the unbiotinylated strand by giving a wash with NaOH. Allele-specific probe complementary to the SNP region is allowed to hybridize with the target genome in the presence of intercalating fluorescent dye. Change in a single nucleotide can cause a change in melting temperature (Tm). Melting curve will be generated based on the fluorescence

once the temperature starts to increase. SNP presence will make a lower curve/ peak than the expected Tm. This technique can be converted to a high-throughput type with the implementation of chip-based methodology and recording the melting curve in a modernized platform (rapid melting curve analysis). However, measurement of change in Tm due to all types of mutations is the chief limitation in this method.

SNP microarray / GeneChip array

It is also a hybridization-based method wherein a small chip, allele-specific probes of 25-mer oligonucleotides are arranged to form a probe array (Rapley and Harbron 2004). Millions of probes can be accompanied in a single array and it can be used for parallel genotyping of 10^4 - 10^5 SNPs (Matsuzaki *et al.* 2004). Amplified regions of SNP from the target genome are allowed to hybridize in the chip containing a probe which are redundant in nature *i.e.*, the probes are designed in such a way to have a SNP complementary site in several different locations to increase the accuracy of SNP detection. Probe and target hybridization can be inferred by the intensity of fluorescence signals (Kennedy *et al.* 2003; Matsuzaki *et al.* 2004).

TaqMan Assay

It combines the hybridization method with enzyme activity to detect the SNP, based on the emission of fluorescence (Holland *et al.* 1991). An allele-specific probe is designed by having a fluorophore (reporter) at 5' end and quencher (non-fluorescent dye) at 3' end. When the probe is intact, the activity of the fluorophore will be restricted by the quencher and there will be no fluorescence signal. In this method, target DNA containing SNP will be amplified using forward and reverse primers. During amplification, if the probes get hybridized with the target, 5' nuclease activity of *Taq* polymerase cleaves fluorophore for further extension of the strand. *Taq* polymerase can cleave only the hybridized probe (Syvänen 2001; McGuigan and Ralston 2002). The released reporter will emit the fluorescence in the absence of the quencher, whereas the strand that doesn't contain SNPs won't emit any fluorescence. If the probe is not perfectly complementary to the target, probe can't bind efficiently as they have a low Tm (Livak 1999).

Oligonucleotide ligation assay

The function of DNA ligase is to join the 3' end of DNA fragment with 5' end of the nearby DNA fragment. In this method, two allele specific probes have been designed so that one probe with its 3' end is located above the SNP site and second probe, which hybridizes with the template strand adjacent to the SNP site (Landegren *et al.* 1988). Ligase is sensitive to mismatch at 3' end. Ligase will join the two adjacent fragments only if the probe's nucleotide is complementary to the SNP. The ligated product can be determined by capillary electrophoresis (using a fluorescent tags such as Combinatorial Fluorescent Energy Transfer (Tong *et al.* 2001) or MALDI-TOF mass spectrometry (described below).

Flap endonuclease

Flap endonuclease is an enzyme that is used to produce a structure-specific cleavage. In this method, an invader is used, which is complementary to the 3' end of the target segment with a mismatch at the SNP site (Olivier 2005). Allele-specific probe is also designed in such a way that its 5' end is complementary to the target site. This leads to the formation of a triplet structure which will be recognized and cleaved by the flap endonuclease only if the allele-specific probe is complementary to the SNP site. Reporter and quencher will be attached to the allele-specific probe and if the cleavage occurs by the flap endonuclease, fluorescence will be emitted (Lyamichev *et al.* 1999).

Pyrosequencing

SNPs are also detected by next-generation sequencing methods (Ronaghi *et al.* 1998). Primers are designed for the regions flanking SNP site. PCR amplifies target DNA and the reaction mixture contains single-stranded amplified product, primers, DNA polymerase, dNTPs, ATP sulphurase, apyrase and luciferase. Primers get attached to the single-stranded DNA at the complementary sequence and they are elongated by adding a nucleotide by DNA polymerase. If the nucleotide added is complementary to the template strand, it will be incorporated and pyrophosphate will be released. Released pyrophosphate will be converted to ATP by ATP sulphurase. Luciferase uses this ATP and produces a colored product called oxyluciferin. The amount of light produced will be detected by the luminometer. Excess dNTPs added during each cycle will be eliminated by apyrase (Ahmadian *et al.* 2000).

RNase H – dependent PCR (rhAmp)

It is also a PCR-based method in which RNase H-dependent PCR primers (rhPCR or simply rhPrimers) are used where a single RNA base and 3' blocking will be present. As usual, during amplification, allele-specific primer gets attached with the target DNA, but elongation is not possible due to blocking at its 3' end. If there exists a correct complementary bonding, then the RNase H enzyme will cleave the single RNA base, and it's blocking moiety. So that elongation can occur and also reverse primer helps produce the strand complementary to the SNP containing strand. As the cycle proceeds, strands with allele-specific

primers are identified using a universal probe that is complementary to the tail sequence present in the allele-specific primers. The probe will have a reporter and quencher. If the probe is attached to the tail sequence in allele-specific primers, a signal from the reporter can be recorded, which results in the detection of SNP (Beltz *et al.* 2018; Broccanello *et al.* 2018).

BeadArray

In BeadArray, about 50,000 individual fibers are combined to form an optical fiber bundle which acts as substrate and to create a well, ends of the each fiber are imprinted (Ferguson *et al.* 2000). Microbeads of different types are made to place in the well which contain a large copy number of specific oligonucleotide sequence complementary to the SNP sites (Oliphant *et al.* 2002). Three probes have been designed, two of which are allele specific and one is complementary to the downstream of the SNP *i.e.,* locus specific. Genomic DNA is prepared and made to hybridize with the probes. Allele specific and locus specific probes get hybridize with genomic DNA and ligase is used to the join the extended 3' end and 5' end of the locus specific primers if they are perfectly complementary to each other. Ligated strand is amplified by PCR using an allele specific primers and locus specific primers with a fluorescent tag like Cy3 or Cy5. Amplified PCR products are made to hybridize with the oligonucleotides on the microbeads and SNPs are detected by fluorescence emitted by Cy3 or Cy5 (Shen *et al.* 2005).

SNPlex

SNPlex is a high-throughput platform similar to BeadArray and as it employs identical steps such as hybridization, primer extension, ligation and PCR amplification. In this method, locus specific primers will be carrying a biotin tag to separate specific strand and it can be hybridized with the allele specific probe with fluorescent tag. It is used for concurrent analysis of 48 SNPs in 96 samples (Tobler *et al.* 2005).

MALDI-TOF-MS

Matrix-assisted laser desorption/ionization-time of flight – mass spectrometry (MALDI-TOF-MS) is also used in SNP genotyping where PCR amplification, hybridization, primer extension and mass detection is sequentially involved (Tost and Gut 2002). In this method, genomic DNA with SNP site is amplified in multiplex PCR and they are attached to individual wells in the chip as a single-stranded DNA. The single-stranded DNA with SNP site is extended using the primers to generate allele-specific extension products. Primer extension can be done in various ways *viz*., i) using several dNTPs and ddNTPs specific to allele, ii) using mass tagged ddNTPs and iii) using biotinylated ddNTPs. After primer extension, they are subjected to MALDI-TOF-MS where primer's mass indicates SNP and mass difference between the primer and its extended form shows the identity of inserted nucleotide and therefore their genotype (Ross *et al.* 1998; Griffin and Smith 2000).

Detection of SNPs and their implications

Generally, in the methods mentioned above, SNPs are detected either based on the estimation of fluorescence or chemiluminescence. The fluorescence method is based on the emission of fluorescence by the employed compound and in the case of chemiluminescence, light is produced by various biochemical or chemical reactions without heat generation (Ahmadian *et al.* 2000). Fluorescent detection is also based on fluorescent polarization, where the change in molecular weight causes the fluorescent dye molecule to produce various polarization which will be detected using different techniques (Kwok 2002). Another method is fluorescence resonance energy transfer, where the energy transfer occurs from excited non-radiative fluorophore to nearby acceptor fluorophore. According to the nearby or far away placements of donor and reporter (Myakishev *et al.* 2001). Thus, understanding the principle of SNP method and its detection chemistry is imperative to design a sound high throughput marker system which has immense potential in plant breeding program in sorghum and other crops as outlined below.

Applications of Molecular Markers in Sorghum

Though there is significant SNPs usage in Sorghum, other molecular marker classes (especially SSRs) have been extensively employed to show their utility in sorghum breeding at various levels. Molecular markers have been expansively applied in DNA fingerprinting, QTL mapping and genetic dissection of agronomically important traits, and marker-assisted breeding in sorghum. This section provides a brief description of each application with a special reference to the usage of molecular markers in the genetic dissection of drought tolerance traits in sorghum and its implications in plant breeding.

DNA Fingerprinting and Genetic Diversity Analysis in Sorghum

DNA fingerprinting is a technique used to identify a particular individual from a group based on its unique pattern in the DNA structure. In sorghum, DNA fingerprinting has been followed in various studies for variety and race identification. For example, CSH-35, an elite cultivar, has been fingerprinted using the SSR markers from other similar sorghum accessions (Gangurde *et al.* 2016). In the same way, several other Ethiopian lines of

sorghum have been identified by employing SSR markers (Mogus and Bantte 2012). Arya *et al.* (2006) studied 37 accessions using RAPDs and found useful markers to differentiate important accessions among the investigated lines. It has also been shown that a combination of just five markers are sufficient to differentiate 24 species of sorghum out of 25 species studied (Dillon *et al.* 2005). Molecular markers have also been employed in several recent studies on diversity analysis of different cultivars/ varieties/ accessions in sorghum (Mehmood *et al.* 2008; Zhu *et al.* 2020; Sapkota *et al.* 2020).

QTL Mapping in Sorghum

In general, all the agronomic traits have shown measurable continuous variation (hence they are called quantitative traits), and they are controlled by a set of genes. The availability of whole-genome sequence of sorghum (Paterson *et al.* 2009) and several genetic maps allows efficient linkage of genomics and phenomics information and paves a new way for genetic improvement of sorghum under water-limited environments. The hurdles in the development of high-density linkage maps have been nowadays circumvented using next-generation sequencing and genomics tools. Similarly, limitations in high throughput phenomics were overcome by using improved ground and aerial phenotyping platforms (Yang *et al*., 2020). Quantitative trait loci (QTL) mapping is the process by which it identifies the region of the chromosome(s) that governs the expression of the quantitative traits and there are different methods that detect the QTLs (Harris-Shultz *et al.* 2019). In order to have a widespread application in plant breeding programs through marker-assisted selection (MAS), identified QTLs should be in a neutral state *i.e.,* QTL which is having an effect on a particular trait in one environment should have the same equal effect on that particular trait in a different environment (Alimi *et al.* 2013; El-Soda *et al.* 2014).

The chief application of QTL mapping is, it can efficiently dissect-out the complex traits such as drought-tolerant component traits at the genetic level, which is essential to fasten the progress of drought tolerance plant breeding as described earlier in this review. Sorghum is having high water use efficiency than maize and other crops. QTLs have been reported for several drought resistance component traits such as CO_2 assimilation rate, transpiration rate and its ratio, stomatal conductance, stomatal density, epicuticular wax, trichome density on adaxial and abaxial side, crown root angle, nodal root angle, root dry weight, root length, root : shoot ratio, root volume, pre-flowering drought tolerance, post-flowering drought tolerance (or stay green trait) in sorghum (Harris-Shultz *et al.* 2019).

Four QTL for nodal root angle (*qRA1_5, qRA2_5, qRA1_8, qRA1_10*), three QTL for root dry weight (*qRDW1_2, qRDW1_5, qRDW1_8*) and eight QTL for root volume, root fresh weight and root dry weight were identified (Mace *et al*. (2012); Rajkumar *et al.* (2013). Two QTLs (*qRT6* and *qRT7*) associated with brace roots have also been mapped on sorghum chromosomes 6 and 7 (Li *et al.* 2014). Additionally, one of the root angle QTL was co-located with QTL for stay-green and grain yield in sorghum (Mace *et al.* 2012).

Among the different drought-tolerant traits, stay green trait is gaining its importance owing to its inherent capacity in imparting drought tolerance in sorghum. For example, five stay green QTLs *viz*., *stg1, stg2, stg3, stg4* and *stg5* have been identified and shown to be potential candidates for drought tolerance improvement in sorghum (Xu *et al.* 2000). Among them, *Stg2* was found to have more contribution which was located in chromosome SBI-03 followed by *stg1, stg3* and *stg4* which were located in the chromosome SBI-03, SBI-02 and SBI-04, respectively (Mace and Jordan 2010; Weers 2011). *Stg5* was found to be in SBI-01 and it was co-localized with dhurrin biosynthesis gene (Hayes *et al.* 2016). Interestingly, these *stg* loci were also found to reduce the canopy size during flowering, reduce tillering and promote the overall root growth (Harris-Shultz *et al.* 2019)

Several other QTL mapping studies revealed that QTLs located at the upper ends on chromosome SBI-06 were contributing mainly to sorghum growth and development in various environments. In SBI-06, QTLs have been mapped for drought response (Mace *et al.* 2012; Phuong *et al.* 2019), thermal response (Chopra *et al.* 2017), cold (Parra-Londono *et al.* 2018) and also for biotic stress such as ergot resistance (Parh *et al.* 2008). Therefore, it may be concluded that this genomic region governs not only the abiotic stress tolerance but also the biotic stress resistance in sorghum. Besides, another striking point of this genomic segment is it has also been shown to be associated with yield. Yield improvement is the ultimate aim of the breeders, which is also considered as a complex trait. Total grain yield could be increased by having an early flowering period with a long grain filling duration and QTLs governing these traits was also dissected out in SBI-06 (Said *et al.* 2018). Thus, it may be concluded that these QTLs would serve as a potential candidate for introgression in sorghum genetic improvement programs through MAS owing to its governance in multiple stress tolerance and yield under changing climatic conditions.

It is well established that phenotypes are continuously changing as they grow and in response to environmental stresses such as water stress.

However, it would be difficult to capture those changes at different time points and to incorporate such information in QTL analysis. Recently, Miao *et al.* (2020) used novel engineering and computer vision technologies to track phenotypic change over time in a set of diversity panels and used functional principal component analysis by employing higher density SNP markers generated for the same population. Such genome-wide association studies can also enable robust time-series mapping analyses in drought tolerance experiments since such effort can increase the accuracy and power of quantitative genetic analyses. However, these studies have to be done in sorghum or any other crop under drought stress.

Further, several other advanced genetic mapping and MAS methods have been proposed since the traditional bi-parental genetic mapping strategy was considered to have the following shortcomings: i) many DNA marker maps are not sufficiently dense to achieve the potential of QTL mapping, since sparse marker maps severely limit the power of QTL mapping ii) even under optimal experimental conditions, multiple QTLs identification on a single linkage group are difficult or impossible to resolve iii) populations must be relatively large in order to uncover minor loci and the biological relevance of loci uncovered depends on the cut-off chosen for statistical significance and iv) environmental factors and genetic background potentially have an enormous impact on results and hence many large, time-consuming experiments need to be carried out to analyze all the QTLs thoroughly (Young, 1999).

Fortunately, the advanced QTL mapping methods (to name a few: Bulked segregant analysis and selective genotyping, advanced backcross – QTL analysis (AB-QTL), association mapping (which is also referred as association analysis, linkage disequilibrium (LD) mapping and structured association mapping), nested association mapping, mapping using multi-parent advanced generation inter-cross (MAGIC) population, Array Mapping, Genome-Wide Association Analysis (GWAS), TILLING and EcoTILLING (Boopathi 2020)) have provided powerful analytical tools to overcome these limitations.

Association mapping is used to identify markers that are located in close proximity to the gene of interest using a natural population. This is because of utilization of the entire recombinant event that happened between the populations in the past for LD analysis. There are two approaches in association mapping *viz.*, GWAS and candidate gene approaches (Singh and Singh 2015). In GWAS, markers used are located throughout the genome eventually. In this method, large numbers of markers are to be used in a large population to identify the markers associated with trait of interest. In such a case, huge comparison of genotype and phenotype has to be done. This limitation can be overcome by using an elite population to increase LD value. When there exists a high LD value, low number of markers is sufficient to genotype the population.

In the candidate gene approach, based on the previous knowledge from comparative genomics, genome sequence annotation, transcript profiling and QTL analysis, the candidate gene(s) is/are fixed and markers in the particular region of the genome are used to reduce the target genome region. The main demerit with this approach is that genes that are not included in the list of candidate gene may be the main contributing factor for the trait of interest and researcher may lose the particular gene associated with it. Association mapping strategy was successfully employed in sorghum to identify the locus associated with grain mold resistance (Nida *et al.* 2019), starch metabolism (Chen *et al.* 2019), male sterility (Girma *et al.* 2019), different quality traits of fodder sorghum (Li *et al.* 2018), phytoremediation and heavy metal stress tolerance (Abou-Elwafa *et al.* 2019) and cold tolerance under chilling condition (Moghimi *et al.* 2019)

However, association mapping is influenced by various factors such as population structure and kinship and they result in false association between marker and QTL. There also exists a difficulty in identifying QTLs with small effect and false assessment of LD value leads to problem in association mapping. Association between the marker and target will be influenced by the allele frequency in the genome and strong association between the trait and population structure may also lead to difficulty in analysis.

Nested association mapping (NAM) take advantage of both linkage map and association map by combining low marker density and allele richness with high resolution map. There are five NAM population available in sorghum, of which 4 NAM focus on sorghum grain and fifth NAM focus on bioenergy (Boyles *et al.* 2019; Grover *et al.* 2019). In linkage equilibrium, association mapping and NAM, recombination is an important event which occurs in slow rate in linkage mapping and NAM. In association mapping, recombination event occurs in huge rate but with a problem of false-positive result.

It can be overcome by using multi-parent advanced generation inter-cross (MAGIC) population, a next-generation mapping source that is developed by using a multiple parents. In sorghum, the first MAGIC population was created in 2015 using 19 diverse lines. These lines were crossed with male-sterile line and 10 generations of random mating. In a random, 1000 fertile plants were identified

and subjected to six generations of selfing to produce 1000 MAGIC inbred lines. Of these, 200 MAGIC inbred lines were subjected to genotyping by sequencing (GBS) and 79,728 SNPs were identified in these sorghum lines (Ongom and Ejeta 2018). Consequently, such high throughput genetic map can be used for efficient identification of QTLs linked to several desirable traits in sorghum.

Thus, it is expected that advances in the QTL mapping strategies, as described above, will eventually lead to providing promising approaches for genetic improvement of drought resistance in sorghum and other economically important crops.

Exploring the Sorghum Genomics and Phenomics Knowledge

With knowledge of markers and QTLs identified in sorghum, several studies have been carried out to introgress the trait controlling a particular gene through marker-assisted back crossing (MABC) and to select the improved line using the marker (Nanaiah and Rakshit 2020). For example, striga resistance gene was introgressed from the resistant source N-13 into different elite cultivating varieties using MABC (Ali *et al.* 2016; Afolayan *et al.* 2019). Another evidence was the development of a shoot fly resistance variety by introgressing the resistance gene from resistance donor J2614 into the popular variety of SPV1411 through MABC (Gorthy *et al.* 2017)

In sorghum, significant advances have been achieved through analyses of natural and induced mutants. Genes inducing the brown midrib (*bmr*) phenotype, which is accompanied by a higher tolerance have primarily been investigated. To date, a total of 8 *bmr* genes have been discovered in sorghum. Three of these genes have been characterized at the molecular level and correspond to enzymes of the lignin biosynthetic pathway. Some of these genes have been extensively used to develop sorghum varieties targeting the feed industry (Hennet *et al.* 2020). In order to decrease the lignin content in sorghum, *bmr6*, a recessive allele that reduces the activity of key enzyme involved in lignin synthesis, was introduced into sorghum line IS23777 through backcrossing from the donor, CMSXS170 (Pinto *et al.* 2019).

As that of the above biotic stress resistance improvement in sorghum using MAS, abiotic stress tolerance improvement has also been shown successfully in several instances. For example, an improved line, RSG03123 was obtained by introgressing stay green QTL from a donor, B35 into R16, a high-yielding cultivar (Kassahun *et al.* 2010). Performance of one of the introgressed line, RSG03123 was compared with its parents and it was found that stay green QTL remain functional in senescence stage with improved resistance to water scarcity (Galyuon *et al.* 2019).

Similarly, studies have been made to introgress the stable QTL for root volume *viz*., *qRV3* and *qRV10* from land race Basavanapada into the recurrent lines, which were already introgressed with three stay green trait QTLs and one water use efficiency QTL for gene pyramiding the lines for drought tolerance with different drought-tolerant traits (Kadam and Fakrudin 2017).

Incorporation of Useful Information

Sorghum breeding is gaining its importance in various aspects, mainly in the case of drought response. Various advanced genomics and phenomics-based approaches like MAS, MAB, MABC *etc*., made rapid improvements in sorghum breeding than the conventional breeding methods. On the other hand, several QTLs that have been identified in various studies need to be validated as they differ in these studies for a particular trait.

To this end, a meta-analysis has to be made to pool all the QTL data set and to identify the exact candidate gene behind the trait of interest. Joint analysis of QTLs of several studies provides a way to combine advantages and avoid the pitfalls associated with these methods. In this context, Daware *et al*. (2020) developed MetaQTL specific regional association analysis and demonstrated its utility to rapidly narrow down previously identified QTL intervals to few candidate genes. This report describes the detailed step-by-step guide for performing MetaQTL specific regional association analysis to identify important genomic regions and underlying potential major effect genes governing traits of agronomic importance in cereals. In sorghum, many genetic, genomic resources and QTLs associated with desirable agronomic traits are available in the public domain. Marker-traits association needs to be validated independently and suitable SNP assay are required to be generated for further sorghum crop improvement.

Detailed structural and functional comparisons of genes involved in various biological processes among plant species revealed that actually orthologous genes exist in plants with similar functions and they represent a basis for ancestors of the evolution of higher plants. The functions of some of the master regulators have been shown to be conserved, at least in some respects, in rice, sorghum, maize, poplar and eucalyptus (Hennet *et al.* 2020) and the same trend is expected in other crops too.

Comparative sequence analysis has significantly altered our view on the complexity of genome organization, gene function and regulatory pathways.

Several advanced methods of online and offline data mining tools integrate structural and functional annotation of published plant genomes with a large set of interactive tools to study gene function and gene and genome evolution. Precomputed data sets cover homologous gene families, multiple sequence alignments, phylogenetic trees, intraspecies whole-genome dot plots and genomic collinearity between species. Such analysis provides a comprehensible and up-to-date research environment to support researchers exploring genome information within the agricultural crop lineage.

Therefore, the genomics and phenomics knowledge base developed in sorghum and in a few other model species constitutes an opportunity to accelerate and facilitate the discovery of genes involved in drought tolerance in sorghum and grasses. Hence, it is suggested that the working plan developed in sorghum for drought resistance improvement can also be applied well to other crops.

Work plan for different TPE

The target population of environments (TPE) is the set of all environments, fields and seasons in which an improved variety is targeted to perform well and owing to its leading role in determining plant performance, complete understanding of the TPE is essential. Jongdee *et al.* (2006) concluded that TPE could be identified and characterized by the use of crop models in terms of incident water stress (which requires historical weather data) or by using the probe and reference genotypes in multi-environment trials (which requires reference genotypes that are known for their adaptation to each target environment). Such effort will be useful to decide which cultivar (for example, early and late-season drought-tolerant cultivars) would be beneficial for this TPE.

Although functional validation is the paradigm toward the proof of QTL or gene function, alternative approaches are also noteworthy to identify the most pertinent genes to use in future breeding schemes that focus on evolving early and late-season drought-tolerant cultivars. Assessment of the expression differences (at transcription, translation and metabolome level) of genotypes harboring different patterns of accumulation and in different environmental conditions (*i.e.*, different TPEs) would probably allow for sharpening up our strategies to maximize their applications in breeding programs in the future. Further, it is essential to increase the resolution of the genomics analyses through the analysis of expression of specific groups of organs or tissues or cells (e.g., flowers and seeds) with several time frame sampling. Combining the recent phenomics approaches (Yang *et al*., 2020) with analysis of the arrangements of nucleotide diversity of these genes may serve to focus the few candidates to track in the future and its efficient introgression into the elite cultivars.

Concluding Remarks and Future Prospects

It is imperative to evolve novel plants that are phenotypically plastic to the changing climatic conditions, including water stress. A gap between germplasm available and its utilization in crop breeding programmes has been realized during the last few decades. This gap should be bridged to overcome the problems of crop production under the current changing climatic compulsions. The creation of a common platform for all the available crop germplasm will enhance breeding efficiency. To this end, powerful statistical tools are required to integrate the results of various experiments in order to come up with informative decisions. Further focus on alternative applications of existing crop produce may also provide useful strategies to cope up the climatic vagaries. For example, sweet sorghum is gaining importance in bioethanol production. Though the drought stress affects the final grain production to a large extent, significant size of biomass can be produced even under severe water stress. However, the main problem is the rapid degradation of sugar upon harvest. Thus, genetic understanding through genomics and phenomics of sweet sorghum under water-limited environments would have multi-fold applications: evolving a suitable variety/hybrid with biotic and abiotic stress tolerance, developing multipurpose sweet sorghum with more sugar content as well as increased grain and fodder yield under water stress.

In recent years, climate-resilient approaches have picked up owing to their importance in alleviating the problems associated with the increasing atmospheric carbon content. It is believed that the progress in increasing the efficiency of photosynthesis in sorghum by employing biotechnological approaches can open up novel avenues in crop production in other economically important crops. Sorghum's C4 photosynthetic system made it stand better when compared with other major cereals due to its inherent ability to overcome drought. Possible effects of drought on sorghum productivity especially due to climate change, mechanisms and genetics underlying drought tolerance and modern genomic strategies to overcome drought and other climate resilience traits were well studied (Nanaiah and Rakshit 2020).

Though SNP assays are gaining its importance, as it offers several advantages over other kinds of markers, still some issues have to be settled: requirement of prior knowledge on SNPs specific to the trait of interest, time-consuming optimizing process, bias in the array due to non-random

sampling of polymorphism in a population of interest and usage of small number of samples in SNP panel. Similarly, there is a scope for improvement of advanced genetic analysis strategies that are proposed in this genomics era. For example, in GWAS, false-positive due to population structure and multiple testing is a problem that needs to be addressed by a powerful statistical approach. It is believed that the upcoming years can realize the potentials of sorghum genomics and phenomics in productivity enhancement not only in sorghum but in other crops too.

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