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# Chapter 6
# Genomic Designing of Pearl Millet: A Resilient Crop for Arid and Semi-arid Environments


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**Abstract** Pearl millet [*Pennisetum glaucum* (L.) R. Br.; Syn. *Cenchrus americanus* (L.) Morrone] is the sixth most important cereal in the world. Today, pearl millet is grown on more than 30 million ha mainly in West and Central Africa and the Indian sub-continent as a staple food for more than 90 million people in agriculturally marginal areas. It is rich in proteins and minerals and has numerous health benefits such as being gluten-free and having slow-digesting starch. It is grown as a forage crop in temperate areas. It is drought and heat tolerant, and a climate-smart crop that can withstand unpredictable variability in climate. However, research on pearl millet improvement is lagging behind other major cereals mainly due to limited investment in terms of man and money power. So far breeding achievements include the development of cytoplasmic male sterility (CMS), maintenance counterparts (*rf*) system and nuclear fertility restoration genes (*Rf*) for hybrid breeding, dwarfing genes for reduced height, improved input responsiveness, photoperiod neutrality for short growing season, and resistance to important diseases. Further improvement of pearl millet for genetic yield potential, stress tolerance, and nutritional quality traits would enhance food and nutrition security for people living in agriculturally dissolute environments. Application of molecular technology in the pearl millet breeding program has a promise in enhancing the selection efficiency while shortening the lengthy phenotypic selection process

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ultimately improving the rate of genetic gains. Linkage analysis and genome-wide association studies based on different marker systems in detecting quantitative trait loci (QTLs) for important agronomic traits are well demonstrated. Genetic resources including wild relatives have been categorized into primary, secondary and tertiary gene pools based on the level of genetic barriers and ease of gene introgression into pearl millet. A draft on pearl millet whole genome sequence was recently published with an estimated 38,579 genes annotated to establish genomic-assisted breeding. Resequencing a large number of germplasm lines and several population genomic studies provided a valuable insight into population structure, genetic diversity and domestication history of the crop. Successful improvement in combination with modern genomic/genetic resources, tools and technologies and adoption of pearl millet will not only improve the resilience of global food system through on-farm diversification but also dietary intake which depends on diminishingly fewer crops.

**Keywords** Biofortification · Climate resilient · Cytoplasmic male sterility · Dwarfing gene · Gene pool · Genomic-assisted breeding · *Pennisetum glaucum*

## 6.1 Introduction

The world population is predicted to be more than 9.6 billion by the middle of twenty-first century with the highest increase projected in Sub-Saharan Africa (SSA) from the current 0.9 billion to 2.2 billion by 2050 (prb.org; fao.org). On the contrary, in the last five decades, the SSA region has recorded only an ~25% increase in cereals yield per unit land as compared to >300% in the developed countries. There could be multiple reasons for the large yield-gap but one of the major reasons is slow or no adoption of modern tools and technologies. The most suboptimal crop production in arid and semi-arid environments covering >40% of the world's land area is characterized by dry and hot weather conditions (Safriel et al. 2005). Food production in such dissolute environments is challenged by low rainfall with erratic distribution and high temperature. The climatic variability experienced in the last few decades is projected to hit the arid and semi-arid regions hard to exacerbate the biophysical and socio-economic stresses. The looming global climate change will further aggravate the drought and high temperature stresses and will adversely impact crop production.

The combined effect of dwindling water resources and competition for water among industries limit the availability of water for irrigation in dry areas. Moreover, the available water for irrigation is preferably used for fruits and vegetables production than grain crops. Therefore, the next productivity increase in arid and semiarid environments is expected to come largely from crop resilience to the stresses imposed by climatic variability.

Pearl millet (*Pennisetum glaucum* (L.) R. Br. syn. *Cenchrus americanus* L.) is one of the climate resilient crops having $C_4$ photosynthetic pathway which is very

efficient in energy production (50% higher photosynthesis efficiency than $C_3$ crops) in hot and dry climate (Wang et al. 2012) and a widely grown cereal crop for food in SSA and the Indian subcontinent since time immemorial. Pearl millet is believed to be domesticated in West Africa about 4000–5000 years ago, probably in the southern margins of the present day Sahara Desert (Manning et al. 2011; Burgarella et al. 2018). Subsequently spread to eastern and southern Africa and introduced to India pearl millet became one of the staple crops. Today, its main center of diversity appears in the Sahel between Senegal and central Sudan (Brunken et al. 1977). Its high photosynthetic efficiency and rapid biomass production potential in harsh climatic conditions make the crop compatible for low soil moisture and soil fertility, and high temperature.

Pearl millet is thought to have originated in the Sahel region from Senegal to central Sudan (Oumar et al. 2008; Burgarella et al. 2018), and it is largely cultivated in West Africa and Asia. The archaeobotanical evidences indicate pearl millet domestication dating back at about 4500 BP (Manning et al. 2011), support the Sahara and Sahel hypothesis of origin and the broad distribution and cultivation in SSA (D'Andrea and Casey 2002). The wild progenitor of cultivated pearl millet has been identified as *Pennisetum glaucum* ssp. *monodii*, is native to the Sahel zone (Brunken 1977). It has widely been suggested that domestication of pearl millet is the result of multiple events (Marchais and Pernes 1985) leading to various morphological, agronomic and physiological modifications for use by the farmers and consumers. Differential selection pressures over centuries modified the crop to suit the needs of growers and consumers.

Pearl millet accounts for more than half of the total millet production in the world (FAOSTAT 2013). It is grown in the most dissolute environment where other major cereals, such as wheat (*Triticum aestivum*), maize (*Zea mays*), and rice (*Oryza sativa*), are likely to fail or produce no economic yield. Today, pearl millet is grown on more than 30 million ha in West Africa and the Indian subcontinent and provides basic sustenance to more than 90 million people in agriculturally marginal areas. Pearl millet has high nutritional value in terms of high levels of energy, dietary fiber, proteins with a balanced amino acid profile, essential minerals, vitamins, and antioxidants (Jukanti et al. 2016). Nevertheless, genetic improvement of pearl millet lags far behind other cereals. This is mainly because of limited resource allocation both from the public and private sector.

Pearl millet is also grown in the United States, Australia, and Brazil predominantly for forage and as a cover crop. Pearl millet was introduced into the United States in early date, grown and consumed by the early settlers until 1875 (Oelke et al. 1990), but with the improvement of other crops, its role for human consumption diminished. It is currently grown in small acreage primarily as a summer grazing forage crop. Until recently, pearl millet grain is widely known as a bird feed. However, with the recognition of its nutritional value, pearl millet is gaining importance slowly. There is a slight increase in productivity of millet since the year 2000, mainly in India as a result of adoption of high yielding hybrids (Fig. 6.1). However, the area has been decreasing over years probably because of an expansion of improved adaptive sorghum varieties into millet areas.

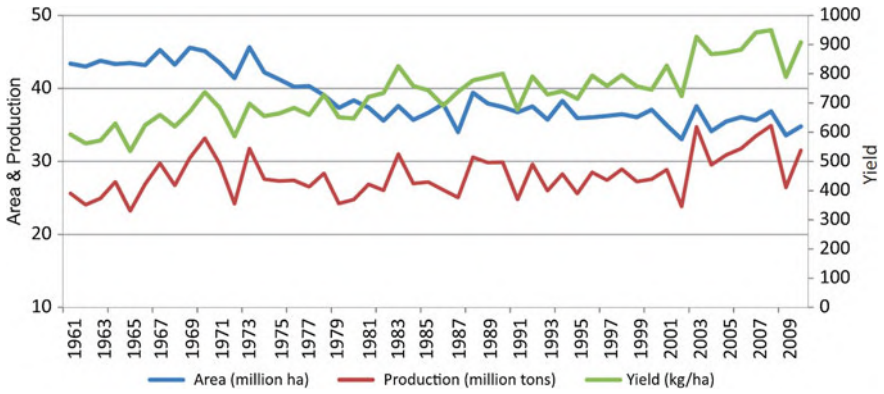


**Fig. 6.1** Global trend in millets area, production, and productivity during 1961–2010 (FAO 2013)

Research on pearl millet improvement in the United States started back in 1936 (Burton and Powell 1968). Since then several breakthrough results have been recorded. The development of cytoplasmic male sterility (CMS) system for hybrid breeding, identification of dwarfing genes for reducing the plant height, development of early maturing photo-neutral genotypes, identification of resistance sources to major diseases such as downy mildew, rust and leaf spot, collection and preservation of diverse germplasm at ICRISAT, construction of comprehensive genetic linkage maps, detection of quantitative trait loci (QTL) for important traits, and the recent release of the genome sequence and re-sequencing based domestication are the important milestones.

To bring a quantum jump in the productivity, there are still big challenges to focus on, especially on improving the genetic yield potential, stress tolerance and seed characteristics for good stand establishment. Its importance as a subsistence crop in harsh environments and potential usefulness for large-scale production in the future, should have drawn much research attention as the yield harvested by farmers is still low. Disease pressure especially downy mildew is forcing most of the farmers in West Africa to stick to the traditional landraces rather than adopting genetically improved cultivars. Comprehensive research program geared towards addressing the priority production constraints is of paramount importance. This chapter portrays the development of milestones that have been made and the future focus on pearl millet improvement as a climate resilient crop in an abridged form.

### *6.1.1 Food, Nutrition, Energy and Environmental Security*

Pearl millet is used to make a number of traditional foods and beverages across the Sahel of Africa and India. The unique characteristics of this cereal are that it can be as creamy as mashed potatoes or as fluffy as rice. In West Africa and the Indian

subcontinent, pearl millet is regarded as one of the major sources of dietary energy and nutritional security. The crude protein content of pearl millet ranges from 9 to 18% with wide variation among genotypes and locations. Investigation into the nature of *Pennisetum* protein showed that only 6.8% of the total nitrogen is of the non-protein type (Swaminathan 1937). The embryo contains about 10% of the total protein, which is seven times as rich in protein as the endosperm on equal weight basis (Swaminathan et al. 1971). It also contains several essential minerals like phosphorus (P), iron (Fe), zinc (Zn), calcium (Ca), and magnesium (Mg). The essential amino acids and vitamins contributed to its therapeutic properties such as treatment of stomach ulcers by reducing excess acidity in the stomach after food intake. The high phosphorus content helps in bone growth and development. Because of its gluten-free nature, pearl millet is recommended as an alternative food for people with gluten allergy. The grain is also high in fiber content and the starch digests slowly. The slow digestion releases glucose into the blood stream at a slower rate than other cereals, and remarkably powerful for controlling diabetes (Kam et al. 2016).

Pearl millet is grown in the areas where it is too hot and too dry for other major cereals. Its production also involves minimum external input. The small-scale farmers in south Asia and SSA use minimum fertilizer and application of agro-chemicals in the form of herbicides, insecticides or fungicides is almost none. As a result it has significant economic and social significance with minimum impact on the environment. These undoubtedly ensure the sustainability of the production system.

### *6.1.2 Global Warming and Climate Change*

Climate change (CC) is continuing as a major threat to sustainable agricultural production by embracing unpredictable extreme climatic events such as fluctuation in temperature and uneven rainfall patterns. This climate variability is expected to increase crop vulnerability in different agro-ecologies with drastic consequences on food security and economic growth. Pearl millet is a climate-smart crop with nutritious grains (Anuradha et al. 2017) and ideal for environments prone to drought and extreme heat, thereby reinforcing the fight against food insecurity in the arid and semiarid environments (Bailey et al. 1979; Buerkert et al. 2001; Jukanti et al. 2016).

A study on the effect of climate change on $C_4$ crop productivity in Africa and India indicated apparent temperature-driven yield reduction across the board with much more uncertainty in arid regions (Berg et al. 2013). Yield losses are induced by higher temperature leading to increased potential evapotranspiration, crop maintenance respiration and acceleration of the phenological cycle (Berg et al. 2013; Sultan et al. 2013) and impact pearl millet yield potential. Therefore, crop adaptation and improved agricultural practices would have a potential contribution in offsetting some of these negative impacts.

In the Sudanian and Sahelian savannas of West Africa, a study was conducted on the impact of climate change on pearl millet productivity (Sultan et al. 2013). This process-based crop model, calibrated and validated over multi-year field trials and surveys at eight contrasting sites in terms of climate and agricultural practices. Simulations under 35 future possible climate scenarios combining −20 to 20% precipitation anomalies and 0 to +6 °C temperature increases, indicated that most of the scenarios have a negative impact on yield than those recorded in the recent past (Fig. 6.2). This study implied up to 41% yield reduction could be incurred with 6 °C increase in temperature and a 20% reduction in rainfall. The simulation study showed the photoperiod-sensitive pearl millet traditional cultivars seems more resilient to future climate conditions than improved cultivars with high genetic yield potential. Its fast growth rate as a result of high radiation use efficiency and large leaf area index result in high potential yield. The water saving, drought tolerance and climate change compliance of pearl millet make it a viable choice for the looming climate variability that will potentially affect crop production. In view of these circumstances, pearl millet cultivation is to be reclaimed by recognizing production options in context to anticipated climate change scenarios.

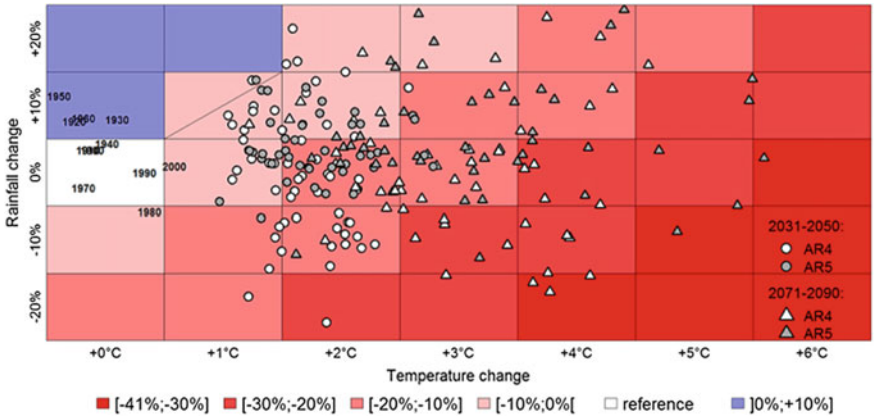


**Fig. 6.2** Effects of rainfall and temperature changes on mean yield changes in pearl millet and sorghum relative to the 1961–90 baseline for 7 temperatures (*x*-axis) and 5 rainfall (*y*-axis) scenarios (Sultan et al. 2013). Results are shown as the average over the 35 stations across West Africa and the 6 cultivars of millet and sorghum. White triangles and circles are the projected anomalies computed by several CMIP3 (Climate Research Program's Coupled Model Inter-comparison Project phase 3 multi-model dataset) GCMs (Global Climate Model) and three Intergovernmental Panel on Climate Change (IPCC) assessment reports (AR) emission scenarios (B1, A1B, A2) for 2071–90 and 2031–50, respectively. Projections from CMIP5 GCMs and three Representative Concentration Pathways (RCPs) (4.5, 6.0 and 8.5) are represented by grey triangles and circles. Past observed climate anomalies from Climatic Research Unit (CRU) data are also projected by computing 10-year averages (e.g. '1940' is for 1941–50). All mean yield changes are significant at a 5% level except boxes with a diagonal line (Adopted from Sultan et al. 2013; Environ Res Lett 8:014040)

### 6.1.3 Limitations of Traditional Breeding

Successful improvement of pearl millet for genetic yield potential, tolerance to biotic and abiotic stresses, and grain quality characteristics would mend food security for millions of people depending on the crop as a staple grain. However, the development of high-yielding cultivars, drought tolerance, downy mildew resistance, and enhanced grain content is much below the demand. This is because of the lengthy conventional hybridization method that takes two fundamental steps that are lengthy in process. The first step involves generation of a breeding population that is highly variable for traits of interest. This is accomplished by identifying two or more parents having complementary traits and cross-pollinating the parents to initiate recombination. The second and longer fundamental step in conventional breeding involves raising several generations of segregating populations and selection of individual plants that combine useful traits of the parents among the population. These steps essentially follow the normal mating process with breeders' intervention with directed mating and selection of plants that suits human needs.

Marker-assisted breeding by and large has gained evident importance to address these long-standing challenges of plant breeding by enhancing the selection efficiency and cutting the lengthy phenotypic selection processes. Nevertheless, the application of molecular markers covers only very special aspects of plant breeding and will not replace the conventional breeding methods completely. Therefore, a systematic complementation of the conventional methods with the advanced genomic techniques is encouraged to lead to more efficient and expedited breeding procedures. The techniques such as 'speed breeding' and RGT (rapid generation turn over) in combination with molecular markers could help to reduce the overall time required in conventional breeding (Ochatt et al. 2002; Watson et al. 2018). The other game changer could be the use of DH (doubled haploid) lines to expedite the homozygosity by reducing the 6–8 generations to 2–3 generation thus saving 5–6 years.

## 6.2 Priority Target Traits

The relative importance of traits may vary across production conditions and the intended use of a crop. As pearl millet is grown for grain as well as forage use, it is commanding to target the traits exclusively for both purposes. In general, two evolutionary steps considerably modified plant phenotype during plant domestication process. The first step being the change in characteristic traits of domestic plants that have occurred under the conditions of primitive cultivation of wild populations (Hillman and Davis 1990). These groups of specific characters in cultivated plants are generally shared by all the components of the domestic gene pool of the same species and defined as "domestication syndrome" (Hammer 1984).

The second step, takes in the diversification of the domestic gene pool as a byproduct of local adaptations to new environments and to the needs and tastes of diverse mankind. The morphological and physiological diversity observed in the domestic gene pool, which largely exceeds what is usually observed in their wild counterparts, is the product of this evolutionary process (Lakis et al. 2012). For instance, population-based resequencing of cultivated barley accessions reveals that flowering time was the main target of this second step of the domestication process to adapt to new environmental conditions encountered during the expansion of the cultivation areas (Jones et al. 2008). Climatic variations dictate the selection for early or late maturing genotypes. The target traits that play important role in adaptation, yield performance, and stress tolerance in pearl millet are discussed hereunder.

### *6.2.1 Photoperiodism*

Days to flowering is one of the most important plant characters that influence the cropping pattern in relation to the natural environment (Takei and Sakamoto 1987). Besides being an adaptive trait, flowering time and photoperiodic sensitivity are influencing yield and yield stability of pearl millet in arid and semi-arid regions of West and Central Africa (WCA). Pearl millet is generally a photoperiod sensitive crop and almost all landraces flower in short-day condition (Dave 1987). As photoperiodism affects the growth and development, it allows for flexible sowing dates by maintaining flowering and grain maturity at the end of the growing season. For instance, pearl millet varieties grown in the Sahel can mature in less than 90 days. In relation with the short duration of the rainy season in the Sahel, photoperiodism has an advantage in such hot and dry condition where the onset of rain varies from year to year.

In temperate regions, the minimum temperature during the light period of 12 h and less are mostly below optimum for pearl millet growth and development. This low suboptimal temperature for the crop can potentially extend the number of days from floral initiation to anthesis and slow down grain maturity (Burton 1965) which exposes the crop to likely frost in the autumn. Therefore, pearl millet breeders are intended to develop early maturing day-neutral varieties. So, the day-neutral cultivars can flower in the long-day condition of the summer by accumulating growing degree days and mature earlier before the onset of frost.

Selection in a flowering time pathway during domestication of pearl millet showed that genes for the trait underwent selection more frequently than expected (Clotault et al. 2012). Significant signatures of selection were found in six pearl millet flowering time genes and higher deviations from neutrality for circadian clock-associated genes, indicating that one category of genes of the flowering pathway were preferentially selected during pearl millet domestication. Crossing a late-maturing with an early maturing genotype revealed that photoperiodism was controlled by several genes with additive and minimal dominance effects (Burton 1965).

A study conducted on geo-referenced ICRISAT collection from a wide range of latitudes revealed that 45.6% of the accessions are photoperiod-sensitive (Upadhyaya et al. 2012). Generally, the late-maturing genotypes are short-day types, whereas the improved early-flowering ones are day-neutral (Burton 1951). Long-day delays the genetic tendency to flower by forcing the plant to wait for a specific signal (Dingkuhn et al. 2006). Latitude of origin of pearl millet genotypes affects photoperiod and day-length responses mainly in West Africa (Sanon et al. 2014). The critical photoperiod and temperature required to trigger flowering is cultivar-specific. As a result, some cultivars flower later than others due to difference in the level of photoperiodism.

Pearl millet varieties widely vary in growth cycle length (early: 45 days to late: 140 days) (Rao et al. 1985). The maturity duration of the cultivars grown in WCA can broadly be classified into three groups: (1) early types (flower in 45 to 70 days) are mostly facultative short-day and grown in the northern dry regions of the Sahel; (2) intermediate types (flower in 70 to 100 days) and (3) late landraces (flower in 100 to 140 days) are more abundant in the wetter southern regions and strictly sensitive to day length (absolute short-day plants). The world collection of pearl millet germplasm assembled at the ICRISAT gene bank, Patancheru, India, is from a wide range of latitudes and includes the typical temperature and photoperiod sensitive and insensitive accessions (Upadhyaya et al. 2012).

Selection during domestication left some regions of the genome depleted of diversity as compared to the wild genome, indicating potential locations for genes associated with domestication syndrome. Three flowering candidate genes, *PgHd3a*, *PgDwarf8*, and *PgPHYC* were cloned and studied for nucleotide diversity in the wild and domesticated pearl millet population (Sehgal et al. 2012). The sequence analysis revealed that the domesticated population has 84% nucleotide diversity found in the wild population which is attributed to gene flow between wild relatives and domesticated pearl millets. A positive selection was evidenced that *PgHd3a* and *PgDwarf8* were likely targeted by selection during domestication. Other genes that experienced the most unusual diversity loss, their putative functions are associated with regulation of response to hormones such as auxin and ethylene, regulation of the circadian clock and morphogenesis, along with transcription factors (Varshney et al. 2017).

### *6.2.2 Root System*

The root system of vascular plants plays the key roles in acquiring water and mineral nutrients required for the survival as well as accumulation of metabolites for yield and nutritional quality. Moreover, the roots play a significant role in holding soil in place, carbon sequestration, reducing emissions of greenhouse gasses, and prevent the eutrophication of water bodies associated with the application of mineral fertilizers (White et al. 2013).

Sustainable crop production requires root systems optimized for growing conditions in the field. Many of the traits related to abiotic stress tolerance such as water and nutrient use efficiency, and yield performance are linked to the root properties. Deeper and profuse root systems could withstand drought effects by tapping extra water from the lower soil profile. Thus, root architecture have long been suggested mainly to improve crop adaptation and performance in water limited environments.

Drought tolerance in pearl millet is in large part related to the root system (McIntyre et al. 1995). Sustained water uptake ability by increasing total root length and maintenance of high leaf water status under soil drying conditions are the main factors for drought tolerance in pearl millet (Matsuura et al. 1996). Therefore, breeding pearl millet varieties with improved root traits promises to deliver benefits in water and nutrient acquisition in dry environments.

The hidden part of the plant, root system makes phenotyping very challenging. Recently, imaging technologies have become available that allow to illuminate the dynamics of root structure and function in the soil. Rhizotrons and micro-computed tomography (microCT) phenotyping approaches characterized early stage pearl millet root system development as a fast growing primary root and three distinct types of lateral roots formation on both primary roots and crown roots (Passot et al. 2016). Pearl millet inbred lines studied for the root system architecture exhibited significant variation in primary root length and lateral root density. It is presumed that primary root growth is associated with early stage drought stress tolerance. Search for genetic markers associated with primary root growth in a large panel of genetically fixed pearl millet inbred lines is a priority research area for drought tolerance. However, there is no genetic or genomic studies conducted on early root development traits to dissecting the genetic determinants controlling these key root phenotypes in pearl millet. Different phenotyping technologies that analyze processes at different spatial and temporal scales such as combined magnetic resonance imaging (MRI) and positron emission tomography (PET) may open up mechanistic understanding of root structure and function of the crop.

### *6.2.3 Cold Tolerance*

Pearl millet is a sun loving plant and requires warmer temperatures (30–35 °C) for normal growth. Its sensitivity to cold weather limits its usefulness to only the hot temperature in the summer. Seed germination is also affected by soil temperature. Pearl millet seeds germinate only when the soil temperature is above 18 °C. Cold weather can stress plants and increase nitrate levels. Frost in the fall is also observed to stop the growth of pearl millet.

Nevertheless, the existence of genetic diversity for relative cold tolerance cannot be ruled out. Landraces from Yemen were found to be potential sources of variation

for cold tolerance (Shivhare and Lata 2016). The existence of a complex gene regulatory network for stress tolerance was witnessed with the identification of about 2,494 differentially regulated transcripts in response to drought, salinity, and cold stress (Mishra et al. 2007).

### 6.2.4 Drought and Heat Tolerance

Drought inflicts an adverse effect on plant growth and development. The key plant biochemical processes such as photosynthesis and transpiration involve water molecules. Reduced water in the plant system also accelerates chloroplast damage (Stone 2001) and affects photosynthetic outputs as a result of reduced light interception and carbon reduction. Water is also required by plants for surface cooling from sun light radiation. About 99% of the water absorbed from the soil is usually evaporated from aerial parts such as- leaves, stems and flowers- into the atmosphere, in trade-off the plant parts remain in normal physiological status and intake $CO_2$.

Drought is the major constraint in pearl millet production as it is almost exclusively grown in the dry semiarid and arid environments. However, adaptive evolution and natural selection made pearl millet relatively the most drought and heat tolerant among other cereals. Morpho-physiological traits were developed through evolution, including both shoot and root characteristics naturally modified for efficient water absorption from the soil and conservation in the system help pearl millet adapt to the dry environment. Stomatal conductance, photosynthetic capacity, timing of phenological phases, starch availability during embryo development, stem reserve mobilization in drought stress, stay green, reduced leaf area, rooting depth and density, cuticular resistance and surface roughness, osmotic adjustment, membrane composition, antioxidative defense, and accumulation of stress-related proteins (Cattivelli et al. 2008) are some of the morpho-physiological traits of drought tolerance and relevant for yield performance. Drought often accompanying by increased air temperature results in reduced reproduction due to heat damage on the pollen grain (Bita and Gerats 2013), and increases sterility (Schoper et al. 1987; Jagadish et al. 2012).

Decreased $CO_2$ diffusion to the chloroplast through limited stomatal conductance affects the rate of photosynthesis (Pinheiro and Chaves 2011). Drought stress prompts plant system to accumulate metabolites such as proline in plant cell to serve as osmolyte, as a metal chelator, an antioxidative defense molecule, and a signaling molecule (Hayat et al. 2012). It also maintains osmotic balance, prevents electrolyte leakage by stabilizing membranes and proteins such as RUBISCO (Ribulose 1, 5-bisphosphate carboxylase) (Hayat et al. 2012). It also controls the reactive oxygen species (ROS) concentration in plants (Paleg et al. 1984; Hare et al. 1998) and maintains the integrity of mitochondrial electron transport complex II (Hamilton and Heckathorn 2001) to impart stress tolerance. However, molecular and biochemical evaluations conducted so far in pearl millet have not considered

the effect of proline accumulation on drought tolerance. There are other numerous metabolic changes and enzymatic activities associated with water deficit in the plant system (Serba et al. 2017).

Traditional landraces from drier regions were good sources of genes for breeding drought tolerance (Kusaka et al. 2005; Yadav 2010). However, drought tolerance is a complex polygenic trait and the various morpho-physiological responses to drought are controlled by many genes and significantly influenced by the environment (Hu and Xiong 2014). Dynamic and complex cross-talk between different regulatory gene networks regulate physiological and morphological adaptation and plant stress responses (Saito and Matsuda 2010). Therefore, there are technical limitations prohibiting the classical breeding program to study plant responses to environmental stress. In that scenario, pre-breeding and genomics-assisted breeding will be very helpful for characterization of germplasm collection and introgression of desirable traits in adopted varieties (Varshney et al. 2018).

The mechanism how plants endure the effect of drought on their growth and development takes various forms. The ability of plants to grow and yield satisfactorily with limited moisture supply or under periodic soil water deficits is termed as 'drought resistance.' It is principally the constitutive plant traits of maintaining high plant water status (dehydration avoidance) that enables plants to survive in water limited environments (Blum 2005). This mechanism has virtuous relevance in terminal drought tolerance of pearl millet in which tolerant genotypes fill their grain and offset any drastic effect on their yield. Since phenotyping dehydration avoidance is difficult, not much study is devoted to understand the genetic bases of drought tolerance in pearl millet. However, a recent genome resequencing of a large number of genotypes shaded light on the genetic basis of drought tolerance in pearl millet. The heat and drought tolerance of pearl millet is associated with a stock of lipid biosynthesis genes for increased cuticular wax synthesis (Varshney et al. 2017). A detailed account of epicuticular wax in pearl millet is presented under the crop-specific traits below.

Root characteristics such as depth, density and architecture are also important traits in drought tolerance. Fine roots and root length density are some of the root traits known to contribute to productivity under drought stress (Comas et al. 2013). Genotypes with such root characteristics can exploit the water available deeper in the soil profile and better tolerate terminal drought. However, no information is available on the genomic regions or genes underlying such root characteristics in pearl millet. Further, any genetic variation in the germplasm for such traits and its relationship with terminal drought tolerance was not studied yet.

However, different researches conducted drought tolerance studies in pearl millet. Evaluation of hybrids against female and male inbred parents under sprinkler irrigation gradient highlighted the significant dry biomass reduction in low water level (Ibrahim et al. 1985). In this evaluation the performance of the hybrids was better than the parents for dry biomass, harvest index, and water use efficiency under severe drought stress.

High temperature stress can affect pearl millet plant at two stages; either at germination when soil temperatures can be very high or at flowering/reproductive stage. Field studies in the Sahel indicated that pearl millet seedlings are most vulnerable to high temperatures during the first 10 days of sowing, this was confirmed by field studies in the Indian Thar Desert (Yadav et al. 2012). Genetic differences in seedling survival under high soil surface temperatures were identified (Peacock et al. 1993). Pearl millet has good degree of tolerance to high temperatures of up to 42 °C during flowering. Hence, it has occupied considerable areas (about 1 m ha) in the hot and dry post-rainy season (locally referred to as summer) in the northern and western parts of India. Heat tolerance is required in summer season when air temperatures of >42 °C coincides with flowering period which leads to reproductive sterility and finally drastic reductions in grain yield. Large genetic variability for seed set at daily maximum air-temperature of $\geq$ 42 °C during flowering in pearl millet was reported (Gupta et al. 2015b). They indicated that seed set in pearl millet started declining when maximum air temperatures reaches 42 °C and decreased in curvilinear fashion to 20% till 46 °C. High temperature stress imposed for season long, or during reproductive stages of development for different durations (18 or 45 days) caused significant decrease in number of seeds per panicle, individual seed weight and seed yield per panicle (Djanaguiraman et al. 2017). Two periods (10–12 and 2–0 days before anthesis) were identified as most sensitive to short episodes (2 d) of high temperature stress, causing maximum decreases in pollen germination percentages and seed numbers per panicle.

### *6.2.5 Salinity Tolerance*

In arid and semiarid regions, the extent of salt affected soils is steadily increasing mainly because of flooding irrigation system. Today, more than one billion hectares of land area are affected by soil salinity around the globe (Saade et al. 2016), and has become one of the major constraints on agricultural production.

Pearl millet is well-suited to grow in harsh conditions including saline soils. It is generally considered as an alternative crop for salt affected areas because of its fair tolerance to salinity (Ali et al. 2006). Research conducted on salt tolerance in semiarid areas found a significant interaction of NaCl concentration and pearl millet varieties (Yakubu et al. 2010). Though both plant growth parameters and nutrient contents significantly decreased with increasing soil salinization, they found a variety named Maiwa that was relatively tolerant to soil salinization as it expressed superior nutrient content and root and shoot growth when compared to the control. But the usefulness of these growth parameters for salinity response with respect to grain and forage yield needs to be further investigated.

### 6.2.6 Disease Resistance

Numerous fungal, bacterial, and viral pathogens that infect pearl millet were discovered decades ago (Ramakrishnan 1971). Downy mildew (*Sclerospora graminicola*), rust (*Puccinia substriata* var. *indica*), Pyricularia leaf spot or blast (*Pyricularia grisea*), and smut (*Moesziomyces penicillariae*) are the most important diseases causing major yield losses and quality reduction of pearl millet. High relative humidity (85–90%) and moderate temperature (20–30 °C) favor infection and disease development (Thakur et al. 2011). As a result, the severity of these diseases varies across different growing regions.

The first report of downy mildew (DM) pathogen on pearl millet was from India (Butler 1907). However, the disease remained sporadic and caused yield losses in poorly drained and low lying areas until the introduction of improved cultivars in 1960s (Singh 1995). DM is now a major biotic constraint in India and West Africa. The use of Tift 23A as a seed parent, increased the susceptibility of the first batch of hybrid cultivars in India. The wide spread of hybrids based on Tift $23A_1$ increased oospore inoculum and resulted in epidemics in 1971–72 (Singh 1995). DM incidence is estimated to cause pearl millet grain yield loss ranging from 10 to 80% (Gupta and Singh 1996) and gained more importance for resistance breeding.

Although several control measures—including seed sanitation, chemical control of the seed, soil, and air-borne inoculums—were suggested, a major emphasis has been placed on host-plant resistance for its effectiveness. Consequently, a great progress has been made in downy mildew resistance breeding in India. Studies in disease epidemiology and pathogen biology (Singh and Williams 1980) enabled development of reliable field and controlled environment screening techniques (Singh et al. 1997). All breeding material passes through the downy mildew-screening nursery and resistant varieties and hybrids have been bred since then. Pathogen isolation procedures, and severity rating scales, have been established for downy mildew and other major diseases such as rust, blast or leaf spot, ergot and smut at ICRISAT (Singh et al. 1997; Thakur et al. 2011). A good number of germplasm and breeding lines with high levels of stable resistance have been identified from West Africa (Singh 1990; Singh et al. 1997). These materials have strategically been used in breeding for resistance at ICRISAT and All India Coordinated Pearl Millet Improvement Program (AICPMIP) centers (Hash et al. 2006; Thakur et al. 2006). Development and commercial deployment of downy mildew resistant HHB 67, a popular hybrid being grown in North India, is the first successful story of marker-assisted breeding (MAB) in field crops in public domain in India (Hash et al. 2006; Thakur et al. 2006). However, the disease still remains the major constraint in West Africa. To offset the disease epidemics, farmers in the region are still growing landraces resistant to downy mildew but low in grain yield potential.

In addition to studying the biology of the pathogen, the *Sclerospora graminicola* genome was recently sequenced at 40x (Nayaka et al. 2017). A total of 299.9 Mb of the genome sequence was assembled and 65,404 genes predicted, of which 38,120

genes were annotated. This resource is important to study the pathogenicity, race dynamics, spread of the pathogen and as a powerful synergy to resistance breeding. It was also reported that endophytic *Trichoderma hamatum* UoM 13 isolated from pearl millet root suppressed downy mildew disease (Siddaiah et al. 2017).

Although sources of resistance are available, the mechanism of host resistance is still barely understood. An attempt was made to test enzyme lipoxygenase (LOX), known to play a role in disease resistance in many host-pathogen systems. LOX activity was tested in seeds of different genotypes of pearl millet with different level of susceptibility to downy mildew (Nagarathna et al. 1992). The LOX activity of seeds assay indicated a positive correlation between enzyme activity of genotypes and DM resistance in the field and thus can be used as a biochemical marker for downy mildew resistance. In a recent study to unravel inheritance, it was observed that resistance to DM is controlled by a single dominant gene in 834B and IP 18294-P1 and by two dominant genes in IP 18298-P1. A test for allelism inferred that a single dominant gene for resistance in 834B is nonallelic to that which governs resistance in IP 18294-1, whereas, one of the two dominant genes for DM resistance in IP 18298-P1 against the test isolates is allelic to the gene for DM resistance in 834B and a second gene is allelic to the resistance gene present in IP 18294-P1 (Raj et al. 2018).

Rust and leaf spot are the two important diseases of pearl millet in the United States (Wilson and Hanna 1992). Rust is known to cause up to 72% yield loss (Wilson et al. 1995). Rust disease control through other methods is more difficult as the pathogen is primarily disseminated by wind, and the spores can survive in the soil, on plant debris, volunteer pearl millet, and alternative hosts. The research for genetic resistance discovered a dominant rust resistance gene, $Rr_1$, in wild grass (*P. glaucum* ssp. *monodii*) from Senegal and introgressed into cultivated pearl millet via backcrossing (Hanna et al. 1985). This introgression only improved rust resistance provisionally because the gene was fast overcome by a shift in the virulence of the pathogen. Germplasm screening against single uredinal isolates of the rust pathogen discovered 10 new races with different level of prevalence (Tapsoba and Wilson 1995). Both race-specific and non-race-specific (horizontal) resistance is prevalent in the germplasm. Then the resistance breeding shifted to broadening the genetic basis of resistance by combining slow rusting genes with race-specific resistance in the development of improved forage pollinator lines (Wilson 2002). A major rust resistance QTL explaining 58% phenotypic variance was mapped on linkage group 1 ($LG_1$) using a $F_7$ recombinant inbred line population from the cross 81B-P6 x ICMP 451-P8 (Ambawat et al. 2016) and was found to confer a durable slow-rusting phenotype.

Pyricularia leaf spot (blast) is another common fungal disease affecting grain and forage pearl millet production in India and the United States. Unlike the rust pathogen, very little has been accomplished on pathogen characterization or management in the United States. Information on germplasm variability for host-plant resistance, precise phenotyping and screening techniques, and genetic mechanism of resistance are limited for this disease. Based on two resistant sources having diverse parentage, resistance to foliar blast in pearl millet is controlled by a single

dominant gene (Gupta et al. 2011). Recently, six blast resistant lines having diverse parentage revealed the presence of single dominant gene governing resistance to two *Magnaporthe grisea* isolates, and were found allelic (Singh et al. 2018).

Variability from African germplasm has been exploited for disease resistance. However, fungal diseases, especially DM and smut, are still inflicting significant yield losses in major pearl millet-growing areas of West Africa. A priority breeding strategy that harness the germplasm available is highly needed to incorporate disease resistance with cultivar yield improvement to enhance acceptable to the growers.

### 6.2.7 Insect Resistance

There are about 100 species of insect pests attacking pearl millet as previously reviewed (Gahukar 1984; Nwanze and Harris 1992). Important insect pests in West Africa include stem borer (*Coniesta ignefusalis* Hampson), grain midge (*Geromyia penniseti* Felt), ear-head caterpillars (*Heliocheilus albipunctella* de Joannis), head beetle (*Rhinyptia infuscata* Burmeister), shoot flies (Athrigona spp), and leaf beetles (*Lema planifrons* Weise and *Chaetocnema tibialis* Illiger). Other general feeders such as armyworms (*Spodoptera exempta* Walker, *S. exigua* Hubner, *S. littoralis* Boisduval, *Mythimna loreyi* Duponchel), aphids (*Rhopalosiphum maidis*, Fitch (Aphididae: Homoptera)), grasshoppers (*Hieroglyphus nigrorepletus* (Bolivar), *H. banian* (Fab), *Colemania spheneroides* (Bolivar), *Chrotogonus spp*. (Acrididae: Orthoptera)), and locusts may cause complete defoilation and severe losses to the crop during prolonged drought early in the season. However, the occurrence of such pests are sporadic, and sometimes localized.

A recent study found that insects belonging to six orders and 11 families were found to herbivore on pearl millet in the southern United States (Obeng et al. 2015). Eastern leaf-footed stinkbug (*Leptoglossus phyllopus* (L.), Hemiptera: Coreidae), American bird grasshopper (*Schistocerca americana* Drury; Orthoptera: Acrididae) and the differential grasshopper (*Melanoplus differentialis* (Thomas: Orthoptera: Acrididae) were the most prevalent and dominant species.

Soil dwelling insects such as white grubs (*Holotrichia* spp, *Anomola* spp, (Melolonthidae: Coleoptera) and termites or white ants (*Odontotermes* spp, *Microtermes* spp, *Macrotermes* spp, (Termitidae: Isoptera) are attacking the root of pearl millet. Particularly, the grubs of *H. consanguinea* cut the roots causing wilting and death of plants in patches and is known to devastate the crop in large areas in central India.

Pearl millet is tolerant or essentially a poor host to sugarcane aphid (SCA) ((*Melanaphis sacchari* Zehntner, (Aphididae: Homoptera)). Field observations by producers, county extension agents, and millet breeders in several areas found little or no SCA in pearl millet field grown for forage. Controlled screening of pearl millet breeding lines along with a resistant and a susceptible grain sorghum (*Sorghum bicolor*) varieties at the K-State Agricultural Research Center-Hays

(ARCH) under controlled condition observed minimum damage (Serba and Michaud, unpublished). Representative seed parents, restorers and the germplasm were included in the study. The statistical analysis data found that there is significant difference among genotypes for aphid feeding damage but not among the different genotype categories (Fig. 6.3). However, none of the pearl millet genotypes affected to the level of susceptible sorghum, which implied pearl millet is a poor host for SCA. With the recent incidence of SCA on forage sorghum, pearl millet is recommended as an alternative to forage sorghums in the Central Great Plains of the United States (Trostle et al. 2015).

Inherent plant traits and its interaction with the external environmental factors affect plants tolerance to herbivory. Genetic tolerance with built-in compensation abilities of plants, climatic factors, and cultural practices are important factors in raising healthy pearl millet and keeping pests in bay.

### 6.2.8 *Grain Micronutrients (Fe and Zn)*

In the past decades, almost entire pearl millet research efforts have been directed towards the development of high yielding lines or cultivars through various breeding methods. Nutritionally, pearl millet is not less than any cereal crop and is rich in certain nutrition qualities, including micronutrients. Pearl millet is a healthy

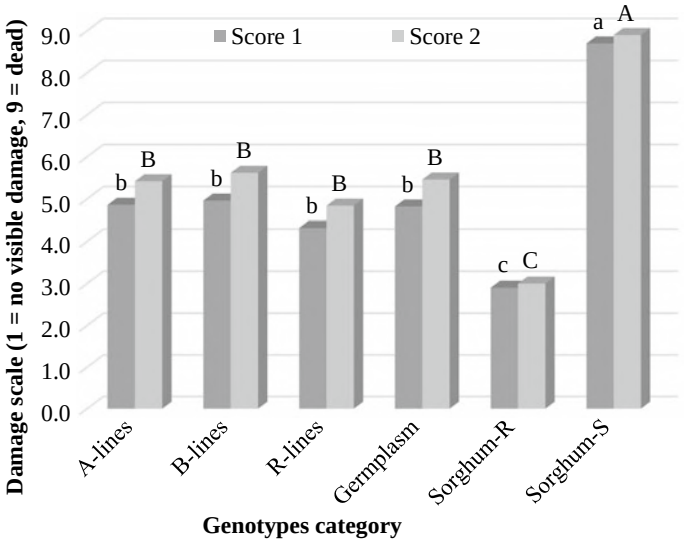


**Fig. 6.3** Average SCA damage score on pearl millet parental lines and germplasm against resistant and susceptible sorghum using 1 to 9 damage scale (score 1 and 2 were made five and eight days after infestation, respectively). Bars labeled with the same later (upper or lowercase) were not significantly different (Serba and Michaud, unpublished)

and versatile grain worthy to add to anyone's diet. It has higher percentages of essential amino acid profile and also exhibit higher contents of Ca, Zn, Fe and Mg than sorghum (Rai and Virk 1999; Iren Leder 2004). Pearl millet is the cheapest source not only for energy and protein but also inexpensive source for Fe and Zn among all cereals and pulses (Rao et al. 2006). However, more research effort is needed to enhance the essential micronutrients in the grain beside yield per se. This will help to addresses the malnutrition of the consumers who mostly rely on pearl millet as a staple food.

Preliminary screening of several mainstream breeding populations at ICRISAT, including released open-pollinated varieties displayed substantial variability for Fe (30–75 mg $kg^{-1}$) and Zn (25–65 mg $kg^{-1}$) (Velu et al. 2007; Gupta et al. 2009; Rai et al. 2012). HarvestPlus-supported pearl millet biofortification research has shown much large genetic variability for grain Fe (31–125 mg $kg^{-1}$) and Zn (35–82 mg $kg^{-1}$) densities among advanced breeding lines, population progenies, hybrid parents. Also several lines and accessions with 90–100 mg $kg^{-1}$ Fe and 70–80 mg $kg^{-1}$ Zn densities were identified, indicating a good prospect of genetic enhancement for nutritional quality (Govindaraj et al. 2016). Considering these, both Fe and Zn become core traits in current ICRISAT pearl millet breeding program and demonstrated with dissemination of first surge of biofortified diverse hybrids parents (seed and restorer) with other preferred traits including diverse cytoplasm (Table 6.1).

Over the last four decades, ICRISAT-bred lines and populations have been extensively used by breeders, both in public and private sector to breed open-pollinated varieties (OPVs) and hybrids. All these available released OPVs and/or commercial hybrids were evaluated to examine the variability for Fe and Zn density (Fig. 6.4). The Fe density in hybrids varied from 46–56 mg $kg^{-1}$ and Zn density from 37–44 mg $kg^{-1}$. In addition to high-Fe control ICTP 8203, very few hybrids viz., Ajeet 38 and 86M86 had high Fe and Zn densities. Systematic research also indicated Iniadi germplasm to be a valuable germplasm resource for Fe and Zn genetic improvement in pearl millet (Rai et al. 2013, 2015). The lines derived from Iniadi can be used for developing mapping populations to identify QTL for high levels of Fe and Zn densities (Kumar et al. 2018). Genomic studies of the Iniadi accessions selected for high Fe and Zn densities would provide useful information on the extent of diversity for genes responsible for high levels of these two micronutrients. Hence, developing micro-nutrient-dense seeds would be of great benefit to farmers by improving nutrient content of harvested grain. Biofortifying pearl millet is also the most cost effective and sustainable approach to enhance the levels of bioavailable micronutrients (Fe and Zn) and nutritional security (Vinoth and Ravindhran 2017).

**Table 6.1** Biofortified diverse seed and restorer parents developed at ICRISAT (data are mean of 4 seasons)

| Line | 50% flowering time (days) | XRF Fe (mg $kg^{-1}$) | XRF Zn (mg $kg^{-1}$) | 1000-grain weight (g) | CMS |
|---|---|---|---|---|---|
| *Seed parents* | | | | | |
| ICMA/B 1501 | 39 | 76 | 42 | 13.2 | A4 |
| ICMA/B 1502 | 43 | 92 | 50 | 13.6 | A1 |
| ICMA/B 1503 | 43 | 69 | 43 | 15.0 | A4 |
| ICMA/B 1504 | 47 | 97 | 55 | 15.5 | A1 |
| ICMA/B 1505 | 41 | 110 | 55 | 15.5 | A1 |
| ICMA/B 1506 | 45 | 96 | 53 | 9.9 | A4 |
| ICMA/B 1507 | 43 | 92 | 50 | 10.1 | A4 |
| ICMA/B 1508 | 53 | 73 | 44 | 15.0 | A1 |
| *Restorer parents* | | | | | |
| ICMR 1201 | 48 | 79 | 41 | 10.5 | A1 |
| ICMR 1202 | 50 | 89 | 47 | 14.2 | A1 |
| ICMR 1203 | 52 | 101 | 58 | 7.9 | A4 |
| ICMR 1301 | 55 | 91 | 52 | 12.7 | A1 |
| ICMR 1501 | 55 | 86 | 42 | 9.9 | A1 |
| ICMR 1502 | 51 | 110 | 62 | 12.4 | A1 |
| ICMR 1503 | 51 | 99 | 47 | 13.7 | A4 |
| ICMR 1504 | 57 | 96 | 51 | 8.8 | A1 |
| ICMR 1505 | 55 | 74 | 41 | 6.9 | A4 |

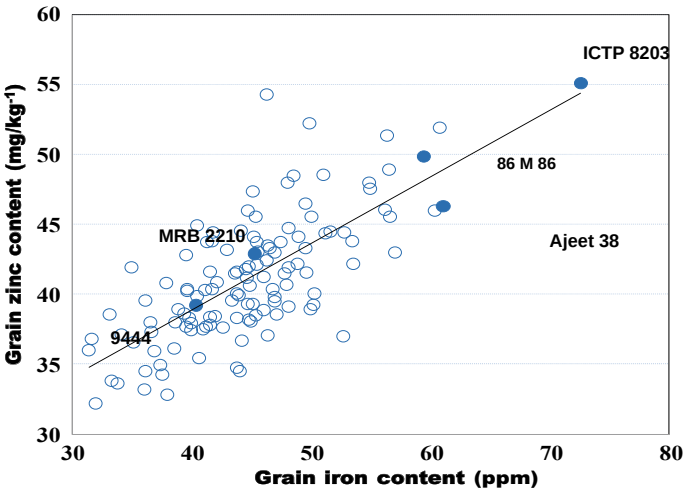


**Fig. 6.4** Variability and relationship between grain Fe and Zn content in commercial hybrids of pearl millet

### 6.2.9 Nutrient Use Efficiency

Of the 16 essential nutrients needed for successful plant growth and development, nitrogen (N), phosphorus (P), and Potassium (K) are the three most important primary nutrients. Assuming soil moisture is conducive, nitrogen is the most important limiting factor in crop production. In fact, N is the important constituent of protein and protoplasm and its shortage leads to chlorosis (leaf yellowing) and slowdown the growth.

N ranks first among the applied inorganic fertilizers to maximize yield in agriculture. The global demand for N fertilizer for agricultural production, which already stands at approximately 110 million metric tons per year, is projected to increase to approximately 250 million metric tons by 2050 (www.fertilizer.org). Because nitrates are very mobile in the soil, a substantial amount (> 50% in some cases) of applied N is lost by leaching, run-off, and de-nitrification. In addition to an increase in production cost, in the long run these processes of N loss not only pollute the ground water and adversely affect soil structure but also have detrimental effects on the environment such as increase in nitric oxide, ozone, etc. Hence, developing crop varieties with improved efficiency for N absorption and utilization will help mitigate these problems to some extent (Frink et al. 1999; Good et al. 2004). In addition to its role in plant growth, N level (organic and inorganic forms) in the soil increases soil water-holding capacity and control wind-erosion (Bationo et al. 1993).

The prevalence of significant genotypic variation in biomass production and nitrogen use efficiency (NUE) were documented in pearl millet (Alagarswamy and Bidinger 1987). This study found that a genotype, Souna B from West Africa had NUE values 32% higher than the less efficient Indian genotype BJ 104, even though both had similar N uptake. In addition to genotype difference, N uptake and utilization in pearl millet was found to be influenced by the growing environment especially soil moisture (Maman et al. 2006).

Soil fertility is marginal and plant-available P is severely limited in the Sahelian west Africa (Lambers et al. 2015). Pearl millet is the cereal grown well to this adverse climatic condition and acid sandy soils. For economic production of pearl millet in such an environment assessment of genetic variability for P uptake, utilization in available germplasm, and identification of potential secondary selection traits are important endeavors. To overcome soil P-deficiency, exploitation of pearl millet genetic diversity is an economically worthwhile and environmentally feasible opportunity. Genetic variation was studied under low and high P level for uptake and utilization in both seedling and mature plants using 180 West African pearl millet inbred lines (Gemenet et al. 2015). Phosphorus utilization efficiency increased in low-P, but total P uptake was more important for grain production than P utilization under low-P conditions. Both seedling and mature plant morphological traits are potentially useful as secondary traits in selection of pearl millet for low-P adaptation. From this study, it was suggested that pearl millet breeding for low-P

tolerance needs to be integrated with other system-oriented research, such as nutrient cycling and modest applications of locally available rock phosphate.

In general, pearl millet germplasm from West Africa unveils significant genetic variation for P-uptake and utilization efficiency, and grain yield under P-limited soils indicating the possibility of breeding P-efficient cultivars (Gemenet et al. 2015). To accelerate the breeding process for P uptake and utilization, mapping the genomic regions through marker-trait association and application of marker-assisted selection (MAS) are vital.

### *6.2.10 Water Use Efficiency*

In water-limited environments, plant productivity is determined jointly by the amount of water available and the water use or evapotranspiration efficiency (Emendack et al. 2011). Yield and water use efficiency (WUE) of pearl millet vary across genotypes (Emendack et al. 2011) and moisture regimes (Sivakumar and Salaam 1999). Improvement in pearl millet WUE is likely surge yield by increasing the number of productive tillers and uniform maturity of the tillers with the main culm. Research conducted on soil nutrient status and WUE under water stressed condition found that, WUE is influenced by available P level in the soil, as cumulative transpiration increased with P level (Payne et al. 1992). In the arid environment of Niger, year effect on pearl millet yield was observed which is primarily because of variations in the amount and distribution of rainfall in relation to the potential demand for water (Sivakumar and Salaam 1999). Increase in soluble soil phosphate due to application of fertilizer increased WUE and yield. The beneficial effect of fertilizers could be attributed to the rapid early growth of leaves, which can contribute to reduction of soil evaporative losses and increased WUE.

WUE is also dependent on root architecture, as nodal and primary roots have distinct responses to soil moisture level (Rostamza et al. 2013). Association of greater nodal root length in pearl millet with increased shoot biomass is attributable to efficient water uptake and WUE. Six times longer nodal roots, mainly from 8-fold increase in branch root length, were observed with 12% soil water content than dry treatments. Enhanced plastic response to moisture around the nodal roots in pearl millet is attributed to faster growth and progression through ontogeny for earlier nodal root branch length and partitioning to nodal root length from primary roots, independent of shoot size. Genotypes with deeper nodal roots can be selected in a breeding program to shape root architecture. Enhanced response to soil moisture and rapid rate of plant development are important traits that favor nodal roots and WUE without any cost to shoot growth.

Reports show that WUE is more influenced by edaphic than climatic condition as significant reduction by waterlogging than drought observed (Zegada-Lizarazu and Iijima 2005). The drought resistance of pearl millet is explained by higher WUE but not by increased water uptake efficiency in deep soil layers as compared to barnyard millet, another drought-resistant millet species. However, there was not

any study on the genetic factor underlying WUE variations in pearl millet. As WUE has a leveraging effect on drought tolerance, it necessitates a new frontier in genomic study.

### 6.2.11 Carbon Sequestration

Soil organic carbon (SOC) accumulation largely depends on vegetation cover. The pearl millet production system is characterized by a little, if any, recycling of organic matter. The crop is grown as a dual purpose crop in the arid and semi-arid areas where the stock is used as a fodder for animals or as a fuel for cooking. A study conducted on the effect of soil organic matter amendment on grain yield in rain-fed production systems in the semiarid tropics of India reported that for every Mg $ha^{-1}$ increase in soil organic carbon stock in the root zone, pearl millet grain yield increases by 170 kg $ha^{-1}$ (Srinivasarao et al. 2014). The grain yield response from pearl millet with the soil organic carbon amendment was the highest when compared to other crops such as groundnut (*Arachis hypogaea*), finger millet (*Eleusine coracana*), sorghum, soybean (*Glycine max*), castor (*Ricinus communis*), cluster bean (*Cyamopsis tetragonoloba*) and rice.

Comparison of five millet species [Japanese millet (*Echinochloa esculenta*), pearl millet, foxtail millet (*Setaria italica*), browntop millet (*Urochloa ramosa*), and proso millet (*Panicum miliaceum*)] for conservation use in the United States recommended pearl millet for fast-growing as a cover crop that can increase organic matter, scavenge residual nutrients, create large amounts of surface mulch, reduce compaction, and reduce root-lesion nematodes (USDA 2014).

### 6.2.12 Genome Plasticity

The adaptation of plants to a dynamic environment is defined by the controlled expression of genes both temporally in response to a change in the environment and regularly for normal growth and development. To survive in a changing environment and reproduce, genome plasticity acquired through inheritance and mutation enable a plant to compete for the resources it needs to grow. Changes in nucleotide sequence that occurs as a result of several forces, chemical and genetic, can alter the genetic content of a plant and genomic plasticity. Transposons, insertion sequence elements, DNA repetitions, introns, and DNA rearrangement are the reserve in the genome for changes in gene expression in response to external factors (Bennett 2004).

Early characterization of pearl millet nuclear genome with respect to its size, buoyant density, sequence organization, and association kinetics estimated the genome size at 0.22 pg $nucleus^{-1}$, GC content at 44.9%, and the melting temperature to be 49.7 °C (Wimpee and Rawson 1979). In other cereals, such as hexaploid wheat, new variation is rapidly generated because of the dynamic nature of wheat

genomes through gene deletions and insertions of repetitive elements into coding and regulatory gene regions (Dubcovsky and Dvorak 2007). Pearl millet has an estimated genome size of 1.79 Gb (Varshney et al. 2017). The percentage of repetitive DNA in pearl millet is estimated at 80% (Wimpee and Rawson 1979; Paterson et al. 2009; Bennetzen et al. 2012), which is close to that of maize. It is presumed that this high level of repetitive DNA is a reflection for a high level of genome plasticity in pearl millet.

Remarkable intraspecific differences in gene expression were documented in pearl millet. Given the absence of detailed studies in pearl millet genome, it is difficult to present a detailed account of the genome organization. Yet it is not sufficiently studied how diversity bottlenecks are compensated. Although the wild relatives are growing in the same environment with the cultivated pearl millet, especially in Sahelian West Africa, the extent and significance of natural gene introgression between species has not been studied well. However, the transfer of CMS from the compatible primary gene pool to pearl millet warrants the gene flow from the wild relatives that enhance genome plasticity of the crop.

### *6.2.13 Other Important Traits*

Pearl millet exhibits some characteristics that are relatively specific to the crop or shared among related species. Some of these specific traits are related to leaf area to extend the duration of active photosynthesis, leaf anatomical structure related to forage digestibility (such as brown mi-rib trait), accumulation of cuticular waxes on the above ground surface to minimize water loss in dry conditions, and purple pigmentation for ornamental value. These key traits are of interest in the improvement of pearl millet for specific usage.

#### 6.2.13.1 Stay Green

Stay green is a characteristic of delayed leaf senescence. Some pearl millet genotypes extend the duration of active photosynthesis by delaying leaf senescence for a longer period of time than others. The relationship between carbon fixation capacity and spending over the life of a leaf alters the timing of senescence initiation and progression. In stay green variants the deconstruction of the photosynthetic apparatus during leaf senescence is partially or completely prohibited (Thomas and Howarth 2000). A complex signaling network that involves mutations activating cytokinin signaling or those suppressing ethylene, abscisic acid, brassinosteroid, and strigolactone signal transduction often result in stay-green phenotype (Kusaba et al. 2013). Also, impairment in the enzymatic steps responsible for chlorophyll breakdown leads to stay-green phenotypes.

A study conducted using some inbred lines found that a SNP in putative acetyl CoA carboxylase gene showed robust association with grain yield, harvest index,

and panicle yield under irrigated and non-irrigated conditions and an InDel in putative chlorophyll a/b binding protein gene was significantly associated with both stay-green and grain yield under drought stress and the later may serve as a functional marker for selecting high yielding genotypes with 'stay green' phenotype (Sehgal et al. 2015). As increase in leaf area, daily duration of photosynthesis or leaf area duration is significantly associated with yield increases achieved in grain crops (Richards 2000), stay green characteristic is important for both yield increase and stress tolerance. A plant that maintains the integrity of its chlorophyll despite the soil water status during reproductive stage can continue photosynthesis and can fill the grain and yield a reasonable grain in drought stress. The semi-dwarf pearl millet inbred lines developed in the United States for hybrid breeding are stay-green. However, a detailed study on locating the genes/genome regions involved is not conducted.

Five scenarios were proposed for change in chlorophyll content and photosynthetic capacity (Kusaba et al. 2013) during the leaf senescence process in stay green leaves (Fig. 6.5). Disabled chlorophyll pigment degradation during the later stage is associated with stay green. Stay green characteristic also has an essential effect for the feed value of the fodder after grain harvested. Therefore, the trait has important especially in dual purpose pearl millet variety development.

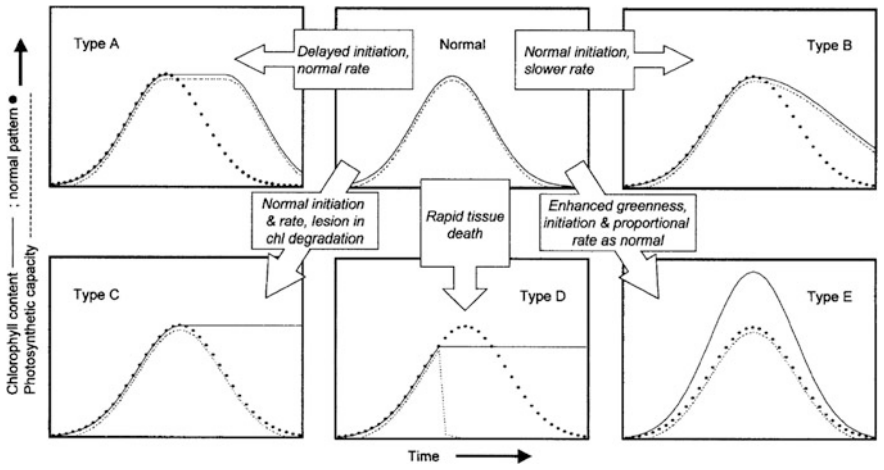


**Fig. 6.5** Five ways to stay-green. Curves show chlorophyll content and photosynthetic capacity (arbitrary scale) for a representative leaf, whole plant or canopy. Type A stay-greens lose pigment and function at the normal rate after a delay in the start of senescence. In Type B, senescence is initiated on schedule, but subsequently proceeds more slowly. Type C stay-greens undergo functional senescence on a normal time-scale, but a lesion in pigment breakdown means they retain chlorophyll indefinitely. Type Ds are stay-green because they are dead. In Type E behavior, the photosynthetic capacity of an intensely green genotype may follow the normal ontogenetic pattern, but comparison of absolute pigment contents identifies it as a stay-green (Adopted with permission from Thomas and Howarth 2000; J. Exp. Bot. 51: 329–337)

#### 6.2.13.2 Brown Mid-Rib (BMR)

Pearl millet is extensively used as a forage for livestock and improvement in digestibility and palatability may enhance feed value. High lignin content is the primary forage digestibility restricting factor by ruminants and reducing the shoot lignin content is the most effective way to increase the digestibility.

A brown coloration of the leaf mid veins (BMR) is associated with reduced lignin content and altered lignin composition of the midrib of maize, sorghum, sorghum x sudangrass, sudangrass, and pearl millet (Sattler et al. 2010). Therefore, brown midrib offers greater digestibility and palatability compared to conventional forage, thereby yielding greater returns in the form of weight gain in beef cattle or milk per ton of forage feed. Two sources of brown midrib lines have been reported in pearl millet (Gupta 1995). Pbmr developed at Purdue University through chemical mutagenesis (Cherney et al. 1988) and SDML 89107 developed by SADC/ICRISAT through germplasm selection from Zimbabwe (Gupta et al. 1993) were the two independent lines. Later, Tift-91 BMR was identified and bulked at the University of Georgia. In vitro dry matter digestibility (IVDMD) of pearl millet BMR was higher than its normal counterpart (Gupta 1995). Lower lignin content (23%) and 4% higher IVDMD was ($p \leq 0.01$) was reported in BMR forage lines (Cherney et al. 1990). BMR allelic inheritance study in pearl millet reported that BMR is controlled by homozygous recessive allele at a locus. The $F_1$s of the crosses between the two BMR and elite normal midrib inbred lines were found entirely normal, $F_2$ segregated 3:1 (normal/bmr) and testcross population segregated 1:1 confirm the trait under a major gene control (Gupta 1995).

#### 6.2.13.3 Cuticular Wax

Abiotic stress arises from exposure to climatic extremes such as drought, heat, cold, and frost. Plants have evolved to exist in conditions which are unideal for maintenance of normal physiology and may be at the limit for survival. The above ground surfaces of terrestrial plants are covered with cuticular wax (Jetter et al. 2007). Epicuticular waxes are complex mixtures of hydrophobic molecules form the outermost layer of the hydrophobic cuticle, which is composed of cutin polyester membrane, and provides the last barrier to prevent uncontrolled water loss (Kosma and Jenks 2007). Cuticular wax plays a significant role in plant abiotic and biotic stress tolerance and has been implicated in defense mechanisms against excessive ultraviolet radiation, high temperature, bacterial and fungal pathogens, insects, high salinity, and low temperature tolerance (Xue et al. 2017). Cuticular waxes consist of homologous series of very-long-chain fatty acids, alcohols, aldehydes, alkanes, esters, and cyclic organic compounds (Cameron et al. 2006). Primary plant surfaces are coated with hydrophobic cuticular waxes to minimize non-stomatal water loss. Wax compositions differ greatly between plant species, organs, tissues, and developmental stages (Guo et al. 2017).

A comparison study between sorghum, maize, and pearl millet leaves showed no glossiness even on the pearl millet twelfth leaf (Traore 1985). The glossy character was correlated with a reduction or absence of observable wax deposits on the leaf surfaces and higher cuticular water loss than non-glossy leaves. A recent genomic study unfolded a substantial enrichment for wax biosynthesis genes (Varshney et al. 2017). Therefore, it is believed that cuticular wax deposition in the leaves that minimizes water loss and reflect radiation probably contributed to heat and drought tolerance in pearl millet. It was also demonstrated that changes in cuticle gene expression in drought exposed plants (Suh et al. 2005) provide evidence for alteration of cuticle traits for drought tolerance.

#### 6.2.13.4 Purple Foliage

Another notable trait in pearl millet is purple foliage. It is known to be controlled by three plant pigments: anthocyanidins-cyanidin, delphinidin, and pelargonidin (Raju et al. 1985). A genetic study revealed that purple color is determined by three alleles at a single locus: $Rp_2$ (red), $Rp_1$ (purple), and *rp* (green) (Hanna and Burton 1992). Red is dominant over purple and normal green, whereas purple is dominant over the normal green ($Rp_2 > Rp_1 > rp$). Red plant can be distinguished at 5 days, whereas, purple can be distinguishable two weeks after emergence. The $Rp_1$-$Rp_2$ locus was independent of the trichomeless (*tr*), yellow ($yn_1$), female sterile (*fs*), and light green ($lgn_1$) loci but linked to the dwarf ($d_2$) locus (about 28% recombination). The $D_2/d_2$ plant height locus and the *P/p* foliage color locus are linked and mapped to pearl millet linkage group 4 (Azhaguvel et al. 2003). A purple foliage pearl millet was identified in a population of 500 plants derived from a combined mutagenic treatment with a 20 Kr dose of gamma rays and 0.1% aqueous solution of ethyl methane sulphonate (EMS) (Varalakshmi et al. 2012).

#### 6.2.13.5 Bristle Panicle

Bristliness in pearl millet is another important trait. The inflorescence consists of a central rachis covered with short hairs and bears fascicles on rachillae. Each fascicle contains spikelets surrounded by a wall of bristles (i.e., involucre). The extent of prolongation of the fascicle axis limits the length of bristles. Long bristles have an advantage in deterring birds feeding on the grain. A comparison of varieties with long and short bristles observed that long bristle showed reduced damage by blister beetle (*Psalydolytta fusca* Olivier) in the Sahel (Gahukar 1988, 1991). Bristled panicle is a dominant trait and hence easy to work in hybrids, when one of the parents is bristled.

#### 6.2.13.6 Dwarfing Genes

Plant height is a vital component for carbon gain strategy (Moles et al. 2009) as it determines a plant's ability to compete for light (Falster and Westoby 2003). On the other hand, plant height has agronomic importance as plants with short stature entail improved lodging resistance. Furthermore, cultivars with short plant height are preferred in modern agriculture for input responsiveness and mechanized harvesting. Dwarfing genes have been successfully utilized in developing short statured cultivars of cereals such as wheat, rice, barley, sorghum, and pearl millet. Semi-dwarf grain cultivars respond better to high levels of nitrogen application than tall plants because of their reduced lodging vulnerability.

Five different sources dwarf plants, named $D_1$–$D_5$, were first discovered in 1960s (Burton and Fortson 1966). Through crossing with the tall counterparts, the dwarfness in source lines were found to be controlled largely by one or two recessive genes. When transferred to near-isogenic backgrounds, dwarfness from source lines $D_1$ and $D_2$ is controlled by single independently segregating recessive genes, designated as $d_1$ and $d_2$. The $d_2$ dwarfing gene is known to reduce plant height by 50% through a reduction in the length of all stem internodes except the peduncle, leading to a higher proportion of leaves (Rai and Hanna 1990). The $d_2$ also has several pleiotropic effects. In addition to increased leaf percentage, improved nutritional quality of the stem fraction of pearl millet forage was attributed to $d_2$ (Burton et al. 1969). Subsequently, $d_2$ has been deployed widely in commercial cultivars grown in India, the United States, and Australia. Comparison of tall and dwarf near-isogenic $F_1$ hybrids found dwarf varieties have virtually no impact on the production that might restrict the release of dwarf pearl millet cultivars (Bidinger and Raju 1990).

After the discovery of dwarfing genes and inheritance studies, the research was focused on knowing the genomic location of the genes, the biochemical function of the candidate genes controlling the trait. Identifying the actual gene is particularly important to develop molecular markers that can be used to screen for the presence of the gene long before the effects of the gene can be visually observed. Using restriction fragment length polymorphism (RFLP) markers in an $F_2$ mapping population developed from a IP19283 x Tift 238D1 cross, the $d_1$ and $d_2$ plant height loci were mapped as morphological traits in pearl millet linkage group 1 (LG1) and linkage group 4 (LG4), respectively (Azhaguvel et al. 2003). QTLs for plant height were detected in parallel on respective linkage groups.

Genetic mapping using large population and haplotype analysis of three tall and three dwarf inbred lines delineated the $d_2$ region in the genome (Parvathaneni et al. 2013). Comparative analysis defines the region in sorghum genome 410 kb with 40 annotated genes. One of the sorghum genes annotated within this region is $ABCB_1$, which encodes a P-glycoprotein involved in auxin transport. This gene had previously been shown to underlie the economically important $dw_3$ dwarf mutation in sorghum. The co-segregation of $ABCB_1$ with the $d_2$ phenotype, its differential expression in the tall inbred ICMP 451 and the dwarf inbred Tift 23DB, and the similar phenotype of stacked lower internodes in the sorghum $dw_3$ and pearl millet $d_2$ mutants suggest that $ABCB_1$ is a likely candidate for $d_2$. ATP binding cassette

subfamily B member 1 (*Abcb$_1$*) gene that plays a role in polar auxin transport, was identified as the likely candidate underlying $d_2$ in pearl millet (Parvathaneni et al. 2013). The *ABCB$_1$* gene encodes a P-glycoprotein and underlies dwarfing traits in maize ($br_2$), sorghum ($dw_3$), and pearl millet ($d_2$) displayed considerable variation in intron composition (Parvathaneni et al. 2017). Physiological analysis confirmed that the gene affects the downward transport of auxin. If this gene is on, the auxin flows freely, and millet will grow to its full height, about 3 m. If it is off, the millet plant may only grow 1–1.5 m in height.

In addition to the five dwarfing genes, 13 dwarf plants were identified from world collection (Appa Rao et al. 1986). $F_2$ from the cross between three (IP 8056, IP 8210 and IP 8214) and tall inbred showed continuous variation for plant height suggesting that dwarfness was controlled by more than one gene. Only two of the 10 crosses with either $d_1$ (Tift 238) or $d_2$ (Tift 23 DB) dwarfs, produced tall $F_1$ hybrids and they segregated for height in $F_2$ indicating that these two new dwarfs were non-allelic to $d_1$ and $d_2$. Reciprocal crosses of these two dwarfs produced tall $F_1$ hybrids and showed a dihybrid segregation of 9:3:4 in $F_2$ indicating that the dwarfing genes of these two parents were non-allelic to each other. These non-allelic dwarfs were recognized as new sources and assigned the gene symbols $d_3$ (IP 10401), and $d_4$ (IP 10402).

## 6.3 Genetic Resources of Climate-Smart Genes

The genus *Pennisetum* consists of approximately 140 highly diverse species (Brunken 1977). The genus is a heterogeneous assemblage of species with four different basic chromosome numbers ($n$ = 5, 7, 8 and 9) (Jauhar 1981); ploidy levels ranging from diploid to octoploid, both sexual and apomictic reproductive behaviors, and annual, biennial or perennial life cycles (Martel et al. 1997). Centered on morphological differences the genus is divided into five sections, namely *Gymnothrix* (P. Beauv.) Benth. & Hook. f., *Brevivalvula* Doll, *Heterostachya* Stapf & C. E. Hubb., *Eupennisetum* Stapf, and *Penicillaria* (Willd.) Benth. & Hook. f. (= *Pennisetum*) (Stapf and Hubbard 1934).

### *6.3.1 Gene pool*

Genetic diversity studies in *Pennisetum* recognized three gene pools and delineated as primary, secondary, and tertiary gene pools. These genepools were first proposed based on genetic relationship among the species (Harlan and De-Wet 1971). Later, these gene pools were identified on the basis of crossability and cross fertility of the wild species with the domesticated diploid cultivated *P. glaucum* and gene transfer complexity between genepools. Some of the sections classified under secondary and tertiary genepools have different life cycle, mode of reproduction, and/or basic chromosome numbers (Table 6.2).

**Table 6.2** Life cycle, reproductive behavior, and chromosome numbers of few species of the genus Pennisetum classified under primary, secondary, and tertiary genepools

| Genepool/section/ species | Life cycle | Mode of reproduction | Chromosome | | References |
|---|---|---|---|---|---|
| | | | (*2n*) | x | |
| *Primary genepool* | | | | | |
| Section Pennisetum | | | | | Jauhar (1981), Martel et al. (1996), Jauhar and Hanna (1998), Robert et al. (2011) |
| *P. glaucum* ssp. *glaucum* | a | Sx | 14 | 7 | |
| *P. glaucum* ssp. *Monodii* | | | | | |
| Ecotype 1. *P. violaceum* | a | Sx | 14 | 7 | |
| Ecotype 2. *P. mollissimum* | a | Sx | 14 | 7 | |
| *Secondary genepool* | | | | | |
| Section Pennisetum | | | | | Jauhar (1981), Martel et al. (2004), Robert et al. (2011) |
| *P. purpureum* | p | Sx, Ap | 28 | 7 | |
| Section Heterostachya | | | | | |
| *P. squamulatum* | p | Ap | 54, 54 | 7, 9 | |
| *Tertiary genepool* | | | | | |
| Section Brevivalvula | | | | | Jauhar (1981), Martel et al. (1997), Jauhar and Hanna (1998), Martel et al. (2004), Robert et al. (2011) |
| *P. pedicellatum* | a | Ap | 36, 54 | 9 | |
| *P. polystachion* | a/p | Sx, Ap | 18, 36, 54 | 9 | |
| *P. hordeoides* | a | Ap | 36, 54 | 9 | |
| *P. subangustum* | a | Sx, Ap | 18, 36, 45 | 9 | |
| *P. setosum* | a | Sx, Ap | 54 | 9 | |
| Section Gymnothrix | | | | | |
| *P. mezianum* | p | Ap | 32 | 8 | |
| *P. hohenacken* | p | Sx, Ap | 18 | 9 | |
| *P. alopecuroides* | p | Sx | 18 | 9 | |
| *P. ramosum* | a, b | Sx, Ap | 10 | 5 | |

Life cycle: a = annual, b = biannual, p = perennial; Reproduction: Sx = Sexual, Ap = apomictic

#### 6.3.1.1 Primary Gene Pool

The primary gene pool includes all forms of cultivated, weedy, and wild diploids ($2n = 2x = 14$). Pearl millet is a diploid with seven pairs of homologous chromosomes ($2n = 2x = 14$), have annual growth habit. Harlan and De-Wet (1971) classified that the cultivated *P. glaucum* and the wild relative *P. glaucum* ssp. *monodii* are the two members of the primary genepool. *P. violaceum* and *P. mollissimum* are currently considered as the two ecotypes of subspecies *monodii,* and are the wild diploids ($2n = 2x = 14$) also belonging to the primary gene pool.

Cytogenetic investigation of these wild relatives along with the cultivated form of this gene pool have shown high similarity between their karyotypes (Khalfallah et al. 1993). And in situ hybridization analysis confirmed similar localization of rDNA among members of the primary gene pool (Martel et al. 1996) that substantiated their conserved high level of genome similarity with the cultivated pearl millet.

On the other hand, *P. schweinfurthii* Pilger is another diploid species that have $2n = 2x = 14$ chromosomes and annual in growth habit (Hanna and Dujardin 1986). Its chromosomes were also reported to be similar in size as those of pearl millet, but non-homologous. Inter-specific hybrids between *P. schweinfurthii* and pearl millet were morphologically intermediate to both species. They were also found as male sterile and partially female sterile. In Sahelian West Africa, the intermediate weedy form, *P. stenostachyum* is widely found.

Enzyme polymorphism study in the *Pennisetum* gene pool reported variations in leaf esterases (EST), 6-phosphogluconate dehydrogenase (PGD), shikimate dehydrogenase (SKDH), leucine aminopeptidase (AMP), phosphoglucomutase (PGM) and malate dehydrogenase (MDH) genes (Lagudah and Hanna 1989). In the primary gene pool, two loci, *Est-1* and *Est-2*, were identified to controlling leaf esterases.

#### 6.3.1.2 Secondary Gene Pool

The secondary pool solely consisted of the tetraploid *P. purpureum* (Shum.) ($2n = 4x = 28$). *P. purpureum* is a fast-growing perennial grass native to the African grasslands. Pearl millet (*P. glaucum*) with AA genome and *P. purpureum* (Napier grass or elephant grass) with A'A'BB genomes are the two economically important species of the genus *Pennisetum*. It is widely grown in tropical and subtropical regions of the world as one of the most important tropical forage crops. The genetic proximity between *P. glaucum* and *P. purpureum* enables triploid hybrids ($2n = 3x = 21$) that yield higher quality forage from both species. Conventional cytogenetic techniques revealed the presence of homeology between the genomes A and A', and with the genome B (dos Reis et al. 2014). Genomic in situ hybridization (GISH) confirmed the homeology between the genomes A of pearl millet and A'B of Napier grass, and showed that there are differences in the distribution and proportion of homologous regions after hybridization (dos Reis et al. 2014).

These days, the apomictic octaploid *P. squamulatum* ($2n = 8x = 56$) is considered as the member of secondary gene pool (Pattanashetti et al. 2016). The perennial *P. squamulatum* is stress tolerant and can be utilized for pearl millet or Napier grass improvement. Genome similarity, crossability, genetic relatedness, and cytology of hybrids supported the placement of *P. squamulatum* in secondary genepool (Kaushal et al. 2008).

#### 6.3.1.3 Tertiary Gene Pool

The broad tertiary gene pool comprises *Pennisetum* species of various ploidy levels and growth habits (Dujardin and Hanna 1989) that are distantly related to primary and secondary genepools. This comprises many among the approximately 140 diverse species of the genus. Gene flow from the tertiary genepool to pearl millet is limited because of strong reproductive barrier with the primary and secondary genepools. The species have either annual or perennial growth habit and have economic importance as a forage, ornamental, or landscaping purposes.

The whole section Brevivalvula belongs to the tertiary genepool. It consists of six morphological taxa: *P. atrichum* Stapf & Hubb., *P. hordeoides* (Lam.) Steud., *P. pedicellatum* Trin., *P. polystachion* (L.) Schult., *P. setaseum* (Swartz) L. Rich., and *P. subangustum* (Schum.) Stapf & Hubb., which together form a polyploid and agamic complex. Four euploid ($x = 9$) and twelve aneuploid chromosome levels have been reported so far. The polyploids are apomictic, while the diploid populations of *P. polystachion* and *P. subangustum* are considered sexual.

### *6.3.2 Races*

Pearl millet is categorized under the section *Pennisetum*. Based on grain shape which follows geographic pattern, the world collection of cultivated pearl millet were classified into four races (*typhoides, nigritarium, globosum,* and *leonis*) (Brunken et al. 1977). Pearl millet domestication has produced many landraces displaying a broad diversity in environmental adaptation, cycle length, morphological traits, genetic yield potential, stresses tolerance, and grain quality characteristics.

Race *typhoides* is mainly cultivated in India and characterized by obovate caryopses that are obtuse and terete in cross section. Inflorescences are mostly cylindrical in shape. Morphologically it is the most variable among the four races and is also most widely distributed. It occurs in entire Africa. It is the only basic race found outside Africa and the predominant race grown in India.

Race *nigritarum* caryopsis is angular in cross-section with three and six facets per grain. Inflorescences are candle-like. The apex of the grain is usually truncate and often tinged purple. The mature grain is generally longer and protrudes beyond the floral bracts. This race is generally found in western Sudan to northern Nigeria (Brunken et al. 1977). Race *nigritarum* is dominantly grown in eastern Sahel.

Race *globosum* is dominantly grown in western Sahel and has spherical shaped caryopsis with each of its dimensions being approximately equal. Depth of the grain always exceeds 2.4 mm. The grain is otherwise terete and obtuse. Inflorescences are candle shaped and often exceed 1 m in length. It is the most common race in central Nigeria, Niger, Ghana, Togo, and Benin (Bono 1973).

Race *leonis* is dominant in coastal regions of West Africa and characterized by an acute, oblanceolate, and terete caryopsis. The most distinct character of the leonis grain is its acute apex, which is terminated by the remnants of the stylar base.

At maturity, approximately one-third of the grain protrudes beyond the floral bracts. Inflorescence shape is candle-like. It is specific to Sierra Leone but also grown in Senegal and Mauritania (Brunken et al. 1977).

## 6.4 Genetic Diversity

Genetic diversity is a reflection of a variety of genes in a given species which are important for survival of natural selection, for tailoring adaptation to a changing environment, and conservation of desired traits. Genetic diversity occurs as an outcome of mutations, recombination, genetic drift, migration, and selection. Natural and human selection processes over thousands of years have generated diverse cultivars of pearl millet adapted to different biophysical conditions, suited to various production systems and socio-economic conditions, and well-matched various consumer preferences (Brunken 1977).

### *6.4.1 Phenotypic Diversity*

Different phenotypic traits such as flowering time, panicle length, grain and stover characteristics, grain nutritional composition, and tolerance to biotic and abiotic stresses in cultivated pearl millet (Bhattacharjee et al. 2007; Amadou et al. 2013) have been used to study the genetic diversity at different times. A study conducted in Nigeria on 25 pearl millet genotypes collected for diverse morphological variation showed that farmers' husbandry practice resulted in the isolation of ideotypes, making landrace names tradeoff for genetic diversity (Danjuma and Mohammed 2014). The results suggested that artificial selection had greater influence in shaping the population than environmental factors. ICRISAT pearl millet breeding program at India has developed diverse range of seed (A-/B- pairs) and restorer parents (R-lines) utilizing diverse sources of germplasm, and characterized 99 A-/B- pairs and 114 restorer parents based on 26 morphological traits (Rai et al. 2009).

Besides per se performance and geographic origin, selection of parents based on genetic diversity yields significant yield improvement. A very recent study into the effect of genetic distance of inbred parents on heterosis of the hybrid reported positive correlation between phenotypic distance of parents and better-parent heterosis for grain yield (Gupta et al. 2018). But the correlation was not strong enough to be used as a major selection criterion for parental selection for heterosis breeding to improve grain and stover yield.

The wide range of climatic conditions in the center of diversity and farmers preferences and utilization habits created landraces with local adaptation that maintained broad genetic variability. The prominent early-maturing and productive landrace from West Africa, Iniadi, contributed desirable traits towards genetic improvement of pearl millet (Andrews and Kumar 1996). The traits contributed by

Iniadi included adaptation, productivity and grain nutritional quality. However, the genetic control and heritability of the nutritional composition of the grain need further investigation for effective bio-fortification with essential micronutrients.

A study attempted to quantify the degree of diversity in 122 commercial hybrids of pearl millet cultivated in India and to understand the relationship among various phenotypic and quality traits, showed large variation for flowering time (42–58 days), tillering (1.1–4.4 panicles/plant), individual grain size (7.6–17.3 mg), plant height (185–268 cm), panicle length (20–33 cm) and grain yield (3.5–7.2 tons $ha^{-1}$). Hybrids resulted in 7 distinct clusters, which highlighted the successful efforts of Indian national program of pearl millet improvement toward genetic diversification of hybrids (Yadav et al. 2016).

### 6.4.2 Genotypic Diversity

Analysis of molecular variation within and between Indian landraces (Bhattacharjee et al. 2002) and among inbred lines derived from WCA landraces (Stich et al. 2010) revealed more than two-fold variation between than within landraces. Population structure analysis among the later, categorized the landraces into five subgroups depicting the diversity of West African germplasm.

Genotypic diversity investigated on 213 parental lines (99 seed and 114 restorer parents) of pearl millet indicated significant diversity (Nepolean et al. 2012). Analysis of 379 hybrid parents developed at ICRISAT (current 166 parents and 213 previously developed hybrid parents) carried out using SSRs detected 12.7 alleles per locus. Also, the seed and restorer parents were clearly separated from each other, indicating existence of two diverse and broad-based pools in hybrid parents of pearl millet (Gupta et al. 2015a). Restorers (R-lines) were found more diverse than seed parents (B-lines), as higher average gene diversity was detected among R-lines (0.70) than B-lines (0.56).

Genetic diversity analysis in pearl millet inbred germplasm association panel (PMiGAP) representing cultivated germplasm from Africa and Asia, elite improved open-pollinated cultivars, hybrid parental inbred lines and inbred mapping population parents showed an average gene diversity of 0.54 and six subpopulations within PMiGAP (Sehgal et al. 2015). The PMiGAP panel along with several other lines were also studied for genome wide diversity (Varshney et al. 2017).

### 6.4.3 Relationship with Other Cultivated Species and Wild Relatives

Pennisetum sect. Penicillaria is a morphologically diverse taxon native to tropical Africa. Following morphological and biosystematic evidence the section is divided into two biological species. *Pennisetum purpureum* Schmach. is a tetraploid,

perennial species of the wet tropics. *Pennisetum americanum* (L.) K. Schum., a native of the arid and semi-arid tropics, is annual and diploid and is segmented into three sub-species each having different adaptations to domestication. Sub-species monodii, a wild taxon from the Sahel of West Africa, is identified as the progenitor of pearl millet. Subspecies stenostachyum is morphologically intermediate between subsp. americanum and monodii and includes the mimetic weeds often found in association with pearl millet. A genetic diversity and species relationship study among eight wild species of the genus *Pennisetum* including pearl millet using RFLP markers revealed wide variation among the species (George et al. 2005).

### *6.4.4 Relationship with Geographical Distribution*

There are many landraces (varieties named by farmers) grown in different ecological niches by small-scale farmers, but the role of sociological factors in the evolutionary dynamics of the crop is not well understood. A study assessed the connection between ethno-linguistic diversity and extent genetic diversity in pearl millet grown in the western side of the lake Chad in Central Africa revealed the existence of a genetic structure concomitant with ethno-linguistic differences (Naino Jika et al. 2017). On the other hand, a high seed and pollen-mediated gene flow among different pearl millet growing regions of Sudan was inferred as molecular variation of pearl millet accessions within the regions was much higher than among the regions (Bashir et al. 2015). As there are no linguistic barriers among the regions, the theory of more seed exchange between villages of the same language group contributed for the variation. This outcome suggests that gene flow is limited between landraces grown by different ethno-linguistic groups probably because of language barriers that limit seed exchange among the farmers' communities. Irrespective of its outcrossing nature, the lack of genetic exchange among the landraces might have also been contributed by physical delineations along the linguistic differences.

This type of strong concurrence between ethno-linguistic boundaries and genetic discontinuities was also observed for cassava (Delêtre et al. 2011) signifying the influence of sociological factors in genetic exchange. Similarly, population genetic structure was also coincided with the main language families for sorghum landraces in Africa (Westengen et al. 2014). A genetic diversity study on maize landraces grown by different ethno-linguistic groups in southern Mexico is emphasizing the role of farmers' local selection in influencing genetic structure rather than environmental factors (Perales et al. 2005).

On the other hand, geographic distance from the center of diversity was associated with high genetic divergence suggesting a major effect of isolation by distance on divergence (Hu et al. 2015). Moreover, adaptation to a new environment can bring novel variations as a result of ecological opportunity and competition

(Andrew et al. 2010). But, the genetic basis of environmental adaptation in pearl millet has not been well understood.

### 6.4.5 *Extent of Genetic Diversity*

Understanding the extent of genetic variability available and bio-geography of genetic resources have paramount importance for optimal improvement and conservation of pearl millet. Moreover, it is an indispensable raw material for success in breeding new cultivars for improved yield, quality, and stress tolerance. Hence, valid estimation and thorough understanding of the array of genetic diversity present in pearl millet would have practical application in breeding for different objectives. Assessment of genetic variability permits to identify contrasting parental materials to enhance hybrid vigor and yield stability in variable climates (Haussmann et al. 2012).

To broaden the extent of genetic variability available to breeders, ICRISAT was engaged in germplasm collection and characterization. By the year 2007, ICRISAT collected more than 20,844 cultivated pearl millet accessions and 750 wild relatives through 76 collection missions in 28 countries (Upadhyaya et al. 2007). Most of these collections were made from the center of diversity, WCA. To facilitate identification of potential parents for a genetic improvement program, a core collection comprising of 2094 accessions ($\sim$10% of the entire collection) were identified using data on 11 quantitative traits (Bhattacharjee et al. 2007). Then, a mini-core collection comprising 238 accessions (1.1% of the collection) was established following proportional sampling strategy, using the same 11 qualitative and 8 quantitative traits data on the accessions (Upadhyaya et al. 2011). This stratified information about the ICRISAT collection warrants a persistent availability of germplasm for further improvement of pearl millet mainly in India.

## 6.5 Association Studies

Dissecting marker–trait relationship using allelic variations accumulated through historical recombination events in natural populations is encouraged for QTL mapping and candidate gene identification. While conventional linkage mapping using bi-parental populations has identified a number of important quantitative traits in pearl millet, the resolution provided by two parent-derived mapping populations is severely limited. Some efforts of association mapping made in pearl millet created valuable information about genetic variability and linkage disequilibrium (LD). It is believed that a good understanding and identification of underlying genes, alleles or QTLs for stress responsiveness and adaptation traits may facilitate breeding for increased climate-resilience (Kole et al. 2015).

### 6.5.1 Extent of Linkage Disequilibrium

Genes that control phenotypic traits can be identified with high resolution using association studies based on LD. However, LD patterns across the pearl millet genome have not been systematically studied yet. Periodic studies targeting a certain genome region or genes have been conducted. From the available information, a phenomenon of rapid decay of inter-loci and intra-locus LD (Hu et al. 2015; Varshney et al. 2017) is observed.

### 6.5.2 Target Gene-Based LD Studies

Several target gene or genome region based assessment of LD was conducted in pearl millet. Assessment of the extent of LD among 1,575 pairs of loci mapped on $LG_2$ in a total of 250 inbred lines of PMiGAP found that a total of 441 (28%) of the marker pairs were in LD ($P < 0.01$) (Sehgal et al. 2015). But, when LD was calculated separately within each of the six sub-populations formed by population structure analysis, the frequency of pairs of loci with significant LD ($P < 0.05$) was reduced by more than half.

The extent of LD in *PgD8*, a gene in gibberellic acid (GA) pathway and conditioning photoperiod in pearl millet was found lower than that of the homologous maize D8 (Li et al. 2018). Likewise, the effect of selection on LD in the vicinity of Phytochrome C gene (*PHYC*) was assessed with a panel of 90 pearl millet inbred lines using 75 markers in 100-kb region identified the best candidate markers on the PHYC gene (Saïdou et al. 2014). However, in genomic regions containing polymorphisms for genes that have been targeted for selection, it has been speculated that LD may extend over a relatively long distance (Saïdou et al. 2009).

Attempt of mining favorable alleles for grain Fe and Zn content through association mapping in pearl millet identified three SSR markers consistently associated with elevated Fe and Zn with more than 11% coefficient of determination ($r^2$-value) (Anuradha et al. 2017). As Fe plays a role in the production of oxygen-carrying proteins hemoglobin and myoglobin its deficiency creates health and developmental problems in the developing world. Lack of sufficient Fe in nutrition can lead to different forms of anemia.

### 6.5.3 Genome-Wide LD Studies

A characterization of two pearl millet diversity panels using genotyping by sequencing (GBS) of *PstI-MspI* reduced representation libraries identified 83,875 single nucleotide polymorphisms (SNPs) (Hu et al. 2015). In this study much faster LD decay in Senegalese landraces compared to global accessions. Rapid LDD of

0.5 kb in B- and R- lines (48 bp) as well as in PMiGAP lines (84–444 bp) was also observed using genome-wide SNPs detected through resequencing (Varshney et al. 2017). This indicated that LD in pearl millet is similar with that of maize, which is also an allogamous species.

In the model species foxtail millet nonlinear regression showed rapid decline of LD with distance and rapid LD decays to half the initial value within ca. 1.2 kb (He et al. 2015). Similarly in maize, LD decayed rapidly with distance between sites within loci, but there was substantial variation among genes (Remington et al. 2001). In this study, predicted $r^2$ values declined to less than 0.1 within 2,000 bp in two-third of the loci.

### 6.5.4 *Potential of Association Studies for Germplasm Enhancement*

Diversity in plant genetic resources (PGR) provides opportunity for plant breeders to develop new and improved cultivars with desirable traits such as genetic yield potential, biotic stress tolerance, environmental adaptation, and quality attributes. Genetic diversity within and between plant populations is usually assessed using morphological, biochemical (allozyme), and molecular marker (DNA) analysis.

In this postgenomic era, abundant single nucleotide polymorphism (SNPs) and the affordability genotyping stimulated the application of genomic-assisted breeding and efficient utilization of genetic diversity for crop improvement. Association mapping, also known as linkage disequilibrium (LD) mapping, utilizes ancestral recombination events in germplasm collections or natural populations to make marker-trait associations attributed to the strength of linkage disequilibrium between markers and functional polymorphisms across a set of diverse germplasm (Zhu et al. 2008). It also offers an alternative means of allele mining by enabling to survey a much larger and diverse genepool having thousands of recombination events, lending high mapping resolution, and potential to identify the causal polymorphism within a gene and/or QTL.

The existence of tremendous phenotypic variability is documented in pearl millet germplasm for many agronomic traits (Bhattacharjee et al. 2007). In spite of the extent of genetic variability available, only a small fraction of the gene pools are used for developing pearl millet varieties and hybrids. This limited utilization of the available germplasm is probably because of lack of access to the resource by the breeders, limited preservation or lack of systematic documentation of the genetic diversity. In pearl millet improvement, utilization of wild relatives as donors of specific traits such as apomixes (Hanna 1987), or resistance to pests and diseases (Wilson et al. 2004) is also scarce. Further, most of the allele mining for agronomically important traits including biotic and abiotic stress resistance has been conducted using bi-parental mapping populations (Yadav et al. 2002, 2004; Bidinger et al. 2007; Sehgal et al. 2012; Vengadessan et al. 2013).

For a suburb germplasm conservation and genetic improvement of pearl millet, understanding the genomic diversity, population structure of the germplasm, and association mapping are the important areas to be assessed. The recent release of genome sequence is expected to facilitate association mapping and genome selection in pearl millet. GWAS across 288 testcross progenies of PMiGAP was also conducted for 20 grain yield and stover yield component trait and identified 1054 strongly significant marker–trait association for 15 of the traits (Varshney et al. 2017). A study of genomic diversity, population structure, and linkage disequilibrium conducted on 398 accessions from different geographic regions provided a noteworthy insight (Serba et al. 2019). Data analysis using a total of 82,112 genome-wide SNPs discovered through GBS revealed hierarchical genetic structure of six subgroups that mostly overlap with the geographic origins (west Africa, east Africa, Southern Africa, the Middle East, India, and lines developed in USA). The result also confirmed that germplasm from west Africa rooted the dendrogram of the phylogeny analysis with much diversity and greater LD decay, indicating a long history of recombination among landraces. Assessment of genetic differentiation between population subgroups and genome-wide patterns of nucleotide variation within each subpopulation indicated that the Indian subpopulation is less differentiated from all other subpopulations ($F_{ST} = 0.006$). This is probably attributed to the strong breeding program led by ICRISAT that utilized diverse germplasm. On the contrary, the Middle East subpopulation was relatively highly differentiated from all others (average $F_{ST} = 0.072$), followed by the inbred lines from US breeding programs ($F_{ST} = 0.060$). The later indicates the limited germplasm utilization by the US breeding program. It is also discovered that the lowest average nucleotide diversity ($\pi = 4.23 \times 10^{-4}$) was found in the inbred lines from the United States as compared to the average genome-wide nucleotide diversity for the whole population ($\pi = 5.0 \times 10^{-4}$).

## 6.6 Molecular Mapping of Genes and QTLs

The application of genomics has become an indispensable component of breeding programs and proven to be useful for identifying novel genes for traits of agronomic importance and stress tolerance. Marker assisted-selection (MAS) has a great practical importance to facilitate gene introgression into desirable genetic backgrounds and crop improvement by shortening the lengthy phenotypic evaluation and by increasing selection accuracy.

Genomic research for pearl millet is however lagging behind the major cereals such as rice, maize, wheat, and sorghum. It is a poor man's crop and the public and private investment towards the improvement of the crop is limited. As a result, addressing the genetic yield barriers with the help of next-generation sequencing (NGS) technology and use of available germplasm is inadequate. Nevertheless, with the continuous efforts of ICRISAT and few national programs especially India, UK, some basic genomic information has become available. Most recently, pearl

millet genome sequence has been released for public use (Varshney et al. 2017) as a reference for further development of genomics-assisted breeding. This genome sequence, provides a genetic blueprint of the species and apparently facilitates the development of genomic tools that would expedite the breeding process and improve selection gains through the application of marker-assisted breeding.

### *6.6.1 Brief History of Mapping*

Pearl millet is one of the orphan crops with minimum public investment in the research and development of the crop. Compared to other cereals such as rice, sorghum, maize, wheat, and barley, research on the development and application of molecular markers is limited in pearl millet. However, development of molecular markers for the improvement of pearl millet as a potential crop for the hot and dry environments was started in 1991 (Gale et al. 2005). As a result, the first genetic linkage map was constructed using RFLP earlier than other orphan crops (Liu et al. 1994). Linkage groups corresponding with the seven haploid chromosome number of the species were successfully formed and this RFLP based map was used as the basis for subsequent pearl millet marker-based studies. Further marker development and mapping efforts utilized different marker systems and mapping populations.

The first pearl millet genetic linkage map constructed using RFLP markers served as a foundation for subsequent mapping using polymerase chain reaction (PCR) based markers and currently using the high throughput SNP markers. Then, significant efforts have been made in developing other molecular markers, mapping the genome, trait-specific QTL and genes in pearl millet. Development of genetic linkage maps using RFLP, simple sequence repeats (SSR), and single nucleotide polymorphism (SNPs), identification of QTL for drought tolerance, downy mildew resistance, grain quality traits, and genes for plant height are among the molecular resources have become available for pearl millet breeding.

A genetic linkage map from four different crosses have been integrated to develop a consensus map of 353 RFLP and 65 SSR markers where extreme localization of recombination toward the chromosome ends that affect transfer of genes controlling important agronomic traits from donor to elite pearl millet germplasm (Qi et al. 2004). To provide improved genome coverage, pearl millet genetic linkage map was integrated with diversity arrays technology (DArT) and SSR markers following PstI/BanII complexity reduction digestion (Supriya et al. 2011). A total of 321 loci (258 DArTs and 63 SSRs) that spanned 1148 cM with an average adjacent-marker interval length of 3.7 cM was constructed.

PCR-based linkage map was constructed on the basis of a recombinant inbred line (RILs) population resulting from a cross between Tift 23DB and PI 536400 (Pedraza-Garcia et al. 2010). The objective of this mapping was to obtain more evenly distributed markers throughout the linkage map and improve genome coverage of the gaps in earlier maps characterized by high concentration of markers in the centromere region than the distal regions of the linkage groups. An average

marker density of per 9.2 cM was achieved with 196 PCR-based DNA markers (66 sequence-related amplified polymorphisms (SRAPs), 63 random amplified polymorphic DNA (RAPDs), 27 inter-simple sequence repeats (ISSRs), 31 pearl millet, 6 sorghum, and 3 maize SSRs) involving 152 recombinant inbred lines (RILs) but mapped into nine linkage groups.

### 6.6.2 Evolution of Marker Types: RFLP to SNPs

After the construction of the first linkage map using RFLP, development and application of other marker systems continued. Construction of a bacterial artificial chromosome (BAC) library using nuclear DNA of pearl millet contains a total of 159,100 clones with an average insert size of 90 kb and corresponding to 5.8 haploid genome equivalents (Allouis et al. 2001) was used as a resource for the isolation of SSR sequences. PCR-based screening of 4.7 haploid genome equivalents using five sequence-tagged site (STS) and six SSR markers identified an average of 5.4 positive superpools.

With the development of PCR, a program at ICRISAT has developed 100 SSR markers and mapped 60 of them (Gale et al. 2005). That facilitated the integration of the pearl millet map in the grass consensus map and subsequently the establishment of the plant genome database MilletGenes. This database provided genome related data (maps, markers, DNA sequences, and images) on pearl millet, finger millet (*Eleusine coracana*), foxtail millet and tef (*Eragrostis tef*) (http://jicbio.bbsrc.ac.uk/cereals, Accessed on 18 August, 2018). Probe information, end-sequences of RFLP probes, RFLP and STS polymorphism data, autoradiograph and gel images, segregation data, genetic maps, QTL data, and morphological data were used to be deposited in this database.

SNP marker development was first conducted using rice genome as a reference (Bertin et al. 2005). Using pearl millet expressed sequence tags (ESTs) and annotated rice genomic sequences, 299 homologues of single-copy rice genes with precise prediction of the intron positions were identified as single-strand conformational polymorphism (SSCP)-SNPs. Analysis of the fragments amplified by PCR primers designed to amplify approximately 500-bp genomic fragments on SSCP gels revealed considerable polymorphism. Further sequence analysis of the fragments over a panel of eight inbred genotypes estimated about one SNP or InDel (insertion-deletion) every 59 bp in the introns, but considerably fewer in the exons.

With the application of NGS, a large number of SNPs markers were developed and applied in mapping and QTL identification (Sehgal et al. 2012; Moumouni et al. 2015; Punnuri et al. 2016), population genomics and genome-wide association mapping (Hu et al. 2015; Sehgal et al. 2015). Application of GBS is proved to be a quick and low cost marker development and genotyping platform. With the current availability of the genome sequence, this approach has a great potential in characterizing the genome and mapping of important traits.

### 6.6.3 Mapping Populations

Genetic linkage maps are necessary for applied genetics and marker-assisted breeding of pearl millet. To this end, significant efforts have been made in constructing genetic linkage maps, improving the genome coverage, marker density, and integrating the map for better resolution. The first linkage map based on RFLP marker used an inter-varietal $F_2$ population (Liu et al. 1994). The first integrated map was constructed using four $F_2$ populations developed from LGD x ICMP 85410, 81B x ICMP 451, ICMB 841 x 863B, and PT 732B x P1449-2 crosses (Qi et al. 2004). The $F_2$ population of the ICMB 841-P3 x 863B-P2 cross was also used to integrate a newly developed SSR markers in previous maps (Senthilvel et al. 2008).

Subsequently, RILs were used for genetic linkage mapping of pearl millet genome. RILs developed from Tift23DB x PI536400 cross (Pedraza-Garcia et al. 2010), H 77/833-2 x PRLT 2/89-33 (Supriya et al. 2011), H77/833-2 x PRLT 2/89-33 (Sehgal et al. 2012) crosses were used for genetic linkage mapping. Four RILs populations developed from ICMB 841-P3 x 863B-P2, H 77/833-2 x PRLT 2/89-33, 81B-P6 x ICMP 451-P8, and PT 732B-P2 x P1449-2-P1 were used to construct a consensus linkage map (Rajaram et al. 2013). An $F_2$ population of a cross between wild pearl millet (116_11-(PS202-14)-121) and a cultivated pearl millet (SOSAT-IBL-197) was also used to map high density map (Moumouni et al. 2015). To circumvent the problems of segregation distortion and masking of minor-effect alleles/QTLs, a set of chromosome segment substitution lines (CSSLs) for all the seven LGs was developed by introgression of overlapping chromosome segments from 863B line into the genetic background of elite ICMB 841 line (Kumari et al. 2014). These are valuable genetic stocks for minor-effect QTL detection, fine mapping, and trait mechanism studies, especially for complex traits in pearl millet.

The second-generation mapping populations such as nested-association mapping (NAM) and multi-parent advanced generation inter-cross (MAGIC) populations are derived from multiple elite breeding lines with a combination of useful traits. These populations are useful for precise QTL mapping and for use in cultivar development. However, their development in pearl millet is not yet emphasized.

### 6.6.4 Genetic Linkage Maps

Obviously, the marker system and speed of genotyping evolved over time. Much improvement has been made in genome coverage, marker density, and resolution since the first map of pearl millet (Table 6.3). The coverage is more than double and the density and distribution of markers dramatically increased. For comparison, the first RFLP based (Fig. 6.6a) and the recent haplotype based (Fig. 6.6b) maps are presented below.

**Table 6.3** List of genetic linkage maps of pearl millet constructed using different populations and marker systems

| Population and cross | Marker system | Loci mapped | Total length (cM) | Marker density (cM) | References |
|---|---|---|---|---|---|
| $F_2$ LGD x ICMP 85410 | RFLP | 181 | 303 | 2 | Liu et al. (1994) |
| RILs (152) Tift23DB x PI536400 | RAPD, SRAP, ISSR, SSR | 196 | 1796 | 9.2 | Pedraza-Garcia et al. (2010) |
| $F_2$ (four) LGD x ICMP 85410, 81B x ICMP 451, ICMB 841 x 863B, PT 732B x P1449-2 | RFLP, SSR | 242[a] | 473 | 2 | Qi et al. (2004) |
| F2 (149) I CMB 841-P3 x 863B-P2 | SSR | 27[b] | | | Senthilvel et al. (2008) |
| RILs (140) H 77/833-2 x PRLT 2/89-33 | DArT, SSR | 321 | 1148 | 3.7 | Supriya et al. (2011) |
| RILs (88) H77/833-2 x PRLT 2/89-33 | EST-SSR, SNP, CISP | 133 | 815.3 | 6.1 | Sehgal et al. (2012) |
| RILs (four) ICMB 841-P3 x 863B-P2, H 77/833-2 x PRLT 2/89-33, 81B-P6 x ICMP 451-P8, PT 732B-P2 x P1449-2-P1 | EST-SSR, gSSR, STS | 174 | 899 | 5.2 | Rajaram et al. (2013) |
| $F_2$ (93) 116_11-(PS202-14)-121 x SOSAT-IBL-197 | SNP | 314[c] | 640 | 2.1 | Moumouni et al. (2015) |
| RILs (150) Tift 99DB x Tift 454 | SNP | 16,650 | 716.7 | 2.1 | Punnuri et al. (2016) |

[a]The information presented is for the consensus map; [b]Newly developed SSR markers integrated in previous maps (Yadav et al. 2004; Bidinger et al. 2007); [c]Non-redundant haplotypes

### *6.6.5 Mapped QTLs*

Prompted by the straight forward diploid genetics and high levels of polymorphism, several QTL controlling important traits in pearl millet have been detected. As drought tolerance is the most important trait of the crop, mapping QTL for terminal drought tolerance (Yadav et al. 2002) has provided a more-targeted approach to

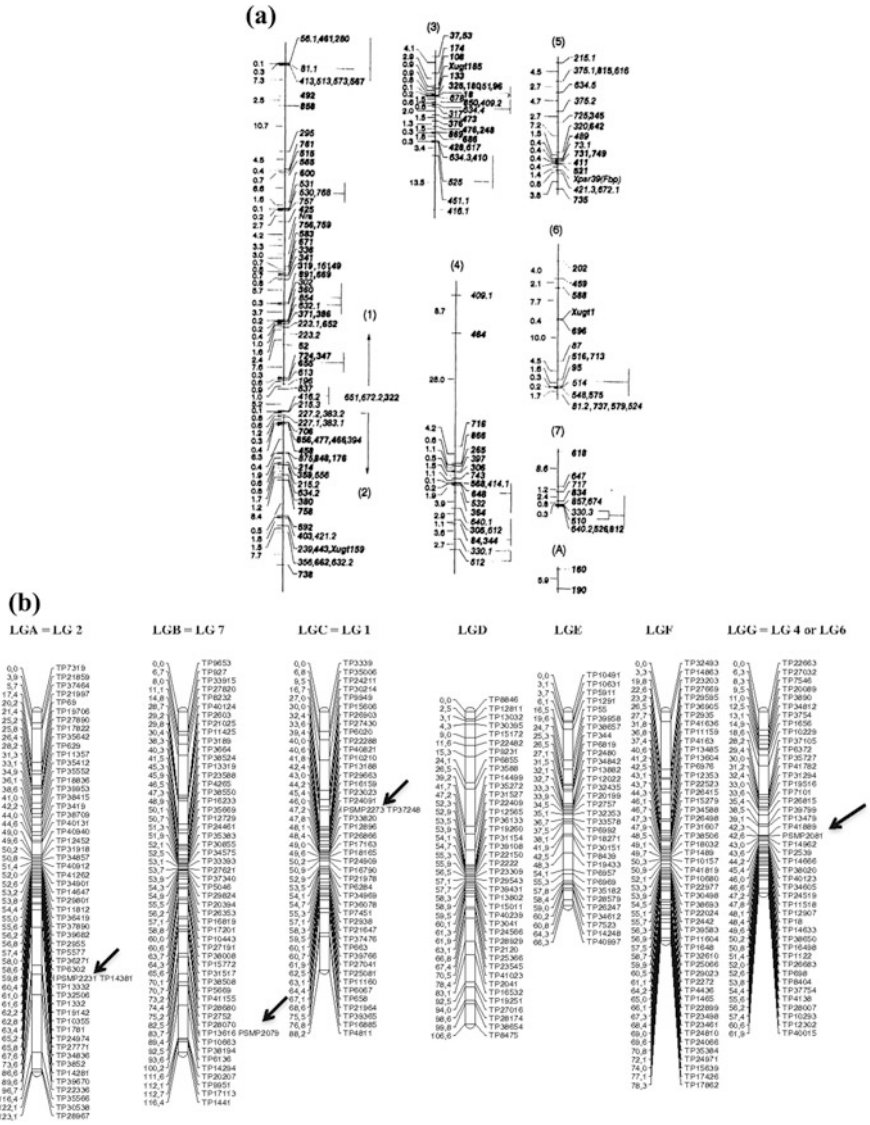


**Fig. 6.6** The first genetic linkage map of pearl millet constructed using RFLP markers in $F_2$ population of LCD-I-B-I0 x ICMP 85410 cross (Liu et al. 1994) (**a**), Haplotype-based genetic map of pearl millet developed using SNP markers developed by genotyping-by-sequencing (GBS) (Moumouni et al. 2015) (**b**) (Adopted with permission from **a** Liu et al. 1994, Theoretical and Applied Genetics 89(4):481–7, and **b** Moumouni et al. 2015, Molecular Breeding 35:5)

improving the drought tolerance and yield in water-limited environments (Howarth and Yadav 2002).

Consequently, some efforts have been made in genome mapping and detection of QTL for specific traits (Table 6.4). With the aim of identifying genes underlying drought tolerance (DT) QTL, facilitate understanding of molecular mechanisms of drought tolerance, and accelerate genetic improvement of pearl millet through MAS, a genetic linkage map based on gene-based markers was constructed (Sehgal et al. 2012). In considerably short period of time, hundreds of pearl millet molecular markers were developed (Liu et al. 1994; Qi et al. 2001; Allouis et al. 2001) and genetic linkage maps were produced (Liu et al. 1994; Devos et al. 2000). These maps became the platform for the detection of QTL for downy mildew resistance (Jones et al. 1995; Jones et al. 2002).

## 6.7 Marker-Assisted Breeding

Valuable genetic improvements have been made to several important traits of pearl millet through conventional breeding. It is presumed that considerable increase in efficiency can be achieved through the application of MAS. Thus far numerous QTL for important traits such as yield and yield components, disease resistance, and drought tolerance have been mapped.

QTL mapping for yield and yield components (Poncet et al. 2000; Yadav et al. 2003), drought tolerance (Yadav et al. 2002), downy mildew (Jones et al. 1995, 2002; Yadav et al. 1999) and rust resistance have been conducted. A major QTL mapped for terminal drought tolerance on linkage group 2 ($LG_2$) (Yadav et al. 2002) was validated through introgression of the QTL region from the donor parent genome to a hybrid parent (Serraj et al. 2005). Similarly, introgression of a downy mildew resistance QTL into an elite parental lines through RFLP-based marker-assisted backcrossing was successfully done (Thakur et al. 2008). Recently there are QTL mapped for different traits using high throughput marker system. This QTL based on high resolution maps and the associated markers are readily available for MAS. It was suggested that development of second-generation mapping population and study of market-trait association studies using high throughput markers will advance the breeding process (Serba and Yadav 2016).

### *6.7.1 Germplasm Characterization and Distinctness, Uniformity, and Stability (DUS)*

It is evident that the extent of genetic variability is the determining factor for plant breeding success. A vast genetic variability is available in pearl millet. Natural and human selection processes imposed since its domestication, resulted in the

**Table 6.4** Major and minor effect QTLs and genes detected for various traits in pearl millet

| Trait | QTL locus/markers | LG | LOD value | PVE/ Effect | References |
|---|---|---|---|---|---|
| Grain yield | *Xpsm592-Xpsm356* | 2 | 6.91 | /−17.27 | Yadav et al. (2003, 2004) |
| | *Xpsm464-Xpsm716* | 4 | 2.32 | /−10.62 | |
| | *Xpsm588-Xpsm514* | 6 | 2.21 | /11.26 | |
| | *Xpsm322-Xpsm2050* | 2 | 6.07 | 33.2/ | |
| | *Xpsmp2064-Xpsm345* | 5 | 2.39 | 16.9/ | |
| Stover yield | *Xpsm295-Xpsm416.3* | 4 | 4.12 | /−21.86 | Yadav et al. (2003), Poncet et al. (2000) |
| | *Xpsm87.1-Xpsm514* | 6 | 4.96 | /23.84 | |
| | *Xpsm322-Xpsm2050* | 2 | 2.82 | 30.0/ | |
| | *Xpsmp2064-Xpsm345* | 5 | 4.36 | 22.7/ | |
| | *Xpsm514-Xwg110* | 6 | 2.86 | 15.5/ | |
| | *Xpsmp2074-Xpsmp2027* | 7 | 6.83 | 40.8/ | |
| Biomass yield | *Xpsm592-Xpsm443* | 2 | 8.22 | /−49.79 | Yadav et al. (2003) |
| | *Xpsm716-Xpsm265* | 4 | 3.84 | /−35.03 | |
| | *Xpsm87.1-Xpsm514* | 6 | 3.62 | /37.72 | |
| Harvest index | *Xpsm618-Xpsm717* | 6 | 2.20 | /−0.76 | Yadav et al. (2003, 2004), Kannan et al. (2014) |
| | *Xpsmp2248_166* | 6 | 4.33 | – | |
| | *Xpsm322-Xpsm2050* | 2 | 5.35 | – | |
| Plant height | $D_1/d_1$ | 1 | – | – | Azhaguvel et al. (2003), Kannan et al. (2014), Kumar et al. (2017), Poncet et al. (2000) |
| | $D_2/d_2$ | 4 | – | – | |
| | *Xpsmp2085_175* | 4 | – | – | |
| | *Xpsmp2224_157* | 7 | – | – | |
| | *Pgpb6112-pgpb9106* | 1 | 6.4 | 30.2/2.9 | |
| | *Pgpb9498-Xipes017* | 1 | 8.54 | 32.3/−3.0 | |
| | *Xipes203-Xpsmp2273* | 1 | 8.8 | 33.0/5.9 | |
| | *Pgpb12094-pgpb10685* | 2 | 6.67 | 28.7/2.1 | |
| | *Xpsmp322-Xipes181* | 2 | 6.45 | 25.5/2.7 | |
| | *Xipes162-Xipes163* | 2 | 5.7 | 22.9/3.6 | |
| | *pgpb7379-Xipes2227* | 3 | 5.91 | 23.6/1.8 | |
| | *Pbpb6901-pgpb8757* | 3 | 9.83 | 39.5/−3.1 | |
| | *Xpsmp2085-Xipes225* | 4 | 8.87 | 34.1/6.1 | |
| | *Xipes207-Xicmp3058* | 6 | 5.78 | 23.2/−0.4 | |
| | *Xipes153-Xpsmp2040* | 7 | 9.7 | 35.8/−6.0 | |
| | *Pbpb8626-Xipes205* | 7 | 6.42 | 25.4/−2.9 | |
| | *Xipes198-Xipes082* | 7 | 11.13 | 39.8/−6.4 | |
| | *Hmax6* | 6 | – | 25.3/ | |
| | *hmax7* | 7 | 2.73 | 16.1/ | |
| Panicle length | *los1* | 1 | 2.45 | 13.2/1.27 | Poncet et al. (2000), Kumar et al. (2017) |
| | *los2* | 2 | 7.37 | 35.9/−2.13 | |
| | *pgpb9498-Xipes17* | 1 | 6.93 | 27.1/−1.4 | |
| | *Xipes226-Xicmp3032* | 1 | 7.27 | 28.2/0.7 | |
| | *Xipes4-Xipes229* | 1 | 6.42 | 25.4/0.4 | |
| | *Xipes7-Xpsmp2088* | 2 | 7.34 | 28.4/1.7 | |
| | *pgpb9647-Xicmp3027* | 5 | 6.08 | 24.4/−0.4 | |
| | *Xipes200-Xicmp3002* | 6B | 8.61 | 32.5/−0.4 | |
| | *pgpb10687-pgpb10299* | 6B | 5.94 | 24.6/0.9 | |
| | *pgpb12322-pgpb8782* | 6B | 5.95 | 26.0/1.1 | |
| | *pbpb9915-Xipes206* | 7 | 6.23 | 24.7/−1.0 | |
| | *pbpb9819-Xpsmp2074* | 7 | 6.65 | 26.2/0.2 | |
| | *Xipes198-Xipes82* | 7 | 7.84 | 30.1/−1.0 | |

(continued)

**Table 6.4** (continued)

| Trait | QTL locus/markers | LG | LOD value | PVE/ Effect | References |
|---|---|---|---|---|---|
| Panicle number | *Xpsm858-Xpsm565* | 1 | 2.65 | /0.68 | Yadav et al. (2003) |
| | *Xpsm592-Xpsm443* | 2 | 8.29 | /−1.87 | |
| | *Xpsm95-Xpsm575* | 6 | 2.34 | /−1.07 | |
| | *Xpsm618-Xpsm717* | 7 | 4.04 | /−1.31 | |
| Flowering time | *Xpsm59-Xpsm443* | 2 | 6.79 | /−26.98 | Yadav et al. (2003), Kannan et al. (2014), Kumar et al. (2017), Poncet et al. (2000), Punnuri et al. (2016) |
| | *Xpsm416.3-Xpsm196.2* | 4 | 3.92 | /−0.75 | |
| | *Xpsm87.1-Xpsm95* | 6 | 9.41 | /1.26 | |
| | *Xpsmp2248_162* | 6 | 5.78 | /0.30 | |
| | *Pgpb6981-Xipes226* | 1 | 8.42 | 32.9/ | |
| | *Xipes236-Xpsmp2059* | 2 | 6.46 | 25.2/ | |
| | *Pgpb11647- Xipes166* | 3 | 5.85 | 23.4/ | |
| | *Pgpb7379-Xpsmp2227* | 3 | 14.68 | 48.8/ | |
| | *Pgpb9967-Xpsmp11527* | 4 | 6.05 | 24.1/ | |
| | *Pgpb10505-Xipes230* | 5 | 10.05 | 36.8/ | |
| | *head5* | 5 | 10.75 | 59.0/10.93 | |
| | *head7* | 7 | 3.68 | 24.2/−5.52 | |
| | *S1_1423-S1_3590* | 1 | 2.61 | 3.03/1.8 | |
| | *S2_1896-S2_2803* | 2 | 4.86 | 6.00/−2.0 | |
| | *S5_0012-S5_1669* | 5 | 2.38 | 4.75/1.5 | |
| | *S7_0244-S7_2067* | 7 | 2.48 | 0.49/1.3 | |
| Panicle threshing percentage | Xpsmp2085_175 | 4 | 2.4 | | Kannan et al. (2014) |
| 1000 grain weight | Xipes200-Xicmp3002 | 6 | 7.36 | 28.5/0.8 | Kannan et al. (2014) |
| Iron (Fe) | Xpsmp2214-Xipes142 | 3 | 4.68 | 20.5/8.5 | Kumar et al. (2016, 2017) |
| | *Xpsmp2214-Xipes142* | 3 | 4.68 | 20.5/8.5 | |
| | *Xipes017-pgpb 12900* | 1 | 6.22 | 9.0/4.0 | |
| | *pgpb10531-pgpb9130* | 1 | 25.36 | 31.9/9.7 | |
| | *Xipes188-pgpb6069* | 3 | 6.59 | 9.5/0.4 | |
| | *pgpb9502-pgpb6039* | 4B | 6.87 | 10.4/−0.6 | |
| | *pgpb11956-pgpb9273* | 7 | 7.25 | 12.5/−1.9 | |
| | *pgpb8427-pgpb13221* | 7 | 8.58 | 12.2/5.3 | |
| | *pgpb11938-pgpb8987* | 7 | 8.83 | 12.5/4.9 | |
| | *pgpb6825-Xipes195* | 7 | 9.70 | 14.0/0.1 | |
| | *pgpb8445-pgpb11206* | A | 7.67 | 12.4/4.0 | |
| | *pgpb10660-pgpb8626* | D | 7.00 | 11.6/1.2 | |
| | *pgpb10727-Xipes179* | E | 9.36 | 14.3/3.1 | |
| Zinc (Zn) | Xpsmp2214-Xipes142 | 3 | 9.66 | 32.3/8.5 | Kumar et al. (2016), 2017) |
| | *pgpb10531-pgpb9130* | 1 | 23.93 | 30.4/6.7 | |
| | *pgpb10397-pgpb10394* | 1 | 6.50 | 9.4/1.7 | |
| | *pgpb9502-pgpb6039* | 4B | 7.33 | 11.1/−0.6 | |
| | *pgpb10483-pgpb11463* | 4B | 6.68 | 11.6/−2.2 | |
| | *pgpb13229-pgpb12681* | 5 | 8.17 | 11.6/2.7 | |
| | *Xipes198-pgpb8427* | 7 | 7.16 | 10.2/2.7 | |
| | *pgpb12329-pgpb9721* | 7 | 7.58 | 10.9/2.8 | |
| | *pgpb8779-pgpb12691* | H | 6.68 | 11.6/2.1 | |

(continued)

**Table 6.4** (continued)

| Trait | QTL locus/markers | LG | LOD value | PVE/ Effect | References |
|---|---|---|---|---|---|
| Downy mildew | *M413-M93*<br>*M543-M380*<br>*M37-M248*<br>*M390-M318* | 1<br>2<br>3<br>5 | 27.4<br>7.2<br>5.0<br>5.4 | | Jones et al. (2002) |
| Rust | $Rr_1$<br>*Rust Res(ICMP83506)* | 3<br>4 | | –<br>– | Morgan et al. (1998) |
| Leafspot | S2_7773-S2_8331<br>S3_0019-S3_4763<br>S5_2145-S5_4145<br>S7_0738-S7_3864 | 2<br>3<br>5<br>7 | 2.18<br>2.25<br>4.56<br>3.01 | 1.78/−0.6<br>1.82/−0.5<br>4.83/0.9<br>5.05/0.9 | Punnuri et al. (2016) |
| Drought tolerance* | M214-M443<br>M716-M416<br>M87.1-M514 | 2<br>4<br>6 | | | Yadav et al. (1999) |

PVE = Phenotypic value explained (%)

development of diverse cultivars adapted to different environments, suited to various production systems, and aligned with different consumer preferences (Brunken 1977). There are wide variations in various morphological traits, phenological, yield, and quality traits often corresponding to adaptation zones especially in the center of diversity.

To ensure the availability of these genetic variations for breeding programs, collection, conservation, characterization, evaluation, and documentation of the germplasm are very important pre-breeding activities. A concerted effort has been made mainly by ICRISAT to collect from different geographic areas and also consolidate collections done by different centers and different groups around the world (Upadhyaya et al. 2007; Yadav et al. 2017). Accordingly, ICRISAT genebank collected and conserved a total of 22,288 pearl millet germplasm. In addition to ICRISAT, Institute of Research for Development (IRD, France) has 3,968 accessions collected from 16 countries, Canadian Genetic Resources Program (Saskatoon, Canada) has 3,821 accessions of cultivated *P. glaucum* and related species, and the US Germplasm Resource Information Network (GRIN) collected and preserved more than 1283 accessions (Serba et al. 2017; Yadav et al. 2017).

## 6.8 BAC Library

With the objective to develop resources from which SSR markers can be developed for pearl millet, a bacterial artificial chromosome (BAC) library was constructed (Allouis et al. 2001). Through a novel way to developing new markers, 25 SSR markers that can anchor individual BACs to the genetic maps were developed from 40 BAC pools, comprising a total of 384 clones (Qi et al. 2001).

## 6.9 Genomics-Assisted Breeding

### *6.9.1 Genome Sequence*

A draft whole genome sequence of 1.79 Gb has been assembled using whole genome shotgun (WGS) and BAC library sequencing of a reference genotype Tift 23D2B1-P1-P5 (Varshney et al. 2017). This genome resource is expected to bring a paradigm shift to pearl millet molecular breeding by providing a foundation for accelerating gene mapping and characterization. The unparalleled heat and drought tolerance has been attributed to the enrichment of wax biosynthesis genes discovered through the genome sequence. In addition to the whole genome sequence, 963 pearl millet inbred lines and 31 wild accessions preserved at ICRISAT were re-sequenced to generate data that give insight into the population structure, genetic diversity, and domestication of the crop. The genome sequence and the resequencing data is expected to facilitate future marker-trait association studies, to define heterotic pools, and predict hybrid performance.

Tift23D2B1, an important ancestral genotype of many seed parents of pearl millet hybrids in use for forage and grain as well as dual-purpose hybrids was used for the genome sequence. Tift23D2B1 was developed at the Coastal Plain Experiment Station (Tifton, Georgia, USA) by introducing $d_2$ dwarfing gene into the genetic background of elite seed parent maintainer line Tift 23B1. The genome features such as repetitive DNA, gene density, and SNPs identified using resequencing of a pearl millet inbred germplasm association panel (PMiGAP) is visualized (Fig. 6.7). A total of 38,579 gene models with mean coding sequence length of 1,014.71 bp were annotated. Higher gene density was observed in the telomeric region than the peri-centromeric region of the pseudomolecules (exact centromere location unknown). Conversely, repetitive DNA density was higher in peri-centromeric region than telomeric region. The average GC content of pearl millet (47.9%) is higher than that of foxtail millet (46.1%), sorghum (44.5%), barley (*Hordeum vulgare*, 44.4%), and rice (43.5%). These resources are important preludes for the application of genomic-assisted breeding to improve this climate-resilient crop for agronomic traits and yield potential.

The assembled pearl millet genome accounts for more than 77% of the repetitive elements. This percentage of its repetitive DNA is consistent with the previous estimate of 80% repetitive DNA of the crop (Paterson et al. 2009; Bennetzen et al. 2012) which is close to the proportion of repetitive DNA found in the 2.3-Gb maize genome (nearly 85%) (Schnable 2009), and considerably higher than 730-Mb of sorghum (~61%) (Paterson et al. 2009), ~400-Mb of foxtail millet (~46%) (Bennetzen et al. 2012) or 466-Mb of rice (~42%) (Yu 2002) genomes. More than 50% of its nuclear genome repetitive DNA is classified as long-terminal repeat retrotransposons (Varshney et al. 2017).

In pearl millet, there were several independent domestication events that resulted in four different races (Brunken et al. 1977). Enriching the genome sequence through resequencing of the populations used in the QTL mapping and in entirety

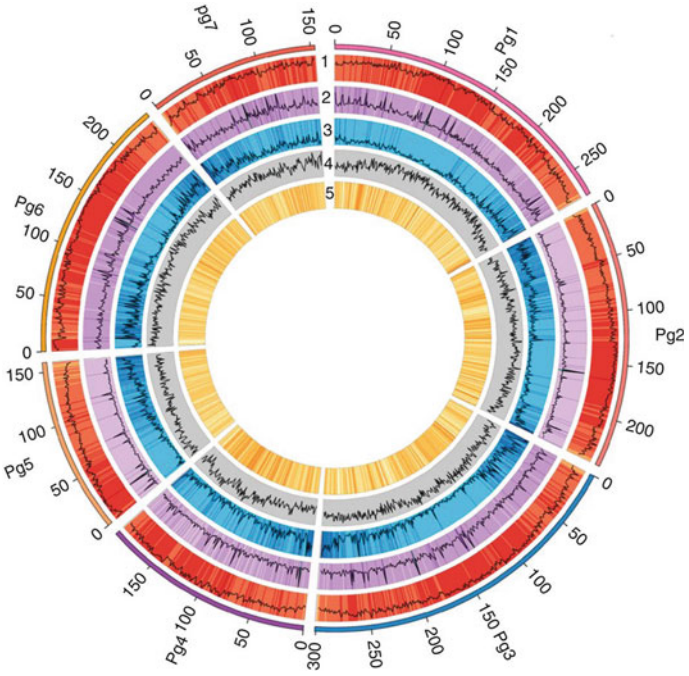


**Fig. 6.7** Genome features in 1-Mb intervals across the seven pseudomolecules (Varshney et al. 2017). Units on the circumference are megabase values of pseudomolecules. (1) Repeat density, (2) tandem repeat density, (3) gene density, (4) GC content and (5) SNPs identified by resequencing PMiGAP lines in 1-Mb bins. The genome assembly furnished an average GC content of 47.9% and contained 38,579 gene models with mean coding sequence length of 1,014.71 bp (Adopted from Varshney et al. 2017, *Nature Biotechnology* 35:969–976)

of independently sequencing a genotype from other race thus becomes highly desirable. To conduct inclusive characterization of the genetic factors responsible for the seed morphological variations used as a basis for the classification, integrating the known races into the genome sequence would broaden the application of the resource.

With the assumption that origin of domestication corresponds with the greatest genetic diversity (Vavilov 1992), domestication origin of pearl millet is recently inferred based on whole genome sequence of a large number of wild forms and cultivated landraces (Burgarella et al. 2018) representative of the geographical diversity of pearl millet (Varshney et al. 2017). The result indicated Western Sahara as the original center of domestication of pearl millet. It was also used to predict the onset of cultivated of pearl millet in Africa back to 4,900 years ago. The genome sequence established wild-to-crop gene flow that increased cultivated genetic diversity leading to diversity hotspots in western and eastern Sahel and adaptive introgression of 15 genomic regions. The result of this study reconciled the genetic and archaeological data available for the crop.

### 6.9.2 Gene Annotation

Based on the genome sequences, 38,579 gene models with an average transcript size of 3,945 bp and an average coding sequence size of 687 bp were estimated (Varshney et al. 2017). A gene function has been assigned to 27, 893 (72.30%) genes, leaving 10,686 (27.70%) genes unannotated. More than 74% of the predicted pearl millet proteins have orthologues in foxtail millet, one of the related species (Varshney et al. 2017). Investigation of the completeness of pearl millet genes was studied in comparison with rice genes. The comparison revealed that more than 90% of the 4,202 rice single-copy genes have homology in pearl millet genome (Varshney et al. 2017).

### 6.9.3 Genomic Selection

Accelerated breeding cycle and increased selection efficiency are the two reasons behind the drive for molecular breeding in crop improvement. Genomic selection (GS) is a system of marker-assisted selection in which genetic markers covering the whole genome are used so that all QTLs are in linkage disequilibrium with at least one marker (Goddard and Hayes 2007). It is advocated to accelerate the breeding cycle and facilitate efficient selection of superior genotypes (Crossa et al. 2017). It is an advanced molecular breeding approach in which high throughput SNPs in the whole genome are indiscriminately used in the genomic-enabled prediction of the breeding value of candidate genotypes for selection. This accelerated genetic gain approach integrated with a precise phenotyping is promising to identify superior alleles in a germplasm. Therefore, it has become a favored genomics-assisted breeding approach that can be integrated in germplasm enhancement and efficient development of climate resilient cultivars (Varshney et al. 2018).

Markers developed from a resequencing data were used to carry out GS to predict grain yield for test crosses in favorable, early stress, late stress, and across environments (Varshney et al. 2017). High prediction accuracy, measured as the Pearson correlation coefficient between the predicted and observed values, standardized with the square root of the heritability ($h = 0.78$), amounting to 0.6 was observed for the performance across environments. As a modeling study in wheat indicates GS with this level of prediction accuracy could substantially improve selection gain per year (Longin et al. 2015).

Another GS study for its application in the hybrid breeding observed that phenotypic data from inbred parents can improve genomic prediction in pearl millet hybrids (Liang et al. 2018). In this study, data from hybrids gave high prediction accuracies for 1000-grain weight (0.73–0.74), days to flowering (0.87–0.89), and plant height (0.72–0.73), followed by grain yield (0.48–0.51). When BLUPs were used to control for the effects of heterosis, the inclusion of inbred phenotypic

datasets moderately improved genomic predictions of the hybrid genomic estimated breeding values. But for traits with little to no heterosis, no changes in prediction accuracy were observed between hybrid only and hybrid/inbred data.

## 6.10 Defining Heterotic Gene Pools

Enhancement of grain and stover yield is the most important breeding objective. Lately there has been a lot of emphasis on the development of high yielding single-cross hybrids. Categorization of hybrid parents into different heterotic groups is a basic prerequisite for maximum exploitation of heterosis in single-cross hybrids. In pearl millet, development of diverse sets of seed (A-/B-) and pollen/restorer (R-lines) inbred parents from genetically distant gene pools are a starting point for the development of a hybrid breeding program. In this direction, a first attempt was made by ICRISAT and partners to identify heterotic groups for grain yield using EST and genomic SSR markers (Ramya et al. 2018). The study revealed that largely the maintainer and restorer lines form two broad gene pools, which can be broken down into sub-groups for maximization of heterosis for grain yield. On the basis of SSR genotyping data, the B- lines were differentiated into 10 sub-clusters (B1 through B10), and R- lines into 11 sub-clusters (R1 through R11). Based on per se performance, high specific combining ability (SCA) effects and standard heterosis, a total of seven heterotic groups were identified. These were B10R5, B3R5, B3R6, B4UD, B5R11, B2R4, and B9R9 (Fig. 6.8).

## 6.11 Role of Bioinformatics

To conduct an integrated genomics research on pearl millet, the utilization of a common communication tool among different centers is crucial. Having a common tool facilitates efficient use of resources that are generated at different centers, such as genome information and sharing of research results. In this regard, bioinformatics plays a vital role not only in boiling down the vast NGS data and generating useful information but also communication. The availability of genome, comparative, gene expression and protein databases provide useful information about the crop.

To empower pearl millet crop improvement genome sequencing and assembly was conducted recently. To unravel population structure, genetic diversity, evolution and domestication history, 994 pearl millet genotypes were re-sequenced. As a result, a genome database (https://www.ncbi.nlm.nih.gov/id=4543) was created.

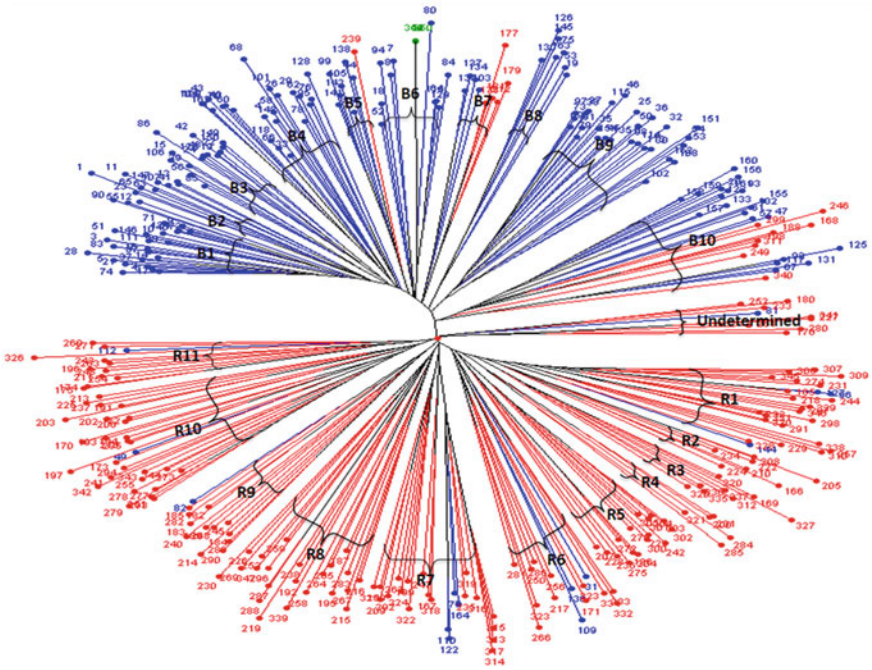


**Fig. 6.8** Unweighted neighbor joining tree based on the simple matching dissimilarity matrix of 88 SSR marker data for 347 hybrid parental lines. Lines in blue are B-lines, line in green is Tift 23D2B1-P1-P5 (B-line, and the world reference genotype), lines in red are R-lines; B1 to B10 are the 10 sub-clusters of the B-lines; while R1–R11 are the 11 sub-clusters of the R-lines (Adopted from Ramya et al. (2018). Front. Plant Sci. 8:1934. https://doi.org/10.3389/fpls.2017.01934

## 6.12 Future Perspectives

Today, fewer crop species are feeding the world than several decades ago. This scenario raises a serious concern about the resilience of the global food system in the wake of global climate change. With lack of on-farm species diversity and dietary intake, more and more people are now exposed to harvest and health failures than ever. Many people are affected by gluten allergy. Gluten free, protein and micronutrient rich crops that can withstand the climatic variability would have a great chance of coming to the main stream production in certain parts of the world where the consequence of climate change would be impactful.

### *6.12.1 Potential for Expansion of Productivity*

Among crops with high potential of production expansion, pearl millet is expected to come to the forefront. This is attributed to its exceptional heat and drought

tolerance and high nutritional value. Productivity has been low; however, some improvements have been made in India. Improved hybrids have been widely adopted by Indian farmers with the result that the crop productivity has gone up from 305 kg $ha^{-1}$ during 1951–1955 to 998 kg $ha^{-1}$ during 2008–2012, registering a 227% improvement which assumes greater significance is being given despite more than 90% of pearl millet is grown as rainfed and often on marginal lands (Yadav and Rai 2013). Launching hybrid breeding in major pearl millet growing countries in west Africa have a glimmer of hope to bring about a paradigm shift in productivity in the region.

### 6.12.2 Potential for Expansion into Nontraditional Areas

A small number of crop species that are being produced in large scale account for overall human diet. These crops have become the key in global food security. With the looming climate change, the current production system based on a limited number of crop species is vulnerable to the effect of climate variability. To avert such anticipated effect of the climate change, diversity in crop species is an ultimate solution.

Pearl millet is a drought resilient crop with heat and salinity tolerance makes it a potential for expansion in dryland as well as agriculturally favorable areas of the world. However, yield advantage of other crops like sorghum is impeding its popularity among the commercial producers in many areas. With the application of genomics, genetic yield potential, biotic and abiotic stresses tolerance, and nutritional quality improvements is expected to progress in the near future. With the expected improvement and sizable production areas of major crops challenged by climatic changes, the prospect of pearl millet to be considered as a major crop is high.

### 6.12.3 Potential for Nutritional Enhancement

Germplasm and breeding lines have much higher nutritional level than farmers growing OPVs or hybrids. So far, millet breeders are focused on productivity of gain like other crops. The genetic enchantment of essential nutrients of new cultivars has to be achieved with no penalty on grain yield productivity. Micronutrient screening is highly expensive to deal with larger germplasm and breeding lines when resources are limited. With availability of X-ray fluorescent (XRF) tool, cost of biofortification breeding will decrease over time, and micronutrient content built into the gene pool will not affect future breeding for yield traits. Apparently micronutrient traits are not affected by genetic erosion (just like $d_2$ gene revolution) and involve little maintenance breeding after the genes are transferred to elite lines. Therefore, to cope with climate change and nutritional importance, diverting

25–30% of breeding investment as part of mainstreaming these traits at NARS and seed companies would be highly helpful in delivering nutritionally-dense pearl millet cultivars to farmers in India and SSA in near future.

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