Chapter 11
Genomic Approaches to Enhance Stress Tolerance for Productivity Improvements in Pearl Millet

Srikanth Bollam, Vijayalakshmi Pujarula, Rakesh K. Srivastava, and Rajeev Gupta

Abstract Pearl millet [*Pennisetum glaucum* (L.) R. Br.], the sixth most important cereal crop (after rice, wheat, maize, barley, and sorghum), is grown as a grain and stover crop by the small holder farmers in the harshest cropping environments of the arid and semiarid tropical regions of sub-Saharan Africa and South Asia. Millet is grown on ~31 million hectares globally with India in South Asia; Nigeria, Niger, Burkina Faso, and Mali in western and central Africa; and Sudan, Uganda, and Tanzania in Eastern Africa as the major producers. Pearl millet provides food and nutritional security to more than 500 million of the world's poorest and most nutritionally insecure people. Global pearl millet production has increased over the past 15 years, primarily due to availability of improved genetics and adoption of hybrids in India and expanding area under pearl millet production in West Africa. Pearl millet production is challenged by various biotic and abiotic stresses resulting in a significant reduction in yields. The genomics research in pearl millet lagged behind because of multiple reasons in the past. However, in the recent past, several efforts were initiated in genomic research resulting into a generation of large amounts of genomic resources and information including recently published sequence of the reference genome and re-sequencing of almost 1000 lines representing the global diversity. This chapter reviews the advances made in generating the genetic and genomics resources in pearl millet and their interventions in improving the stress tolerance to improve the productivity of this very important climate-smart nutri-cereal.

Keywords Genomics · Markers · Molecular breeding · Nutrition · Pearl millet · Stress tolerance

S. Bollam · V. Pujarula · R. K. Srivastava (✉) · R. Gupta (✉)
International Crops Research Institute for the Semi-Arid Tropics (ICRISAT),
Patancheru, India
e-mail: r.k.srivastava@cgiar.org; g.rajeev@cgiar.org

11.1 Introduction

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is one of the principal staple food and earliest used global cereal crops cultivated in the semiarid regions of the sub-Saharan Africa and the central Asian countries. Worldwide about 31 million ha area was covered in 2016 by millet crop with a total production of 28.35 million tonnes (Fig. 11.1). Major pearl millet-producing countries include India, Niger, China, Mali, Nigeria, and Sudan (FAO stat: http://www.fao.org/faostat/en/#search/Millet). India is the largest producer of pearl millet; within the country it occupies 7.14 million ha area under cover with 8.06 million tonnes production with an average productivity of 1132 kg/ha (http://www.aicpmip.res.in/pmnews2017.pdf) and continues to play a critical role in food and livelihood security of millions of people in India (Fig. 11.2). It is the main source of calorie intake for the 500 million people of Asia and Africa. It is a small seeded, highly cross-pollinated, C4 panicoid annual crop
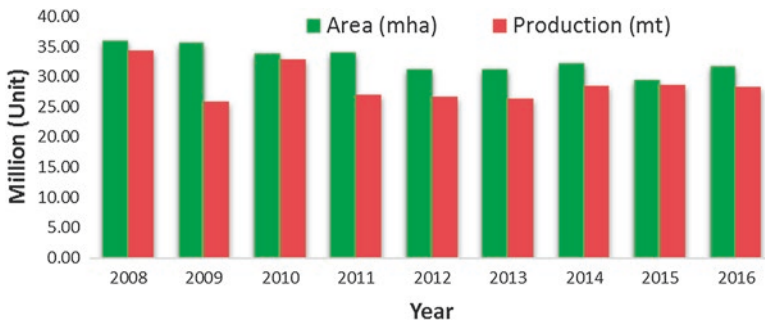

Fig. 11.1 Global millet production and area harvested from the year 2008 to 2016. (Source: FAO Stat)
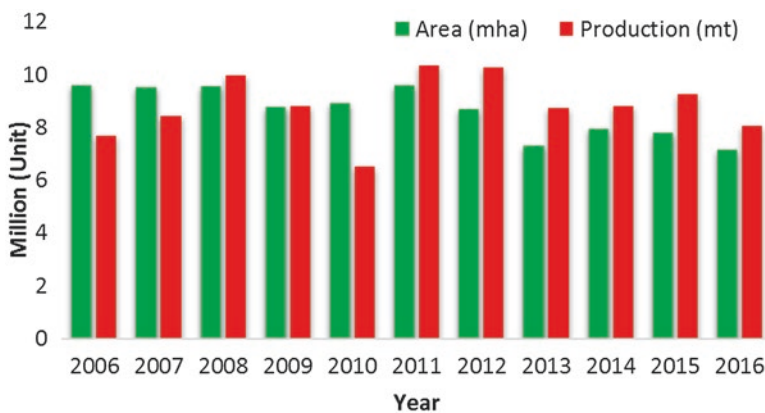

Fig. 11.2 India's pearl millet production and area harvested from the year 2006 to 2016. (Source: AICPMIP, 49th Annual review meeting, 2014 and AICPMIP News letter, 2016 and 2017)

with a genome size of ~2400 Mb and short life cycle (Vadez et al. 2012). Pearl millet is a warm season coarse grain and can be well adapted in the infertile soils with low water-holding capacity, low rainfall, high temperature, and droughty environments, where other cereals would not survive (Vadez 2014; Shivhare and Lata 2016). Apart from its hardy nature to withstand harsh environments, least input demand and outstanding nutritional values make the pearl millet as a highly desirable crop for the arid region farmers. The utility of this crop is diverse, ranging from food to feed, fodder, forage, brewing, biofuel, building material, and fuel for cooking, in dryland areas.

Pearl millet grain has relatively higher energy and contains proteins, vitamin A, B, and carbohydrates; it also contains a higher nutritive value with higher protein (8–60%), carbohydrates (60–78%), fat (3.0–4.6%), and 40% higher in lysine than in feed corn. Pearl millet is a rich source of micronutrients such as iron and zinc with higher quantity than other cereals (Souci et al. 2000). Furthermore, it has higher energy density than sorghum, wheat, and maize (Hill and Hanna 1990). Pearl millet feeding trials in India also confirmed its nutritional values and are superior to maize and rice (DeVries and Toenniessen 2001; Lardy et al. 2004). The biological value of the pearl millet grain protein is higher than wheat, and it is free of gluten and tannin; it contains 5–7% oil, higher protein and energy levels, and more balanced amino acid profile than maize or sorghum (Rai et al. 2008). Apart from human consumption, it is used as animal feed in dairy and poultry industries and alcohol, processed food, and starch industries along with export demand (Basavaraj et al. 2010).

Pearl millet is considered as an important crop species for nutritional security of the poor. The recent advances in tools and technologies and currently available genetic and genomic resources offer immense scope in crop improvement by accelerating the rate of genetic gains. This chapter reviews the recent advances in genomic resources, tools, and technologies and their interventions to address stress tolerance in pearl millet to enhance its yield stability.

11.2   Recent Advances in Genomic and Genetic Resources

In the recent years, whole genome scanning emerged as a potential technology and expanded its utility from cereals to millets. Recently, sequence of reference genome and re-sequencing of 994 pearl millet lines representing the global diversity were accomplished by an international consortium led by ICRISAT (Varshney et al. 2017). In this study, nearly 30 million single nucleotide polymorphisms (SNPs) were identified in pearl millet inbred germplasm association panel (PMiGAP, a panel of 348 lines), in which a total of 450,000 high-quality SNPs were reported after principal component analysis and constructing a neighbor-joining tree. From these released sequenced data, a total of 88,256 SSR markers were identified, in which primers designed for 74,891 SSR markers. These markers will be used for future genetics and breeding application and also help the pearl millet scientists to better understand the trait variation and expedite the genetic improvement of pearl

millet (Varshney et al. 2017). In an earlier study, a total of 83,875 SNPs were identified by using genotyping by sequencing (GBS) in 500 pearl millet lines which have included 252 global accessions and 248 Senegalese landraces (Hu et al. 2015). Moumouni et al. (2015) identified a total of 3321 SNPs in F_2 population of 93 progenies from a wild x cultivated pearl millet cross, out of which 2809 were exhibited high-quality SNPs, a minor allele frequency ≥0.3. A total of 314 nonredundant haplotypes for which a single representative SNP marker was used for map construction. These SNPs were evenly distributed over seven linkage groups with an average density of 0.51 SNP/cM. Recently GWAS were performed with 22 SNPs and 3 indel markers in pearl millet inbred germplasm association panel (PMiGAP) comprising 250 inbred lines for drought-tolerant traits (Sehgal et al. 2015). Sehgal et al. (2012) observed the high SNP polymorphism between two parent lines in gene-based DNA sequence in pearl millet. Kumari et al. (2014) developed a set of chromosome segment substitution lines in pearl millet for all the seven linkage groups (LGs). These lines may serve as valuable genetic resources to dissect the genetic effects of complex traits such as yield. Some of the important mapped traits, QTLs and developed markers in pearl millet, were presented in Fig. 11.3, Tables 11.1 and 11.2.

11.3   Genomic Interventions for Abiotic Stress Tolerance

Abiotic stresses pose a serious threat for plant growth and development and causing more than 50% yield reduction in major crops (Bray 2000). Only 10% of the global arable agricultural land fall under non-stress category, and the remaining cultivable lands are prone to abiotic stresses (Dita et al. 2006). The crops affected by abiotic stress were also easily prone to various insects, weeds, and pathogens which in turn add up to the yield losses considerably (Reddy et al. 2004). Pearl millet cultivation is majorly practiced on marginal lands with irregular, untimely rainfall, and environmental stresses; drought stress is the major constraint that ultimately affects the grain yield in low-input farming systems. Besides drought conditions, high temperature and salinity are the other major abiotic stress conditions which affect the pearl millet productivity.

Plant stress response, adaptation, survival, and subsequent yielding in stress conditions are complex mechanisms and are regulated by several cellular, molecular networks along with physiological factors (Ahuja et al. 2010). Therefore, understanding the molecular mechanisms of plant stress tolerance and its adaptation is needed of the hour to overcome the present-changing climatic situations to address the yield stability. Conventional breeding approaches for drought tolerance are more difficult due to location-specific stress factors. Therefore, effective integration of breeding with the support of advanced and cutting-edge "Omics" technologies could be the promising approach to dissect and manipulate the genetic architecture of adaptation to abiotic stresses in crop plants (Langridge and Fleury 2011). In recent times, Omics approaches were extensively utilized in cereals in order to
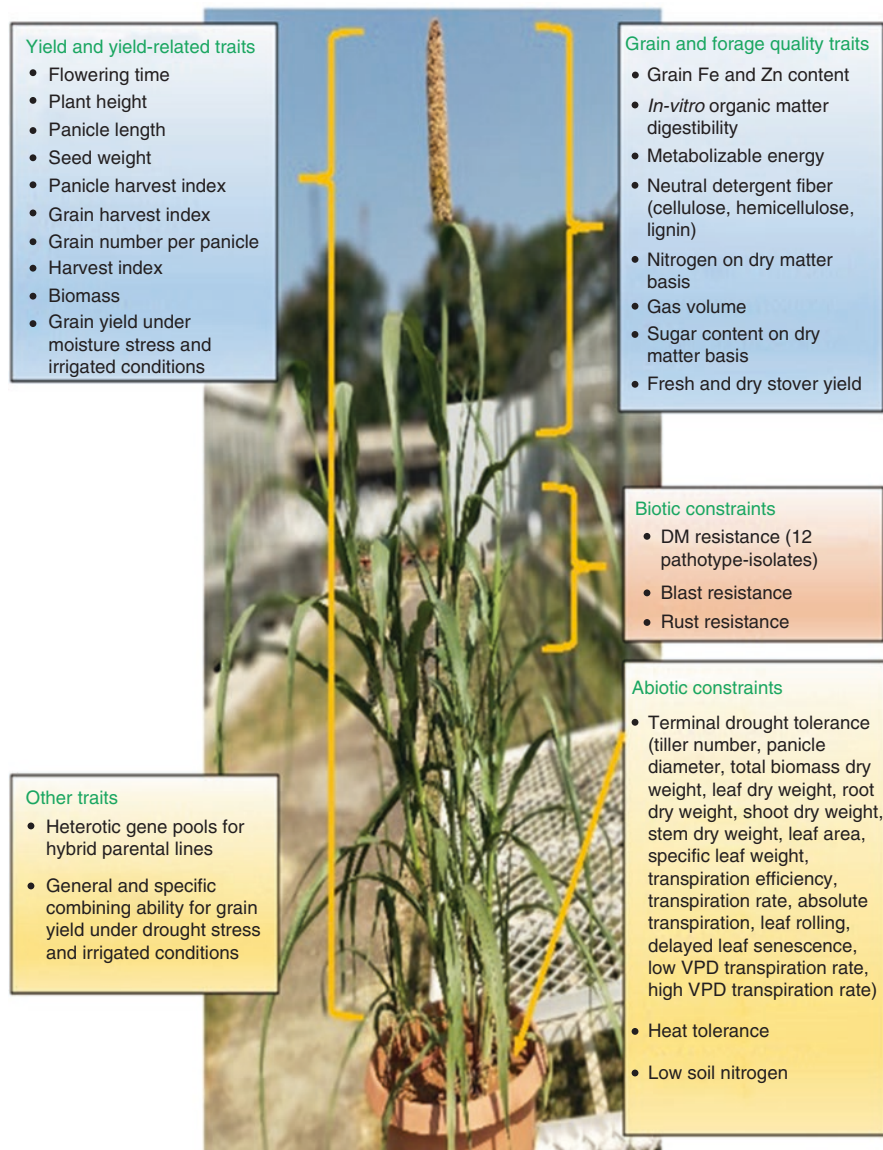
Yield and yield-related traits
- Flowering time
- Plant height
- Panicle length
- Seed weight
- Panicle harvest index
- Grain harvest index
- Grain number per panicle
- Harvest index
- Biomass
- Grain yield under moisture stress and irrigated conditions

Grain and forage quality traits
- Grain Fe and Zn content
- *In-vitro* organic matter digestibility
- Metabolizable energy
- Neutral detergent fiber (cellulose, hemicellulose, lignin)
- Nitrogen on dry matter basis
- Gas volume
- Sugar content on dry matter basis
- Fresh and dry stover yield

Biotic constraints
- DM resistance (12 pathotype-isolates)
- Blast resistance
- Rust resistance

Abiotic constraints
- Terminal drought tolerance (tiller number, panicle diameter, total biomass dry weight, leaf dry weight, root dry weight, shoot dry weight, stem dry weight, leaf area, specific leaf weight, transpiration efficiency, transpiration rate, absolute transpiration, leaf rolling, delayed leaf senescence, low VPD transpiration rate, high VPD transpiration rate)
- Heat tolerance
- Low soil nitrogen

Other traits
- Heterotic gene pools for hybrid parental lines
- General and specific combining ability for grain yield under drought stress and irrigated conditions

Fig. 11.3 Diagrammatic representation of some of the important traits which are mapped in pearl millet

dissect the genetic architecture of stress adaptation, and quantitative response of abiotic stress tolerance and several QTLs, genes, and genomic loci controlling the adaptive response to harsh environmental conditions was identified, namely, drought adaptive traits (Tuberosa 2012); abscisic acid concentration (Rehman et al. 2011); stay-green (Borrell et al. 2014), canopy temperature traits (Lopes et al. 2014); root

Table 11.1 Some of the important genomic resources and markers associated with different traits in pearl millet

S. no.	Type of markers	Reference
1	450,000 high-quality SNPs, 74,891 SSRs	Varshney et al. (2017)
2	7 SSR markers for iron and zinc	Anuradha et al. (2017)
3	Linkage map constructed with 229DArT and 57 SSR markers developed by genotyping of a RIL population	Ambawat et al. (2016)
4	37 SSRs and CSIP markers, 22 SNPs, and 3 indels for abiotic stresses	Sehgal et al. (2015)
5	745 ESTs in response to drought	Choudhary and Padaria (2015)
6	ISSR-derived SCAR markers for downy mildew	Jogaiah et al. (2014)
7	75 SNPs and CISP developed from available ESTS	Sehgal et al. (2012)
8	100 plus EST-SSRs (developed and mapped in one or more of four pearl millet RIL populations)	Rajaram et al. (2010, 2013)
9	250–280 DArT markers (screened in three pearl millet RIL populations)	Senthilvel et al. (2010)
10	11 finger millet EST-derived SSRs	Arya et al. (2009)
11	4 EST-SSRs and 9 CISPs (polymorphic in populations)	Yadav et al. (2008)
12	21 EST-SSRs and 6 genomic SSRs	Senthilvel et al. (2008)
13	19 EST-derived SSRs	Yadav et al. (2007)
14	16 EST-derived SSRs	Mariac et al. (2006)
15	SSCP-SNP markers by rice and pearl millet EST sequences	Bertin et al. (2005)
16	36 Genomic library-derived SSRs	Qi et al. (2004)
17	18 Genomic library-derived SSRs	Budak et al. (2003) Allouis et al. (2001)

architecture (Lynch et al. 2014); and water-soluble carbohydrate accumulation and its partitioning to storage organs (Rebetzke et al. 2008). Recently, Serba and Yadav (2016) and Shivhare and Lata (2017) have reviewed studies on abiotic and biotic stresses in pearl millet. There is an imminent need for crop plants with improved abiotic stress tolerance to ensure the improved yield stability in pearl millet and thereby contributing to the sustainable dryland agriculture. So far QTLs have been identified, for important traits on different linkage groups were presented in LG1 to LG4 (Fig. 11.4a) and LG5 to LG7 (Fig. 11.4b).

11.3.1 Drought Stress

Abiotic stress, in particular drought stress, causes variations in grain yield loss based on stress intensity, duration, and timing. Among millets, however, foxtail millet, pearl millet, and to lesser extent finger millet have lately started gaining some importance among the research community wherein "omics" have played an

Table 11.2 Quantitative trait loci (QTLs) associated with some of the important traits in pearl millet

S. no.	QTLs and traits	Chromosome	Reference
1	QTLs for agronomic traits	LG1, LG2, LG3, and LG6B	Kumar et al. (2017)
2	QTLs for iron and zinc	LG3	Kumar et al. (2016)
3	QTL for rust resistance	LG1	Ambawat et al. (2016)
4	QTLs for reduced salt uptake	LG2	Sharma et al. (2011, 2014)
5	QTLs for drought tolerance	LG2	Sehgal et al. (2012)
6	QTLs for grain yield in late stress	LG3, LG4, and LG6	Bidinger et al. (2007)
7	QTLs for terminal drought	LG2	Sehgal et al. (2009), Bidinger et al. (2005, 2007), Yadav et al. (2002, 2004)
8	QTLs for grain yield in early stress	LG2 and LG5	Bidinger et al. (2007), Yadav et al. (2004)
9	QTLs for downy mildew	LG1 and LG4	Jones et al. (1995)

important role apart from conventional plant breeding. In pearl millet, significant progress was achieved by using genetic and genomic approaches to identify several genes and their regulatory networks not only to understand the relationships between pearl millet and different cereal crops (Devos and Gale 2000) but also utilized in introgression of QTLs for drought adaptation components (Kholová et al. 2012), terminal drought tolerance, and grain and stover yield (Yadav et al. 2002, 2003, 2004). Several genetic maps for various traits were developed and utilized in breeding of promising QTLs of significant importance (Morgan et al. 1998; Jones et al. 2002; Serraj et al. 2005; Nepolean et al. 2006; Gulia et al. 2007; Bidinger et al. 2007; Yadav et al. 2011).

Drought is the major constraint for pearl millet production in the driest regions of Africa and South Asia. Traditional pearl millet landraces are an excellent source of drought adaptation and able to produce higher grain, biomass, and stover than elite populations (Yadav 2008). Under harsh drought stress conditions, small-panicled and high-tillering landraces produce increased grain yield than landraces with low tillering and large panicles (Van Oosterom et al. 2006). Genotypes with drought tolerance use more water post-anthesis than pre-anthesis, resulting in higher grain yield (Vadez et al. 2013). Post-flowering drought stress is the most critical environmental stress which considerably affects the grain yield and yield stability in pearl millet and leads to crop failure in dryland production systems (Mahalakshmi et al. 1987). Drought stress during grain filling (terminal drought stress) causes severe damage to pearl millet productivity than vegetative stage stress, as pearl millet's rapid growth and asynchronous tillering allow the plant to recover from vegetative stage drought stress but fails in the case of terminal drought stress (Bidinger et al. 1987). In pearl millet under terminal drought stress conditions, significant
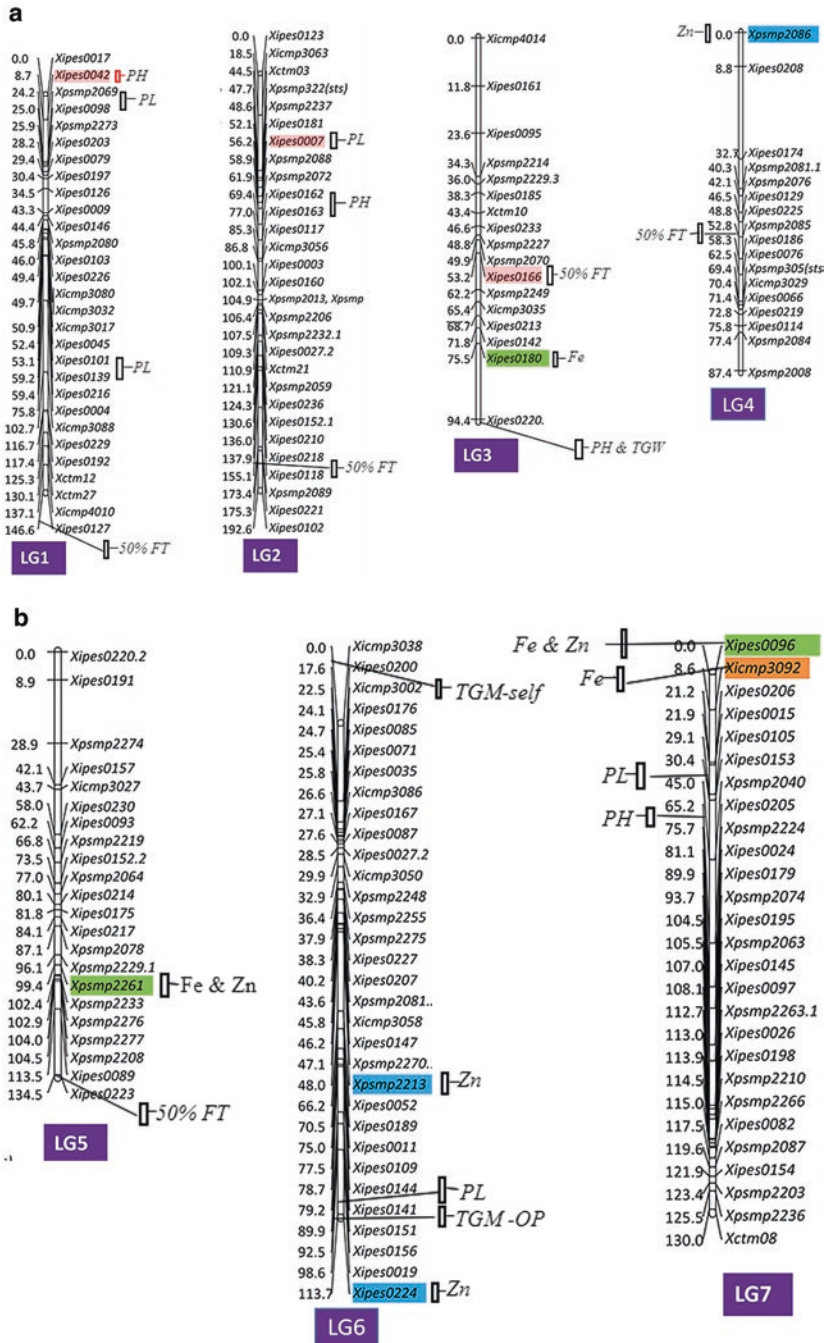
Fig. 11.4 (**a**, **b**) Consensus map with QTL positions for important traits. (Source of linkage map, Anuradha et al. 2017). FT, time to 50% flowering (d); PH, plant height (cm); PL, panicle length (cm); TGW_self, self-pollinated 1000-grain weight (g); TGW_OP, open-pollinated 1000-grain weight (g); Fe, grain iron; Zn, zinc

progress was made in mapping QTLs for components of stover and grain yield, along with yield stability using progeny of two sets of mapping populations, namely, H 77/833–2 and PRLT 2/89–33 and the other from a cross between elite inbred parents (ICMB 841 and 863B) (Yadav et al. 2002, 2003, 2004; Hash et al. 2003; Bidinger et al. 2007). These two crosses identified a major QTL and confer to terminal drought tolerance on linkage group 2 (LG2) with 23% of the variation explained (PVE). In the same LG2 region, QTLs for biomass and harvest index were also colocated which suggest that increased terminal drought tolerance might enhance the dry matter partitioning to the grain (Yadav et al. 2002) due to this genomic segment. Kholova et al. (2011) studied the physiological (transpiration efficiency and ABA concentration) and biochemical (contents of the photosynthetic pigments and reactive oxygen species (ROS) enzyme activities) activities in drought-sensitive and drought-insensitive pearl millet lines and QTL NILs for dissecting the drought QTL and concluded that the pigment content and ROS machinery are not playing their vital role in pearl millet terminal drought stress. It was further concluded that the DT-QTL on LG2 is crucial for constitutive water storage mechanism and helps the plant at the time of grain filling during terminal drought stress (Kholova et al. 2011). The positive effect of this QTL on yield and other yield components under saline and alkaline conditions was also tested and confirmed (Sharma et al. 2014). Recently, multiple efforts are actively going on to introgress these important QTLs into elite lines using marker-assisted selection (MAS) to develop drought-tolerant pearl millet (Srivastava, Gupta et al. unpublished).

11.3.2   Nitrogen Use Efficiency (NUE)

The global demand for nitrogen (N) fertilizer for agricultural production, which already stands at ~110 million metric tonnes per year, is projected to increase to ~250 million metric tonnes by the year 2050 (FAO.org; Frink et al. 1999; Good et al. 2004; Tilman 1999; Tilman et al. 2011). India is the second largest producer and consumer of chemical fertilizers in the world (Abrol et al. 2017). Globally about 50% of human population relies on N fertilizer for food production (Smil 2001). Nitrogen (N) is the major fertilizer input, and it is the key nutrient element for crop productivity. For the complete realization of the higher yield potential, enhanced fertilizer N application is compulsory (Roberts et al. 2009). It is estimated that >40% of the human-consumed protein-nitrogen was derived from nitrogen fertilizer (Smil 2001). About 60% of the world's N fertilizer is used for producing three major cereals, namely, rice, wheat, and maize (Ladha et al. 2005). Because nitrate is very mobile in the soil, substantial amount (>50% in some cases) of applied N is lost by leaching, runoff and denitrification. In addition to increase in cost of crop production, in the long run these processes of N loss (Raun and Johnson 1999; Good et al. 2004; Hakeem et al. 2011; Prasad 2013) not only pollute the ground water and adversely affect soil structure but also have detrimental effects on environment such as increase in nitric oxide, ozone, etc. (Ramos 1996). 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). Nitrogen is an essential component of cellular physiology and present in several compounds including nucleoside phosphates and amino acids which are basic building blocks of nucleic acids and proteins, respectively. In general, N most often limits plant growth and agricultural productivity because naturally soil particles do not contain many N-containing minerals, and in addition N can be readily lost from the rooting environment (Duvick 2005). Most of the plant roots absorb N from the soil in the form of nitrate, which, aside from being assimilated to a minor extent into amino acids in the root, is transported to the leaf for reduction and assimilation (Crawford and Glass 1998). Nitrate is reduced to nitrite by nitrate reductase (NR) in cytosol, whereas nitrite is further reduced to ammonium by nitrite reductase (NiR) in chloroplast, and ammonium is then assimilated into amino acids or other compounds (Buchanan et al. 2000). Influx of nitrate into the root cells is accompanied by efflux, which is favored because the interior of the cell is negatively charged, and nitrate, being an anion, is taken up against an electrochemical gradient. Efflux goes up significantly as the concentration of nitrate around the root surface increases, accounting for up to 30% of the total nitrate absorbed (Volk 1997). Absorption and reduction of nitrate into ammonium are highly energy-intensive process suggesting that crops with improved NUE will save lot of energy thus the biomass and yield. Improving nitrogen use efficiency (NUE) in crops is one of the major initiatives for both private and public researchers because the outcome will not only increase the profit margins for the farmers by reducing the input costs but also save the environment from degradation by reduced use of chemical fertilizers.

Being its complex nature, NUE can be influenced by both internal and external factors. In India, the current average NUE in the field is approximately 33%. Through efficient N management practices, NUE can be improved if the cultivar is responsive. Thus, developing high NUE genotypes becomes the major objective for pearl millet breeding programs in the current agricultural scenario. High NUE cultivars can be defined by their ability to produce higher grain yields under low N inputs (Ladha et al. 1998). The core idea is to get the maximum possible yield with optimal nitrogen inputs which can compensate the compromised yield with economic and environmental benefits.

With the priority of low nitrogen input and sustainable and environment-friendly agriculture, NUE of pearl millet becomes very important in modern agriculture. However, increase in global yield was not kept in pace with the excessive fertilizer usage and also subsequent increase of the cost of cultivation and immediate negative environmental impacts. The primary goal of utilizing nitrogen fertilizers is to increase in yield per a unit land area, for this achievement fertilizer nitrogen usage must be within the optimum and economic level (Firbank 2005). However, the fertilizer demand of high yielding varieties and hybrids was high; N fixation and organic nitrogen recycling were not in line with the food requirements of the growing global population. In the past few years, conventional breeding practices improved the crop productivity as well as the NUE. Thus, to minimize the loss of nitrogen, a comprehensive approach to reduce or optimize nitrogen usage, improvement of NUE for genotypes, and their N management strategies were urgently needed. Several critical reviews elaborated the challenges associated with excess fertilizer nitrogen usage

Fig. 11.5 Differential N response of pearl millet lines under field condition at ICRISAT during summer season 2018

and the need for developing the NUE (Pathak et al. 2008; Garnett 2009). It was proposed that annual increase of 1% NUE could save $1.1 billion (Meena et al. 2016). Therefore, it is important to develop and cultivate the crop varieties with higher NUE for reducing nitrogen loss to the environment for sustainable agriculture. Several workers have made attempts to study the genetic differences of nitrogen uptake and yield per unit nitrogen application in crop plants such as rice, wheat, maize, sorghum, and barley (Ortiz-Monasterio et al. 1997; Le Gouis et al. 2000; Presterl et al. 2003; Anbessa et al. 2009; Namai et al. 2009). In pearl millet systematic attempts are in progress to understand the regulatory mechanisms, which control nitrogen usage and metabolism, particularly in N-limited conditions. As part of "Cambridge-India Network for Translational Research in Nitrogen" (CINTRIN) project, scientists from ICRISAT are trying to identify nitrogen use efficient lines under low and high N conditions for the marginal and favorable ecologies, respectively. A total of 400 pearl millet cultivars including the association mapping panel (PMiGAP) and parents of mapping populations were grown in the precision field of ICRISAT at three (0%, 50%, and 100% of the recommended nitrogen doses) nitrogen levels in alpha lattice design with two replications during multiple seasons in the recent years (Fig. 11.5). The trials were evaluated for different morphological (five traits), physiological (five traits), agronomic (five traits), and yield characters (six traits). In two seasons, wide variations were observed among the measured traits. Irrespective of genotypes, in all measured traits, the highest grand mean was observed in N100, followed by N50 and N0. A few genotypes were shown good performance in minimum fertilizer condition. Data compilation and analyses are under way (CINTRIN, unpublished data). Some of the genotypes showing better chlorophyll content in all the three treatments, namely, IP16120, Jakhranas 8–35-2-P2, H77/833–2-P5, W504–1-1, and IP3201. Few mapping population parents, including Jakharana S8–35-2-P2/RIB 334/74-P1, are showing substantial differences in terms of chlorophyll content under N-0 conditions during summer 2017. These mapping populations seem promising for the mining of QTL/gene/genomic regions associated with chlorophyll content and can be effectively used for mapping studies (CINTRIN, unpublished data). Data obtained from the 400 pearl millet cultivars from the two seasons will be utilized for genome-wide association studies (GWAS). Based on the genotypic performance under three nitrogen levels, nitrogen-sensitive (NS) and nitrogen-insensitive (NIS) pearl millet genotypes will be used for transcriptome studies to identify nitrogen use efficiency genes in pearl millet.

11.3.3   Salinity Stress

Salinity is one of the major abiotic stresses in arid region crops where water evaporation is high and makes it unavailable to the plants. Salinity stress is severe in the regions affected by extreme drought and high-temperature stress which increase the capillary water to the upside and the soluble salts to the root zone (Várallyay 1994). Pearl millet is cultivated in less fertile soils with a minimum amount of organic matter and low levels of phosphorus (Yadav and Rai 2013). Pearl millet is a hardy plant that can survive in the saline lands for forage and grain production, though it can be considered as significant abiotic stress in the west and north zone of central Asia. Only limited information is known about salinity stress in pearl millet; there is an imminent need to understand the molecular and physiological mechanisms in order to develop the salinity tolerance in pearl millet. In pearl millet reduction in shoot nitrogen content and increase in K+ and Na + content are associated with salinity tolerance (Dwivedi et al. 2012). At vegetative stage, shoot biomass ratio and Na+ contents were considered as potential traits for screening salinity tolerance in pearl millet (Krishnamurthy et al. 2007).

11.3.4   High–Temperature Stress

In pearl millet, high-temperature stresses at both seedling and reproductive stages have a significant impact on crop establishment and its productivity. Pearl millet is well adapted to the arid regions, and most of the growth stages such as seed germination, photosynthesis, and coleoptile elongation require 35 °C (Garcia-Huidobro et al. 1985). The temperature above 35 °C could be harmful to the development of major cereals, but pearl millet could sustain growth as well as yield potential in the hot environmental conditions. It was reported that pearl millet seed germination occurs at 35–45 °C, further increase in temperature to 47 °C it decreases and more or less stops at 50 °C (Garcia-Huidobro et al. 1982). Increase in the temperature of seedbed causes poor plant stand which ultimately affects yield. It was reported that the pearl millet seedlings of the first 10 days of sowing are more vulnerable to temperature stress (Stomph 1990). Pearl millet breeding line H77/833–2 is tolerant to high-temperature stress and sensitive to drought; hybrids of it are extensively used in northwestern region of India (Yadav et al. 2014). Mapping population developed by this cultivar was used to map terminal drought tolerance, grain yield, and stover yield and yield component QTLs in pearl millet (Nepolean et al. 2006). Recently, it was reported that there is genetic variation for heat tolerance at seedling and reproductive stage among germplasm; the reproductive stage heat-tolerant breeding line, IP 19877, has shown same seed set as that of 9444, a tolerant check (Gupta et al. 2015). Multiple efforts are underway at ICRISAT to dissect heat tolerance trait in pearl millet at genetic and molecular levels.

11.4 Genomic Interventions for Biotic Stress Tolerance in Pearl Millet

Like any other crops, pearl millet is also susceptible to several biotic stresses. Major biotic stresses include fungal infections such as downy mildew, blast, rust, ergot, and smut which impact the pearl millet production and grain yield.

11.4.1 Downy Mildew (DM)

DM is the most devastating yield constraint for pearl millet production, caused by *Sclerospora graminicola*, an obligate biotrophic pathogen, which results in exhaustive yield loss up to 80% (Singh et al. 1993; Howarth and Yadav 2002). DM majorly attacks pearl millet panicles, and its effect ranged from minor symptoms to disasters when major fields have been destroyed. Moderate temperature (20–30 °C) and high relative humidity (85–90%) favor the DM disease incidence, and it is clearly identified by leaf chlorosis, leafy inflorescence, and seed set failure (Thakur et al. 2008). This disease was first reported in India and was considered a minor impact of pearl millet yield production until 1970. However, in 1970–1971 estimated annual grain yield of popular Indian pearl millet hybrid (HB3) is approximately 8.2 million metric tonnes (Singh 1995). In the following year (1971–1972), severe yield loss of HB3 has been observed to about 4.6 million metric tonnes due to epidemic of DM (Dwivedi et al. 2012). So far, different techniques and resources have been developed for the identification and screening of virulent traits for DM at different ICRISAT centers. Further, effective phenotypic methods are also developed for DM field screen (Jones et al. 2002; Thakur et al. 2008). Recently Siddaiah et al. (2017) published the draft genome sequence of pathotype 1, which is one of the most virulent pathotypes of *S. graminicola* from India. This sequence information will be useful for breeding program to develop DM resistance varieties. Development and commercialization of new hybrid cultivars have been increased in the last 10 years, which give raises to new strains of DM pathogens. Almost 10 years back, in India six major DM pathotypes have been reported (Thakur et al. 2006). However, recently Thakur et al. (2011) reported about 20 virulent pathotypes of *S. graminicola* suggesting that in the coming years, new more virulent strains could be identified. The inheritance of DM resistance is a quantitative character. DM resistance shows dominance over susceptibility and recessive traits, though part of host plant resistance is governed by one or several genes along with modifiers (Hash and Witcombe 2001; Breese et al. 2002; Dwivedi et al. 2012). DM resistance QTLs have been mapped by screening *S. graminicola* pathogen and F4 mapping populations (from India, Nigeria, Niger, and Senegal), namely, LGD-1-B-10 x Blast ICMP 85410UK and 7042(S)-1 x and P 7–3 by field and greenhouse screening in India and the UK; it was also used to test the efficiency of greenhouse screen resistance indicators in field conditions. Two DM resistance QTLs, which are consistent, were

detected on LG1 and LG2 in field and glasshouse screens of the UK and India; however, LG1 QTL is showing up to 60% higher variation than LG2 QTL (up to 16%) (Jones et al. 1995, 2002). Number of loci which contributed about 17.4% and 47.7% of the total inheritance of the resistance to DM incidence and severity in pearl millet was reported (Angarawai et al. 2009). Jones et al. (1995) mapped a major DM resistance QTL detected on LG1 from India, against the Nigeria and Niger pathogen populations on LG4, and also on LG2, against Senegal pathogen population. Interestingly there was no QTL effective against all the four pathogen populations, suggesting that pathotype-specific resistance is a major mechanism of DM resistance in this cross (Jones et al. 1995). Liu et al. (1994) used F2 population plants to construct the map using RFLP markers. Several other workers used RFLP markers for DM resistance QTL mapping on these LGs (Breese et al. 2002; Gulia et al. 2007). SCAR marker was developed from inter-simple sequence repeats associated with DM resistance linkage group in pearl millet (Jogaiah et al. 2014). Hash et al. (2006) reported that gene pyramiding could be the promising approach to enhance the resistance toward diverse isolates of DM; in his study, pearl millet inbred lines (48) were studied against nine different *S. graminicola* isolates from five geographical regions across India to confirm the hypothesis. Developing DM resistance varieties has become the highest priority for pearl millet breeders. Resistance to DM has been reported in some pearl millet germplasm accessions and hybrids. Singh (1995) reported that ICMH451 and Pusa 23 are popular resistant hybrids; likewise, a top cross hybrid ICMH 88088 was developed by ICRISAT, highly DM resistant and also producing high yield. In India, four widely cultivated OPVs such as WC-C75, ICMS7703, ICTP8203, and ICMV155 are resistant to DM (Shivhare and Lata 2017). ICRISAT also developed a few DM resistance varieties for Western Africa, namely, ICMV1 and ICMV2 for Senegal and IKMP2, IKMP3, and IKMV 8201 for Burkina Faso (Singh et al. 1993). Development and commercial deployment of downy mildew-resistant version of HHB 67, a popular hybrid being grown in North India, are the first successful story of marker-assisted breeding (MAB) in field crops in public domain in India (Hash et al. 2006). A number of quantitative trait loci (QTLs) for downy mildew resistance have been identified on different linkage groups, and some of them are specific to different pathotypes (Hash and Witcombe 2001; Jones et al. 2002). DNA markers have been identified for about 60 different putative DM resistance QTLs in pearl millet (Breese et al. 2002; Hash and Witcombe 2002). A number of downy mildew-resistant QTLs effective against diverse Indian pathotypes of *S. graminicola* have been transferred to the commercial B-lines (843B, 81B) and R-lines (H 77/833–2, ICMP 451). In a recent study, Jogaiah et al. (2016) suggested the potential of the biocontrol agents to resist DM in pearl millet. A recent study using transcriptome analysis of DM-resistant and susceptible genotypes upon infection and control using NGS platform reported that, in the resistant genotype, upregulation of pathways for secondary metabolism especially phenylpropanoid pathway was observed. Upregulation of defense responsive transcripts, namely, R genes, HR-induced proteins, PR proteins, and plant hormonal signaling transduction proteins were also observed. In resistant genotype, the

Fig. 11.6 Downy mildew resistance and high grain iron (Fe) and zinc (Zn) content double QTL introgression hybrid trial at Bikaner, India, during rainy season (2017)

transcripts for V-type proton ATPase, purothionin, and skp1 proteins showed highest expression. This study also suggested the potentials of systemic acquired resistance and hypersensitive response as possible machinery operating the defense mechanism in pearl millet DM resistance (Kulkarni et al. 2016). During rainy season (2017), hybrid trial has shown high level of downy mildew resistance by introgression of double QTL (Fig. 11.6).

11.4.2   Rust

Rust is one of the major constraints for pearl millet production across the world and not only causes up to 76% yield loss but also affects fodder yield and its quality (Wilson et al. 1996). This disease was first reported on pearl millet in India during 1904 as being caused by *Puccinia substriata var. indica* (Wells et al. 1973). Effect of rust can be austere ranging from death of the affected young plant from early infection to desiccation or death of leaves with later infection. Several studies suggested that green yield, dry matter, and in vitro digestibility are negatively correlated with the rust incidence (Monson et al. 1986; Wilson et al. 1991, 1996). Several attempts have been made to identify the markers/ QTLs controlling rust resistance in pearl millet. It was reported that rust resistance controlled by a single dominant gene individually named as Rpp1, Rr1, Rr2, and Rr3, respectively, and rust susceptibility controlled by its recessive allele, with numerous diverse sources of the major gene and its quantitative resistance have been extensively studied, identified,

and exploited (Hanna et al. 1985; Singh and Singh 1987; Singh 1990; Wilson 1993, 1994; Wilson et al. 2001, 2006). A combination of RFLP and RAPD markers was used to map the Rr1 gene on LG3 in wild pearl millet (Morgan et al. 1998) though gene resistance was overcome by the pathogen in the southeast USA soon after its introgression in popular grain and forage hybrids followed by its backcross with a hybrid parent maintainer background Tift 85D2A1/85D2B1 (Hanna et al. 1987; Wilson 1993, 1994). Three segregating populations were screened with RAPD (random decamer) primers and RFLPs using a core set of single-copy markers (probes detected) on the pearl millet genetic map (Liu et al. 1994). Similarly, Morgan et al. (1998) also identified rest resistance genes in three segregating populations with RFLP and RAPD markers and resulted in *Rr1* rust resistance gene from the pearl millet subspecies *P. glaucum* ssp. *monodii* which was linked 8.5 cM from the RAPD OP-8350. In the same study, genetic linkage map was constructed with RFLP markers in both Tift 89D2 and ICMP 83506, while rust resistance genes were located linkage group 4. The linkage of two RFLP markers, Xpsm108 (15.5 cM) and Xpsm174 (17.7 cM), placed the Rr1 gene on linkage group 3 of the pearl millet map. Another RFLP marker Xpsm716 closely linked to rust resistance gene in ICMP 83506 effective against race PS92–1, but the gene was not found effective against Patancheru isolate of *P. substriata var. indica* in India (Sharma et al. 2009). In a recent study, an integrated high-density genetic linkage map was used to map the QTLs for rust in pearl millet by using DArT and SSR markers in a RIL population comprising of 167 F7 plants which are segregating for rust resistance developed from a cross between 81B-P6 (susceptible) and 9 ICMP 451-P8 (resistant). Out of the 167 RILs, 32 lines were resistant, 18 moderately resistant, 73 moderately susceptible, and 40 susceptible, and the remaining 4 lines were highly susceptible to rust. A major QTL for rust with 58% phenotypic variation was mapped on linkage group 1 with LD score of 27, and two small effect putative modifiers were also detected on LG4 and LG7 explaining 9 and 8.3% of the observed phenotypic variance. It was also reported that the novel rust resistance QTL which was identified on LG1 is also associated with the slow-rusting phenotype of importance (Ambawat et al. 2016). Few rust-resistant varieties have been reported in some pearl millet germplasm accessions and breeding lines (Wilson 1993; Singh et al. 1997). However, lines that were resistant in India became susceptible in the USA indicating the existence of different physiological races in India and the USA (Wilson et al. 1991; Tapsoba and Wilson 1996). Sharma et al. (2009) identified the four resistance lines including one B-line (ICMB 96222) and three R-lines (ICMR 0699, ICMP 451-P8, and ICMP 451-P6), and other four R-lines were susceptible to rest.

11.4.3 Blast

Blast or *Pyricularia* leaf spot is the second most serious fungal foliar disease of pearl millet in India and the USA causing substantial yield and forage losses. This disease is caused by *Pyricularia grisea*. Symptoms include grayish, water-soaked foliar lesions that further enlarge and become necrotic, causing extensive chlorosis and premature drying of young leaves (Wilson et al. 1989). Blast disease develops maximum during humid conditions specifically with dense plant stands (Hanna and Wells 1989). Pearl millet leaf blast was negatively correlated with dry matter yield, green-plot yield, and digestible dry matter (Wilson and Gates 1993). In rice, it was reported that blast affects the reduction in the overall growth, leaf area, 1000 kernel weight, dry matter accumulation, and grain yield (Bastiaans 1993). Resistance to blast in pearl millet was derived from the *P. glaucum ssp. Monodii* accession in which the rust gene (Rr1) was also identified (Hanna et al. 1987). Blast resistance in monodii accession was studied as three independent and dominant genes (Hanna and Wells 1989), though Tift 85DB, with resistance from monodii, was presented to have a lone resistance gene (Wilson et al. 1989) which is effective against all the tested *Pyricularia grisea* isolates. Several other sources of *Pyricularia* leaf spot resistance have been identified from Burkina Faso landraces. Each has been characterized as having dominant, single-gene resistance that is independent of the *monodii* resistance gene. Three pearl millet-segregating populations were screened for RAPDs (random decamer) primers and RFLPs using a core set of probes which detects single-copy markers on the map. In this study, one RAPD marker (OP-D11700, 5.6 cM) was linked to *Pyricularia* leaf spot resistance, and two other RFLP markers were linked to rust resistance genes (Morgan et al. 1998). In finger millet, Babu et al. (2014) developed 58 functional SSR markers for crucial blast resistance genes by using comparative genomic analysis with rice for population structure, genetic diversity, and association mapping approaches. Recent advancements in the pearl millet genome sequencing of reference genotype Tift 23D2B1-P1-P5 and resequencing of 994 pearl millet genotypes (Varshney et al. 2017) could be a great resource for breeders and genomics researchers, and will ultimately accelerate the efforts for identifying and mapping the resistant genes of important biotic and abiotic stresses. Two hybrid parent lines such as ICMB 06444 and ICMB 97222 were shown high level of blast resistance (Sharma et al. 2013.). These lines can be used for the development of high-yielding, blast-resistant pearl millet hybrids in India. Recently, hybrid HHB 146 has shown a high level of blast resistance by introgression of LG4 blast resistance QTL from 863B-P2 (Fig. 11.7).

Fig. 11.7 Blast-resistant LG4 QTL introgression hybrid HHB 146 improved (right) against the original blast susceptible hybrid HHB 146 (left)

11.4.4 Ergot and Smut

Ergot and smut are other fungal diseases in pearl millet. These two diseases are tissue specific which mainly infect the floral part of the plant (Thakur and Williams 1980). The causal agent of this disease (ergot disease) is *Claviceps fusiformis*, an ascomycete fungus, and symptoms of ergot include honeydew (cream to pink mucilaginous droplets) oozing from infected florets of panicle; these droplets contain asexual spores called conidia. This disease not only reduces the yield but also poisonous to humans, chicks, birds, and other animals (Kumar and Manga 2010). Ergot resistance is a recessive polygenic trait with notable cytoplasmic and nuclear interaction. The hybrid to be resistant for ergot, both parents, needs to carry the similar resistant alleles (Rai and Thakur 1995; Thakur and Rai 2003).

Smut is also an important floral disease of pearl millet in India, Western Africa, USA, and other countries growing pearl millet. It is caused by *Tolyposporium penicillariae* and reported to cause 5–30% yield losses in farmers' fields (Rachie and Majmudar 1980). So far there is no epidemics that have been reported, and the extent of yield losses caused by smut disease is quite variable (Thakur and King 1988). Panicle which is infected by the disease appears green, shining smut sori in place of grains on panicles; the sori mature within 2 weeks, turn into brown, and burst to release dark-brown to black spores which further infect healthy ear heads. Few studies reported that the resistance to smut is dominant and simply inherited (Phookan 1987). In another study, Chavan et al. (1988) reported that both dominant and additive gene effect for smut resistance, but the additive genetic effect was larger than dominance effect.

11.5   Future Prospects

In the current scenario of changing and un-predictive climatic conditions/events and global warming, pearl millet is going to be one of the crops of the future. Recent advances in the genetic and genomic resources of pearl millet have put it at par with other cereals like rice, maize, and wheat. ICRISAT led whole genome sequencing of the reference line and re-sequencing of nearly 1000 lines from all over the globe, and availability of more than 38 million SNPs on the world association mapping panel (PMiGAP) has thrown immense opportunities to mine and map novel alleles for both biotic and abiotic stress tolerance QTLs/genes. These newly developed ddRAD and WGRS-SNPs can be used in forward breeding in the development of hybrid parental lines and populations and in marker-assisted backcross (MABC) breeding programs. Some of the newly developed EST and genomic SSR markers have also shown promise in deciphering heterotic gene pools in pearl millet (Ramya et al. 2017). Knowledge on heterotic gene pools in combination with high-throughput genotyping and precision phenotyping platforms for important biotic (downy mildew, blast, rust, ergot, smut, *Striga*) and abiotic (drought, heat, salinity, low soil fertility) constraints may lead to an efficient pipeline for faster development of hybrid parental lines and improved populations suited to specific agroecologies of India, Africa, and other parts of the world. Pearl millet improvement programs need to constantly match itself with the evolving biotic and abiotic constraints. There is a need to keep pace with the shifting regional priorities as well. The genomics and breeding pipelines need to be constantly upgraded and aligned to deliver improved hybrid parental lines (A-/B- and R-lines) and populations tailored specifically to match the needs of specific agroecologies globally. An integrated approach combining modern genomic, molecular, statistics and data management tools and technologies is needed of the hour to expedite the breeding cycle to accelerate the rate of genetic gains by enhancing the yield and yield stability under stress.

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