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Abstract With an alarming concern of global climate change and increasing demand for drought-tolerant cereal staples, pearl millet, a widely exo-geographically adopted member of the millet family, has caught the attention for a robust genetic improvement. This chapter focuses on the various gene transfer technologies, both horizontal and vertical, employed so far in pearl millet to improve the strategy of introgression of newer traits and validation of gene function through transgenic development. This chapter also compares the different gene transfer technologies based on their exploitation in pearl millet development. It also accounts for the details of genes transferred so far, especially for conferring biotic and abiotic stress tolerance, in this crop. This chapter also discusses the future possibilities regarding the introgression of genes of new traits and technologies already utilized in other millets, which are hitherto unexploited for pearl millet.

Keywords Abiotic stress • *Agrobacterium*-mediated transformation • Biolistics • Biotic stress • Gene transfer • Pearl millet

4.1 Introduction

Millets, which rank as the world's sixth most important tropical food cereal (see FAOSTAT at www.fao.org), are grown mostly in the semi arid West Africa and India. Pearl millet is the only major cereal that reliably produces both grain and forage on poor, sandy soils under hot and dry conditions. In the drier regions of Africa and Asia, this crop is a staple food grain, besides being extensively utilized as a summer

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annual grazing crop in the southern USA (Hanna et al. 1997). The release of new high-quality forage and grain cultivars, combined with pearl millet's natural drought resistance, is gradually increasing the US commercial growers' interest in this underexploited, multipurpose crop.

Millet crops are grasses that belong to the family Poaceae of the monocotyledon group. Millets are staple foods that supply a major proportion of calories and protein to large segments of populations in the semiarid tropical regions of Africa and Asia (O'Kennedy et al. 2006). The semiarid tropics are characterized by unpredictable weather, limited and erratic rainfall, and nutrient-poor soils and suffer from a host of agricultural constraints (Sharma and Ortiz 2000). The projected food demand for 2025 will require the yield of cereals, including millets, to rise from 2.5 to 4.5 t/ha (Borlaug 2002). Production of millets, besides abiotic stresses, is also constrained by fungal diseases; so host plant resistance through alien gene transfer as well as genetic engineering is essential for conferring high level of resistance to improve the yield of millets (Ceasar and Ignacimuthu 2008).

4.2 Taxonomy and Gene-Pool Concept

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is a highly cross-pollinated monocot belonging to the Poaceae. It is one of the most widely cultivated drought- and high-temperature tolerant C4 cereals, being grown for forage, grain, and stover under dryland, rainfed, and irrigated conditions in drought-prone regions of the arid and semiarid tropics and subtropics and as a mulch in conservation tillage production systems in the humid and subhumid tropics. It is especially important as a staple food grain and source of feed and fodder for livestock, in hot, dry marginal agricultural production environments of Africa and South Asia that are home to hundreds of millions of the world's poorest farmers. The genus *Pennisetum* consists of more than 140 species of various ploidy levels that display annual and perennial life cycles and sexual, asexual, and apomictic reproductive behavior (Hanna 1987). Sexual reproduction through outbreeding is the most common mode of reproduction in cultivated pearl millet and its wild and weedy relatives. Harlan and de Wet (1971) classified *Pennisetum* germplasm into three gene pools on the basis of crossability relationships and following the biological concept of species. They reported that the primary gene pool consists of all the cultivated forms, their wild progenitor (*Pennisetum glaucum* ssp. *violaceum* (= *monodii* Maire)), and its weedy form (*Pennisetum glaucum* ssp. *stenostachyum* Kloyasch ex. A. Br., and Bouche). Species belonging to this gene pool are all diploid with $2n = 14$. Secondary gene pool comprises of only elephant or Napier grass (*Pennisetum purpureum* Schumach.), which is an allotetraploid ($2n = 4x = 28$) and rhizomatous perennial. All other species are part of the tertiary gene pool.

The classical botanists have tried to establish the genetic relationships between wild and cultivated pearl millets. Based on crossing of two wild \times cultivated crosses, annual wild and cultivated species have been grouped into a single biological species (Bilqueza and Lecomte 1969). Other study based on the excellent fertility

observed in hybrids derived from three wild×cultivated crosses also concluded these species as a single biological species but with three botanical subspecies corresponding to cultivated, wild, and intermediate types (Brunken et al. 1977). However, cultivated pearl millet has been classified into four basic races (*typhoides*, *nigritarum*, *globosum*, and *leonis*) on the basis of seed shape (Brunken et al. 1977). In subsequent studies, another series of hybrids (wild×cultivated) were studied, which did not show any divisions within the biological species (Belliard et al. 1980; Pernes et al. 1980). These authors defined it as a “domestication syndrome” which refers to the type of genetic organization of the characters differentiating wild and cultivated genotypes.

4.3 Genetic Resources

The International Crops Research Institute for the Semiarid Tropics (ICRISAT) is the only major repository of *Pennisetum* germplasm and has 21,191 accessions from 49 countries, including 20,503 accessions of cultivated types, 334 accessions of *ssp. monodii*, and 354 accessions of 22 other species. This collection is from diverse geographical areas including Africa, stretching from South Africa to Somalia in the east and Senegal in the west and Asia. In addition to this, few other centers also hold sizeable *Pennisetum* germplasm. Among these, there are three gene banks in the USA with a total of 6,637 accessions (including 805 accessions of 31 wild relatives) (Hanna and Lovell 1995), ORSTOM (Institut Français de Recherche Scientifique pour le Développement en Coopération) at Bondy in France (2,700 accessions), and ISRA at Bambey in Senegal (2,400 accessions) (Rai et al. 1997). Western Africa is the primary center of diversity for *Pennisetum*.

4.4 Vertical Alien Gene Transfer Using Cross-Compatibility

4.4.1 Conventional Approach

The utilization of wild germplasm for transferring the desirable alien genes has not been used widely owing to problems in cross-compatibility. Pearl millet has been hybridized with a number of wild species, including *P. orientale* L. C. Rich (Hanna and Dujardinm 1982), *P. schweinfurthii* Pilger (Hanna and Dujardinm 1986), and *P. setaceum* Forsk. Chiov. (Hanna 1979). Subsequently, cross-compatibility between diploid and tetraploid species was studied in detail (Dujardin and Hanna 1989). They reported that interspecific hybrids between pearl millet and *P. ramosum*, *P. megianum*, *P. macrourum*, *P. pedicellatum*, and *P. polystachion* showed cross-incompatibility. However, partial seed development in diploid pearl millet×*P. pedicellatum* or *P. polystachion* crosses indicated possibilities of using embryo culture technique for recovering interspecific hybrids. Though wild species *P. setaceum* showed cross-compatibility with pearl millet, this culminates in a dead end for the

breeders because of complete male sterility and poor female fertility with obligate apomixis. The use of *P. orientale* has also been limited for alien gene transfer due to lack of recombination between the genomes of the two species (Dujardin and Hanna 1989). The wild species *P. squamulatum* has been identified as a valuable species for transfer of important traits to pearl millet due to its high frequency as well as the high level of male fertility. In general, species with $x=9$ of the tertiary gene pool (*P. setaceum*, *P. orientale*, and *P. squamulatum*) have been shown more readily crossing with pearl millet than the $x=5$ (*P. ramosum*) and $x=8$ (*P. meianum*) species (Dujardin and Hanna 1989). Transfer of alien genes for rust resistance to pearl millet has been accomplished from a wild subspecies *P. glaucum* (L.) R. Br. ssp. *monodii* (Maire) due to chromosome homology (Hanna et al. 1985). Alien genes controlling earliness, long inflorescences, leaf size, and male fertility restoration have also been transferred from Napier grass, *P. purpureum* Schumach. (a wild allopolyploid species) for improving pearl millet. It could be possible due to one genome similarity with the genome of pearl millet (Hanna 1983).

Few efforts have been made at Tifton, Georgia, for using accessions of wild species, namely, *Pennisetum glaucum* ssp. *monodii* for rust and leaf spot resistance and cytoplasmic male sterility, *P. Purpureum* for greater stalk strength and male fertility restorer genes, and *Pennisetum squamulatum* for apomictic genes (Hanna 1992). More recently, the wild grassy subspecies *Pennisetum glaucum* (L.) R. Br. ssp. *monodii* (Maire) Brunken has been used as a source of germplasm for improved disease resistance and cytoplasmic diversity for improving pearl millet [*P. glaucum* (L.) R. Br.] cultivars. The hybrids for forage produced by crossing between the accessions of wild-species *monodii* from Niger, Mali, Senegal, and Burkina Faso and Tift 85DA1, a cytoplasmic-nuclear-male-sterile (CMS) pearl millet, showed significant increase in dry matter. This research indicates that alien genes from wild grassy subspecies *monodii* have enhanced the level and distribution of yield in cultivated pearl millet (Hanna 2000). The completely male-sterile lines were also produced in the wild cytoplasm background (*Violaceitum* and *P. mollissimum*). These were produced following the backcross method and resulted in inbred lines that resembled greatly to the cultivated parent. Restoration tests of this new male-sterile line indicate that it is different from the three known male-sterile sources A1, A2, and A3. Wild forms seem to possess a higher frequency of restorer alleles than the cultivated forms, regardless of their geographical origins (Marchais and Pernes 1985). Genetic diversity based on new molecular tools showed differences between the cultivated and wild groups in Niger. This study also showed introgression of cultivated alleles into wild accessions in the central region of Niger, while wild alleles were introgressed in cultivated species in the western, central, and eastern parts of Niger (Mariac et al. 2006).

4.4.2 Marker-Based Vertical Gene Transfer

In recent years, molecular marker-based alien gene transfer from wild species is being widely utilized in crop plants. In pearl millet, molecular markers have been

used to identify the genes for abiotic stress, and efforts were made to transfer such genes into cultivated background. Moisture stress is one of the important abiotic stresses that affects at various growth stages, but yield losses are maximum when moisture stress coincides with grain filling stage (i.e., terminal water stress). Genetic differences in tolerance to salinity and high temperature at both seedling and grain filling stages have been established and screening techniques standardized (Yadav et al. 2010). Use of conventional approaches to improve drought tolerance in pearl millet has been difficult despite the use of some novel approaches such as use of adapted germplasm, genetic diversification of adapted landraces through introgression of suitable elite genetic material, and exploitation of heterosis to amalgamate drought tolerance and high yield for enhancing yield under drought environments.

Molecular marker-based genetic linkage maps of pearl millet are available and genomic regions determining yield under drought environments have been identified. These efforts have paved the way for precise introgression of drought-tolerance-related traits through marker-assisted selection. The first molecular marker-based genetic linkage map of pearl millet, comprising largely of RFLP loci supplemented by a few isozyme loci, was reported by Liu et al. (1994). In subsequent years, the linkage map was expanded with SSR markers (Qi et al. 2004) and DArT markers (Supriya et al. 2011). A number of studies on quantitative trait loci (QTLs) for drought tolerance (Yadav et al. 2002, 2004), components of drought adaptation (Kholova et al. 2012), flowering time, and grain and stover yield (Yadav et al. 2003) have been mapped, and effective marker-assisted selection for several of these traits has been demonstrated (Serraj et al. 2005; Hash et al. 2003). These studies clearly showed that molecular markers can also be used to map the genes in the background of wild species, which can be precisely introgressed in cultivated background through marker-based selection (Rajaram et al. 2013).

4.5 Horizontal Alien Gene Transfer

Traditional breeding has been the main avenue for crop improvement in pearl millet. However, genetic transformation is a valuable tool useful to transfer alien genes for resistance to diseases, insects, and herbicides from those sources which are inaccessible through traditional plant breeding approaches. Using this approach several cereal crops have been transformed (Repellin et al. 2001). This approach can be used to test the effect of putative apomixes gene(s) from a wild relative (*Pennisetum squamulatum*; Ozias-Akins et al. 1998).

4.5.1 Biotic Stress

Fungal diseases are a major constraint in crop production imparting high yield loss. Significant yield losses occur in most of the agricultural and horticultural species due to fungal attacks. In Indian context, fungal diseases are rated either the most

important or the second most important factor contributing to yield losses in major cereal, pulse, and oilseed crops (Grover and Gowthaman 2003). Several successful attempts have been made to develop fungus-resistant cereal crops through genetic engineering. The chitinase gene has been considered best candidate for defending crop plants from fungal diseases. The first transgenic pearl millet expressing functionally active foreign gene conferring resistance to fungal disease (downy mildew) has been produced by Girgi et al. (2006). They used the antifungal protein (*afp*) gene isolated from the ascomycete, *Aspergillus giganteus*, using immature zygotic embryos as target for biolistic transformation. Transformation of pearl millet was confirmed by molecular analysis; the disease resistance also increased up to 90 % when compared to non-transformed control plants. Latha et al. (2006) have also developed a transgenic pearl millet conferring resistance to downy mildew disease by inserting a chemically synthesized prawn antifungal protein encoding gene (*pin*); embryogenic calli were used as target explants for bombardment. However, only two fertile transgenic pearl millets expressing functional foreign genes have been reported so far (Girgi et al. 2006; Latha et al. 2006). Chitinase and glucanase genes have also been shown promising against fungal pathogens. Hence, in the near future, these alien genes could be transferred in pearl millet in order to offer the prospect of conferring high levels of resistance against fungal pathogens and thereby improving pearl millet production.

4.5.2 *Abiotic Stress*

Significant genetic diversity exists among plant species for stress adaptation, hence elucidating the transcriptome from abiotic stress-adapted species. Several reports have shown that genes from stress-adapted species are functionally more efficient in imparting tolerance (Bartels and Salamini 2001; Whittaker et al. 2001; Mundree et al. 2002). Stress-adapted species and resurrection plants with very high tolerance threshold may possess mechanisms and genes which make them survive extreme conditions. A few recent studies have been initiated to understand the drought-tolerant traits in terms of identification of QTLs in pearl millet, exploiting the available pearl millet EST sequences to generate a mapped resource of 75 new gene-based markers for pearl millet. These demonstrated its use in identifying candidate genes underlying a major DT-QTL in this species. The reported gene-based markers represent an important resource for identification of candidate genes for other mapped abiotic stress QTLs in pearl millet (Sehgal et al. 2012). They also provide a resource for initiating association studies using candidate genes and also for comparing the structure and function of distantly related plant genomes such as other Poaceae members. Also, these candidate genes can be used for thoroughly elucidating its role in drought tolerance in other species and to develop transgenics. Being an abiotic stress-tolerant crop, it is of not much relevance to transfer any other stress gene homologue from allied species into millets. On contrary, the abiotic stress tolerance genes of millets, although very little characterized and reported, are of great importance for enhancing stress tolerance in other plant species—indeed, a good example of bioprospecting.

4.6 Conclusions and Future Prospects

The wild gene pool contains important genes that might be transferred to cultivated pearl millet and utilized in creating useful genotypes. However, interspecific hybridization requires great efforts depending upon the relationships of the species crossed. In general, use of wild species for transferring the alien genes has not been accomplished widely in pearl millet as compared to other cereal crops. Therefore, more efforts are required to study species relationships and utilization of many different accessions of many species. This can result in identification of readily crossable accessions of wild species. The efforts can also be made on attempting many crosses before producing one or a few interspecific hybrids.

Genetic transformation is an important approach that can be used to transfer alien gene from wild species as well as other sources. Therefore, efforts made in the *Agrobacterium*-mediated transformation of other cereals need to be extended to millets in the near future to produce transgenic millets expressing agronomically important foreign genes. This will greatly help to improve millet production by conferring resistance to biotic and abiotic stresses.

Conservation of phenological diversity is an important aspect for the future pearl millet improvement and sustainable use. Orthologous genes have been identified in pearl millet for flowering on the basis of candidate gene-based approach. This approach can also be extended to identify the agronomically important genes in the background of cross-incompatible wild species. It well known that future of pearl millet improvement through genetic engineering largely depends upon discovery of novel genes attributing the potent water deficit stress tolerance property of this important crop. As the recent addition of millet improvement, the foxtail genome analysis discovered a family of 586 genes functionally annotated as “response to water,” which further indicates that foxtail millet-specific genes might be related to adaptation of foxtail millet to semiarid environments. Thus, identification and isolation of alien genes/novel genes through recent genomics tools and techniques can help to use them in pearl millet improvement using transgenic approach (Lakis et al. 2012).

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