Review Article

Current Status and Future Prospects of Marker Assisted Breeding for Genetic Improvement of Minor Millets

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A B S T R A C T

Millets, a powerhouse of nutrients, hold great potential in contributing substantially to food and nutritional security of the developing country. They are climate resilient crops and due to their unique nutritional characteristics, they contribute significantly in increasing demand for nutritious food and feed. Recently the government has renamed jowar, bajra, ragi and other millets as "Nutri Cereals", instead of "coarse cereals". These Nutri Cereals includes Sorghum (Jowar), Pearl Millet (Bajra), Finger Millet (Ragi/Mandua), Minor Millets: Foxtail Millet (Kangani/Kakun), Proso Millet (Cheena), Kodo Millet (Kodo), Barnyard Millet (Sawa/Sanwa/ Jhangora), Little Millet (Kutki) and two Pseudo Millets (Black-wheat (Kuttu) and Ameranthus (Chaulai) which have high nutritive value for production, consumption and trade point of view. Among millets, the sequencing of whole genomes of two millets sorghum and foxtail millet has been done. It will speed up the genomic selection of better performing millets by marker assisted breeding, QTL mapping, gene tagging etc. Marker assisted breeding can be speed up by doing multiplexed genotyping, next generation sequencing and targeted resequencing. Advance genomic tools can be applied in minor millets for analysis of germplasm resources, allele mining, QTL mapping, fine mapping, gene tagging, foreground and background selection, genome wide marker trait association for further improvement of millets. Thus detail analysis on all omics information i.e., genomics, transcriptomics, proteomics, and metabolomics could promote millets as model systems for biofortification of staple crops and enhance targeted nutritional values.

Introduction

Nutritional insecurity is a major threat to the increasing world's population that is highly dependent on cereals based diet which is deficient in micronutrients. Small millets are very promising agricultural entity and are the primary sources of energy in the semi-arid tropics and drought-prone regions of Asia and Africa. They gained remarkable importance in agriculture due to their resilience to climatic changes and drought tolerant nature. Millets are nutritionally superior as their grains contain high amount of proteins, essential amino acids, vitamins and minerals. With close genetic-relatedness to cereals,

comparative genomics has helped in deciphering quantitative trait loci and genes linked to protein quality in finger millet. Recently, transgenic expression of zinc transporters resulted in the development of high grain zinc while transcriptomics revealed various calcium sensor genes involved in uptake, translocation, and accumulation of calcium in finger millet. Biofortification in millets is still limited by the presence of antinutrients like phytic acid, polyphenolic compounds, and several tannins. To reduce these antinutrients recent RNAi and genome editing tools [zinc finger nucleases (ZFNs), transcription activator like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeats (CRISPR)] needs to be employed (Vinoth *et al*., 2017) to get significant nutrients in millets. DNA markers have enormous potential to improve the efficiency and precision of conventional plant breeding via marker-assisted selection (MAS) (Collard *et al*., 2008). Several hundrade pearl millet SSR markers were developed using available sequence information from 3520 expressed sequence tags (ESTs) (Senthilvel *et al*., 2008). The molecular research in little millet is very limited and very less number of molecular markers has been reported. For construction of genetic maps and MAS more genome wide analysis of minor millets are required. The genome sequence of millets can be used for rapid identification of simple- sequence repeats (SSRs), InDels and single nucleotide polymorphisms (SNPs). Improved nutritional quality, resistance to biotic and abiotic stresses and resistance to fungal infection are major thrust area for millet improvement programme and it can be achieved faster by applying recent biotechnological tools.

Minor millets as great source of nutrition

Millets are commonly referred as "small seeded grasses" and currently referred as "Nutri Cereals" due to their extraordinary nutritional values. Several classes of minor millets has been included in that category i.e., pearl millet (*Pennisetum glaucum* L), finger millet (*Eleusine coracana* L), foxtail millet (*Setaria italica* L), barnyard millet (*Echinochloa* spp.), kodo millet (*Paspalum scrobiculatum*), proso millet (*Panicum miliaceum* L.), and little millet (*Panicum sumatrense*) (Fig. 1). Among them, pearl millet occupies 95% of the production (Agricultural Statistics, Government of India, 2014), Foxtail millet [*S. italica* (L.) P. Beauv] is the second largest crop among the millets, Finger millet is the sixth largest crop under cultivation, Proso millet is a short-season crop, and Barnyard millet is the fastest growing among the millets with short harvesting period of 6 weeks. Millets are comparable to rice and wheat in protein and fat and are superior to them in other nutrients as they contain a high amount of proteins, dietary fibers, vitamin, calcium, iron, zinc, phosphorus, potassium, and other essential amino acids. According to the Indian Council of Medical Research (ICMR), compared to rice Foxtail millet has 81% more protein, Little millet has 840% higher fat, 350% higher fiber and 1,229% higher quantity of iron. There has been demand to declare these crops as Nutri Cereals to boost its demand and allow farmers to get higher prices. But the presence of antinutrients like phytates, polyphenols, and tannins reduce the mineral bioavailability by chelating multivalent cations like Fe, Zn, Ca, Mg and K.

Recent advance in marker assisted millets breeding

It is well known that germplasm resources are rich source of useful genes and have been successfully used in traditional breeding efforts to improve several crop plants. Trait-specific germplasm characterization is primary requirement to identify genotypes contrasting for desirable traits. In a study, the Solexa

sequencing technology and the Genome Analyzer ii (GA ii) were employed to re-sequence the genome of a foxtail millet landrace, Shi-Li-Xiang (SLX) and to analyze its genetic structures (Bai *et al*., 2013) and provided a rich tag library for future genetic studies and molecular breeding of foxtail millet and its related species. Most agronomically important traits are determined by several genes or multiple loci, named quantitative trait loci (QTL). It is important to rapidly identify each locus of QTL for efficient crop breeding by molecular markers, which can be rapidly done by high-throughput sequencing-based genotyping technologies on the whole-genome scale. Currently, there are many methods used in QTL identification, including genome-wide association studies (GWAS), QTL-seq, MutMap, SHOREmap, NGS etc. With the further rapid development in sequencing technology, marker/genomics-assisted crop breeding can be done in a quick and cost-effective manner, which will speed up millet genetic improvements. Gimode *et*

al., (2016) have identified SNP and SSR Markers in Finger Millet by Next Generation Sequencing Technologies using both Roche 454 and Illumina technologies. Wang *et al.,* (2017) reported a high-density genetic map and QTL analysis of agronomic traits in foxtail millet [*Setaria italica* (L.) P. Beauv.] using RAD-seq and identified 11 major QTLs for eight agronomic traits and five co-dominant DNA markers were also developed. A finger millet genotype ML-365 sequenced recently using Illumina and SOLiD sequencing technologies (Hittalmani *et al.*, 2017). Recently Kumar *et al.,* (2018) reported large effect iron (Fe) and zinc (Zn) content quantitative trait loci (QTLs) using diversity array technology (DArT) and simple sequence repeats (SSRs) markers to generate a genetic linkage map using 317 recombinant inbred line (RIL) population. Many other studies have been focused on drought tolerance and fungal diseases in pearl millet using marker assisted selection approaches.

Fig.1 Different classes of minor millets

Future prospects for millet improvement

Millets are highly nutritious crops feeding poor populations suffering from malnutrition in Asia and Africa. Further improvement in millets can be done by good grain qualities and significant amounts of nutrients having essential amino acids, minerals, and vitamins, bioavailability of nutrients need further improvement by reduction of antinutrients or by the use of novel promoters. Identification of novel germplasm of millet with appropriate nutrients can be done faster by using molecular markers. Identification of gene based trait specific molecular markers specially linked to nutritional traits will help in controlling these traits. Advent of next-generation sequencing platforms favors rapid sequencing of millet genome and there trait specific characterization. Omics information on millets should advance more rapidly as cereal crops in order to enhance their utilization in the fight against micronutrient malnutrition. Recent advancement in RNA interference and genome editing tools like clustered regularly interspaced short palindromic repeats (CRISPR) needs to be employed to reduce antinutrients of millets to make it more beneficial to feed growing population.

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