

Biofortification of Millets

Simranjeet Kaur and Chandanpreet Kaur

Chandigarh Group of Colleges, Jhanjeri, Mohali

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Introduction

Millets are commonly referred as “small seeded grasses” which include pearl millet [*Pennisetum glaucum* (L.) R. Br.], finger millet [*Eleusine coracana* (L.) Gaertn], foxtail millet [*Setaria italica* (L.) Beauv], proso millet (*Panicum miliaceum* L.), barnyard millet (*Echinochloa* spp.), kodo millet (*Paspalum scrobiculatum*), and little millet (*Panicum sumatrense*). Among the millets, pearl millet occupies 95% of the production.



Figure 1: Millet Map of India

Millets are nutritionally superior as their grains contain high amount of proteins, essential amino acids, minerals, and vitamins. Bio fortification of staple crops is proved to be an economically feasible approach to combat micronutrient malnutrition. Harvest Plus group realized the importance of millet bio fortification and released conventionally bred high iron pearl millet in India to tackle iron deficiency. Molecular basis of waxy starch has been identified in foxtail millet, proso millet, and barnyard millet to facilitate their use in infant

foods. 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. Bio fortification in millets can be achieved through two strategies: (1) by enhancing the accumulation of nutrients in milled grains and (2) by reducing the antinutrients to increase the bioavailability of minerals.

Characterization of Millet Germplasm for Grain Nutrients:

Success of bio fortification program lies in the sustainable utilization of PGRs for nutritional enhancement. International Crop Research Institute for Semi-Arid Tropics (ICRISAT) contains the largest collection of millet germplasm representing 27.4% of total crop accessions in the genebank (Figure 2). Of this, pearl millet constitutes the vast majority of germplasm represented by 23,092 accessions including landraces, cultivars, genetic stocks, breeding lines, and wild relatives. Finger millet germplasm consisting of 6,084 accessions is grouped under two subspecies, africana and coracana on the basis of morphology of inflorescence. Foxtail millet is a self-fertilizing species including 1,542 accessions from 23 different countries. Foxtail millet accessions are classified into three races, namely indica, maxima, and moharia and 10 subraces. Barnyard millet germplasm comprises of 749 accessions mainly from Japan and India. The major collections of kodo millet from India and USA account for 665 accessions. India is the prime contributor of little millet germplasm with 473 accessions.

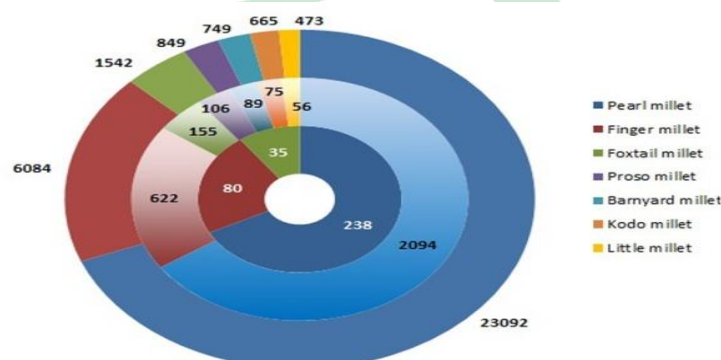


Figure 2: Germplasm collection of millet accessions in ICRISAT genebank. The outer concentric circle represents the entire collection of millets followed by reduced subsets of core and minicore collections in the inner circles.

Core and Minicore Collection:

Germplasm characterization at ICRISAT has led to the establishment of core and minicore collections for pearl millet, finger millet and foxtail millet while only core collections were established for other small millets.

Trait-specific germplasm characterization is a prerequisite to identify genotypes contrasting for desirable traits. Screening of pearl millet, foxtail millet, and finger millet accessions for grain nutrients revealed sufficient genetic variability. Multi-location on farm trials identified nutritionally superior lines with farmer preferred traits such as earliness to flowering and grain yield, adapted to local environments. This process accelerates the pace of breeding in millets by studying the inheritance pattern and genotype–environment interaction for grain nutrients. A thorough evaluation of small millets core collections for nutritional traits is the need of the hour to bring genetically diverse parents into mainstream breeding for generating recombinant inbred lines suitable for biofortification. The following section details the utilization of millet germplasm in biofortification in millets under the major headings: macronutrients, micronutrients, and antinutrients.

Macronutrients

1. **Starch:** Millets are the primary source of carbohydrates in tropics and semi-arid tropics of India. Grain starch typically comprises of two polymers, amylose (15–30%) and amylopectin (70–85%). Based on the amylose content, millet accessions are classified into two major phenotypes, waxy and non-waxy. Waxy grains containing 0% amylose and nearly 100% amylopectin are glutinous in nature, easily digestible and therefore recommended as food for infants under 6 years of age. Amylose synthesis in millets is controlled by a single dominant waxy allele (Wx), while the recessive loss-of-function allele (wx) leads to the waxy phenotype with near 0% amylose content. The waxy gene product named as granule-bound starch synthase 1 (GBSS 1) is the key enzyme catalyzing the formation of amylose. Mutations in GBSS 1 result from insertions/deletions (InDels), transposable elements, and single base pair mutations. In millets, Wx gene was found to contain 14 exons and 13 introns.
2. **Proteins and Amino Acids:** Cereal proteins contain 1.5–2% lysine and 0.25–0.5% tryptophan while estimated average requirement is 5% and 1.1% for lysine and tryptophan. Finger millet on the other hand is high in essential amino acids than

cereals. High lysine and tryptophan in finger millet is attributed to the transcriptional regulation of amino acid catabolism genes by Opaque2 (o2), a basic leucine zipper (bZIP) transcription factor. o2 modifiers (Opm) downregulate lysine ketoglutarate reductase dehydrogenase and upregulate aspartate kinase resulting in free lysine and tryptophan in endosperm. Molecular characterization of Opm alleles using SSRs and SNPs can effectively identify quantitative trait loci (QTLs) influencing amino acid content.

Micronutrients

1. **Iron (Fe):** Iron (Fe) deficiency is reported in 79% of pre-school children of India and 56% of Indian women are reported to be anaemic. Fe supplementation program in India since 1970 failed to address the issue of iron deficiency. Recognizing biofortification as a feasible alternative for Fe delivery, HarvestPlus has developed high Fe pearl millet by conventional breeding. The first step in breeding crops for better nutrition is to evaluate the genetic diversity of available germplasm for target nutritional trait. ICRISAT, a member of Harvest Plus undertook the process of screening pearl millet germplasm for sources of high Fe density. High Fe bio fortified pearl millet provides twofolds higher iron than modern wheat varieties. This led to increase in iron absorption by 5–10% in around 35 million people consuming biofortified pearl millet. Feeding trial conducted in 2013 revealed that consumption of 232 g iron biofortified pearl millet flour/day resolved 65% more iron deficiency in Indian school children.
2. **Zinc (Zn):** Genetic enhancement of grain Zn content is possible by modulating the metal transporters that facilitate their uptake, translocation, and storage. Members of Zn-regulated transporters and Iron (Fe) regulated transporter-like protein (ZIP) family contribute to Zn homeostasis by either uptake or remobilization in intracellular compartments (Figure 2). Cereals and millets with high Zn seeds can be engineered by seed-specific expression of ZIP transporters. Initial success in transgenic development for seed Zn accumulation was recorded in rice. Recently, high zinc accumulating finger millet transgenic plants were produced by over expression of OsZIP1 driven by constitutive (35S) and endosperm-specific promoters (Bx17). Seeds of T1 transgenic

plants showed 10–15 mg/kg higher Zn than wild type and the difference further increased to 20 mg/kg in T2 generation.

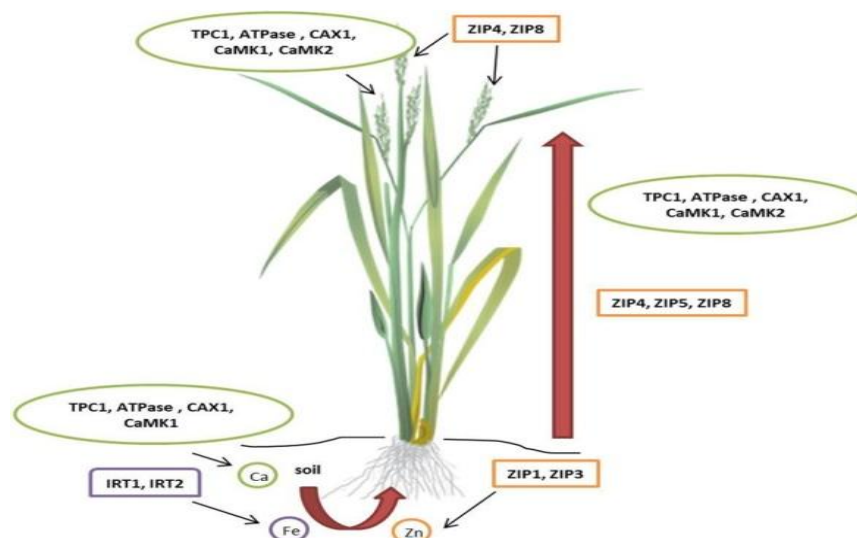


Figure 2: Molecular targets for micronutrient accumulation in millets

3. Calcium: Elucidation of role of calcium transporters in plants favours the development of Ca biofortified cereals. Finger millet containing about 5–30 times higher Ca than wheat and rice serves as a model system to understand seed calcium accumulation. Transcriptomics approach characterized calcium sensor gene family from the developing spikes of finger millet using Illumina paired-end sequencing methods which included characterization, identification, classification, phylogeny, and pathway analysis of calcium sensor genes of two genotypes, GP-1 (low calcium) and GP-45 (high calcium). In total, 82 calcium sensor proteins identified in the transcriptome of finger millet spikes were grouped into 25-calmodulin (CaM) and calmodulin-like proteins (CaML), 9-CDPK-related protein kinases (CRK), 9-calcineurin B-like protein (CBL), 23-CBL interacting protein kinases (CIPK), and 14-Ca²⁺-dependent and CaM-independent protein kinases (CDPK) genes. Comparative phylogenetic analysis of calcium sensor gene family in finger millet identified 12 calcium sensor genes.

Conclusion

Millets are highly nutritious crops feeding poor populations. As millets exhibit cross-genera transferability, introgression of nutrient-linked genes into other cereals can become feasible by the use of molecular breeding or genetic engineering. Advent of next-generation sequencing platforms favours rapid sequencing of millet genome. Omics information on



millets should advance more rapidly as cereal crops in order to enhance their utilization in the fight against micronutrient malnutrition. Thus, integration of knowledge on genomics, transcriptomics, proteomics, and metabolomics could promote millets as model systems for advancements in biofortification of staple crops.

