Comparative Genomics of Finger Millet

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Abstract

High synteny existing among the closely related genomes of cereals with millets paved the way for comparative genomics approaches in unraveling the genetic mechanisms and identification of orthologs and paralogs in the related species like finger millet. Recent publication of the finger millet draft genome helped in comparative genomics studies aiming at bridging the yield gaps in finger millet cultivation by finding the genes of important economic traits of interest. The present chapter describes the comparative genomic approaches for biotic and abiotic stress resistance as well as for quality traits. Also discussed is the comparison among the genomes of closely related species like pearl millet, foxtail millet and also with other crops like rice and maize.

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7.1 Introduction

Ragi is a highly nutritious crop having 18 chromosomes with tetraploid in nature, an important nutraceutical millet crop under the family Poaceae and the Eragrostideaetribe. It is normally called as ragi in India, and with other names in different places (Sood et al. 2017). It is a crucial food crop grown to some extent in dry parts of the world, especially in Asian and East African countries viz., India, Sri Lanka and China (Fakrudin et al. 2004). It is observed that the finger millet was evolved tentatively dated to the third millennium BC as per the oldest archaeological record, suggesting that domestication could have occurred in East Africa approximately around 5,000 years back. The finger millet crop is cultivated mainly by marginal and poor farmers which serves as a crop with more nutritional value crop due to its high calcium and fiber values and drought resistance nature (Dida et al. 2007; Kumar et al. 2012; Sood et al. 2019). Though the crop is neglected to a large extent, the production and productivity of finger millet crop is stagnating between 400 and 2,000 kg/ha (Dida et al. 2007). Earlier days conventionally the genetic changes in crops are characterized by

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variation in the morphological parameters in plants. Several studies were also proved by cytological analysis for depicting the genetic variation. With the advancement of molecular marker technology, considerable amount of growth in research of marker-assisted selection (MAS) was occurred in major crops except in minor millets, like finger millet. In crops like rice, maize and wheat these molecular tools are in wide use for analysis of molecular characterization, mining of quantitative trait loci (QTLs), association studies and MAS studies (Babu et al. 2018a; Kumar et al. 2016a, b).

7.2 Comparative Genomics

Finger millet has genome coverage of 1,593 Mb (Goron and Raizada 2015) and is monocot crop, cultivated by low income people of the world. But due to its nutritive properties, it is being considered recently as a high value crop by most of the rich people and is widely recommended for consumption by diabetic patients. It has wealthy nutrient composition of in comparison to rice, wheat and sorghum (Sharma et al. 2017; Gupta et al. 2017). Ragi is well recognized for remarkable more calcium (Ca) content (0.34%) of total seeds as against with cereals (0.01–0.06%) (Kumar et al. 2016a; Panwar et al. 2011; Gupta et al. 2017). The ragi is having rich amount of amino acids, fiber content, iron and trypsin inhibitory factors (Ceasar et al. 2018; Babu et al. 2014c; Sood et al. 2016; Chandra et al. 2016). In comparison to the major cereals like rice, the finger millet genome sequence has been postponed and was sequenced second after the foxtail millet genome, leaving it at back among cereals. The first draft genome for rice was released in the year 2005 (International Rice Genome Sequencing 2005) with the complete annotation in 2013 (Kawahara et al. 2013). Till 2013 foxtail millet is the one having whole genome sequence (WGS) among the millet crops. In the year 2012 only two genotypes of foxtail millet whole genome sequence were released (Bennetzen et al. 2012; Zhang et al. 2012). However, a big gap has taken place to release the second draft WGS of any millet that is finger millet, where the prime draft sequnce of finger millet was out in 2017 (Hittalmani et al. 2017), which is almost over a decade after release of rice genome and half a decade after foxtail millet genome published. Because of that very few genomic studies were conducted and in-depth high resolution analysis is yet to carry out after the sequencing in finger millet.

Comparative genomics studies will help to identify key genes of abiotic and biotic stress tolerance genes and nutrient strengthening into other crops. Prediction and identification of genes and pathway analysis using KEGG automatic annotation server shown that the carbohydrate and amino acid metabolism-related genes are more expressed in finger millet (Ceasar et al. 2018; Subramani and Manikandan 2019). This drought-tolerant crop can grow well even under harsh climatic conditions, due to its efficient carbon assimilating mechanism through the C4 pathway. The KEGG metabolic analysis combined with bioinformatics on ragi genome may help to be acquainted with key characters like abiotic traits and nutrient parameters, which can be applied to crops like rice and wheat (Subramani and Manikandan 2019). High synteny was revealed by genome colinearity among the cereals like rice and finger millet followed by foxtail millet by the workers (Subramani and Manikandan 2019). However, less synteny was observed between finger millet and maize (Subramani and Manikandan 2019). Though 60 million years of evolution have taken place for major grass families, it was shown that comparative genome analysis resulted in high synteny between grass genomes belonging to Pooideae, Panicoideae, and Ehrhartoideae (Subramani and Manikandan 2019).

7.3 Comparative Genomics with Other Species

Due to high colinearity and similarity in the grass genome sequences belonging to cereals, comparative genomics has been playing a key role in utilizing the crucial information present in the high value crops like millets especially, finger millet (Gale and Devos 1998; Moore et al. 1995). The impact of comparative genomics analysis is also important in major cereals as well as in minor cereals which paves the way for identifying the important metabolic pathways for yield and other characters. Proof for analogous genome sequences are already well established in wheat (Roder et al. 1995) and rice (Zhao and Kochert 1993), legumes (Weeden et al. 1992) and crucifers (Lagercrantz et al. 1996). These results are road maps for identification of major QTLs or genes of significant economic and morphological traits in minor cereal or millets like finger millet. The first report on syntenic regions between finger millet and rice was observed (Srinivasachary et al. 2007) using genic and genome-wide microsatellite markers. It was found that on an average 85% resemblance existed between these two genomes by using 218 markers. High syntenic relation observed among rice, finger millet and foxtail millet might be due to similarity in the gene sequences of these crops. It was also found that genome of finger millet remains greatly preserved though it diverged from a familiar predecessor of grass genomes like rice long ago 60 million years. The circus maps representing comparative analysis of finger millet and rice showed that chromosome number differences between these two genomes and rearrangements had taken place (Subramani and Manikandan 2019).

The similarity analyses between millets like finger millet, sorghum, foxtail millet, Brachypodium, and cereals showed maximum similarity, which has evidence of more closeness during evolution of these crops. Nearly 1592 similar regions were identified among rice and ragi; whereas among foxtail millet and finger millet it is 1709 and 436 between finger millet and Brachypodium, representing 97, 98, and 82% of genome of ragi (Hittalmani et al. 2017). These syntenic relations will help in mapping the orthologous genes/QTLs of interest. These QTLs will be further used for MAS in transfer of desirable traits into most desirable genotypes. Babu et al. (2007) studied the cross transferability of 345 rice genomic simple sequence repeat (SSR) markers into 12 finger millet accessions for their applicability in genomics studies. Among them, 58.6% of SSR nearly 202 showed its applicability. They observed higher synteny or similarity for yield-related traits followed by leaf and root traits (Fig. 7.1). Among the amplified microsatellites, polymorphism was observed with thirteen microsatellite markers among GPU48 and VR708, however five were found polymorphic among PRM801 and GE86. They also studied similar kind of analysis for cross transferability, identification of polymorphic markers and genetic diversity of finger millet between maize and finger millet using 64 maize SSRs. Out of 64 microsatellites, 43 (67%) were present in the finger millet cultivars. Similarities were observed between genetic diversity analysis in differentiation of finger millet geno types using markers of maize and ragi. Likewise, few finger millet and maize microsatellites at the genomic regions were used for their application in other millets like barnyard millet for genetic diversity, population studies and for other genomic studies. It was observed by the researchers that thirty-nine SSRs were generated reproducible amplicons in the barnyard millet cultivars.

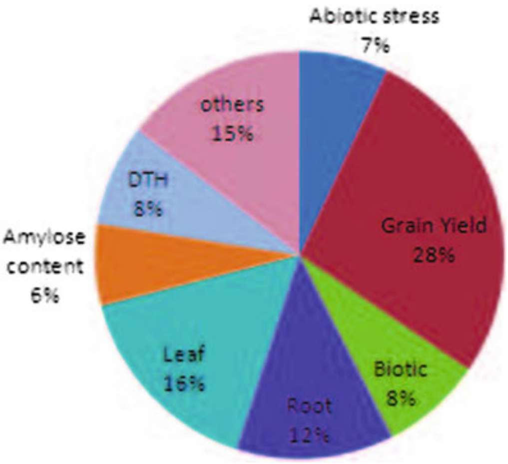


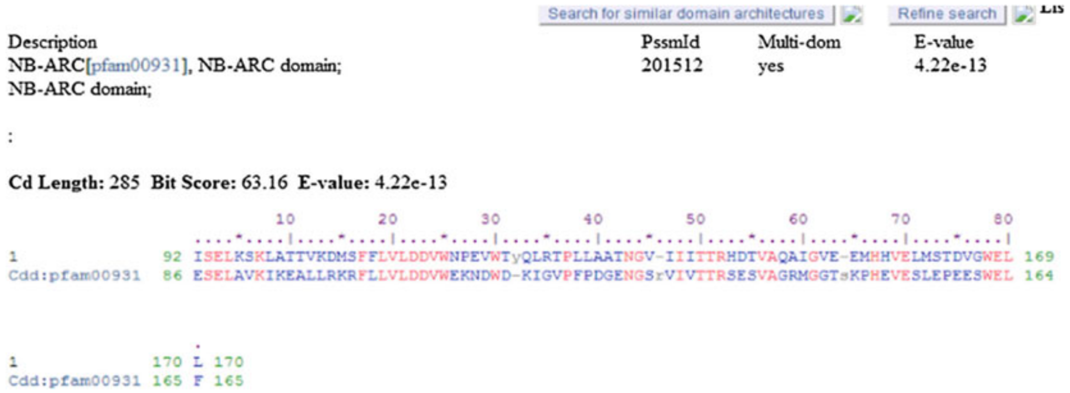
Fig. 7.1 Syntenic regions between finger millet and rice using transferability (Babu et al. 2017)

7.4 Comparative Genomics for Biotic Stress Resistance

Biotic stress conditions like pest and disease incidence play major role in influencing the yield levels of any crop including finger millet. In general *AVIRULENCE(Avr)* and *RESISTANCE (R)* genes govern the disease resistance in most of the crop species which codes for certain recognition factors. These hypersensitive responses are responsible for gene action combined by the restriction of development of pathogen colonization (Carine et al. 2008). They cloned nearly R-genes from many plant species using different molecular biology approaches (Okuyama et al. 2011). The nucleotide binding site—leusine rich repeat (NBS–LRR) also plays significant task in the disease resistance mechanism. These genes belong to major group of gene families and they are highly varied, depends on distinctive N-terminal features. These are used to isolate and classify motifs related to different amino acids which encode important genes. The ascomycetes, *Magnaporthegrisea*is the causal organism for finger millet blast which is a devastating disease. The approximate yield loss due to this disease is around 28%, but under severe conditions, it may be up to 80–90% (Babu et al. 2018b). The most common stages for pathogen attack are neck, finger and seedling stages. Till now, in case of finger millet very less or no R-genes were identified or mapped. Sequencing of NBS-LRR alleles specific to the resistant and susceptible genotypes was done by Babu et al. (2012) using the ABI 3130XL genetic analyzer. It was found kinase-2 and kinase 3a motifs of R-genes might be playing important role in finger millet defence mechanism (Fig. 7.2). So, these identified motif sequences of NBS region may be effectively used in molecular biology applications to trace out the defence-related genes.

7.5 Comparative Genomic Analysis for Blast Resistance Genes

Very few genomic studies were conducted in Finger millet and very much ignored crop. Data available on expressed sequence tags (ESTs) is very scarce in finger millet. In such cases the comparative genomics aids to compare with fully sequenced rice or maize genome to identify the similar genes related to economically important. Preliminary studies on molecular aspects on blast resistance were studied by Babu et al. (2014a) where they used few microsatellites representing different QTLs or genes responsible for biotic stress like blast resistance in finger millet. They were identified in the coding regions, untranslated regions of 5′ and 3′ end in the expressed sequences of finger millet and rice. These expressed sequence were found to belong to NBS-LRR region of disease resistance like blast in finger millet such as *Pi2, Pi16, Pi21* etc. They found that SSR marker FMBLEST32 in finger millet and RM262 loci in rice were strongly



Description	PssmId	Multi-dom	E-value
NB-ARC[pfam00931], NB-ARC domain; NB-ARC domain;	201512	yes	4.22e-13

:

Cd Length: 285 Bit Score: 63.16 E-value: 4.22e-13

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                           10        20        30        40        50        60        70        80
                   ....*....|....*....|....*....|....*....|....*....|....*....|....*....|....*....|
1               92 ISELKSKLATTVKDMSFFLVLDDVWNPEVWTyQLRTPLLAATNGV-IIITTRHDTVAQAIGVE-EMHHVELMSTDVGWEL 169
Cdd:pfam00931   86 ESELAVKIKEALLRKRFLLVLDDVWEKNDWD-KIGVPFPDGENGSrVIVTTRSESVAGRMGGTsKPHEVESLEPEESWEL 164


                   .
1              170 L 170
Cdd:pfam00931  165 F 165
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Fig. 7.2 The NBS-LRR domains in finger millet as revealed by in silico analysis (Babu et al. 2014d) associated for blast resistance QTLs at a *P*-value of less than 0.007. This marker was developed from the key candidate gene *Pi5* which is reported to be responsible for resistance to *M. griseae* in rice (Wang et al. 1994). It was found that *Pi5* gene is a dominantly inherited gene which shows resistance to minimum of six races belonging to four lineages. The results also found that *Pi5* gene displayed resistance to diverse isolates and found to have broad-spectrum resistance. The results from the comparative studies showed that similar types of pathogen races may be responsible in causing the disease. So, based on these findings, the *Pi5* gene can be a potential or key candidate which may be playing important role in finger blast disease resistance. This gene in general codes for LRR motif, N-terminal coiled coil (CC), and NB domain.

7.6 Comparative Genomics for Abiotic Stress Management

Abiotic stress is a complex trait which is influenced by several parameters. Finger millet is a sturdy crop with tolerance to abiotic stress conditions like drought conditions which will act as a model crop in unraveling the genes responsible for drought tolerance. Several works identified that Pfam-dependent regulatory genes for drought stress discovered that many genes were found in more than nineteen Pfam domains. Even now, insufficient amount of researches were accomplished on the classification of key genes in ragi for key agronomic and economic traits. For instance, in case of tobacco, it was found that heterologous expression of *NAC1* gene of finger millet resulted in drought and salinity tolerance. It was also found that *PIN1a* plays major role in improvement of root hairs and lateral roots in finger millet. For acquisition of phosphorous from soil it was found that four phosphate transporter1 (PHT1) genes, such as *EcPHT1 1, 1;2, 1;3,* and *1;4* were involved. Different candidate genes are involved in several pathways like protein kinases (PF00069), BTB/POZ (PF00651), protein tyrosine kinases (PF07714), U-box (PF04564), universal stress protein family (PF00582), NAD-dependent epimerase/dehydratase family (PF01370), were mainly spread in ML-365 genome (Hittalmani et al. 2017). These linked quantitative loci were mostly associated with drought-tolerant genes like MYB, MYC, AREB and NF-Y transcription factors, (Singh et al. 2002; Vinocur and Altman 2005). Application of these transcription factors (TFs) is to study the association of transcription regulators and studying cis-acting elements for perceptive study on drought tolerance. So, characterization and prophecy of factors involving cis elements by promoter examination is an important aspect in finding the signaling networks and functional properties. Now-a-days novel technique like clustered regulatory interspaced short palindromic repeats (CRISPRs), RNAi, overexpression, transcription activator-like effector nucleases (TALENs), and zinc finger nucleases (ZFNs), are available for understanding the function of regulatory factors. The technical tools will have chief blow in creating resistant or tolerant varieties for abiotic stress breeding (Gaj et al. 2013; Rabara et al. 2014). Among the minor cereals and other millet crops, finger millet has rising quantity of calcium, tryptophan, sulphur, methionine, and fiber-containing amino acids. Adding up, it has mechanism of C4 carbon assimilation photosynthesis, exploits water and nitrogen resourcefully under arid regions with no harshly upsetting production (Hittalmani et al. 2017). They did sequencing and annotation of whole genome procedure of ML-365 finger millet at a size of 1196 Mb covering just about 82% of total genome. The finger millet genome was thought to be more similar or collinear with rice and foxtail millet compared to other crop plants (Hittalmani et al. 2017).

7.7 Comparative Genomics for Quality Trait Improvement

The major breakthrough happened in the finger millet improvement after the availability of finger millet rough draft genome sequence, published by Hittalmani et al. (2017). In their report, they made comprehensive description and identification of genes for different functions and prediction of the molecular mechanism of finger millet. This paved the way for validating the calcium and other nutrient transport and regulatory genes which are involved in grain filling for future research activities. In other words, in total the draft genome sequence will be a great source to meet the food and nutritional security especially in the developing and developed countries. Genes for nutritional parameters like Calmodulin gene (CaM) were cloned by molecular techniques from ESTs of *Eleusine coracana* which are freely accessible in dbEST database (www.ncbi.nih.com). It was found that 613 bp obtained from PCR reactions were consistently present in most of the cereals and millets except *Setari-aitalica*. Researchers like Manoj et al. (2010) eluted the amplified PCR products from gel, cloned, sequenced and did similarity search through BLASTn analysis. They also did multiple sequence alignment, motif prediction and analysis, and phylogenetic construction of tree. From these phylogenesis results, it was found that CaM genes were similar to all cereals except *Triticum aestivum*. The translation of CaM amino acid sequences confirmed that they also have conserved amino acids of 110 which were consistently pragmatic across many poaceace crops except sorghum. The CaM genes protein motif revealed a very close evolutionary relationship between different cultivars or varieties of finger millet like PRM1, PRM801 and PRM 701. They also showed relationship with *Hordeum vulgare* for their higher amount of seed calcium than rice and other millets. These works showed that dissimilarity at structure level in CaM genes has direct influential role in the calcium build up pathway. The in silico 3D-structural patterns identified revealed comparable model and high degree of preservation in CaM in terms of structure and relations with calcium ions, thus reflecting to additional inspection into the role of CaM same forms, with transport mechanism drawn in calcium assimilation (Manoj et al. 2010; Yadav et al. 2014, 2020).Yadav et al. (2014) developed SSR markers from the coding and non-coding sequences of for factors related to calcium and transport genes viz. calcium-binding proteins, and calcium-regulated protein kinases in sorghum and rice. They found a conserved behavior from corner to corner of the finger millet genotypes representing the mineral transport which remains preserved in plants and even microsatellite differences in them remain hidden all through the track of development.

It was also evident that structural closeness among the CaM genes in their probable functional activities and accumulation of nutrients like calcium. The similarity was also observed with reference to the finger millet varieties viz. brown, golden and white. Researchers amplified identical set of CaM genes through in silico analysis of PCR amplified products and cloned sequences (Nirgude et al. 2014). They were also checked with finger millet EST primers along with other EST-designed primers of sorghum, rice, maize and millets. All the cloned EST sequences were deposited to GenBank database and obtained the accession numbers.

The protein content of cereals is in general deficient in lysine and tryptophan amino acids which are highly essential. Generally, most of the cereals consisted of 1.5–2% lysine and 0.25–0.5% tryptophan which need more amount for healthier human metabolic activities. Young et al. (1998) opined that 5% lysine and 1.1% tryptophan are required for optimal human nutrition. The seeds of cereals and millets contain zein proteins which are resulted from mutation in the *opaque2* gene which resulted in reduced synthesis of certain seed storage proteins which belong to multigene family. Fascinatingly, a rice cDNA encoding a similar bZIP transcriptional activator, RISBZ1, was obtained from tissues of seeds (Onodera et al. 2001), demonstrating that the *o2* gene arise much early than the splitting of maize and rice evolution. The finger millet quality parameters like high tryptophan and lysine influenced by *opaque2* modifier genes will be improved by using molecular tools. Some researchers found SSR markers linked to *o2* genes in maize and rice. These were designed from 3’UTR, 5’UTR, CDS and intron regions of the sequences (Babu et al. 2014b). These EST sequences were selected from *opaque2* modifier genes of maize, and rice RISBZ sequences factors and sorghum *opaque2* modifier genes. The results of this study linked SSR locus FMO2-EST1 to protein QTLs at a *P*-value of 0.002. This marker was obtained from the rice gene, RISBZ1 which was found to regulate tryptophan content to maximum extent. The tryptophan and protein are inversely related and this marker may be down regulating the *o2* modifier genes (Babu et al. 2014b). RISBZ1 is a bZIP transcriptional activator sourced from seeds tissues of rice, and given a hint that orthologous genomic regions of *o2* modifiers may be present in the ancestors of cereals and millets before they evolute (Babu et al. 2014b).

7.8 Future Strategies

Though the finger millet genome has been sequenced recently, it led to many questions on the effective analysis of the whole genome, and its comparison with relative species at genome level. There is need to focus on identifying the specific genes for drought tolerance, important nutritional traits, and biotic stress resistance traits for their use in breeding better cultivars with more yield. This also will pave the way for enriching the genomics-assisted selection for drought tolerance in the related species. At the same time, researchers need to focus on improving the yield levels of finger millet, since the yield levels are very low and there is need to produce high-yielding cultivars.

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