A Nutritional Crop Factory of Quality Seed Storage Proteins in Finger Millet for Combating Malnutrition

10

Apoorv Tiwari, Supriya Gupta,
Pramod W. Ramteke, and Anil Kumar

Abstract

Finger millet is a nutrition-rich crop to combat starvation, malnutrition, and water security for peoples. This enables to explore opportunities to increase finger millet production with enormous capacity for agriculture aimed at enhancing the health of malnourished people. Implementation in basic and translation studies by newer approaches has resulted in the overwhelming growth of scientific data. Strategic innovations in agriculture, i.e., enough availability of food has led to the growth and choice of high-yield varieties with the availability of quality proteins having enriched amount of essential amino acids, i.e., >40% of eight essential amino acids in the building blocks of seed storage proteins (SSPs) as per the recommendation of World Health Organization. SSPs offer nutritional proteins for people and animals as well as a source of nitrogen and sulfur for germinating seedlings. Therefore, SSPs are closely related to our life. Currently, OMICS and other high-throughput platforms have been used for the characterization of high-quality proteins in cereals and pulses. Considering the nutritional significance of finger millet, attempts were made first time in our lab to identify the seed storage proteins of finger millet using genomics-transcriptomics transition approaches and identified many variants of albumin, globulin, glutelin, and prolamin proteins with four proteins were having more than 40% essential amino acids. Gene sequence of two SSPs, albumin (*fima1) and* prolamin *(fimp2)* having 9% lysine and 15.8% methionine, respectively, were cloned and recombinants proteins have been expressed in *E. coli* system. This chapter provides an overview of SSPs, including their categorization, accumulation in seed cells, and breakdown of SSPs during seed germination. Multi-OMICS and in silico approaches are

A. Tiwari
Department of Computational Biology and Bioinformatics, Jacob Institute of Biotechnology and Bio-Engineering, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj, Uttar Pradesh 211007, India

A. Tiwari · S. Gupta · A. Kumar (✉)
Department of Molecular Biology & Genetic Engineering, G.B. Pant Univ. of Agriculture and Technology, U.S. Nagar, Pantnagar, Uttarakhand 263145, India
e-mail: anilkumar.mbge@gmail.com

A. Kumar
Rani Laxmi Bai Central Agricultural University, Uttar Pradesh, Jhansi, Uttar Pradesh, India

P. W. Ramteke
Department of Molecular Biology & Genetic Engineering, RTM Nagpur University, Nagpur, Maharashtra, India

Department of Life Sciences, Mandsaur University, Mandsaur, Madhya Pradesh, India

A. Kumar et al. (eds.), *The Finger Millet Genome*, Compendium of Plant Genomes,
https://doi.org/10.1007/978-3-031-00868-9_10

helpful to identify the SSPs in finger millet along with the association studies to pin point the role of genes/alleles involved in grain protein accumulation besides prospects to produce bulk amount of recombinant proteins with nutritional superiority to use as nutraceuticals.

10.1 Introduction

Global cereal production is projected to expand by 375 Mt (Metric Tons), to reach 3054 Mt in 2029, mainly driven by higher yields (OECD-FAO Agricultural Outlook 2020–2029). Several short-term interventions have increased productivity but spoilt sustainability and eroded the resource base's ability itself, which leads to nutrient-deficient salty soil and a reduction in water supplies (Raza et al. 2019). Approximately one-third of the world's population lives in dried soil, which makes up 40% of the world's area. The World Bank report suggests that 815 million individuals globally face hunger as a challenge. In an agriculture-based country like India, the number of farmer suicides has risen to more than 50 a day on average, which depicts the sternness of the agriculture crisis even after a good production (Thomas and Taveriner 2017).

To satisfy world starvation (cereal demand) and increase farmers' incomes, sustainable crop substitutes are required and therefore the importance of millets in ensuring long-term food security cannot be overstated. The content of protein in millets is regarded to be equivalent or superior to the proteins of wheat (*Triticum aestivum*), corn (*Zea mays*), sorghum grain (*Sorghum bicolor*), and rice (*Oryza sativa*) (Kumar et al. 2018). Millets play a part in the design of contemporary foods such as multigrain and gluten-free cereals. Due to its richness in polyphenols and other bioactive compounds, millets are considered medicine for heart disease, diabetes, and higher stress and is also considering lower fat inclusion, releases sugars slowly (low glycaemic index) (Ugare et al. 2014). Finger millet is the sixth largest crop in provinces of Central, East Africa, and southern Indian regions, supplying vital nutrition (Vinoth and Ravindhran 2017). Finger millet is a grass belonging to Poaceae family and is frequently referred to as ragi in India. It has evolved as high nutritional value crop than other cereals and millets, primarily because of its elevated nutrients, vitamins, minerals, fiber, and quality proteins. Finger millet proteins have not been reported as allergenic as wheat. Finger millet can be produced and used as preventive medicine against osteoporosis and has calcium as high as 450 mg/100 g of grains (Puranik et al. 2017). Finger millet has numerous advantages like a large food in the livestock industry (Bhagwat 2019). This enables to explore opportunities to increase finger millet production in fields with huge capacity for agriculture aimed at enhancing the health of small-scale producers (Gupta 2017) (Fig. 10.1).

10.2 Food and Nutritional Security Require Adequate Protein

Proteins are substances that contain nitrogen and are composed of amino acids and serve as the muscle as well as other tissues' major structural component of the body. These are also used for the processing of hormones and hemoglobin. Proteins can also be used as fuel, but as an energy source, they are not the primary choice and required to be metabolized in the simpler form of amino acids for proteins to be used by the body (Hoffman and Falvo 2004). It has been identified that 20 amino acids are necessary for development and metabolism process. Among them 12 amino acids are considered non-essential, indicating the body will synthesize them and do not need to be ingested in the diet and the remaining 8 amino acids are not able to be synthesized in the body and are defined as necessarily implying to our diets (Lopez and Mohiuddin 2021). In order to fulfil the daily requirement for essential amino acids by the human body, a balanced intake of quality proteins are required in our diet. According to the recommendation of World Health Organization (WHO), the proteins having >40% essential amino acids in its structural composition are

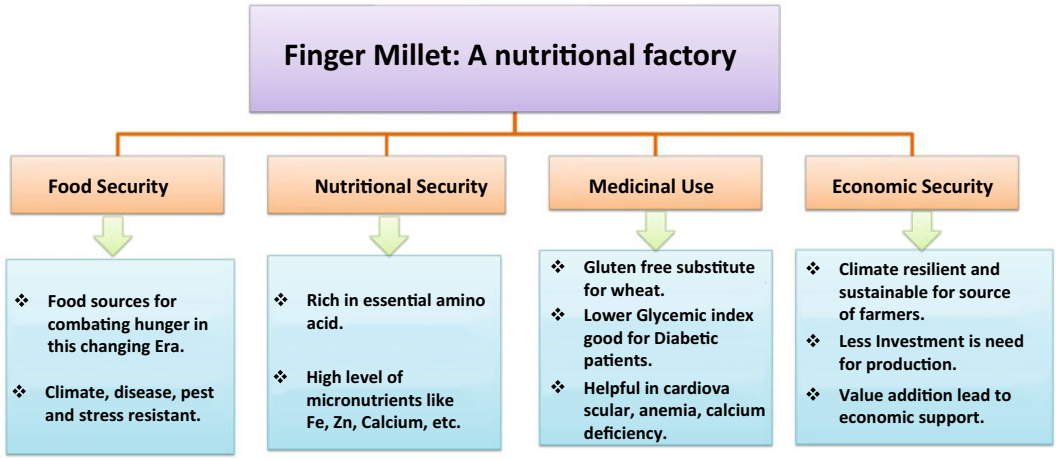

Fig. 10.1 Finger millet as a nutritional and economic security crop

referred to as quality proteins and nutritionally superior for not only combating protein energy malnutrition but also in turn augmenting the health security (Hoffman and Falvo 2004).

10.3 Protein for Life

Dietary methods for health promotion and independence sustenance in lives are required with a growing population partly by preserving muscle weight and endurance when individuals become old. New proof indicates that existing nutritional fiber intake suggestions may not be enough to accomplish this objective and that people may profit from enhancing their intake and incidence of high-quality protein consumption (Dwyer et al. 2015). However, there are doubts about the economic impacts of increased manufacturing of animal food, and substitute, more viable forms of food must be regarded. Protein is more required than other major macronutrients and has been uncertain if diets of plant protein impact the appetite of elderly people who are likely to become malnourished. In the last decade, a lot of attention was paid to plant nutrition (Lonnie et al. 2018). The protein requirements of aging populations (>40 years old), sustainable protein supplies, and dietary appetite-related consequences of plant protein were analyzed.

The ever-increasing demand for inherently protein-rich products forms a portion of an ecological discussion about encouraging more viable alternatives. In advanced nations, the elevated share of animal-based protein consumption increases safety and environmental concerns. The first of these is the enhanced danger of obesity, diabetes, cardiovascular disease death, and certain cancers in food-borne models characterized by elevated intakes of animal protein (Anand et al. 2015). However, it should be stressed out that the dietary model describes the whole diet and that all components in animal-based patterns (e.g., meat, fish, eggs, and dairy) have an equal and harmful effect on health cannot be stated (Battaglia et al. 2015; Birol et al. 2015; Campbell 2017).

The protein substance and the arrangement of amino acids differ between plant and cereal plant protein, methionine and cysteine are restricted, oats (lysine, tryptophan), nuts vegetables (cysteine, methionine, threonine), and ocean growth (histidine).

Investigating elective protein sources and progressing toward increasingly manageable, plant-based protein regimens has been an ongoing exploration need (Henchion et al. 2017). As indicated by the Food and Agriculture Organization (FAO) definition, practical weight control plans have “low natural effects which add to

nourishment and sustenance security and solid life for the present scenario." It has been very much reported that a plant-based diet can bring down the danger of cardiovascular disorder, sicknesses diabetes, hypertension, weight, metabolic disorder, and mortality, just as anticipate explicit kinds of malignant growth. Human food safety needs the processing of enough protein and dietary energy of high quality (Petrie et al. 2018).

10.4 Seed Storage Proteins of Finger Millet

Seed storage proteins are proteins that accumulate considerably in the growing seed and act as a reserve for nitrogen, carbon, and sulfur. During seed germination, these proteins are rapidly mobilized and provide the primary source of reduced nitrogen for developing seedlings. Storage proteins are not commonly involved in enzymatic functions. Despite the fact that storage proteins from different plants have different architectures, they all share some similar characteristics (Fujiwara et al. 2002). Storage proteins have a higher proportion in some tissues at a specific stage of development, which is one of their major features. Protein bodies are membrane-bound organelles that accumulate proteins and storage proteins are kept separate from the cell's metabolic compartments by being sequestered within protein bodies. Seed storage proteins are among the first proteins to be identified and are the primary source of plant proteins in the human diet. For example, in 1745 (Beccari 1745), wheat gluten was first extracted, and in 1859, Maschke had crystallized Brazil nut gluten. Nonetheless, literature on protein storage from seed goes back to the turn of the century, when Osborne (1924) classified them based on their solubility in water (albumins), distilled saline (globulins), fatty acid mixtures (prolamin), and diluted acids or alkaline solutions (glutyls). These were listed in classes and albumins, globulins, and prolamins are the key storage proteins for seeds (Shewry et al. 1995). In certain stages of development, plants produce storage substances such as starch, lipids, and proteins. The retention of proteins in the preservation of vegetative and reproductive tissues acts as a repository for the potential use of plants. SSPs are a group of proteins produced primarily during seed development and stored for the creation of embryos during germination in seeds that serves as nitrogen sources. The cereal grain's total protein content is 10–15% of its dry mass. SSPs normally occur inside the membrane of the vesicle (protein bodies, aleurone grains) in an aggregated state. Cereal seeds are synthesized as a nitrogen supply for germinating plants and used as a source of human and livestock nutrients. Because the SSP accounts for a considerable portion of the whole seed nutrition, the nutritional efficiency of the seed is linked to the value of the SSP and the structure. Soybean globulins are nutritionally and practically useful in the food industry (Tandang-Silvas et al. 2011). The protein content of seeds ranges from 10% (in cereals) to 40% (in nuts) (Shewry et al. 1995). The genes encoding the Triticeae's primary storage proteins have two origins: an ancient gene family found in the progenitor of monocotyledonous and dicotyledonous plants and a sequence of more recently evolved repetitions. Cereal seeds have a high concentration of storage proteins, which are degraded during germination to supply nutrients to the seedling (Gaur et al. 2018a). The cereals, which typically account for more than 50% of the total endosperm proteins, are analyzed in more detail. Prolamin storage proteins are usually maintained and organized into protein bodies within the ER (Endoplasmic reticulum) (called zeins in Zea mays and kafirins in sorghum) which are also abundant in seeds of crop plants with large amount of leucine and alanine (Otegui et al. 2006).

Because of the abundance and influence on seed consumption, seed storage proteins are the most studied plant proteins. These proteins differ greatly in structure and characteristics across and between species. Nonetheless, the great majority

are classified into only four categories based on their solubility and sedimentation factors. The most extensively distributed seed proteins are globulins, which constitute the majority of storage protein fractions in dicotyledonous plants as well as certain cereals (oats, Avena Sativa, and rice) (Liu et al. 2017). Because both forms of protein are stored in certain species, the comparable structures of 7S and 11S globulins may enable their packing together in the same protein bodies. In some species, however, either one or both kinds of globulin are stored with prolamins (cereals) or 2S albumins. Prolamins and 2S albumins are also members of a same protein superfamily, with the majority of members sharing a conserved pattern of cysteine residues in a tightly folded helical domain (Gell et al. 2017). However, there is limited overlap between 2S albumins and prolamins. The 2S albumins are abundant in dicotyledonous seeds but have yet to be identified in monocotyledonous plants, despite the fact that their existence in fern spores indicates an origin prior to that of monocotyledonous and dicotyledonous plants (Table 10.1).

10.4.1 Prolamins

Initially, the prolamin superfamily was defined on the basis of the preserved cysteine residue sequence in sulfur-rich seed storage prolamins, monocotylase-trypsin, and 2S storage albumins inhibitors. Some low-molecular-weight allergy proteins, including soybean hydrophobic protein, non-specified lipid transfer protein, and α-globulins, have also been described as belonging to this superfamily (Kawakatsu et al. 2010). A group of eight cysteine residues conserved cysteine skeletons, with a distinct Cys–Cys and Cys–X–Cys motif (X representing any other residue). In the case of alpha-amylase/trypsin inhibitors, there are two extra cysteine residues. Members of this superfamily share a common 3D structure, in addition to the prolamins for seed storage that characterize the insertion of an extensive repetitive domain. This contains a bundle of four disulfide bonds in the nsLTPs that are stabilized by a lipid-binding tunnel, which has degraded into 2S albumin structures. Many of these proteins can lead to their protection

Table 10.1 Properties of seed storage proteins

S. no	SSPs	Properties
1	2S Albumins	• Soluble in water • Molecular weight (Mw) typically ~10,000–15,000 • These proteins are post-translationally processed to produce large and small subunits having two intra-chain bonds inside the large subunit and two inter-chain disulfide bonds. They are not glycosylated, and certain constituents are high in methionine
2	7S Globulins	• These proteins are soluble in the dilute salt solutions • Typically trimeric proteins of Mw 15,0000–19,0000 • Subunits can be glycosylated and proteolytically digested • Cystine and methionine levels are low or non-existent
3	11S Globulins	• In dilute salt solutions, it is soluble. Mw 30,000–45,0000 hexameric proteins are typical. Mw 60,000 subunits are generally post-translationally processed to yield Mw 40,000 (acidic) and Mw 20,000 (basic) chains linked by one inter-chain disulfide bond • Cystine and methionine levels are low • Rarely glycosylated
4	Prolamins	• Structures vary greatly, with component Mw ranging from 10,000 to 10,000,000. Monomeric forms and high Mw polymers stabilized by inter-chain disulfide bonds are examples. Proline and glutamine are abundant, while lysine and, in certain circumstances, tryptophan, threonine, and methionine are deficient. There will be no glycosylation or proteolytic processing • When native and/or reduced, it is soluble in alcohol/water combinations • A source of cysteine and methionine

against proteolysis by maintaining their allergenic properties. As a consequence of the repetitive domain abundant in the amino acids of proline and glutamine, the cysteine skeleton and α-helical arrangement that are normally typical of the prolamin superfamily have degraded in seed storage prolamins (Balakireva and Zamyatnin 2016). The physical–chemical properties of these repetitive domains of the seed storage prolamins are dominated by a loose spiral structure made up of a complex ensemble of unfolded and secondary structures containing overlapping structures including β-turns and poly-L-proline II. Proteins are normally insoluble in diluted salt, either in their native state or after inter-chain disulfide bonds have been reduced (Kawakatsu and Takaiwa 2010).

10.4.2 2S Albumins

2S albumins are a leading class of seed storage proteins usually synthesized as single chains of 10–15 kda in the seed, which can be processed in a translational manner such that small and large subunits are commonly combined with disulfide bonds. The system of processing differs depending on species, with sunflower albumins being single-chain albumins and Brazil nut albumins being two-chain albumins. They can act as both industrial (due to dust inhalation) and dietary allergens (Moreno and Clemente 2008).

10.4.3 Lipid Transfer Proteins

The name of these proteins derives from the fact that they were found in plants because of their capacity to transport lipids in vitro, but their exact biological function in plants is unclear. They can play a role in plant defence because their expression is regulated by abiotic stress and they belong to pathogenesis-related protein group 14. They are present in plant outer epidermal tissues such as the peel of peach or apple fruits, and their lipid-binding properties have led to the theory that they are active in transporting the cutin and suberin monomers to plant outer tissues, where they polymerize to form outer waxy layers. They are the most widely distributed type of prolamin, found in a variety of plant organs such as seeds, fruit, and vegetative tissues, and have been labeled pan-allergens. As a result, in addition to being included in a variety of fruits and nuts, they have also been reported as allergens in the pollen of many plant species (Vergnolle et al. 1992).

10.4.4 Bifunctional Inhibitors

This class of allergens is limited to cereals, individual subunits that serve as inhibitors of, amylases from insects, trypsin or both, giving rise to the word bifunctional allergen. These proteins can serve as allergens in wheat flour allergies, such as baker's asthma, or in food sensitization through the gastrointestinal tract. They were first found in extracts made up of chloroform and water and are often referred to as CM proteins, but they are often soluble in water, dilute salt solutions, or alcohol and water mixtures (Radauer et al. 2008).

10.5 What Are the Quality Proteins from Plant Sources?

Adequate human nutrition is dependent on the consumption of a variety of nutrients included in the food. Proteins, which include essential amino acids, are necessary macronutrients. The nutritional quality of a protein supply might vary in terms of digestibility, amino acid composition, and bioavailability containing cysteine and methionine. Thus, with an ever-increasing global population, a contemporary problem is the consumption of low-cost, easily available proteins that fulfil environmental and social requirements. Several well-known plant protein sources may feed the human diet and aid in overcoming the population growth issue (Henchion et al. 2017). Plant proteins, depending on the source, may be lacking in some important amino acids. Cereals often have low Lys levels, whereas legumes have a sulfur amino acid deficit (Met and Cys).

However, pseudo-cereals (such as amaranth and quinoa) are high in Lys. Also, due to changes in climate and soil diversity, geographic altitude and latitude, precipitation levels, agricultural techniques, and varietal/cultivars, the same plant might vary in composition (e.g., macronutrients, such as protein and oil content, and amino acid profile) (Cremer et al. 2014).

Millets contain significant levels of essential amino acids, including Lys, beyond the amounts suggested by WHO/FAO/UNU for humans, making it a good alternative for increasing protein intake in diets. Finger millet is an important source of protein for India's rural population. As a result, the protein composition and quantity of this cereal are critical. Prolamin and glutelin are the main protein fractions based on solubility fractionation. White finger millet genotype has more prolamin but less glutelin than brown finger millet. The increase in protein content of finger millet varieties is mostly due to an increase in the grain's prolamin fraction, as has previously been documented in a few other types of cereal. The amino acid composition of finger millet protein reflects the usual tendency for prolamin rise with grain protein content increase (Sachdev et al. 2020). The negative association seen between lysine levels and protein content of finger millet can be explained to the prolamin fraction's significant lysine deficit when compared to the other protein fractions. Varietal variations in glutamic acid, proline, valine, leucine, and isoleucine levels are also consistent with variances in prolamin content. Differences in the amino acid content of different maize types and sorghum have been attributed mostly to rising percentages of prolamins as total protein increases. The essential amino acid content of a protein can be used to assess its nutritional quality (Huang et al. 2004). Finger millet protein is relatively low in lysine but has acceptable levels of other necessary and related amino acids. However, when finger millet is the only source of protein in the diet, the quantity of protein in the grain is insufficient. As a result, it is profitable to produce finger millet genotype with increased protein content (Zamaratskaia et al. 2020).

10.6 Synthesis, Deposition, and Regulation of Seed Storage Proteins

SSPs accumulate in the endosperm from the early accumulation storage stage to the late accumulation storage stage. SSPs are produced on the rough endoplasmic reticulum (ER) (Fig. 10.2), although the timing of protein production changes throughout seed development. Albumin and globulin protein fractions are synthesized in the early stages of seed development and are deposited in the abaxial cotyledon surface of embryo and aleuronic tissue, whereas prolamin and glutelin proteins are synthesized in the later stages of seed maturation and are deposited in the protein bodies and protein matrix (Fontanini and Jones 2002).

During seed growth, the regulatory network of transcription factors, hormones, miRNA, and other genes organizes the accumulation of SSPs. The endoplasmic reticulum (ER) produces seed storage proteins, which are then transferred to protein storage vacuoles (PSV), where they are predominantly kept. The mature SSPs are delivered in two ways: Golgi-dependent and Golgi-independent. Seed growth and development are inextricably linked to the buildup of seed storage reserves. The seed growth method begins with twofold fertilization. The embryo and endosperm are protected by maternal integuments and a possible seed coat. Organic nutrients like sucrose, amino acids, and potassium are imported from the maternal tissue to the seeds during the early stages of development via vascular tissue within the funiculus, which terminates in the chalazal portion of the seed coat. The released nutrients are largely stored in the endosperm, which provides sustenance to the growing embryo. Transcriptional regulation of SSP accumulation occurs during the seed development phase. The transcription factors (TFs) LEC1, LEC2, FUS3, and ABI3 are known to play a role in seed development, SSP aggregation, and regulating the expression of several other metabolic pathway regulators in Arabidopsis (Gacek et al. 2018) (Fig. 10.3).

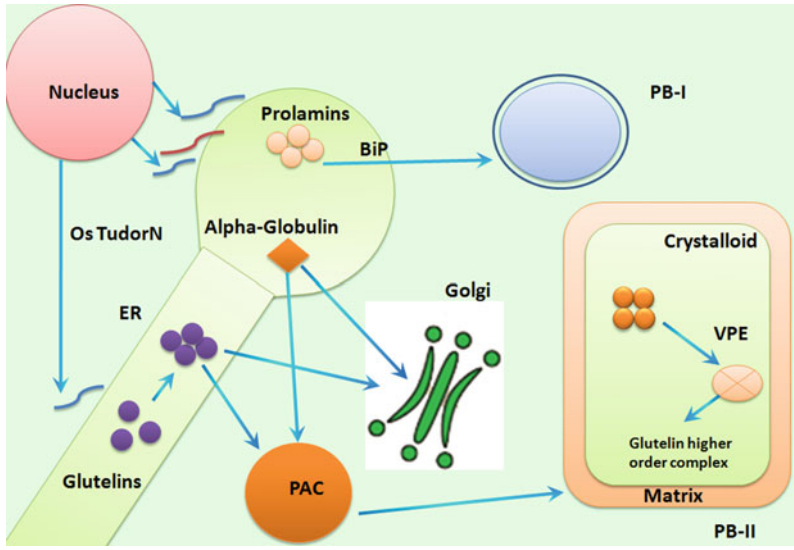

Fig. 10.2 Synthesis, trafficking, and deposition of different SSPs

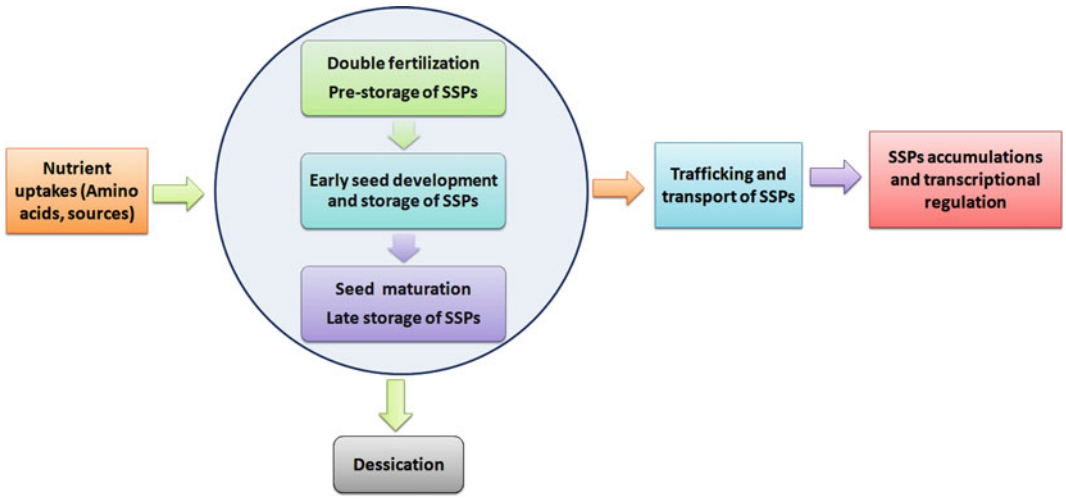

Fig. 10.3 Transcriptional regulation, accumulation, and translocation of seed storage proteins (SSPs)

10.7 Use of Omics Approaches for Studying Seed Storage Proteins

Advent of *OMICS* approaches has revolutionized the field of nutritional biology and use of such genomics-transcriptomics transition, proteomics, metabolomics, genotyping by sequencing, and other high-throughput technologies are being used in deciphering complex process of seed development, biology, and nutritional traits for understanding the mechanisms of accumulation of nutrients and SSPs during the different stages of seed development (Kumar et al. 2015).

10.7.1 Genomics for Identification of SSP Genes and Cloning of Quality Protein Genes

Seeds are the sources of a wide range of foods, magnesium, and amino acids, including phosphates and leucine**.** Different genomic resources/properties for finger millet have been made available in NCBI. Plant breeders will benefit from the genome sequencing initiative in terms of allele selection, genetic mapping, and recognition of candidate genes for agronomically significant traits. The availability of finger millet genomic resources will expand novel breeding opportunities to solve future problems in finger millet improvement. (Hittalmani et al. 2017). PBF (prolamin box-binding factor) transcription factors regulate the seed storage proteins of cereals (Gupta et al. 2011). However, advancements in laboratory techniques like as high-throughput sequencing and mass spectrometry have resulted in an increase in the output of protein information. This has demanded precise annotation, categorization, characterization, and decoding of these sequences biological function (Radhika and Rao 2015). Recently, the genome of finger millet is sequenced and efforts have been done to find and annotate the genes coding for SSPs. The experimental findings from the whole genome sequencing and assembly procedure of a finger millet cultivar yielded 1196 Mb, which accounts for approximately 82 percent of the overall predicted genome size. The existences of 85,243 genes were discovered by genomic technologies, and one-half of the genome is repetitive in nature. When compared to other Poaceae plants, the finger millet genome had higher co-linearity with foxtail millet and rice. Functional annotation and transcription factor mining showed that the finger millet genome contains a significant number of drought tolerance genes. In a recent study, we have identified 18 SSPs using genomic and transcriptomic approaches. Using NGS applications, a set of 18 SSP genes were identified in finger millet transcriptome and their physical location in the genome was defined. A comprehensive analysis was done by annotation and characterization of identified SSPs, while their expression pattern was analyzed by calculating fragments per kilobase of transcript per million mapped reads (FPKM). Molecular functions of all 18 SSPs were predicted using the gene ontology approach by mapping the blast, InterPro results. It was found that the genes were involved in different molecular functions like nutrient reservoir activity, molecular function regulation, enzyme regulatory-inhibitory activity, lipid, ion binding, and immunoglobulin binding (Hittalmani et al. 2017 and unpublished data).

10.7.2 Transcriptomics for Studying the Expression of SSPs in Developing Spikes

The finger millet has a volume of transcriptome series acquired under various stress conditions and for the consistency of the crop. There have hardly been attempts to combine specific genotypes, such as dry and saline effects, into the transcriptome (Rahman et al. 2014). In our lab, we also attempted for the first time to describe the de novo assembly of transcriptome data from two-finger millet genotypes that differed in seed protein and calcium content. The transcriptome data collected may be utilized to uncover genes expressed throughout spike formation as well as to build useful molecular markers. The annotated genes are characterized using transcriptomics and proteomics techniques based on the transcriptome data (Kumar et al. 2014). The data of developing spikes transcriptome is quite useful in the identification and annotation of seed storage protein (SSP) genes. A total of 10 SSP genes were identified rich in essential amino acids. The recent advancement of the molecular biology approach became useful to find out novel and multifunctional genes from the transcriptome data and further cloning. The two out of four quality proteins identified using developing spikes transcriptome later their presence in genome of finger millet and having >40% essential amino acids were cloned in our labs and expressed in *E.coli* expression system (filed

patent no. 201711006104). The four quality proteins identified in developing seeds of finger millet have been depicted in Fig. 10.4. One such gene has 9% lysine and another having 15.8% methionine in its amino acid composition and can be harnessed for nutritional quality improvement of other staple cereals lacking lysine and methionine as one of the essential amino acids (patent no. 201811033469) (Fig. 10.4).

Attempts were made to clone as per transcriptome data, the full open reading frame sequence of the lysine-rich albumin *fimA; fi:* finger, *m:* millet, *A-1:* albumin *1* and methionine-rich *fimP2; fi:* finger, *m:* millet, *P-2:* prolamin, high methionine-variant 2, containing 9% and 15.7% methionine, respectively. After cloning, the expression construct was made for heterologous expression. The recombinant proteins *FIMA1* and *FIMP2* expressed in the bacterial system can be further purified for nutraceutical purposes. The unique properties of the protein were disclosed by using computer-based in silico tools and validated subsequently through experimental approaches. Such gene can be employed for the development of nutraceuticals through genetic engineering and heterologous expression in a bacterial system (Le et al. 2016). The cloning and expression strategies have been given in Fig. 10.5.

Two patents have been filed for both the *FIMA1* and *FIMP2* storage proteins. The amino acid analysis of cloned genes revealed 45 and 46.5% EAAs including lysine (9%) and methionine (15.8%) in the cloned SSPs. The recombinant protein was expressed in bacteria and it was isolated and purified. Albumin-1 and Prolamin-2 are further characterized for its physicochemical properties. The importance of the protein having high lysine and methionine content is the target entity to use as a nutraceutical for improving human nutrition. Further in silico approaches were utilized to identify its unique properties. The tissue-wide expression analysis was performed using quantitative PCR analysis for determining the specificity of temporal and spatial expression (Fig. 10.5). Functional properties of the protein taken in the diet of

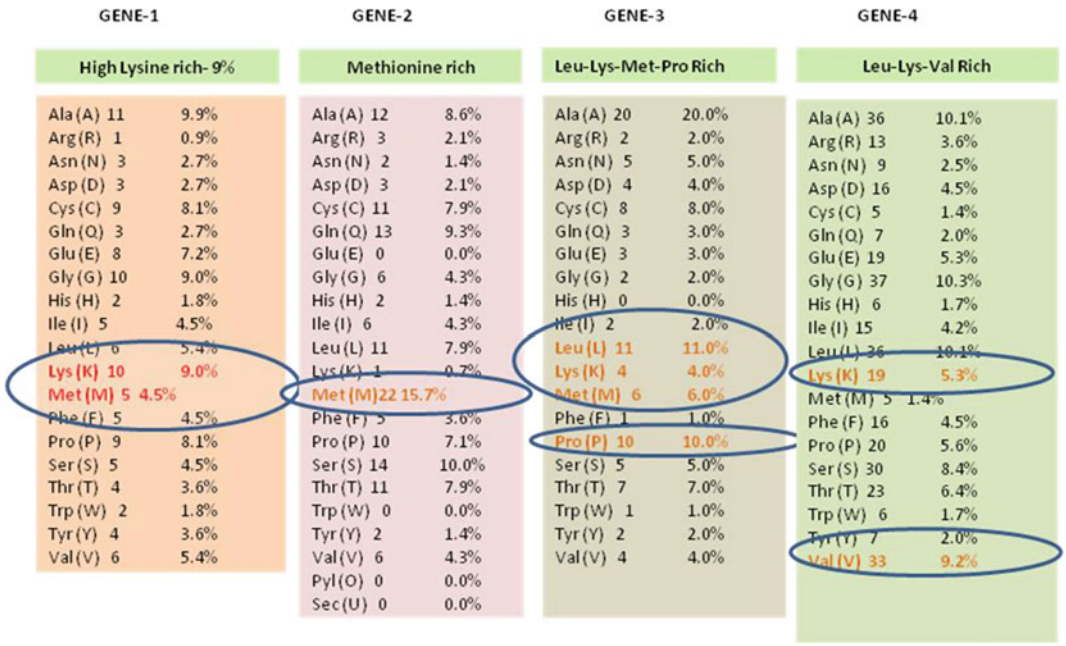

Fig. 10.4 Identification of candidate gene(s) having nutritional potential (high amount of essential amino acids in finger millet proteins)

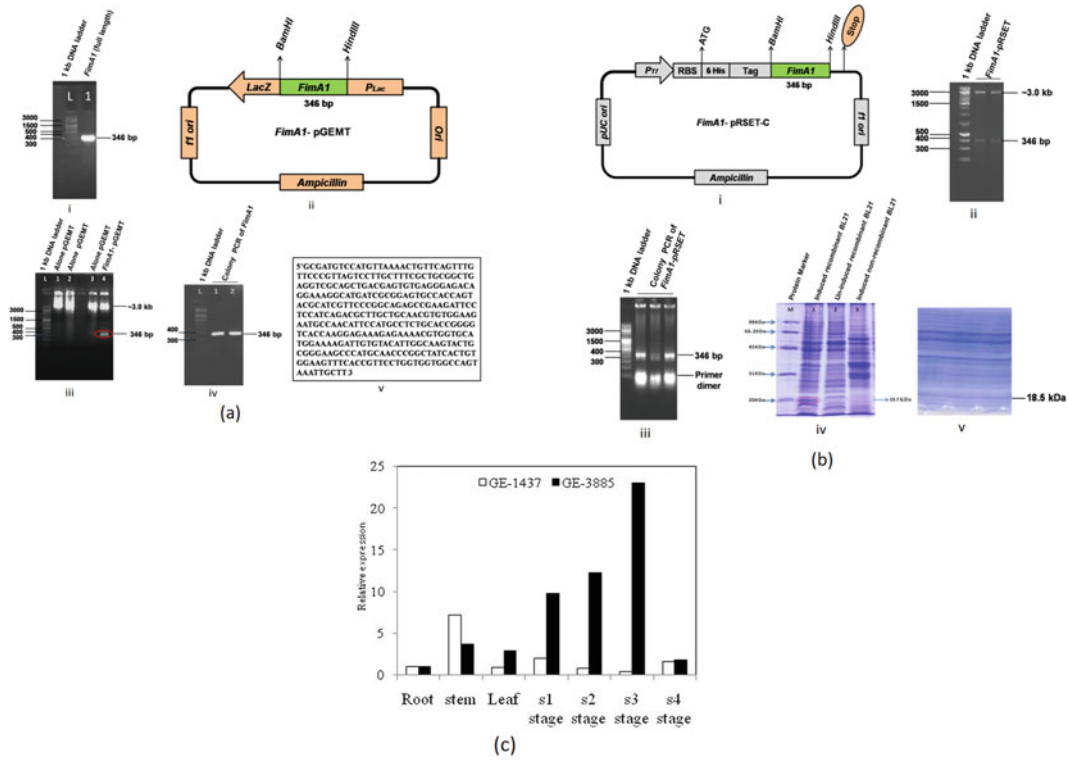

Fig. 10.5 Cloning of seed storage proteins enriched in essential amino acids in an expression vector; (**a**) schematic representation of the *fimA1*-pGEMT clone harboring the full-length *fimA1*; (**b**) schematic representation of the *fimA1*-pRSET-C clone harboring the full length *fimA1*, III and IV represent *BamHI* and *Hind III* restriction sites, respectively; (**c**) expression of FIMA1 protein in different stages of developing spikes of finger millet

the human system can be further elaborated by advanced research methods, of nutritional science and molecular biology.

In our lab, we identified and described the mRNA expressing *OPAQUE2* (O_2) similar TF from finger millet (FM) (*Eleusine coracana*) (*EcO2*). Full-length *EcO2* mRNA was derived by utilizing conserved primers developed by aligning O_2 mRNAs from other cereals, as well as 3′ and 5′ RACE (rapid amplification of cDNA ends). A 1248-nt ORF in the full-length *EcO2* mRNA codes for the 416 amino acid O_2 protein. Domain research showed the existence of the BLZ and bZIP-C domains, which are common in O_2 proteins (Gaur et al. 2018b). *EcO2* protein shared high sequence similarity with barley BLZ1 protein, according to phylogenetic study of *EcO2* protein with other bZIP proteins found using finger millet transcriptome data and O_2 proteins from other cereals. *EcO2* transcripts were used in the root, stem, leaves, and seed growth stages. In addition, the expression profiles of *EcO2* and a prolamin gene were examined during the seed development stages of two FM genotypes (GE-3885 and GE-1437), which differed in grain protein content (13.8 and 6.2 percent, respectively) grown under increasing nitrogen inputs to investigate nitrogen responsiveness and the role of *EcO2* in regulating seed storage protein gene expression. *EcO2* gene was more abundant throughout the S2 stage of seed development, and its expression increased as nitrogen intake increased. At all nitrogen inputs, the Ec-prolamin gene was significantly mediated in the high-protein genotype (GE-3885). These findings point to the existence of nitrogen responsiveness regulatory elements, which may play a role in protein accumulation in FM

genotypes by regulating *EcO2* expression in response to plant nitrogen status (Gupta et al. 2018). In addition to this DOF transcription family, well-characterized plant-specific transcription factors having diverse roles, has also been identified in finger millet. The expression profile of these *EcDof* transcription factors has shown their role during different stages of plant development (Gupta et al. 2018).

10.7.3 Proteomics for Sequential Extraction and High-Throughput Approaches for Analyzing SSPs

In our lab, we compared the seed proteomes of finger millet and rice to learn more about the nutritional and stress-related proteins that accumulate during the growth of finger millet seeds. Using Liquid Chromatography-Mass Spectrometry/Mass Spectrometry (LC-MS/MS), seed proteins from finger millet and rice were analyzed, and total 453 and 437 proteins were detected, respectively. Comparative analyses found that finger millet and rice both have 25 and 9 proteins that are special to them. Seventeen and five of these unusual proteins were seed storage proteins, respectively (SSP). These SSPs in finger millet were the gliadins, zeins, and avenins. The remaining special proteins in both crops were implicated in abiotic and biotic stress resistances. Of the 428 common proteins, 175 had a higher relative abundance in finger millet (unpublished data).

A BLAST-based homology search was used to identify the full-length gene of *Eleusine coracana* alpha prolamin (Ec-prolamin). Phylogenetic study of Ec-α-prolamin and associated prolamin genes from various cereals and millets reveals Ec-α-prolamin clustering in a separate cluster. Secondary structure prediction shows that Ec-α-prolamin has 59.4% alpha helix structure, which is a structural hallmark. Aside from that, the protein contains a balanced proportion of all essential amino acids. According to qPCR expression study, the accumulation of Ec-α-prolamin transcripts in developing finger millet seeds rises before seed maturity. Western blotting with a monospecific anti-prolamin antibody confirmed the presence of a 22 kDa band in the S3 and S4 phases of growing spikes. The heterologous production of isolated full-length Ec-prolamin might be exploited to develop nutritionally enhanced functional food items as well as value-added industrial goods (Fig. 10.6).

Further expression of calcium-binding proteins was also analyzed in developing spikes to investigate their functional role in the accumulation of calcium in developing seeds (Kumar et al. 2014; Singh et al. 2016).

To further explain the remarkable high grain calcium buildup in finger millet grains, a calmodulin (*CaM*) gene that is strongly expressed during growing spikes of the high grain calcium genotype was reported. Using 5'-3' RACE, the full-length *CaM* open reading frame (ORF) was extracted, and the predicted protein sequence indicated the presence of four unique EF motifs. Phylogenetic research revealed that the finger millet *CaM* (*Eleusine coracana* calmodulin [*EcCaM*]) is related to the rice *CaM 1-1*. Southern hybridization indicated the presence of at least four copies of the *CaM* gene in different locations of the "AABB" finger millet genome. According to immune detection utilizing monospecific polyclonal anti-*EcCaM* antibodies, *EcCaM* is localized in the embryo and aleurone layer and accumulates in greater levels in the high grain calcium genotype compared to the low grain calcium genotype. *In silico* analysis also found that *EcCaM* interacts with aquaporin, implying that calcium is most likely supplied to growing spikes by mass flow of water. These findings imply that enhanced *CaM* expression can stimulate the downstream calcium transport mechanism in the aleurone layer, resulting in larger calcium buildup in grains of high grain calcium genotype (Puranik et al. 2017) (Fig. 10.7).

A clearly visible blue color band of 48 kDa stained by Stains-all was eluted and identified as calreticulin using nano-liquid chromatography-tandem mass spectrometry (nano-LC-MS) (CRT). Based on the top hits of peptide mass

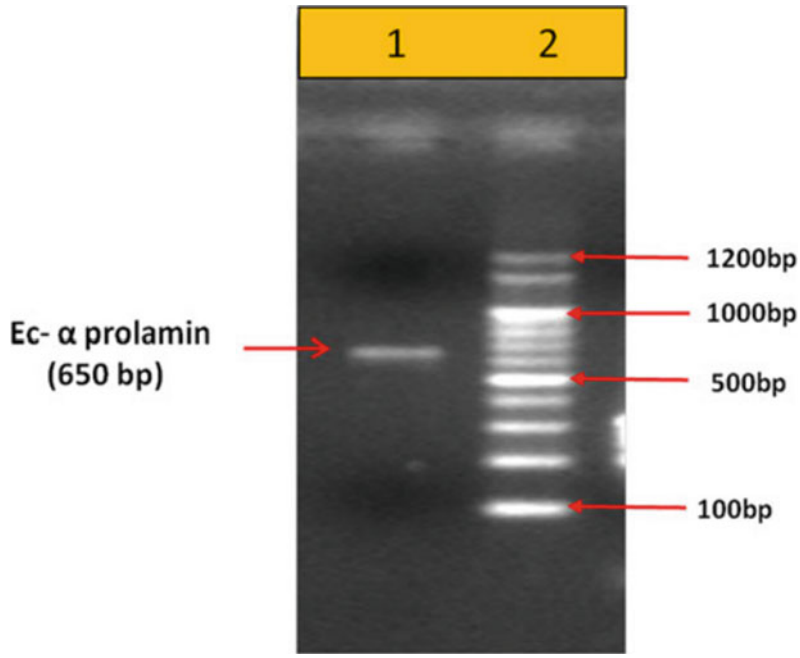

Fig. 10.6 PCR-based amplification of the Ec-prolamin gene from the finger millet GE-3885 genotype. Lane 1 represents a 100-bp DNA ladder, whereas Lane 2 represents a 650-bp amplified gene

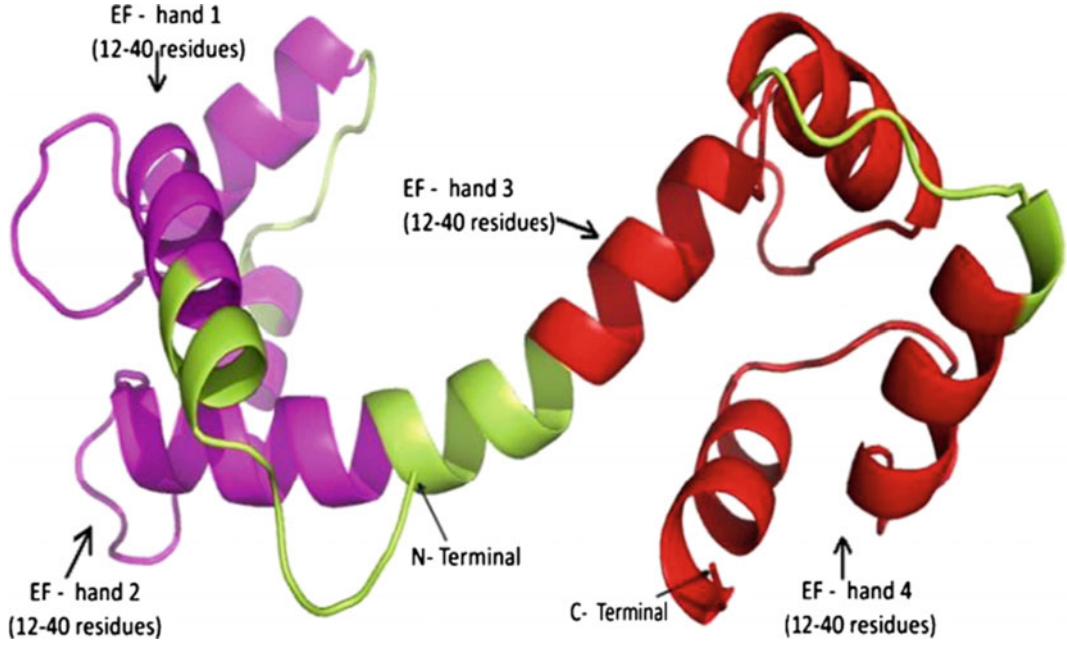

Fig. 10.7 Using Modeller 9.10, a ribbon model of the protein encoded by the isolated EcCaM gene (finger millet Calmodulin protein) was created

fingerprinting data, conserved primers were constructed to isolate the CRT gene from finger millet utilizing calreticulin sequences from other cereals. The deduced nucleotide sequence analysis of a 600 bp amplicon revealed up to 91% similarities to *CRT* gene(s) of rice and other plant species and was named *EcCRT1*. *EcCRT1* transcripts profiling revealed varying levels of relative expression at various stages of spike growth. *EcCRT1* transcripts and protein were identified to be more abundant in later stages of growing spikes, which might be ascribed to enhanced translational synthesis of *EcCRT1* protein during finger millet seed development. Higher synthesis of this *CaBP* at later phases of grain filling may be responsible for calcium sequestration in the endoplasmic reticulum of finger millet grains (Singh et al. 2016) (Fig. 10.8).

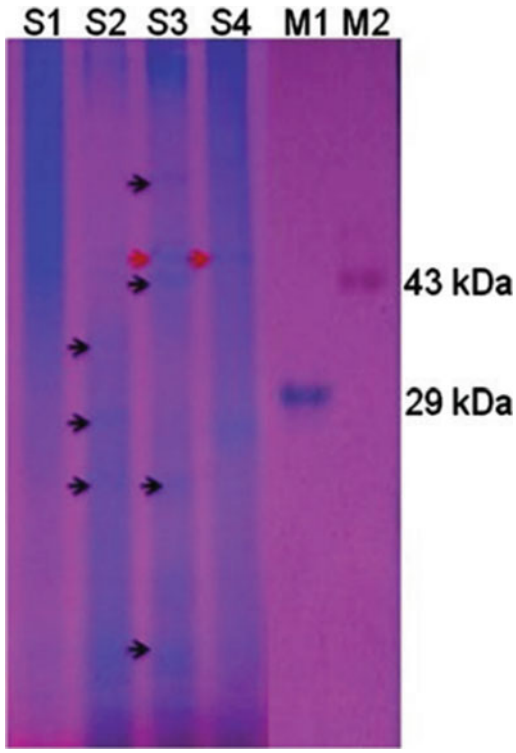

Fig. 10.8 Protein profiling of CaBPs in finger millet genotype GPHCPB-45 spikes at various phases of development using Stains-all staining. (M1, M2: native PAGE molecular weight protein marker; S1, S2, S3, and S4: spike development phases.)

10.7.4 Molecular Marker-Assisted Breeding

Continuous research efforts in the fields of molecular breeding, plant biotechnology, and genomics have resulted in massive amounts of data being generated using high-throughput sequencing technologies in recent years (Fig. 10.9).

Furthermore, genome-wide genetic variation study has aided in understanding the molecular and genetic foundation of nutritional quality characteristics in finger millet (Sharma et al. 2018). Because of their uniform distribution, ubiquitous nature, biallelic character, and high heritability, SNPs (single nucleotide polymorphisms) are thought to be the best option among all molecular markers. Furthermore, alterations in gene function may result from mutations at a single nucleotide level within genes, which may be responsible for phenotypic variances. As a result, identifying SNPs associated to essential nutritional characteristics such as SPC (seed protein content) and related traits such as DM (days to maturity) and GY (grain yield) (Tiwari et al. 2020).

Protein content phenotypic diversity was found high in different finger millet genotypes. However, no attempts have been undertaken too far to study both the genetic architecture and functional significance of genetic loci controlling SPC in finger millet. To unravel the complex genetic architecture of SPC and related characteristics, genomics-assisted breeding was used to identify and introgress candidate genes/QTLs (quantitative trait locus) regulating this essential quality attribute in various crop plants. However, little emphasis has been paid to the discovery of SPC-regulating genes/QTLs that may be exploited in marker-assisted genetic improvement of finger millet (Tiwari et al. 2020).

A study by Tiwari et al. (2020) was designed to identify genes/QTLs regulating SPC and associated characteristics in finger millet by utilizing SNPs identified by GBS (genotyping by sequencing) of a naturally varied population of finger millet. One of the main methods used to identify and develop specific qualities is

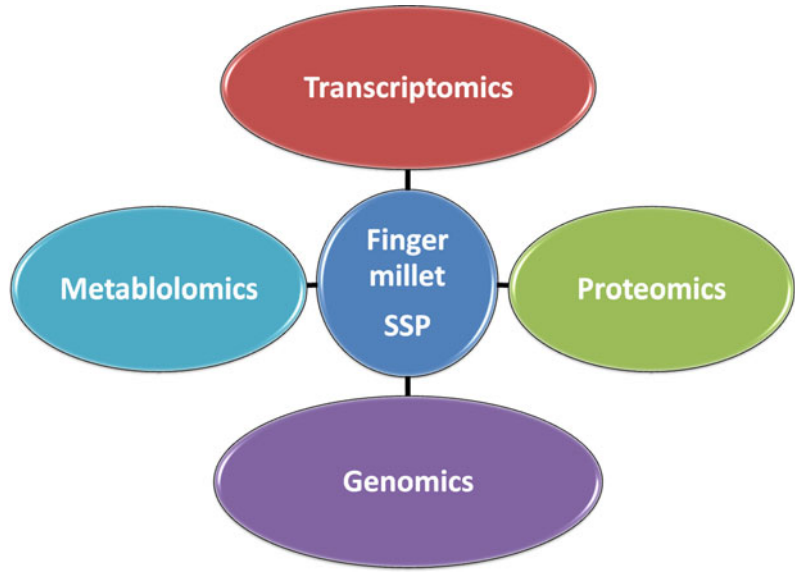

Fig. 10.9 Different NGS approaches to annotate the SSPs of finger millet

molecular markers. An extensive range of molecular marker systems has been identified by DNA markers, which are typically used as part of the plant breeding program (Jiang et al. 2015). The use of molecular markers has revolutionized the speed and reliability of plant genetic analysis which has allowed the molecular replication of crops in effect. The development of marker mechanisms and their respective methods have advanced immensely over the last three decades. The center of the molecular genetics stage was quickly increased in recent years by markers based on SNPs due to their abundant genomes and their comfort to high-performance detectives and platforms. The sequence of knowledge in public libraries dominates SNP discovery methods. A few studies for analyzing genetic ability and QTL in finger millets with molecular markers are available. The analysis of the strong hereditary heterogeneity is important for improving the yield, as it exposes the sensitivities of the hereditary relations and offers inspections of reproductive populations**.** Hereditary and efficient variety study recognizes inherited genotype ties around the world and assists in identifying appropriate genotypes for reproductive systems (Babu et al. 2017). For finger millet produced in Asia and Africa under various climatic conditions, analyses for genetic diversities require genome variability between genotypes and subsequently increase the population.

10.7.5 Identification of QTLs for SSP

A study of the complex genetic architecture of SPC (seed protein content) and related traits such as DM and GY of 113 diverse finger millet genotypes was carried out in our lab at two geographically different locations in India (Uttarakhand), i.e., Almora (E1) and Pantnagar, using an integrated genomic-based breeding strategy and significant variations between SPC, DM and GY genotypes were found in both places (Tiwari et al. 2020).

For the MTA (marker-trait association) study, a set of genome-wide SNPs has been identified by genotyping. NCBI blasts of common SNP markers identified 5, 3, and 5 most effective genomic areas for SPC, DM, and GY, respectively. The SNP encoding the aspartyl protease gene exhibited the greatest SPC interaction and was chosen as the most promising candidate for protein content variation in finger millet. As ATP was revealed to be the influential gene, the ATP synthase gene linked by GY and DM catalyzes the addition of a phosphate to ADP, collecting energy from the proton gradient. Five SPC-

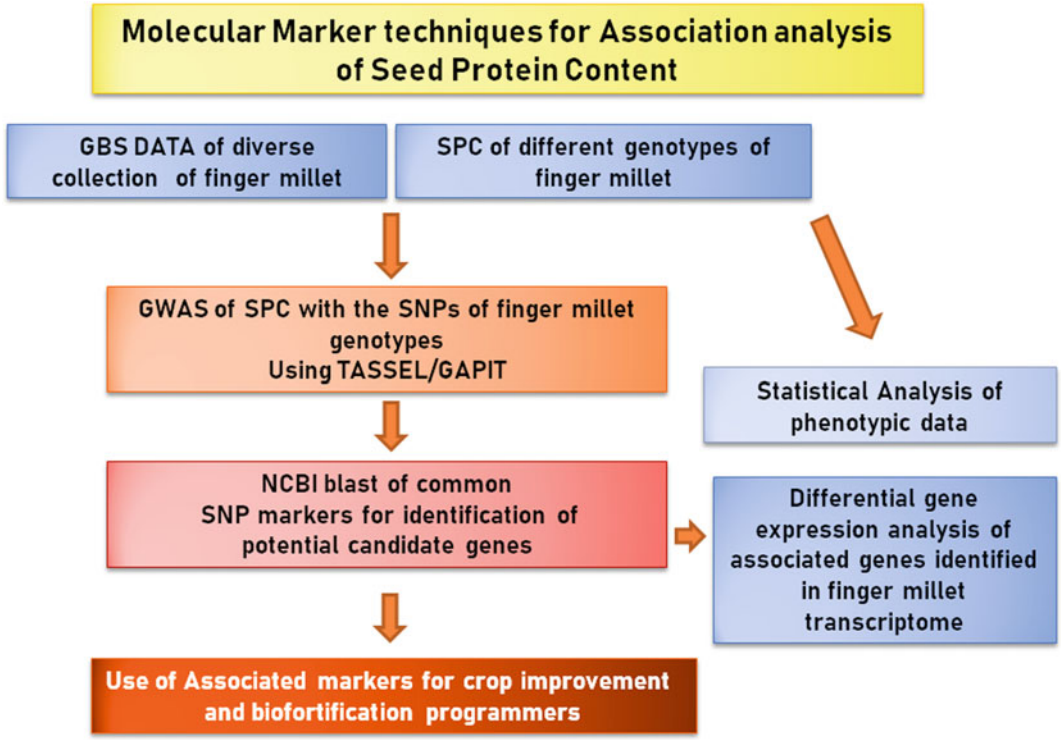

Fig. 10.10 A sequential flow of association studies for SSP trait in finger millet

related genes had higher expression levels in the high protein-containing genotype than in the low protein-containing genotype (Fig. 10.10) (Tiwari et al. 2020).

10.8 Bioactive Peptides

Dietary-derived peptides have the potential to be functional food components that may be included into health-promoting diets aimed at the prevention and management of a variety of chronic illnesses. The discovery of bioactive peptides (BPs) in intact proteins that have a favorable impact on bodily processes will have an impact on health. BPs derived from millet seeds such as sorghum bicolor, foxtail, buckwheat, finger millet, pearl millet, chia, and quinoa, as well as their biological activities such as antimicrobial peptides, anticancerous peptides, antioxidant peptides, antidiabetic peptides, and antihypertensive peptides, have been reported (Orona-Tamayo et al. 2019).

The BPs are inert inside the parent protein sequence but become active once released. Peptides can be released in a variety of ways, including breakdown by digestive enzymes, proteolytic bacteria, and enzymes generated from microbes or plants (Udenigwe et al. 2012). The usefulness and efficacy of BPs, such as their small intestine absorption and bioavailability in target tissues, are largely determined by their size, intrinsic amino acid composition, sequence, and other characteristics such as charge, hydrophobicity, and rate of hydrolysis. As a result, their generation/production is a critical phase that requires more research and attention (Chai et al. 2020).

In another interesting study using extraction of finger millet functional ingredients (FFI) using

different protein extraction buffers and isolated ingredients and their enzymatic hydrolyzed fraction were used to access its influence on calcium uptake studies using Fura 2 fluorescent dyes under in vitro intestinal mimicking environment of $CaCO_2$ cell culture system. The results were found that FFI in its hydrolyzed fraction is promoting higher calcium uptake compared to whole FFI indicating that finger millet seeds not only contain higher calcium contents but also have calcium-binding proteins which facilitate higher uptake of calcium through human gut (unpublished data).

The traditional in vivo techniques for generating, identifying, and validating BPs are time-consuming and labor intensive. Because of its focused approach, low time consumption, quick pace of results collection, cost-effectiveness, and bioinformatics/in silico analysis might be a strong tool for BPs discovery. Bioinformatic techniques enable the identification of possible BPs among dietary proteins. Elucidating protein sequences is an important stage in the development of BPs, and molecular docking is used to assess the therapeutic potential of novel BPs produced from food and to monitor protein–ligand interactions (Panyayai et al. 2019). Many peptide databases are now available for BPs like FeptideDB which is a computational aid for assessing peptide bioactivities (Panyayai et al. 2019). Identification of derived BPs could be a potential source for targeting many diseases.

10.9 Concluding Remark and Future Prospects

Seeds are source of wide range of foods, magnesium phosphates, and amino acids, including leucine and lysine. Finger millet is an annual herb widely cultivated which provides a large amount of protein as well as high calcium. Identification of seed protein contents provides an improved biofortification path and boosts the finger millet nutritional value for cultivation. These are the beginnings that need to be explained by modern approaches to the biology of computational systems, including genomics, transcriptomics, and proteomics to improve the finger millet crop's nutritional quality. Thus, *OMICS*, high-performance genotyping, and sequencing technologies are expected to contribute not only toward improving breeding practices but also improving the quality and quantity of such crops. Gene sequence of two SSPs, albumin (*fima1), and* prolamin *(fimp2)* having 9% lysine and 15.8% methionine, respectively, was cloned and recombinant proteins have been expressed in *E. coli* system which is a remarkable output of these *OMICS* technologies. The functionally annotated SNPs have been used to identify genes that regulate important agricultural traits in finger millets. This information would be an asset for potential finger millet breeding research. The SNP genotyping allows breeders to choose parents and to introgress rare germplasm alleles. The SNPs identified and associated with these genes could be used for the cloning of full gene sequences, precise mapping, and ultimate reproductive support programs for the marker to incorporate alleles in local genotypes. The genomic regions found may be targeted in finger millet for marker exploration. Using the GWAS (genome-wide association studies), new essential genetic information for these agronomic characteristics was discovered. In the potential biofortification of finger millets, the established loci and candidate genes will serve as promising targets. The recent findings would become a milestone for selection of genotypes containing high proportions of these seed storage proteins or analysis of their amounts by genetic engineering which could lead to improved nutritional quality of finger millet. Further, identification of active bioactive peptides in finger millet using bioinformatics tools could be a potential source for targeting many diseases.

Competing Interest The authors declare that there are no competing interests.

References

Anand SS, Hawkes C, de Souza RJ (2015) Food consumption and its impact on cardiovascular disease: importance of solutions focused on the globalized food system: a report from the workshop convened by the World Heart Federation. J Am Coll Cardiol 66 (14):1590–1614

Babu BK, Sood S, Agrawal PK, Chandrasekhar C, Kumar A, Kumar A (2017) Molecular and phenotypic characterization of 149 finger millet accessions using microsatellite and agro-morphological markers. Proc Natl Acad Sci India Sect B Biol Sci 87(4):1217–1228

Balakireva AV, Zamyatnin AA (2016) Properties of gluten intolerance: gluten structure, evolution, Pathogenicity and Detoxification Capabilities. Nutrients 8(10):644

Battaglia RE, Baumer B, Conrad B, Darioli R, Schmid A, Keller U (2015) Health risks associated with meat consumption: a review of epidemiological studies. Int J Vitam Nutr Res 85(1–2):70–78

Beccari (1745) De Frumento De Bononiensi Scientiarum et Artium Instituto atque Academia Commentarii, II. Part I:122–127

Bhagwat VR (2019) Safety of water used in food production. Food Safety Human Health 219–247

Birol E, Meenakshi JV, Oparinde A (2015) Developing country consumers' acceptance of biofortified foods: a synthesis. Food Sec 7:555–568

Campbell TC (2017) Nutritional renaissance and public health policy. J Nutritional Biol 3(1):124–138

Chai KF, Voo AYH, Chen WN (2020) Bioactive peptides from food fermentation: a comprehensive review of their sources, bioactivities, applications, and future development. Compr Rev Food Sci Food Saf 19:3825–3885

Cremer JE, Bean SR, Tilley MM, Ioerger BP, Ohm JB, Kaufman RC, Wilson JD, Innes DJ, Gilding EK, Godwin ID (2014) Grain sorghum proteomics: integrated approach toward characterization of endosperm storage proteins in kafirin allelic variants. J Agric Food Chem 62(40):9819–9831

Dwyer JT, Melanson KJ, Sriprachy-anunt U (2015) Dietary treatment of obesity. In: Feingold KR, Anawalt B, Boyce A et al (ed) South Dartmouth (MA): MDText.com, Inc

Fontanini D, Jones B (2002) SEP-1—a subtilisin-like serine endopeptidase from germinated seeds of Hordeum vulgare L. cv Morex. Planta 215:885–893

Fujiwara T, Nambara E, Yamagishi K, Goto DB, Naito S (2002) Storage proteins. The arabidopsis book 1: e0020

Gacek K, Bartkowiak-Broda I, Batley J (2018) Genetic and molecular regulation of Seed Storage Proteins (SSPs) to improve protein nutritional value of Oilseed Rape (Brassica napus L.) Seeds. Front Plant Sci 9:890

Gaur VS, Kumar L, Gupta S, Jaiswal JP, Pandey D, Kumar A (2018a) Identification and characterization of finger millet OPAQUE2 transcription factor gene under different nitrogen inputs for understanding their role during accumulation of prolamin seed storage protein. 3 Biotech 8(3):163

Gaur VS, Sood S, Tiwari S, Kumar A (2018b) Genome-wide identification and characterization of seed storage proteins (SSPs) of foxtail millet (Setaria italica (L.) P. Beauv.). 3 Biotech 8(10):415

Gell G, Kovács K, Veres G, Korponay-Szabó IR, Juhász A (2017) Characterization of globulin storage proteins of a low prolamin cereal species in relation to celiac disease. Sci Rep 7:39876

Gupta N, Gupta AK, Singh NK (2011) Differential expression of PBF Dof transcription factor in different tissues of three finger millet genotypes differing in seed protein content and color. Plant Mol Biol Rep 29:69–76

Gupta S, Pathak RK, Gupta SM, Gaur VS, Singh NK, Kumar A (2018) Identification and molecular characterization of Dof transcription factor gene family preferentially expressed in developing spikes of *Eleusine coracana* L. 3 Biotech. 8(2):82

Henchion M, Hayes M, Mullen AM, Fenelon M, Tiwari B (2017) Future protein supply and demand: strategies and factors influencing a sustainable equilibrium. Foods (Basel, Switzerland) 6(7):53

Hittalmani S, Mahesh HB, Shirke MD (2017) Genome and Transcriptome sequence of Finger millet (*Eleusine coracana* (L.) Gaertn.) provides insights into drought tolerance and nutraceutical properties. BMC Genomics 18:465

Hoffman JR, Falvo MJ (2004) Protein—which is Best? J Sports Sci Med 3(3):118–130

Huang S, Adams WR, Zhou Q, Malloy KP, Voyles DA, Anthony J, Kriz AL, Luethy MH (2004) Improving nutritional quality of maize proteins by expressing sense and antisense zein genes. J Agric Food Chem 52 (7):1958–1964

Jiang GL (2015) Molecular marker-assisted breeding: a plant breeder's review. In: Al-Khayri J, Jain S, Johnson D (eds) Advances in plant breeding strategies: breeding, biotechnology and molecular tools. Springer, Cham

Kawakatsu T, Takaiwa F (2010) Cereal seed storage protein synthesis: fundamental processes for recombinant protein production in cereal grains. Plant Biotechnol Jouurnal 8(9):939–953

Kumar A, Gaur VS, Goel A (2015) De Novo assembly and characterization of developing spikes transcriptome of finger millet (*Eleusine coracana*): a minor crop having nutraceutical properties. Plant Mol Biol Rep 33:905–922

Kumar A, Mirza N, Charan T, Sharma N, Gaur VS (2014) Isolation, characterization and immunolocalization of a seed dominant CaM from finger millet (*Eleusine coracana* L. Gartn.) for studying its functional role in differential accumulation of calcium in developing grains. Appl Biochem Biotechnol

Kumar A, Tomer V, Kaur A (2018) Millets: a solution to agrarian and nutritional challenges. Agric Food Secur 7:31

Le DT, Chu HD, Le NQ (2016) Improving nutritional quality of plant proteins through genetic engineering. Curr Genom 17(3):220–229

Liu Y, Wu X, Hou W (2017) Structure and function of seed storage proteins in faba bean (Vicia faba L.) 3 Biotech 7:74

Lonnie M, Hooker E, Brunstrom JM, Corfe BM, Green MA, Watson AW, Williams EA, Stevenson EJ, Penson S, Johnstone AM (2018) Protein for life: review of optimal protein intake, sustainable dietary sources and the effect on appetite in ageing adults. Nutrients 10(3):360

Lopez MJ, Mohiuddin SS (2021) Biochemistry, essential amino acids. In: StatPearls [Internet] Treasure Island (FL): StatPearls Publishing

Moreno FJ, Clemente A (2008) 2S Albumin storage proteins: what makes them food allergens? Open Biochem J 2:16–28

OECD-FAO Agricultural Outlook 2020–2029

Orona D, Valverde ME, Paredes-López O (2019) Bioactive peptides from selected latin american food crops —a nutraceutical and molecular approach. Crit Rev Food Sci Nutr 59(12):1949–1975

Otegui MS, Herder R, Schulze J, Jung R, Staehelin LA (2006) The proteolytic processing of seed storage proteins in Arabidopsis embryo cells starts in the multivesicular bodies. Plant Cell 18(10):2567–2581

Panyayai T, Ngamphiw C, Tongsima S (2019) FeptideDB: A web application for new bioactive peptides from food protein, Heliyon 5(7)

Petrie JR, Guzik TJ, Touyz RM (2018) Diabetes, hypertension, and cardiovascular disease: clinical insights and vascular mechanisms. Can J Cardiol 34 (5):575–584

Puranik S, Kam J, Sahu PP, Yadav R, Srivastava RK, Ojulong H, Yadav R (2017) Harnessing finger millet to combat calcium deficiency in humans: challenges and prospects. Front Plant Sci 8:1311

Radauer C, Bublin M, Wagner S, Mari A, Breiteneder H (2008) Allergens are distributed into few protein families and possess a restricted number of biochemical functions. J Allergy Clin Immunol 121(4):847–52.e7

Radhika V, Rao VS (2015) Computational approaches for the classification of seed storage proteins. J Food Sci Technol 52(7):4246–4255

Rahman H, Jagadeeshselvam N, Valarmathi R, Sachin B, Sasikala R, Senthil N, Sudhakar D, Robin S, Muthurajan R (2014) Transcriptome analysis of salinity responsiveness in contrasting genotypes of finger millet (*Eleusine coracana L.*) through RNA-sequencing. Plant Mol Biol 85(4–5):485–503

Raza A, Razzaq A, Mehmood SS, Zou X, Zhang X, Lv Y, Xu J (2019) Impact of climate change on crops adaptation and strategies to tackle its outcome: a review. Plants 8(2):34

Sachdev N, Goomer S, Singh LR (2020) Foxtail millet: a potential crop to meet future demand scenario for alternative sustainable protein. J Sci Food Agric 101 (3):831–842

Sharma D, Tiwari A, Sood S, Jamra G, Singh NK, Meher PK, Kumar A (2018) Genome wide association mapping of agro-morphological traits among a diverse collection of finger millet (*Eleusine coracana L.*) genotypes using SNP markers. PloS One 13(8)

Shewry PR, Napier JA, Tatham AS (1995) Seed storage proteins: structures and biosynthesis. Plant Cell 7 (7):945–956

Singh M, Metwal M, Kumar VA, Kumar A (2016) Identification and molecular characterization of 48 kDa calcium binding protein as calreticulin from finger millet (*Eleusine coracana*) using peptide mass fingerprinting and transcript profiling. J Sci Food Agric 96(2):672–679

Tandang- MR, Tecson EM, Mikami B, Utsumi S, Maruyama N (2011) Molecular design of seed storage proteins for enhanced food physicochemical properties. Annu Rev Food Sci Technol 2:59–73

Thomas G, De Tavernier J (2017) Farmer-suicide in India: debating the role of biotechnology. Life Sci Soc Policy 13(1):8

Tiwari A, Sharma D, Sood S, Jaiswal JP, Pachauri SP, Ramteke PW, Kumar A (2020) Genome-wide association mapping for seed protein content in finger millet (*Eleusine coracana*) global collection through genotyping by sequencing. J Cereal Sci 91:102888

Ugare R, Chimmad B, Naik R, Bharati P, Itagi S (2014) Glycemic index and significance of barnyard millet (*Echinochloa frumentacae*) in type II diabetics. J Food Sci Technol 51(2):392–395

Vergnolle C, Arondel V, Jolliot A, Kader JC (1992) Phospholipid transfer proteins from higher plants. Methods Enzymol 209:522–530

Vinoth A, Ravindhran R (2017) Biofortification in millets: a sustainable approach for nutritional security. Front Plant Sci 8(29)

Zamaratskaia G, Gerhardt K, Wendin K (2020) Biochemical characteristics and potential applications of ancient cereals—an underexploited opportunity for sustainable production and consumption. Trends Food Sci Technol 107:(114–123)