



Seed Biology and Packaging of Finger Millet Using Omics Approaches for Nutritional Security

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Abstract

Seeds act as storage organs for nutrition, bio-energy, processing, and other essential bio-molecules, as well as a distribution mechanism for genetic information to be passed on to future generations. Research into deciphering the complex system of gene regulation and pathways related to seed biology and nutrient partitioning is still in its early stages. There has been a renewed interest in the cultivation and consumption of nutraceuticals in recent years, especially small millets such as *Eleusine coracana* (L.) Gaertn (finger millet; FM). It could be an ideal model system for studying nutraceutical properties using multi-pronged molecular approaches to decode seed biology, processes

of nutrient partitioning, and biologically relevant molecule packaging. To comprehend the complexities of seed biology and packaging, it is essential to understand the genes and pathway(s) involved in biomolecule homeostasis and accumulation at the time of seed development. Multi-layered “Omics” methods and high throughput platforms have recently been used to distinguish the genes and proteins involved in different metabolic and signaling pathways and their regulations for understanding the molecular genetics of biosynthesis and homeostasis of bio-molecules in many model organisms such as *Arabidopsis* and rice. However, there is very little information existing in scientific domain about the molecular biology of seed development in FM. This can be accomplished by combining multi-omics data with systems biology to better understand the complexities of seed developmental biology and nutrient partitioning. Via pathway engineering and biotechnology, this information can be used to improve biologically essential chemicals for large-scale development of nutrients in seeds and nutraceuticals.

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9.1 Introduction

In India, the govt. is setting in situ Associate in Nursing Initiative for biological process Security through Intensive Millet Promotion (INSIMP),

whereas within the developed world millets and alternative ancient grains have appeared on the shelves of specialized outlets. Vocation them as “climate-resilient crops” and “powerhouse of nutrients” government of India has declared eight millet crops (sorghum, pearl millet, finger millet, barnyard millet, foxtail millet, proso millet, kodo millet and little millet) and 2 pseudocereals (amaranth and buckwheat) as “nutricereals” (The Gazette of India, thirteenth April 2018). Finger millet may be a tiny cereal grain big within the semi-arid sub-tropical and tropical regions of Africa and Asia wherever it's one among the cereal staples (ICRISAT/FAO 1996; Obilana and Manyasa 2002). The America National analysis Council (1996) states, “Despite its importance, FM is grossly neglected each scientifically and internationally”. FM is sort of entirely a subsistence crop and in Africa, it's used primarily for the assembly of ancient foods, virtually none of them square measure commercial (ICRISAT/FAO 1996), whereas the use of sorghum, another less common cereal, is being inflated by victimization to supply novel business merchandise like food, bread, cookies, and snack foods (Taylor et al. 2006), identical is extremely restricted with FM. Wheat is the ideal cereal for manufacturing food as a result of it contains protein proteins, that square measure essential for the standard of the merchandise, however it grows well in cooler climates and therefore countries in hotter regions import half or all of the wheat victimization the scarce exchange. Partial substitution of wheat with FM in bakeshop merchandise like cookies could have several benefits together with a high biological process worth, saving exchange, and health-promoting (due to the phenolic resin antioxidants).

Seeds square measure one among the key materials of nutraceutical and prescription drugs resources consisting of all attainable sorts of nutritionally necessary and bioactive molecules that embrace varied soluble carbohydrates, storage proteins, starch chemical compound and

lipids required for our daily diet. Aside from this, they conjointly operate as necessary delivery system of genetic info from generation to generation. Seeds square measure one among the key materials of nutraceutical and prescription drugs resources consisting of all attainable sorts of nutritionally necessary and bioactive molecules that embrace varied soluble carbohydrates, storage proteins, starch chemical compound and lipids required for our daily diet. Aside from this, they conjointly operate as necessary delivery system of genetic info from generation to generation. This chapter describes grain structure and composition, seed development, factors poignant seed development, nutrient partitioning and completely different ‘omics’ tools and alternative branches that square measure united to make the foremost engaging space of analysis toward establishing the seeds as organic chemistry factories for human health and nutrition.

9.2 Seed Development: Transition from Vegetative Stem to Developing Spikes to Seed Development

On the basis of morphology and development stage of ovary and anthers, four different developmental stages of the spike, inflorescence immergence or booting, anthesis, grain filling, and grain maturation or ripening, were identified and designated as (a) S1 stage, i.e. booting or inflorescence immergence when florets are compact, androecium and gynoecium are very small, closely arranged, and (c) S3 stage, i.e. grain filling, occurs when there is a noticeable increase in particles in the liquid endosperm, and the caryopsis is crushed between fingers (d) The S4 stage, which occurs after 50% of the spikelets have ripened and the caryopsis has hardened to the point where it is difficult to divide by thumb-nail, was critical for determining the expression of putative transporters and their regulatory genes (Fig. 9.1).



Fig. 9.1 Developmental stages of finger millet spike namely, spike, booting or inflorescence immergence, anthesis, grain filling and grain ripening or maturation were identified on the basis of morphology and

development stage of ovary and anthers and were designated as S1, S2, S3 and S4, respectively. (Adapted from Mirza et al. 2014)

9.2.1 Factors Influencing Seed Developmental Machinery

9.2.1.1 Environment

Herbivores or a lack of water can alter maternal influences on seed development. It has the potential to reduce the resources available for

seed development and to impact the critical regulatory event that occurs during and after pollination (Gehring and Delph 2006; Diggle et al. 2010). During seed development, resource lamination might induce mother plants to produce smaller seeds. However, the molecular mechanisms of maternal control are poorly understood. The paternal part also influences the

developmental mechanisms of seeds in two ways: in the first, paternal alleles contribute to embryo and endosperm vigor, resulting in variation in seed size and mature embryo; in the second, paternal alleles contribute to embryo and endosperm vigor, resulting in variation in seed size and mature embryo (Kigel 1995).

9.2.1.2 Nutrition

Nutrient translocation during seed development into the seed coat, endosperm, or embryo is still unknown. Only a few plant species have been thoroughly examined so far. Plants use atypical transport systems for embryo feeding, according to previous research, because there is no vascular connection between the embryo and the vascular bundle. Nutrients can be transported via two different pathways: apoplast and symplast. CO₂ and O₂ can be transferred through the middle layer of the parenchyma's intercellular spaces (Kigel 1995). Glycolysis, gluconeogenesis, amino acid biosynthetic pathways, energy, and pyruvate metabolism are among the routes involved with seed biology and their involvement in nutrition. Seeds are also linked to hormone signaling pathways such as IAA/ethylene, jasmonate, and gibberellin production (Sreenivasulu and Wobus 2013; Li et al. 2015). Through a systems biology approach, progress has recently been achieved in identifying the main components of seeds that control nutrient loading during their maturation process (Zhang et al. 2007; Kumar et al. 2015a, b).

9.2.1.3 Physiology

A better understanding of plant physiological characteristics is one of the most important concepts in building a biology background for predicting seed growth. Single fusion pairings are observed using micromanipulation and video microscopy. In synergids, genes involved in pollen discharge or pollen tube guidance, as well as genes with differential expression in sperm, central cells, and eggs before and after fertilization, have been identified (Raghavan 2000). The continual perception and transmission of signals by endosperm, seed coat, and the embryo is essential for the coordination of development, maturation,

and differentiation processes. Furthermore, the ratio of phytohormones in certain signals controls specific stages of seed development (Locascio et al. 2014). In addition, numerous methods have been developed, such as infrared thermography, to decode biophysical and biochemical changes associated with imbibition, germination, and overall seed vitality (Kranner et al. 2010; Macovei et al. 2017). Reactive chemical element species (ROS) are thought to play an important role in seed development as both a signaling and a harmful molecule (Kumar et al. 2015a, b). A tight link between phytohormones, reactive oxygen species (ROS), and DNA repair have been predicted at all stages of seed development, from embryogenesis to germination (El-Maarouf-Bouteau et al. 2013; Kumar et al. 2015a, b). The seed's hydration state, on the other hand, is a significant element that influences ROS signaling and DNA repairing (Bewley et al. 2012).

During seed germination and imbibition, ROS as signaling molecules is found in a variety of signaling pathways, including the pentose phosphate pathway and mitogen-activated protein kinases (Diaz-Vivancos et al. 2013; El-Maarouf-Bouteau et al. 2013). Several antioxidative enzymes are either activated or inhibited during seed imbibitions, in addition to these (Balestrazzi et al. 2011). On the other hand, several phytohormones have been found to influence ROS production during germination and seed development, such as ABA, which reduced ROS accumulation in rice and sunflower (Zhang et al. 2013), barley (Ishibashi et al. 2012), and gibberellic acid, which increased ROS generation in *Arabidopsis* (Lariguet et al. 2013). Given this critical information, it is imperative that omics approaches be used efficiently to unravel several complex mechanisms involved in seed development.

9.2.1.4 Epigenetic Modifications

In flowering plants and mammals, genomic imprinting is an independent and self-contained phenomena. Imprinted genes are determined to be responsible for the regulation of nutrition transfer to the developing offspring at the site of embryo-nourishing organs, such as the placenta

and endosperm. The antagonistic activities of Polycomb group-mediated histone methylation and DNA methylation have a significant impact on the regulation of many imprinted plant genes (Jiang and Köhler 2012). Long-term selection can preserve imprinted expression at some loci because genes with imprinted expression are conserved between monocots and dicots (Ohnishi et al. 2014). A harmonization of the genetic route, HAIKU (IKU), with epigenetic regulators limits genome dosage in Arabidopsis. Actually, the seed size is determined by DNA methylation and the deposition of trimethylated lysine 27 on histone H3 (H3K27me3) (Li et al. 2013). The regulatory implications of DNA methylation in rice seed maternal integument development have recently been investigated, with a whole genome bisulfite deep sequencing technique utilized to identify differentially methylated areas and genes driving this important process (Wang et al. 2017). According to another study, a seed-specific transcription factor gene LEAFY COTYLEDON 1 reverses the silent state inherited from gametes by encouraging the early development of an active chromatin state and activating its expression *de novo* at FLOWERING LOCUS C (Tao et al. 2017).

9.2.1.5 Small Regulatory RNA

Small non-coding RNAs (sncRNAs), together with microRNAs (miRNAs) and short meddlesome RNAs (siRNAs), are vital monitors of organic phenomena at the transcriptional and post-transcriptional stages, and have also been found to play critical roles in seed biological processes and germination (Rodrigues and Miguel 2017). Because studies have focused solely on miRNAs, which are typically 21-nt length, the biological significance of the presence of different siRNA profiles in seeds is not yet understood (Rodrigues and Miguel 2017). miRNA serves a critical function in seed growth and germination by down-regulating target genes (Rogers et al. 2013). In Arabidopsis mutants, differential expression of miRNAs in tissues has been proven (Zhai et al. 2014). Artificial miRNA (ami-RNA) was created and expressed to target a specific factor, resulting in abnormal embryogenesis. The mechanical

device Like-1 (DCL-1) factor is in charge of catalyzing the creation of the right structure from the secondary precursor (Nordine and Bartel 2010). Similarly, *emb76*, a DCL-1 deficient mutant, caused embryo arrest and did not prevent the development of a kind of cell known as a suspensor cell (Bewley et al. 2013). More research into the *dcl1-5* and *dcl1-10* mutants revealed that DCL1 mutations have an early influence on the 8-cell embryonic stage, most likely due to up-regulation of miR156-targeted SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL) ten and eleven (Xu et al. 2016). The involvement of alkyl enzyme has been identified for the aim of reading miRNA biogenesis and maturation (Pluskota et al. 2011). The temporal order of seed maturation is likewise controlled by miRNAs (Willmann et al. 2011). In *dcl15* mutants, up-regulation of FUS3, LEC2, L1L, NF-YB6, MYBs, and bZIPs was observed, while down-regulation of Arabidopsis 6B-INTERACTING PROTEIN1-LIKE1 (ASIL1) and ASIL2 and simple protein deacetylase HDA6/SIL1 and simple protein deacetylase HDA6/SIL1 was observed. Furthermore, miRNAs have been implicated in seed dormancy and germination transition. MiR156 and miR172 are both involved in seed germination, with miR156 inhibiting germination in lettuce and Arabidopsis and miR172 promoting it (Huo et al. 2016). In Arabidopsis, miR159, which targets MYB33 and MYB101, is known to fully regulate ABA responses required for the shift from seed dormancy to germination (Nonogaki 2017). Furthermore, a recent study on rice plants demonstrated the presence of different junction events of secret writing and long non-coding ribonucleic acid (lncRNA) throughout the biological process stage of seed via comparison of immature seed with embryo as well as reproductive structure of mature seed during the biological process stage of seed. It was projected that developing seeds would have more different junction events, i.e. 5.8–57 times more than root, leaves, buds, flowers, and procreative meristems. As a result, additional research is needed to understand lncRNA's labyrinthine process during seed development (Kiegle et al. 2018).

9.3 Finger Millet Grain Structure and Composition

A fruit coat or pericarp covers the seed in most cereal grains, which comprises of an embryo or germ and an endosperm wrapped by a nucellar epidermis and testa or seed coat (Hoseney 1994). A wheat grain's structure can be either a caryopsis or a utricule (Angold 1979). The pericarp or fruit coat surrounds the seed in a caryopsis and attaches closely to the seed coat, but in a utricule, the pericarp surrounds the seed like a sac and is linked to the seed at a single point. Caryopses are the primary cereal grains wheat, maize, barley, sorghum, rice, oats, and rye, whereas utricles are the millets finger, proso, and foxtail millets (Hoseney 1994; McDonough 2000).

9.3.1 Structure of the Finger Millet Grain

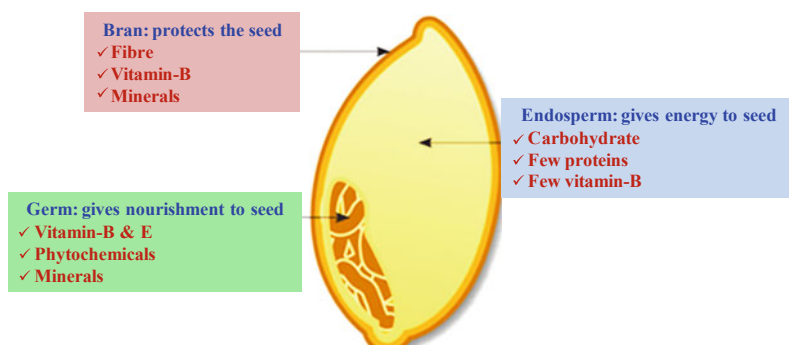
The utricular structure of the FM kernel (grain) was suggested by Angold (1979) and elucidated by McDonough et al. (1986). The FM kernel is roughly globular to oval (1–1.5 mm in diameter) in shape with average weight of 2.64 g of 1000 kernel (Angold 1979; McDonough et al. 1986). The FM kernel contains outer layers, the starchy endosperm and germ (Fig. 9.2).

9.3.2 Outer Layers

The outer layers of the FM kernel, according to McDonough et al. (1986), consist of a membranous cover that is loosely connected to the kernel

at maturity and a testa that overlays an aleurone protein layer (Fig.9.3a). The authors determined that the cover is a flimsy membranous covering that is not attached to the epispem at any specific location and can be removed by rubbing or laundering, similar to what Angold (1979) and Hilu et al. suggested previously (1979). The cover appeared to be made up of several layers of tissue (McDonough et al. 1986). McDonough et al. (1986) also noted that the FM epispem layer's outward appearance was distinctive and distinct from that of other cereals. They discovered that FM epispem has five unique layers and range in color from red to purple. The initial layer was 1.5 m thick and autofluorescent blue, indicating that ferulic acid or a polymer was present. The second layer seemed striated and was made up of mound-like formations made up of portions of “interlocking” tissue. When the epispem was seen in cross-section, junctions were seen between the mound-like structures within the outer epispem layers, and the junctions were assumed to correlate to the interlocking sections seen from the surface. The second layer (5.5–17.5 m) was the thickest, had darker coloration than the lower layers, and most likely contained more phenolic resin components than the others. The third and fourth layers were approximately the same thickness (1.4–2.1 m) and color tone. The third layer (Fig. 9.3b) was characterized by unique wave forms throughout, but the fourth layer was mostly straight, with a few isolated wave patterns. The fifth layer was one meter thick and had a color that was noticeably distinct from the previous ones. The authors stated that the majority of the FM grain phenolics, as well as the tannins, were targeted

Fig. 9.2 Example of nutrient distribution and partitioning in developed seed (Adapted from: www.precisionnutrition.com/all-about-grains)



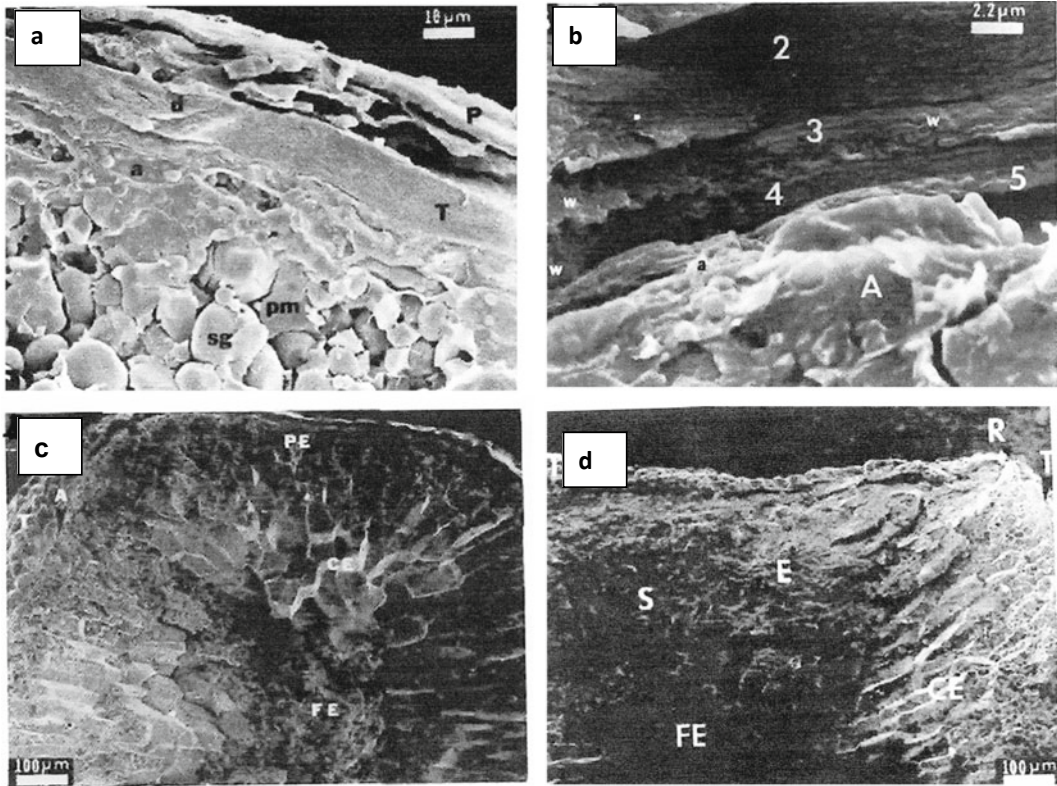


Fig. 9.3 Structure of anatomical parts of the finger millet grain (adapted from McDonough et al. 1986). **a** Pericarp, testa, aleurone layer and peripheral endosperm layers. P = pericarp; T = testa; a = aleurone; pm = protein matrix; sg = starch granule. **b** Four of the five testa layers, showing wave formations and contour striations. 1–5 = testa layers; w = wave formation; A = aleurone

cell; a = aleurone cell wall. **c** Three discrete layers of the starchy endosperm. A = aleurone layer; PE = peripheral endosperm; CE = corneous endosperm; FE = flourey endosperm. **d** Cross-section of the germ. R = ridge; T = testa; E = embryonic axis; S = scutellum; CE = corneous endosperm; FE = flourey endosperm

within the episperm layer, although the location of the tannins was unknown.

9.3.3 Endosperm

The endosperm makes up the majority of the FM kernel's weight (McDonough et al. 1986). The aleurone layer of FM was discovered to be quite similar to that of maize, sorghum, and pearl millet. It was one cell layer thick and completely encircled the FM starchy endosperm. There are many aleurone bodies present, but no starch granules. The cell walls of aleurones autofluorescent, indicating the presence of

phenolic chemicals. FM starchy endosperm contained separate flourey and corneous layers, according to Angold (1979). Similar to what was discovered in sorghum, pearl millet, and maize, McDonough et al. (1986) identified three separate forms of starchy endosperm, the peripheral, corneous, and flourey endosperm (Fig. 9.3c). The peripheral endosperm cells were the tiniest of the endosperm cells. The peripheral endosperm cell contents were firmly packed. A vast number of protein structures were contained in a protein matrix and linked to compound and simple starch granules (diameter: 8.0–16.5 m). Corneous endosperm made up the majority of the endosperm. It was mostly made up of compound

starch granules (3.0–19.0 μ m in diameter), with some simple starch granules thrown in for good measure. The starch granules were linked to patches of a protein matrix. Compound starch granules about 11–21 μ m in diameter make up the floury endosperm. There were only a few protein bodies and a protein matrix to be found. The starchy endosperm cell walls fluoresced brightly, showing the presence of phenolics.

9.3.4 Germ

Millet contains a comparatively small germ (270 \times 980 μ m), according to McDonough (2000). McDonough et al. (1986) proposed that the FM germ was located in a deep depression surrounded by a distinctive ridge that wrapped around the entire germ (Fig. 9.3d). The hilum was placed adjacent to the germ, and the stylar was placed on the kernel's opposite facet. The scutellum stratum separated the scutellum from the tiny reproductive structure. The macromolecule bodies were found in the scutellum. It's worth noting that the FM grain may be difficult to mill due to its small size and the fact that its testa is securely attached to the endosperm (McDonough 2000).

9.4 Composition of the Finger Millet Grain

FM grain is supposed to be more nutritious than other cereal grains in terms of amino acids, minerals, and dietary fiber, according to the US National Research Council (1996). As previously stated, FM grain includes numerous phenolic chemicals, which may be beneficial to one's health due to their antioxidant characteristics (Dykes and Rooney 2006). Both genetics and the environment influence the chemical composition

of FM grain (McDonough 2000). Despite the fact that Table 9.1 shows mean values, the proportions of grain chemical composition might vary greatly.

9.4.1 Carbohydrates and Dietary Fiber

Carbohydrates account for 70–76% of the total weight of the FM grain, according to Obilana and Manyasa (2002), with 7.9% cellulose, 61.8% starch, 0.8% reducing sugars, 4.9% pentosans, and 0.5% dextrins. In the endosperm, starch is usually found in the form of simple or complicated granules (McDonough et al. 1986). The major components of FM starch are amylose and amylopectin, which have similar MW to other cereal starches (Serna-Saldivar and Rooney 1995). Sucrose, raffinose, glucose, maltose, and fructose are among the sugars contained in FM grain (McDonough 2000). Glucose and sucrose account for 12.5 and 33% of the soluble sugars in FM grain, respectively.

FM grain has a higher total dietary fiber content (22.0%) than other cereal grains, which contain 12.6%, 4.6%, 13.4%, and 12.8% wheat, rice, maize, and sorghum, respectively. The fiber components of FM grain, like those of other cereal grains, are found in the cell walls (mostly endosperm and pericarp cell walls) (Serna-Saldivar and Rooney, 1995). Dietary fiber comprised 18.6% of the FM grain, according to Kamath and Belavady (1980), which included 4.6% cellulose, 6.1% non-cellulosic polysaccharides (1.5% water-soluble and 4.7% water-insoluble), and 7.9% lignin. FM grain has 1.4% soluble dietary fibre and 15.7% insoluble dietary fiber, according to Chethan and Malleshi (2007). Shobana and Malleshi (2007) found 22.0% total dietary fiber, 19.7% insoluble dietary fiber, and 2.5% soluble dietary fiber in their study. The non-cellulosic polysaccharide components of FM grain dietary

Table 9.1 Chemical composition of finger millet grain

Nutrients				Non-nutrients
Major nutrients (g/100 g ^a)	Minerals (mg/100 g ^c)	Amino acids (g/100 g protein ^d)	Vitamins (mg/100 g ^c)	Phenolic compounds ^d
Moisture: 12.0	Calcium: 358.0	<i>Essential amino acids</i>	Vitamin A (RE): 6.0	<i>Phenolic assay</i> ^f
Carbohydrate: 74.0	Chlorine: 84.0	Phe: 6.2	Thiamin: 0.2	Folin/Ciocalteu: 0.55–0.59
Protein: 7.3	Copper: 0.5	His: 2.6	Riboflavin: 0.1	Vanillin-HCl: 0.17–0.32
Fat: 1.3	Iodine (µg): 10.0	Ile: 5.1	Niacin: 1.0	Phenolic acids ^g
Total dietary fibre: 22.0 ^b	Iron: 9.9	Leu: 13.5	Vitamin C: 1.0	Protocatechuic: 23.1
Ash: 2.6	Magnesium: 140.0	Lys: 3.7		Gentisic: 61.5
	Manganese: 1.9	Met: 2.6		p-OH Benzoic: 8.9
	Molybdenum (µg): 2.0	Thr: 5.1		Vanillic: 15.2
	Phosphorus: 250.0	Val: 7.9		Caffeic: 16.6
	Potassium: 314.0	<i>Non-essential amino acids</i>		Syringic: 7.7
	Sodium: 49.0	Asp: 7.9		Coumaric: 56.9
	Zinc: 1.5	Glu: 27.1		Ferulic: 387.0
		Ala: 8.0		Cinnamic: 35.1
		Arg: 5.2		
		Cys ^e : 1.6		
		Gly: 4.8		
		Pro: 6.7		
		Ser: 6.9		
		Tyr: 3.6		
		Trp ^e : 1.3		

^a Obilana and Manyasa (2002)

^b Shobana and Malleshi (2007)

^c US National Research Council (1996)

^d McDonough (2000)

^e cysteine and tryptophan are not essential amino acids, but they can spare the requirement for methionine and phenylalanine, respectively

^f mg/100 mg catechin equivalents, dry weight basis

^g µg/mg

fiber appear to be predominantly non-starch polysaccharides arabinoxylans (pentosans), with glucose, xylose, galactose, and arabinose as important sugar contents, while mannose and rhamnose are minor constituents (Nirmala et al. 2000; Rao and Muralikrishna 2001).

9.4.2 Protein

Due to water availability, genotype, temperatures, soil fertility, and environmental factors present during grain development, the total protein content of FM grain varies from 4.9 to

11.3% (McDonough 2000). (Serna-Saldivar and Rooney 1995). When compared to brown FM grain variations, white FM grain varieties are said to contain higher protein (Virupaksha et al. 1975; Rao 1994). FM has a protein level of 7.3% (Table 9.1), which is similar to rice (7.9%) and lower or similar to other millets, sorghum, and wheat (11.0, 9.6, 9.0, 12.6 and 7.9%, respectively, pearl millet, teff, fonio, wheat, and sorghum) (Klopfenstein 2000; Obilana 2003). Prolamins are the most abundant protein component in FM grain, followed by glutelins (Serna-Saldivar and Rooney 1995). Prolamins are also the most abundant protein fraction in sorghum and other millets (Serna-Saldivar and Rooney 1995). (foxtail, pearl and proso millets). These fractions (glutelins and prolamins) are mostly found in the starchy endosperm's protein bodies and protein matrix, respectively (Serna-Saldivar and Rooney 1995). Lysine and other critical amino acids are abundant in albumin, glutelin, and globulin fractions (Serna-Saldivar and Rooney 1995). When compared to other millets, FM proteins are said to be more nutritionally balanced (Ravindran 1992). "Eleusinin," the primary protein fraction of FM grain, contains adequate quantities of cystine, tryptophan, methionine, and total aromatic acids, which are sometimes low in other cereals (US National Research Council 1996). FM is notably high in methionine, accounting for about 5% of total amino acid content (US National Research Council 1996). However, lysine is limited in FM grain, as it is in other cereals, but pearl and FM millets have the greatest lysine (McDonough 2000). Anti-nutritional factors such as phenolic chemicals (condensed tannins) and trypsin inhibitors, which may be found in FM grain, might reduce protein bioavailability (Serna-Saldivar and Rooney 1995; McDonough et al. 2000).

9.4.3 Lipids

Sorghum and millets, according to Serna-Saldivar and Rooney (1995), include a variety of lipids, including phospholipids, glycolipids, triglycerides, phytosterols, carotenoids, and

tocopherols, which make up a minor fraction of the grains' proximate composition. In most cases, lipids are found in the scutellum. Polar, non-polar, and non-saponifiable lipids can be found in millets and sorghum, and they can be found as free, bound, or structured lipids. The non-polar lipids, which include triglycerides (fat/oil), are the most abundant. FM grain has a total lipid content of 5.2%, with the primary elements being oleic, palmitic, and linoleic acids (McDonough et al. 2000). FM grain has a lower fat content (1.3%) than sorghum and other millets, and is similar to 4.8, 2.0, 1.8, 1.1, and 2.8% pearl millet, tef, fonio, wheat, and sorghum, respectively (Obilana 2003). (Table 9.1). Because of its thin germ, FM grain has a low fat content (1.3%) (Serna-Saldivar and Rooney 1995). FM's low fat content may be important in that the grain has better storage qualities due to a lower tendency to go rancid.

9.4.4 Minerals

FM is a good source of minerals, especially calcium, which is 5–30 times higher than in other cereals (US National Research Council 1996). Iron, potassium, copper, magnesium, phosphorus, and sodium are also abundant (Obilana and Manyasa 2002) (Table 9.1). Minerals abound in the aleurone layer, pericarp, and germ (Serna-Saldivar and Rooney 1995). However, due to their interaction with anti-nutritional substances such as oxalic acid, phytic acid, and condensed tannins found in FM grain, the bioavailability of some of these minerals (e.g. divalent metal ions and phosphorus) may be reduced (Serna-Saldivar and Rooney 1995; McDonough 2000).

9.4.5 Vitamins

FM is high in water-soluble and lipid-soluble vitamins [thiamin, riboflavin, niacin, and maybe vitamin C, as well as tocopherols (vitamin E)]. (Serna-Saldivar and Rooney 1995; Obilana and Manyasa 2002; Serna-Saldivar and Rooney 1995; Obilana and Manyasa 2002) (Table 9.1).

The dried grain, on the other hand, is devoid of vitamin C. (Serna-Saldivar and Rooney 1995). Water-soluble B vitamins are concentrated in the aleurone layer and germ, while liposoluble vitamins are primarily found in the germ (Serna-Saldivar and Rooney 1995).

9.4.6 Phenolics, Flavonoids and Tanins

Phenolic acids, flavonoids, and tannins are among the health-promoting phenolic substances found in FM. The most common polyphenols are phenolic acids and tannins, with flavonoids making up a tiny percentage of the total. These compounds have recently attracted more attention due to their antioxidant and other nutraceutical characteristics. FM grains contain 0.3–3% polyphenols, with the majority (90%) localized in the seed coat, but a tiny fraction being present in the endosperm.

9.5 Nutrient's Partitioning

It observes specific differentiation of seed tissues into embryo, endosperm, and protein layer, and consequently, partitioning of the various nutritionally necessary bio-molecules, nutrient partitioning phenomena within the seed tissues may be a new area of interest to improve the standard of seeds (Nadeau et al. 1995). Previous research in the field of traditional genetics and breeding has made a significant contribution to the identification of factors affecting crop growth and grain filling. In addition to genetic and epigenetic variables, the environment has a significant impact on seed development. The primary bio-molecules such as carbohydrates, proteins, lipids, and others are dispersed throughout the seed tissues; nonetheless, it is important to determine whether these components influence nutritional partitioning. Nonetheless, for a better knowledge of seed biology, genetic variables should primarily be investigated at the molecular level (Xie et al. 2015; Jing et al. 2016).

9.5.1 Nutrient Partitioning and Omics Approaches

The various omics-based approaches are the best method for understanding seed biology at the molecular to systems level, because they provide holistic views of complex biological phenomena in the seed system by interpreting the behavior of each constituent (gene, protein, and metabolites) and their interactions in seed. It can also identify a variety of innovative features that can be applied to seed biology studies (Fukushima and Kusano 2013; Kumar et al. 2015a, b). The following section of this chapter offers molecular insights into the complexities of nutrient partitioning and seed biology.

9.5.2 Molecular Status for Dissecting the Complexity of Seed Biology and Nutrients Partitioning

Seed development research has developed as a critical area of study in plant biological process biology (Lohe and Chaudhury 2002). Seed development is controlled by a number of genes and their interactions with their targets. B3 or HAP3 domains are found in seed development regulators that interact with the basic essential amino acid zipper (bZIP) and APETALA2 (AP2) transcription factors (TFs). Homeotic gene, NAM, ATAF, and CUC (NAC), Myeloblastosis (MYB), and plant hormone Response issue (ARF)-domains-domains are some of the TFs that play an important part in this strategy (Agarwal et al. 2011). Many genes play important roles, including LEAFY COTYLEDON2 (LEC2) (Stone et al. 2001), MYB (Dumas and Rogowsky 2008), GNOM (Grebe et al. 2000), SHOOT MERISTEMLESS (STM) (Long and Barton 2000), MONOPTEROS (Hardtke and Berleth 1998), and FACKEL. The ALTERED MERISTEM PROGRAMMING1 (AMP1) gene was discovered to play an important role in embryogenesis during pattern formation (Lohe and Chaudhury 2002), and the LEAFY COTYLEDON (LEC)

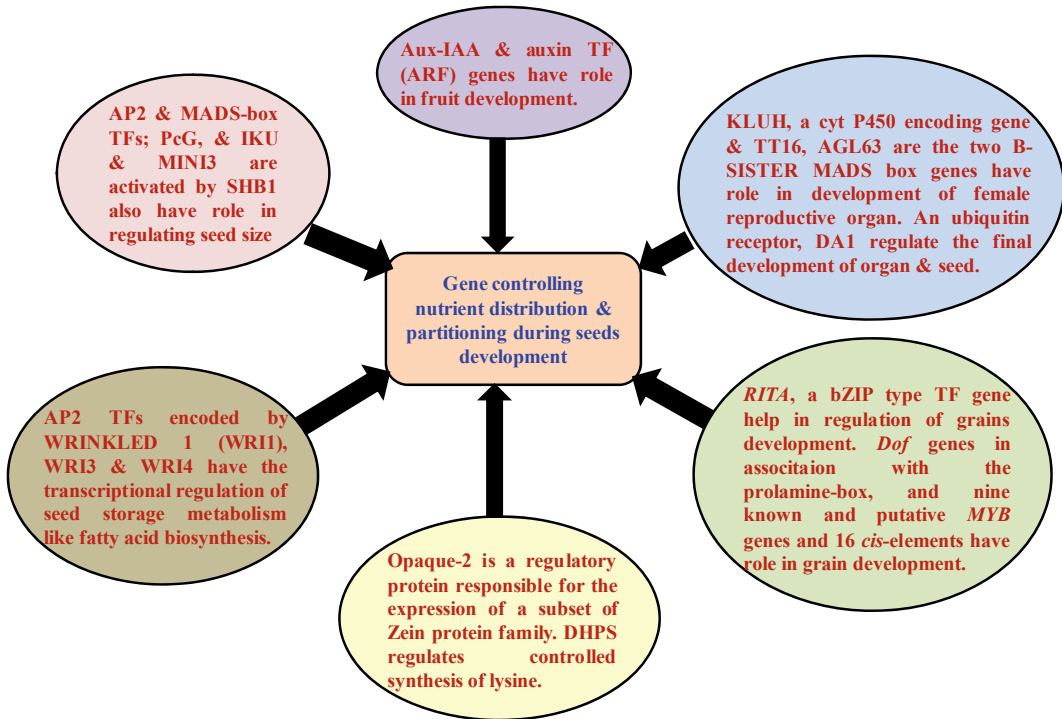


Fig. 9.4 Gene controlling nutrient distribution and partitioning during seeds development

genes LEC1, LEC1-LIKE, LEC2, and FUS3 are seed-specific TF genes that regulate seed developmental processes (Le et al. 2010) (Fig. 9.4).

Because seed developmental mechanisms differ across monocot and dicot plants, high-throughput transcriptome data analysis provides a first step toward understanding the molecular networks and pathways that act during seed development in specific compartments. In dicots and monocots, comparative systems-based co-expression network analyses will define evolutionarily conservative (FUS3/ABI3/LEC1) and divergent (LEC2) networks (Sreenivasulu and Wobus 2013).

Homeotic genes play an important part in the differentiation and development of the plant's various organs. In *Arabidopsis*, for example, AP2 is in charge of floral homeotic gene expression regulation. AP2 is expressed at the RNA level in all four types of floral organs, including petals, sepals, carpels, stamens, and developing ovules, in accordance with its genetic roles (Jofuku et al. 1994). B-sister genes have

been demonstrated to be necessary for the proper differentiation of the ovule/seed in angiosperm species. Many genes, particularly those expressed in the seed, are still in the characterization or uncharacterized stage, and their roles remain uncertain. As a result, identification of seed-specific genes that could be exploited in biofortification programs to provide food and nutritional security for our civilization faces more obstacles. As a result, contemporary systems biology methodologies can help us gain a better understanding of seed biology.

9.5.2.1 A Hypothetical Model for Defining the Role of Various Calcium Transporter Genes Involved in Calcium Accumulation in Seeds

In the absence of a genomic resource in FM, rice and sorghum MPSS data were used to investigate the expression of different calcium transporter genes at various stages of seed development, and

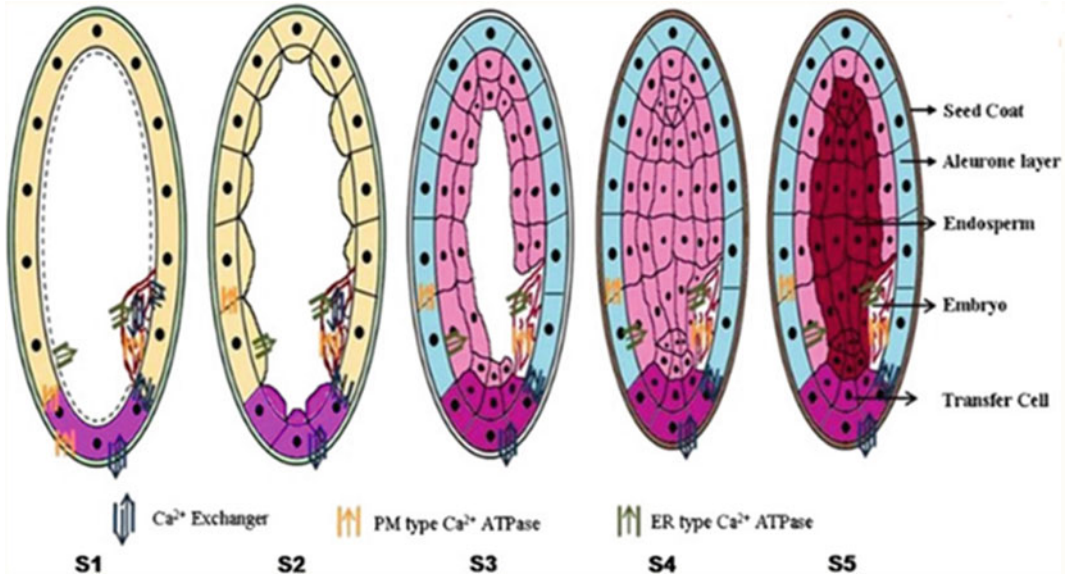


Fig. 9.5 A hypothetical model to show the possible presence of Ca²⁺ transporter genes that may be responsible for calcium accumulation in cereal grains during grain filling. (Adapted from Goel et al. 2012)

the results were used to identify the major transporters expressed at various stages of FM spike development (Goel et al. 2011). A possible model for calcium buildup in cereal grain seeds can be given based on expression analyses of calcium transporter genes and data from the literature (Fig. 9.5). In cereals, the $3n$ endosperm cell multiplies repeatedly after fertilization without establishing a plasma membrane, giving birth to the syncytium. A big vacuole's nucleus extends all the way around its edge. The plasma membrane is generated in the following cellularization stage, enveloping each nucleus. After that, the cells divide periclinally to create aleurone layer cells on the exterior and endosperm cells on the inside, completely covering the vacuole. Before undergoing apoptosis, the endosperm cells aggressively express seed-specific genes, such as TF genes, regulatory proteins, and genes involved in seed storage protein, glucose, and fatty acid accumulation. The aleurone cells create layers of cells to cover the endosperm and stay alive until germination. The majority of calcium transporter genes expressed in seed are expressed during the early stages of seed development and drop subsequently, indicating that the majority of calcium is mobilized through the transporters during the

early stages of seed development. At the S1 stage of seed development, however, members of all three transporters (type IIA and type IIB Ca²⁺ + ATPases and calcium exchangers) were present, and expression of all genes was reduced, with the exception of the two ER type ATPases, which had significant levels of expression until the S5 stage. We assumed that there must be channels in the membranes of transport cells (TC) at the basal region of the developing endosperm because they act as an entrance point for solutes from the mother plant to the developing endosperm. However, because no calcium channel genes have been expressed at this point, this activity could be performed by PM-type Ca²⁺ + ATPases or exchangers. Three PM-type Ca²⁺ + ATPases and four Ca²⁺ exchangers are expressed during the S1 stage, and calcium enters the TCs via some of these transporters found in the plasma membrane of TCs on the maternal tissue side. The incoming calcium is pumped into the developing coenocyte by ATPases and exchangers found in the PM of TCs on the other side (i.e., toward the syncytium). Calcium is pumped into the large vacuole by the action of ER-type ATPases located in the vacuolar membrane when the calcium concentration in the coenocytes

reaches a certain level, and because calcium cannot move back into the maternal plant, it is pumped into the large vacuole by the action of ER-type ATPases located in the vacuolar membrane. Calcium exchangers may potentially carry calcium from the syncytium to the developing embryo. Furthermore, other ER-type ATPases with high expression levels may pump calcium into the vacuoles of developing embryonic cells. There could also be Ca²⁺ ATPases with a similar function. One of the PM-type ATPases is expressed at high levels in the S1 and S2 phases, whereas the other two are expressed at lower levels. Because there is no aleurone layer at the S1 stage, higher-expressing ATPase cells are present in the TCs and are engaged in calcium transfer into the developing coenocytes. The other two ATPases are likely situated on the plasma membrane side of the aleurone layer, where they pump calcium into the aleurone layer from the beginning of the S3 stage until the conclusion of the S5 stage, or until the seed maturation stage (Goel et al. 2012). However, more experimental evidence is needed to determine whether the increase in calcium concentration detected in the seed coat is due to transporters present in the aleurone layer directing calcium to the seed coat or from the maternal tissue.

9.5.3 Potential Promises of Omics Approaches

The potential of “Omics” techniques open the door to new technologies that can aid in gaining an advantage by studying various components of biological systems that are essential for normal cell function. The application of integrative omics-based techniques is predicted to aid in the knowledge of seed biology and developmental processes (Fig. 9.6). These omics-based approaches include the use of genomics, transcriptomics, proteomics, metabolomics, glycomics, lipidomics, and other omics sciences to investigate biological molecules involved in the proper functioning of cells and their physiological mechanisms, as well as their responses to various environmental changes (Kumar et al. 2015a, b).

9.5.4 Phenomics: Characteristics Features of Seeds

Phenomics, which uses a modeling approach to obtain unbiased data from biological systems, will be critical in understanding the complexities of seed biology (Navarro et al. 2016). Seeds contain complicated traits that can be difficult to quantify (Gustin and Settles 2015). These are now required to understand the genetic basis of agriculturally relevant traits and to harness quantitative phenotypes. In resource-constrained contexts, screening for germplasm with high performance attributes would be easier. Plant phenomics has recently introduced and integrated new tools to better describe complex plant phenotypes. With the development of high-throughput phenotyping devices, it is now possible to obtain non-destructive phenotypic data from plants (Rahman et al. 2015). Researchers have used computational algorithms to decipher the complexity of phenomics data such as plant senescence progression by analyzing distorted and blurred color images under high throughput conditions (Cai et al. 2016), as well as phenomic prediction of maize hybrids to obtain a viable alternative for genomic and metabolic prediction of hybrid performance (Cai et al. 2016; Edlich-Muth et al. 2016).

9.5.5 Genomics and Transcriptomics

The availability of whole genome sequence information for model plants such as *Arabidopsis* has enabled the development of tools to facilitate systems level integration of genes into functional units in massive interconnected biological networks and speed crop improvement (Venglat et al. 2014; Gupta et al. 2017). System biology techniques may now compare transcriptional networks across species that act throughout embryo and seed development, providing deeper insights into conserved and divergent gene networks (Bevan and Uauy 2013). It's also a good resource and reference for other seed systems, such as FM (Dean et al. 2011).

Crop plants have yet to fully leverage the global genetic and metabolic pathways involved

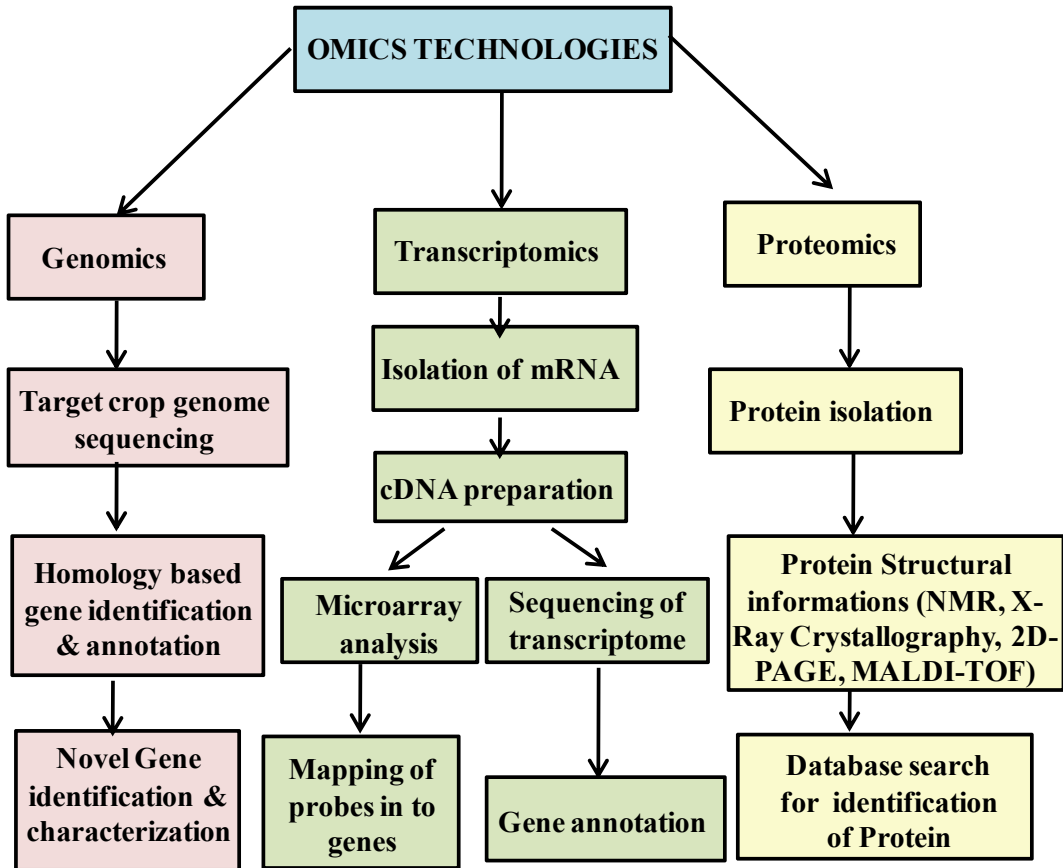


Fig. 9.6 Omics approaches for deciphering the secret of biochemical factories in seeds

in seed development and contribute to seed genetic improvement. The work is being progressed by gaining insights into molecular and biochemical pathways linked with gene expression, protein, and metabolite profiles during seed development in model and crop plants. Comprehensive datasets are being produced as a result of these integrated systems techniques and studies (Venglat et al. 2014). Seed size is governed by genetic variables that have been under continual selection during the evolution of various seed systems in flowering plants, as previously documented (Westoby et al. 2002). The expression of numerous known and suspected TF genes was analyzed during grain filling to investigate the involvement of TFs in the transcriptional

regulation of grain filling. A set of TF genes with expression characteristics comparable to those of known grain filling genes was found using cluster analysis. RITA, a bZIP type TF gene, is one of the genes in this group that is strongly expressed in aleurone and endosperm tissues and may have a role in regulating gene expression in developing rice grains. Dof genes, whose gene products can activate the expression of seed storage protein genes by binding to the prolamine-box, and nine known and probable MYB genes are also included in this cluster (Kumar et al. 2009; Gaur et al. 2011; Gupta et al. 2011, 2017).

The size of the final seed is also influenced by the growth of the endosperm and seed coat. The transcription factors AP2 and MADS-box play

essential roles in Arabidopsis seed size regulation. The *ap2* mutant's endosperm cellularization is delayed, resulting in larger embryo sacs and embryos with increased cell quantity and size. This larger seed characteristic was handed down through the maternal sporophyte and endosperm genome (Fang et al. 2012). Polycomb (PcG) protein-encoding genes are the second group of genes implicated in seed size regulation. This contains genes that make up the FERTILIZATION INDEPENDENT SEED (FIS) complex, which acts as a transcriptional repressor by methylating histones, as well as imprinting genes (DNA METHYLTRANSFERASE1 and DECREASE IN DNA METHYLATION2), which methylate the PcG genes (Hennig and Derkacheva 2009). HAIKU (IKU) and MINI-SEED3 (MINI3), which are activated by SHORT HYPOCOTYL UNDER BLUE1, are two genes that govern seed size (SHB1). In monocot crops, however, little is known about the molecular pathways that regulate endosperm cellularization (Figueiredo and Köhler 2014). The inner integument of developing ovules is heavily influenced by the expression of *KLUH*, a cytochrome P450 expressing gene. The two B-SISTER MADS-box genes *TT16* and *AGL63*, which play roles in Arabidopsis female reproductive organ development, have been discovered to be negative regulators of seed coat differentiation. Larger seeds result from mutations in them (Prasad et al. 2010). Arabidopsis has a ubiquitin receptor called *DA1* that controls the final seed and organ size, as well as the cell proliferation period. Seed size is reduced when *DA1* is mutated, whereas seed size is increased when it is overexpressed. The function of *WRINKLED 1* (*WRI1*), *WRI3* and *WRI4* in the control of fatty acid synthesis in numerous plant species, for example, has been shown by genetic study (Li et al. 2008).

9.5.6 Loss and Gain of Gene Function

The introduction of functional genomics technologies such as loss and gain of function in mutants has created a fantastic platform for studying seed biology. The Clustered Regularly Interspaced Short Palindromic Repeats-Cas9 (CRISPR-Cas9) technology has effectively changed the investigation of key gene activities (Rajendran et al. 2015). In seed biology, the method has more potential for identifying functional mutants of rate-limiting enzymes and critical regulatory targets of seed storage components, as well as revalidating models for building seed sinks for nutritional and health benefits (Sreenivasulu 2017).

9.5.7 Quantitative Trait Loci (QTLs) and Genome-Wide Association Studies (GWAS)

Quantitative trait loci analysis (QTLs) has been a precise and powerful way to determine loci and genes that manage essential and complex aspects connected with seed development in the post-genomic era (Macovei et al. 2017). While genome-wide association studies (GWAS) have lately gained popularity due to their ability to overcome numerous constraints of QTL analysis, allowing for a quantitative measurement of the connection between each genotyped marker and a phenotype of interest (Macovei et al. 2017). Several QTLs associated with seed dormancy have been studied in cereals (Wan et al. 2005; Gu et al. 2008; Hori et al. 2007; Sato et al. 2016; Imtiaz et al. 2008; Ogonnaya et al. 2008) and need to be examined in FM.

9.5.8 Proteomics

The presence of high-abundance storage proteins limits the dynamic resolution of seed protein samples. Several methods for protein extraction have been developed over the last decade (Gupta and Misra 2016), and the use of proteomics approaches will aid in gaining a better understanding of seed biology. Key proteomics technologies such as MALDI-TOF, Edman sequencing, Q-TOF, LC-MS/MS, and others have emerged as the most important players in protein analysis (Deng et al. 2013). Proteomics is particularly useful for crops since it may reveal not just nutritional benefits, but also yield and how adverse conditions affect various aspects (Salekdeh and Komatsu 2007). Detailed proteomic analysis of rice leaf, root, and seed tissues using two independent technologies, 2DE followed by LC-MS/MS and MudPIT, resulted in the discovery of a large number of novel proteins (Ramalingam et al. 2015) involved in central metabolic pathways, transcription control, and mRNA and protein biosynthesis. Furthermore, because the protein composition and functional quality of cereal flour are significantly associated, the proteomics approach is ideal for discovering flour-making protein markers for suitable cultivars.

9.5.9 Metabolomics

Metabolomics has become a helpful diagnostic tool for phenotyping biological systems' biochemical phenotypes. Transcriptomics and metabolomics studies combined revealed similar variations in metabolite and transcript expression levels in diverse settings. DELAY OF GERMINATION (DOG) 1 is a seed-expressed gene that is necessary for dormancy induction. DOG1's function is not limited to dormancy, according to transcriptomics and metabolomics investigations, and it may possibly play many functions during seed development by interfering with the ABA signaling components in *Arabidopsis thaliana* (Dekkers et al. 2016). During tomato seed germination, however, considerable genetic diversity in metabolite abundance was identified,

demonstrating that metabolic composition is linked to germination phenotypes and overall seed performance (Kazmi et al. 2017).

A considerable amount of data pertaining to mass spectra, compound names and structures, statistical/mathematical models, metabolic pathways, and metabolite profile data has been produced and maintained in the form of databases over the last few decades. Although data resources are distributed over the Internet, such databases complement each other and facilitate efficient growth in metabolomics (www).

Available metabolome datasets aid in summarizing the current state of related tool development, with an emphasis on the plant metabolome. Data sharing would pave the path for more accurate metabolomics interpretation and advancements in plant systems biology. Such a large amount of data can be used to extract key information about seed systems, which can then be used to engineer metabolic pathways responsible for nutrient production. It can also be used to develop strategies to increase or decrease the synthesis of high-energy compounds or the manufacturing of any other nutrients in the seed, depending on our needs (Fukushima and Kusano 2013; Kumar et al. 2015a, b).

9.5.9.1 Lipidomics

Lipidomics strives to quantitatively represent the many types of lipids in biological systems, as well as their molecular species. Lipidomics advancements allowed for a new degree of sensitivity and precision in quantitative lipid analysis. The ability of MS-based lipidomics to address the complexity of cell biology inquiries is critical (Brügger 2014; Horn and Chapman 2014). Recent research on *Arabidopsis* transgenic seeds has revealed where dihydroxy ascorbate (DHA) is collected and joined with other neutral and phospholipid fatty acids in growing as well as mature seeds (Zhou et al. 2014). Lipidomics techniques have the ability to examine the complexities of seed biology from a molecular to a systems level, allowing for the quantification of seed developmental processes that can be used to improve seed nutritional content through genetic engineering.

9.5.9.2 Glycomics-Thermodynamics Approach

Glycomics is the study of all the glycan structures found in a cell, tissue, or organism on a systemic level. It is one of the most promising developing technologies in the post-genomic era for characterization of biological systems. Studies using mass spectrometry-based glycomics and glycoproteomics techniques on N-glycan structures of lotus seed identified 19 N-glycan structures with high mannose (20%), paucimanosidic (40%) and complex forms (40%) and lotus convicilin storage protein 2 (LCP2), which has high mannose N-glycans and serves as a model system for deciphering the role of seed proteins and their glycosylation in food allergy (Dam et al. 2013). The seeds of plants with a high quantity of biomolecules such as carbohydrates, which include complicated information for conveying biopolymers, are gaining popularity among scientists (Hu et al. 2015). Glycosylation alterations have been observed throughout a number of important events, including embryogenesis and differentiation, and it may potentially play a role in seed formation. Understanding the role of glycan during seed development may therefore be useful in identifying important molecules that can be used to better understand the complicated biology of seeds and their developmental processes.

9.5.9.3 Vitamin Analysis

Vitamin analysis, like that of other chemicals present in seed tissues, can be done using a variety of detectors depending on their biochemical structures. Water-soluble vitamins can be determined simultaneously using an ion-pair reversed-phase HPLC separation method and an ESI-MS/MS detection system. It has also been demonstrated that it is effective in extracting complete vitamin content from meals. It has also been demonstrated that thiamine (B1), riboflavin (B2), nicotinic acid, nicotinamide, pyridoxine (PN), pyridoxamine (PM), piridaxal (PL), thiamine-monophosphate (TMP), riboflavine-5'-phosphate (FMN), and piridoxal-5'-phosphate

(PLP) can all be separated using 0,05% (v/v) (Engel 2009). These approaches can be used to detect the numerous vitamins contained in the seed, as well as their concentrations, which can help in the production of agri-food items with high nutritional value.

9.5.9.4 Minerals Analysis

The presence of several micro and macro nutrients in seed tissues makes them an important source of nutrition for humans. Mineral analysis is a widespread activity in the agricultural research field. Synchrotron X-ray microfluorescence was used to examine the in vivo mineral distribution profiles in rice grains, as well as alterations in those distribution patterns during advanced phases of germination (XRF). Mineral translocation from certain seed sites during germination was element specific as well. The mobilization of K and Ca from grains to growing roots and leaf primordia was found to be high, while the flow of Zn to these expanding tissues was found to be lower than that of K and Ca. At least during the first several days after germination, the mobilization of Mn or Fe was modest.

9.6 Systems Biology: A Holistic Approach to Seed Biology Data Integration and Analysis

In any organism, a system is made up of a set of components or elements such as genes, proteins, metabolites, and so on. These system components are designed to promote the flow of knowledge, either directly or indirectly, and to preserve the system's balance, which is essential for its survival and achievement of its goal. Systems biology is a holistic approach to studying its behavior through the integration, annotation, modeling, and analysis of high-throughput omics data provided by available omics platforms (Pathak et al. 2013; Kumar et al. 2015a, b).

Progress in "omics" technologies have produced a massive amount of molecular data about seed, and substantial efforts have been made to

decode biological systems as true systems in order to conduct affordable and cost-effective research in systems biology. Bioinformatics methods involving database handling, modeling, network analysis, and simulation result in significant improvement in systems level understanding of biological systems when high-throughput omics data is analyzed (Kumar et al. 2012; Gupta and Misra 2016; Pathak et al. 2017).

Systems biology is critical for dissecting the intricacy of each component and its particular function in seed developmental processes, as well as assisting in the increase of nutrient output through metabolic engineering, biofortification, and other biotechnological approaches that will lead to increasing contents of nutrients in the seed (Fig. 9.7).

The embryonic development of seeds is a large-scale metabolic conversion process. Photosynthates and amino acid precursors are

imported and converted into oil, protein, and storage polysaccharides in this process. The developmental processes are genetically predetermined and influenced by external factors (Li et al. 2015). Current biological network models depend upon accumulation data, which makes it impossible to predict the entire picture of cellular metabolism (Gurwitz 2014). Previous omics-based studies using systems biology methods have looked into a group of unique genes and their molecular mechanisms by combining transcriptomics and metabolomics to decipher the global developmental and metabolic networks that find out the structural features and examined the biochemical composition of mature seeds of soybean (Li et al. 2015). Such research may aid in the identification of nutritional protein contained in seeds to combat hidden hunger, starvation, and preserve health, as well as the production of nutraceuticals products for societal gain.

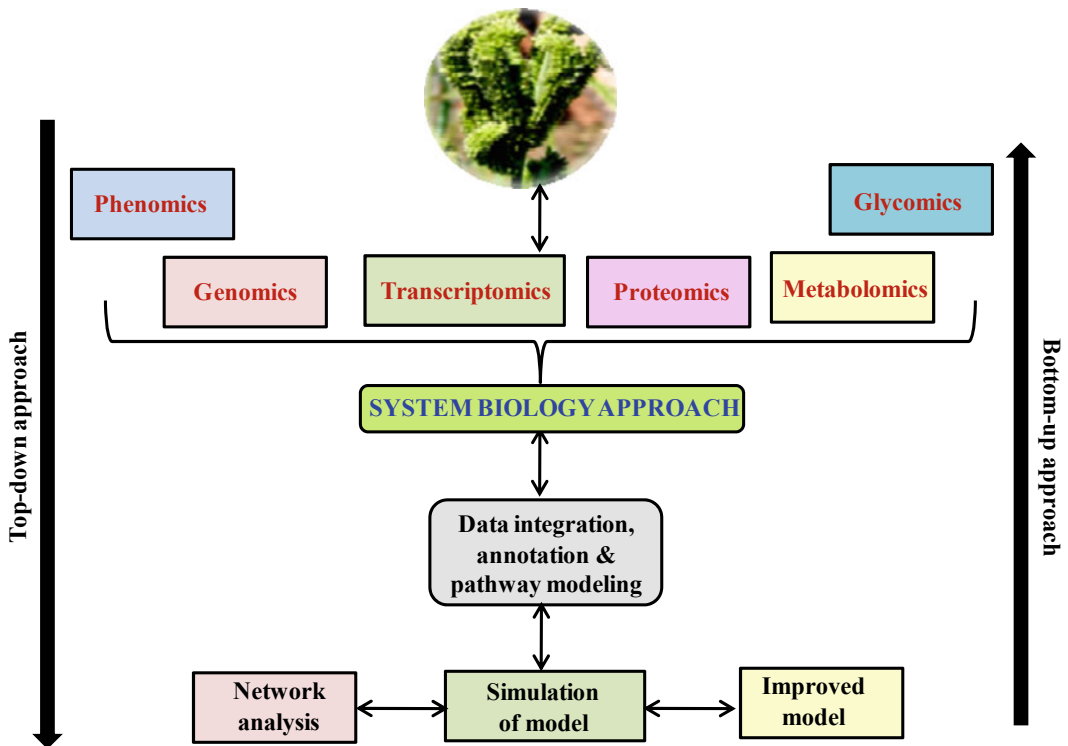


Fig. 9.7 Systems Biology methods are being used to decode the dynamics of seed and its developmental mechanism

9.6.1 Integrative Seed Systems Biology

It starts with sample collection followed by wet-lab experimentation using high-throughput omics platforms including genomics, transcriptomics, metabolomics, phenomics, glycomics, lipidomics, and data analysis, and continues with analytical methods, data integration and interpretation, and investigation of useful information. It aims to develop integrative models focused on data analysis and system organization at the molecular level, with a focus on detailed queries at specific scales. This strategy is also regarded as a “top-down” approach (Kumar et al. 2015a, b; Gupta and Misra 2016).

Recent advances in omics platforms have begun to provide information on a few primary genes/metabolites needed for seed development. mRNA abundance analyses and visualizations of during seed growth, maturation, and beyond are revealing global gene expression patterns that decide the final seed structure and composition in various crops (Watson and Henry 2005; Li et al. 2005; Furutani et al. 2006; Gallardo et al. 2007; Collakova et al. 2013; Palovaara et al. 2013). Accessible public domain information/data can be used for integration and analysis to explore the dynamics of seed biology at the molecular level in order to gain a full image of seed developmental processes.

9.6.2 Predictive Seed Systems Biology

Predictive systems biology or “Bottom up” approach focuses on the molecular mechanism and role of various components in biological/seed systems by building mathematical models and formulating hypotheses based on the detailed data provided by top-down approaches. Data retrieval from various database resources, literature mining, pathway modeling, network design, and perturbation analysis using systems biology techniques to predict the behavior of all the components present in

systems are the key steps in the bottom up approach (Kumar et al. 2015a, b; Pathak et al. 2013; Gupta and Misra 2016).

The majority of currently available biological network models are focused on transcript accumulation data, which does not provide a full picture of cellular metabolism (Gurwitz 2014). As a result, combined omics dataset analysis is needed, and software is being developed to resolve the challenges of these “big data” analyses (Nadeau et al. 1995; Hur et al. 2013). Recent systems-based studies have clearly demonstrated the incorporation of transcriptomics, metabolomics, and metabolic flux analyses (MFA) for a deeper understanding of the metabolic and regulatory network that regulates soybean seed composition (Li et al. 2015). The MetNet systems biology platform was used to analyze data in order to establish hypotheses and predictive models related to the organization and control of metabolic networks (Wurtele et al. 2007; Sucaet et al. 2012). Additionally, 37,593 Glycine max probes were annotated as model systems for studying soybean seed biology using sequence homology with *Arabidopsis* (Li et al. 2015).

9.6.3 Intermediate Approach in Seed Biology

The scientific community faces challenges in comprehending the complexities of biological processes. As omics-based high-throughput experiments assess the complexity of systems at multiple levels such as intercommunicating cell groups, interaction of multiple molecules in active pathways, and altered states to generate profiling data. This information will be used to bridge the difference between top-down and bottom-up approaches. Intermediate methods are used to integrate different biomolecules in an organized way from the cell to the system stage, allowing for the quantification of biological processes and the filling of unknown information for better understanding of biological systems (Butcher et al. 2004; Gupta and Misra 2016).

9.6.4 Tools and Databases for Seed Systems Biology

The development of databases resources and software packages for the study of biological systems is the most challenging field today because it requires a multidisciplinary team of researchers with strong backgrounds in fields such as biological sciences, physical sciences, chemical sciences, mathematical and statistical

sciences, and computational sciences to create effective and user-friendly tools and databases. We identify some key computational systems biology databases and tools that should be used to decode the complexities of seeds at the molecular to systems level for characterization of important genes/proteins for the development of nutraceuticals and functional foods as well as other applications (Table 9.2).

Table 9.2 List of some important tools and databases of computational and systems biology

Softwares/databases	Application	Availability
R and bioconductor	It is a well-known software program for decoding the complexity of biological systems using next-generation sequencing data, functional genomics, network biology, and other applications	https://www.r-project.org/ , https://www.bioconductor.org/
Cell designer	CellDesigner is a tool for designing, visualizing, and simulating biological systems' pathways	http://www.celldesigner.org/
Cytoscape	It is well-known systems biology software for high-throughput data integration and modeling, as well as visualization and analysis of biological networks	http://www.cytoscape.org/
Biological networks	It's a network systems biology tool for integrating and modeling multi-scale data and other applications	http://biologicalnetworks.net/Software/index1.php
Matlab	Matlab is a well-known program for biological system modeling and simulation	http://in.mathworks.com/products/matlab/?requestedDomain=www.mathworks.com
CLC genomic workbench	CLC Genomics Workbench is a piece of software that allows you to examine and visualize high-throughput omics data	http://www.clcbio.com/
PMR (Plant/Eukaryotic and Microbial Systems Resource)	It's a database of plant and eukaryotic microbe metabolomics data	http://metnetdb.org/PMR/
KEGG	KEGG is a well-known database resource for gaining a better grasp of the complicated functions and applications of biosystems	http://www.genome.jp/kegg/
GEO	GEO is a functional genomics data repository that houses gene expression data generated by high-throughput omics platforms. These data can be reused by the scientific community	http://www.ncbi.nlm.nih.gov/geo/
Array express	It's a functional genomics experimentation database that stores gene expression data from microarray and other high-throughput sequencing tools for researchers to reuse	https://www.ebi.ac.uk/arrayexpress/
Plant metabolic network	PMN is a database that contains information about plant metabolic pathways	http://www.plantcyc.org/

9.6.5 Application and Expected Outcomes

Genome sequencing of crop plants on a large scale, helped by high-throughput omics technologies, data integrations, modeling, and visualization of important genes and proteins, and by studying critical components present in seed with structural and functional details, simulation analysis methodologies have opened up new paths for bridging the gap in seed systems biology and identifying its undiscovered processes. When we use diverse genomic, transcriptomic, and other omics data about seeds for analysis through integrative and predictive approaches to develop seed as a model with complete information of each component present in the seed, and that information can be used to develop nutraceutical products, systems-based approaches will be very useful. Furthermore, it may contribute to future food and nutritional security (Kumar et al. 2015a, b).

9.7 Conclusions

To encourage translational research to engineer seed systems, it is critical to study the omics data of seeds, their integration, and annotation for identification of key components associated with nutrient loading during seed developmental processes, followed by their validation, using effective and precise molecular and systems biology techniques, which would provide a forum for researchers to investigate the secrets of biochemical seed factories in order to better understand their existence and produce sustainable seeds that can thrive in a variety of environments and geographical regions. Several genes and transcription factors affect the growth of the seed tissue system. The nutrient distribution event is governed by a complex network, which these omics-based tools are successfully exploring. System biology has tremendous potential in designing of research program for nutritional biology. From a very basic standpoint, we now have a well-established forum on which to continue seed biology research in order to

improve nutritional quality of seed. The model plant system has sparked ideas for a wider prospectus and methods to make this more predictable. Thus, a comprehensive informational study is needed, which will open up a new horizon of molecular and developmental biology studies to aid nutrition biology research.

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