Chapter 2

Decoding the Transcriptome of Rice Seed During Development

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Additional information is available at the end of the chapter

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Abstract

Rice seed development is a continuous process wherein it undergoes complex molecular and tissue reprogramming. It is a collective effect of embryo and endosperm development, each of which undertakes its own developmental paths, with endosperm development significantly affecting embryo. Understanding the mechanistics of the regulatory networks administrating this process is the building block for any future research on grain yield and quality. High-throughput transcript profiling and small RNA profiling studies have proved useful in providing information about the molecular changes occurring in various tissues associated with seed development. Transcriptome sequencing studies have highlighted the significant genes and pathways that are operating during seed development. The involvement of TFs and hormones has also been implicated in regulating key aspects of seed development, including embryo patterning and seed maturation. This chapter will review the information provided by high-throughput sequencing studies on various aspects of rice seed development, highlighting the developmental complexities of embryo and endosperm.

Keywords: cell cycle, embryo, endosperm, hormones, rice, seed development, transcription factors, transcriptome

1. Introduction

Seed development is a unique attribute of plants providing them the privilege of perpetuating genetic information over generations by safeguarding against environmental atrocities. Physiologically, it is a combined effect of two complex developmental processes, embryo and endosperm development. In case of dicots, majority of the seed volume is formed by the

embryo at maturity and the endosperm is consumed by the embryo during the course of seed development. The structure of monocot seed, such as rice, is different from a dicot seed by the presence of a starchy endosperm which occupies most of the space inside the seed coat and the embryo is positioned at the ventral side. Furthermore, the seed is covered entirely by the husk, which is formed by drying of the lemma and the palea. Seeds serve as the storage factories for synthesizing carbohydrates, proteins and lipid molecules, hence act as nutrition suppliers to the germinating seedling as well as to animals and humans. Rice seeds, in particular, are the major calorie providers constituting about 20% of the human nutrition worldwide [1, 2]. Therefore, it becomes imperative to understand seed development in rice to produce varieties with improved nutritional content and yield.

Seed development in rice incorporates development of the embryo and the endosperm and occurs in a systematic and sequential manner followed by desiccation and seed dormancy. The entire process of seed development in rice has been summated into five different stages from S1 to S5, categorized as 0–2, 3–4, 5–10, 11–20 and 21–29 days after pollination (DAP) seeds, respectively. Developmental period of the seed consisting of post-fertilization to middle globular embryo constitutes the first stage followed by embryo patterning and endosperm cellularization in second stage. The third stage is concerned with embryo morphogenesis, formation of a milky endosperm and initiation of endoreduplication. In the maturation phase, the milky endosperm transits from soft dough and hard dough stages in S4, and the seeds progress towards dormancy and desiccation in S5 stage [3, 4]. These developmental changes are channelized impeccably through the skillful operation of several genes and complex regulatory networks upon perception of internal and external stimuli [4–6]. Recent technological advances have facilitated the identification of genes responsible for guiding various steps of seed development. High-throughput mRNA profiling studies or transcriptomics is one such technology that has helped in deriving vital information about a myriad of molecular events that orchestrate seed development [7, 8]. Transcriptome profiling of a wide range of rice tissues, including vegetative and reproductive tissues, have proved beneficial in providing primary information about the genes expressed during seed development including their levels, patterns and molecular functions [6, 9–11]. With the aid of advanced bioinformatics platforms, transcriptome data is now being processed to derive more complex interpretations including pathways and regulatory networks that provide more complete picture of the molecular changes regulating seed development [12–15].

2. Expression atlases capture the dynamicity of transcriptome during seed development

Seed development is a continuous process and is effected by the participation of many tissues that undergo various developmental changes over a course of time. Such dynamic alterations would be difficult to be depicted entirely by studying tissues in isolation. Gene expression atlases incorporate transcriptome profiles of a wide range of cell types and/or developmental stages. Such global profiling studies become important when tracing

the complex transcriptional changes associated with transition of tissues from one phase of development to another. Several high-throughput studies of this nature have been conducted in rice, employing MPSS, microarray and transcriptome sequencing, which span both vegetative and reproductive tissues [6, 9–11, 16, 17]. The primary information obtained from expression atlases is about the transcriptional status of the tissues/organs with respect to one another. For instance, the steady increase in the number of down regulated genes in seed tissues with respect to vegetative tissues indicates gradual decrease in transcriptional activity with the progression of seed development [6]. The similarity and disparity in the transcriptome profiles has also been used to assess the relatedness of tissues [16]. Through microarray analysis of 39 vegetative and reproductive tissue types in rice, it has been shown that rice endosperm forms a separate cluster and exhibits relatively lesser gene expression than other tissues including panicle stages. However, the expression levels of these genes are significantly high and a large portion of these are endosperm-specific. Also, most of the development related genes show variation in expression levels among different tissues revealing the fluctuations at the molecular level that these genes experience as they pass from one stage of development to another. These observations provide several important inferences regarding seed development, such as transcriptional dynamics and tissue-specificity. Also, an inevitable inference from here is that the transcriptome undergoes intense spatiotemporal reprogramming during transition from vegetative to seed development in order to manifest the expression of seed-specific/preferential genes [11]. Atlases also provide information about the contributions of various tissues in seed development. Transcriptome study encompassing various vegetative and reproductive tissues of rice indicate that out of the total seed-specific genes obtained, the proportion of endosperm-specific genes is higher than that of embryo-specific genes. This might imply that the endosperm has more disparate transcript profile shifting the balance towards the role of endosperm in seed development in comparison to embryo [10]. Nevertheless, conclusions from such studies can be subjective and will vary according to the tissues and developmental stages under investigation and the methods of data analysis. For instance, the discovery of seed-specific genes will be influenced by the variety of vegetative tissues that are being considered for assessing specificity or the parameters set to call a gene expressed or differentially expressed. In a study published by our group, encompassing 19 vegetative and reproductive tissues, the number of seed-specific genes has been found to vary when expression is considered against different vegetative controls such as root and mature leaf [6]. This indicates that a single transcriptome data can emanate various circumstantial biological interpretations. However, to minimize erroneous accounting it is necessary to exercise caution while sampling and have precise knowledge of the query that is being pursued.

3. Functional intricacies of seed development revealed by atlases explain the conundrum of seed development

Functional annotations of the enormous data generated by atlases further elaborate on the predominant activities occurring during seed development including their mode of

regulation. The enrichment of genes involved in embryonic development in the transcriptome of endosperm suggests a possible communication between the embryo and the endosperm [11]. This exchange of information between the embryo and the endosperm highlights that a certain level of cross-talk might be necessary for their growth. Induction of expression of seed-specific genes after 5 days of flowering, when the endosperm development is accelerated and starch accumulation is initiated, suggests that majority of the seed-specific genes are associated with later stages of seed development and are required for seed filling and maturation [10]. Information about the key processes, pathways and genes involved in seed development can also be identified by comparative transcript profiling of tissues from various stages of development. Seed-specific proteins, seed allergens, genes involved in starch biosynthesis/degradation and ubiquitin-mediated protein degradation pathway show specific expression throughout the seed stages in comparison to four vegetative stages [6]. Transcription factors (TFs) have been reported to be enriched in both early and later stages of seed development suggesting their involvement throughout the process. In a study involving 48 organs of a j*aponica* rice variety, it has been found that out of 41 tissue-specific TFs obtained, 29 are seed-specific. These include several members of MADS, NAC, AP2-EREBP and CCAAT families that are expressed in a seed-specific manner with predominant expression either in the endosperm or the embryo and this expression is also driven by the stage of the tissues (**Figure 1**). TFs such as *VP1* and *LEC1*, which have been reported to be active during seed maturation, have been found to be present in the seed-specific category whereas AP2-EREBP TFs are found to express mostly in the embryo through its entire development [10]. In our study on an *indica* rice variety, it has been seen that 27 TFs families have higher number of members expressing in the seed stages, which include MYB, NAM, HSF, MADS, POZ, and bZIP. About 47 TFs are found to be specific to seed stages, of which most have specificity to S2 stage [6]. Such preferential expression patterns observed in various studies would imply that these TFs are administrating seed development by regulating downstream genes and pathways required for individual growth and development of the embryo and the endosperm at specific time points and stages.

Hormonal regulation is another indispensable component of the multi-faceted regulation of seed development. Rhythmic fluctuations in levels of hormones such as auxins, gibberellic acid (GA) and abscisic acid (ABA) have been observed during the course of seed development suggesting a complex interplay between these [18]. Different hormones have been known to be controlling different modules of seed development such as organ patterning, cell enlargement, desiccation and dormancy [19–22]. Seed-specific differential regulation of various hormones has been encountered in several reports. Auxin biosynthesis genes have been found to be induced during early stages of seed development [23]. This emphasizes on the role of auxins in the initial seed development processes that are mainly associated with active cell division in the embryo and the endosperm and organ initiation [4]. Along with auxins, implications for the role of gibberellin and ethylene have also been proposed. Genes associated with ent-kaurene biosynthesis, a precursor of gibberellin biosynthesis, were found to be up regulated in the S1 stage of seed development indicating the role of gibberellin in early phases of seed development [23]. A negative regulator of gibberellin signaling, *SLRL2* and a putative ethylene receptor that negatively regulates ethylene signaling, *OsETR2;2*, have been seen to be showing seed-specific expression [10]. These findings enumerate the significance of differential

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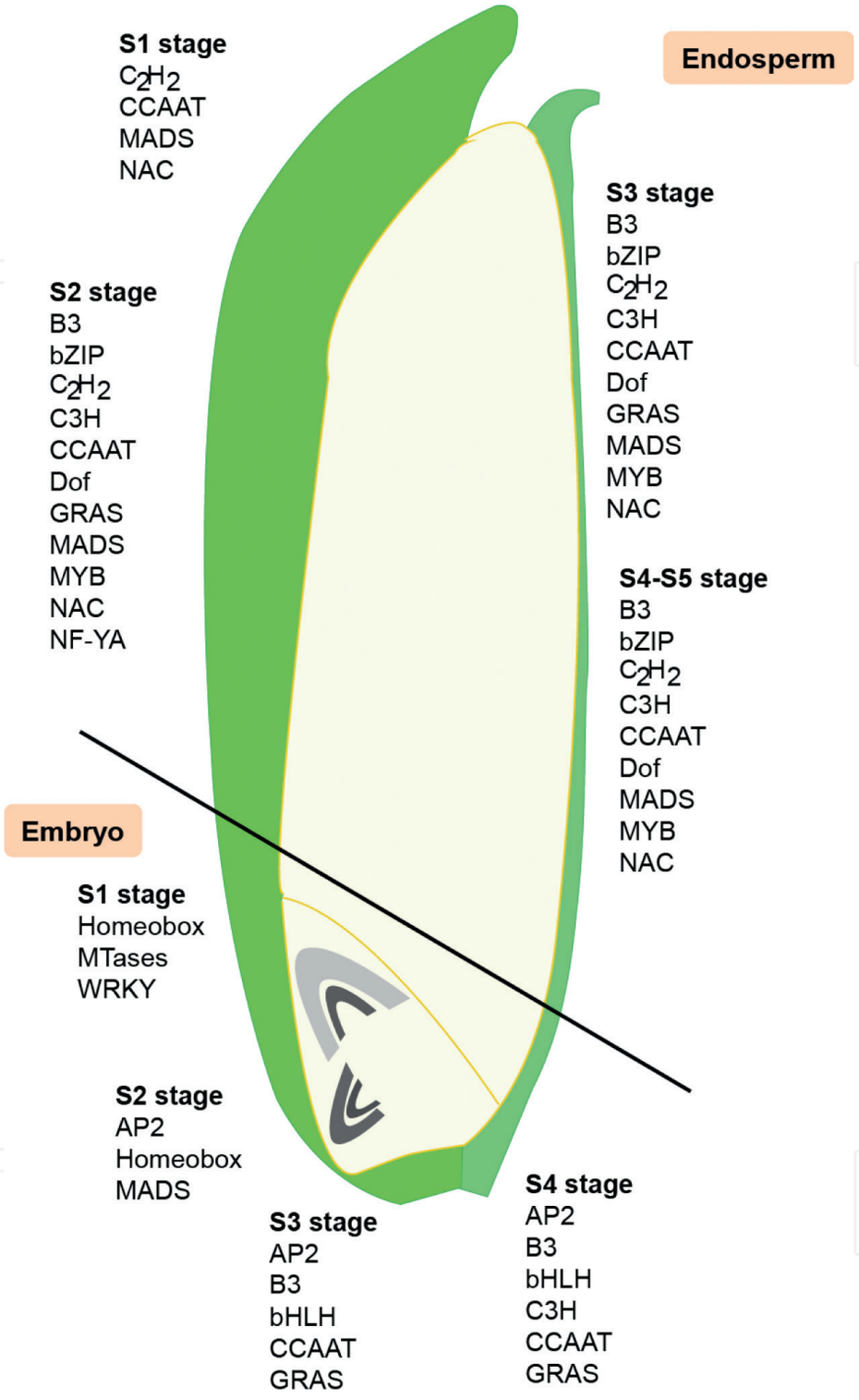

Figure 1. Schematic representation of TFs involved in rice seed development. TF families whose members express during the five stages of seed development in the endosperm and embryo have been mentioned above and below the solid line, respectively.

regulation of these hormones in materializing seed development. The localized expression of different phytohormone-related genes during embryo development as well as their critical role in endosperm development and grain filling has been discussed in further sections.

4. Genes involved in polarity establishment and organ initiation are expressed in the embryo

Embryogenesis is an important aspect of seed development, which involves cellular division and establishment of the embryo body plan. The process of embryo development is classified into ten major stages in rice. The first stage is the zygotic stage formed at 0 days after fertilization (DAP). The initial stages of post-zygotic development are characterized by the repeated cellular division without morphogenesis. This leads to the formation of a globular embryo, which can be observed from 1 to 3 DAP. It is the stage at which embryo specifies its body axis. Subsequently, formation and proper positioning of the shoot apical meristem (SAM), coleoptile primordium and the radicle primordium occurs at 4 DAP. This is followed by the origin of leaf primordium, which initiates at 5 DAP and is completed by 8 DAP, by the formation of all the three leaf primordia. With the enlargement of various organs, embryo morphogenesis is completed in rice by 10 DAP. This is followed by a stage of maturation which lasts till about 20 DAP, and thereafter, it enters into a dormant state [4, 5]. Profiling the RNA expression levels has greatly broadened the understanding of rice embryo development. Unlike animal embryos, both maternal and paternal genomes contribute equally in the process of embryogenesis and the plant embryonic development is majorly under zygotic control. Maternal-to-zygotic transition (MZT) initiates 50 hours after fertilization [24–26]. Thus, zygotic genome gets "switched on" almost immediately after fertilization.

Microarrays coupled with laser microdissection (LMD) have made available the expression profiles of developing female gametophyte from the pre-meiotic to the mature embryo-sac stages in rice [27]. Similarly, expression profiles of stigma as well as embryo sac are also available from high-throughput RNA-Seq technology [28]. These cell-type specific expression analyses have been compared with various developmental stages of embryo to identify genes that are differentially expressed during the post fertilization period. Identification of such genes expands our idea of embryogenesis as many of them can be candidates for maintaining various stages or aspects of embryo morphogenesis. Microarray-based comparison of gametic and zygotic tissues has identified a total of 325 genes up regulated in zygote in comparison with the egg cell. Majority of these up regulated genes are involved in DNA/chromatin organization and their assembly is probably involved in the induction of genes participating in zygotic development. Further, methyltransferase 1 (MET1) and different TFs belonging to the homeobox proteins are highly up regulated in the zygote, apparently affecting polarity or asymmetric division in zygote. Specific METs show higher expression during early rice seed development and are essential for cytosine methylation, regulating the genes involved in various developmental processes [29]. Also, microarray analysis shows a significant transcript accumulation of one such member, *OsMET1–2* during the early seed stages [23]. This is an indication of the role played by various DNA methyl transferases (MTases) in gene regulation during early seed development (**Figure 1**). Microarray analysis has also shown that a total of 94 genes are down regulated in the zygotic stage. Subsequent gene ontology (GO) and pathway analyses suggest the involvement of these genes in metabolic pathways possibly associated with suppression of the maternal genes [26, 29]. Thus, various transcript analyses indicate the existence of an active zygotic genome during early seed development.

A systematic expression profiling at three developmental stages of the embryo categorized as early (3–5 DAP), middle (7 DAP) and late (14 DAP) have shown the expression of about 20,856 common genes, suggesting their role in housekeeping functions. However, many genes show specific expression in each category suggesting their involvement in imparting functions unique to the stage. These genes belong to different functional categories as metabolic processes, binding and cell part and cellular processes. About 1131 genes show specific expression at 3–5 DAP, possibly involved in determining the embryo axis. Polarized expression of different TF and transcription regulator (TR) genes has been identified at the apical-basal and dorsal-ventral axis in the globular embryo. This spatiotemporal expression of specific TFs and TRs might be involved in the establishment of early embryo patterning in rice [30]. Different phytohormone-related genes including GA biosynthetic genes, auxin efflux *PIN* genes, cytokinin A-type response regulators, and brassinosteroid (BR)-perception genes also show an embryonic axis-dependent expression. The repressor of GA-signaling *OSSLR1* shows a preferential expression in the basal region of the embryo. On the other hand, a GA biosynthetic gene, *OsGA20ox1* is expressed in the apical-dorsal region. Apical to basal auxin transport is initiated at the early globular stage by the auxin transport proteins, thus, regulating various aspects of embryonic pattern formation. Transcript accumulation for cytokinin response-regulator occurs in the apical-ventral region whereas BR and ethylene biosynthesis occurs in the basal region [31]. Among TFs, homeobox gene family members show differential expression during different phases of embryo development suggesting their inevitable roles during the process [4, 30]. Different MADS-box transcripts show seed-preferential expression with about 12 of them showing a specific expression in the seed, including Arabidopsis ABCDE class gene orthologs, suggesting their involvement in early embryo development [30, 32].

A gradual transition in the transcript profile from early to late stages of embryo has been observed. A large number of genes are shared between the early and middle stages of embryo development although, unique expression of different genes is also observed [30, 31]. The genes up regulated in the early and middle stages of embryo development are majorly involved in amino acid, lipid and energy metabolism, nucleic acid replication/processing, signal transduction and transcriptional regulation. The enrichment of these pathway genes provides the energy required for the early developing embryo. As the embryo progresses towards the middle stage, additional genes as ribosomal protein components, translational machinery components are up regulated. Thus, the protein biosynthesis genes show a greater expression during the middle phase. There occurs a significant enhancement in the expression of genes belonging to different categories as the embryo progresses towards the maturation phase. Many of these genes show differential expression between 7 and 14 DAP embryos. Pathway and gene ontology studies suggest significant differences in the physiological processes that occur during early and late stages of rice embryogenesis [30]. Further, the maturation phase is characterized by the accumulation of protein modification and starch biosynthesis genes. Auxin related *Aux/IAA*, *OsIAA18*, shows a significant up regulation during the middle and late stages of embryo development. Auxin-biosynthetic genes have been shown to be induced during different stages of embryo development [6, 14]. Also, the biosynthesis of ethylene is down regulated during embryo development by the enhanced level of ABA. Thus, the two phytohormones ABA and ethylene function antagonistically during embryo development in rice [14]. Additionally, GA also functions in the seed development process during maturation. Seed maturation process

is majorly determined by the GA/ABA ratio [22]. To add, profiling studies have also identified the accumulation of long-lived mRNAs between 10 and 20 DAF within the embryo. Long-lived mRNAs present in the mature dry seeds are required for proper seed germination. These majorly code for proteins related to the signaling of ABA, calcium ions and phospholipids as well as a heat shock protein HSP DNA J, essential for rice seed germination [33].

5. Rice endosperm shows structural and developmental complexities

The endosperm of rice occupies a major portion of the seed and defines the shape of the grain. It is the storehouse of nutrients including carbohydrates, lipids and storage proteins and serves as an important source of nutrition for the developing embryo. The formation of the endosperm starts with triple fusion, wherein the male nucleus fertilizes the bi-nucleate central cell to produce a triploid cell. Thereafter, it sequentially undergoes events of cell division, cell fate determination, tissue differentiation and programmed cell death (PCD) to produce the mature endosperm. Structurally, the endosperm features four major types of cells, the starchy endosperm, the aleurone cells, the transfer cells and the cells in the vicinity of the embryo [34]. The cells in the peripheral region, except those near the vascular tissue, form the aleurone layer which varies in thickness from one to five cell layers [35]. Cells immediately above the vascular bundles form the transfer cells. Cells enclosed within these two cell layers form the starchy endosperm. The embryo surrounding cells create the cavity in which the embryo is housed. These differentiated cell layers perform specific functions which are required by the embryo during its growth and afterwards for seed germination. Photosynthate (sucrose) produced in the leaves (source) is transported into the endosperm (sink) via the transfer cells [36, 37]. Endosperm surrounding region separates the embryo and the endosperm and might also be involved in providing nutrition to the embryo by apoplastic transport [38, 39]. Starchy endosperm stores starch and proteins that start accumulating soon after cellularization is complete [39]. The aleurone layer is composed of terminally differentiated cells that produce proteolytic, hydrolytic and cell wall degrading enzymes that digest the starch and proteins stored in the endosperm into sugars and amino acids for utilization by the growing embryo during seed germination [40, 41]. Hence, the development of endosperm is complex and singular owing to the modifications in its structure occurring through a short span of time and the accumulation of reserve materials and cell cycle activities that are switched on and off at precise time points.

The functional uniqueness of the endosperm is reflected in its transcriptome which has been found to be quite distinct from several other tissue types including reproductive stages and embryo [10]. Transcriptome analysis of three developmental stages of endosperm, spanning from during this time, has shown an overall decline in gene expression during this time. Even more down regulation has been observed in the later stages [12]. Studies also indicate that in the young endosperm stages (0–4 DAP), the number of specific genes increases with age suggesting that the complexity of molecular changes rapidly increases with progression of endosperm development [9]. In another study involving 7, 14 and 21 DAP endosperm tissue, it has been observed that the expression of specific genes can be clustered into distinct patterns. About 79 genes are expressed in all the three stages suggesting that they are constitutively required

throughout endosperm development. A set of 32 genes express highly in 14 and 21 DAP indicating their role in nutrient accumulation and PCD. About 22 genes and 15 genes show higher expression in 7 DAP alone and in both 7 and 14 DAP, respectively. These genes can be presumed to be regulating cell proliferation and cellularization during initial development and synthesis and accumulation of storage compounds [42]. Thus, amalgamation of such information from transcriptome data with knowledge from previous developmental studies can be useful in generating knowledge about the functions performed by various genes in different stages.

Endosperm involves the precise operation of several transcription factors throughout its course of development. About 1118 transcription factors belonging to 55 families have been reported to be expressing in early stages of endosperm development [12]. TFs have emerged as a major functional category in later stages (7–21 DAP) of endosperm development [42]. Expression pattern of TFs has also been indicated to be subjected to temporal regulation. Members of the transcription factor families such as, MADS, NAC, AP2-EREBP, MYB and CCAAT, have been observed to show higher expression in the endosperm (**Figure 1**). Out of these, *MADS* genes are expressed through the early stages (1–14 DAP), *AP2-EREBP* and *MYB* are expressed during early through middle stages (7–21 DAP), whereas, *NAC* and *CCAAT* are expressed through all stages (2–42 DAP) of endosperm development [10, 42]. MADS TFs have been shown to regulate endosperm development by a mechanism affecting the cytokinin level. Overexpression of *MADS29* activates the genes involved in starch biosynthesis and promotes the differentiation of proplastids to starch-containing amyloplasts [43]. In our study encompassing five different rice varieties, three NAC TFs exhibit seed-specific/preferential expression with significantly higher expression in S3-S5 stages suggesting their role in accumulation of storage reserves. They also show significant association with seed traits emphasizing their role in regulation of seed development [44]. Similarly, genome-wide analysis of 14 vegetative and reproductive tissues has indicated the expression of 21 C_2H_2 proteins in seeds of which 12 are specific to seed tissue. The expression of these genes shows variable pattern among the five stages. Some of them are expressed from S1–S5, while most of them show higher expression in the later stages of seed development implying their function in both initial seed development and seed maturation [3]. In another report including three endosperm stages covering 3–10 DAP, different types of expression patterns of the transcription factors have been observed. Six TFs families including Dof are up regulated through 3–10 DAP. Three transcription factor families including GRAS are down regulated from 3 to 6 DAP then up regulated till 10 DAP. NF-YA family members on the other hand are up regulated from 3 to 6 DAP then down regulated until 10 DAP [12]. In summary, TFs are expressed throughout the development of the endosperm and their expression is highly preferential. The heterogeneity in the expression patterns of TFs is an indicator of the intricate molecular regulation of endosperm transcriptome probably required for proper completion of a stage and subsequent transition to another.

As mentioned previously, hormones are known to be regulators of embryo development and this raises the possibility of them being key ingredients in the regulatory network of endosperm development. In this context, it has been observed that several hormone response *cis*-elements are present in the promoters of endosperm-specific genes that are expressed from 7 DAP to 21 DAP. The most abundant *cis*-elements belong to abscisic acid responses, including ABADESI1 and ABREMOTIFAOSOSEM. Since abscisic acid is a well-known hormone for

desiccation and dormancy, which is associated with seed maturation, the aforesaid observation would imply that these processes are very eminent in endosperm and are initiated from the middle stages of development [45]. Along with ABA, *cis*-elements for gibberellic acid, such as GARE1OSREP1 and PYRIMIDINEBOXOSRAMY1A, auxins, ARFAT, and ethylene-responsive element, such as ERELEE4 have also been observed [42]. The simultaneous expression of the genes regulated by hormones unambiguously indicates the significance of hormonal interplay in the growth of endosperm. Although, the specific effects of these hormones can only be understood from detailed functional characterization studies.

One eccentric yet indispensable feature of endosperm development is the occurrence of two types of cell cycles at different stages of development. First is the free nuclear division without cellularization leading to syncytium formation in the initial stages of development (0–5 DAP), and second is endoreduplication that occurs in the later stages (8–10 DAP) and is associated with increasing cell size and endosperm volume [4]. In coherence with this information, two CDKs, *CDKB;1* and *CDKB;2*, have been found to be showing higher expression in early stages of endosperm (1–2 DAP). Also, one A-type cyclin and four B-type cyclins exhibit patterns of expression overlapping with these CDKs. It is noteworthy that cell cycle defects associated with the endosperm can influence the growth of the embryo. Knockdown of a rice cyclin gene, *CycB1;1*, results in the formation of a large embryo and abortive endosperm suggesting that normal mitotic activity of the endosperm is imperative for the development of the embryo as well [46].

One of the primary objectives of the endosperm is stocking of nutrients which will eventually be assigned to various metabolic pathways required for seed development process. Bulk of the endosperm is constituted by starch and prolamin storage proteins [47–49]. Transcriptome profiling studies advocate that genes associated with accumulation of starch and sugars are significantly up regulated in the endosperm. Genes related to starch metabolism and storage protein biosynthesis have been found to be among the highly up regulated genes as development progresses from 3 to 10 DAP. Also, 11 members of Dof TFs have been found to be up regulated in endosperm [12]. Dof TFs are known to be associated with synthesis of storage proteins in the endosperm [50]. Pathway studies have also indicated that in the endosperm, starch and sugar metabolism are highly up regulated followed by amino sugar and nucleotide sugar metabolism and carbon fixation by photosynthesis. It has also been observed that as endosperm moves from 3 DAP to 10 DAP most of the genes and pathways are down regulated except for those related to accumulation of storage materials [12]. Functional annotation of endosperm-specific genes from 7 DAP to 21 DAP have shown that seed storage protein, carbohydrate and energy metabolism, seed maturation, protein metabolism, lipid metabolism and transport emerge as the major categories. Seed storage proteins, including prolamins, glutelins and globulins have been reported to constitute the third largest category of endosperm-specific genes after transcription factors and stress responsive genes. Apart from this, overrepresentation of CATGCA motif or the RY element has been seen in the promoters of the endosperm-specific genes expressed from 7 DAP to 21 DAP. These genes show varied molecular function, including hydrolase activity, nutrient reservoir activity and transcription factor activity [42]. From these findings, it can be concluded that the endosperm starts gathering storage material quite early in its development which continues till maturation.

This continuous process is controlled by the collaborative efforts of several genes, pathways and regulatory networks, which are primarily associated with synthesis and accumulation of starch and proteins.

Towards the end of its development, after the complete size has been attained and storage materials are being accumulated, the endosperm undergoes programmed cell death (PCD) which is initiated from 16 DAP in cereal seeds [4, 51, 52]. This results in degeneration of the storage cells of the endosperm surrounded by living cells of the aleurone layer. Although PCD has been less explored in rice endosperm, some reports of PCD genes from pollen tissues and rice protoplasts are available [11, 53, 54]. Transcriptome studies of endosperm have detected several PCD related genes. *AIP5*, a positive regulator of PCD in the tapetum, has been found to be up regulated, whereas, *hsp70*, a negative regulator of PCD in rice protoplasts, is down regulated in the endosperm. Apart from these, 11 PCD related genes have also been found to express in the endosperm [12]. PCD has also been implicated to be influenced by hormones. Ethylene and gibberellic acid have been suggested to be positive regulators and abscisic acid has been shown to be a negative regulator of cell death [51, 55]. Up regulation of six gibberellic acid pathway genes and down regulation of 20 abscisic acid pathway genes has been observed in the 6 and 10 DAP endosperm tissue [12]. These results provide additional support to the earlier reports and emphasize on the active involvement of these hormones in the regulation of PCD in the endosperm.

6. Expression profiling unravels the complex molecular machinery involved in grain filling

Quality of rice grains, the major human calorie provider is very significant in the present scenario of ensuring global food security. Quality and quantity of grain production is majorly dependent on the synthesis and storage of various macromolecules and minerals during the grain filling stage. In rice, grain filling happens in the endosperm tissue and is regulated by highly coordinated and synchronous pathways [4]. Endosperm acts as the nutrient reservoir for the developing embryo initially and to the germinating embryo over the course of time. Endosperm functions in the supply of nutrients to the growing embryo right from its syncytial state. Growth and expansion of the endosperm cells are limited by programmed cell death in mature seeds. Thus, the accumulation of storage reserve is dependent on the life span of endosperm cells [56]. Understanding the intricate machineries involved in grain filling is imperative in the identification and manipulation of the key regulatory pathways aimed at improving the quality and productivity of the crop varieties available. Expression analyses serve as a promising tool facilitating the identification of candidate genes regulating grain filling process in rice.

Major reserves accumulating in seeds include carbohydrates, storage proteins and lipid compounds. Biosynthesis of these storage macromolecules are coordinately controlled by different TFs and other TRs. Expression profiling has shown the co-expression of different TF genes including *bZIP*, *Dof* and *MYB* with many grain filling genes in rice [57]. Members belonging to these protein families have shown to play significant roles in the regulation of

storage protein and starch biosynthesis [57–59]. Additionally, genes involved in the biosynthesis of macromolecules, various transporters for amino acid, sugar, phosphate, peptide, nitrate and ABC transporters show enrichment in the grain filling stage [57]. Transporter genes are essential for the uptake of nutrient and precursor molecules from the source tissues. Expression profiling also gives information regarding the genes involved in specific pathways. Furthermore, a detailed analysis can be useful in the identification of *cis*-elements enriched in specific process. Many of the grain filling genes contain a conserved *cis*-element 'AACA' in their promoters, suggesting its importance in the process. AACA element is essential for conferring the expression in rice seed [57, 60]. Transcript profiling has also shown that the milling yield and eating quality of rice grains depends on the proportion of starch and proteins in the grain. High quality rice grains contain a high composition of starch and protein biosynthetic transcripts. Massively parallel signature sequencing (MPSS) and sequencing by synthesis (SBS) have shown a higher level of alternative splicing and antisense transcripts for different metabolic genes in the high milling yield and eating quality varieties. These transcripts belong to starch, aspartate amino acid, storage protein and allergenic protein metabolism genes, indicating the complex transcriptional cascade involved in the regulation of rice grain quality [61]. Thus, expression profiling has not only improved our understanding in the grain filling process but also identified different transcripts essential for the process. Many of these genes can also serve as potential markers for the identification of superior rice varieties.

Grain chalkiness is another important agronomic trait influencing the market value of rice. It negatively affects the consumer preference and culinary quality. Analyses have shown the differential expression of a large repertoire of genes involved in signal transduction, cell rescue/defense, transcription, protein degradation, carbohydrate metabolism and redox homeostasis in a high chalky rice variety. Out of the different metabolic genes, starch metabolism genes can be considered as the major reason for grain chalkiness because of their opposite expression pattern in varieties showing varying levels of chalkiness. The sucrose and starch biosynthetic genes show up regulation in the chalky variety. Moreover, the non-starchy polysaccharide transcripts show significant down regulation. Thus, the expression profiling suggests a positive correlation of the starchy polysaccharide transcripts with the chalky phenotype in rice grains. Additionally, the genes involved in oxido-reductive homeostasis also show significant up regulation in the chalky rice variety [62]. Thus, transcript profiling has the potential for the identification of candidate genes underlying a phenotype, including grain chalkiness.

A delay in the expression of various genes involved in the transformation of sucrose to starch has been identified as the major reason for poor grain filling in the inferior spikelets located on the lower secondary panicle branches. RNA-Seq analysis shows the lower expression of these genes in the inferior spikelets at an early stage of grain filling in comparison with the superior spikelets. However, it was reversed during the later stages of grain filling process. Low capacity of the sink tissue and the associated limited carbohydrate supply at the later stages of grain filling has been proposed as the probable reason for the poor filling of grains [63]. Thus,

profiling of seed transcripts has greatly deepened our understanding of the molecular machinery involved in seed filling and panicle branching. This has served to identify the cascade of TRs involved in the process. It will definitely pave way for the identification of candidate genes and their introgression for the production of improved variety with better consumer preference.

7. Dissecting out the effect of temperature stress on seed filling at the molecular level using expression profiling

Plants are sessile organisms and show ideal growth and development only when they are grown under optimal conditions. Temperature is one of the most important environmental factors which has a great influence on the growth and development of various plant species. It has got much attention because of the recent global warming. Rice grain weight and yield have been shown to be greatly reduced by the environmental temperature. Grain filling process is more affected by the increase in night temperature than by the day temperature, particularly. High temperature reduces grain yield by impairing the filling process. Transcriptome profiling has greatly helped in revealing the underlying mechanism of yield loss associated with high temperature stress.

An increase in the temperature affects the grain development process right from the opening of the glumes till the grain filling stage. At high temperature, the glumes remain unclosed which affects the germination rate as well as commercial quality of the seeds. Genes involved in cell wall metabolism and response to water and carbohydrate metabolism are up regulated following high temperatures stress [64]. A large number of genes show differential expression in heat sensitive and tolerant cultivars in the early milky stage of the rice seed. These genes are majorly involved in oxidation-reduction, metabolic, transport, transcript regulation, defense response and photosynthetic processes. It has been shown that high temperature disrupts the mitochondrial electron transport system. This further induces a higher concentration of hydrogen ions in the matrix, which affects the functional state of different enzymes involved in TCA cycle and other metabolic pathways [65]. Further, the elevated temperature has been shown to negatively affect the grain filling process. Impairment in grain filling has been shown to result from the shortage of storage material. RNA profiling shows the down regulation of different genes involved in sucrose import and starch biosynthesis. Concomitantly, many of the genes involved in starch degradation show up regulation in the heat sensitive variety. Further, high temperature also results in the inhibition of the respiratory chain. This leads to the inefficient production of energy/ATP, eventually leading to the poor filling in rice grains [66]. An elevation in temperature can negatively affect the development of rice grains in different ways as non-closure of glumes or poor grain filling. Sequence profiling show the down regulation of different genes involved in carbohydrate synthesis, sucrose transport and ETC genes, altogether resulting in poor energy production in the system. Thus, profiling studies have not only helped in understanding the mechanisms associated with poor grain filling and elevated temperature but also serve as a promising tool for the identification of candidates for improving the heat tolerant character in rice.

8. Summary and concluding remarks

Transcriptome studies have gained momentum in the last decade by the advancements in sequencing techniques, bioinformatics tools and functional genomics. This has strengthened our knowledge of the seed development program by boosting data generation and interpretation. The outlook from these high-throughput studies is predominantly determined by the tissues under investigation. Seed development process can be studied in entirety through expression atlases. They provide information about the dynamics and specificity of transcriptomes across a range of tissues/organs associated with the growth and development of rice seed. Larger proportion of endosperm-specific genes obtained in comparative studies between vegetative and seed stages indicate the amount of reprogramming happening at the molecular level. The extent of involvement of various tissues in seed development as well as their molecular relatedness can also be assessed by means of transcript profiling of tissues in groups. This helps in visualizing the molecular changes occurring during progression of development in rice seed through subsequent stages by highlighting the factors that undergo change or remain constant. Several TFs and hormone-related genes show specific or preferential expression in seed tissues indicating their active involvement with the process. Molecular interactions between tissues, such as cross talk between embryo and endosperm, can also be revealed by transcriptome atlases. However, for obtaining detailed information about the developmental changes related to a particular tissue, it will be beneficial to study that tissue in isolation, as it will be cost effective and less time consuming. Transcriptome studies of rice embryo and endosperm have identified the genes and pathways that control various phases of their development. Several MTases, including MET1, genes associated with DNA/chromatin remodeling and homeobox TFs are up regulated in the initial stages of embryo development indicating induction of genes associated with zygotic development and organ formation. Polarized expression of various TFs, TRs and phytohormones, including auxin, GA and cytokinin, suggests their role in establishing apical-basal polarity in the embryo. In the early and middle stages of embryo development, pathways related to amino acid metabolism, lipid and energy metabolism, nucleic acid replication/processing and signal transduction are up regulated, while in the later stages, pathways related to starch biosynthesis and protein modification are up regulated. Also, up regulation of ABA biosynthesis and down regulation of ethylene biosynthesis in later stages suggests their antagonistic role in embryo maturation. Endosperm development starts little later than embryo in seed development. Higher expression of cell cycle related genes in the initial stages suggest that early endosperm development is mostly concerned with cell division and expansion. Variable expression pattern of TFs, such as MADS, AP2-EREBP, NACs and CCAAT, throughout different stages of endosperm development emphasizes their specific roles throughout the process. Up regulation of PCD related genes, starch and storage protein synthesis genes and carbohydrate and energy metabolism genes in the middle and later stages suggests that these stages of endosperm development are active in accumulation of storage reserves and programmed cell death.

Expression profiling studies performed till now have provided information about important genes and pathways, interrelatedness of various processes and cross talks between the key players. Nevertheless, obtaining a fully functional knowledge of this complex development

process would require the enormous task of consolidation of the data generated by various studies in a single platform uniting all the pathways and regulatory networks controlling different aspects of seed development and the functional validation of these genes.

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