



ORIGINAL ARTICLE

Temporal Gene Expression Profiles From Pollination to Seed Maturity in Sorghum Provide Core Candidates for Engineering Seed Traits

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Received: 30 April 2024 | **Revised:** 12 August 2024 | **Accepted:** 19 August 2024

Funding: This study was supported by Science and Engineering Research Board (SERB) and Department of Science & Technology (DST), Government of India, grant/award numbers: CRG/2019/001695; IFA17-LSP90; CRG/2020/003466; and STR/2022/000013) and Indo-German Science & Technology Centre (IGSTC), WISER grant/award number: IGST/WISER2023/RS/39/2023-24/771).

Keywords: grain filling | seed development | seed size | seed storage proteins | sorghum | starch content | transcriptome

ABSTRACT

Sorghum (*Sorghum bicolor* (L.) Moench) is a highly nutritional multipurpose millet crop. However, the genetic and molecular regulatory mechanisms governing sorghum grain development and the associated agronomic traits remain unexplored. In this study, we performed a comprehensive transcriptomic analysis of pistils collected 1–2 days before pollination, and developing seeds collected –2, 10, 20 and 30 days after pollination of *S. bicolor* variety M35-1. Out of 31 337 genes expressed in these stages, 12 804 were differentially expressed in the consecutive stages of seed development. These exhibited 10 dominant expression patterns correlated with the distinct pathways and gene functions. Functional analysis, based on the pathway mapping, transcription factor enrichment and orthology, delineated the key patterns associated with pollination, fertilization, early seed development, grain filling and seed maturation. Furthermore, colocalization with previously reported quantitative trait loci (QTLs) for grain weight/size revealed 48 differentially expressed genes mapping to these QTL regions. Comprehensive literature mining integrated with QTL mapping and expression data shortlisted 25, 17 and 8 core candidates for engineering grain size, starch and protein content, respectively.

1 | Introduction

Sorghum is a highly nutritious, stress-resilient millet crop and therefore, an excellent candidate for addressing global malnutrition and food security challenges (Hao, Li, et al. 2021; Mathur et al. 2017; Tanwar et al. 2023). It is a diploid C4 grass with a genome size of approximately 730 Mb (Paterson et al. 2009). Sorghum is used as food, animal feed and biofuel feedstock (Zheng, Dang, and Sui 2023). Due to multiple health

benefits of sorghum and changing dietary requirements, the demand for sorghum as food is expected to rise. Sorghum grains comprise about 70%–80% carbohydrates, more than two-thirds of which is amylose, 8%–18% proteins and 2.5%–3.8% fats (Tanwar et al. 2023; Zhang et al. 2022). Besides, sorghum grains are an excellent source of phosphorous, zinc, thiamin, niacin, vitamin B6, selenium, iron, copper, manganese and magnesium (Tanwar et al. 2023; Zhang et al. 2022). Sorghum is also gluten-free, hence an excellent substitute for patients suffering from

gluten sensitivity. High dietary fibre, low glycaemic index and antioxidant properties due to bioactive substances present in the whole grain further add to the health benefits of sorghum (Espitia-Hernández et al. 2022). However, due to the use of low-quality varieties and the presence of antinutritional factors, the use of sorghum in the food industry has been limited. Understanding molecular and genetic factors associated with seed development is essential to enhance the yield and nutritional attributes of sorghum.

Transcriptomic studies from temporal stages of seed development have greatly improved our understanding of molecular networks underlying seed development in major cereal crops including rice (Chen et al. 2022; Chettry, Chrungoo, and Kulkarni 2019; Gao et al. 2013; Liu et al. 2010; Ren et al. 2023; Sharma, Agarwal, et al. 2012; Shen et al. 2018; Sun et al. 2015; Wu et al. 2020; Xu, Gao, and Wang 2012; Xue, Zhang, and Xue 2012), wheat (Chi et al. 2019; Guan et al. 2022, 2019; Pellny et al. 2012; Rangan, Furtado, and Henry 2017; Singh et al. 2014; Wan et al. 2008; Wei et al., 2015; Xiang et al. 2019; Yamasaki et al. 2017; Yang et al. 2020; Yu et al. 2016), maize (Chen et al. 2014; Doll et al. 2020; Kim et al. 2017; Li et al. 2014, 2023; Lu et al. 2013; Ming et al. 2021; Qu et al. 2016; Teoh et al. 2013; Wang, Nie, et al. 2022; Yi et al. 2019; Zhang et al. 2024) and barley (Bian et al. 2019; Kovacik et al. 2024; Tang et al. 2017; Zhang et al. 2016). However, sorghum, despite being the fifth most important staple crop worldwide, significantly lags behind in this aspect. Although morphological, anatomical and biochemical studies have been carried out during seed development stages in sorghum (Glennie, Liedenbergh, and Van Tonder 1984; Ma 1975; Ravi Shankar and Dayanandan 2020; Subramanian, Jambunathan, and Seetharama 1983; Zheng et al. 2015); in-depth examination of genetic and molecular pathways regulating grain development and associated agronomic traits remain unexplored. Cruet-Burgos and Rhodes (2023) earlier reported transcriptome sequencing of seed samples collected at three stages from four contrasting sorghum accessions for carotenoid content (Cruet-Burgos and Rhodes 2023). However, they only focused on a priori candidate genes associated with carotenoid biosynthesis, degradation, and carotenoid precursor methylerythritol phosphate pathway. Recently, Costes et al. (2024) reported transcriptional profiling from three stages of sorghum seed development harvested at 7, 13 and 30 days after pollination (DAP). However, the study only focuses on the expression dynamics of cell wall development-associated genes specifically in the endosperm and outer layers extracted using laser microdissection (Costes et al. 2024).

Here, we report a systematic analysis of the transcriptional dynamics in pistils before pollination and fertilization, and four temporal stages of seed development postfertilization in *Sorghum bicolor* M35-1, an Indian grain sorghum cultivar. In addition to the global gene expression dynamics during seed development, we highlight the dominant expression patterns, pathways and transcription factor (TF) families associated with pollination/fertilization, early embryogenesis, grain filling, and seed maturation. QTL mapping, and orthology analysis of key genes, that have been experimentally demonstrated to regulate seed development in other plant species, helped us shortlist high-priority candidates to engineer grain weight and other nutritional attributes of sorghum.

2 | Materials and Methods

2.1 | Growth of Plants and Tissue Sampling

S. bicolor variety M35-1 plants were grown at Jawaharlal Nehru University, New Delhi, India, from February to June 2021 under open field conditions. Seeds were planted with a row-to-row distance of approximately 50 cm and a plant-to-plant distance of 15 cm, with 10 plants per row. The plants were monitored for pollination by observing anthesis marked by protrusion of anthers from glumes. With anthesis starting from the top spikelet and progressing downwards, the sorghum panicle takes 2–3 days for complete anthesis. Panicles in which about half-way spikelets had undergone anthesis were harvested to sample pistil tissue before and after anthesis. Before-anthesis-pistils were collected from spikelets expected to undergo anthesis the following day or the day after. Hence, before-anthesis-pistils represent pistils collected 1–2 days before pollination (DBP). The post-anthesis-pistils were collected from spikelets of the same panicle that had undergone anthesis on the same or the previous day. Hence, the first stage of seed samples collected after pollination represents about 0–2 days after pollination pistils. Developing seeds were harvested at 5, 10, 15, 20, 30 and 40 DAP. The average fresh weight was determined from five biological replicates of seed samples comprising 20 seeds each.

2.2 | Isolation of RNA and Transcriptome Sequencing

Pistils collected 1–2 DBP and developing seeds collected 0–2, 10, 20 and 30 DAP were used for transcriptome sequencing. Freshly extracted samples were snap-chilled and stored at -80°C until further use. Total RNA was extracted from the sampled tissues using TRIzol reagent as described previously (Dhaka et al. 2020) with some modifications. Briefly, 80–100 mg seeds were ground with a prechilled mortar and pestle using liquid nitrogen. Grounded sample was mixed with 1 mL of RNA extraction buffer containing 100 mM Tris-HCL (pH 8.0), 25 mM EDTA (pH 8.0), 2.5% of PVP, 25 mM sodium chloride and 2.5% β -mercaptoethanol. After 5 min incubation at room temperature (RT), 2.5% SDS was added to the tubes and centrifuged at 15 000 rpm for 5 min at 4°C to remove the debris. An equal volume of TRIzol (Ambion) reagent was added and mixed with the supernatant by inverting the tubes. After 5 min incubation at RT, half a volume of phenol:chloroform:isoamyl alcohol (25:24:1) was added and mixed gently, followed by incubation for another 5 min at RT. The samples were then centrifuged at 15 000 rpm for 5 min at 4°C . Half-volume chloroform was added to the supernatant and mixed gently, followed by centrifugation at 15 000 rpm for 5 min at 4°C . The supernatant was collected in fresh tubes and 1/4 volume of 2 M lithium chloride, 1/10 volume of 3 M sodium acetate (pH -4.8) and 1.5 volume of 100% chilled ethanol was added. The tubes were stored at -20°C overnight for RNA precipitation. The following day, samples were centrifuged at 13 000 rpm for 10 min at 4°C . The pellet was washed with 500 μL of 2 M chilled lithium chloride and 500 μL of 75% chilled ethanol, dried at RT and dissolved in nuclease-free water. RNA quantity and quality were assessed using Nanodrop Spectrophotometer (Nanodrop 2000), Qubit fluorometer (Thermo Fisher Scientific, USA) and Agilent 2200

TapeStation. RNA extracted from three biological replicates each of the pistil and seed stages with the RIN value > 7 were used for library preparation. The library preparation was performed using TruSeq Stranded Total RNA Kit for the 1–2 DBP and 0–2 DAP stages, and NEBNext Ultra II Directional RNA Library Prep Kit (NEB, USA) for the 10, 20 and 30 DAP stages, following the manufacturer's instructions. Paired-end RNA-seq libraries were prepared and sequenced on an Illumina HiSeq 2500 for the first two stages and Illumina NovaSeq 6000 platform for the last three stages, with an average read length of 100 and 150 bp, respectively.

2.3 | Transcriptome Data Analysis

The raw sequencing data obtained in FASTQ format was filtered to remove adaptor sequences and low-quality reads with a Phred score < 30 using NGS QC Toolkit v.2.3 using default parameters (Patel and Jain 2012). High-quality reads were mapped to *S. bicolor* v5.1 (https://phytozome-next.jgi.doe.gov/info/Sbicolor_v5_1) reference genome using HISAT2 v.2.2.1 with the `-rna-strandness RF` option, specifying strand-specific information (Kim et al. 2019). The raw counts were computed using FeatureCounts v.2.2.0 (Liao, Smyth, and Shi 2014) with default parameters. Subsequently, the raw counts were normalized to transcripts per million (TPM; Wagner, Kin, and Lynch 2012). The correlation coefficient among biological replicates was calculated using the R package as Spearman's correlation coefficient.

To estimate the variance in gene expression across temporal stages of development, pairwise differential expression analysis between consecutive stages was carried out using the DESeq2 R package (v.1.42.0; Love, Huber, and Anders 2014). Genes with an absolute $\log_2$ fold change ≥ 1 (upregulated genes) or ≤ -1 (downregulated genes) with Benjamini–Hochberg adjusted *p*-values cut-off ≤ 0.05 were considered differentially expressed.

2.4 | Functional Analysis of Differentially Expressed Genes (DEGs)

The DEGs with a $\log_2$ (TPM + 1) > 1 in at least one of the stages and exhibiting differential expression in consecutive stages were categorized into distinct groups based on k-means clustering using Idep 0.94 (<http://bioinformatics.sdstate.edu/idep94/>; Ge, Son, and Yao 2018). The expression patterns were plotted using log-transformed TPM values with MultiExperiment Viewer software (Howe et al. 2010).

Pathway mappings were obtained through MapMan4 from Mercator4 v6.0 (https://www.plabipd.de/mercator_main.html; Schwacke et al. 2019) and enrichment analysis was performed using the hypergeometric test with a *p*-value cut-off of ≤ 0.05 , followed by Benjamini–Hochberg correction with an adjusted *q*-value cut-off of ≤ 0.05 (false discovery rate). TFs were downloaded from the Plant Transcription Factor Database (PTFDB v5.0; <http://planttfdb.gao-lab.org/download.php>) and enrichment analysis was performed using hypergeometric test with the *p*-value cut-off of ≤ 0.05 , followed by Benjamini–Hochberg

correction with a *q*-value cut-off of ≤ 0.05 . Further, sorghum orthologs of *Arabidopsis*, maize, and rice genes were extracted from the Phytozome database (<https://phytozome-next.jgi.doe.gov/>). To identify genes with experimentally demonstrated roles in seed development, we retrieved the functional descriptions associated with *Arabidopsis* genes from TAIR (<https://www.arabidopsis.org/index.jsp>), maize genes from MaizeGDB (<https://www.maizegdb.org/>) and rice genes from FunRice-Genes (<https://funricegenes.github.io/>) databases.

2.5 | Mining of Candidate Genes

The candidate genes were identified using an exhaustive literature survey. The candidate genes for grain size and weight were retrieved from Chen, Łyskowski, et al. (2021); Gasparis and Miłoszewski (2023); Li, Xu, et al. (2018); Li et al. (2022); Long et al. (2023) and multiple other studies. The starch biosynthesis genes were extracted from Hashimoto et al. (2023) and Li et al. (2022), and protein content genes were obtained from Li et al. (2022).

2.6 | Colocalization of Grain Weight QTLs With Sorghum Candidate Genes

The chromosomal coordinates of QTLs associated with grain weight in sorghum were downloaded from the sorghum QTL Atlas database (<https://aussorgm.org.au/sorghum-qtl-atlas/>). The physical coordinates of DEGs in consecutive stages of seed development were obtained from Phytozome database (Sbicolor_313_v3.1.gene.gff3.gz, <https://phytozome-next.jgi.doe.gov/>). The QTLs were mapped on respective chromosomes using Mapchart v.2.32 (Voorrips 2002).

2.7 | Coexpression Network of Sorghum Candidate DEGs Colocalizing With Grain Weight QTLs

DEGs related to grain size and weight that colocalized within the QTLs were utilized to construct the coexpression network. This network was constructed using expression values and converted into Pearson's correlation matrix with a significance threshold set at ≥ 0.8 . Subsequently, the coexpression network was visualized using Cytoscape v.3.10.2 (Shannon et al. 2003). Furthermore, the topological properties of network components (nodes and edges) were analysed to predict the hub genes in this coexpression network using the Network Analyzer plugin of Cytoscape (Assenov et al. 2008).

3 | Results and Discussion

3.1 | From Initiation to Maturation: Key Stages Associated With Seed Development in Sorghum

Seed development commences with flowering, followed by effective pollination and fertilization. The fertilized ovaries undergo a series of cell divisions, expansion, morphogenetic

and differentiation events, leading to the formation of the embryo and the endosperm. Based on the texture of the endosperm, seed development is often categorized into milky, soft dough and hard dough stages in cereals. Sorghum was earlier reported to reach the soft dough stage at approximately 10 DAP. During 10–15 DAP, grain filling transitions from soft to hard dough stage, accumulating up to 90% of the matter with dry matter accumulation reaching a peak between 15 and 25 DAP (Roozeboom and Prasad 2019).

To identify the key stages of seed development and grain filling in M35-1 cultivar of Indian sorghum, we analyzed the fresh weight of seeds collected at 5-day intervals from 5 to 40 DAP. The moisture content of seeds increases during initial developmental stages and grain filling, but declines as the seeds attain physiological maturity (Roozeboom and Prasad 2019). As expected, average fresh seed weight increased sharply from 8 mg at 5 DAP to 39 mg at 20 DAP, followed by a slight decrease, with an average seed weight of 35 mg at physiological maturity (Figure 1a). This pattern clearly reflected that grain filling progresses rapidly immediately after pollination and peaks at 20 DAP, while desiccation commences after 30 DAP (Figure 1a). To explore the temporal patterns of gene expression underlying pre- and postfertilization events associated with seed development, we collected pistil samples 1–2 DBP and 0–2 DAP. Developing seeds were collected at soft dough (10 DAP), hard dough (20 DAP) and at physiological maturity (30 DAP) stages (Figure 1b).

3.2 | Global Expression Dynamics During Fertilization and Seed Development in Sorghum

The transcriptome sequencing of 15 libraries comprising three biological replicates each of 1–2 DBP, 0–2 DAP, 10 DAP, 20 DAP and 30 DAP stages resulted in > 627 billion paired-end reads (Table S1). Of these, > 570 million reads were retained as high-quality reads with Q value > 30. These high-quality reads were mapped to the *S. bicolor* reference genome (v5.1), resulting in an average mapping of 90% (Table S1). One biological

replicate of the 30 DAP stage showed low mapping (12.6%) to the reference genome and was hence removed from further analysis. The Spearman correlation coefficients ranged from 0.93 to 0.98 between biological replicates of each stage, confirming a high level of correlation among the biological replicates (Figure S1).

Subsequently, a total of 31 337 unique genes with a normalized expression value > 0 TPM in all replicates of at least one developmental stage were considered as expressed. The highest number of genes were expressed at 0–2 DAP stage followed closely by 1–2 DBP stage indicating higher diversity in transcripts expressing at early stages of seed development (Figure 2a). Further analysis of their expression amplitude revealed a very peculiar pattern across the temporal stages of development. In 1–2 DBP and 0–2 DAP stages, the proportion of genes exhibiting low expression (TPM ≤ 10) was comparable to those exhibiting medium to high expression (TPM > 10), but from 10 DAP onwards, the proportion of genes exhibiting high expression declined with the stage of development. For example, 41% (11 935) and 40% (12 239) genes exhibited medium to high expression in 1–2 DBP and 0–2 DAP stages, whereas only 34.3% (9490) genes in 10 DAP, 26.4% (7293) genes in 20 DAP and 20.5% (5041) genes in 30 DAP stage exhibited medium to high expression (Figure 2a). This conforms to the high transcriptional activity during pollination, fertilization and early embryogenesis, whereas, as the seed transitions from grain filling to maturity, the transcription repertoire and amplitude declines.

Further comparative profiling of 31 337 expressed genes revealed that 23 243 genes expressed commonly in all five stages, while 199, 600, 57, 54 and 28 genes were exclusive to 1–2 DBP, 0–2 DAP, 10 DAP, 20 DAP and 30 DAP stages, respectively (Figure 2b, Table S2). Most of these stage-specific genes exhibit low expression. Functional significance could be assessed for only one of these stage-specific genes, *Sobic.009G088900*. Its rice ortholog, *BRASSINOLIDE ENHANCED GENE 3 (OsBLE3)*, is reported to regulate meristem development (Mathew et al. 2020). Further characterization of the other

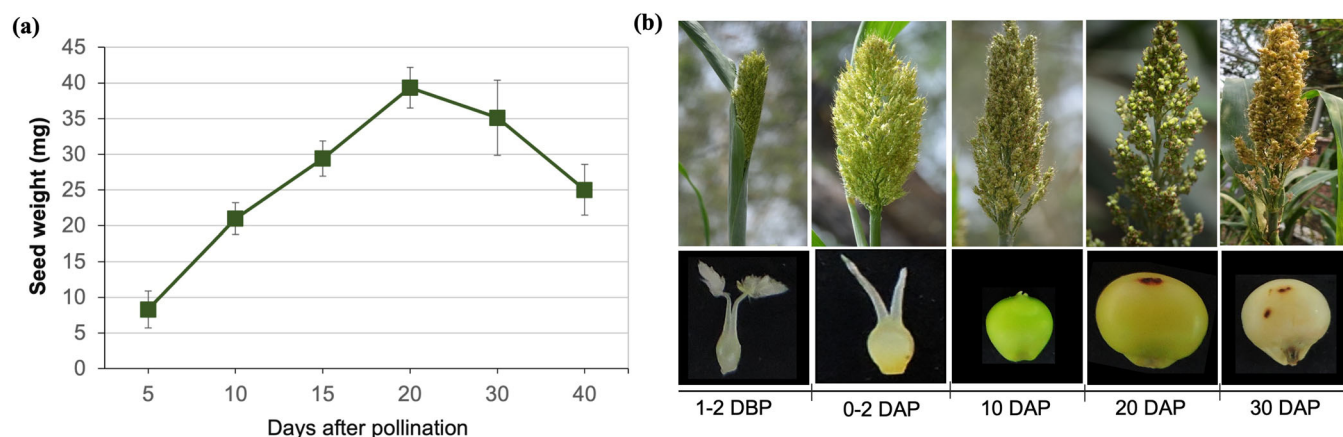


FIGURE 1 | Sample collection and phenotypic analysis of seeds. (a) Analysis of fresh seed weight (mg) of seeds collected from 5 to 40 days after pollination (DAP). The single seed weight shown here represents an average of 20 seeds. The error bars indicate the standard deviation. (b) Representative pictures of panicles (upper panel) and single seed (lower panel) of *Sorghum bicolor* M35-1 used for collecting the pistil and seed samples. Pistils collected 1–2 days before pollination (1–2 DBP), and seeds collected 0–2, 10, 20 and 30 DAP were used for this study.

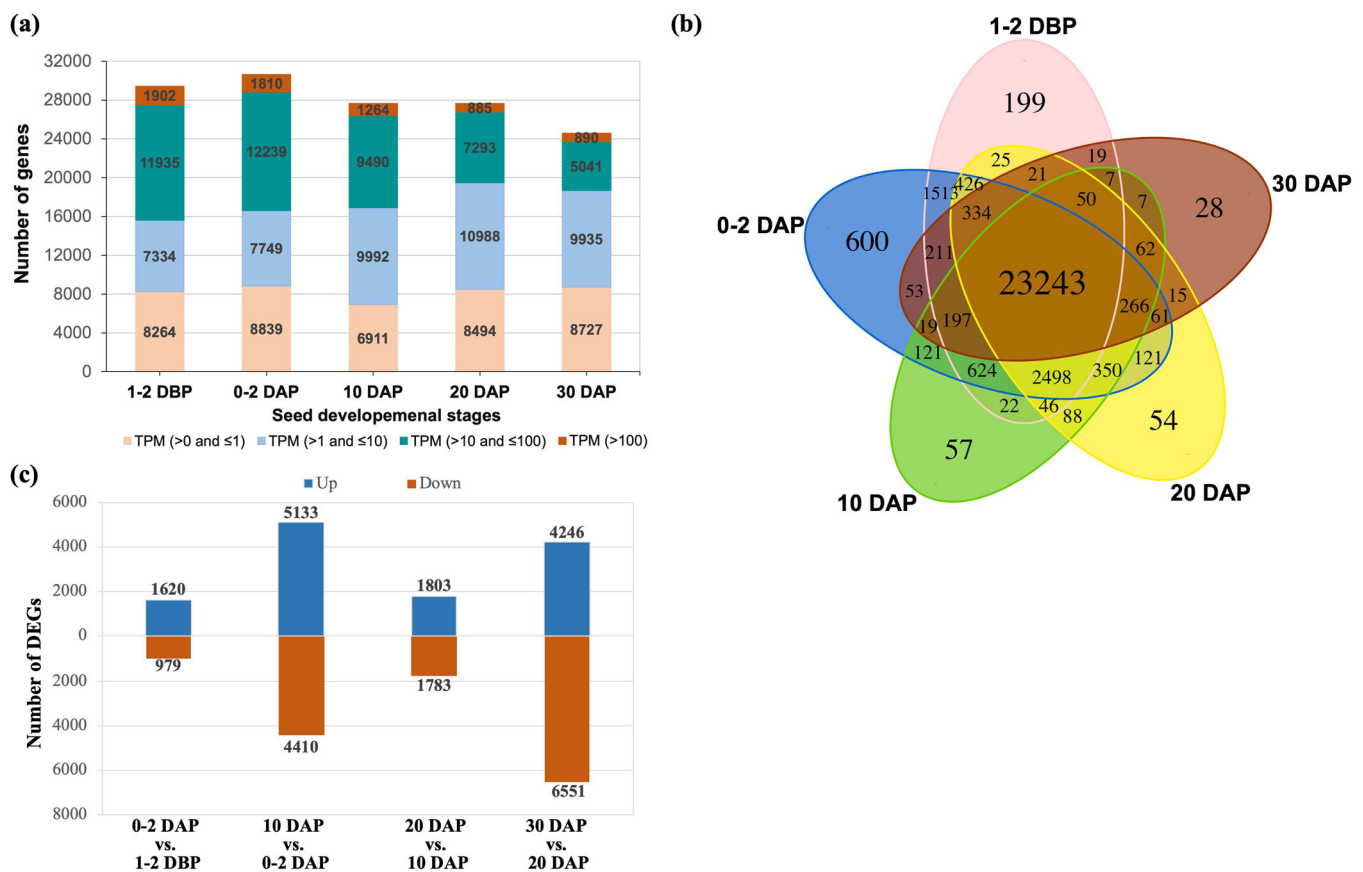


FIGURE 2 | Temporal expression dynamics from pollination to seed maturity. (a) Distribution of genes based on transcript per million (TPM) values during five stages of seed development is shown. (b) Venn diagram showing the overlap between number of genes expressed in each stage. (c) Results of the pairwise differential expression analysis across consecutive stages of seed development: 0–2 DAP versus 1–2 DBP, 10 DAP versus 0–2 DAP, 20 DAP versus 10 DAP and 30 DAP versus 20 DAP. The blue bars represent upregulated differentially expressed genes (DEGs), while orange bars indicate downregulated DEGs. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1111/pe.15134)]

stage-specific genes identified here would be required to gain insights into their roles in seed development.

Comparative analysis between consecutive stages revealed maximum overlap between 1–2 DBP and 0–2 DAP stages, with 1513 genes expressed commonly in both stages, followed by 121 genes commonly expressed in only 0–2 DAP and 10 DAP stages, 88 genes common to 10 DAP and 20 DAP stages and only 15 genes commonly expressed in 20 DAP and 30 DAP stages (Figure 2b). These results confirm distinct expression profiles during seed maturation. A similar pattern was observed after differential expression analysis. DEGs at each stage were identified by taking the preceding stage as a reference so that temporal changes in expression could be observed (Table S3). For example, to identify DEGs in the 0–2 DAP stage, the 1–2 DBP stage was taken as a reference; for 10 DAP, 0–2 DAP was taken as a reference, and so on. The maximum number of DEGs were detected at the 30 DAP stage, taking 20 DAP stage as a reference, with 4246 genes upregulated and 6551 genes downregulated (Figure 2c, Table S3). The second highest number of DEGs was observed in the 10 DAP stage, with the 0–2 DAP stage as a reference. These observations indicate a major transition in seed development stages and corresponding transcriptomes during the 0–2 to 10 DAP and then the 20–30 DAP stage progression (Figure 2c). The 1–2 DBP to 0–2 DAP stage transition mainly involves the differential accumulation of

genes associated with pollination, fertilization, and early embryogenesis. 0–2 DAP to 10 DAP transition involves endosperm cellularization and grain filling until the 20 DAP stage, while the transition from the 20 DAP to the 30 DAP stage represents the culmination of grain filling followed by physiological maturity. Thus, we could delineate 0–2 DAP to 10 DAP as the early seed development phase, 10–20 DAP as the grain filling phase, and 20–30 DAP as the seed maturation phase.

3.3 | Clustering of DEGs Reveals Dominant Patterns, Pathways and Associated Genes Concerning Seed Development

Out of 16 856 genes exhibiting differential expression compared to the preceding stage, 12 804 exhibiting mean $\log_2$ (TPM + 1) > 1 in at least one developing seed stage were used to identify the dominant expression patterns associated with grain development progression by employing k-means clustering (Table S4). This additional filtering step was implemented to prioritize DEGs with considerable expression levels throughout grain development.

A total of 10 clusters were delineated and designated as Groups 1–10 based on the peak expression stage/s. These groups were further analyzed for functional relevance. Out of 12 804 DEGs, 7377

mapped to 41 different Mapman pathways, while 793 genes could be assigned to 51 TF categories (Table S4). Group-wise enrichment of pathways, TFs and orthology analyses revealed the functional significance of these expression patterns.

3.3.1 | Group 1–3 Genes With Peak Expression in Prepollination and Postfertilization Pistils Guide Anthesis, Pollination, Fertilization and Early Embryogenesis

Groups 1–3 genes exhibit peak expression in 1–2 DBP, 1–2 DBP and 0–2 DAP and, 0–2 DAP stages, respectively, important for regulating anthesis, pollination, fertilization and early embryogenesis (Figure 3a). Group 1 contains 915 genes with peak expression in prepollination pistils and significant enrichment of RNA biosynthesis, plant organogenesis, external stimuli and hormonal pathways, including auxin, jasmonic acid (JA) and abscisic acid (ABA) (Figure 3a,b, Table S4 and S5). Expression of auxin biosynthesis genes in pre- and postpollination ovaries and their role in initiating seed development has been well documented in both dicot and monocot species (Figueiredo and Köhler 2018; Jain et al. 2023). Similarly, JA has been earlier reported to regulate pollen maturation, gynoecium function and anthesis (Jain et al. 2023; Niwa et al. 2018). Expression of the ABA biosynthetic gene, *SINCE1* encoding 9-*cis*-

epoxycarotenoid dioxygenase, of tomato has also been reported to peak 1 day before bloom, and regulate pistil development and seed set by modulating pistil-specific zinc finger TFs, indicating the involvement of ABA biosynthesis in similar functions in sorghum as well (Kai et al. 2019). In fact, TFs belonging to B3 and ZF-HD are also enriched in Group 1 (Figure 3c, Table S6). Orthologs of some of the genes from this group have previously been demonstrated to play an important role in key events associated with flowering, pollination and fertilization. For instance, rice ortholog of *Sobic.001G314400*, *MADS-BOX TRANSCRIPTION FACTOR 56* (*OsMADS56*), regulates long day-dependent flowering in rice (Ryu et al. 2009). Interestingly, *OsMADS56* is also the causal gene for the QTL controlling thousand grain weight (TGW) in rice, and hence is an important candidate for engineering flowering time and grain weight in crop plants (Zuo et al. 2021). Similarly, *Sobic.001G119200* from this group is an ortholog of rice ZF-HD TF gene, *UCLACYANIN LIKE PROTEIN 8* (*UCL8*), that regulates pollen–pistil interaction. Loss of function of *UCL8* negatively impacts the embryo sac and seed development (Zhang et al. 2018). Another gene, *Sobic.001G119000*, is the ortholog of an *Arabidopsis* plastocyanin gene that regulates anther dehiscence, pollen tube guidance into style and seed set (Dong, Kim, and Lord 2005). In addition, rice orthologs of 12 genes from this group have been reported to directly control grain weight/size (Table 1). For example, rice ortholog of



FIGURE 3 | Clustering of differentially expressed genes (DEGs) and functional analysis. (a) Results of clustering show 10 different groups of DEGs with distinct temporal expression profiles. (b) Identification of enriched MapMan pathway subcategories among DEGs in each group. (c) Identification of enriched transcription factor families in each group. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1111/pcr.15134)]

TABLE 1 | Sorghum genes shortlisted as candidates for grain weight/size, starch and protein content based on literature mining and differential expression analysis. The genes colocalizing with grain weight QTL are highlighted with *.

S. No.	Candidate gene	Sorghum gene ID	Rice ortholog	Groups	Regulation	Function	References
<i>Grain weight/size</i>							
1	<i>bZIP47</i>	Sobic.010G110100*	LOC_Os06g15480	Group 1	Negative	Suppresses cell proliferation and regulates grain size and weight negatively	Hao, Wang, et al. (2021)
2	<i>GRX8/WG1</i>	Sobic.006G054600*	LOC_Os02g30850	Group 1	Positive	Regulates grain weight and width by encoding CC-type glutaredoxin protein which enhances cell proliferation	Hao, Wang, et al. (2021)
3	<i>HDR3</i>	Sobic.001G425100	LOC_Os03g16090	Group 1	Positive	Positively regulates cell proliferation and enhances grain growth	Gao et al. (2021)
4	<i>PUB3</i>	Sobic.003G339500	LOC_Os01g60860	Group 1	Positive	It encodes U-box E3 ubiquitin ligase which enhances cell elongation to increase grain size and width	Wang, Zhang, et al. (2022)
5	<i>GIF1</i>	Sobic.001G101700*	LOC_Os03g52320	Group 1	Positive	Transcriptional cofactor <i>GIF1</i> interacts with <i>GRF4</i> and enhance grain size	Li, Gao, et al. (2016) and Wang et al. (2008)
6	<i>BG1</i>	Sobic.001G485400*	LOC_Os03g07920	Group 1	Positive	Overexpression positively regulates grain size due to increased cell proliferation	Liu et al. (2015) and Lo et al. (2020)
7	<i>TSG1/FIB</i>	Sobic.003G052700*	LOC_Os01g07500	Group 1	Positive	<i>TSG1</i> positively regulates grain size and yield by enhancing auxin level	Guo et al. (2020)
8	<i>GL10/MADSS6</i>	Sobic.001G314400*	LOC_Os10g39130	Group 1	Positive	It encodes <i>MADSS6</i> in glume cells which positively regulates cell elongation and eventually increases grain length and weight	Zhan et al. (2022)
9	<i>SLR1</i>	Sobic.001G120900*	LOC_Os03g49990	Group 1	Negative	<i>SLR1</i> negatively affects grain size via reducing GA signaling	Lan et al. (2020)
10	<i>GL6/SG6</i>	Sobic.010G220800	LOC_Os06g45540	Group 1	Positive	Encodes PLATZ TF which enhances cell proliferation during early panicle development	Wang, Hou, et al. (2019)
11	<i>SPL18</i>	Sobic.002G257900*	LOC_Os09g32944	Group 1	Positive	<i>SPL18</i> mutants show reduced grain width, thickness and number of grain due to decreased cell proliferation in spikelet hull	Yuan et al. (2019)
12	<i>RAV6</i>	Sobic.004G280500	LOC_Os02g45850	Group 1	Negative	Affects BR pathways and shows negative impact on grain size	Zhang et al. (2015)
13	<i>DEP1/qPE9</i>	Sobic.002G216600*	LOC_Os09g26999	Group 2	Positive	Encodes <i>Gγ</i> subunit which increases grain size and starch content by enhancing duration of grain filling	Huang et al. (2009) and Zhou et al. (2009)

(Continues)

TABLE 1 | (Continued)

S. No.	Candidate gene	Sorghum gene ID	Rice ortholog	Groups	Regulation	Function	References
14	<i>GW2</i>	Sobic.004G107300*	LOC_Os02g14720	Group 2	Negative	E3 ubiquitin ligase encoded by GW2 reduces grain width, weight and yield by ubiquitin–proteasome pathway	Song et al. (2007)
15	<i>SPL14</i>	Sobic.007G210200*	LOC_Os08g39890	Group 2	Positive	Increases grain size and shape	Jiao et al. (2010) and Miura et al. (2010)
16	<i>SMG2/MKKK10/YDAI</i>	Sobic.006G176800	LOC_Os04g47240	Group 2	Positive	Enhances expression of downstream genes and forms MKKK10-MKK4-MAPK6 complex to increase grain size and weight	Xu et al. (2018)
17	<i>D11/CYP724B1</i>	Sobic.006G114600*	LOC_Os04g39430	Group 2	Positive	Positively regulates BR synthesis and enhances grain size	Tanabe et al. (2005), Wu et al. (2016) and Zhou et al. (2017)
18	<i>LARGE1/OML4</i>	Sobic.004G158400*	LOC_Os02g31290	Group 2	Negative	Mutants show increased grain size by enhancement of cell expansion in spikelet hull	Lyu et al. (2020)
19	<i>DLT/D62/GS6/SMOS2</i>	Sobic.010G017400*	LOC_Os06g03710	Group 2	Negative	Encodes GRAS family protein which reduces grain size by affecting BR signaling pathway	Tong et al. (2009)
20	<i>BRI1/D61</i>	Sobic.003G277900*	LOC_Os01g52050	Group 2	Positive	Acts as a BR receptor kinase, playing crucial role in cell elongation which increases grain size	Morinaka et al. (2006)
21	<i>OFPI</i>	Sobic.003G010700	LOC_Os01g12690	Group 2	Positive	<i>OFPI</i> interacts with downstream genes <i>GSK2</i> and <i>DLT</i> to increase grain size	Xiao et al. (2017)
22	<i>OFP3</i>	Sobic.003G286400*	LOC_Os01g53160	Group 2	Negative	<i>OFP3</i> negatively regulates cell expansion and BR synthesis which results in small grains	Xiao et al. (2020)
23	<i>D2/SMG11</i>	Sobic.003G030600*	LOC_Os01g10040	Group 2	Positive	Mutant of <i>D2</i> suppress BR biosynthesis and results in small seeds	Fang et al. (2016)
24	<i>XIAO</i>	Sobic.006G187000	LOC_Os04g48760	Group 2	Positive	Encodes for leucine-rich repeat receptor-like kinase which regulates seed length positively	Jiang et al. (2012)
25	<i>ARF12</i>	Sobic.006G262100*	LOC_Os04g57610	Group 2	Positive	Overexpression promotes cell expansion which increases grain weight and grain filling	Zhao et al. (2023)
26	<i>ARF6</i>	Sobic.004G051900	LOC_Os02g06910	Group 2	Negative	Enhances <i>AUX3</i> promoter expression and <i>ARF6-AUX3</i> complex reduces auxin content which suppresses grain length and weight	Qiao et al. (2021)
27	<i>GDD1/BC12/YD1</i>	Sobic.002G143200	LOC_Os09g02650	Group 2	Positive	Encodes kinesin-like protein BC12, which enhances enzymes involved in GA biosynthesis and promotes cell elongation	Li, Jiang, et al. (2011)

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TABLE 1 | (Continued)

S. No.	Candidate gene	Sorghum gene ID	Rice ortholog	Groups	Regulation	Function	References
28	<i>GSR1/GW6/GASR7</i>	Sobic.010G111200*	LOC_Os06g15620	Group 2	Positive	Mutants show reduced GA content and decreased grain width and weight	Shi et al. (2020)
29	<i>AGO2</i>	Sobic.006G215000	LOC_Os04g52540	Group 2	Positive	Overexpression promotes cell expansion and proliferation resulting larger seeds and better yield by modulating cytokinin levels	Yin et al. (2020)
30	<i>PAO5</i>	Sobic.006G261600*	LOC_Os04g57560	Group 2	Negative	Negatively regulates ethylene level which affects cell proliferation and reduces grain weight and yield	Lv et al. (2021)
31	<i>SMOS1/SHB</i>	Sobic.009G124200*	LOC_Os05g32270	Group 2	Positive	Encodes AP2 TF which regulates cell expansion, BR biosynthesis and auxin signaling. It positively regulate grain size	Aya et al. (2014)
32	<i>APG</i>	Sobic.009G036400*	LOC_Os05g04740	Group 2	Negative	It negatively regulates grain length by reducing cell elongation in hull cells	Heang and Sassa (2012a)
33	<i>PGL2/BUL1</i>	Sobic.004G237000*	LOC_Os02g51320	Group 2	Positive	In BR biosynthesis pathway, <i>BUL1</i> upregulates <i>BGL1</i> which increases grain length	Heang and Sassa (2012b) and Jang and Li (2017)
34	<i>GL7/GW7</i>	Sobic.002G367300*	LOC_Os07g41200	Group 2	Positive	Encodes a TONNEAU1-recruiting motif protein which enhances cell elongation resulting larger grains with improved quality	Wang, Xiong, et al. (2015)
35	<i>SPL12</i>	Sobic.010G254200	LOC_Os06g49010	Group 2	Positive	Overexpression causes cell elongation in spikelet cells and eventually increases grain length and width	Zhang, Yang, et al. (2021)
36	<i>SPL16/qGW8</i>	Sobic.007G193500*	LOC_Os08g41940	Group 2	Positive	Enhances cell proliferation which increases grain width and grain filling	Wang et al. (2012) and Wang, Li, et al. (2015)
37	<i>qTGW1a</i>	Sobic.001G341700*	—	Group 2	Negative	<i>qTGW1a</i> encodes a G-protein subunit which negatively regulates grain size	Zou et al. (2020)
38	<i>ASPI</i>	Sobic.007G051700*	LOC_Os08g06480	Group 4	Negative	Encodes a transcriptional co-repressor which affect branching and spikelet development due to which grain size is reduced	Yoshida et al. (2012)
39	<i>BRD1</i>	Sobic.001G172400*	LOC_Os03g40540	Group 4	Positive	<i>BRD1</i> encoded protein catalyses the C-6 oxidation step to produce active BR which increases cell proliferation and expansion in grains	Hong et al. (2002) and Mori et al. (2002)

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TABLE 1 | (Continued)

S. No.	Candidate gene	Sorghum gene ID	Rice ortholog	Groups	Regulation	Function	References
40	<i>BZR1</i>	Sobic.002G353200*	LOC_Os07g39220	Group 4	Positive	<i>BZR1</i> act as BR-signaling factor and elevates starch accumulation in developing seed and improves grain yield	Zhu et al. (2015)
41	<i>GS5</i>	Sobic.009G053600*	LOC_Os05g06660	Group 4	Positive	Encodes a serine carboxypeptidase which positively regulates mitosis in panicle and increases grain length and width	Li, Fan, et al. (2011) and Xu et al. (2015)
42	<i>GS9</i>	Sobic.002G220700*	LOC_Os09g27590	Group 4	Negative	Interacts with OVATE-family members and forms a transcriptional complex to control cell proliferation inside glume and suppresses grain size	Zhao et al. (2018)
43	<i>OFP8</i>	Sobic.003G366000	LOC_Os01g64430	Group 4	Negative	Acts in BR pathway and negatively affects grain size by regulating cell proliferation	Yang et al. (2016)
44	<i>GASR9</i>	Sobic.002G360900*	LOC_Os07g40240	Group 4	Positive	Encodes a Gibberellic acid-stimulated transcript (GAST) family protein which facilitates cell elongation resulting in longer grains	Li et al. (2019)
45	<i>CKX2</i>	Sobic.003G029900*	LOC_Os01g10110	Group 4	Negative	Reduces cytokinin level which decreases cell proliferation resulting reduces grain size	Yeh et al. (2015)
46	<i>FBK12</i>	Sobic.001G488500*	LOC_Os03g07530	Group 4	Positive	Acts as repressor for downstream gene <i>SAMS1</i> and reduces ethylene level and cell proliferation but increases grain length by increasing cell size in spikelet hull	Chen et al. (2013)
47	<i>AFG1/MADS6</i>	Sobic.004G281000	LOC_Os02g45770	Group 4	Positive	Encodes MADS-box protein which acts as transcriptional activator for downstream genes which enhance grain cell proliferation and expansion	Yu et al. (2020)
48	<i>MADS1/qLGY3</i>	Sobic.001G455900*	LOC_Os03g11614	Group 4	Negative	Encodes <i>MADS1</i> TF which interacts with <i>DEP1</i> and affects grain size	Liu et al. (2018) and Yu et al. (2018)
49	<i>MADS29</i>	Sobic.004G056000	LOC_Os02g07430	Group 4	Positive	Overexpression enhances cytokinin level which increases cell expansion and proliferation in grain	Nayar et al. (2013) and Yin and Xue (2012)
50	<i>SRS5/TID1</i>	Sobic.001G107100*	LOC_Os11g14220	Group 4	Positive	Induces cell elongation in spikelet cells and produces longer grains	Segami et al. (2012, 2017) and Sunohara et al. (2009)

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S. No.	Candidate gene	Sorghum gene ID	Rice ortholog	Groups	Regulation	Function	References
51	<i>OFP22</i>	Sobic.009G173400	LOC_Os05g39950	Group 5	Negative	Suppresses signaling pathways of GA and BR, resulting in decreased cell elongation and eventually reduces grain size	Chen, Yu, et al. (2021)
52	<i>DLT2</i>	Sobic.001G109100*	LOC_Os03g51330	Group 5	Negative	A GRAS-family member which enhances transcriptional activity of <i>DLT2-DLT-BZR1</i> complex to modulate BR pathway signaling and reduce grain size	Zou et al. (2023)
53	<i>LG3</i>	Sobic.001G481400*	LOC_Os03g08470	Group 5	Positive	Encodes TF which facilitates cell elongation and increases grain size	Yu et al. (2018)
54	<i>NF-YC10</i>	Sobic.007G054700*	LOC_Os01g24460	Group 5	Positive	Positively regulates cell division in spikelet hull cells and endosperm resulting in increased grain width, and grain weight	Jia et al. (2019)
55	<i>DG1</i>	Sobic.001G446800*	LOC_Os03g12790	Group 5	Positive	Activates starch synthesising genes in developing seeds and ensure grain filling.	Qin et al. (2021)
56	<i>GL4</i>	Sobic.006G261200*	LOC_Os04g57530	Group 5	Positive	Overexpression induces cell elongation in inner and outer glume cells which eventually increases grain size	Wu et al. (2017)
57	<i>AUX3/qGL5</i>	Sobic.009G156600*	LOC_Os05g37470	Group 6	Negative	It negatively regulate grain size and weight by reducing cell elongation	Qiao et al. (2021)
58	<i>NF-YB1</i>	Sobic.004G254400*	LOC_Os02g49410	Group 6	Positive	Overexpression induces cell proliferation in endosperm during early seed development stage and upregulates grain size	Bello et al. (2019) and Xu et al. (2021)
59	<i>YUC11</i>	Sobic.008G064100	LOC_Os12g08780	Group 6	Positive	Encodes flavin-containing monooxygenase essential for auxin biosynthesis. It maintain grain size, starch biosynthesis and grain filling in endosperm	Xu et al. (2021)
60	<i>ERF115</i>	Sobic.007G205200*	LOC_Os08g41030	Group 6	Positive	It enhances cell proliferation and elongation in spikelet and endosperm cells resulting increased grain size, width and weight	Liu et al. (2022)
61	<i>GSAI</i>	Sobic.001G084400*	LOC_Os03g55040	Group 6	Positive	Mutants show reduced cell proliferation and expansion in spikelet cells resulting in reduced grain size	Dong et al. (2020)

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TABLE 1 | (Continued)

S. No.	Candidate gene	Sorghum gene ID	Rice ortholog	Groups	Regulation	Function	References
62	<i>BG3/PUP4</i>	Sobic.003G227400*	LOC_Os01g48800	Group 7	Positive	Encodes purine permease which maintains cytokinin distribution and positively regulates grain size	Xiao et al. (2019) and Yin et al. (2020)
63	<i>PUP7</i>	Sobic.009G227300	LOC_Os05g48300	Group 7	Positive	Regulates cytokinin levels and cell division which positively regulates grain size	Qi and Xiong (2013)
<i>Starch content</i>							
1	<i>SBEIII</i>	Sobic.003G213800	LOC_Os06g26234	Group 2	Positive	Involved in upregulation of starch metabolism pathway and starch biosynthesis	Kang et al. (2013)
2	<i>SSIIB/SSII-2</i>	Sobic.004G2238600	LOC_Os02g51070	Group 2	Positive	Encodes starch synthase protein which regulates starch metabolism and accumulation in endosperm	Li, Huang, et al. (2018) and Xu et al. (2020)
3	<i>SBEIIa</i>	Sobic.006G066800	LOC_Os04g33460	Group 4	Positive	Encodes important starch branching enzyme that upregulates the formation of amylopectin in starch biosynthesis pathway	Sawada, Itoh, and Nakamura (2018)
4	<i>SBEI</i>	Sobic.010G2273800	LOC_Os06g51084	Group 4	Positive	Enhances accumulation and branching of amylopectin in endosperm during starch biosynthesis	Satoh et al. (2003)
5	<i>PHO1</i>	Sobic.001G083900	LOC_Os03g55090	Group 4	Positive	Plays essential role in initiation of glucan synthesis and also regulates starch synthesis in endosperm	Satoh et al. (2008)
6	<i>Sweet2-1</i>	Sobic.001G377600	LOC_Os03g22200	Group 4	Positive	Acts as sugar transporter in panicle throughout grain development	Mizuno, Kasuga, and Kawahigashi (2016)
7	<i>ISA2</i>	Sobic.009G127500	LOC_Os05g32710	Group 5	Positive	ISA2 along with ISA1 forms heteromeric debranching enzyme during seed development and upregulates amylopectin synthesis	Utsumi et al. (2011)
8	<i>SSI</i>	Sobic.010G047700	LOC_Os06g06560	Group 5	Positive	Plays catalytic role in formation of amylopectin from ADP-glucose and also upregulates other enzymes involved in starch biosynthesis in endosperm	Fujita et al. (2006)
9	<i>PK2/PKpα1</i>	Sobic.002G054400	LOC_Os07g08340	Group 5	Positive	Encodes plastidic pyruvate kinase which takes part in biosynthesis of starch in endosperm, formation of compound granule and grain filling	Cai et al. (2018)

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TABLE 1 | (Continued)

S. No.	Candidate gene	Sorghum gene ID	Rice ortholog	Groups	Regulation	Function	References
10	<i>Bt1/SBT1</i>	Sobic.004G085100	LOC_Os02g10800	Group 6	Positive	Encodes amyloplast membrane protein and acts as sugar transporter in endosperm. It also controls seed dormancy through glucose metabolism	Cakir et al. (2016) and Li et al. (2017)
11	<i>Bt2/AGPS2</i>	Sobic.007G101500	LOC_Os08g25734	Group 6	Positive	Involved in starch biosynthesis and signaling. It also facilitates grain filling in endosperm	Lee et al. (2007)
12	<i>SE1</i>	Sobic.005G199300	—	Group 6	Positive	Acts as GA and ABA regulator and controls starch and polysaccharide metabolism in seed kernels	Zhang, Mogel, et al. (2019)
13	<i>Sh1</i>	Sobic.010G072300	LOC_Os06g09450	Group 6	Positive	Downregulation of <i>Sh1</i> results in lower production of starch. It is involved in sucrose synthesis and metabolism	Chourey and Nelson (1976)
14	<i>Sh2/APL2</i>	Sobic.003G230500	LOC_Os01g44220	Group 6	Positive	Encodes large subunit of ADP-glucose pyrophosphorylase. It act as starch biosynthetic enzyme which suppresses starch biosynthesis pathway	Hannah and Nelson (1976)
15	<i>ISA1/SU</i>	Sobic.007G204600	LOC_Os08g40930	Group 6	Positive	Main isoamylase-type debranching enzyme in amylopectin biosynthesis. It also impacts starch formation via starch biosynthesis pathway	Shufen et al. (2019)
16	<i>PUL</i>	Sobic.006G015800	LOC_Os04g08270	Group 6	Positive	Involved in debranching of amylopectin	Fujita et al. (2009)
17	<i>SBEIIB/SBE3</i>	Sobic.004G163700	LOC_Os02g32660	Group 6	Positive	Mainly involved in biosynthesis of amylopectin. Mutation causes higher amylose and resistant starch content in rice endosperm	Baysal et al. (2020)
18	<i>SSIIta/SSI-3/ALK</i>	Sobic.010G093400	LOC_Os06g12450	Group 6	Positive	Transfers glucose to α -1,4-glucan chain from ADP-glucose during starch biosynthesis	Chen, Yan, et al. (2020), Chen, Lu, et al. (2020) and Zhang et al. (2020)
19	<i>bHLH144/ZOU-1</i>	Sobic.004G171500	LOC_Os04g35010	Group 6	Positive	Upregulates starch accumulation	Bello et al. (2019)
20	<i>NAC20/26</i>	Sobic.002G192600	LOC_Os01g01470	Group 6	Positive	It positively regulate the genes involved in starch and storage protein biosynthesis.	Wang, Chen, et al. (2020)
21	<i>NF-YC12/NF-YC11</i>	Sobic.005G089101	LOC_Os10g11580	Group 6	Positive	Encodes for nuclear factor-Y-transcription factor subunit C which regulates genes that involved in transport of sucrose to aleurone layer	Bello et al. (2019) and Xiong et al. (2019)

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TABLE 1 | (Continued)

S. No.	Candidate gene	Sorghum gene ID	Rice ortholog	Groups	Regulation	Function	References
22	<i>Wx</i>	Sobic.010G022600	LOC_Os06g04200	Group 6	Positive	Encodes for granule-bound starch synthase (GBSS) which plays role in amylose biosynthesis in rice endosperm	Zhang, Yang, et al. (2021)
23	<i>Sweet4-3</i>	Sobic.004G136600	LOC_Os01g18290	Group 6	Positive	Sugar transporter which facilitates starch unloading in phloem during accumulation phase	Mizuno, Kasuga, and Kawahigashi (2016)
24	<i>UGE3</i>	Sobic.002G271200	LOC_Os09g35800	Group 6	Positive	Shows UDP-galactose/glucose epimerase activity that facilitates substrates for polymerization of polysaccharides	Tang et al. (2022)
25	<i>TPI</i>	Sobic.003G352300	LOC_Os01g62420	Group 6	Positive	Acts as catalyst in glycolysis and is also reported to be involved in photosynthetic carbon-fixation	Sharma, Mustafiz, et al. (2012)
26	<i>PDC1</i>	Sobic.009G169000	LOC_Os05g39310	Group 6	Negative	Encodes for the enzyme which converts glucose to pyruvate through glycolysis	Agarwal et al. (2007)
<i>Protein content</i>							
1	<i>AAP10</i>	Sobic.004G256800	LOC_Os02g49060	Group 5	Positive	Encodes amino acid permease which loads amino acid in endosperm	Wang, Yang, et al. (2020)
2	<i>Glb1</i>	Sobic.009G186400	LOC_Os05g41970	Group 6	Positive	Encodes 26-kDa α -globulin protein which regulates the formation and accumulation of storage protein during grain filling.	Wu et al. (1998)
3	<i>GluA1</i>	Sobic.009G007100	LOC_Os01g55690	Group 6	Positive	It encodes glutelin A1 protein which facilitates protein storage	Qu et al. (2008)
4	<i>GluA2/qGPC-10</i>	Sobic.009G007100	LOC_Os10g26060	Group 6	Positive	Codes for glutelin type A2 precursor protein and is a positive of seed protein content	Yang et al. (2019)
5	<i>GluB1a</i>	Sobic.009G007100	LOC_Os02g15178	Group 6	Positive	It encodes for glutelin type B-protein which facilitates protein storage in endosperm	Wu et al. (1998)
6	<i>GluB1b</i>	Sobic.009G007100	LOC_Os02g15169	Group 6	Positive	Encodes for glutelin type B-protein which positively regulates protein storage during grain filling	Wu et al. (1998)
7	<i>GluB4</i>	Sobic.009G007100	LOC_Os02g16830	Group 6	Positive	It also encodes for glutelin type B-4 protein which facilitates protein storage	Qu and Takaiwa (2004)
8	<i>GluB5</i>	Sobic.009G007100	LOC_Os02g16820	Group 6	Positive	Encodes for glutelin type B-5 protein and positively regulates seed storage protein	Qu et al. (2008)

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TABLE 1 | (Continued)

S. No.	Candidate gene	Sorghum gene ID	Rice ortholog	Groups	Regulation	Function	References
9	NAC20/26	Sobic.002G192600	LOC_Os01g01470	Group 6	Positive	Regulates protein storage in endosperm. It also regulates expression of genes involved in protein biosynthesis like—GluA1	Wang, Chen, et al. (2020)
10	AAP6	Sobic.003G376000	LOC_Os01g65670	Group 7	Positive	Positively regulates seed protein content and quality	Peng et al. (2014)

Sobic.001G485400 from this group, *BIG GRAIN 1* (*OsBG1*) regulates auxin transport. Overexpression of *OsBG1* leads to increased grain size, while knockdown results in smaller grains (Liu et al. 2015).

Group 2 is the largest, comprising 4949 genes with peak expression in the 1–2 DBP and 0–2 DAP stages (Figure 3a, Table S6). Pathway mapping revealed protein modification, solute transport, cell division, chromatin organization, cytoskeleton organization, vesicle trafficking, organogenesis and photosynthesis as significantly enriched categories in this group (Figure 3b, Table S5). Enrichment of cell division and chromatin organization confirms that the transition from ovule to fertilized seed is marked by a rapid surge in cell proliferation. Among TFs enriched in this group, MYB-related, HD-ZIP, auxin response factor (ARF) and TCP are predominant (Figure 3c, Table S6). Genes belonging to the aforementioned pathways and TF families are well-known to regulate cell division required for embryo formation and patterning. One of the genes in group 2, Sobic.007G071400, is orthologous to *Arabidopsis EMBRYONIC FACTOR 19* (*FAC19*), which encodes for a pentatricopeptide repeat (PPR) protein critical for zygotic embryogenesis (Yu et al. 2012). Similarly, Sobic.004G156200 is an ortholog of *Arabidopsis TILTED 1* (*TIL1*), essential for embryo development by regulating cell division (Jenik, Jurkuta, and Barton 2005). Further, *Arabidopsis* ortholog of Sobic.001G292900 corresponds to a gene *OPENER* (*OPNR*) that is essential for cell division and seed development, and loss of function mutants are embryo lethal, with embryo defects detected as early as 2 DAP stage (Wang, Zhang, and Niittylä 2019).

It has been experimentally demonstrated in sorghum that the prefertilization stage of ovary development just before anthesis is critical for determining grain weight as larger kernel size leads to more endosperm cells and hence enhanced grain filling (Yang et al. 2009). Orthologs of 24 genes (*POSITIVE REGULATOR OF GRAIN LENGTH 2: PGL2*, *BRITTLE CULUM 12: BC12*, *DENSE AND ERECT PANICLE 1: DEP1*, *BRASSINOSTEROID-INSENSITIVE 1: BRI1*, *ARGONAUTE 2: AGO2*, *AUXIN RESPONSE FACTOR 12: ARF12*, *LYSINE-HISTIDINE-TYPE TRANSPORTER 1: LHT1*, *SQUAMOSA PROMOTER BINDING PROTEIN-LIKE 14: SPL14*, *GRAIN WIDTH 6: GW6*, *DWARF 2: D2*, *D11*, *SMALL ORGAN SIZE 1: SMOS1*, *YODA: YDA1*, *XIAO*, *OVATE FAMILY PROTEIN 1: OFP1*) from this group have been shown to regulate grain weight/size in other plant species (Table 1). For instance, *PGL2* suppresses *ANTAGONIST OF PGL1* (*APG*), a negative regulator of grain length, and thus positively regulates grain size (Heang and Sassa 2012b). *DEP1* positively regulates the number of endosperm cells and starch accumulation in rice by modulating phytohormone signaling (Zhang, Zhang, et al. 2019). In rice, *DEP1* regulates the duration of grain filling, with increased expression of *DEP1* during the middle and late stages of grain filling, increasing the number of endosperm cells and hence the final grain size (Zhang, Zhang, et al. 2019). *SMALL GRAIN 2/MITOGEN-ACTIVATED PROTEIN KINASE KINASE KINASE 10* (*SMG2/MKKK10*) in rice interacts with *MITOGEN-ACTIVATED PROTEIN KINASE KINASE 4* (*MKK4*) to positively regulate grain size by enhancing cell proliferation (Xu

et al. 2018). On the other hand, *GW6* does not impact the cell number but regulates cell size (Shi et al. 2020). The upstream regulator of *GW6*, *HOMOLOG OF DA1 ON RICE CHROMOSOME 3 (HDR3)*, positively regulates cell proliferation by stabilizing *GW6* (Gao et al. 2021), and *HDR3* is expressed in early seed stages in our data. Further, a total of 205 genes from Group 2 comprise cell division-related genes which have not been previously demonstrated to be associated with seed weight (Table S4). Of these, 17 exhibit an average TPM of > 100 in 1–2 DBP and 0–2 DAP stages, indicating their role in cell proliferation during initial seed development (Table S4). These would make excellent novel candidates for engineering seed weight in sorghum.

Group 3 has 412 genes with the highest expression in the 0–2 DAP stage, which gradually declines in later stages (Figure 3a, Table S4). This group is enriched in pathways associated with enzyme classification, protein modification, solute transport, cell wall organization, strigolactone, auxin action and NAC and TALE TF families (Figure 3b,c, Tables S5 and S6). Although the direct involvement of strigolactones in seed development is not established yet, a recent study in an oilseed crop, *Xanthoceras sorbifolium*, indicated the role of strigolactones in ovule development after fertilization by regulating invertase-mediated release of hexoses (Zhou, Zhou, and Cai 2024). The enrichment of solute transport pathways in groups 2 and 3 also suggests their involvement in transporting photoassimilates from the source (leaves) to sink (developing seeds) which is essential for grain filling (Lim et al. 2006).

3.3.2 | Groups 4 and 5 Genes With Predominant Expression in 0–2 and 10 DAP Stages Drive Embryo and Endosperm Development

Group 4 comprises 1342 genes with the highest expression in 0–2 and 10 DAP stages that involve early morphogenetic seed development of embryo and endosperm (Figure 3a, Table S4). The enriched pathway categories in Group 4 include enzyme classification, photosynthesis, lipid metabolism, cytoskeleton organization, chromatin organization, cell wall organization and carbohydrate metabolism (Figure 3b, Table S5). TFs belonging to MADS-like, YABBY, SBP, CO-like, and so forth, are enriched in Group 4 (Figure 3c, Table S6). Rice and *Arabidopsis* orthologs of several genes in this group are known to regulate embryo development. For instance, *Sobic.001G234200* and *Sobic.001G309200* are orthologous to *Arabidopsis ACTIN-RELATED PROTEIN 7 (AtARP7)* and *STEROL C4-METHYL OXIDASE 1-2 (AtSMO1-2)*, respectively, with both genes demonstrated to regulate embryogenesis (Kandasamy et al. 2005; Song et al. 2019). Similarly, *Sobic.004G149800* is orthologous to rice *REPRESSOR OF SILENCING 1 (OsROS1)*, which regulates male and female gametogenesis and aleurone layer development (Lang and Gong 2018). Orthologs of 13 genes, including *GRAIN SIZE ON CHROMOSOME 5: GS5*, *DWARF*, *F-BOX KELCH REPEAT PROTEIN 12: FBK12*, *BRASSINAZOLE-RESISTANT 1: BZR1*, *GIBBERELLIC ACID STIMULATED TRANSCRIPT-RELATED 9: GASR9*, and *MADS1* from this group, are known to regulate grain size/weight (Table 1). *BZR1* modulates

brassinsteroid (BR) signaling and regulates cell division and grain size (Yan et al. 2024; Zhang, Bai, and Chong 2014). *GASR9* regulates grain size and weight by modulating gibberellin (GA) response in rice (Li et al. 2019), whereas *OsFBK12* is involved in ethylene-mediated regulation of grain size (Chen et al. 2013).

Group 5 comprises 2085 genes with peak expression in the 10 DAP stage (Figure 3a, Table S4). Pathway categories associated with RNA biosynthesis and processing, protein biosynthesis, homeostasis and translocation, cellular respiration and carbohydrate, amino acid and nucleotide metabolism are enriched in Group 4 (Figure 3b, Table S5). Among TF families, MYB and MYB-related TFs are particularly enriched in Group 5 (Figure 3c, Table S6). *Sobic.004G160700* is one of the highest-expressing genes in this group. Its rice ortholog, *OsTudor-SN*, regulates endosperm development, seed storage protein accumulation and protein body formation in rice (Chou et al. 2019). Another interesting candidate in this group is *Sobic.008G088200*, which is highly expressed in all stages with peak expression in 10 DAP. Its rice ortholog *FLOURY ENDOSPERM 11 (FLO11)* exhibits a similar pattern in rice and has been shown to regulate starch granule formation in the amyloplasts during endosperm development (Zhu et al. 2018). Orthologs of six genes from this group have been experimentally associated with grain size/weight. Among these, *LONG GRAIN 3 (LG3)* and *NUCLEAR FACTOR Y SUBUNIT C 10 (NF-YC10)* are the best candidates. *NF-YC10* is well evidenced in rice to exhibit peak expression in the endosperm during the 5–10 DAP stage and to positively regulate cell proliferation, thereby increasing grain width and weight (Jia et al. 2019). *OsLG3* is also an *APE-TALA2 (AP2)* TF and is a major determiner of grain size in rice by positively regulating cell division (Yu et al. 2017). Another positive regulator, *GRAIN SIZE AND ABIOTIC STRESS TOLERANCE 1 (GSA1)*, from this group, increases cell proliferation and expansion in an auxin-responsive manner in rice (Dong et al. 2020).

3.3.3 | Group 6 and 7 Genes With Predominant Expression in 10 and 20 DAP Stages Are Crucial for Grain Filling

Group 6, with 842 genes, exhibits peak expression in both the 10 and 20 DAP stages, with a decline in the 30 DAP stage, while group 7 contains 203 genes with peak expression in the 20 DAP stage (Figure 3a, Table S4). These stages correspond to grain filling stages with maximum seed weight attained in the 20 DAP stage. Concomitant with this, the pathway categories, including enzyme classification, RNA biosynthesis, plant reproduction, auxin action, external stimuli response, cell wall organization, phytohormone action through signalling peptides, clade-specific metabolism, and carbohydrate metabolism, are enriched in Group 6. Conversely, solute transport, carbohydrate metabolism, amino acid metabolism, and redox homeostasis pathways are enriched in group 7 (Figure 3b, Table S5). TFs belonging to ERF and NAC families are enriched in Group 6, while C2H2 and LBD family TFs are overrepresented in Group

7 (Figure 3c, Table S6). One of the prominent candidates in Group 6 is *Sobic.002G367600*, an ortholog of rice *CYP78A13*, which performs a unique role of regulating the size balance between embryo and endosperm (Nagasawa et al. 2013). Grain weight peaks around 20 DAP, and the Group 7 genes with a peak in the 20 DAP stage harbours two crucial positive regulators of grain size, *PURINE PERMEASE 4 (PUP4)*, and *PUP7*, by regulating cytokinin CK transport and cell division (Qi and Xiong 2013; Xiao et al. 2019; Yin et al. 2020).

Signalling peptides have recently emerged as crucial candidates for mediating communication between developing embryo and endosperm in angiosperm seeds once the symplastic connections between different compartments are lost (Ingram and Gutierrez-Marcos 2015). Enrichment of the phytohormone-mediated signalling peptides in the 10 and 20 DAP stages aligns with the suggested role in apoplastic signalling during seed development.

Moreover, enrichment of carbohydrate and amino acid metabolism genes in Groups 6 and 7, coincides with our observation that grain filling peaks at the 20 DAP stage (Figure 3b, Table S5). Orthologs of several positive regulators (*NF-YB1*, *ZHOUP1-1: ZOU-1*, *ETHYLENE RESPONSE FACTOR 115: ERF115*, *YUCCA (YUC) FLAVIN-CONTAINING MONOOXYGENASE 11: YUC11*) of grain size were detected in the 10 and 20 DAP stages (Table 1). *NF-YB1* is an endosperm-specific gene crucial for cell proliferation during early seed development in rice (Sun et al. 2014). *ZOU-1* is a basic-helix-loop-helix (bHLH) TF, and serves as an endosperm-specific regulator pivotal for coordination developmental processes between endosperm and embryo cuticle formation, as well as nutrient remobilization in *Arabidopsis*, maize and rice (Doll et al. 2023; Dou et al. 2018; Grimault et al. 2015). *ERF115* is an ethylene-regulated AP2 TF that is expressed during the early and middle stages of seed development in rice and promotes cell division and elongation, thus positively impacting grain size and weight (Liu et al. 2022). *YUC11* modulates auxin homeostasis, endosperm development and grain size (Xu et al. 2021).

Since all these genes are positive regulators of cell division and are upregulated during grain filling in sorghum, they are good candidates for improving grain weight and size of sorghum varieties.

Starch is the major component of sorghum grain endosperm and, thus, the major edible part (Kang et al. 2023). It consists of two polymers amylose (linear) and amylopectin (highly branched). Starch quantity and quality directly impact the nutritional quality of cereal grains (Li et al. 2022). Starch synthesis uses glucose-1-phosphate as the primary substrate, which is used to generate ADP glucose. Through a series of enzymatic reactions, ADP-glucose can serve as a substrate for forming both amylose and amylopectin. Amylose is synthesised via granule-bound starch synthase I (GBSSI), while amylopectin is synthesized via a series of enzymes including soluble starch synthases (SSSs), starch branching enzymes (SBEs) and debranching enzymes (DBEs). As expected, most of these carbohydrate metabolism genes were enriched in Groups 6 and 7 (Figures 3b and S2, Table S5).

Ortholog of one of the most crucial genes for regulating starch metabolism, *Waxy (Wx; Sobic.010G022600)*, is also in Group 6 (Figures 4 and S2, Table 1). It is among the highly expressed (~2000 TPM) genes in 10 and 20 DAP stages. We also detected an important starch metabolism gene *SUGARY (SbSu; Sobic.007G204600)* in Group 6 (Figure 4 and Figure S2, Table 1). *SbSu* is recently characterized for its regulatory role in starch synthesis in sorghum during grain filling (Hashimoto et al. 2023). Further analysis of Group 6 carbohydrate metabolism genes showed that apart from these two master regulator genes, sorghum orthologs of eight other starch biosynthesis-related genes from rice (*UDP-GALACTOSE/GLUCOSE EPIMERASE 3: UGE3*, *ADP-GLUCOSE PYROPHOSPHORYLASE LARGE SUBUNIT 2: APL2*, *TRIOSE PHOSPHATE ISOMERASE: TPI*, *STARCH BRANCHING ENZYME IIb: SBEIIb*, *AGPASE SMALL SUBUNIT 2: APS2*, *FLOURY ENDOSPERM 4: FLO4*, *PYRUVATE DECARBOXYLASE 1: PDC1*, *SOLUBLE STARCH SYNTHASE IIa: SSIIa*) and *Arabidopsis (SOLUBLE STARCH SYNTHASE 3: SS3*, *SUCROSE SYNTHASE 4: SUS4*) are also present in Group 6, while remaining six carbohydrate genes of Group 6 (*Sobic.002G123100*, *Sobic.002G190500*, *Sobic.002G381600*, *Sobic.006G015800*, *Sobic.006G070830*, *Sobic.007G181600*) are novel candidates for regulation of starch metabolism (Figures 4 and S2, Table S4). Apart from carbohydrate metabolism genes, Group 6 also contains other crucial regulators of starch metabolism, like *NF-YB1*, *NAC20/26*, *BRITTLE 1 (BT1)*, *SWEET4-3*, *ZOU-1*, *NFYC12*, *SUGARY ENHANCER 1 (SE1)*, *YUC11* (Table 1). These genes can be targeted to manipulate starch structure and composition in sorghum for specific industrial uses.

Protein biosynthesis has also been shown to peak during grain filling in sorghum (Mrid Ben et al. 2018). Sorghum grains have a unique protein composition compared to other cereals, with ~80% of the total seed storage proteins being kafirins. These are prolamins that negatively impact the protein digestibility of sorghum seeds (Duressa et al. 2018). To identify kafirin genes in our data, we used a previous study in sorghum to extract the information on kafirin genes (Duressa et al. 2018). Of the 27 kafirin genes reported by Duressa et al. (2018), 22 were differentially expressed in our data (Table S7). Interestingly, all these 22 genes belong to Group 6, suggesting that 10 and 20 DAP stages are most critical in determining kafirin deposition in sorghum seeds (Figure S3, Table S7). Also, these genes were among the highest expressed genes in our data, with *Sobic.005G184700* having TPM value > 67 000 and can be targeted to manipulate protein content and digestibility. In fact, *Sobic.009G001600*, a beta-kafirin gene, has previously been modified through a synthetic approach and introduced in sorghum cultivar Tx430 to attain increased protein content with concomitant improvement in protein digestibility (Liu et al. 2019). Further, 10 and 20 DAP stages were also marked by upregulation of genes characterized for their roles in protein homeostasis during grain filling (*AMINO ACID PERMEASE 10: AAP10*, *NAC20/26*, *AAP6*, *GLUTELIN TYPE-A1: GLUA1*, *GLOBULIN 1: GLB1*). For instance, *AAP6* and *AAP10* exhibit high expression in the endosperm of rice seeds, and their loss of function decreases protein content but improves taste value in rice (Wang, Yang, et al. 2020).

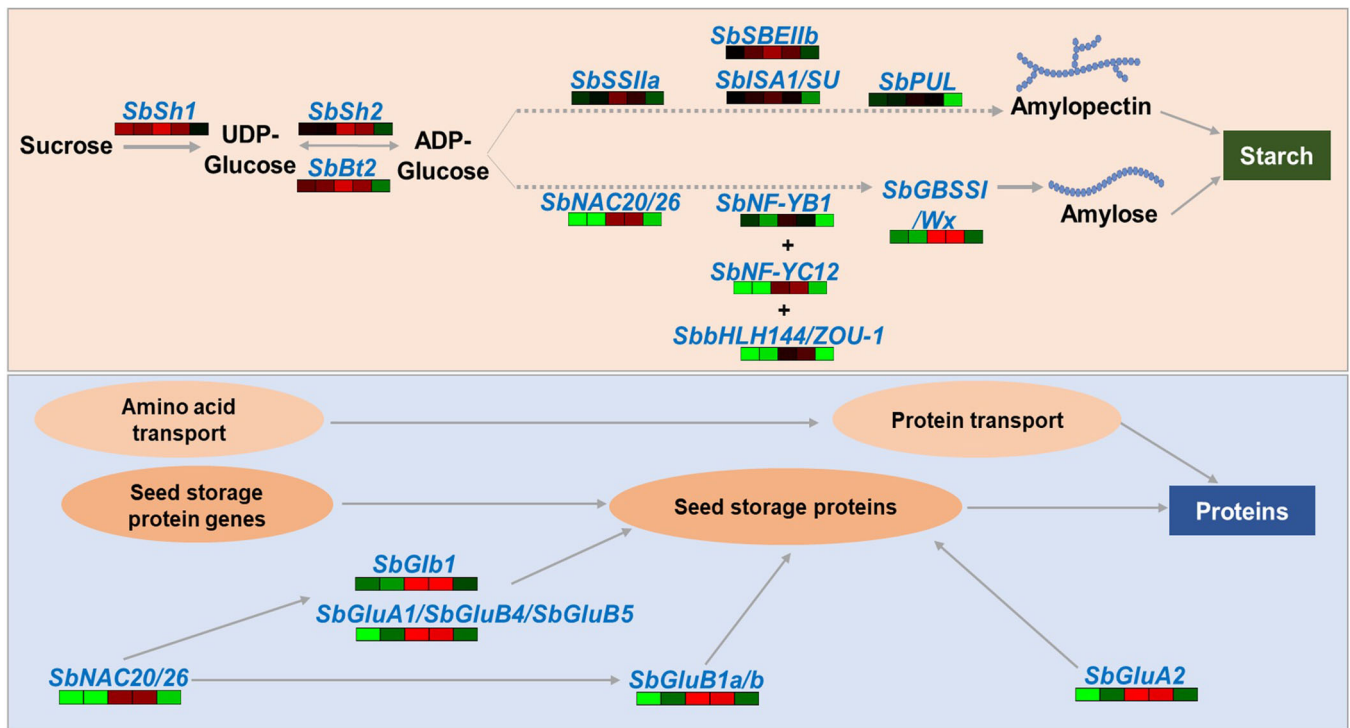


FIGURE 4 | Sorghum candidate genes for regulation of starch biosynthesis and protein content and quality during grain filling. The information of starch and protein biosynthesis genes was retrieved from rice as discussed in previous studies (Hashimoto et al. 2023; Li et al. 2022). Differentially expressed orthologs in sorghum that belong to Group 6 associated with grain filling have been given. *bHLH144/ZOU-1*, *BASIC-HELIX-LOOP-HELIX 144/ZHOUP1-1*; *Bt2*, *BRITTLE 2*; *GBSSI/Wx*, *GRANULE-BOUND STARCH SYNTHASE I/Waxy*; *Glb1*, *GLOBULIN 1*; *GluA1*, *GLUTELIN A1*; *ISA1/SU*, *ISOAMYLASE 1/SUGARY*; *NF-YB1*, *NUCLEAR FACTOR Y SUBUNIT B1*; *PUL*, *PULLULANASES*; *SBEIIB*, *STARCH BRANCHING ENZYME IIB*; *Sh1*, *SHRUNKEN 1*; *SSIIa*, *SOLUBLE STARCH SYNTHASE IIa*. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

3.3.4 | Group 8 and 9 Genes With Peak Expression in 20 and 30 DAP Stages Regulate Seed Maturation and Dormancy

Group 8 has 177 genes with peak expression in the 20 and 30 DAP stages, while Group 9 comprises 1120 genes with peak expression in the 30 DAP stage (Figure 3a, Table S4). The expression patterns of these genes indicate a potential role in seed maturation. Only three pathway categories, including enzyme classification, lipid metabolism and chromatin organization, are enriched in Group 8 (Figure 3b, Table S5). In contrast, Group 9 includes several enriched categories, including enzyme classification, RNA biosynthesis, protein biosynthesis, coenzyme metabolism, cellular respiration, cytokinin action and redox homeostasis (Figure 3b, Table S5). Although cytokinins have been shown to impact source-sink relations during seed development, their precise role during seed maturation remains elusive (Roitsch and Ehneß 2000). In addition, TFs belonging to ERF, bZIP, EIL and trihelix families are enriched in group 9 (Figure 3c, Table S6). *Sobic.003G302466* of group 8 corresponds to *Arabidopsis* and maize *DELAY OF GERMINATION 1* (*DOG1*), critical for seed dormancy and germination. Among group 9 genes, *Sobic.002G356300* (*SEED DORMANCY 4: SDR4*) and *Sobic.003G398200* (*VIVIPAROUS 1: VPI*) have also been reported to underlie dormancy-related QTL in sorghum (Guo et al. 2015). In fact, *VPI* in rice is known to act as a master regulator of seed maturation and regulates *SDR4* to promote seed dormancy (Chen, Wang, et al. 2021). *Sobic.002G356300* (*SDR4*) and *Sobic.003G398200* (*VPI*) have also been reported to underlie dormancy-related QTL in sorghum (Guo et al. 2015). In sorghum, expression of *VPI* has been

found to peak in mature seed stage in a previous study, and unlike rice in which it peaks at 16 DAP, *VPI* expression continues to increase till physiological maturity (Guo et al. 2015). This finding further strengthens the expression patterns that we observed in our data.

3.3.5 | Group 10 Genes With Peak Expression in 1–2 DBP and 30 DAP Stages Include Genes Required for Protein Biosynthesis

Group 10, with 759 genes, exhibits a unique expression pattern with peak expression in 1–2 DBP and 30 DAP stages (Figure 3a, Table S4). This group is particularly enriched in protein biosynthesis, RNA processing, protein translocation, coenzyme metabolism, clade-specific metabolism and amino acid metabolism, indicating that these genes are required for the translational shift that occurs during fertilization and seed maturation and associated protein synthesis (Figure 3b, Table S5). TFs belonging to *GLABROUS1* enhancer-binding protein (GeBP) and double B-box (DBB) are enriched in group 10 (Figure 3c, Table S6). The members of GeBP and DBB family have been shown to regulate cytokinin response and circadian-associated events, respectively, in other plant species (Chevalier et al. 2008; Kumagai et al. 2008).

Although in this study, we have mainly focused on genes likely responsible for grain weight, starch and protein content,

previous reports involving transcriptional profiling of sorghum grains focused on genes regulating cell wall biosynthesis and carotenoid content (Costes et al. 2024; Cruet-Burgos and Rhodes 2023). A total of 385 cell wall and 59 carotenoid related candidate genes have been highlighted in these studies. Expression profiling of these genes in our data revealed differential expression 237 cell wall and 29 carotenoid accumulation-related genes in our data (Table S10 and S11). Notably, majority of these genes exhibit peak expression in early seed stages in our data conforming with the accumulation of carotenoids and active cell wall biosynthesis during early seed development.

3.4 | Mapping of Candidate Genes With Grain Weight QTLs in Sorghum

Numerous QTLs have been identified for grain weight in sorghum (Mace et al. 2019), but only *qTGW1a* and *SbDEP1* have been cloned so far (Tao et al. 2021; Zou et al. 2020). Similarly, only one gene, *SbSu*, has been validated in sorghum for its role in starch metabolism. Fine mapping and map-based cloning are laborious and time-consuming; therefore, existing QTL studies

must be leveraged by integrating transcriptional profiles of candidates.

We utilized the previous information of grain weight QTLs in sorghum (<https://aussorgm.org.au/sorghum-qtl-atlas/>) to identify the candidate genes for grain weight that overlap with these QTLs using the information of physical coordinates. We obtained 147 grain weight QTL from this data set (Table S8). The analysis of colocalization of DEGs which are candidates for regulating grain size/weight as evidenced from the literature, showed that 48 candidate genes were differentially expressed in our data and showed colocalization with 57 QTLs in our data set (Table S9). Several candidate genes were detected within a single QTL. For example, in QTL *QGWGT1.12* located on chromosome 01, four differentially expressed candidate genes *SbBG1*, *SbMADS1*, *SbDG1* and *SbLG3* were observed. Alternatively, the same candidate gene could also be found in different QTLs. For example, *SbGIF1* (Sobic.001G101700) was located in various QTL regions like *QGWGT1.18*, *QGWGT1.25*, *QGWGT1.26* and *QGWGT1.31* (Figure 5a, Table S9).

qTGW1a, is a significant grain weight QTL, consistently detected in sorghum for negative regulation of grain weight. Its

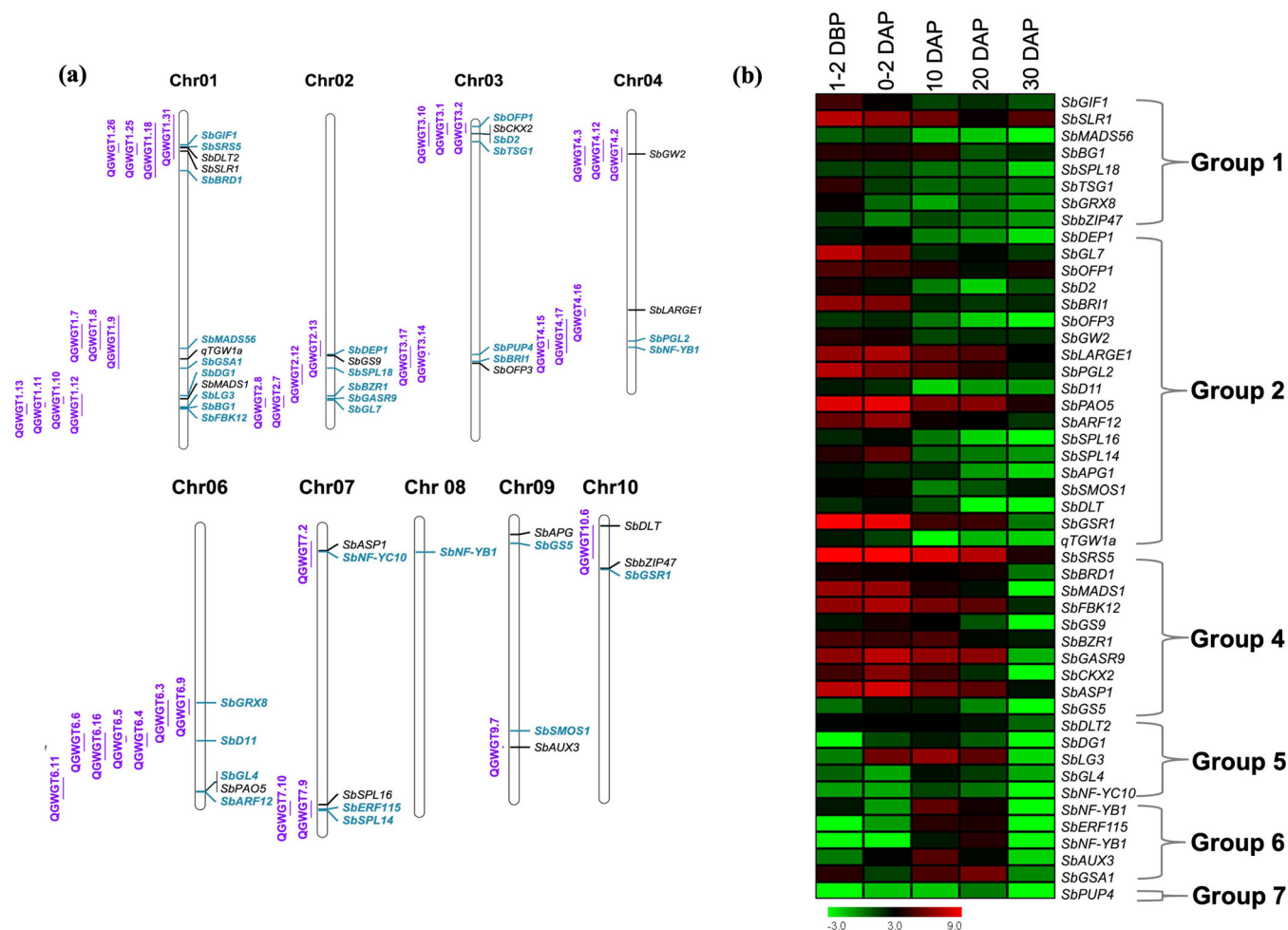


FIGURE 5 | Colocalization of candidate DEGs and grain weight quantitative trait loci (QTLs) in *Sorghum bicolor*. (a) Candidate genes regulating grain weight and/or size, which exhibit differential expression in our data, as well as colocalization with previously detected sorghum grain weight QTLs, are shown. Gene color coding: blue represents positive regulators, black represents negative regulators. (b) Heatmap showing the expression pattern of these candidate genes. The groups to which these DEGs belong are also marked. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

underlying causal gene was identified as SORBI_3001G341700. It codes for a G-protein and is also a causative gene for grain size in other cereals like rice, maize and wheat (Chen, Yan, et al. 2020; Chen, Lu, et al. 2020; Li et al. 2010; Wenhui et al. 2019). In our data, this gene (Sobic.001G341700) belongs to group 2, with peak expression in 1–2 DBP and 0–2 DAP stages (Figure 5b; Zou et al. 2020). This is in agreement with its previously reported expression patterns in sorghum and rice (Zou et al. 2020). Due to its negative regulation and consensus detection in multiple cereal crops, it is an ideal candidate to improve M35-1 grain weight via genome editing mediated loss of function. Another sorghum grain weight QTL causal gene, *SbDEP1* (Sobic.002G216600), also belongs to Group 2 in our data, conforming with the role of early seed-associated genes in regulating grain weight (Figure 5b; Tao et al. 2021). Furthermore, network analysis of these 48 candidates based coexpression identified five genes (*SbMADS1*, *SbD11*, *SbBRI1*, *SbSPL18* and *SbSRS5*) as hub genes with potential role in regulating grain weight (Figure S4). In a recent study, variant analysis of grain size and weight-related genes in 15 accessions of sorghum revealed six grain size-related candidates exhibiting polymorphism in their coding sequences (Ananda et al. 2023). Authors suggested that these variants likely contribute to observed variation in seed size and weight in sorghum accessions. Three of these candidates, Sobic.001G335800 (*qGW7/GL7*), Sobic.001G341700 (*GS3*) and Sobic.004G107300 (*GW2*), are differentially expressed and fall in group 2 in our study with peak expression in the 1–2 DBP and 0–2 DAP stages. These are also colocalizing with grain weight QTLs indicating high possibility of their involvement in regulating seed size/weight. Though identifying functional markers directly associated with phenotypic variations for precision breeding is difficult (Baloch et al. 2023), the data generated here will be a highly beneficial resource for developing functional markers to enhance sorghum breeding efforts.

3.5 | Conserved and Unexplored Roles of Phytohormone-Associated Genes and TF Families in Seed Development

Phytohormones play crucial role in seed set from pollination to maturation (Jain et al. 2023). In our data, we observed significant enrichment of JA and ABA-related genes in prepollination ovaries conforming with their reported role in pollen maturation, pistil development, anthesis and seed set (Kai et al. 2019; Niwa et al. 2018). The role of auxins has been well documented in triggering endosperm development, regulating zygote to embryo transition, embryo polarity and patterning, and initiation of seed development in diverse plant species (Figueiredo and Köhler 2018). Significant enrichment of auxin biosynthesis and signalling genes from prepollination pistils to grain filling stage in our data conform with their conserved role in regulating seed development in sorghum as well (Figueiredo and Köhler 2018; Locascio et al. 2014).

Strigolactones-associated genes were enriched in postfertilization ovaries, conforming with their earlier suggested role in seed set by regulating hexose levels for carbon partitioning in developing seeds (Zhou, Zhou, and Cai 2024). Cytokinin-related

genes were enriched in the 30 DAP stage, representing mature seeds. This contrasts previous reports where cytokinin biosynthesis genes have mainly been shown as enriched in early seed stages. Although there is an indication of role of cytokinins in maintaining source-sink relationship, precise function of cytokinins during seed maturation remains unexplored (Roitsch and Ehneß 2000).

TFs play regulatory roles in conjunction with phytohormones during plant growth and development. The same TF family members are often associated with similar pathways and regulatory functions. Conforming to this, we observe the enrichment of specific TF families in distinct stages of seed development. For instance, the members of the B3 family are particularly overrepresented in the 1–2 DBP stage. A B3 family TF, *VERDANDI* (*VDD*) has previously been shown as essential for pollen tube-synergid communication during fertilization (Matias-Hernandez et al. 2010). B3 is a plant-specific TFs superfamily comprising ARF, LAV, RAV and REM families. ARF family is enriched in Group 2 with peak expression in both 1–2 DBP and 0–2 DAP stages and has been associated with early seed development by regulating cell division and organ growth (Li, Xie, et al. 2016). The other two families enriched in Group 2, TCP and HD-ZIP have also been shown to regulate embryogenesis by regulating cell division and embryo patterning in an auxin-dependent manner (Challa, Rath, and Nath 2019; Izhaki and Bowman 2007). MYB and MYB-related TFs enriched in groups 2 and 5 have been implicated in differentiation of transfer cells at the base of endosperm for solute transport and pigment accumulation in seed coat (Flores et al. 2022; Gómez et al. 2009). MADS-MIKC family of TFs are enriched in Group 4 and have been extensively reported to play crucial roles in regulating diverse aspects of seed development including seed morphogenesis, starch accumulation, endosperm formation, seed coat development and maintain hormone homeostasis during seed development (Ehlers et al. 2016; Malabarba et al. 2017; Mizzotti et al. 2012; Nayar et al. 2013). NAC family TFs, enriched in Group 6 genes associated with grain filling, have been documented to regulate grain filling in rice and wheat by regulating starch biosynthesis and seed storage proteins (Mathew et al. 2020; Sharma et al. 2019; Shen et al. 2021). The members of bZIP family enriched in Group 9 have been shown to regulate early seed maturation (Agarwal, Kapoor, and Tyagi 2011). The ERF family TFs are enriched in Groups 6 and 9, suggesting their role from grain filling to the maturation phase. Although ERF family genes have been widely implicated in seed germination and stress response, their role in grain-filling stages and seed maturation remains unexplored.

All of the TF families, except the ERF family, enriched in the seed stages conform with their previously reported roles in other plant species, indicating their conserved role in regulating seed development and associated traits. Further exploration of ERF family members in the context of seed development and grain filling would provide required insights into gene functions.

4 | Conclusion and Future Perspectives

The genome editing technologies leveraging CRISPR/cas system have revolutionized the prospects of plant biotechnology in crop improvement. However, the critical step in genome editing

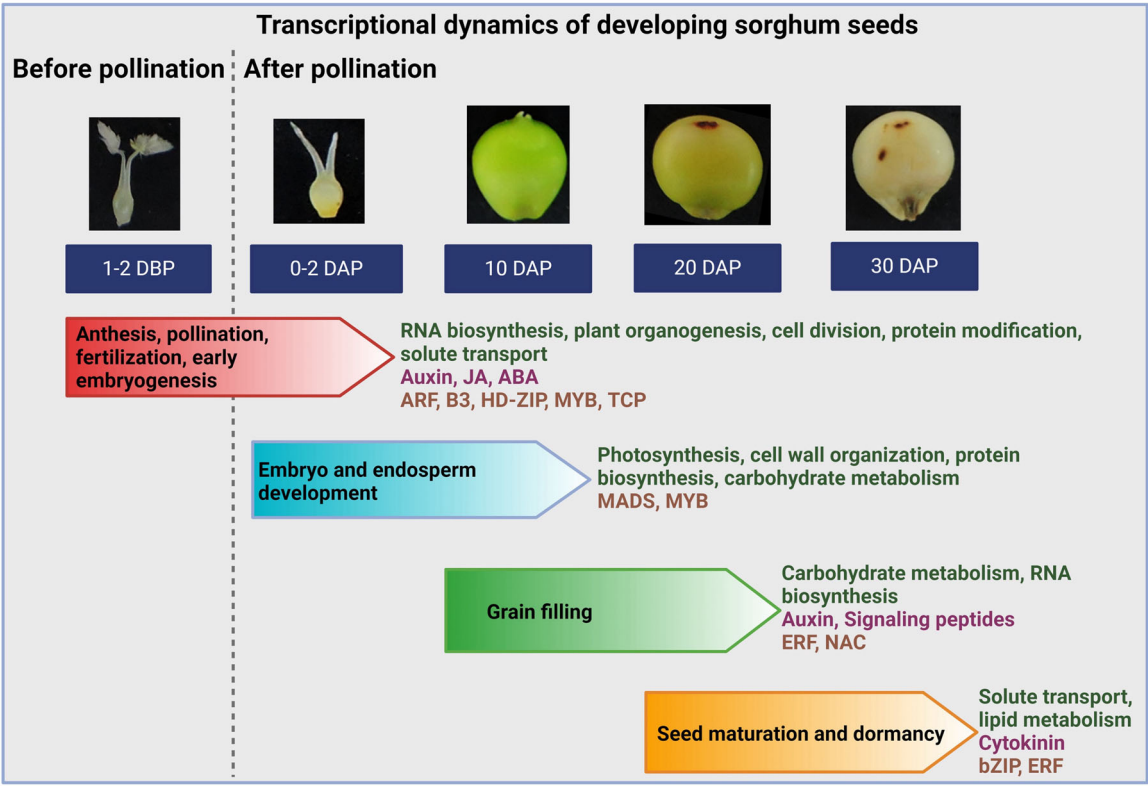


FIGURE 6 | Transcriptional dynamics in developing seeds of *Sorghum bicolor*. Overall scheme depicting the stages of seed development used in this study and respective landmark biological events, enriched pathways (green), phytohormones (magenta) and transcription factors (brown) (created with [BioRender.com](https://www.biorender.com)). [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1111/pcel.15134)]

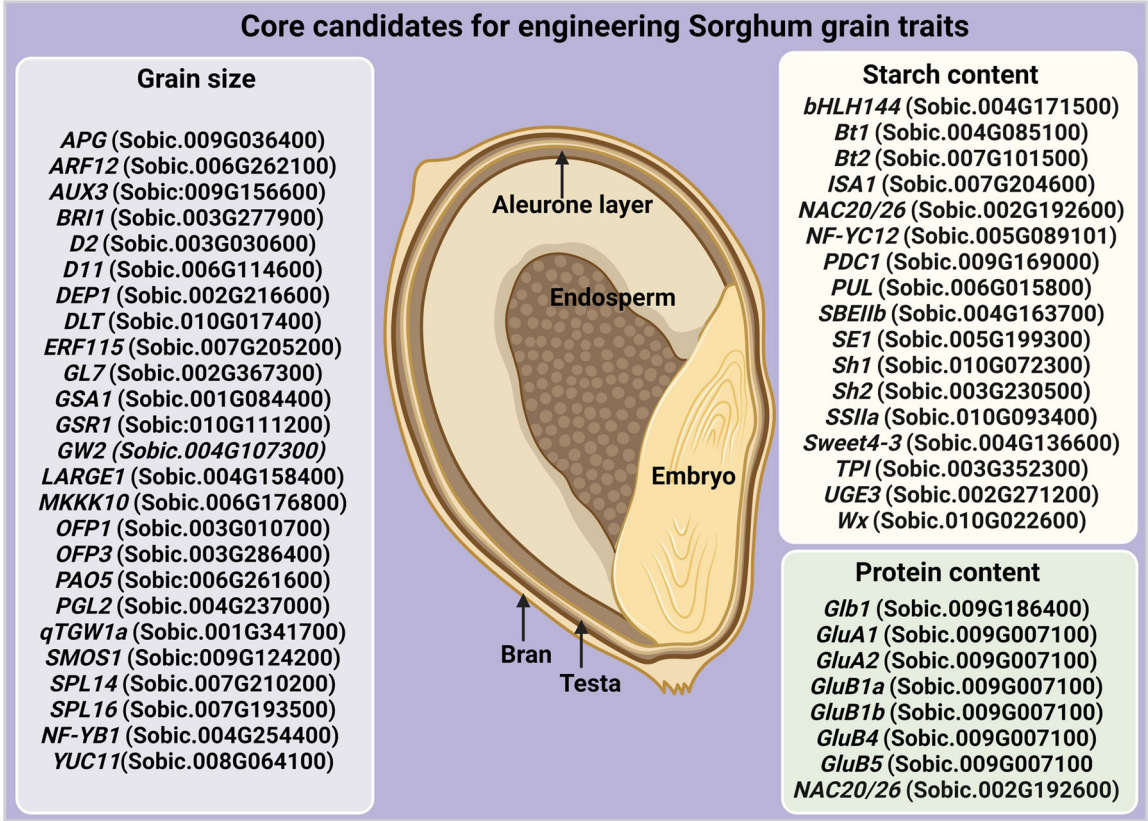


FIGURE 7 | Core candidates for engineering grain size, starch and protein content in sorghum (created with [BioRender.com](https://www.biorender.com)). [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1111/pcel.15134)]

studies is the selection of a suitable candidate gene. Analysis of gene expression patterns in temporal stages of development has proven to be a very useful strategy for quickly determining the association of leading pathways and associated genes with the key developmental transitions. Extensive literature mining followed by mapping of orthologous genes further helps to narrow down the candidates with conserved functions across diverse plant species. This approach adds more confidence to expression-based selection and allows translation of the findings in multiple crops. We have used this approach for candidate gene mining during seed development in sorghum.

Our study explored transcriptional dynamics in five temporal stages encompassing pollination to seed maturity (Figure 6). The study not only verified conserved functions of phytohormones and TFs in regulating major events associated with grain development in sorghum but also highlighted some of the unexplored territories in the context of seed development. For instance, the molecular and physiological basis of the enrichment of strigolactones in the early seed stage and cytokinin pathways in the seed maturation phase remains elusive. Similarly, role of ERF family TFs in grain filling and maturation is unclear. Conversely, the enrichment of phytohormone-mediated signalling peptides during grain filling opens new and exciting avenues of research with potential applications in engineering grain filling. Moreover, extensive analysis of orthologs in rice and colocalization with grain size QTL showed 48 candidates for grain size. Of these, 25 genes belonged to Group 2 and Group 6 (Figure 7, Table 1), which were found to be most crucial for regulating initial cell proliferation and grain filling based on expression analysis and can, therefore, be considered as core candidates for engineering grain weight/size in sorghum. Further evidence from previous genomic analysis supported Sobc.001G335800 (*qGW7/GL7*), Sobc.001G341700 (*GS3*) and Sobc.004G107300 (*GW2*) as key candidates for engineering grain weight. Moreover, analysis of dominant expression patterns revealed the grain filling stage as the most crucial for regulating starch and protein content, providing 17 and 8 candidates for regulating starch and protein content, respectively (Figure 7, Table 1). These genes provide a highly beneficial resource for engineering grain weight/size, starch, and protein composition and digestibility. Since we have identified the candidate genes using an elite grain sorghum cultivar, allelic diversity in these genes may be assessed using sorghum gene pool to identify superior alleles for enhancing the grain traits.

Acknowledgements

N.D. thanks research grant from the Science and Engineering Research Board (SERB), (CRG/2019/001695) and DST INSPIRE grant IFA17-LSP90 by Government of India. R.A.S. thanks CRG/2020/003466 and STR/2022/000013 and Indo-German Science & Technology Centre (IGSTC), WISER grant/award number: IGST/WISER2023/RS/39/2023-24/771. R.J. acknowledges financial assistance through an ICMR-SRF fellowship. G.Y. thanks CSIR-UGC for the junior research fellowship. P.P. thanks SERB for fellowship through the manpower component of the CRG/2019/001695 research grant. The authors are thankful for the facilities provided by Jawaharlal Nehru University, New Delhi and Central University of Haryana, Mahendergarh.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Raw data have been submitted to NCBI-GEO and can be accessed using the series record GSE206876. The data that support the findings of this study are openly available in NCBI-GEO at <https://www.ncbi.nlm.nih.gov/geo/>, reference number GSE206876.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.