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Research article

Comparative regulatory network of transcripts behind radicle emergence and seedling stage of maize (*Zea mays* L.)

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ABSTRACT

The transition from radicle emergence to seedling growth in maize is a crucial phase in the plant's life cycle, where rapid physiological and biochemical changes occur to facilitate successful development. In this study, we conducted a comparative transcriptomic analysis to gain a deeper understanding of the molecular processes driving this critical transition. The early divergence in gene expression patterns highlighted the upregulation of a substantial number of genes during radicle emergence. During radicle emergence, gene ontology (GO) term enrichment analysis unveiled active participation in biological processes such as chromatin assembly, cellular response to abiotic stress, and hormone signaling. This indicates that the initial stages of growth are marked by cellular expansion and adaptation to environmental stimuli. Conversely, in the seedling growth stage, GO analysis demonstrated a shift toward processes such as photosynthesis, nitrogen metabolism, and secondary metabolite biosynthesis, reflecting a transition to energy production and enhanced growth. In contrast, seedling growth was characterized by pathways related to photosynthesis and the production of gibberellins, crucial for robust seedling development. Hormonal regulation and starch metabolism were also prominent during radicle emergence, with various hormones, including auxins, diterpenoids, and brassinosteroids, driving processes like cell enlargement and stem growth. Moreover, starch and sucrose metabolism genes were expressed to mobilize stored reserves for energy during this stage. These findings offer valuable insights into the dynamic regulation of genes and pathways during this critical phase of maize development.

1. Introduction

Zea mays L. is a prominent commercial and staple crop grown all over the world. The rate and uniformity of field crop establishment, which eventually determines the yield, particularly for maize, has a direct link to the early stages of plant growth i.e., seed germination and seedling establishment [1]. The mature seed restarts growth throughout the intricate process of germination,

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changing its development plan from one driven by maturity to one that promotes seedling growth [2]. A dry mature seed is expected to quickly absorb water (phase I), doing so until all of the seed tissues are moistened. Following phase II is a low intake of water, but phase III shows an increase in water uptake that coincides with the end stage of germination. The most important phase is Phase II, which is linked to many kinds of cellular and physiological functions, as well as DNA repair and the translation of both newly generated and stored mRNAs [3–5]. In fact, the final stage of seed germination is described as the extension of the embryonic axis, which often refers to radicles emerging out of the encasing seed layers [6].

The cell cycle must be activated for seedling development during the germination stage. Cell expansion in the embryonic axis is what largely drives seedling growth from the embryo. The majority of a plant's growth prospects after germination depend on cell divisions that take place in the adult plant embryo's root and shoot meristems. A crucial step in the development of a seedling is the proliferation of the embryo root meristem, which is required for the beginning of root growth [7,8]. It has been shown in the past that the start of the mitotic cell cycle occurs in the shoot and root meristems at the last stages of seed germination and is regulated by plant hormones. These modifications may be seen in the gene expression patterns, which abruptly show a transition from a germinative to a developmental program [9].

ISTA has certified the RE test (early single count of radicle emergence) to measure maize seed vigor [10]. In many crop species, the RE test has been linked to seedling emergence potential [11,12]. The underlying physiology of the RE test is the time frame (lag period) between imbibition and radicle emergence, which reveals the restart of gene expression from the embryo's resting condition to establish a functioning genetic program allowing the new plant to develop. It includes signal transduction, cell wall remodeling and modification, phytohormone metabolism, a surge in metabolic pathways for energy availability, and the synthesis of macromolecules, mRNAs, and proteins that allow cells to survive and thrive [13]. The activation of the embryo root meristem is a critical phase of the post-germination stage, where radicles continue to elongate and cotyledons appear and expand. This is during which photosynthesis and energy metabolism processes are interconnected, leading to the establishment of the seedling [14]. Seed tissues perform distinct activities during germination, which lasts around 66 h in maize [15]. As a result, investigations of particular tissues at certain time points must be focused on to analyze the development process at the molecular level. In this situation, transcriptomics has shown to be a potent tool for examining certain biological functions of development in maize. Since the tissues that will grow and develop to make a new plant are present at both the initial radicle emergence stage and the final seedling stage in maize, an overall study of the expression at both steps has been carried out by RNA sequencing to set special emphasis on the various genes controlling several regulatory mechanisms during these crucial stages.

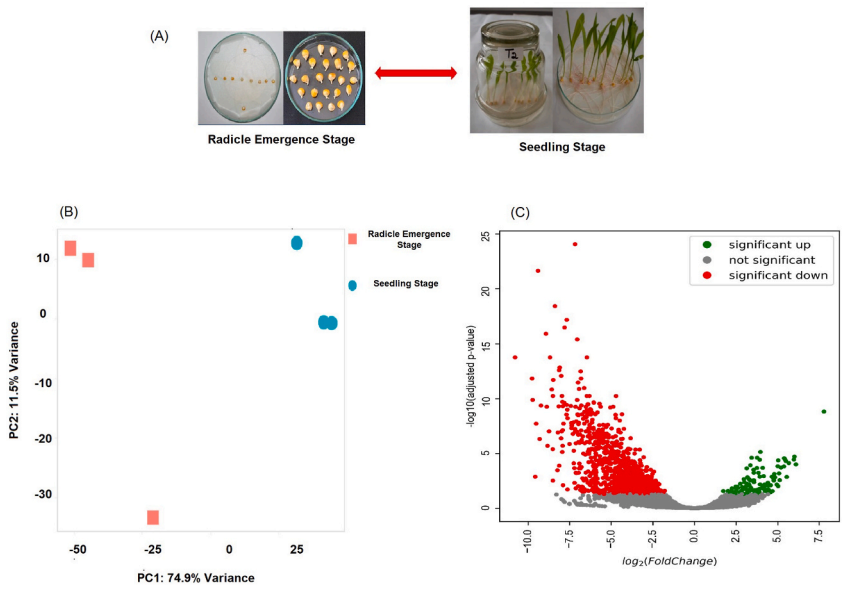

Fig. 1. (A) Pictorial representation of tissues selected for transcriptomic analysis during the radicle emergence stage and seedling stage, (B) Principal component analysis (PCA) plot of all expressed genes in the RNA−seq data. The X−axis indicates the first principal component; the Y−axis indicates the second principal component. The percentage of variance explained by each PC is shown in each case. 1–3, biological replicates, (C) Volcano plot of expressed genes of seedlings compared to radicle emergence stage. The y-axis illustrates −log10 p values and the x-axis corresponds to a log2-fold change of gene expression between seedlings and the radicle emergence stage. Significantly up and downregulated genes are highlighted in green and red colors, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

2. Materials and methods

2.1. Seed germinating condition and sample phenotyping

In our research study, we employed seeds of the maize (*Zea mays* L.), specifically the hybrid MAH 14-5, as the biological system under investigation. These maize seeds were sourced from the AICRP on Seed (Crops), University of Agricultural Sciences, GKVK, Bengaluru. Notably, the seeds used in our experimentation were procured from a newly harvested, untreated seed lot, ensuring the freshness and integrity of the plant material. This deliberate choice of untreated seeds aimed to maintain the seed's natural state and characteristics, which is pivotal for unbiased scientific analysis.

After being surface-sterilized in 70% (v/v) ethanol for 15 min, seeds were then three times rinsed in sterile distilled water [16]. Seeds were kept for germination in a sterile glass plate covered with glass jars for 48–66 h by water imbibition on moisturized Whatman filter paper at 27 ± 1 °C in a growth chamber. Three biological replicates were prepared and ten seeds were mixed for each sample. The embryonic axes were excised manually from the radicle emergence stage; whereas in 7-day-old seedlings root and shoot samples were collected separately for RNA isolation. The RNA was extracted as per the manufacturer's protocol using the Trizol reagent (Takara) as shown in Fig. 1 (A).

2.2. RNA isolation and transcriptome sequencing

RNA isolation was done using a Trizol reagent. About 100 mg of stored (−80 °C) maize tissue (embryonic axis of pre-germinated seeds; shoot and root tissues of 7-day-old seedlings) was taken and immediately tissues were placed in liquid nitrogen and ground thoroughly. Tissue powder was transferred to 2 ml Eppendorf tube and 1 ml of Trizol reagent was added and kept at room temperature for 5 min. It was mixed thoroughly by pipetting and kept on ice for 15 min. Centrifuged at 10,000 rpm for 5 min at 4 °C and transferred liquid phase to new Eppendorf tube. 200 μl of chloroform (chilled) was added and vortexed for 30 s followed by centrifugation at 13,000 rpm for 15 min at 4 °C. Pipetted aqueous phase to new Eppendorf tube and added 200 μl of chloroform and centrifuged at 13,000 rpm for 15 min at 4 °C. The aqueous phase was pipetted to a 1.5 ml Eppendorf tube and 500 μl of ice-cold isopropanol was added and incubated at −20 °C for overnight. Centrifuged at 13,000 rpm for 15 min at 4 °C and the pellet formed was washed with 70% ethanol (twice) at 13,000 rpm for 15 min at 4 °C and air dried the pellet in ice for 30 min. Finally, the pellet was dissolved in nuclease-free water (50 μl). The quality of extracted RNA was verified using a Qubit 4.0 fluorometer (Thermofisher #Q33238) and an RNA HS test kit (Thermofisher #Q32851) according to the manufacturer's procedure. To ensure the purity of the extraction, the concentration was measured on a Nanodrop. Finally, RIN values were obtained by running RNA through the Tapestation using HS RNA screen tape.

2.3. Gene quantification and differential-expression analysis

Illumina Novaseq 6000 (Molsys Lab Pvt Ltd, Bangalore, India) was used for transcriptome sequencing and the quality of raw fast reads from the sample was assessed using FastQC v.0.11.9 [17]. Fastp v.0.20.1 was used to pre-process the raw fast reads, and FastQC was used to re-assess the quality [18]. The *Zea mays* genome was indexed using hisat2-build [19]. Using Hisat2, processed high-quality reads were mapped to the reference genome assembly (Zm-B73-REFERENCE-NAM-5.0). Aligned reads from each sample were evaluated using feature count v.0.46.1 to derive gene counts [20]. These gene counts were fed into DESeq2 [21], for differential expression estimates. Protein sequences from differentially expressed genes are obtained from the maize protein dataset, compared to nrdb using Diamond blastp, then annotated using blast2go [22].

2.4. GO and KEGG analysis of differentially expressed genes

Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) were performed on the ShinyGO [23] and iDEP 96 [24] enrichment tool based on the DAVID database to analyze and graphically represent the data. This online bioinformatics resource is a free database that analyzed genes utilizing three terms: Molecular Function (MF), Biological Process (BP), and Cellular Component (CC).

2.5. Statistical analysis of data

All data sets had three biological replicates. Differentially expressed genes were defined as genes with false discovery rate (FDR) < 0.05 and fold change $>$ twofold. An adjusted p value < 0.05 was considered significant when identifying enriched GO terms, and an adjusted p value < 0.05 was considered indicative of significantly enriched KEGG pathways.

3. Results

3.1. Comparative transcriptomic profiling of maize during radicle emergence to seedling transition

In the study of maize seed germination and seedling growth, we speculated that a series of rapid physiological and biochemical reactions occurred during the very early stage of seedling development. The principal component analysis (PCA) demonstrated a high level of differences between the radicle emergence and seedling stage. The percentage of the explained dispersion for the PCA model

was 86.4% (74.9% for PC1 and 11.5% for PC2) and shown in Fig. 1 (B). The differentially expressed transcripts profile in the heatmap clearly showed that a greater number of genes were upregulated in the radicle emergence stage compared to the seedling stage (Fig. S1). Similarly, the volcano plot showed that a total of 1382 DEGs were expressed, among which 219 DEGs were up-regulated and 1163 were down-regulated in the seedling stage compared to the radicle emergence stage, which depicts that the gene expression was very strong at the initial radicle protruding stage (Fig. 1 (C) and Fig. S2).

3.2. Characterization and functional annotation of DEGs in the maize during radicle emergence and seedling stage

By mapping the GO database using the ShinyGO 0.76 toolbox and using a false discovery rate (FDR values 0.05) as the cutoff, DEGs during the radicle emergence and seedling growth stage were utilized to examine the highly enriched genes compared with the genomic background. The findings revealed that when compared to the maize seedling stage, a total of 34 significant GO terms were found among the three gene ontologies in the up-regulated DEGs of a radicle emergence stage. The main classes contributing to the "biological process" were "chromatin assembly and remodeling", "cellular response to abscisic acid stimulus", "cellular response to lipid", "Response to hormone", and "response to abiotic stimulus". "Nucleosome", "DNA packing complex", and "protein-DNA complex" were the enriched GO terms in the cellular-component category. In the molecular-function category, the primary class was "DNA-binding transcription factor activity", which is shown in Fig. 2 (A-C). The top molecular networks associated with each of the significantly altered genes appear to be linked to key mediators during radicle protrusion (Fig. S3).

In the up-regulated DEGs of the seedling growth stage when compared to the radicle emergence stage, a total of 76 significant GO terms were found among the three GO categories, with "tricarboxylic acid biosynthesis process", "glutamine biosynthesis process", "gibberellin biosynthesis process", "carbon fixation", "photosynthesis-light reaction", "electron transport chain", and "generation of precursor metabolites-energy" in the biological process category. "Photosystem", "thylakoid", "chloroplast", "cell wall", "photosynthetic membrane" and "organelle envelope" in the cellular component group; and "peroxidase activity", "heme binding", "transporter activity", "tetrapyrrole binding" and "antioxidant activity" in a group of molecular function Fig. 3 (A-C). Furthermore, their associated networks link closely to the photosynthetic pathway at the seedling level as shown in Fig. S4.

3.3. Functional regulatory network analysis (KEGG pathway enrichment) of maize during radicle emergence and seedling stage

Specifically detected DEGs in the embryonic axis with radicle outgrowth and in seedlings of 7- days old were subsequently searched against the KEGG pathway database to clarify the unique biochemical processes during the radicle emerging and seedling stage of maize. The key mechanisms elevated during the radicle protrusion period include "biosynthesis of secondary metabolism," "starch and sucrose metabolism," "gluconeogenesis," "MAPK signaling pathway," "plant hormone signal transduction," "carotenoid biosynthesis," and "benzoxazinoid biosynthesis" (Fig. 4). As for those genes identified in seedlings of maize, eight pathways were significantly enriched, i.e., "diterpenoid biosynthesis", "nitrogen metabolism", "photosynthesis", "metabolic pathways", "biosynthesis of secondary metabolites", "phenylpropanoid biosynthesis", "arginine and proline metabolism", and "alpha-linolenic acid metabolism" (Fig. 5), as

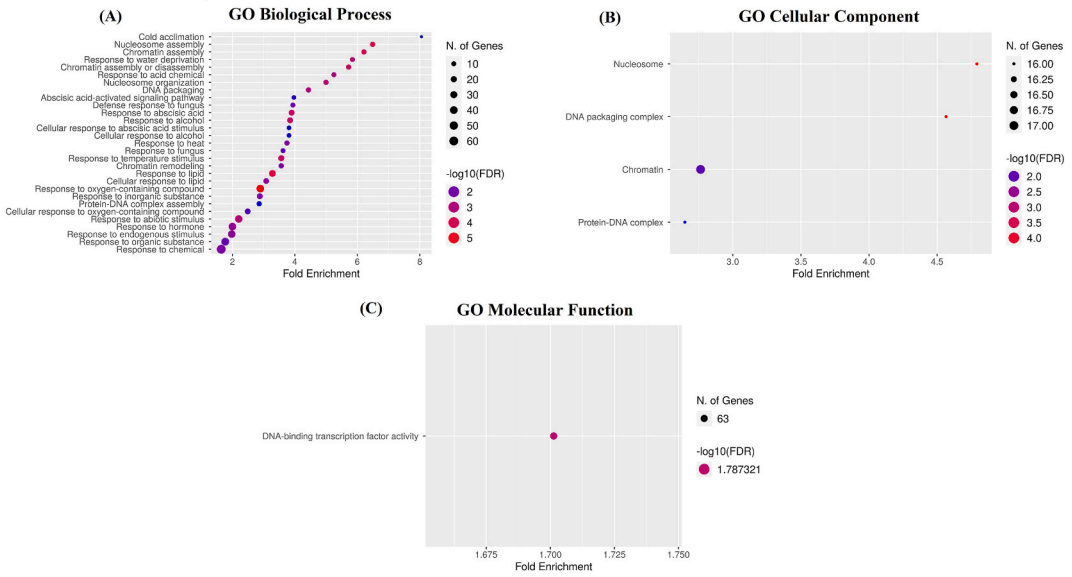

Fig. 2. The Dotplot chart showing functional annotation of (A) Biological process, (B) Cellular component, and (C) Molecular function enrichment of up-regulated genes in the radicle emergence stage at a p-value cut-off (FDR) of 0.05 (Fold enrichment represents how drastically genes of a certain pathway are overrepresented). X-axis shows the fold enrichment; the y-axis shows the GO terms. The node size represents the number of genes involved in a particular function.

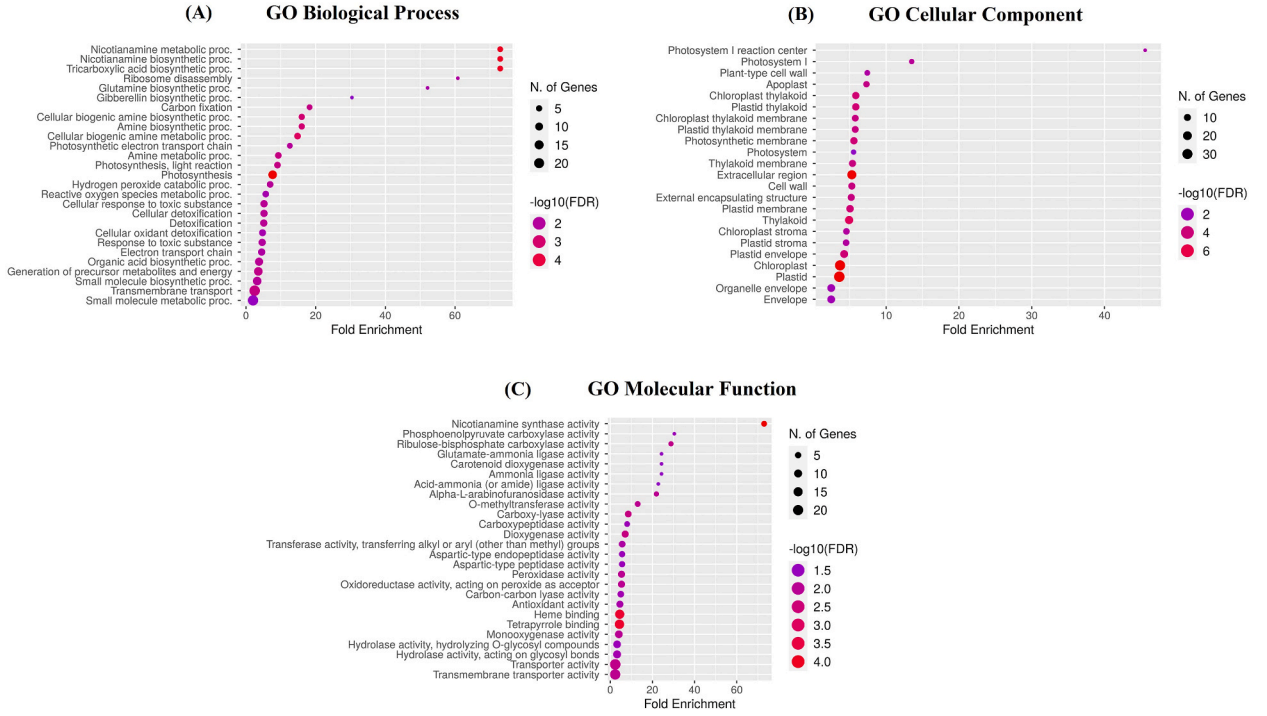

Fig. 3. The Dotplot chart showing functional annotation of (A) Biological process, (B) Cellular component, and (C) Molecular function enrichment of up-regulated genes in the seedling growth stage at a p-value cut-off (FDR) of 0.05 (Fold enrichment represents how drastically genes of a certain pathway are overrepresented). X-axis shows the fold enrichment; the y-axis shows the GO terms. The node size represents the number of genes involved in a particular function.

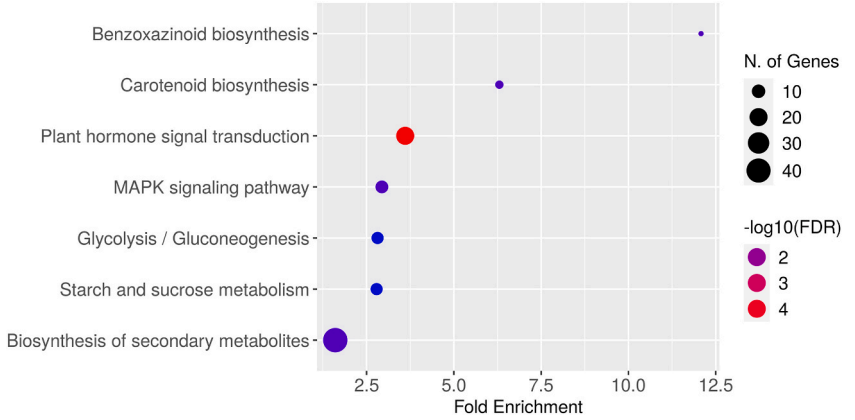

Fig. 4. KEGG pathway enrichment analysis among up-regulated genes of radicle emergence stage at a p-value cut-off (FDR) of 0.05 (Fold enrichment represents how drastically genes of a certain pathway are over represented). The node size represents the number of genes involved in a particular function.

per the selection criteria of FDR values < 0.05.

3.4. Hormonal regulation and starch metabolism of radicle emergence stage

To propose a pathway involved in the process of a radicle protrusion, we first highlighted the DEGs in the plant hormone signal transduction. These were divided into a series of hormone signaling pathways, of which auxin-responsive (*AUX/IAA* and *SAUR*) were expressed to promote cell enlargement. In the diterpenoid signaling pathway, the gene encoding *DELLA* was upregulated promoting stem growth, while the brassinosteroid signaling pathway showed *BZR1/2* expression supporting cell division and elongation. The carotenoid biosynthesis, α-linolenic acid metabolism, and phenylalanine metabolism showed the genes producing proteins involved in

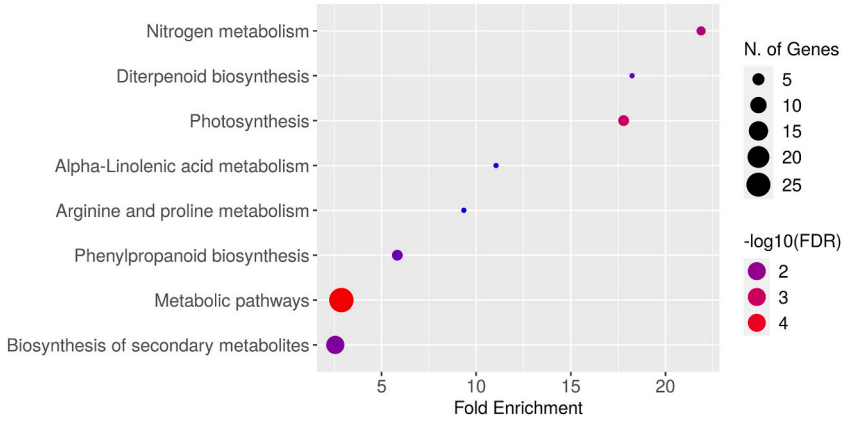

Fig. 5. KEGG pathway enrichment analysis among up-regulated genes of seedling growth stage at a p-value cut-off (FDR) of 0.05 (Fold enrichment represents how drastically genes of a certain pathway are over represented). The node size represents the number of genes involved in a particular function.

resistance to biotic and abiotic stresses (Fig. 6 and Table S1). In the starch and sucrose metabolism pathway, genes encoding proteins that are counterparts in hydrolyzing stored reserves during the radicle emergence stage like beta-amylase, ADP-glucose pyrophosphorylase, beta-glucosidase, endoglucanase, trehalose-phosphate phosphatase, and sucrose synthase were expressed (Fig. S5 and Table S2).

3.5. Formation of photosynthetic system and diterpenoid biosynthesis promotes rapid growth of maize seedlings

For analyzing seedling growth and development, the DEGs involved in the photosynthesis based on the KEGG pathway annotation from those upregulated DEGs specifically detected in the 7-day-old seedlings were used. This identified some six key genes producing proteins that are crucial components of photosynthesis including cytochrome b6-f complex iron-sulfur subunit, plastocyanin major isoform chloroplastic, ferredoxin-3, photosystem I H subunit 1, photosystem I reaction center subunit XI (Fig. 7). Additionally, diterpenoid biosynthesis had three key genes that produce proteins having a role in supplementing gibberellin production which is essential for seedling growth like CPP synthase, cytochrome P45088A1-like, ent-copalyl diphosphate synthase (Fig. S6).

3.6. Identification of transcription factors responsive during radicle emergence and seedling stage

Additionally, the DEGs encoding the transcription factor (TF) were analyzed. Numerous transcription factors that regulate the radicle emergence were found like SCREAM2 (*LOC103652960, LOC103652960*), GRAS (*LOC100281784, LOC100281386, LOC100281386*), WRKY (*LOC100193434, LOC100193434*), HSF (*LOC109940795, LOC100286298*), bZIP (*LOC103643147, LOC103637889), MYB (LOC103645799, LOC103642827, LOC103631701, LOC103645799, LOC103634904, LOC103631701, LOC103645799, LOC103642827, LOC541969, LOC103634904*), GRFs (*LOC100101539, GRF-1*), ABA response element binding factor (*LOC100285149*), ERF (*LOC109939235, LOC103642046, LOC109939235, LOC100278463*), Trihelix transcription factor GTL2 (*LOC100273304, LOC100273304, LOC100273304, LOC100273304*), GATA (*LOC103637652*) were seen. Most of the identified DEGs in seedling stage encoded members of the bHLH (*LOC118476620, LOC103627392, LOC103627392*), EMB (*LOC103631852*), NAC (*LOC107403159*), protein IRON-RELATED TRANSCRIPTION FACTOR 2 (*LOC103636279*) related TF families.

3.7. Change in enzyme activity in response to radicle emergence and seedling growth

Key enzymes involved in various biosynthetic pathways of maize during radicle emergence were seen. Some important enzymes that were noticed include protein phosphatase, E3 ubiquitin ligase, ATPase, phosphoenolpyruvate carboxylase kinase, glycosyltransferase, beta-carotene hydroxylase, chitinase, oxidoreductase, transferase, cytokinin-O-glucosyltransferase, gibberellin 2-beta-dioxygenase, peroxidase, beta-amylase. In the seedling growth stage of maize, some key enzymes like peroxidase, plastoquinol-plastocyanin reductase, lipid phosphatase, and polygalacturonase were upregulated (Tables S3 and S4).

3.8. Overview of metabolism

Further to study the various pathway that is interacting, MapMan analysis of 7-day-old seedlings compared to the radicle emergence stage was performed to observe the changes in the metabolism. The radicle emergence to the seedling stage transition was related to biosynthesis of cell wall components, photosynthesis, secondary metabolism, metabolism of sugars, lipids, and redox status. Starch and sucrose metabolism, light reaction, and photorespiration were enhanced. The genes involved in the maintenance of the tetrapyrrole structure of chlorophyll, light reaction pathway, and tricarboxylic acid cycle (TCA) cycle that are essential for optimal

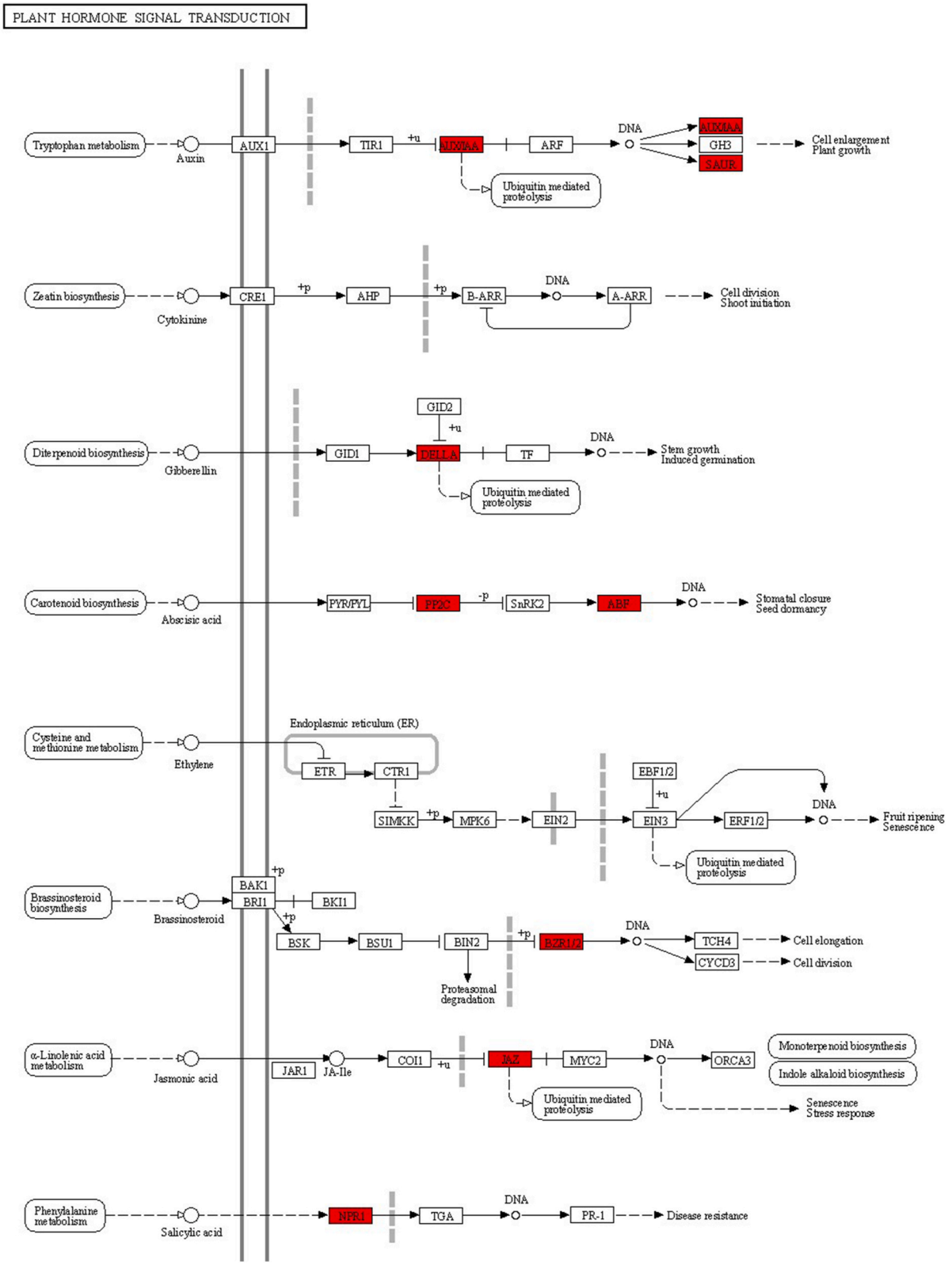

Fig. 6. Pathways in plant hormone signal transduction during radicle emergence stage from KEGG with query genes highlighted in red. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

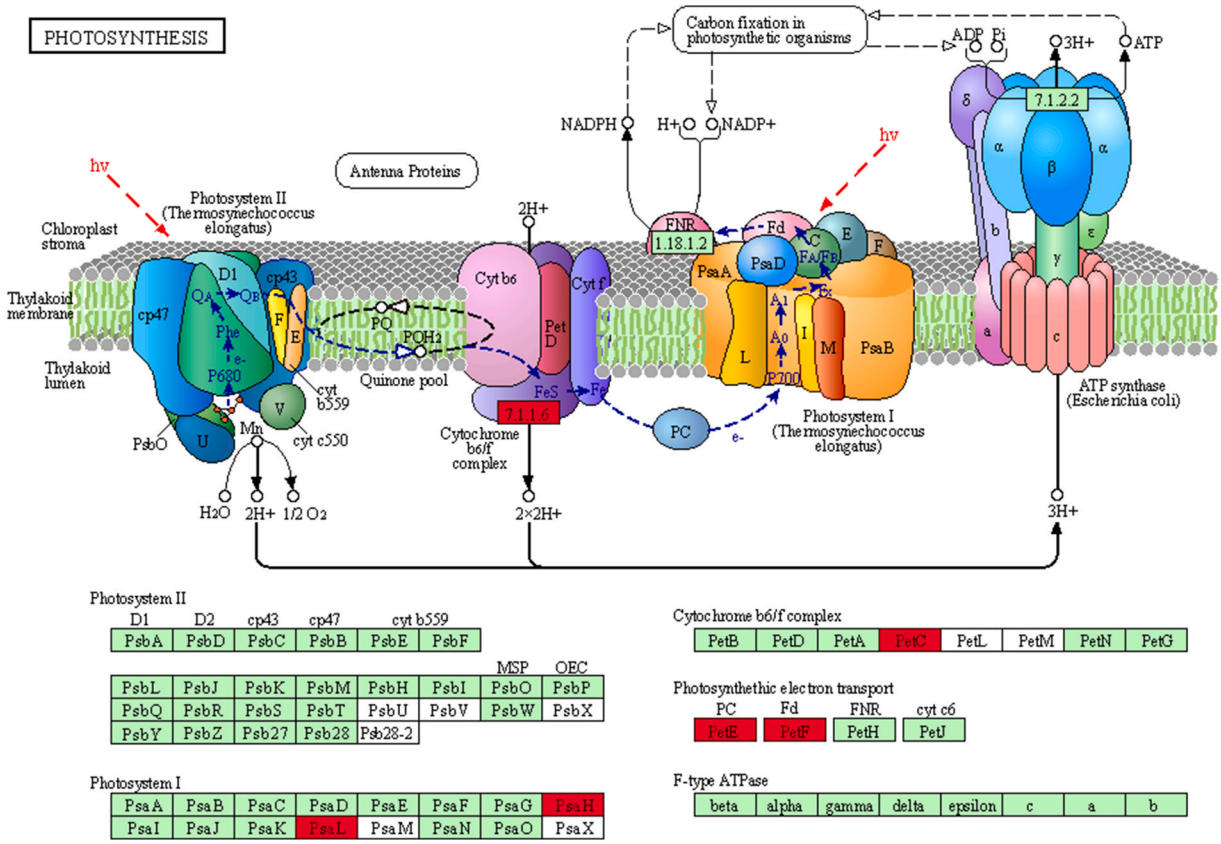

Fig. 7. Pathways in photosynthesis during seedling growth stage from KEGG with query genes highlighted in red. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

functioning of the primary carbon metabolism in plants were expressed (Fig. S7).

4. Discussion

Here, we made progress in our understanding of the physiological expression that occurs in the embryonic axis during the radicle emergence stage as well as in the seedlings. In addition, we proposed the use of some of the transcripts studied here as a tool to clarify the role of stored mRNAs in the reconstruction of the translational machinery needed for radicle emergence and the start of photosynthesis in seedlings by differentiation of photosynthetic apparatus soon after the completion of stored reserves.

The seed initiates a multitude of repair and response mechanisms during germination including the intricate networks of DNA damage control [25]. To deal with DNA damage and maintain chromosomal integrity, the radicle protrusion process depends on cell cycle regulating mechanisms [26] and activation of DNA and protein repair machinery, which ultimately determines the vigor of the seed [27]. The mobilization of seed storage proteins is seen as a post-germinative process that takes place during seedling establishment [28,29]. In contrast, Gallardo et al. [30] proved the fact that this mobilization may commence during the imbibition stage of *Arabidopsis* seed germination. The MAPK pathway can influence cell cycle progression, which is crucial for radicle emergence, and modulate the activity of proteins involved in cell cycle checkpoints ensuring vigor. The MAPK pathway may regulate the mobilization of stored nutrients within the seed, such as carbohydrates and lipids, supporting germination and initial growth which correlates to the present results.

Radicle emergence is regulated by the weakening of the seed coat tissues by enzymatic action before an active embryo becomes visible [31,32]. Here, we found some of the genes regulating cell wall modifying enzymes like alpha-galactosidase, towards the promotion of radicle outgrowth. Extremely high levels of the phosphorylated sugars, fructose-6-phosphate, and glucose-6-phosphate show that catabolism has been activated by remobilizing stored starch granules from endosperm to growing embryos [33–35]. In this work, two beta-amylase genes that were significantly expressed were engaged in the metabolism of starch into sucrose. This suggests that beta-amylase plays a crucial part in the starch breakdown process at the germination phase. Genes encoding beta-glucosidase, which converts maltose to glucose, were also noticeably elevated. Additionally, hexokinase gene expression was increased, which aided in the conversion of glucose to glucose-6-phosphate (G6P). The enzymes of sucrose synthase will then catalyze the subsequent resynthesis of sucrose. Two genes expressing sucrose synthase were boosted in this work [36]. During the early stages of seed germination, plant hormone signals like gibberellin (GAs) are crucial because they inhibit ABA. Additionally, it has been shown that

GA affects the expression of the α-amylase gene during the expansion of aleurones, needed for radicle emergence and early seedling growth. Transcripts involved in plant hormones signal transduction are upregulated contributing to cell division and cell enlargement, which is in line with earlier findings [4,37]. Auxin signal transduction pathway genes showed higher expression levels during the radicle emerging stage, demonstrating their involvement in it [38]. Expansin transcripts reflect a stronger correlation with radicle emergence in maize seeds than the other target genes examined in our study. Similar findings were also shown in soybean, where a radicle-derived development pattern occurred and Expansin-like A1 expression strongly correlated with the expansion of the embryonic axis [39].

The capacity of photosynthesis is taken into account for the ability of seedlings to repair and generate new cells. PSII, which has significant activity in the seedling stage, is widely recognized to be the most sensitive mechanism in photosynthesis [40,41]. Undifferentiated proplastids in meristematic tissue give rise to chloroplasts and photosynthetic machinery, linking the development of leaves to chloroplast biogenesis, which plays a key role during the transition from primary to secondary morphogenesis in leaves [42,43]. Additionally, many crucial metabolic processes, including the production of fatty acids, amino acids, and tetrapyrroles, as well as the main carbon metabolism, are found in plastids. Similar transcripts involved in the photosystem were markedly elevated in our study. These transcripts serve as the central hub of a transcriptional network linking many activities such as hormones, nutrition, and other signaling pathways, and they control the light-responsive coupling of shoot development and photosynthesis with root growth and nutrient uptake [44–46]. By activating several genes involved in developmental processes such as cell elongation, pigment deposition (chlorophyll), and root growth, it plays a crucial role in seedling development as well as reactions to light [47]. TCA cycle genes have a significant role in many aspects of plant growth and development, including root formation [48], and leaf development [49]. Nitrogen embedded in seeds has a vital ecological function in seedling growth. It has also been proposed that the metabolites contained in seeds signal certain metabolic pathways and may control gene expression, determining growth and development [50]. Transitions from meristem to shoot growth, juvenile to adult leaf stage, and vegetative growth are triggered by gibberellic acid (GA) diterpenoid biosynthesis, a plant hormone that stimulates plant growth and development [51].

Reactive oxygen species (ROS) are known to be scavenged by antioxidant enzymes like peroxidase, superoxide dismutase, and others in plants, and their activity may be raised in response to stress [52]. In the present study, the peroxidase activity in maize seedlings considerably enhanced, promoting plant development by quenching free radicals. The “metabolic and catabolic process” appeared to be amplified during the seedling growth phases, as per GO enrichment analyses. A large portion of the DEGs were implicated in metabolic pathways that aid in seedling development, and they were also enriched with genes involved in the biosynthesis of secondary metabolites, under our transcriptome data. Transcription factors (TFs) play an important role in many pathways, which regulate the expression of specific genes involved in germination and seedling growth. Among them, MYB, GRFs, ERF, EMB, bZIP, WRKY, etc., are involved in seed germination, secondary metabolism, stress response, and growth [53,54]. Overall, the information given here offers a molecular foundation for a greater comprehension of the important biochemical and physiological mechanisms influencing the radicle emergence of maize embryos for germination as well as the seedling development stage.

5. Conclusions

The study revealed the transcriptional level at two different stages of plant growth that are radicle emergence (germination) and seedling growth. The unveiling of this network filled a critical gap in our understanding of the intricate biological process: how the mature seed begins to germinate by extending radicles out of the protective seed covering tissues and the genes that control it. The principal component analysis clearly delineated significant disparities between these developmental stages, with the initial radicle protrusion stage exhibiting pronounced gene expression activity. DEGs have unveiled that during radicle emergence, the upregulated genes were predominantly linked to hormonal regulation and starch metabolism, facilitating cell enlargement for radicle protrusion. In contrast, the seedling stage was marked by a strong emphasis on genes related to photosynthesis and diterpenoid biosynthesis, crucial for the rapid growth of seedlings. Transcription factors provided valuable insights into the regulatory network orchestrating the events of radicle emergence and seedling development. In essence, this comprehensive study unraveled the genomic intricacies underlying the transition from radicle emergence to the seedling stage in maize, providing a robust foundation for understanding the molecular nuances of early plant development.

Author contributions

Nethra Nagarajappa: Writing – review & editing, Writing – original draft, Visualization, Supervision, Project administration, Funding acquisition, Formal analysis, Conceptualization. Siddegowda Rajendra Prasad: Conceptualization, Methodology, Supervision, Writing – review & editing. Roopashree Byregowda: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. M. K. Prasanna Kumar: Writing – review & editing, Supervision, Methodology, Formal analysis, Data curation, Conceptualization

Data availability statement

The raw sequencing reads generated during the current study have been deposited in the NCBI Sequence Read Archive Database with the Accession Numbers: SRR26688494, and SRR26688495 under the BioProject with the accession number PRJNA1035513.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e25683.

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