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Aged sweet corn (*Zea mays* L. *saccharata* Sturt) seeds trigger hormone and defense signaling during germination

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ABSTRACT: Seed ageing during storage or shipping can affect the yield and cost of agricultural production. Sweet corn is a naturally mutated corn cultivar with weak seed vitality during storage. In this study, we have shown that endogenous hormone level dynamics of fresh and aged sweet corn seeds were different during early geminating process. Transcriptomic assembling uncovered several novel transcripts during early stage of sweet corn germination in signal transduction and RNA processing pathways. Comparative transcriptomic profiling revealed that expression of many genes was activated earlier in aged seeds than fresh ones. Functional analyses showed that, as two major networks, plant hormone signaling and defense pathways were different between fresh and aged sweet corn seeds. Auxin, cytokinin, gibberellin, salicylic acid and jasmonic acid signaling pathways were activated earlier in aged seeds than fresh ones on the first day after imbibition. These results provided knowledge for understanding the ageing process of sweet corn seeds and several candidate genes for genetic study of seed ageing mechanism.

Index terms: hormone concentration, seed ageing, seed germination, transcriptome.

RESUMO: O envelhecimento da semente durante o armazenamento ou transporte pode afetar o rendimento e o custo da produção agrícola. O milho doce é um mutante natural com fraca vitalidade da semente durante o armazenamento. Neste estudo, mostramos que a dinâmica do nível de hormônio endógeno de sementes de milho doce recém-colhidas e envelhecidas foi diferente durante o processo de geminação precoce. A análise transcriptômica revelou vários novos transcritos durante o estágio inicial da germinação do milho doce nas vias de transdução de sinal e processamento de RNA. O perfil transcriptômico comparativo revelou que a expressão de muitos genes foi ativada mais cedo nas sementes envelhecidas do que nas recém-colhidas. As análises funcionais mostraram que, como duas redes principais, a sinalização de hormônios vegetais e as vias de defesa eram diferentes entre sementes de milho doce recém-colhidas e envelhecidas. As vias de sinalização de auxina, citocinina, giberelina, ácido salicílico e ácido jasmônico foram ativadas mais cedo nas sementes envelhecidas do que nas recém-colhidas no primeiro dia após a embebição. Esses resultados forneceram conhecimento para a compreensão do processo de envelhecimento de sementes de milho doce e vários genes candidatos ao estudo genético do mecanismo de envelhecimento de sementes.

Termos para indexação: concentração hormonal, envelhecimento da semente, germinação da semente, transcriptoma.

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INTRODUCTION

Corn (*Zea mays* L.) is a staple crop which is cultivated all over the world. Sweet corn (*Zea mays* L. *saccharata* Sturt) is a member of maize family that serves as an important vegetable for human (Lertrat and Pulam, 2007). Sugar content of sweet corn endosperm is higher and its starch content is lower than normal corns (Hu et al., 2021). Good taste and high nutrition value of sweet corn have made it popular in fresh food markets. Thus, the cultivated areas of sweet corn are continuously increased in many countries.

Higher sugar contents and lower starch contents of sweet corn are due to the recessive mutation of SE1 gene which is expressed in endosperm (Zhang et al., 2019). Mutations also results in imperfect and shrinking corn grains, which reduce the quality of sweet corn seeds. Such hypogenetic seeds have weaker seed vitality and lower seedling emergence percentage. Moreover, the vitality of sweet corn seeds was decreased faster than that of normal corn seeds during storage, which indicated rapid ageing rate of sweet corn seeds. These features increased the cost of producing commercial sweet corn seeds and reduced the agricultural production of fresh sweet corn.

Plant hormones are important molecules playing key roles in regulating plant growth and development, including seed germination and ageing (Santner et al., 2009; Khan et al., 2014; Blázquez et al., 2020; Agostini et al., 2022). Different hormones act in various metabolic pathways to regulate the germination of seed and physiological status of seedlings (Eisvand et al., 2010). For example, auxin promotes seedling emergence while gibberellin (GA) enhances chlorophyll concentration in seedlings (Peng and Harberd, 2002; Jonsson et al., 2021). Crosstalk between different hormone signaling pathways is another important function during seed germination. Auxin regulates *ABSCISIC ACID INSENSITIVE 3* (*ABI3*) expression, a key regulator in abscisic acid (ABA) signaling pathway, to control plant seed dormancy (Liu et al., 2013). ABA can regulate the dormancy and germination of seed in ethylene signaling pathway (Arc et al., 2013). In addition, GA and ABA signaling pathways are regulated by jasmonic acid (JA) during seed dormancy (Singh et al., 2017) . Therefore, regulation of seed germination and ageing by endogenous hormone contents is a critical task, but is a complicated phenomenon.

Gene expression is the fundamental driving force of plant development. Next-generation sequencing technology can profile gene expression genome-widely at one time. In this study, we employed RNA-seq for the evaluation of gene expression dynamics in sweet corn during early stage of seed germination. The transcriptomic profiles of fresh and aged sweet corn seeds were compared to uncover molecular variations during seed storage. Potential genes related to seed deterioration were characterized based on their expression patterns and their functional significances were investigated. Our findings may be useful for advancing agriculture and seed technology production.

MATERIAL AND METHODS

Plant material and growth conditions

Sweet corn seeds from“Yongzhen #7” cultivar were used. Fresh seeds were harvested in 2018 and aged seeds were harvested in 2015. Both seed lots were tested with > 90% germination rates in corresponding years. Before germination, seeds were sterilized with 0.1% NaClO for 10 min and washed 3 times with ddH_2O. Sterilized seeds were embedded in germination paper and incubated in growth chamber at 25 °C following 12 h light/12 h dark light period for germination tests. Germination tests were conducted in 2018. Samples for hormone content study and transcriptomic profiling were harvested in 2018.

Measurement of hormone contents

Embryos of seeds on days 0, 1, and 2 after imbibition were dissected and frozen with liquid nitrogen. 0.1 g samples were grinded to fine powder and resuspended in 1 ml 80% MeOH. Lysates were incubated at 4 °C for 1 h, following centrifugation at 15000 xg for 10 min at 4 °C. Supernatants were transferred to new tubes and pellets were extracted again with 1 mL 80% MeOH. Two fractions of supernatants were mixed and dried in a nitrogen blow down dry evaporator.

After complete drying, 30% MeOH was added to every tube and homogenized at 4 °C for 1 h. The products were further centrifuged at 15000 xg for 10 min at 4 °C and supernatants were preserved for UHPLC-ESI-MS/MS analyses on ABI 5500 Q-Trap system according to a previously published method (Šimura et al., 2018) . Mobile phase was set at 0.3 $mL.min^{-1}$ with MeOH and H_2O. In this way, levels of GA1, GA3, JA, ABA, salicylic acid (SA) and indole-3-acetic acid (IAA) were measured.

RNA-seq library preparation

Total RNA was extracted by TRIZOL. About 2 µg qualified total RNA was used for RNA-seq library preparation. Poly(A)+ mRNA was enriched by oligo(dT) beads and sheared by fragmentation buffer. Reverse transcription was conducted with oligo containing 6 N random hexamers. DNA polymerase I was applied for the synthesis of 2 nd cDNA strand. The obtained product was purified by AMPure XP beads to eliminate excessive oligos and reagents. Purified cDNA was end-repaired and A-tailed for adaptor ligation. The size of product was controlled to 200-500 bp by AMPure XP beads and amplified to generate library for sequencing. Qubit 2.0, Aglient 2100 bioanalyzer and qPCR were applied to qualify sequencing library before loading on Illumina NovaSeq platform.

RNA-seq data processing

Raw reads were subjected to adaptor clipping by FASTX-Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/links.html). Reads containing >50% low quality nucleotides (< q20) or >50% N nucleotides were discarded. Clean reads were mapped to maize B73 RefSeq Genome (https://www.ncbi.nlm.nih.gov/genome/12?genome_assembly_id =310773) by HISAT2 (Kim et al. 2015). Transcripts were assembled by StringTie (Pertea et al., 2016) and compared to B73 annotation reference for identifying novel transcripts. Gene expression was detected by feature Counts (Liao et al., 2014). DEGs were identified by edgeR package in R (Robinson et al., 2010) England with thresholds of $|\log_2 FoldChange \geq 1$ and FDR < 0.05. Significant enrichment of gene ontology, Cluster of Orthologous Groups of proteins (COG), Kyoto Encyclopedia of Genes and Genomes (KEGG) and Pfam were filtered with a threshold of $p < 0.05$.

Statistics

Germination rates and level of hormones were presented by mean values. The error bars were calculated based on standard deviation. Paired *t* test was used for comparing global expression difference of gene clusters.

Data availability

RNA-seq data have been deposited in Beijing Institute of Genomics Data Center (http://bigd.big.ac.cn) with accession CRA004989.

RESULTS

Aged seeds of sweet corn showed much darker color than fresh seeds (Figure 1A). The fresh seeds appear more shrunken than aged seeds, which indicates fresh seeds were dehydration enough. Germination experiments showed that germination rate of aged seeds was about 20% lower than fresh seeds (Figure 1B). From day 0 to day 2, the slopes of germination rate of fresh seeds were distinctly higher than that of aged seeds. While from day 3 to day 6, the slopes became less difference between fresh and aged seeds. The results indicate that day 1 and day 2 are crucial for establishing higher germination rate of corn seeds. To evaluate the potential role of hormones in regulating seed gemination, contents of GA, JA, SA, ABA and IAA in embryo of fresh and aged seeds were measured during the first two days after imbibition. Results showed that GA1, GA3, JA and SA were synthesized in both fresh and aged seeds, whereas ABA and IAA were mostly degraded during two days after seed imbibition (Figures 2A-F).

GA1 and GA3 contents in fresh seeds were greatly increased on day 1 (Figures 2A and B). GA1 content in fresh seeds was maintained higher than aged seeds on day 2; however, GA_3 content in fresh seeds was decreased which was

consistent with aged seeds. In aged seeds, GA1 content was not upregulated on day 1 but on day 2 and GA3 content was steadily increased during days 1 and 2.

JA and SA play important roles in plant defense systems. It has been shown that fresh and aged seeds maintain lower contents of JA and SA on first day after imbibition. However, significant synthesis of JA and SA was witnessed in aged seeds, with higher contents than fresh seeds on day 2 (Figures 2C and D).

Furthermore, after imbibition, ABA content in fresh seeds was significantly higher than that in aged seeds (Figure 2E). ABA was almost degraded in both fresh and aged seeds after 1 day of imbibition and was maintained at very low-level during day 2. IAA content was also decreased during 2 days after imbibition of fresh or aged seeds (Figure 2F). In addition, IAA content of ageing seeds was higher than that of fresh seeds.

These results indicated that GA, JA and SA were significantly synthesized in early two days after the imbibition of sweet corn seeds. However, ABA and IAA were mainly consumed in early two days after the imbibition of sweet corn seeds. Difference in hormone content dynamics of fresh and aged seeds might suggest the roles of different hormones in regulating seed gemination.

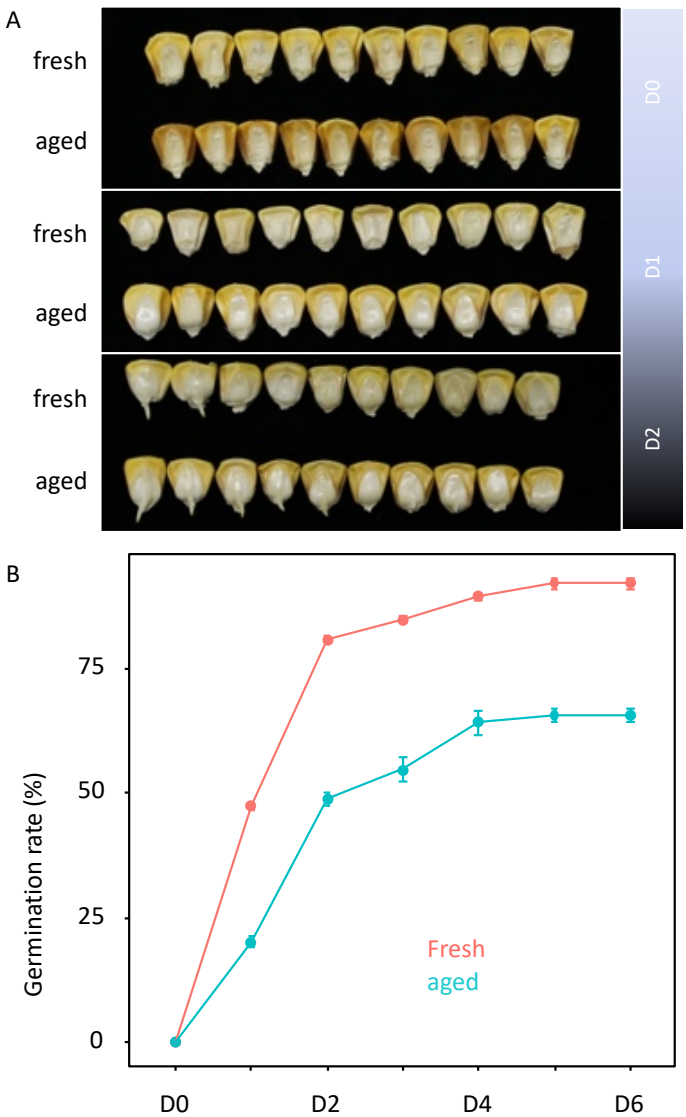

Figure 1. Germination rate of fresh and aged seeds. A, appearance of fresh and aged seeds on day 1 (D1) and day 2 (D2) after imbibition. Upper row is fresh seeds, lower row is aged seeds. B, germination rates of fresh and aged seeds from D1 to D6. Error bars indicates standard deviations.

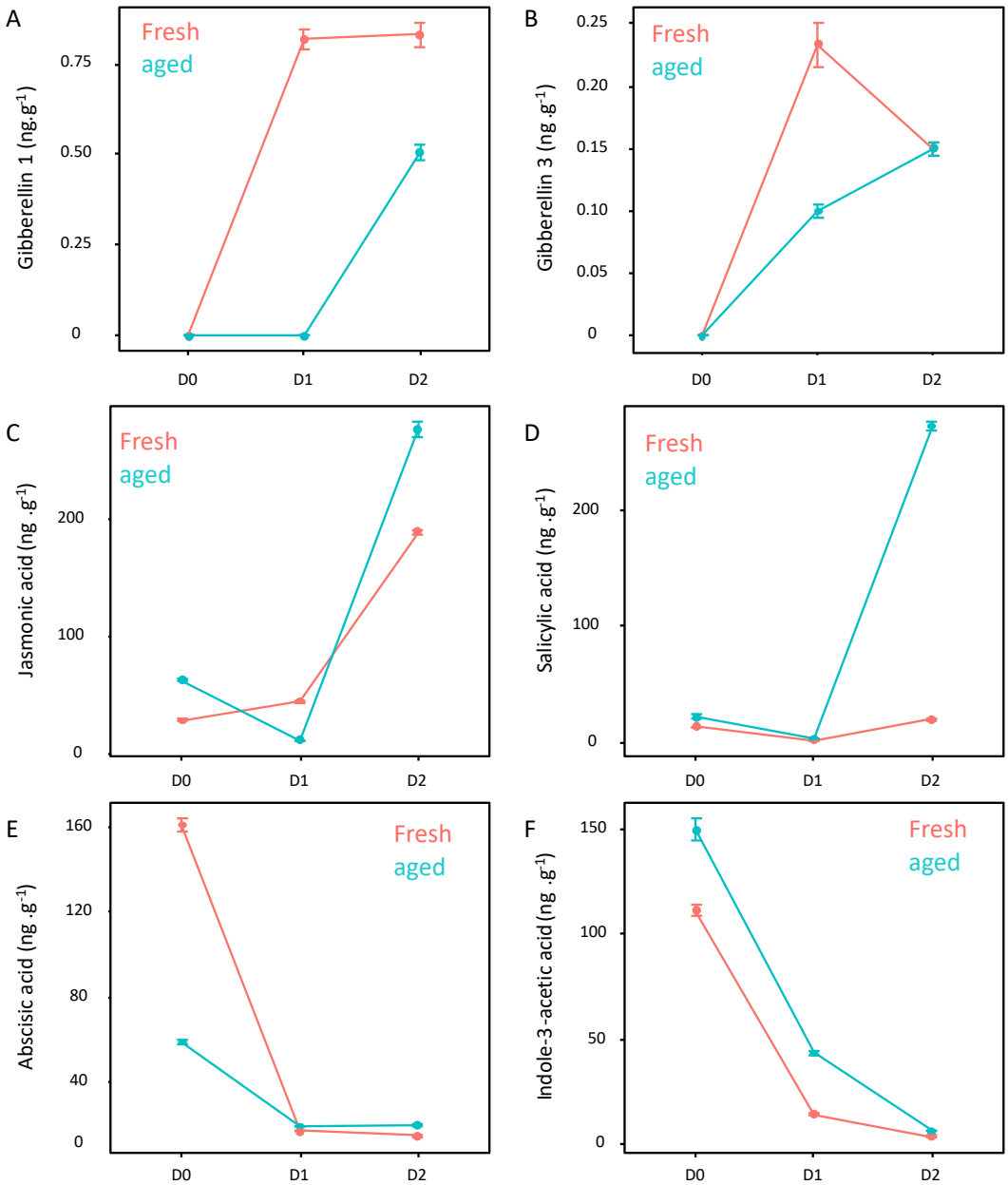

Figure 2. Endogenous hormone contents during seed germination in fresh and aged seeds. D0: day 0, D1: day 1 after imbibition, D2: day 2 after imbibition. Error bars indicates standard deviations.

To investigate molecular differences between fresh and aged seeds, RNA-seq was employed for profiling the transcriptome of sweet corn seeds on days 1 and 2 after imbibition. After adaptor trimming and quality filtration, about 80% of qualified reads could be mapped to B73 corn genome (Table 1). Statistical results showed that about 90% of the mapped reads were aligned to exon region, and about 10% were from intronic or intergenic regions (Table 1).

In total, RNA-seq identified 47148 genes with variable functions from 12 sweet corn samples (Table 2). Among them, 4293 novel genes referring to B73 annotation file were identified and they were predicted to have various roles either in biological processes and molecular functions (Figure 3A-D). COG cluster revealed that novel genes were mainly involved in the regulation of translation, post-translation process, and cell cycles (Figure 3A). Many of them acted as nucleotide binding or protein kinase/phosphatase locating in cytoplasmic part and in signal transduction or RNA processing pathway (Figures 3B and C). Pfam enrichment also supported these findings (Figure 3D).

Table 1. Statistics of sequencing reads and mapping rates.

Sample	Qualified reads	Mapping rate	Exonic	Intergenic	Intronic
aged-D1-1	40464332	76.92%	31,018,942 (88.13%)	1,969,753 (5.6%)	2,207,071 (6.27%)
aged-D1-2	41051114	82.29%	33,535,110 (89.55%)	1,672,770 (4.47%)	2,239,421 (5.98%)
aged-D1-3	40340230	82.32%	33,063,934 (89.76%)	1,671,492 (4.54%)	2,102,478 (5.71%)
aged-D2-1	62974895	86.84%	55,532,274 (91.02%)	2,095,030 (3.43%)	3,381,630 (5.54%)
aged-D2-2	45626647	87.44%	40,342,823 (91.22%)	1,480,388 (3.35%)	2,402,459 (5.43%)
aged-D2-3	62196413	87.75%	54,852,713 (91.03%)	2,058,953 (3.42%)	3,347,157 (5.55%)
fresh-D1-1	55440642	85.64%	48,045,954 (90.46%)	1,999,180 (3.76%)	3,065,434 (5.77%)
fresh-D1-2	59496067	86.02%	50,952,177 (89.19%)	2,363,356 (4.14%)	3,814,353 (6.68%)
fresh-D1-3	47459383	86.55%	41,199,017 (90.56%)	1,661,319 (3.65%)	2,633,385 (5.79%)
fresh-D2-1	53302620	87.04%	47,382,503 (91.67%)	1,631,762 (3.16%)	2,672,519 (5.17%)
fresh-D2-2	57983575	87.24%	51,681,903 (91.81%)	1,755,336 (3.12%)	2,858,039 (5.08%)
fresh-D2-3	34211660	87.23%	30,638,806 (92.21%)	1,021,061 (3.07%)	1,567,476 (4.72%)

Notes: D1 indicates day 1, D2 indicates day 2.

Table 2. Annotation of transcripts identified in sequencing data.

Anno_Database	Annotated_Number	300<=length<1000	length>=1000
COG_Annotation	14003	2956	10799
GO_Annotation	29486	6857	21466
KEGG_Annotation	9010	2326	6237
KOG_Annotation	23268	5785	16725
Pfam_Annotation	29947	5877	23702
Swissprot_Annotation	35047	9067	25036
nr_Annotation	47099	14494	29842
All_Annotated	47148	14520	29850

Interestingly, novel genes were mainly expressed on the first day after the imbibition of sweet corn seeds, which only showed 8 genes overlap between novel genes of day 1 and day 2 (Figure 3E). Moreover, unique novel genes on day 1 from aged seeds were expressed more significantly than those in fresh seeds (Figure 3F).

Principal component analysis (PCA) was used for the calculation of general variations between aged and fresh seeds. Principal component 1 (PC1) represented 51.41% variation, which mainly distinguished day 1 seeds and day 2 seeds (Figure 4A). About 13.4% variation between fresh and aged seeds were observed on PC2, which indicated that the basic transcriptomic difference between the two types of seeds on day 1 was apparently larger than that on day 2 (Figure 4A).

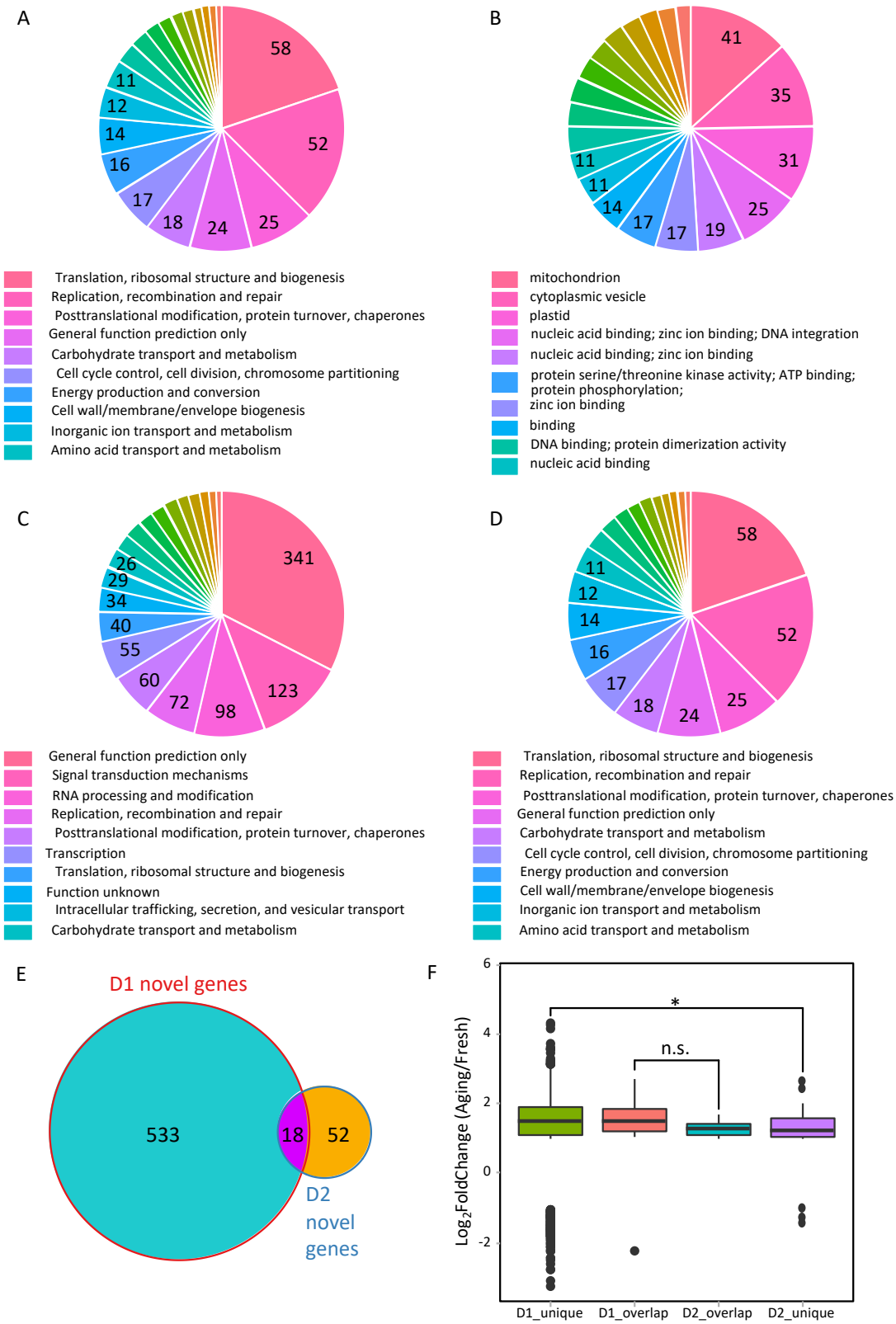

Figure 3. Function annotation of novel genes identified in RNA-seq. A: COG annotation. B: Gene Ontology annotation. C: KEGG pathway annotation. D: Pfam annotation. E: overlap of novel expressed genes on day 1 (D1) and day 2 (D2). F: comparison of the expression of novel genes on D1 and D2. Top 20 terms were selected for pie chart plots. Gene counts of top 10 terms were presented on each pie. "*" indicates p value < 0.05, "n.s." indicates no significant difference.

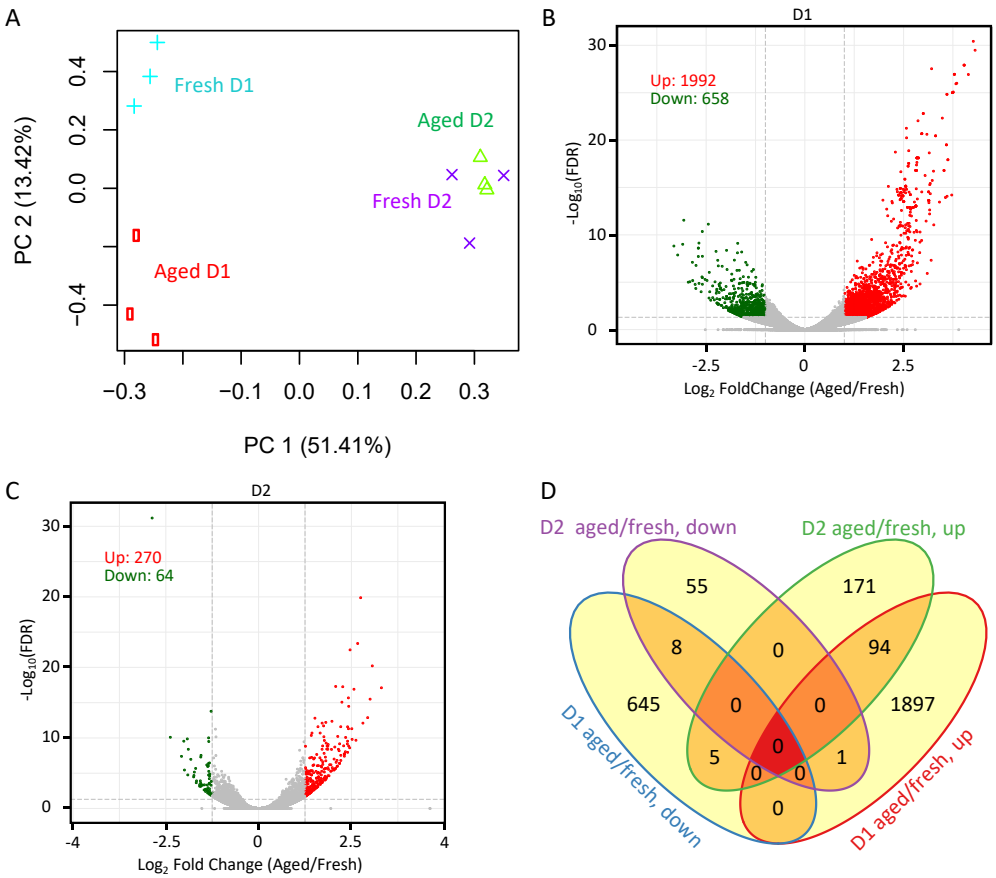

Figure 4. Comparison of transcriptomic profiling between fresh and aged seeds. A: Principal component analysis of different samples. B: volcano plot of differential expressed genes (DEG) between fresh and aged seeds on day 1 (D1). C: volcano plot of DEG between fresh and aged seeds on day 2 (D2). D: venn diagram of DEG. Up indicates DEG was upregulated in aged seeds and down indicates DEG was down-regulated in aged seeds.

To compare the transcriptomes of aged and fresh seeds, DESeq2 was employed to identify differential expressed genes (DEGs) with |log2(Fold Change)|≥ 1 and FDR < 0.05 thresholds. A total of 2650 DEGs were uncovered on day 1 between aged and fresh seeds. Among them, 1992 DEGs were upregulated and 658 genes were down-regulated in aged seeds (Figure 4B). Numbers of DEGs were dramatically decreased on day 2, which only identified 270 up-regulated and 64 down-regulated DEGs in aged seeds (Figure 4C). Results showed that many genes in aged seeds were overrepresented during germination. Venn diagram showed that few DEGs were overlapped between day 1 and day 2 (Figure 4D). Only 94 and 8 DEGs maintained the up-regulated and down-regulated expression trends in aged seeds from day 1 to day 2, respectively. In addition, 5 DEGs shifted their expression patterns from down-regulated to up-regulated in aged seeds from day 1 to day 2. Apart from these, most of DEGs were unique on day 1. In summary, results showed that transcriptome of aged seeds on day 1 after imbibition was distinct with that of fresh seeds on day 2.

To illustrate biological functions of DEGs during the germination of aged seeds, gene ontology and KEGG enrichments were applied. Gene ontology enrichment showed that DEGs were mainly enriched in metabolic process, cellular process, biological regulation and response to stimulus (Figure 5A and B). Some DEGs on day 1 were enriched in immune system process and growth, which were distinct from the enrichment of DEGs on day 2. The GO enrichment pattern of day 1 and day 2 are similar. Thus, difference of transcriptomic between aged and fresh seeds on day 1 may reflects the molecular features of seed status. Furthermore, KEGG enrichment showed that DEGs on day 1 were significantly enriched in 7 pathways such as plant hormone signal transduction, phenylpropanoid biosynthesis, MAPK

signaling pathway, etc. (Figure 5C, dots). However, DEGs on day 2 were only significantly enriched in plant-pathogen interaction pathway (Figure 5C, triangle). These results suggested that genes functioned in plant development and defense were spatially differential expressed between the germination of fresh and aged seeds.

In day 1, 35 genes were enriched in plant hormone signal transduction pathway (Figure 6A). We found that most of these genes were up-regulated in aged seeds, including *AUX9*, *ZIM10* and *PP2C14*. By mapping DEGs to KEGG pathway, we found that up-regulated DEGs were involved in major hormone signal transduction pathways such as auxin, cytokinin, and gibberellin (Figure 6B). Interestingly, 4 components in ABA signaling pathway were all over-represented in aged seeds.

On day 2, there were 5 DEGs enriched in plant-pathogen interaction pathway (Figure 6C). The expression level of 5 DEGs in aged seeds were about 2 times higher than that in fresh seeds. These genes mainly functioned as calcium associated proteins, CDPK and CaMCML, and contributed to plant defense (Figure 6D).

In all, higher expression levels of genes involved in hormone signal transduction and plant defense indicated different cellular status of aged seeds during germination compare to fresh seeds.

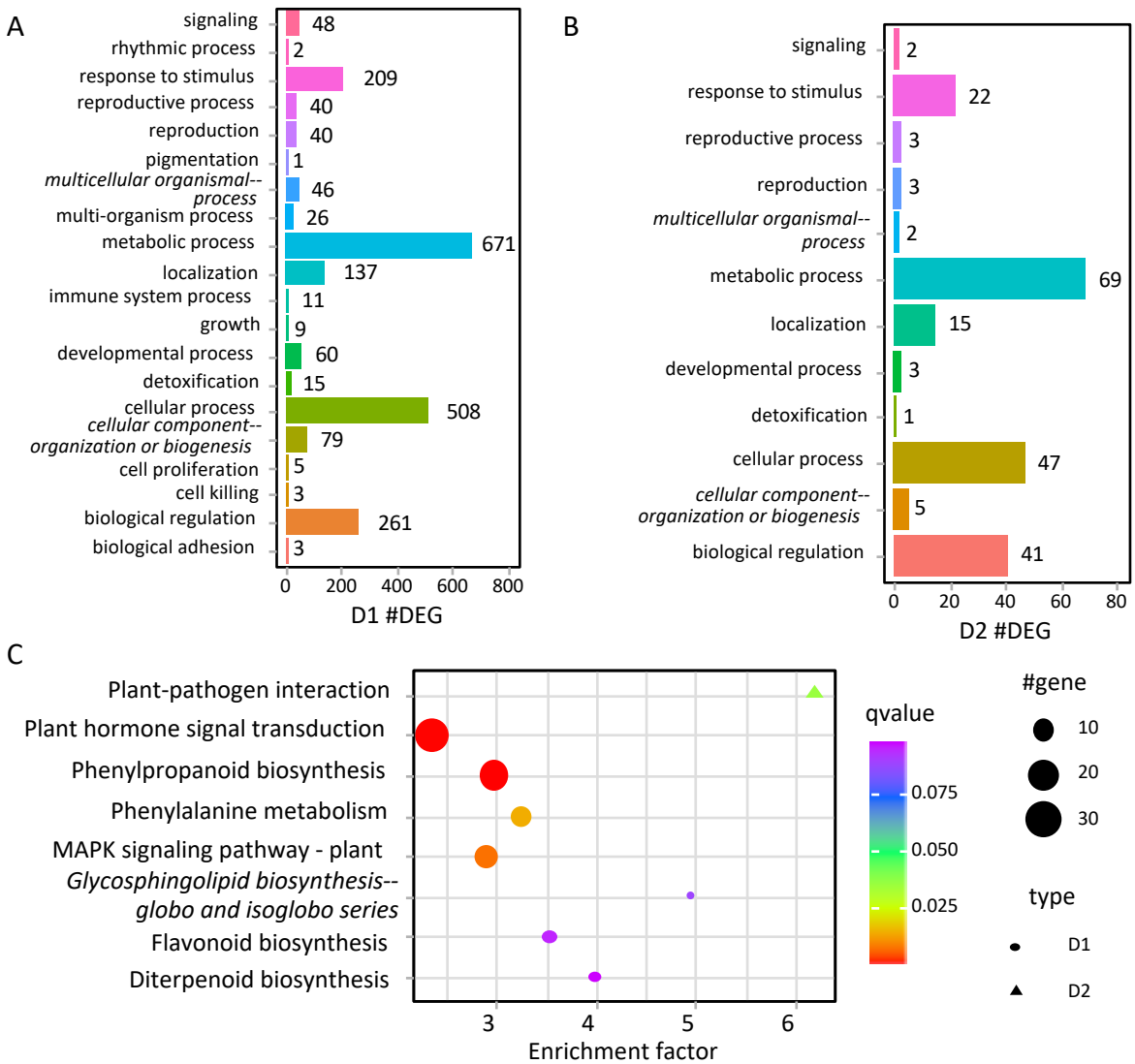

Figure 5. Function enrichment of differential expressed genes (DEGs). A, GO enrichment of DEG in day 1 (D1). B: GO enrichment of DEG in day 2 (D2). C: KEGG pathway enrichment of DEG in D1 and D2.

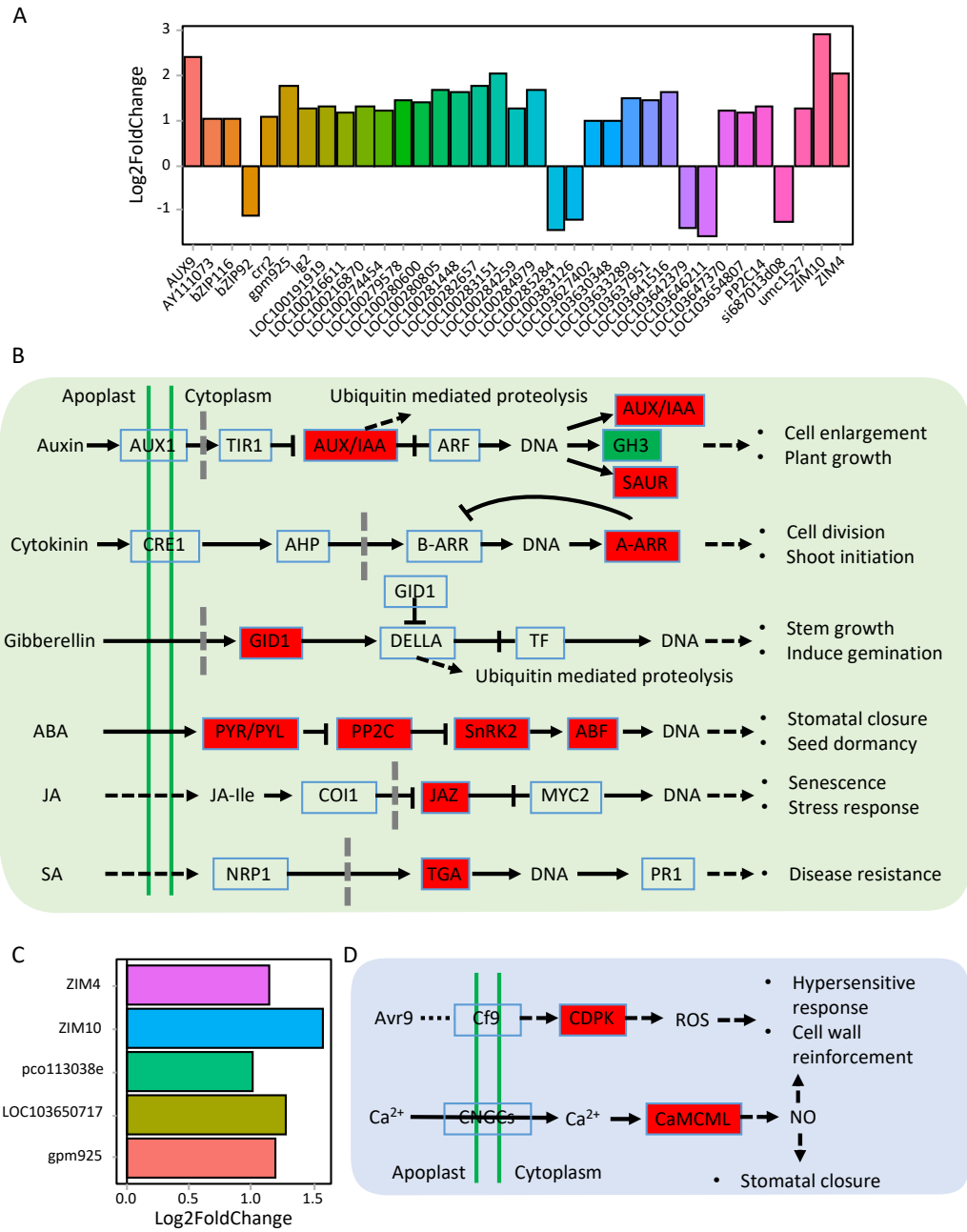

Figure 6. Pathway mapping of key differential expressed genes (DEGs). A: expression of DEGs enrich in plant hormone signal transduction pathway of aged seeds compared to fresh seeds on day 1 (D1). B: pathway mapping of DEGs on D1. C: expression of DEGs enrich in plant-pathogen interaction pathway of aged seeds compared to fresh seeds on day 2 (D2). D: pathway mapping of DEGs on D2. Red rectangles in B and D panel represent up-regulated DEGs. Green rectangle in B panel represents down-regulated DEGs. Light blue rectangles indicate proteins. Green lines in B and D panels represent plasma membrane. Grey dashed lines in B and D panels indicate nuclear membrane.

DISCUSSION

Previous studies have revealed that plant endogenous hormones were critical for seed germination and ageing (Finch-Savage and Footitt 2017; Carrera-Castaño et al., 2020). GA, IAA and CK were found to promote seed germination (Ge et al., 2020). Overexpression of JA biosynthesis gene increased seed germination rate (Singh et al., 2017). ABA inhibited seed germination by suppressing GA biosynthesis; hence, the ratio of GA/ABA correlated with seed germination rate (Seo et al., 2006; Shuai et al., 2017; Binenbaum et al., 2018). Therefore, endogenous hormone balance could regulate seed deterioration. We found that hormone contents in aged seeds during germination were different from fresh sweet corn seeds. Apparently, ABA content in aged seeds before imbibition (day 0) was significantly higher than that in fresh seeds and GA content in aged seeds after imbibition (day 1) was much lower than that in fresh seeds.

The ratio of GA/ABA was increased very fast in fresh seeds from day 0 to day 1 (Figures 1A, B and E), which was correlated with higher germination rate of fresh seeds. These results are consisting with the GA/ABA ratio theory addressed above, and reflect that physiological variations between aged and fresh seeds are associated with their germination rate. Moreover, in aged seeds, IAA content was higher than that in fresh seeds, which suggested that the regulation role of auxin during seed germination was a dose dependent complicated mechanism (Shinkle and Briggs 1984; Grones et al., 2020). JA and SA are known as important hormones functioning in plant defense pathways (Robert-Seilaniantz et al., 2011; Tamaoki et al., 2013).

The contents of JA and SA in aged seeds on day 2 after imbibition were higher than those in fresh seeds. This suggested that aged seeds could be subjected to biotic stress during germination. Hence, seedlings from aged seeds required to balance defense response and development. Previous studies do not speculate the interaction of multiple (more than 3) hormones during seed germination, and the mechanisms are very complicate. In this study, we profiled 5 main hormones during seed germination, and their difference between fresh and aged seeds. Although the transcriptomic differences were side-by-side uncovered in this study, the detail mechanisms underlying seed germination difference between fresh and aged seeds are still required large efforts to reveal.

By comparing to B73 annotated genes, we identified several novel transcripts on day 1 after seed imbibition. A set of novel transcripts were annotated to play roles in post-translational regulation. Post-translational regulation is generally observed in hormone signal transduction pathways (Hill, 2015; Lee and Yaffe, 2016). Results indicated that hormone signal transduction process could be different between aged and fresh seeds during germination. Different hormone contents between two type of seeds also supported this deduction. Moreover, some novel transcripts functioned in RNA processing pathway. Different gene activities in RNA processing could result in different transcriptomic diversities. Therefore, novel transcripts were abundant in early germination stage. This also implicated substantial alternative splicing or other post-transcriptional modification events during seed germination (Bai et al., 2019; Xue et al., 2021).

Due to the defective accumulation of starch in endosperm, the ageing rate of sweet corn seeds was faster than other normal corn cultivars. We found that plant hormone signal transduction was an important different pathway between aged and fresh seeds on day 1 after imbibition. Most of the hormone signaling pathways were over-represented on day 1. This coincided with our finding that hormone contents were differed between two types of seeds. Thus, hormone content was a main aspect for preventing seed deterioration during storage (Eisvand et al., 2010). Furthermore, maintaining a suitable expression level of genes involved in hormone signaling was critical for seed germination. Interestingly, we found that plant defense pathway was more active in aged seeds that in fresh ones on day 2 after imbibition. This might mainly contribute by higher contents of JA and SA in aged seeds on day 2 and indicated different biotic stress response between aged and fresh seeds.

Overall, several differential expressed genes between aged and fresh seeds were identified in this study which provided a candidate gene list for further genetic studies to reveal seed ageing mechanism.

CONCLUSIONS

The germination rate of aged seeds is mainly affected by the endogenous hormone content in seeds. Sweet corn seeds contain many novel transcripts which play roles in important molecular and biological pathways. More genes in aged are overrepresented during germination stage and defense signaling can be triggered earlier in aged seed than fresh seeds.

ACKNOWLEDGMENTS

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REFERENCES

AGOSTINI, R.T.; ABRANTES, FL.; MACHADO-NETO, N.B.; CUSTÓDIO, C.C. Ethanol and hormones in physiological conditioning on germination and seed dormancy of Urochloa humidicola cv. Llanero. *Journal of Seed Science*, v.44, e202244022, 2022. http://dx.doi.org/10.1590/2317-1545v44261411

ARC, E.; SECHET, J.; CORBINEAU, F.; RAJJOU, L.; MARION-POLL, A. ABA crosstalk with ethylene and nitric oxide in seed dormancy and germination. *Frontiers in Plant Science*, v.4, p.63, 2013. https://www.frontiersin.org/article/10.3389/fpls.2013.00063

BAI, B.; VAN DER HORST, S.; CORDEWENER, J.H.G.; AMERICA, T.A.H.P.; HANSON, J.; BENTSINK, L. Seed-Stored mRNAs that are specifically associated to monosomes are translationally regulated during germination. *Plant Physiology*, v.182, n.1, p.378-392, 2019. https://doi.org/10.1104/pp.19.00644

BINENBAUM, J.; WEINSTAIN, R.; SHANI, E. Gibberellin localization and transport in plants. *Trends in Plant Science*, v.23, n.5, p.410-421, 2018. https://doi.org/10.1016/j.tplants.2018.02.005

BLÁZQUEZ, M.A.; NELSON, D.C.; WEIJERS, D. Evolution of plant hormone response pathways. *Annual Review of Plant Biology*, v.71, p.327-353, 2020. https://www.annualreviews.org/doi/abs/10.1146/annurev-arplant-050718-100309

CARRERA-CASTAÑO, G.; CALLEJA-CABRERA, J.; PERNAS, M.; GÓMEZ, L.; OÑATE-SÁNCHEZ, L. An updated overview on the regulation of seed germination. *Plants,* v.9, n.6, p.703, 2020. https://pubmed.ncbi.nlm.nih.gov/32492790

EISVAND, H.R.; ALIZADEH, M.A.; FEKRI, A. How hormonal priming of aged and nonaged seeds of bromegrass affects seedling physiological characters. *Journal of New Seeds*, v.11, n.1, p.52-64, 2010. https://doi.org/10.1080/15228860903584523

FINCH-SAVAGE, W.E.; FOOTITT, S. Seed dormancy cycling and the regulation of dormancy mechanisms to time germination in variable field environments. *Journal of Experimental Botany*, v.68, n.4, p.843-856, 2017. https://doi.org/10.1093/jxb/erw477

GE, W.; BU, H.; WANG, X.; XIA, Y.; MARTINEZ, S.A.; WANG, X.; QI, W.; LIU, K.; DU, G. Changes in endogenous hormone contents during seed germination of Anemone rivularis var. flore-minore. *Global Ecology and Conservation*, v.24, e01200, 2020. https://www.sciencedirect.com/science/article/pii/S2351989420307411

GRONES, P.; MAJDA, M.; DOYLE, S.M.; VAN DAMME, D.; ROBERT, S. Fluctuating auxin response gradients determine pavement cell-shape acquisition. *Proceedings of the National Academy of Sciences of the United States of America*, v.117, n.27, p.16027-16034, 2020. https://www.pnas.org/content/pnas/117/27/16027.full.pdf

HILL, K. Post-translational modifications of hormone-responsive transcription factors: the next level of regulation. *Journal of Experimental Botany*, v.66, n.16, p.4933-4945, 2015. https://academic.oup.com/jxb/article/66/16/4933/499938

HU, Y.; COLANTONIO, V.; MÜLLER, B.S.F.; LEACH, K.A.; NANNI, A.; FINEGAN, C.; WANG, B.; BASEGGIO, M.; NEWTON, C.J.; JUHL, E.M.; HISLOP, L.; GONZALEZ, J.M.; RIOS, E.F.; HANNAH, L.C.; SWARTS, K.; GORE, M.A.; HENNEN-BIERWAGEN, T.A.; MYERS, A.M.; SETTLES, A.M.; TRACY, W. F.; RESENDE, M.F. R. Genome assembly and population genomic analysis provide insights into the evolution of

modern sweet corn. *Nature Communications*, v.12, n.1, p.1227, 2021. https://doi.org/10.1038/s41467-021-21380-4

JONSSON, K.; LATHE, R.S.; KIERZKOWSKI, D.; ROUTIER-KIERZKOWSKA, A.-L.; HAMANT, O.; BHALERAO, R.P. Mechanochemical feedback mediates tissue bending required for seedling emergence. *Current Biology*, v.31, n.6, p.1154-1164.e1153, 2021. https://www.sciencedirect.com/science/article/pii/S0960982220318364

KHAN, M.; ROZHON, W.; POPPENBERGER, B. The role of hormones in the aging of plants - a mini-review. *Gerontology*, v.60, n.1, p.49-55, 2014. https://www.karger.com/Article/FullText/354334

KIM, D.; LANGMEAD, B.; SALZBERG, S.L. HISAT: a fast spliced aligner with low memory requirements. *Nature Methods*, v.12, n.4, p.357-360, 2015. https://doi.org/10.1038/nmeth.3317

LEE, M.J.; YAFFE, M.B. Protein Regulation in Signal Transduction. *Cold Spring Harbor Perspectives in Biology*, v.8, n.6, 2016. https://cshperspectives.cshlp.org/content/8/6/a005918.long

LERTRAT, K.; PULAM, T. Breeding for Increased Sweetness in Sweet Corn. *International Journal of Plant Breeding*, v.1, n.1, p.27-30, 2007. http://www.globalsciencebooks.info/Online/GSBOnline/images/0706/IJPB_1(1)/IJPB_1(1)27-30o.pdf

LIAO, Y.; SMYTH, G.K.; SHI, W. featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics*, v.30, n.7, p.923-930, 2014. https://academic.oup.com/bioinformatics/article/30/7/923/232889

LIU, X.; ZHANG, H.; ZHAO, Y.; FENG, Z.; LI, Q.; YANG, H.-Q.; LUAN, S.; LI, J.; HE, Z.-H. Auxin controls seed dormancy through stimulation of abscisic acid signaling by inducing ARF-mediated ABI3 activation in Arabidopsis. *Proceedings of the National Academy of Sciences of the United States of America*, v.110, n.38, p.15485-15490, 2013. https://www.pnas.org/content/pnas/110/38/15485.full.pdf

PENG, J.; HARBERD, N.P. The role of GA-mediated signalling in the control of seed germination. *Current Opinion in Plant Biology*, v.5, n.5, p.376-381, 2002. https://linkinghub.elsevier.com/retrieve/pii/S1369526602002790

PERTEA, M.; KIM, D.; PERTEA, G.M.; LEEK, J.T.; SALZBERG, S.L. Transcript-level expression analysis of RNA-seq experiments with HISAT, StringTie and Ballgown. *Nature Protocols*, v.11, n.9, p.1650-1667, 2016. https://doi.org/10.1038/nprot.2016.095

ROBERT-SEILANIANTZ, A.; GRANT, M.; JONES, J.D.G. Hormone crosstalk in plant disease and defense: more than just jasmonate-salicylate antagonism. *Annual Review of Phytopathology*, v.49, n.1, p.317-343, 2011. https://www.annualreviews.org/doi/abs/10.1146/annurev-phyto-073009-114447

ROBINSON, M.D.; MCCARTHY, D.J.; SMYTH, G.K. edgeR: a bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics*, v.26, n.1, p.139-140, 2010. https://doi.org/10.1093/bioinformatics/btp616

SANTNER, A.; CALDERON-VILLALOBOS, L.I.A.; ESTELLE, M. Plant hormones are versatile chemical regulators of plant growth. *Nature Chemical Biology*, v.5, n.5, p.301-307, 2009. https://doi.org/10.1038/nchembio.165

SEO, M.; HANADA, A.; KUWAHARA, A.; ENDO, A.; OKAMOTO, M.; YAMAUCHI, Y.; NORTH, H.; MARION-POLL, A.; SUN, T.-P.; KOSHIBA, T.; KAMIYA, Y.; YAMAGUCHI, S.; NAMBARA, E. Regulation of hormone metabolism in Arabidopsis seeds: phytochrome regulation of abscisic acid metabolism and abscisic acid regulation of gibberellin metabolism. *The Plant Journal*, v.48, n.3, p.354-366, 2006. https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1365-313X.2006.02881.x

SHINKLE, J.R.; BRIGGS, W.R. Auxin concentration/growth relationship for avena coleoptile sections from seedlings grown in complete darkness. *Plant Physiology*, v.74, n.2, p.335-339, 1984. https://doi.org/10.1104/pp.74.2.335

SHUAI, H.; MENG, Y.; LUO, X.; CHEN, F.; ZHOU, W.; DAI, Y.; QI, Y.; DU, J.; YANG, F.; LIU, J.; YANG, W.; SHU, K. Exogenous auxin represses soybean seed germination through decreasing the gibberellin/abscisic acid (GA/ABA) ratio. *Scientific Reports*, v.7, n.1, p.12620, 2017. https://doi.org/10.1038/s41598-017-13093-w

ŠIMURA, J.; ANTONIADI, I.; ŠIROKÁ, J.; TARKOWSKÁ, D. E.; STRNAD, M.; LJUNG, K.; NOVÁK, O. Plant hormonomics: Multiple phytohormone profiling by targeted metabolomics. *Plant Physiology*, v.177, n.2, p.476-489, 2018. https://doi.org/10.1104/pp.18.00293

SINGH, P.; DAVE, A.; VAISTIJ, F.E.; WORRALL, D.; HOLROYD, G.H.; WELLS, J.G.; KAMINSKI, F.; GRAHAM, I.A.; ROBERTS, M.R. Jasmonic acid-dependent regulation of seed dormancy following maternal herbivory in Arabidopsis. *New Phytologist*, v.214, n.4, p.1702-1711, 2017. https://nph.onlinelibrary.wiley.com/doi/abs/10.1111/nph.14525

TAMAOKI, D.; SEO, S.; YAMADA, S.; KANO, A.; MIYAMOTO, A.; SHISHIDO, H.; MIYOSHI, S.; TANIGUCHI, S.; AKIMITSU, K.; GOMI, K. Jasmonic acid and salicylic acid activate a common defense system in rice. *Plant Signaling & Behavior*, v.8, n.6, e24260-e24260, 2013. https://pubmed.ncbi.nlm.nih.gov/23518581

XUE, X.; JIAO, F.; XU, H.; JIAO, Q.; ZHANG, X.; ZHANG, Y.; DU, S.; XI, M.; WANG, A.; CHEN, J.; WANG, M. The role of RNA-binding protein, microRNA and alternative splicing in seed germination: a field need to be discovered. *BMC Plant Biology*, v.21, n.1, p.194, 2021. https://doi.org/10.1186/s12870-021-02966-y

ZHANG, X.; MOGEL, K.J.H.V.; LOR, V.S.; HIRSCH, C.N.; DE VRIES, B.; KAEPPLER, H.F.; TRACY, W.F.; KAEPPLER, S.M. Maize sugary enhancer1 (se1) is a gene affecting endosperm starch metabolism. *Proceedings of the National Academy of Sciences of the United States of America*, v.116, n.41, p.20776-20785, 2019. https://www.pnas.org/content/pnas/116/41/20776.full.pdf