Plant Molecular Biology (2024) 114:94
https://doi.org/10.1007/s11103-024-01486-1

Deciphering physiological and transcriptional mechanisms of maize seed germination

Yaqi Jie[1] · Wei Wang[1] · Zishan Wu[1] · Zhaobin Ren[1] · Lu Li[1] · Yuyi Zhou[1] · Mingcai Zhang[1] · Zhaohu Li[1] · Fei Yi[1] · Liusheng Duan[1,2]

Received: 21 January 2024 / Accepted: 14 July 2024

Abstract

Maize is a valuable raw material for feed and food production. Healthy seed germination is important for improving the yield and quality of maize. Seed aging occurs relatively fast in crops and it is a process that delays germination as well as reduces its rate and even causes total loss of seed viability. However, the physiological and transcriptional mechanisms that regulate maize seeds, especially aging seed germination remain unclear. Coronatine (COR) which is a phytotoxin produced by *Pseudomonas syringae* and a new type of plant growth regulator can effectively regulate plant growth and development, and regulate seed germination. In this study, the physiological and transcriptomic mechanisms of COR-induced maize seed germination under different aging degrees were analyzed. The results showed that 0.001–0.01 μmol/L COR could promote the germination of aging maize seed and the growth of primary roots and shoots. COR treatment increased the content of gibberellins (GA_3) and decreased the content of abscisic acid (ABA) in B73 seeds before germination. The result of RNA-seq analysis showed 497 differentially expressed genes in COR treatment compared with the control. Three genes associated with GA biosynthesis (*ZmCPPS2*, *ZmD3*, and *ZmGA2ox2*), and two genes associated with GA signaling transduction (*ZmGID1* and *ZmBHLH158*) were up-regulated. Three genes negatively regulating GA signaling transduction (*ZmGRAS48*, *ZmGRAS54*, and *Zm00001d033369*) and two genes involved in ABA biosynthesis (*ZmVP14* and *ZmPCO14472*) were down-regulated. The physiological test results also showed that the effects of GA and ABA on seed germination were similar to those of high and low-concentration COR, respectively, which indicated that the effect of COR on seed germination may be carried out through GA and ABA pathways. In addition, GO and KEGG analysis suggested that COR is also highly involved in antioxidant enzyme systems and secondary metabolite synthesis to regulate maize seed germination processes. These findings provide a valuable reference for further research on the mechanisms of maize seed germination.

Keywords Seed germination · Seed aging · Coronatine · RNA-seq · Hormone

Yaqi Jie, Wei Wang and Zishan Wu are share first authorship.

✉ Fei Yi
yifei56@cau.edu.cn

✉ Liusheng Duan
duanlsh@cau.edu.cn

Yaqi Jie
bai481082280@163.com

Wei Wang
wangwei2021@cau.edu.cn

Zishan Wu
zswumhb@163.com

Zhaobin Ren
madfrog@cau.edu.cn

Lu Li
lilu0523@163.com

Yuyi Zhou
zhouyuyi@cau.edu.cn

Mingcai Zhang
zhangmingcai@cau.edu.cn

Zhaohu Li
lizhaohu@cau.edu.cn

[1] State Key Laboratory of Plant Physiology and Biochemistry, Engineering Research Center of Plant Growth Regulator, Ministry of Education & College of Agronomy and Biotechnology, China Agricultural University, No.2 Yuanmingyuan West Road, Haidian, Beijing 100193, China

[2] College of Plant Science and Technology, Beijing University of Agriculture, Beijing 102206, China

Published online: 30 August 2024

Abbreviations

ABA	Abscisic acid
BR	Brassinosteroid
CDPKs	Calcium-dependent protein kinases
CFA	Coronafacic acid
CHYB	β-Carotene hydroxylase
CMA	Coronamic acid
COR	Coronatine
CPS	*ent*-Copalyl diphosphate synthase
CTK	Cytokinin
DEG	Differentially expressed gene
ELISA	Enzyme-linked immunosorbent assay
ETH	Ethylene
FDR	False discovery rate
FPKM	Fragments per kilobase of transcript per million mapped reads
GA	Gibberellin
GA20ox	GA20oxidase
GID1	Gibberellin insensitive dwarf1
GO	Gene ontology
IAA	Auxin
JA	Jasmonic acid
KAO	*ent*-Kaurenoic acid oxidase
NCED	9-Cis-epoxycarotenoid dioxygenase
PCA	Principal component analysis
PP2C	Protein phosphatase 2C
SA	Salicylic acid
SDR	Short-chain dehydrogenase/reductase
TTC	Triphenyltetrazolium chloride
TF	Triphenyl formazone
ZR	Zeatin

Introduction

Maize (*Zea mays* L.) is an important grain, feed, and raw material crop in China, and in 2022, the area and production of maize planted in China exceeded that of wheat and rice (China 2023), and the production of maize is of great importance for economic and social stability. All three aspects of seed quality, including seed germination, vigor, and size, may affect crop yields through indirect and direct effects (Ellis 1992). Seed growth at germination directly affects plant growth and ultimately maize yield (Li et al. 2008). Rapid germination of maize seeds after sowing, early seedling emergence, seedling homogeneity, and seedling strength, for the crop quality and high yield laid a solid foundation (Epstein 1994).

At the onset of germination (known as suckering), the seed rapidly absorbs water, causing the seed coat to swell and soften at an optimal temperature. Subsequently, the rupture of the seed coat allows the emergence of the radicle and germ. The seed then activates its internal physiology and begins to respire (Gallardo et al. 2001). This is the lag phase of seed germination. The process of seed germination is influenced by a variety of exogenous and endogenous factors, including temperature, water, oxygen, various nutrients and plant hormones, and even magnetism (Florez et al. 2007; Shine et al. 2012). Among these factors, reactive oxygen species (ROS) is an important factor in seed germination (Mitsuya et al. 2010; Liu et al. 2022). ROS are by-products of aerobic metabolism and are mainly produced by mitochondria, chloroplasts, peroxisomes, and NADPH oxidase (NOX) (Mittler et al. 2004). Senescence-induced elevated levels of ROS cause oxidation of cellular components, damaging the structure of DNA, proteins, and biofilms, and impeding metabolic activities, thereby affecting seed germination (Hussain et al. 2018). The presence of catalase and other antioxidant enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX), and reduced glutathione (GSH) could scavenge ROS produced in peroxisomes (Mitsuya et al. 2010; Liu et al. 2022), which is conducive to seed germination.

In recent years, the stock of maize seed each year has been high in China; because the cost of stock is lower than the cost of seed production, a larger proportion of the seed used for input into field production is stocked (Zhao and Zhong 2016; Gao et al. 2018; Chen et al. 2019). Seed aging is bound to occur in the process of seed storage, and the aging seeds have reduced vigor, uneven seedling emergence, and weak seedling growth, which directly affects the later field management and even reduces the yield and quality of maize (Ellis 1992; Kijak and Ratajczak 2020), and the physiological quality of the seed with the prolongation of the storage period and decline (Bonner 1990). Therefore, it is crucial to improve seed germination of aging seed.

Currently, there are many successful examples of techniques for triggering the germination of aging seeds. Seed priming is a technique that triggers events related to seed germination by sucking water or various chemicals (e.g., vitamins, antioxidants, hormones, etc.) into the seed before radicle protrusion, and is widely used to enhance vigor and promote germination and the emergence of aging seeds (Salah et al. 2015). It has been shown that melatonin is widely present in plants and plays an important role in promoting seed germination, root regeneration, and plant growth and development (Posmyk et al. 2009; Sarropoulou et al. 2012; Sun et al. 2016). Sun et al. reported that the exogenous addition of the basic element silicon improved the germination and vigor index of maize seeds and promoted seedling growth (Sun et al. 2021). In addition, as a signaling molecule for plant signal transduction, NO also has an important role in ending dormancy and promoting seed germination (Arasimowicz and Floryszak-Wieczorek 2007), and appropriate concentrations of NH_3 can accelerate maize seed germination and seedling growth (Li et al.

2023). Seed hormonal priming is a type of seed priming. Hormonal priming can effectively improve the state of seed germination, promote seedling growth and yield increase, and enhance plant resistance.

There has been abundant research on the effects of various plant hormones on seed germination and their regulatory mechanisms. Among various plant hormones, gibberellin (GA) and abscisic acid (ABA) are the main endogenous factors regulating seed dormancy and germination (Leubner-Metzger 2003; Graeber et al. 2012). McCarty et al. (1991) identified a homologous gene *Vp1* of Arabidopsis *ABI3* in maize, which is involved in the regulation of ABA-mediated maize seed dormancy; Song et al. (2011) found that transcripts of 15 GA metabolism genes could be detected during maize seed germination using expression profiling, of which two important enzymes in the GA biosynthesis pathway, endogenous root-kaurene oxidase (KO) and endogenous root-kaurenoic acid oxidase (KAO) were increased after seed water absorption; 72 h after germination, the transcript levels of GA20-oxidase (GA2ox), GA3-oxidase (GA3ox) and GA2-oxidase (GA2-oxidase, GA2ox) were higher than that at the beginning of seed germination. Liu et al. (2013) results showed that auxin (IAA)- and GA-related genes involved in cell division and differentiation, as well as transcriptional regulatory genes, were up-regulated during mid-seed germination. In addition, genes related to lipid metabolism, protein degradation, and synthesis, mitochondrial electron transfer, ATP synthesis, and glycolysis were also highly expressed in the early stage of seed germination. These results provide further evidence that an increase in active GA synthesis in the embryo is required to trigger maize seed germination.

Coronatine (COR) is a non-host-specific phytotoxin produced by the pathogenic bacterium *Pseudomonas syringae lilaca* and is a structural analog of the phytohormone methyl jasmonate (MeJA) (Ichihara et al. 1977; Bender et al. 1999). Both COR and jasmonic acid (JA)/MeJA have a cyclopentadecane structure, and many of their physiological functions are similar (Greulichi et al. 1995; Bender et al. 1999; Schüler et al. 2004; Shiraishi et al. 2014). Moreover, the physiological activity of COR is more than 100~1000 fold higher than that of MeJA, making it a novel plant growth regulator with a wide range of applications (Koda et al. 1996; Tamogami and Kodama 2000; Onrubia et al. 2013). Studies have shown that COR can affect plant growth and development, induce defense-related secondary substance metabolism, and regulate adversity stress (Bender et al. 1999; Wang et al. 2006).

Many studies have also found that low concentrations of COR seed dipping can promote seed germination. Ai Lin et al. (2008) reported that a low concentration of COR (0.01 μmol/L) soaking treatment could significantly promote the growth of the initial roots of upland rice seeds. Xie et al. (2012) showed that seed soaking with a low concentration of COR (0.01 μmol/L) could improve cotton seed vitality and germination rate under salt stress. Luo et al. (2021) showed that COR could improve soybean seed germination and seedling emergence. It has also been shown that COR at low concentrations (≤ 0.01 μmol/L) can promote the germination of maize seeds, and it has been shown that the trend of the effects of MeJA and COR on seed germination rate is consistent, both showing similar dose effects (Wang et al. 2008). However, most of the current studies on the effects of plant growth regulators, including COR, on seed germination have been conducted mainly under normal physiological conditions, while not many studies have been conducted on the viability of aged seeds.

Therefore, in this study, we applied exogenous COR to maize seeds, comprehensively analyzed their physiological and transcriptomic characteristics, and explored the role of COR in promoting seed germination, to reveal the molecular mechanism of COR treatment in promoting germination of aging maize seeds. The results showed that COR could promote the germination of aging maize seeds and the growth of primary roots and shoots. Based on transcriptome and hormone data, key genes of hormone signaling pathways ABA and GA were focused on. The results are not only of practical value for solving the current production problem of economic loss due to seed aging caused by long-term storage but also of theoretical significance for the next elucidation of the role of COR in the phytohormone interaction network.

Materials and methods

Plant material and treatments

Maize hybrids ZD958 (2018, 2019), Xianyu 335 (2017, 2018), and hybrid Gannong 118 (2012) from Henan Dr. Jin Seed Co. Ltd, Shandong Denghai Seed Pioneer Seed Co., and Gansu Furnong High-Tech Seed Co. Ltd were used in this experiment. The hybrid Qianfeng 339 (2015), hybrid QF689 (2015), and hybrid Qianfeng 6 (2015) of Jinchang Qiankun Toyota Seed Industry Co. were used in this experiment. The maize inbred line, B73, was harvested at Shangzhuang Experimental Station of China Agricultural University, Haidian District, Beijing, China, in 2017 and 2020, respectively. The seeds of 2020 are 1 year after harvest, the seeds of 2019 are 2 years after harvest, the seeds of 2018 are 3 years after harvest, the seeds of 2017 are 4 years after harvest, the seeds of 2015 are 6 years after harvest, and the seeds of 2012 are 9 years after harvest.

COR was fermented and purified by the Engineering Research Center of Plant Growth Regulators of the Ministry of Education, China Agricultural University. The purity of COR (purification standard was > 99%) was determined by high-performance liquid chromatography

(Milford, MA, USA). COR was dissolved with methanol and diluted and configured to 1 μmol/L (70% methanol) mother liquor.

GA, from Sinopharm Chemical Reagent Co Ltd (Item No. G0029). ABA, from Sinopharm Group Chemical Reagent Co.

Standard germination test

After selecting uniform and consistent seeds with full grains and washing them 3 times with water in oscillation, they were sterilized with disinfectant (2% sodium hypochlorite or 3% hydrogen peroxide) for 10-15 min, and then cleaned with deionized water for 6 times, and after sucking out the water on the surface of the seeds, they were immersed for 24 h in darkness in the immersion solution, and then the immersion solution was poured off. After germination boxes were cleaned and sterilized with 75% ethanol solution and ultraviolet light, 2 layers of sterilized and dried germination paper were laid down, each germination box was moistened with 5 mL of sterilized water, and seeds were placed along the side wall of the germination box with the embryo side facing upward, and 30 seeds in each box were placed in a light incubator for dark incubation (temperature 25°C, humidity 29%), with three replications for each treatment. The number of germination was recorded every 12 h (with radicle growth reaching seed length as the germination criterion), and representative time points were selected and photographed before counting the length of primordial roots or young shoots of maize using the image processing software ImageJ (v 1.53, NIH, USA).

Artificial aging experiment

Seed artificial aging experiments were done in the Physiology Laboratory of the Seed Science Center, College of Agriculture, China Agricultural University (CAU), and the operation was improved according to the artificial aging method of Hu Jin (1988) and the materials available in the laboratory. A perforated partition (the partition does not touch water) was stuck in the middle of an uncovered plastic box containing fresh water, a small mesh pocket with seeds was placed on the partition (the number of seeds was appropriate for the mesh pocket to be able to lay flat on the partition), the plastic box was sealed with plastic wrap (the wrap did not touch the seeds), and then placed in an oven (temperature 42°C) to create a high-temperature and high-humidity (humidity 100%) aging environment, and the seeds were taken out on the second, fourth, and sixth days, respectively., day 4 and day 6 to remove the three batches of seeds, respectively.

Tetrazolium assay

Referring to the methods of Zhu Nanwen (1996) and Qiao Yanxiang (2003). 10 seeds were removed from each of the three biological replicates of the control and treatment groups, respectively, and cut along the embryo in the longitudinal direction, half of them were taken, put into test tubes, and added with 10 mL of 0.1% TTC solution, and stained for 2 h under dark conditions. At the end of staining, pour off the staining solution and rinse with water three times to absorb the water on the surface of the seeds. Pour the seeds into a dry test tube, add 10 mL of anhydrous ethanol, seal with tin foil, place in a water bath at 80°C, stop extraction when the embryo turns white, cool down, and then determine the absorbance value with a spectrophotometer at a wavelength of 485 nm.

Determination of hormone content

The contents of five hormones, GA, IAA, zeatin (ZT), ABA, and JA, were determined by enzyme-linked immunosorbent assay (ELISA) in B73 seeds soaked with water and 0.005 μmol/L COR, respectively. After 24 h of soaking, the seeds were drained of surface water and the samples were quickly frozen in liquid nitrogen and stored in a refrigerator at − 80°C. After grinding at low temperature, the seeds were extracted with BHT-80% methanol extract and then nitrogen blown, and then the samples were assayed. Four biological replicates were set up for each treatment, and the weight of each sample was about 500 mg. After reading the colorimetric values of the hormone standard samples and each sample to be tested on the enzyme marker, the logit value of each colorimetric value was calculated by using the Formula (1) (*B0* is the colorimetric value of the 0 ng/mL wells, and *B* is the colorimetric value of the other concentrations), and the natural logarithm of the concentration (ng/mL) of each hormone standard sample was taken as the horizontal coordinates. The natural logarithm of each concentration (ng/mL) of the hormone sample was used as the horizontal coordinate, and the logit value of each concentration was used as the vertical coordinate to make a standard curve. The hormone concentration (ng/mL) of the sample to be tested was calculated from the standard curve based on the logit value of the color rendering value.

$$\mathrm{Logit}\left(\frac{B}{B0}\right) = \ln\left(\frac{B}{B0 - B}\right) \quad (1)$$

RNA extraction and preparing library

Total RNA for all samples was extracted using Trizol (manufactured by Invitrogen) according to the manual. Total RNA was then purified using a magnetic scaffold (Invitrogen). Purified total RNA aliquots were stored in a − 80°C refrigerator. Sequencing libraries were constructed with 5 μg of total RNA using the TruseQ™ RNA Sample Preparation Kit (Illumina Inc., San Diego, USA) according to the manufacturer's instructions. End repair, phosphorylation, and 'A' base addition were performed on the synthetic cDNA according to the library construction protocol (Illumina). After 15 cycles of polymerase chain reaction with Neb's Phusion DNA polymerase, cDNA target fragments were size-selected on 2% low-range super agarose (Bio-RAD). The cDNA target fragment size was 200–300 bp. The libraries were then quantified with TBS380 PicoGreen (Invitrogen). All paired-end sequencing libraries were sequenced using HISEQ XTEN (2 × 150 bp read length) (Illumina Inc., San Diego, USA).

RNA-seq data analysis

To ensure the alignment and quality of paired-end reads, we employed SeqPrep (https://github.com/jstjohn/SeqPrep) and Sickle (https://github.com/najoshi/sickle) to trim the paired-end reads and filter the Illumina reads, respectively. Subsequently, we used Hisat2 (Kim et al. 2019) to map the reads to the maize reference genome obtained from MaizeGDB (https://maizegdb.org). The uniquely mapped reads were then processed with Cufflinks (V2.2.0) software (Ghosh and Chan 2016). The number of transcript fragments per kilobase per million mapped reads (FPKM) was used to indicate gene expression levels. The gene expression level was indicated using PFKM. The R^2 value was calculated to measure the correlation between biological replicates. Correlation plots were generated using the prcomp function of R software (Team 2022), with the default settings, to visually represent the correlation among all samples using $\log_2(\text{FPKM}+1)$. For PCA analysis, we utilized the prcomp function in R software (Team 2022) with its original parameters to conveniently display the relationship among all samples. The $\log_2(\text{FPKM}+1)$ values of the genes were utilized for the PCA analysis performed in R (V 3.6.1).

Three biological replicates were set up for each treatment (water and 0.005 μmol/L COR). To ensure the reliability of the analysis results, genes with FPKM values greater than 0 in all six samples were selected for correlation mapping. Based on the correlation value between every two biological replicates, we eliminated one set of data in each treatment (CK3, COR3).

Principal component analysis (PCA) analysis was performed using the prcomp function in the R software (Team 2022), and the raw parameters were easy to graphically display the correlation between all samples. The $\log_2(\text{FPKM}+1)$ of the genes was used for R(V3.6.1) analysis of PCA. The K-means algorithm in MEV (V4.9) software was used for cluster analysis (data were $\log_2$ processed and Z-score normalized).

The advanced volcano plot and the bubble chart were performed using the OmicStudio tools at https://www.omicstudio.cn/tool.

Statistical analysis

The statistical analysis was conducted using GraphPad Prism 8.3.0 software (GraphPad, San Diego, CA, USA). The significance of differences was tested using *t*-tests, one-way analysis of variance (ANOVA), and two-way ANOVA. Dunnett's method was employed for comparisons between one control group and multiple other treatment groups. The Tukey's Honestly Significant Difference (HSD) method was utilized to compare all possible group pairs to determine significant differences. A *p*-value of less than 0.05 was considered statistically significant.

Result

Effects of COR treatment on seed germination of maize inbred.

To investigate the role of COR in maize seed germination, a germination test was conducted with different concentrations of COR (0, 0.001, 0.005, 0.01, 0.05, 0.1 μmol/L) on maize inbred B73 seeds harvested in 2020 (1-year after harvesting and stored at 25°C and protected from light) after 24 h of imbibition, and the seed germination rate was observed (Fig. 1A). The results demonstrated that at low concentrations of COR (0.001, 0.005, and 0.01 μmol/L), the germination rate of seeds increased in comparison to the control. The optimal results were observed at the soaking concentration of 0.005 μmol/L, with a germination rate of 85%. This rate exhibited a declining trend with the increase in COR concentration (Fig. 1B).

The germination test was carried out by soaking B73 seeds with water and 0.005 μmol/L COR for 24 h. Changes in the germination rate of maize from 0 to 96 h as well as the statistical results of root length at the two-time points of 60 and 84 h of germination were observed. The results showed that 0.005 μmol/L COR treatment enhanced the germination rate of maize seed B73, resulting in a 26.8% increase in germination rate at 48 h and an 8.4% increase at 60 h. At 60 h of germination, COR treatment resulted in 100% germination (Fig. 1C), and the root length of maize seeds in the COR-treated group was significantly longer than that of the control

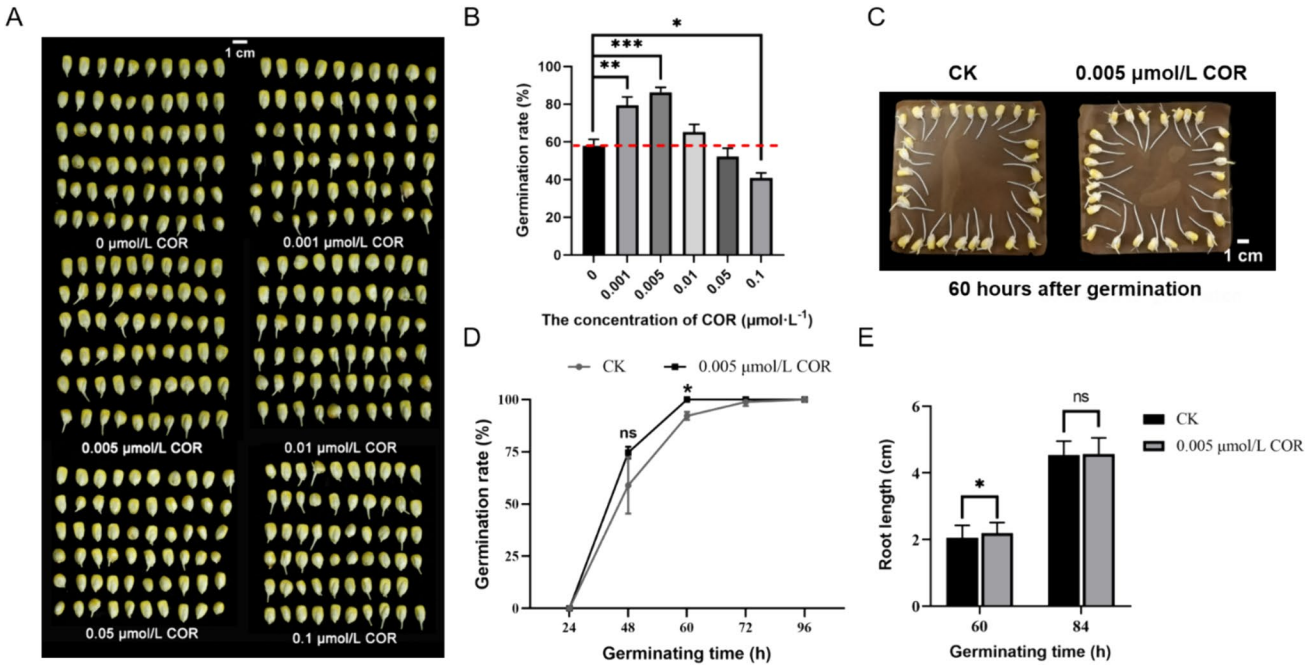

Fig. 1 Effect of COR treatment on maize seed germination. **A** The phenotype of B73 seed germination with COR priming at concentrations of 0.001–0.1 μmol/L. **B** Germination percentage calculated on the 24 h of germination. Each bar represents the mean germination rate of four biological replicates, with standard deviation (±SD), each biological replicate derived from 11 corn seeds. A one-way analysis of variance (ANOVA) was employed for multiple comparisons. **C** The phenotype of B73 seed growth after 60 h of germination with COR priming at concentrations of 0.005 μmol/L. **D** Germination percentage calculated on 0–96 h of germination. The bars show the mean germination rate of three biological replicates (±SD), each biological replicate based on 30 maize seeds. A *t*-test compared treatment and control group means. (E) Root length of seeding at 60 h and 84 h of germination. The bars show the mean root length of corn seeds (n = 80), recorded as mean ± SD. A *t*-test compared treatment and control group means. Significance levels were denoted in B, D, E as ns, *, **, and ***, corresponding to probabilities of no significant difference, 0.05, 0.01, and 0.001, respectively

group by 5% (Fig. 1D, E), and by 84 h of germination, the difference between root lengths of the treated group and that of the control group was not significant (Fig. 1E).

To investigate the effect of COR on the germination of aging B73 seeds, germination tests were conducted on maize-inbred B73 seeds harvested in 2017 (harvested for 4 years and stored at 25°C away from light) by soaking the seeds in water (CK) and 0.005 μmol/L COR for 24 h and observing the germination phenotypes at 60 h as well as 72 h (Fig. 2A). We found that the germination rate of 0.005 μmol/L COR-treated seeds was consistently higher than that of the control group until 72 h (Fig. 2B), and the germination rate increased by 39.1%, 9.8%, and 8.8% at 36 h, 48 h, and 60 h, respectively. In addition, the difference in seed root length between the 60 h to 72 h to 84 h COR treatment group and the control group gradually increased (Fig. 2C), exceeding the control group by 19.5%, 32%, and 53.8%, respectively (Table S1). The results indicated that 0.005 μmol/L COR could promote the germination of aged maize inbred B73 seeds and significantly promote the growth of seed roots after germination.

The above results showed that 0.005 μmol/L COR increased the germination rate more in the pre-emergence period of seeds, which could increase the unaged B73 (2020) and naturally aged B73 (2017) seeds by 26.8% and 9.8%, respectively, at 48 h, and 8.4% and 8.8%, respectively, at 60 h. The results of 0.005 μmol/L COR treatment on the root length of B73 (2020) and B73 (2017) were not consistent with the results of 0.005 μmol/L COR treatment. Whereas, the effect of 0.005 μmol/L COR treatment on the root length of B73 (2020) and B73 (2017) was inconsistent. At 60 h of germination, root length was significantly increased in all treatment groups, by 5% and 19.5% in B73 (2020) and B73 (2017), respectively. Whereas at 84 h of germination, the difference in root length of the B73 (2020) treatment group was not significant compared to the control group, but the B73 (2017) treatment group showed a 53.8% increase in root length compared to the control group.

Effects of COR treatment on seed germination of maize hybrid

Given the ubiquitous use of hybrid varieties in agricultural contexts and the regional divergence in the hybrid selection, our study aims to scrutinize the efficacy of COR application in field production. Accordingly, we opted for the widely

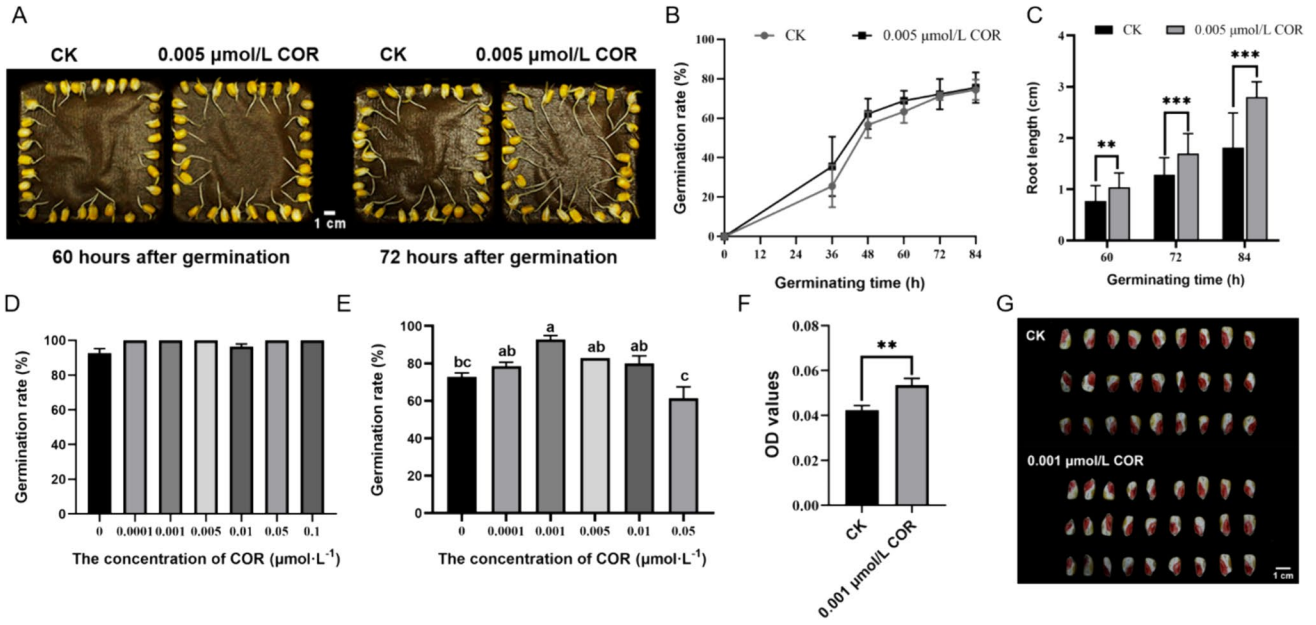

Fig. 2 Effect of COR treatment on germination of aging maize seeds. **A** The phenotype of B73 seed germination with intrinsic aging and further COR priming at concentrations of 0.005 μmol/L. **B** Germination percentage was calculated on 36–84 h of B73 seed germination with intrinsic aging and further COR priming at concentrations of 0.005 μmol/L. The bars depict the average germination rate of three biological replicates, with SD values provided, each biological replicate based on 30 maize seeds. **C** Root length of B73 seeding at 60 h, 72 h, and 84 h of germination with intrinsic aging and further COR priming at concentrations of 0.005 μmol/L. The vertical error bars represent SD values (n = 20) and a *t*-test was employed to compare the means of the treatment and control groups for significant differences. **D** Germination percentage was calculated on the 48 h of ZD958 seed germination with intrinsic aging and further COR priming at concentrations of 0.0001–0.1 μmol/L. Each bar represents the mean of three biological replicates (SD indicated), with each replicate comprising the average germination rate of 30 maize seeds. **E** Germination percentages were assessed after 48 h of ZD958 seed germination following artificial aging and COR priming at concentrations ranging from 0.0001 to 0.1 μmol/L. Each bar represents the mean of three biological replicates (SD indicated), each consisting of 30 maize seeds. Significance at the 0.05 probability level was determined by Tukey test, with differing lowercase letters denoting significance. **F** Results of TCC staining of artificially aging ZD958 seeds after 24 h of imbibition by 0.001 μmol/L COR. Vertical error bars indicate SD values (n = 3), with significance assessed using a *t*-test to compare treatment and control group means. **G** Artificially aged ZD958 seeds were stained with TCC, and the staining product TTF was extracted with anhydrous ethanol. Colorimetry at 485 nm was conducted using a spectrophotometer. Significance levels were denoted in C, F as ** and ***, corresponding to probabilities of 0.01 and 0.001, respectively

utilized hybrid variety ZD958 as our experimental subject for comprehensive investigation. To verify whether COR treatments would have the same action effect on hybrids, we chose ZD958 harvested in 2019 (2 years after harvesting and stored at 25°C and protected from light) as the experimental material and conducted a germination test with different concentrations of COR solutions on ZD958 seeds after 24 h of imbibition to observe the germination rate at 48 h. Since the ZD958 seeds of 2019 were more vigorous, the germination rate of all treatment groups was close to 100% at 48 h. The results showed that different concentrations of COR imbibition had no significant effect on the germination rate of ZD958 seeds harvested in 2019 (Fig. 2D).

To obtain aged seeds, ZD958 seeds harvested in 2018 (3 years after harvesting and stored at 25°C and protected from light) were subjected to high temperature and high humidity treatments (see Materials and methods section for details of the procedure), and seeds aged for 2, 4 and 6 days were obtained. Sand cultivation experiments were carried out on these seeds to verify the effect of artificial aging of seeds. The results showed that the seedling emergence rate was 87.5% without aging, while it decreased sequentially after 2, 4, and 6 days of age to 60.4%, 39.6%, and 4.2%, respectively. The results showed that artificial aging reduced the seedling emergence of 2018 ZD958 maize seeds and the longer the aging time, the lower the seedling emergence rate and the lower the seed viability (Supplementary Fig. 1).

Since the seedling emergence rate of 4-day-aging seeds was significantly reduced but still retained a certain degree of vigor, 4-day-aging seeds were selected for germination tests after being soaked with different concentrations of COR, and the results showed that low concentrations (0.0001–0.01 μmol/L) of COR soaking could improve the germination rate of artificially aging ZD958 seeds, with the 0.001 μmol/L COR treatment increased the germination rate by 27.4% (Table S2), which was significantly different from the control (Fig. 2E).

Dehydrogenase activity was significantly and positively correlated with each germination index, which is an important indicator for determining seed vigor, and dehydrogenase can accurately and rapidly respond to the reduction capacity of seed embryo cells. A study (Qiao et al. 2003) showed that dehydrogenase activity gradually decreased with the aging time of maize seeds. To further verify the effect of 0.001 μmol/L COR on the viability of aging seeds, we dipped 4-day-aging ZD958 seeds in water (CK) and 0.001 μmol/L COR, respectively, for 24 h and then stained the embryonic parts of the longitudinally cut seeds with TTC, and after extracting the staining reaction product TTF with anhydrous ethanol, we measured the absorbance at 485 nm using a spectrophotometer. We found that seeds treated with 0.001 μmol/L COR showed a significant increase in TTF absorbance value of 26% and deeper TTC staining than CK treatment. This result indicated that the seed dehydrogenase activity was higher after 0.001 μmol/L COR soaking, that is, it showed that 0.001 μmol/L COR could significantly enhance the viability of artificially aged ZD958 seeds (Fig. 2F, G). These data suggest that COR treatment can enhance the viability of artificially aged ZD958 seeds.

Changes of phytohormone content in aging seeds under COR treatment

To investigate the mechanism of the effect of COR treatment on maize seed germination, we chose the seeds of inbred line B73 harvested in 2020 (1 year after harvesting) as the experimental material, and treated the B73 seeds with water (CK) and 0.005 μmol/L COR for 24 h, respectively, and then took samples for grinding and extracting hormones in the kernels, and then determined the hormone content (GA_3, IAA, ZT, ABA and JA) in the seeds by enzyme-linked immunosorbent assay (ELISA). The results showed that the GA_3 content of seeds treated with 0.005 μmol/L COR was significantly higher than that of the control (Fig. 3A) and was 9.2% higher than that of the control. On the contrary, ABA content was lower than the control (Fig. 3B) and 24.2% lower than the control, while the content of other hormones remained almost unchanged (Fig. 3C–E). These results indicate that COR treatment may regulate seed germination by altering ABA and GA content in seeds.

Overall analysis of transcriptome in response to COR treatment

To further investigate the molecular mechanisms of maize seed germination, we used RNA-seq to study the effect of COR (0.005 μmol/L) on the gene expression levels of 2020 maize seeds (1 year after harvesting) 24 h after treatment. A total of four libraries were constructed, each containing two biological replicates, and 32.24 GB of high-quality reads were obtained. The efficiency of clean reads mapped in the reference genome sequence using the Hisat 2 tool ranged from 93.65% to 94.08% for each sample, with an average of 71.47% of reads being uniquely mapped (Table S3). A comparison of the two biological replicates showed that the expression values were highly correlated between them (mean $R^2 = 0.93$, Supplementary Fig. 2A and B). Therefore, we used the average FPKM values of the two replicates as the expression levels of the treated and control groups. To minimize the effect of transcriptional noise, we defined genes with FPKM values ≥ 1 as expressed genes. In total, 18,988 genes including 1371 TFs were found to be expressed in at least one of the groups. The data from the four samples were analyzed by plotting scatter plots, PCA, cluster analysis, and volcano plots, and the results showed that between-group differences were greater than within-group differences

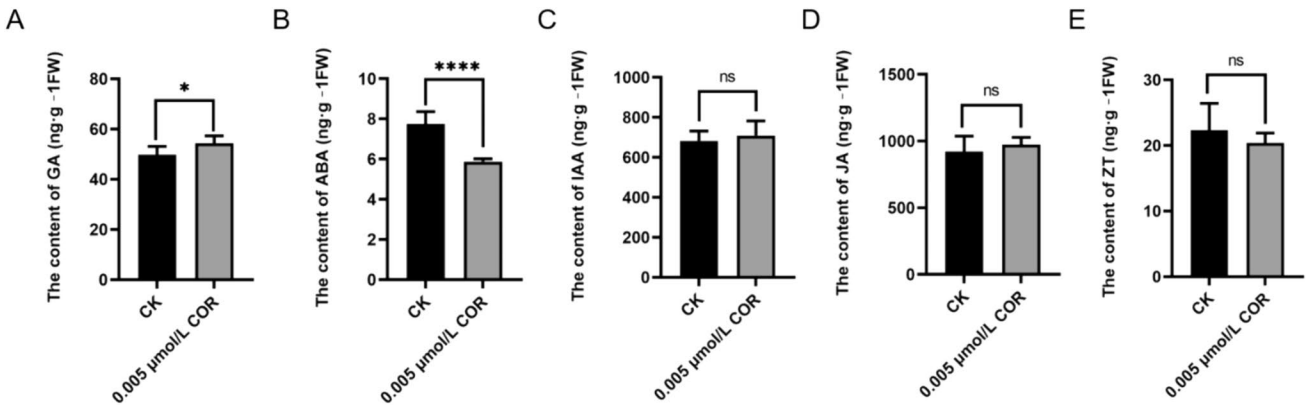

Fig. 3 Effects of COR on contents of endogenous hormones of B73 seeds. **A** Content of GA. **B** Content of ABA. **C** Content of IAA. **D** Content of JA. **E** Content of ZT. Values were recorded by the means ± SD (n = 6). *, **** indicate that the probability levels are 0.05 and 0.001, respectively. "ns" indicates no significant difference

(Supplementary Fig. 2C, D). The above findings suggest that the results of transcriptome sequencing are sufficient to support further data analysis.

Analysis of DEGs induced by COR in the germination process of maize seeds

We used *p*-value less than 0.05, fold change ≥ 2 or ≤ 0.5, and expressed in at least one sample as the criteria for screening differentially expressed genes, and a total of 497 DEGs were identified. 301 of these genes were up-regulated, and 196 were down-regulated. (Supplementary Fig. 2E, Data Set 1).

The 497 DEGs were analyzed by Gene Ontology (GO) enrichment. GO annotation showed that the 497 differential genes were classified into three categories: molecular functions, cellular components, and biological processes. Among the biological processes, cellular carbohydrate biosynthesis process, carotenoid biosynthesis process, cell wall polysaccharide biosynthesis process, cellular catabolism process, chlorophyll biosynthesis process, glycosyl compound biosynthesis metabolism process, lipid biosynthesis process, nucleic acid metabolism process, protein modification process, hormone response processes, seed dormancy processes, terpene metabolic processes account for the major role (Data Set 2). The results of Liu et al. (2013) showed that genes related to lipid metabolism were up-regulated and genes related to protein degradation and synthesis were enriched in the module during early seed germination. Consistent with this, we observed eight differential genes related to protein degradation, *Zm00001d017612*, *Zm00001d017612*, *Zm00001d007915*, *Zm00001d025310*, *Zm00001d050798*, *Zm00001d051849*, *Zm00001d031601* and *Zm00001d017532*, which are mainly associated with ubiquitin E3 SCF (Skp1-Cullin-F-box protein) and RING (really interesting new gene) domain proteins. In addition to genes in mitochondrial electron transport, ATP synthesis, and glycolysis were overexpressed, suggesting an increase in energy expenditure. GO enrichment analysis showed that DEGs contained 14 genes related to ATP metabolism, and the expression of 9 genes was up-regulated, which was consistent with the high energy demand during seed germination. *Zm00001d022356* (*IDP3835*) encodes a DEAD-box ATP-dependent RNA helicase. Its homologous gene in Arabidopsis, *AtRH57*, is involved in feedback inhibition of glucose-mediated ABA accumulation during seed germination and early seedling development (Hsu et al. 2014).

Inside the first 20 pathways enriched, the main ones included response to abiotic stimuli, response to chemicals, response to hormones, cell wall organization and biosynthesis, and seed germination (Fig. 4A). Among them, the phrase seed germination was enriched with many genes, such as *Zm00001d033612* (*ole4*) encoding oleosin, which was down-regulated; *Zm00001d026037* and *Zm00001d024414* encoding seed maturation proteins, which were down-regulated; and *Zm00001d024414*, which encode seed maturation proteins, were down-regulated; and the expression of *Zm00001d053696*, which is involved in seed coat development, was up-regulated. This indicates that seed coat plays a critical role in initiating the germination process.

Phytohormone-related genes are involved in the germination regulation of maize seeds

Seed germination is regulated by a complex crosstalk of phytohormones, and ABA, IAA, GA, and Brassinosteroid (BR) are key components in these processes (Martin et al. 2010; Weitbrecht et al. 2011). We investigated the effect of COR on ABA-, IAA-, GA-, and BR-related genes among 497 differential genes by GO enrichment. A total of 35 ABA-related genes, 16 IAA-related genes, 13 ethylene (ETH)-related genes, 12 salicylic acid (SA)-related genes, 10 BR-related genes, 9 GA-related genes, 8 JA-related genes, and 5 cytokinin (CTK)-related genes were found. Further analysis showed that the ABA, BR, and GA pathways were significantly enriched (Fig. 4B). The results showed that ABA, BR, and GA-related genes differed significantly after COR treatment, and then we focused on the regulation of GA and ABA-related genes by COR.

KEGG enrichment analysis showed that differentially expressed genes were significantly enriched in seven categories ($p < 0.05$) (Fig. 4C). Among them, diterpenoid biosynthesis related to GA synthesis (zma00904) ($p = 0.0045$), peroxisomes, an essential pathway for glyoxylate cycling in plants (zma04146) ($p = 0.0051$), biosynthesis of benzoxazines, which play a role in plant defense (zma00402) ($p = 0.0066$), pentose and glucuronide interactions (zma00040) ($p = 0.0179$), carbon fixation in photosynthesis (zma00710) ($p = 0.0202$), metabolic pathways (zma01100) ($p = 0.0299$), and biosynthesis of secondary metabolites (zma01110) ($p = 0.0370$) were significantly enriched (Data Set 3), suggesting that the mechanism by which COR enhances maize seed germination is related to the synthesis of secondary metabolites, antioxidant enzyme systems, sugar metabolism and phytohormones. Among them, many genes are enriched in metabolic pathways, such as *Zm00001d001820* (*pcr1*), *Zm00001d027511* (*cat2*), and *Zm00001d011097* (*opr4*). Catalase (CAT; EC 1.11.1.6) is an important component of the antioxidant enzyme system in cereals. The expression of *ZmCat2*, which encodes a catalase isozyme in maize seeds, was up-regulated. Catalase can remove ROS in seeds and promote the germination process of seeds (Mitsuya et al. 2010; Liu et al. 2022).

GA plays an important role in the control of plant growth and development including seed germination, stem elongation, leaf extension, and flower and seed (Yamaguchi 2008). Our transcriptome results showed that most of the

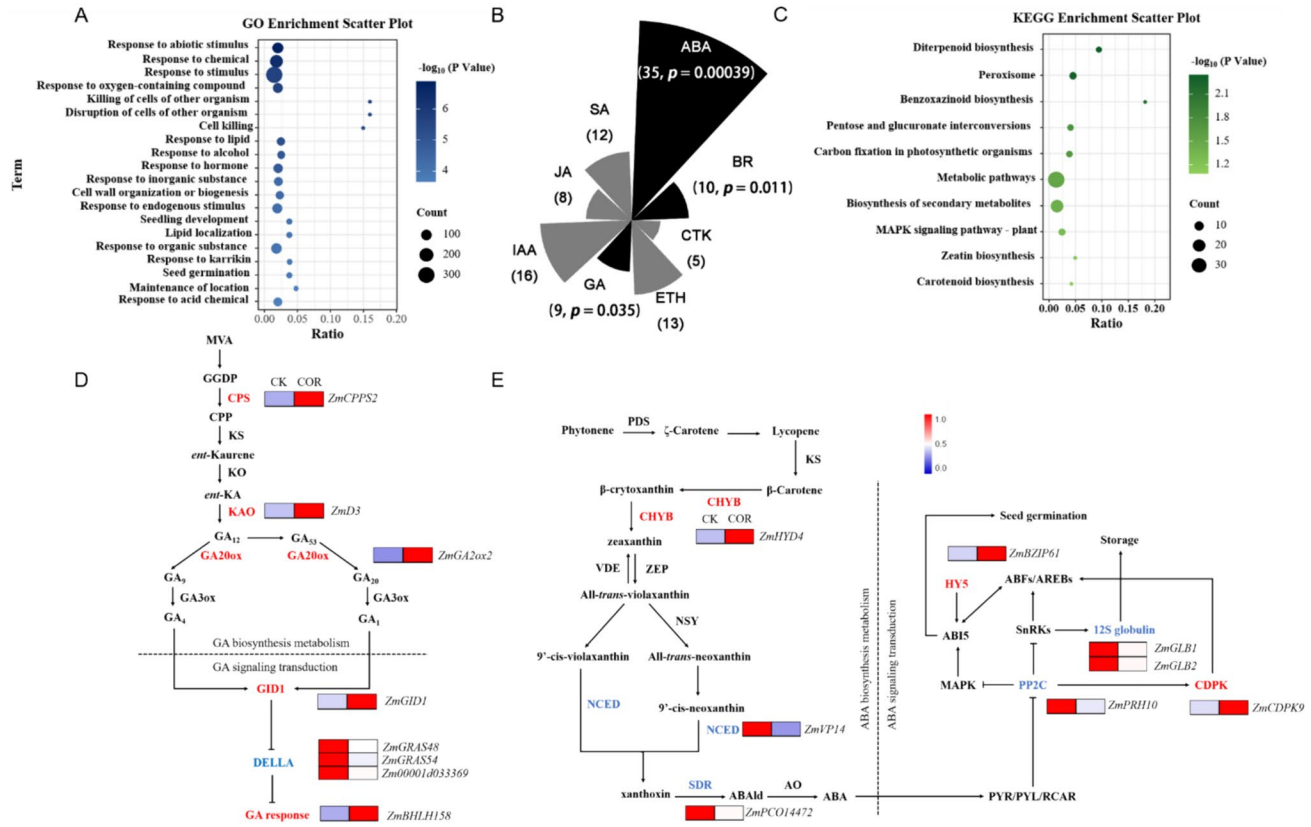

Fig. 4 Analysis of differentially expressed genes in COR-induced maize inbred line B73. **A** GO enrichment analysis of differentially expressed genes. **B** Quantitative analysis of hormone-related differentially expressed genes. Black indicates a *p*-value ≤ of 0.05, and grey indicates a *p*-value > 0.05. **C** KEGG enrichment analysis of differentially expressed genes. **D** Effect of COR on GA pathway genes. Red indicates up-regulation of the gene encoding the protein, and blue indicates down-regulation of the gene encoding the protein. Enzymatic reaction pathways are indicated by arrows. **E** Effect of COR on ABA pathway genes

genes associated with GA biosynthesis metabolism were up-regulated. Three genes encoding positive regulators of GA biosynthesis, *ZmCPPS2*, *ZmD3*, and *ZmGA2ox2*, were upregulated by 193.0%, 160.3%, and 253.8%, respectively. Previous GWAS results on corn seed germination showed that *ZmCPPS2*, *ZmD3*, and *ZmGA2ox2* were likely related to the regulation of GA on seed germination (Song et al. 2011). The gene *ZmGID1*, encoding a positive regulator in the GA signaling pathway, was upregulated by 137.4%. Within the bHLH transcription factor (TF) family, *Zmbhlh158*, *Zmbhlh154*, *Zmbhlh127*, *Zmbhlh104*, *Zmbhlh94*, *Zmili1*, and *ZmTIDP4580* were upregulated by 193.7%, 115.2%, 175.1%, 355.0%, 212.5%, 311.4%, and 146.4%, respectively. The overexpression of *Zmili1* enhances catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) activities (Dou et al. 2024), which is conducive to seed germination (Mitsuya et al. 2010; Liu et al. 2022). The GRAS family TFs, *ZmGRAS48*, *ZmGRAS54*, and *Zm00001d033369*, were downregulated by 49.8%, 52.9%, and 48.9%, respectively (Fig. 4D).

ABA plays an important role in many stages of the plant life cycle, including seed development and dormancy, as well as in plant responses to various environmental stresses (Seo and Koshiba 2002). Signaling our results showed that two positively regulated proteins encoding ABA biosynthesis, *ZmVP14* and *ZmPCO144726*, were downregulated by 70.2% and 48.7%, respectively (Fig. 4E). In addition, the expression of two genes (*ZmGLB1* and *ZmGLB2*) encoding embryo-specific storage proteins in ABA signaling pathways was down-regulated by 48.55% and 48.34%, respectively. These storage proteins are degraded during seed germination to provide energy for seed germination (Chibani et al. 2006). It was shown that COR could enhance the germination of aged maize seeds through ABA and GA signaling pathways.

Effects of ABA and GA on germination of aging seeds

To investigate whether the effect of COR on seed germination is related to the effect of ABA and GA, we conducted

treatment experiments with different concentrations of plant growth regulators. First, we used hybrid seeds of different varieties harvested from 2015 to 2019 for germination tests, and found that maize seeds of ZD958 in 2018 and 2019 germinated quickly, germination rate was close to 100% by 72 h, and primary root growth was very good; seeds of XY335 in 2017 had a higher germination rate and better root growth; seeds of QF6 in 2015 had a low germination rate and root growth was weak; XY335 (2018), QF689 (2015) and QF339 (2015), which are in the middle of germination rate and root growth, can be used as experimental materials for subsequent experiments to observe the effect of COR on seed viability of naturally aged maize seeds, considering the workload problem, we finally chose the slower germination, but still has about 65% germination rate after 72 h of QF689 (2015) (6 years after harvest, stored at 25°C and protected from light) as the material for subsequent experiments (Supplementary Fig. 3).

In this experiment, 0.001 µmol/L, 0.01 µmol/L, 0.1 µmol/L and 1 µmol/L of COR, 0.15 µmol/L (50 mg/L), 0.58 µmol/L (200 mg/L) and 0.87 µmol/L (300 mg/L) of GA, and 0.5 µmol/L of ABA were used as experimental group treatment, and water was used as the control group treatment. The germination test was conducted 24 h after soaking the seeds of QF689 (2015) with the above concentrations of growth regulators. The results of germination percentage at 24 h of germination and length of seed primordial roots and shoots at 48 and 72 h were counted as shown in Fig. 5A and Table S4.

The germination rate statistics of QF689 seeds germinated for 24 h showed that 0.5 µmol/L ABA inhibited seed germination (Fig. 5B), which reduced the germination rate by 27.3% (Table S4), and the treatment effect was close to that of 1 µmol/L COR. 0.58 µmol/L (200 mg/L) of GA promoted the germination of seeds, which increased the rate of germination by 22.7%, and the treatment effect was close to that of 0.001 µmol/L COR. The statistical results of the primary root length of seeds at 48 h (Fig. 5C) showed that low concentrations of COR (0.001–0.01 µmol/L) promoted the growth of primary roots of naturally aged QF689 seeds, in which both 0.15 µmol/L GA3 and 0.01 µmol/L COR significantly promoted the root growth, which resulted in a 12.4% and 14.8% increase in root length, respectively (Table S4). 1 µmol/L COR significantly inhibited the growth of primary roots at the early stage of germination, decreasing root length by 12.7%.

The statistical results of root length and shoot length of each treatment group at the 72nd hour of germination test showed that the treatments of GA (0.15 µmol/L, 0.58 µmol/L and 0.87 µmol/L) and low concentration of COR (0.001–0.01 µmol/L) showed longer root length and shoot length than that of the CK group as a whole (Fig. 5D, E). Among them, root length of 0.001 µmol/L COR, 0.01 µmol/L COR and 0.1 µmol/L COR treatment groups exceeded that of the control group by 22.3%, 26.5% and 21.5%, respectively. The 0.15 µmol/L GA treatment resulted in the optimum increase in root length and shoot length by 22.5% and 24.2%, respectively (Table S4). The 0.1 µmol/L COR treatment optimally increased root and shoot length by 21.5% and 32.2%, respectively. On the contrary, 0.5 µmol/L ABA treatment reduced root and shoot length significantly by 14% and 21.2%, respectively. The results also revealed that 1 µmol/L COR had an inhibitory effect on root length and a promotional effect on shoots, suggesting that shoots may be less sensitive to COR than roots.

Then we test the seeds under ABA + COR, the results were as expected, COR promoted seed germination, ABA inhibited seed germination, and the effect of the combination of COR + ABA was intermediate between the effect of their individual application (Supplementary Fig. 4 A–F). As shown in Supplementary Fig. 4C, by 48 h, 0.01 µmol/L COR treatment increased germination rates by 31.42% compared to the control (CK), while ABA concentrations of 0.1 µmol/L, 0.5 µmol/L, and 1 µmol/L inhibited germination rates by 40.00%, 35.72%, and 40.00%, respectively. Co-administration of 0.01 µmol/L COR with each ABA concentration notably boosted germination rates by 38.09%, 54.67%, and 37.14% compared to individual ABA treatments (Supplementary Fig. 4C). Furthermore, combined treatment with 0.01 µmol/L COR and ABA concentrations resulted in increases in root length by 106.16%, 77.95%, and 73.27% compared to individual ABA treatments (Supplementary Fig. 4E). At 72 h, 0.01 µmol/L COR continued to increase root length by 22.96% compared to CK, while 0.1 µmol/L ABA increased root length by 23.91%. Other treatments exhibited no significant effects on root length (Supplementary Fig. 4F). These findings suggest that 0.01 µmol/L COR mitigates the inhibitory impact of ABA on root elongation.

In summary, the results demonstrated that low concentrations of COR (0.001–0.1 µmol/L) acted similarly to low concentrations of GA_3 (0.15 µmol/L, 0.58 µmol/L), which not only promoted seed germination but also facilitated the growth of roots and shoots during the germination process. The effect of a high concentration of COR (1 µmol/L) was comparable to that of 0.5 µmol/L ABA, which could inhibit seed germination and the growth of roots and shoots during the germination process. Furthermore, low concentrations of COR (0.01 µmol/L) could mitigate the inhibitory effect of ABA on seed germination. This further suggests that COR can regulate seed germination by regulating the contents of GA and ABA.

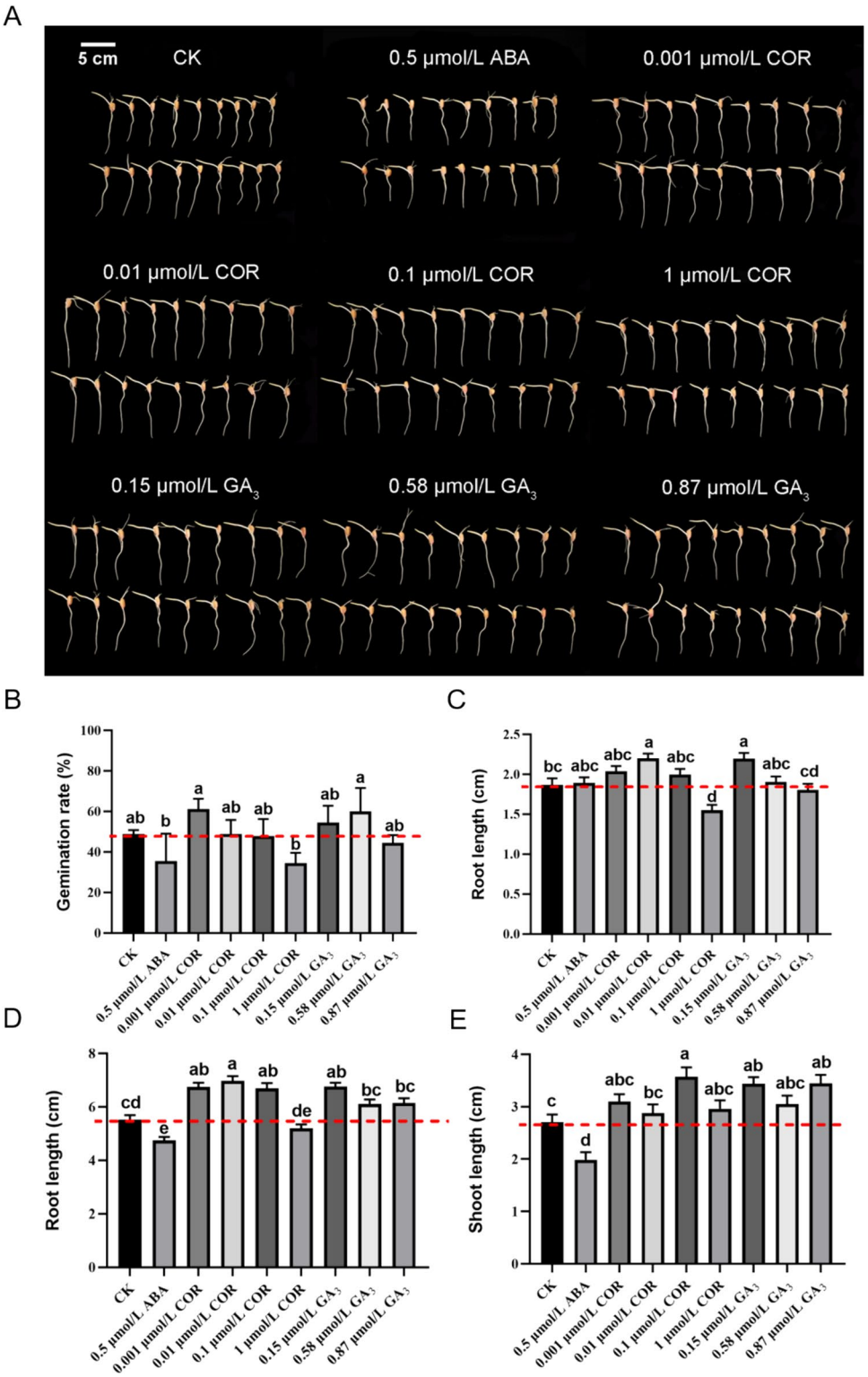

Fig. 5 Results of COR, ABA, and GA regulation on germination and the length of primary roots and shoots of QF689 seeds. **A** The phenotype of seed germination in nine treatment groups of CK, 0.5 μmol/L ABA, 1 μmol/L COR, 0.1 μmol/L COR, 0.01 μmol/L COR, 0.001 μmol/L COR, 0.15 μmol/L GA_3, 0.58 μmol/L GA_3 and 0.87 μmol/L GA_3 at 72 h of germination test. **B** Influence of different growth regulators on the 24 h germination percentage of QF689 (2015) seeds. Bars represent mean germination rates of three biological replicates, with SD values indicated. Each replicate comprises the mean germination rate of 30 maize seeds. **C** Average root length of all seeds in each treatment group at the 48 h of the germination test. The vertical error bars represent the standard deviation (SD) values (n = 70). **D** Average root length of seeds in each treatment group at 72 h of germination test. he vertical error bars represent the standard deviation (SD) values (n = 18). **E** Average shoot length of seeds in each treatment group at 72 h of germination test. The vertical error bars represent the standard deviation (SD) values (n = 18). Tukey test was used for multiple comparisons. Different lowercase letters indicate significance at the 0.05 probability level

Discussion

Promoting seed germination and cultivating flush and strong seedlings is one of the effective measures to improve corn yield (Han et al. 2023). Seed aging is one of the limiting factors for seed germination. However, the physiological and transcriptional mechanisms regulating the germination of maize seeds, especially aged seeds, remain unclear. As a new type of plant growth regulator, COR has been reported to promote the seed germination process. Therefore, this

study recorded the effects of COR on the germination of corn seeds with different aging degrees, combined with the acquisition of phytohormone content and transcriptome data, to analyze the physiological and transcriptional mechanisms of corn seeds, especially aging seeds.

Regulation of phytohormones in maize during seed germination progression

Seed germination is a complex physiological and biochemical process, which is affected by temperature, water, oxygen, and various small molecules, especially phytohormones (Xue et al. 2021). The level of ABA in maize seed was closely related to the process of seed germination (Matakiadis et al. 2009; Frey et al. 2012; Kim et al. 2013). In this study, 0.005 μmol/L COR treatment significantly promoted seed germination, and the ABA content decreased significantly during this process. In 497 DEGs, there were 9 ABA-related genes, and the expression profiles of 5 genes were consistent with the changes in ABA levels. Including 2 key genes (NCED and SDR) involved in ABA synthesis and signal transduction. NCED is a key enzyme involved in ABA biosynthesis, converting 9-cis-epoxy carotenoids to C15. SDR is also an important enzyme in ABA synthesis, which can reduce xanthin aldehyde to ABA aldehyde (Chen et al. 2020). In this study, the expression of one NCED gene (*ZmVP14*) and one SDR gene (*ZmPCO14472*) were down-regulated after COR treatment, and the biosynthesis of ABA was inhibited during seed germination (Fig. 4E).

Studies have confirmed that GA could break seed dormancy and promote seed germination by antagonizing the action of ABA (Gubler et al. 2005; Graeber et al. 2012). Elevated GA content or activation of the GA signaling pathway in seeds can promote seed germination. In this study, the content of GA increased significantly in the process of seed germination promoted by COR treatment The expression of three genes encoding GA biosynthesis (*ZmCPPS2*, *ZmD3*, and *ZmGA2ox2*) was significantly upregulated (Fig. 4D), suggesting that GA biosynthesis was activated during seed germination after COR treatment. GA regulates signal transduction by GID1-DELLA-SCFSLY1/GID2 (Lin et al. 2015). One GID1 gene (*ZmGID1*) was up-regulated after COR treatment, while the expression of three genes encoding DELLA protein (*ZmGRAS48*, *ZmGRAS54* and *Zm00001d033369*) was down-regulated. DELLA protein is a negative regulator of the GA signaling pathway.

In addition, the experimental results of different concentrations of COR, ABA, and GA on seed germination showed that the effect of a high concentration of COR on inhibiting seed germination was similar to ABA, while the effect of a low concentration of COR on promoting seed germination was similar to GA (Fig. 5). These results suggest that COR may regulate the germination process by affecting the synthesis and signal transduction pathways of ABA and GA (Fig. 6).

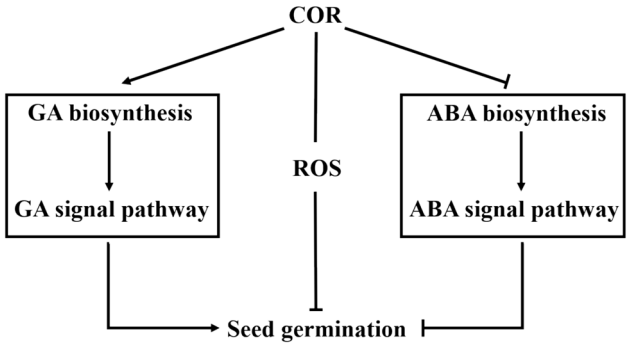

Fig. 6 Model graph of effects of COR treatment on seed germination. COR treatment promoted seed germination mainly by promoting GA synthesis and signaling, enhancing peroxidase accumulation and ROS scavenging, while inhibiting ABA synthesis and signaling. An arrow indicates a promotive effect and a bar a repressive effect

Promoting maize seed germination through other pathways

ROS produced in peroxisomes has a great negative effect on seed germination, and the removal of ROS can significantly promote seed germination (Mitsuya et al. 2010; Liu et al. 2022). Consistent with this, KEGG-enriched pathway "peroxisomes" were significantly enriched in seed germination-responsive genes induced by COR (Fig. 4B), in which COR treatment significantly induced the expression of several peroxisomal genes, *ZmAI795367*, and *ZmCAT2* (Data Set 4), which may help the plants to accumulate more peroxidases, thereby reducing the damage from seed aging-induced ROS bursts.

Studies have shown that plant secondary metabolites (e.g. terpenes, flavonoids, phenolic and polyphenolic compounds, benzoxazines) play important ecological roles in crop defense, attraction and stimulation (e.g. nutrient sequestration, pollination), and against abiotic stresses (Godlewska et al. 2022). Benzoxazines are important secondary compounds for plant defense against abiotic stresses (Brunetti et al. 2015; Makowska et al. 2015). Benzoxazines are not present in dried maize seeds (Cambier et al. 2000), but they have been found in abundance in seedlings after germination (Cambier et al. 2000; Köhler et al. 2015). In this study, COR seed dipping had a significant induction of benzoxazin biosynthesis in senescent seeds, such as the up-regulation of BX family genes *ZmBX5* and *ZmBX4* (Data Set 4), which are involved in benzoxazin biosynthesis. It is suggested that COR may resist senescence-induced stress by activating benzoxazine metabolism. In addition, COR has significant regulatory effects on other metabolic processes, such as the

interconversion of pentose and glucuronic acid, carbon fixation in photosynthetic organisms, and biosynthesis of secondary metabolites.

In summary, COR treatment promoted seed germination by elevating the expression level of genes encoding regulatory proteins in the process of GA synthesis, thereby increasing the content of GA and activating the GA signaling pathway. Meanwhile, COR treatment reduced the expression level of genes encoding the regulatory proteins of ABA biosynthesis and inhibited the synthesis of ABA, thereby decreasing the content of ABA and attenuating its effect of promoting seed dormancy. In addition, the COR treatment significantly induced the expression of multiple peroxisomal genes, facilitating the accumulation of more peroxidases in seeds, enhancing the capacity for ROS scavenging, thereby promoting seed germination. In essence, the COR treatment primarily regulates seed germination by enhancing the GA synthesis pathway, inhibiting the ABA synthesis pathway, and regulating ROS levels (Fig. 6).

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11103-024-01486-1.

Acknowledgements We would like to acknowledge the help of Ruihong Tang in the preliminary trial process.

Author contributions L.D. and F.Y. designed and conceived the experiments. Y.J., W.W., Z.W., Z.R., and L. L. carried out the experiments and analyzed and interpreted the data. Y.J., W.W., Z.W., and F.Y. prepared the manuscript. Y.Z., M.Z., and Z.L. conceived the study and participated in its design. L.D. and F.Y revised the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding This work was supported by the National Key Research & Development Program (Grant Number: 2023YFD1700600).

Data availability The original contributions presented in the study are publicly available. The transcriptome data can be found at: NCBI, BioProject, PRJNA1046141. Further inquiries can be directed to the corresponding author.

Declarations

Conflict of interest The authors declare no conflict of interest.

References

Ai L, Li Z, Li J, Tian X, Wang B, Zhai Z, Duan L (2008) Physiological effect of coronatine on seed germination of upland and lowland rice. Acta Agric Boreali-Sin 23(3):77–80

Arasimowicz M, Floryszak-Wieczorek J (2007) Nitric oxide as a bioactive signaling molecule in plant stress responses. Plant Sci 172(5):876–887. https://doi.org/10.1016/j.plantsci.2007.02.005

Bender CL, Alarcón-Chaidez F, Gross DC (1999) *Pseudomonas syringae* phytotoxins: mode of action, regulation, and biosynthesis by peptide and polyketide synthetases. Microbiol Mol Biol R 63(2):266–292. https://doi.org/10.1128/MMBR.63.2.266-292.1999

Bonner FT (1990) Storage of seeds: potential and limitations for germplasm conservation. Forest Ecol Manag 35(1–2):35–43. https://doi.org/10.1016/0378-1127(90)90230-9

Brunetti C, Guidi L, Sebastiani F, Tattini M (2015) Isoprenoids and phenylpropanoids are key components of the antioxidant defense system of plants facing severe excess light stress. Environ Exp Bot 119:54–62. https://doi.org/10.1016/j.envexpbot.2015.04.007

Cambier V, Hance T, de Hoffmann E (2000) Variation of DIMBOA and related compounds content in relation to the age and plant organ in maize. Phytochemistry 53(2):223–229. https://doi.org/10.1016/S0031-9422(99)00498-7

Chen K, Li GJ, Bressan RA, Song CP, Zhu JK, Zhao Y (2020) Abscisic acid dynamics, signaling, and functions in plants. J Integr Plant Biol 62(1):25–54. https://doi.org/10.1111/jipb.12899

Chen Y, Wang Q, Xiang Y (2019) Analysis of China's maize production status, advantages and self-sufficiency rate. Chinese J Agric Resour Reg Plann 40(01):7–16

Chibani K, Ali-Rachedi S, Job C, Job D, Jullien M, Grappin P (2006) Proteomic analysis of seed dormancy in *Arabidopsis*. Plant Physiol 142(4):1493–1510. https://doi.org/10.1104/pp.106.087452

Dou D, Sun J, Abou-Elwafa SF, Guo X, Guo Y, Wang D, Ding C, Alotaibi NM (2024) *ZmILI1* confers salt stress tolerance by regulating genes of phytohormone response in maize. Environ Exp Bot. https://doi.org/10.1016/j.envexpbot.2024.105673

Ellis RH (1992) Seed and seedling vigor in relation to crop growth and yield. Plant Growth Regul 11(3):249–255. https://doi.org/10.1007/BF00024563

Epstein E (1994) The anomaly of silicon in plant biology. Proc Natl Acad Sci 91(1):11–17. https://doi.org/10.1073/pnas.91.1.11

Florez M, Carbonell M, Martinez E (2007) Exposure of maize seeds to stationary magnetic fields: effects on germination and early growth. Environ Exp Bot 59(1):68–75. https://doi.org/10.1016/j.envexpbot.2005.10.006

Frey A, Effroy D, Lefebvre V, Seo M, Perreau F, Berger A, Sechet J, To A, North HM, Marion Poll A (2012) Epoxycarotenoid cleavage by NCED5 fine-tunes ABA accumulation and affects seed dormancy and drought tolerance with other NCED family members. Plant J 70(3):501–512. https://doi.org/10.1111/j.1365-313X.2011.04887.x

Gallardo K, Job C, Groot SPC, Puype M, Demol H, Vandekerckhove J, Job D (2001) Proteomic analysis of *Arabidopsis* seed germination and priming. Plant Physiol 126(2):835–848. https://doi.org/10.1104/pp.126.2.835

Gao Y, Ji G, Jiao J (2018) China maize seed market supply and demand analysis. China Seed Ind 37(10):4–8. https://doi.org/10.19462/j.cnki.1671-895x.20180930.019

Ghosh S, Chan CK (2016) Analysis of RNA-Seq data using Tophat and cufflinks. Methods Mol Biol 1374:339–361. https://doi.org/10.1007/978-1-4939-3167-5_18

Godlewska K, Pacyga P, Szumny A, Szymczycha-Madeja A, Wełna M, Michalak I (2022) Methods for rapid screening of biologically active compounds present in plant-based extracts. Molecules 27(20):7094. https://doi.org/10.3390/molecules27207094

Graeber K, Nakabayashi K, Miatton E, Leubner Metzger G, Soppe WJJ (2012) Molecular mechanisms of seed dormancy. Plant, Cell Environ 35(10):1769–1786. https://doi.org/10.1111/j.1365-3040.2012.02542.x

Greulichi F, Yoshihara T, Ichihara A (1995) Coronatine, a bacterial phytotoxin, acts as a stereospecific analog of jasmonate type signals in tomato cells and potato tißues. J Plant Physiol 147(3–4):359–366. https://doi.org/10.1016/S0176-1617(11)82168-1

Gubler F, Millar AA, Jacobsen JV (2005) Dormancy release, ABA and pre-harvest sprouting. Curr Opin Plant Biol 8(2):183–187. https://doi.org/10.1016/j.pbi.2005.01.011

Han Z, Jin Y, Wang B, Guo Y (2023) Multi-omics revealed the molecular mechanism of maize (*Zea mays* L.) seed germination

regulated by GA_3. Agronomy 13(7):1929. https://doi.org/10.3390/agronomy13071929
Hsu YF, Chen YC, Hsiao YC, Wang BJ, Lin SY, Cheng WH, Jauh GY, Harada JJ, Wang CS (2014) AtRH57, a DEAD-box RNA helicase, is involved in feedback inhibition of glucose-mediated abscisic acid accumulation during seedling development and additively affects pre-ribosomal RNA processing with high glucose. Plant J 77(1):119–135. https://doi.org/10.1111/tpj.12371
Hu J (1988) Evaluation of different accelerated aging methods for seeds. Seed World 6(09):23–26
Hussain HA, Hussain S, Khaliq A, Ashraf U, Anjum SA, Men S, Wang L (2018) Chilling and drought stresses in crop plants: implications, cross talk, and potential management opportunities. Front Plant Sci 9:393. https://doi.org/10.3389/fpls.2018.00393
Ichihara A, Shiraishi K, Sato H, Sakamura S, Nishiyama K, Sakai R, Furusaki A, Matsumoto T (1977) The structure of coronatine. J Am Chem Soc 99(2):636–637. https://doi.org/10.1021/ja00444a067
Kijak H, Ratajczak E (2020) What do we know about the genetic basis of seed desiccation tolerance and longevity? Int J Mol Sci 21(10):3612. https://doi.org/10.3390/ijms21103612
Kim D, Paggi JM, Park C, Bennett C, Salzberg SL (2019) Graph-based genome alignment and genotyping with HISAT2 and HISAT-genotype. Nat Biotechnol 37(8):907–915. https://doi.org/10.1038/s41587-019-0201-4
Kim W, Lee Y, Park J, Lee N, Choi G (2013) HONSU, a protein phosphatase 2C, regulates seed dormancy by inhibiting ABA signaling in *Arabidopsis*. Plant Cell Physiol 54(4):555–572. https://doi.org/10.1093/pcp/pct017
Koda Y, Takahashi K, Kikuta Y, Greulich F, Toshima H, Ichihara A (1996) Similarities of the biological activities of coronatine and coronafacic acid to those of jasmonic acid. Phytochemistry 41(1):93–96. https://doi.org/10.1016/0031-9422(95)00517-X
Köhler A, Maag D, Veyrat N, Glauser G, Wolfender JL, Turlings TCJ, Erb M (2015) Within-plant distribution of 1,4-benzoxazin-3-ones contributes to herbivore niche differentiation in maize. Plant, Cell Environ 38(6):1081–1093. https://doi.org/10.1111/pce.12464
Leubner-Metzger G (2003) Functions and regulation of β-1,3-glucanases during seed germination, dormancy release and after-ripening. Seed Sci Res 13(1):17–34. https://doi.org/10.1079/SSR2002121
Li M, Liu X, Tao B (2008) Effect of protective agent soaker on germination of maize seeds and their physiological properties. J Northeast Agric Univ 52(09):21–24. https://doi.org/10.19720/j.cnki.issn.1005-9369.2008.09.005
Li Z, Lu X, Chen J (2023) Gasotransmitter ammonia accelerates seed germination, seedling growth, and thermotolerance acquirement in maize. Plant Sign Behav 18(1):2163338. https://doi.org/10.1080/15592324.2022.2163338
Lin Q, Wu F, Sheng P, Zhang Z, Zhang X, Guo X, Wang J, Cheng Z, Wang J, Wang H, Wan J (2015) The SnRK2-APC/CTE regulatory module mediates the antagonistic action of gibberellic acid and abscisic acid pathways. Nat Commun 6(1):7981–7981. https://doi.org/10.1038/ncomms8981
Liu W, Chang Y, Chen SC, Lu C, Wu Y, Lu MJ, Chen D, Shih AC, Sheue C, Huang H, Yu C, Lin H, Shiu S, Sun-Ben KuM, Li W (2013) Anatomical and transcriptional dynamics of maize embryonic leaves during seed germination. Proc Natl Acad Sci USA 110(10):3979–3984. https://doi.org/10.1073/pnas.1301009110
Liu Z, Li Z, Wu S, Yu C, Wang X, Wang Y, Peng Z, Gao Y, Li R, Shen Y, Duan L (2022) Coronatine enhances chilling tolerance of tomato plants by inducing chilling-related epigenetic adaptations and transcriptional reprogramming. Int J Mol Sci 23(17):10049. https://doi.org/10.3390/ijms231710049
Luo X, Dai Y, Song Y, Lv H, Yong T, Wang X, Liu W, Shu K (2021) Effect of three plant growth regulators on growth and development and yield of soybean. J Nucl Agric Sci 35(04):980–988
Makowska B, Bakera B, Rakoczy-Trojanowska M (2015) The genetic background of benzoxazinoid biosynthesis in cereals. Acta Physiol Plant 37(9):1. https://doi.org/10.1007/s11738-015-1927-3
Martin RC, Liu PP, Goloviznina NA, Nonogaki H (2010) MicroRNA, seeds, and darwin?: Diverse function of miRNA in seed biology and plant responses to stress. J Exp Bot 61(9):2229–2234. https://doi.org/10.1093/jxb/erq063
Matakiadis T, Alboresi A, Jikumaru Y, Tatematsu K, Pichon O, Renou J, Kamiya Y, Nambara E, Truong H (2009) The *Arabidopsis* abscisic acid catabolic gene *CYP707A2* plays a key role in nitrate control of seed dormancy. Plant Physiol 149(2):949–960. https://doi.org/10.1104/pp.108.126938
McCarty DR, Hattori T, Carson CB, Vasil V, Lazar M, Vasil IK (1991) The *Viviparous*-1 developmental gene of maize encodes a novel transcriptional activator. Cell 66(5):895–905. https://doi.org/10.1016/0092-8674(91)90436-3
Mitsuya S, El-Shami M, Sparkes IA, Charlton WL, De Marcos LC, Johnson B, Baker A (2010) Salt stress causes peroxisome proliferation, but inducing peroxisome proliferation does not improve NaCl tolerance in *Arabidopsis thaliana*. PLoS ONE 5(2):e9408. https://doi.org/10.1371/journal.pone.0009408
Mittler R, Vanderauwera S, Gollery M, Van Breusegem F (2004) Reactive oxygen gene network of plants. Trends Plant Sci 9(10):490–498. https://doi.org/10.1016/j.tplants.2004.08.009
NBOS China (2023) Statistical bulletin of the people's republic of China on national economic and social development, 2022. China Inform News 03:12–29. https://doi.org/10.38309/n.cnki.nzgxx.2023.000173
Onrubia M, Moyano E, Bonfill M, Cusidó RM, Goossens A, Palazón J (2013) Coronatine, a more powerful elicitor for inducing taxane biosynthesis in *Taxus* media cell cultures than methyl jasmonate. J Plant Physiol 170(2):211–219. https://doi.org/10.1016/j.jplph.2012.09.004
Posmyk MM, Bałabusta M, Wieczorek M, Sliwinska E, Janas KM (2009) Melatonin applied to cucumber (*Cucumis sativus* L.) seeds improves germination during chilling stress. J Pineal Res 46(2):214–223. https://doi.org/10.1111/j.1600-079X.2008.00652.x
Qiao Y, Gao P, Ma J, Zhou J, Ma J (2003) Study on physiological characteristics and seed activity changes of two maize inbreds in aging course. Acta Agron Sin 54(01):123–127
Salah SM, Yajing G, Dongdong C, Jie L, Aamir N, Qijuan H, Weimin H, Mingyu N, Jin H (2015) Seed priming with polyethylene glycol regulating the physiological and molecular mechanism in rice (*Oryza sativa* L.) under nano-ZnO stress. Sci Rep 5(1):14278. https://doi.org/10.1038/srep14278
Sarropoulou V, Dimassi-Theriou K, Therios I, Koukourikou-Petridou M (2012) Melatonin enhances root regeneration, photosynthetic pigments, biomass, total carbohydrates and proline content in the cherry rootstock PHL-C (*Prunus avium* × *Prunus cerasus*). Plant Physiol Bioch 61:162–168. https://doi.org/10.1016/j.plaphy.2012.10.001
Schüler G, Mithöfer A, Baldwin IT, Berger S, Ebel J, Santos JG, Herrmann G, Hölscher D, Kramell R, Kutchan TM, Maucher H, Schneider B, Stenzel I, Wasternack C, Boland W (2004) Coronalon: a powerful tool in plant stress physiology. Febs Lett 563(1–3):17–22. https://doi.org/10.1016/S0014-5793(04)00239-X
Seo M, Koshiba T (2002) Complex regulation of ABA biosynthesis in plants. Trends Plant Sci 7(1):41–48. https://doi.org/10.1016/s1360-1385(01)02187-2
Shine MB, Guruprasad KN, Anand A (2012) Effect of stationary magnetic field strengths of 150 and 200 mT on reactive oxygen species

production in soybean. Bioelectromagnetics 33(5):428–437. https://doi.org/10.1002/bem.21702
Shiraishi K, Konoma K, Sato H, Ichihara A, Sakamura S, Nishiyama K, Sakai R (2014) The structure-activity relationships in coronatine analogs and amino compounds derived from (+)-coronafacic acid. Agric Biol Chem 43(8):1753–1757. https://doi.org/10.1080/00021369.1979.10863680
Song J, Guo B, Song F, Peng H, Yao Y, Zhang Y, Sun Q, Ni Z (2011) Genome-wide identification of gibberellins metabolic enzyme genes and expression profiling analysis during seed germination in maize. Gene 482(1–2):34–42. https://doi.org/10.1016/j.gene.2011.05.008
Sun Q, Zhang N, Wang J, Cao Y, Li X, Zhang H, Zhang L, Tan DX, Guo YD (2016) A label-free differential proteomics analysis reveals the effect of melatonin on promoting fruit ripening and anthocyanin accumulation upon postharvest in tomato. J Pineal Res 61(2):138–153. https://doi.org/10.1111/jpi.12315
Sun Y, Xu J, Miao X, Lin X, Liu W, Ren H (2021) Effects of exogenous silicon on maize seed germination and seedling growth. Sci Rep-Uk 11(1):1014–1014. https://doi.org/10.1038/s41598-020-79723-y
Tamogami S, Kodama O (2000) Coronatine elicits phytoalexin production in rice leaves (*Oryza sativa* L.) in the same manner as jasmonic acid. Phytochemistry 54(7):689–694. https://doi.org/10.1016/s0031-9422(00)00190-4
Team RC (2022) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/
Wang B, Li Z, Zhai Z, Duan L (2006) Coronatine and its physiological functions. Plant Physiol J 56(3):503–510. https://doi.org/10.13592/j.cnki.ppj.2006.03.043
Wang B, Li Z, Duan L, Zhai Z (2008) Effect of coronatine and methyl jasmonate on maize seed germination and seedling growth. Mod Agric Sci Technol 37(12):163–166
Weitbrecht K, Müller K, Leubner-Metzger G (2011) First off the mark: early seed germination. J Exp Bot 62(10):3289–3309. https://doi.org/10.1093/jxb/err030
Xie Z, Li M, Du M, Li Z, Tian X, Duan L (2012) Ameliorating effect of the phytotoxin coronatine on seedlings of transgenic insect-resistant cotton variety under salt stress. Cotton Sci 24(06):511–517
Xue X, Du S, Jiao F, Xi M, Wang A, Xu H, Jiao Q, Zhang X, Jiang H, Chen J, Wang M (2021) The regulatory network behind maize seed germination: effects of temperature, water, phytohormones, and nutrients. Crop J 9(4):718–724. https://doi.org/10.1016/j.cj.2020.11.005
Yamaguchi S (2008) Gibberellin metabolism and its regulation. Annu Rev Plant Biol 59(1):225–251. https://doi.org/10.1146/annurev.arplant.59.032607.092804
Zhao C, Zhong Y (2016) The challenges and solutions of corn overstocking in China. Res Agric Modern 37(05):824–830. https://doi.org/10.13872/j.1000-0275.2016.0088
Zhu N, Min H, Chen M, Zhao Y (1996) The study of determination on TTC-dehydrogenase activity. China Biogas 14(02):3–5