BMC Plant Biology

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Ultrasonic treatment can improve maize seed germination and abiotic stress resistance

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Abstract

Constant-frequency ultrasonic treatment helped to improve seed germination. However, variable-frequency ultrasonic treatment on maize seed germination were rarely reported. In this study, maize seeds were exposed to 20–40 kHz ultrasonic for 40 s. The germination percentage and radicle length of maize seeds increased by 10.4% and 230.5%. Ultrasonic treatment also significantly increased the acid protease, α-amylase, and β-amylase contents by 96.4%, 73.8%, and 49.1%, respectively. Transcriptome analysis showed that 11,475 differentially expressed genes (DEGs) were found in the ultrasonic treatment and control groups, including 5,695 upregulated and 5,780 downregulated. Metabolic pathways and transcription factors (TFs) were significantly enriched among DEGs after ultrasonic treatment. This included metabolism and genetic information processing, that is, ribosome, proteasome, and pyruvate metabolism, sesquiterpenoid, triterpenoid, and phenylpropanoid biosynthesis, and oxidative phosphorylation, as well as transcription factors in the NAC, MYB, bHLH, WRKY, AP2, bZIP, and ARF families. Variable-frequency ultrasonic treatment increased auxin, gibberellin, and salicylic acid by 5.5%, 37.3%, and 28.9%, respectively. Abscisic acid significantly decreased by 33.2%. The related DEGs were upregulated and downregulated to varying degrees. Seed germination under the abiotic stress conditions of salt stress (NaCl solution), drought (PEG solution), and waterlogging (water-saturated sand bed) under ultrasonic treatment were promoted, radicle length was significantly increased by 30.2%, 30.5%, and 27.3%, respectively; and germination percentage by 14.8%, 20.1%, and 21.6%, respectively. These findings provide new insight into the mechanisms through ultrasonic to promote maize seed germination.

Keywords Maize, Ultrasonic, Seed germination, Transcriptome, Abiotic stress

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Introduction

Maize (*Zea mays* L.) is one of the world's major food crops and an important pharmaceutical and industrial raw material [1]. A stable increase in maize production is crucial for economic development and agricultural production. Seed germination is a key stage in the crop growth cycle and an important indicator affecting crop yield [2]. Seeds with high germination percentage allow more plants to establish, increasing crop yield [3]. The physiological response of seeds before germination is activated and can be used for seedling growth.

Owing to the advantages of environmental protection, low cost, and high speed, physical methods to improve seed germination percentage have attracted increasing attention in modern agriculture, including high pressure [4], ultrasonic [5], ultraviolet light [6], magnetic fields [7], and electromagnetic waves [8]. High pressure induces amorphization and promotes starch hydrolysis during germination. Ultraviolet light treatment can stimulate the seed coat to produce cracks, improve the metabolic activity of the cells, and effectively enhance seed germination. The activities of α-amylase, dehydrogenase and protease in seeds can be enhanced by magnetic field and electromagnetic wave, thus promoting seed germination. Ultrasonic treatment can create mechanical pressure on the seed, resulting in some pores on the surface of the seed, creating more opportunities for water and oxygen absorption [9]. The elastic mechanical wave induced by ultrasonic treatment has a positive effect on the structure and function of plant cells, the metabolic activities of plants, and the enzymes and physiological reactions that promote cell division. These changes help to improve cell viability and accelerate plant growth and development [10]. In addition, the treatment of seeds by ultrasonic also increased the permeability of cell membranes, promoted the expansion of seeds and the decomposition of starch, thereby increasing crop yield by increasing the number of grains [11].

Ultrasonic treatment can regulate the germination energy and metabolism, thereby increasing the seed germination percentage. Ultrasonic treatment promotes seed germination and seedling development, where germination related enzymes, such as α-amylase, have increased activity after ultrasonic treatment [12]. Transcriptome analysis showed that differentially expressed genes (DEGs) in ultrasonic treatment were significantly enriched in plant hormone signal transduction and transcription factors (TFs), and the contents of salicylic acid (SA) and abscisic acid (ABA) were significantly increased [13]. Ultrasonic can promote the germination of *Pinus tabuliformis* seeds through lipid metabolism. It induces changes in cellular ultrastructure, which enhances the release of cell wall enzymes and biochemical metabolism, further increasing germination and emergence rates [14]. Therefore, ultrasonic produces physical stimulation of the seed coat and plant cells through mechanical action and can regulate seed germination and seedling growth through signal transduction, metabolism, and TF.

Seed germination is the initial stage of the plant life cycle and the most sensitive to abiotic stresses. Salt, drought and waterlogging stresses are the most common abiotic stress problems affecting seed germination. The seed germination percentage plays a decisive role in crop yield. Salinity can inhibit seed germination because the osmotic effect prevents the seeds from absorbing water, or because the toxic substances have a negative impact on the germinated seeds. Drought stress limits germination and growth by directly affecting the absorption of water by seeds and indirectly affecting physiological and biochemical processes (enzyme activity, hormone levels, antioxidant substances, etc.). Waterlogging stress reduces oxygen availability, limiting normal seed respiration and inhibiting seed germination. This study evaluated the germination of maize seeds treated with variable-frequency ultrasonic under salt, drought and waterlogging stress.

Further studies have been conducted on the use of fixed-frequency ultrasonic waves to improve seed germination [15–17]. However, there have been few reports on the effects of variable-frequency ultrasonic treatment on maize seed germination from a transcriptomic perspective. We hypothesized that (1) variable-frequency ultrasonic can increase the content of hydrolase, promote the hydrolysis of macromolecular substances, and provide energy for seed germination; (2) variable-frequency ultrasonic promotes seed germination and adaptability to abiotic stresses by regulating multiple pathways such as plant hormones, metabolism and genetics. The findings can play a key role in deepening our understanding of the mechanisms of variable-frequency ultrasonic regulation in maize seed germination.

Materials and methods

Experimental setup

The maize seeds *ZD958* (*Zea mays* L.) were obtained from a seed distributor in Beijing city of China and used for all studies. Seeds were placed on a stainless-steel plate in a tunnel ultrasonic processor (5ZCG-T6; Guangzhou Jindao Agricultural Technology Co., Ltd., Guangzhou, China), and then ultrasonic treated for 10 s to 60 s at room temperature (20–25℃). In this experiment, ultrasonic waves with variable frequencies (20–40 kHz) were used to treat maize seeds and that without ultrasonic treatment were used as CK. The germination of seeds was tested immediately after ultrasonic treatment. Seven days after sowings, the ultrasonic treatment with the highest germination percentage and the longest radicle length was named as US.

Seed germination

Seeds were soaked in 1% NaClO for 10 min and then rinsed with distilled water for 30 s (repeated three times). One hundred seeds per treatment, repeated three times. Seeds were placed in trays and the experiment was conducted in a climatic chamber (day / night temperature: 25 °C / 20 °C; photoperiod: 12 h). The percentage of germinated seeds in 100 seeds was calculated at 3, 5 and 7 d after sowing. When a white embryo protuberance was observed, the seed was considered to have germinated [18]. Germination percentage were calculated using the following formula:

$G = a / b \times 100$.

G is the germination percentage (%), a is the number of germinated seeds, and b is the total number of seeds provided in the experiment. The acid protease, α-amylase, and β-amylase content were determined using a commercial kit (Suzhou Grace Biotechnology China). At 7th day after sowing, ultrasonic samples and control samples were collected, quickly frozen in liquid nitrogen, and ground into powder. Each sample (100 mg) was dissolved in phosphate-buffered saline. The mixture was then vortexed for 10 min and centrifuged for 5 min at 12,000 rpm and 4 °C. Enzyme activity of the supernatants was assayed at the indicated wavelengths using a 96-well microplate reader (Thermo Scientific, Pittsburg, PA, USA) according to the manufacturer's instructions.

Detection of plant hormones

7 days after sowing, phytohormones were detected by LC-MS / MS in seedlings treated with variable frequency ultrasound (20–40 kHz, 40 s) and those without ultrasound treatment. Seedling samples from both groups were pulverized, freeze-dried, and then 500 mg of the samples were added into 10 mL centrifuge tubes. Then 5 mL of extraction solution (methanol: water: formic acid; 15: 4: 1; 0.5% BHT) was added and milled for 5 min. Ultrasonic oscillation was conducted for 30 min before the samples were left to stand at −4 °C for 60 min. Centrifugation was performed at 12,000 rpm for 10 min, and the supernatant was then removed.

The plant hormone extracts were separated using ultra-high-pressure liquid chromatography (Waters Acquity UPLC; Allwegene, China). Mobile Phase A was water (0.05% formic acid), and Mobile Phase B was acetonitrile (0.05% formic acid). The column temperature (40 °C), mobile phase flow rate (300 μL/min). The run time (10 min), injection volume (6 μL), and sample gradient elution program were as follows: the gradient ratios of Mobile Phase B were: 0–1 min, 10%; 1–7 min, 70%; 7–8 min, 70%; 8–9 min, 10%; and 9–10 min, 10%. Mass spectrometry was performed using a 5500 Qtrap-MS system (AB SCIEX). Source temperature: 450 °C, ion source gas 1 (GAS1): 55 arb, ion source gas 2 (GAS2): 55 arb, curtain gas (CUR): 35 arb, ion spray voltage float (IonSpray voltage): 4500 V, and multiple reaction monitoring (MRM).

RNA isolation and sequencing

7 days after sowing, total RNA was extracted from three independent biological replicates of seedlings. RNA extraction was conducted using TRIzol reagent (Tiangen Biotech, Beijing, China). For RNA-seq library preparation, the NEBNext® Ultra™ RNA Library Prep Kit for Illumina® (NEB, USA) was used according to the operating instructions. Subsequently, the libraries were subjected to deep sequencing on an Illumina HiSeq 4000 platform by Beijing Allwegene Technology Co., Ltd. (Beijing, China). Raw data (raw reads) were processed to remove reads containing adapter sequences, poly-N, and low-quality (Q score < 20) reads. The generated clean reads were then mapped to the assembled maize genome using STAR (v2.5.2b) with default parameters. Fragments per kilobase per million (FPKM) were used to calculate gene expression levels. The two groups (CK and US) were analyzed for differential expression using the DESeq R software package (1.10.1). Genes exhibiting DEGs were identified based on a Benjamini–Hochberg adjusted $p < 0.05$. Gene functions were annotated based on the Gene Ontology (GO) [19] and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases [20] The differentially expressed genes (DEGs) were clustered by GO terms and KEGG enrichment analysis using the GO seq R package (version 1.22) and the KOBAS software (version 2.0.12).

Abiotic stress

Abiotic stress treatments were as follows: (1) salt treatment: 100 mmol/L NaCl solution [21]; (2) drought treatment: PEG 6000 (15%) solution [22]; (3) waterlogging: sand beds saturated with water holding capacity [23]. Each abiotic stress treatment group had 100 seeds, and three replicates were set. The environmental conditions for seed germination are the same as (Sect. 2.2). Abiotic stresses continued from planting until the end of the experiment (ensure that the tray remains moist and pick out moldy seeds). The germination percentage and radicle length of each treatment group were recorded on the 3, 5 and 7 d after seed sowing.

Statistical analysis

Data were analyzed using SPSS 21 (IBM Corp. Armonk, NY, USA) and the $2^{-\Delta\Delta Ct}$ method. Data were analyzed using a two-tailed Student's t-test for single comparisons. Differences between experimental treatments were evaluated using the least significant difference test (LSD) at $p < 0.05$. GraphPad Prism 9 (GraphPad Software, USA) and Origin 2021 (Origin Lab, USA) were used to create graphs.

Results

Effect of US on seed germination, amylase, and acid proteases

Maize seeds were grown for 7 days (Fig. 1). Figure 2A shows that germination percentage increased with time, and the germination percentage of 94.1% was obtained at 7th day after sowing in US (40 s). At the 3rd day, the highest germination percentage was 71.9% under US for 40 s, compared to CK (35.8%). At the 5th day, the germination percentage was 93.7%in US (40 s), compared to Control (72.0%). At the 7th day, compared to CK (83.7%), the germination percentage in US (40 s) was significantly increased by 10.4%. At the 3rd day, the radicle length in US (40 s) was 10.3 mm, whereas CK was 4 mm. At the 5th day, the radicle length was 34.9 mm in US (40 s), whereas the radicle length in CK was 11.2 mm. At the 7th day, the radicle length was 82.9 mm in US (40 s). This represents a significant increase of 230.5% compared with 25.1 mm in CK (Fig. 2B).

US increased the amylase and acid protease activities during maize seed germination (Fig. 2C). Compared to CK, the acidic proteinase (ACP) activity in US significantly increased by 96.4%. After ultrasonic treatment, the activity regulation of α-amylase (α-AL) and β-amylase (β-AL) demonstrated certain differences. Compared with CK, the activity of α-AL increased by 73.8% in US, showcasing higher enzyme activity. Meanwhile, β-AL also experienced a significant increase of 49.1% in US compared with CK.

Transcriptome profiling of US and CK

7 days after seed sowing, maize from the ultrasonic treatments (40 s) (US1, US2, and US3) and control treatments (CK1, CK2, and CK3) was used for RNA-Sep analysis. The results from the sequencing data showed that filtering sequences with adapters and low-quality reads resulted in a total of 47.19 G clean reads. More than 94.7% of the total reads were localized to reference sequences in the maize genome. This indicated that the sequencing output data were of sufficient quality for subsequent bioinformatic analyses. PCA showed that the three repeated sample groups were extremely similar for both US and CK, but the groups of the two treatments were significantly separated (Fig. 3A). At the same time, correlation analysis indicated distinctly different relationships (Fig. 3B).

DEGs in US and CK

To screen for candidate genes involved in maize seed germination, DEGs in US were selected by $|\log_2 FC| > 1$ compared to CK, which detected 11,475 DEGs (Fig. 3C). In total, 5,695 upregulated and 5,780 downregulated genes were identified after ultrasonic treatment (Fig. 3D).

The 11,475 DEGs were categorized into three GO classifications, that is, biological processes, molecular functions, and cellular components (Fig. 4A). The top ten GO enrichments for three groups included cellular components, cellular anatomical entities, intracellular and biological processes, cellular processes, metabolic processes, molecular functions, binding, and catalytic activity.

The top 20 enriched terms for KEGG pathway terms are shown in the innermost circle of the circular enrichment diagram (Fig. 4B). The second circle shows the upregulated and downregulated genes and the third circle shows the background gene number in the pathway and the Q values. The outermost circle represents the value of the enrichment factor for each pathway. The most enriched KEGG terms were ribosome (zma03010), proteasome (zma03050), pyruvate metabolism (zma009620), phenylpropanoid biosynthesis (zma00940), sesquiterpenoid and triterpenoid biosynthesis (zma00909), oxidative

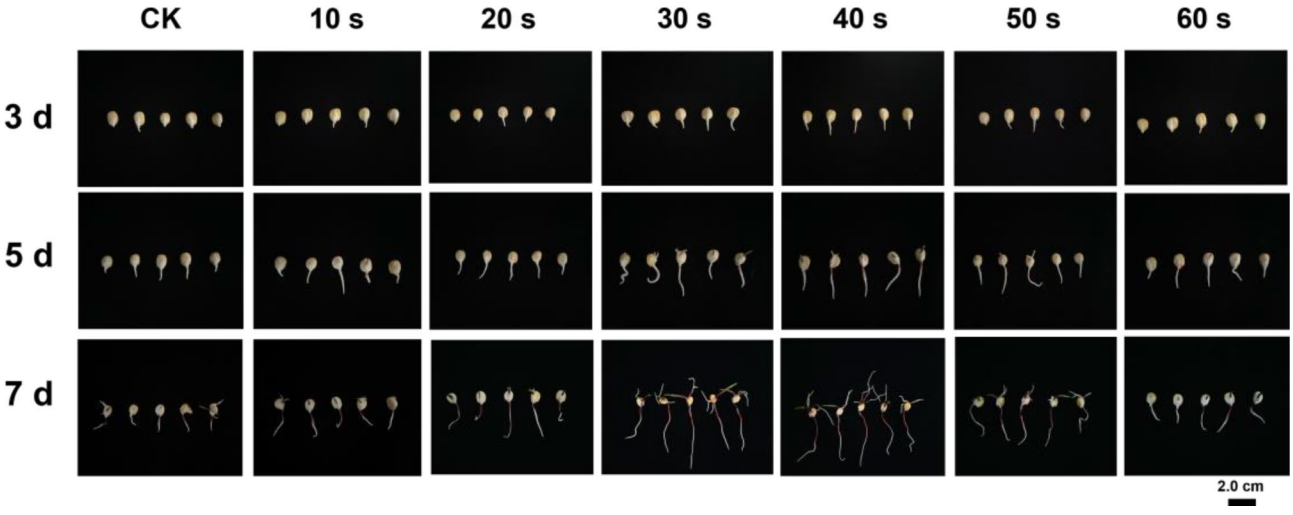

Fig. 1 Different stages of maize germination. The CK and US with ultrasonic time (10 s, 20 s, 30 s, 40 s, 50 s, and 60 s)

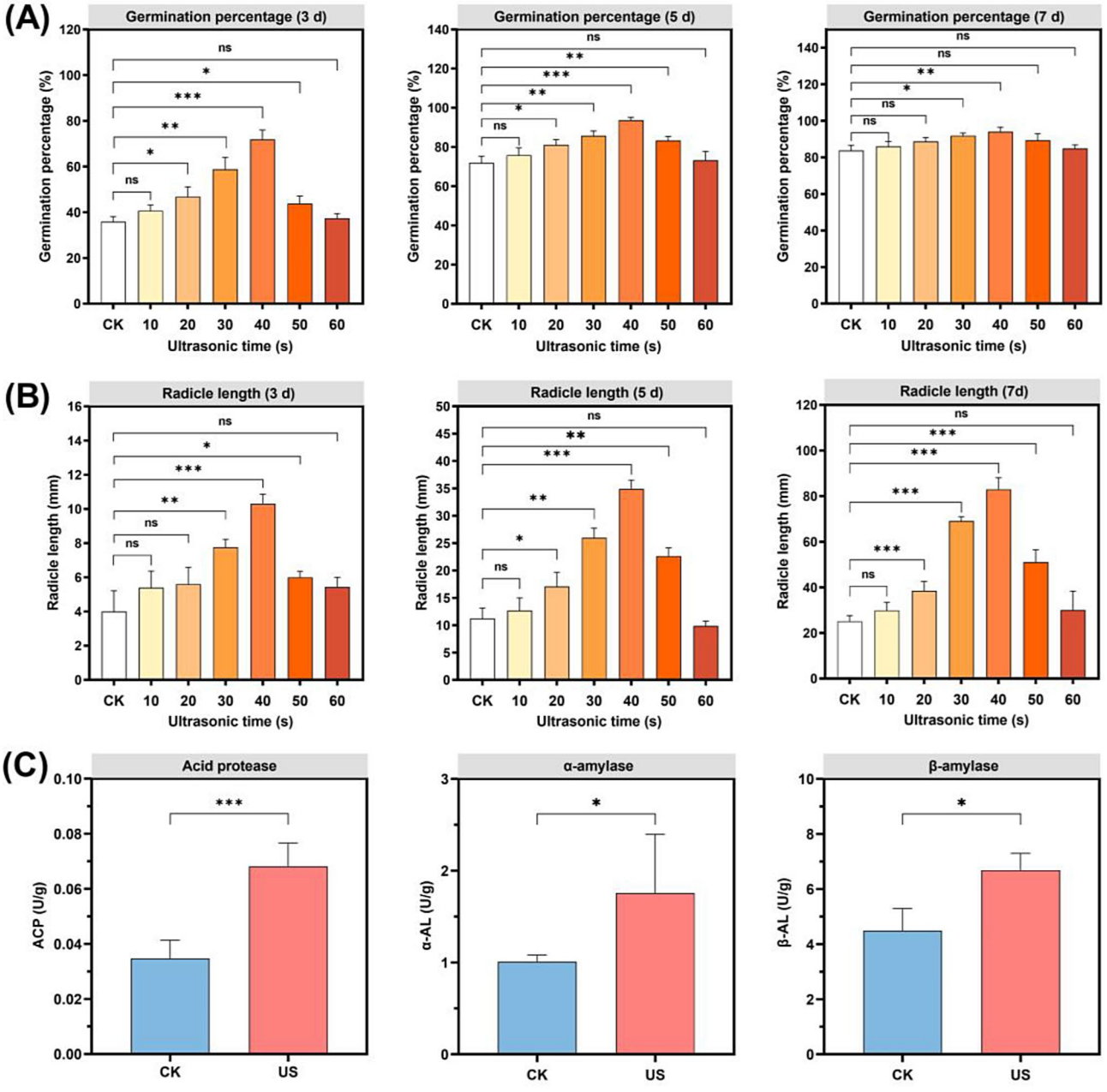

Fig. 2 (**A**) Effect of ultrasonic treatment (0–60 s) on maize germination at different time after sowing (3 d, 5 d, and 7 d). (**B**) Effects of ultrasonic treatment (0–60 s) on radicle length at different time after sowing (3 d, 5 d, and 7 d). (**C**) The content of acid protease (ACP), activity of α-amylase (α-AL) and β-amylase (β-AL) at 7th day. *$p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

phosphorylation (zma00190), and glycosaminoglycan degradation (zma00531).

TF analysis

TF plays a regulatory role in seed germination. Therefore, we analyzed the major TFs involved in maize seed germination. Figure 4C shows the family of maize TFs that were subjected to ultrasonic treatment. The factor N-acetylcysteine (NAC) was enriched for 75 genes (56 up-regulated expression, 19 down-regulated expression). Myeloblastosis-related genes (MYB) was enriched for 69 genes (49 up-regulated expression, 20 down-regulated expression). In basic helix-loop-helix (bHLH) was enriched for 52 genes (26 for both up- and down-regulated expression). WRKY domain containing proteins included 48 genes (46 up-regulated, 2 down-regulated genes). 44 genes were enriched in apetala2/ethylene-response factor (AP2), including 30 up-regulated, 14 down-regulated genes. 41 genes (25 up-regulated genes, 16 down-regulated genes) were enriched in basic leucine

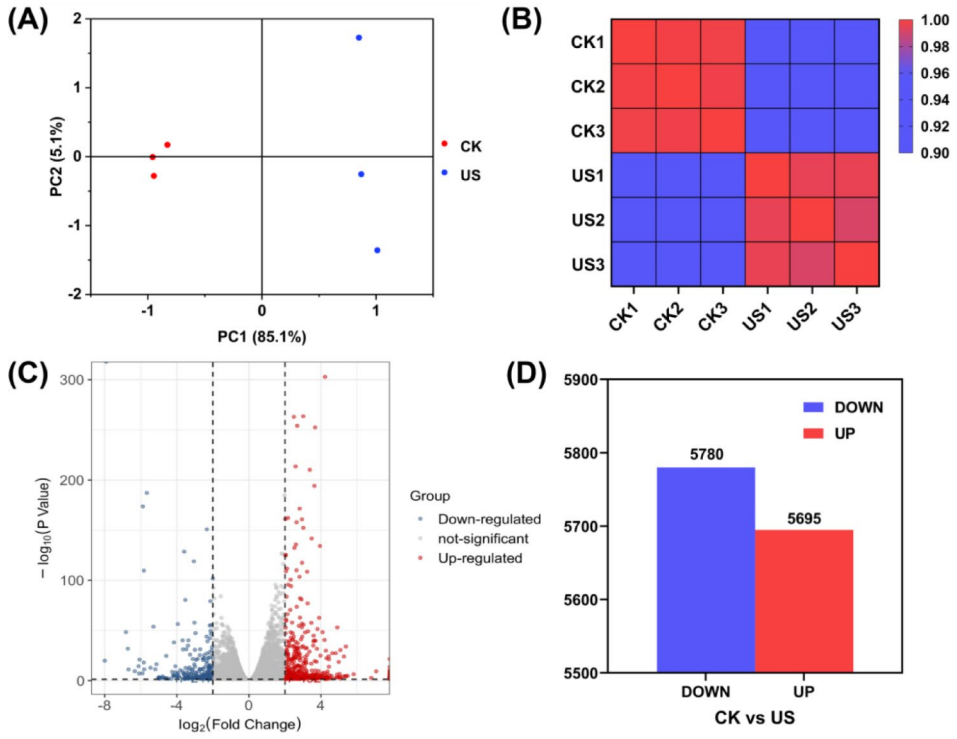

Fig. 3 (**A**): Principal component analysis of identified genes. (**B**): Heatmap of correlation analysis between CK and US; colors show the level of correlation for each sample, from low (blue) to high (red). (**C**): Volcano plot of DEGs; upregulated (red); downregulated (blue); unchanged (gray) between CK and US. (**D**): Compared with CK, the numbers of upregulated and downregulated genes in US

zipper (bZIP), and 21 genes (15 up-regulated, 6 down-regulated genes) were enriched in Auxin response factors (ARFs). These appear to be pivotal in regulating the expression levels of genes responsible for maize seed germination.

Plant hormone detection and related DEGs

In this study, DEGs involved in the synthesis of plant hormones (IAA, ABA, GA and SA) were upregulated and downregulated to different degrees (Fig. 5E).

Among these, 66 DEGs (46 up-regulated and 20 down-regulated) were associated with IAA (Fig. 5A). 17 DEGs (10 up-regulated and 7 down-regulated) associated with GA (Fig. 5C). Two DEGs were upregulated in the SA group (Fig. 5D). Compared with CK, the IAA, GA, and SA content in US increased to varying degrees (Fig. 5F and H, **and** Fig. 5J). 25 DEGs (15 up-regulated and 10 down-regulated) were associated with ABA (Fig. 5B). Compared with CK, the ABA content decreased significantly after ultrasonic treatment (Fig. 5G). Compared with the CK, the ABA/GA ratio also increased significantly after ultrasonic treatment (Fig. 5I).

Seed germination under different abiotic stresses

The germination of maize under salt stress (NaCl), drought stress (PEG) and waterlogging stress (ML) at the 3rd, 5th and 7th day were depicted in Fig. 6. Under salt stress, the radicle length of seeds in US was significantly increased by 33.6% and 30.2% at the 5th and 7th day, respectively. Under drought stress, compared with CK, the radicle length of seeds in US was significantly increased by 30.5% at the 7th day. Under waterlogging stress, compared with CK, the radicle length of seeds in US was significantly increased by 27.3% at the 7th day (Fig. 6B). Under salt stress, the germination percentage of seeds in US was significantly increased by 14.8% at the 7th day. Under drought stress, the germination percentage of seeds in US were significantly increased by 18.7% and 20.1% at the 5th and 7th day, respectively. Under waterlogging stress, the germination percentage of seeds in US were significantly increased by 17.6% and 21.6% at the 5th and 7th day, respectively (Fig. 6C).

Discussion

Constant ultrasonic has been used previously to treat seeds and improve their germination. The application of constant frequency ultrasonic at 20 kHz for 3 min improved the germination of wheat seeds [24], constant frequency ultrasonic at 45 kHz for 30 min improved the germination of maize seeds [25], constant frequency ultrasonic at 40 kHz for 30 min improved the germination of maize seeds [26], and the germination percentage of rice seeds was increased by ultrasonic treatment at 20–40 kHz for 1.5 min [27]. A shorter duration (40 s)

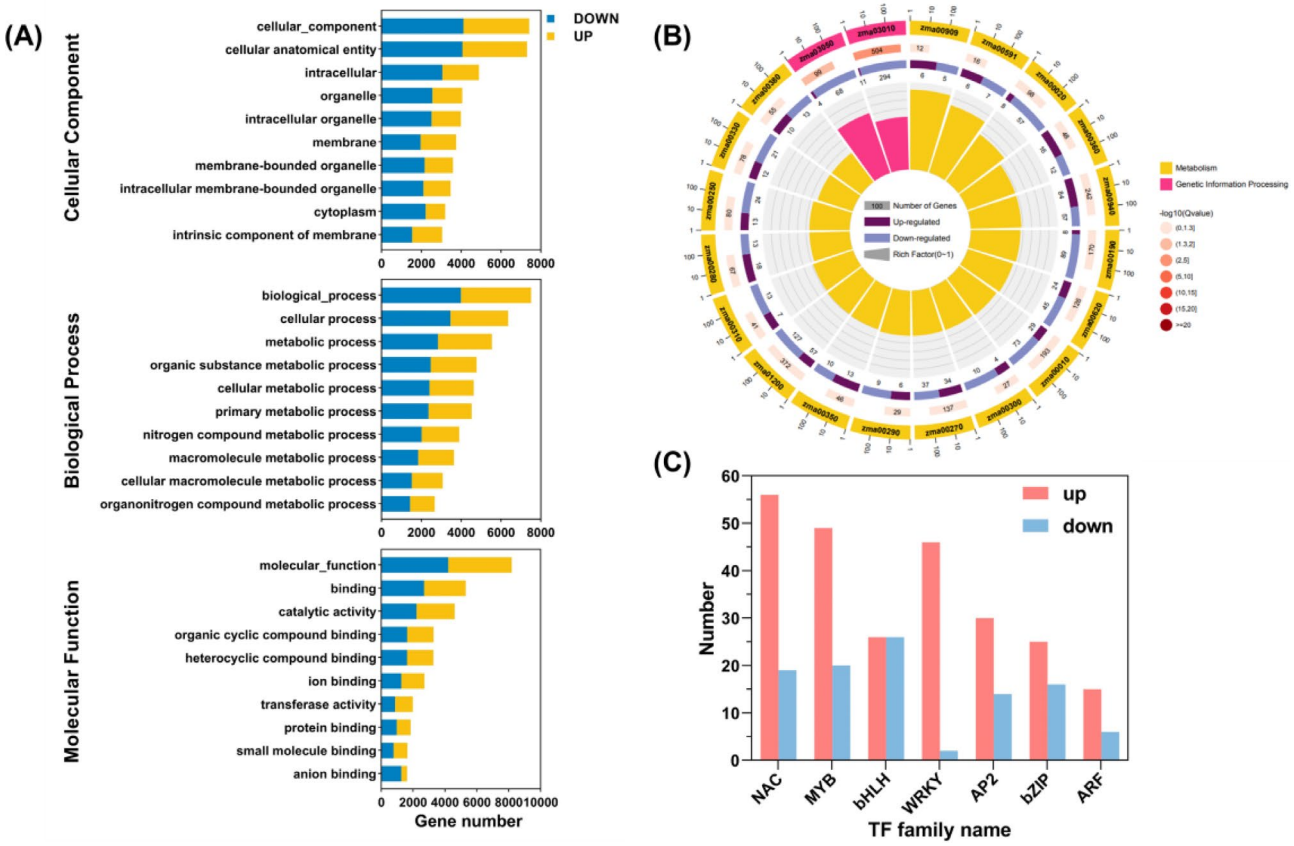

Fig. 4 (**A**): GO enrichment. (**B**): Top 20 KEGG enriched pathways involving 11,475 DEGs. (**C**): Seven transcription factor (TF) families

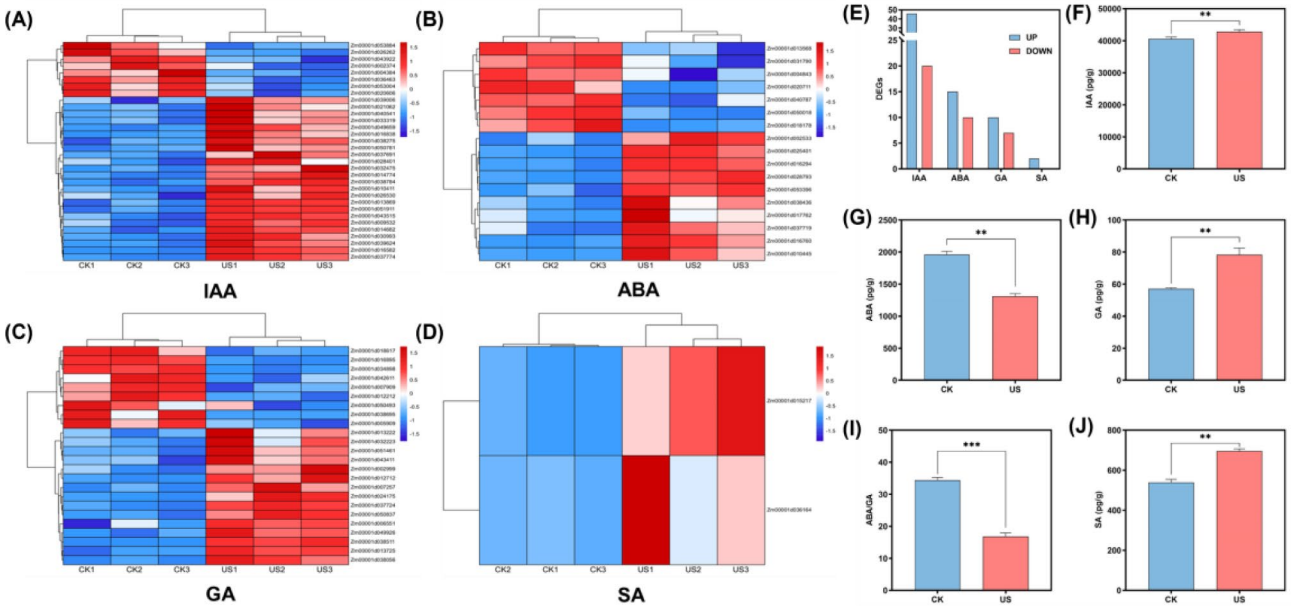

Fig. 5 (**A**): Differentially expressed genes (DEGs) of CK and US on auxin (IAA), (**B**): DEGs of CK and US on abscisic acid (ABA), (**C**): DEGs of CK and US on gibberellin (GA), (**D**): DEGs of CK and US on salicylic acid (SA). (**E**): DEGs regulating plant hormones. (**F**): The effect of ultrasonic treatment on auxin (IAA), (**G**): abscisic acid (ABA), and (**H**): gibberellin (GA). (**I**): The ratio of ABA/GA and (**J**) salicylic acid (SA). *$p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

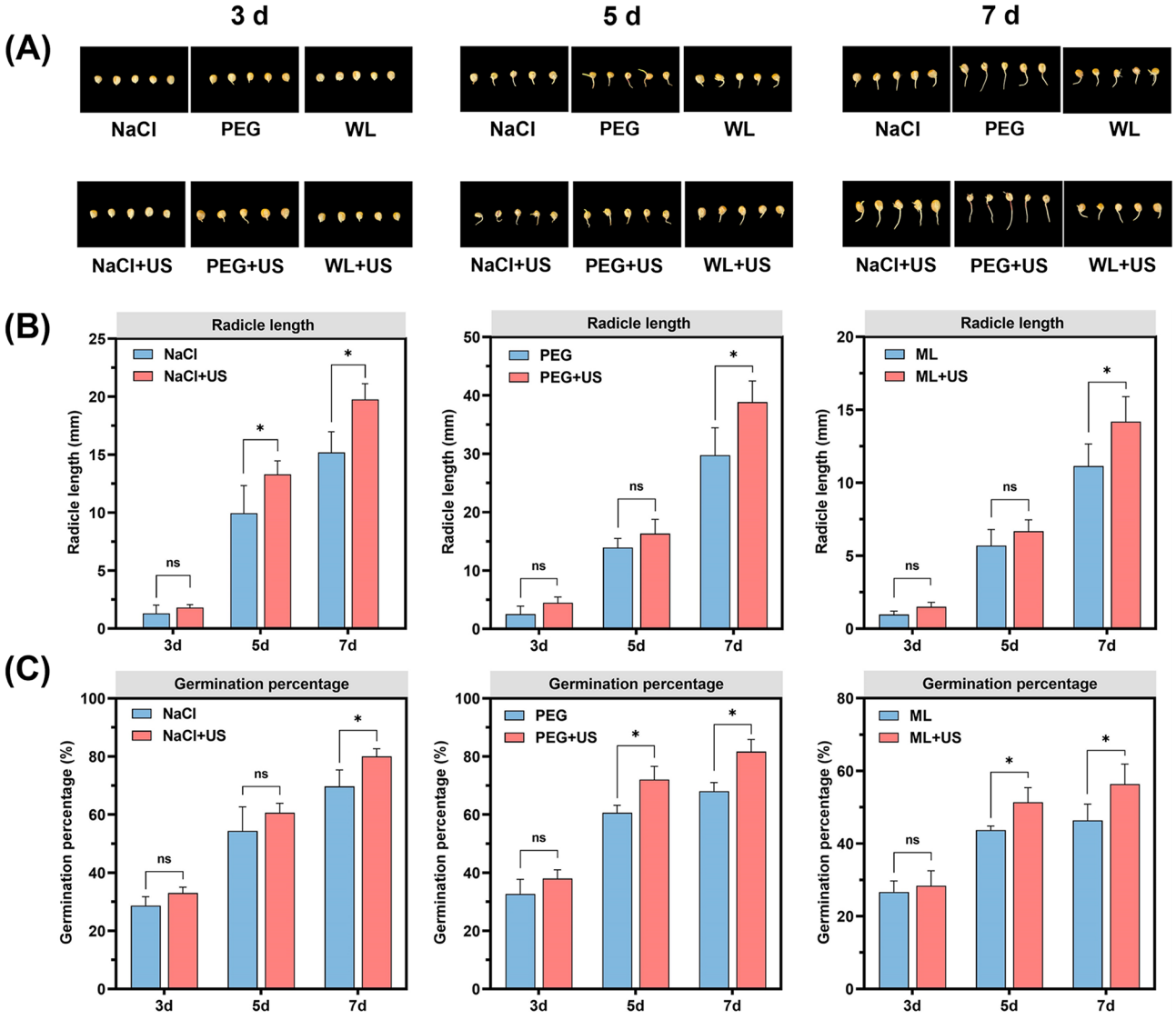

Fig. 6 (**A**): The seed germination pictures under different stress conditions, at the 3rd, 5th and 7th day. (**B**): The radicle length of the seeds under different stress conditions at the 3rd, 5th and 7th day. (**C**): The seed germination percentage under different stress conditions at the3rd, 5th and 7th day. *$p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

of variable-frequency ultrasonic (20–40 kHz) treatment of maize seeds significantly increased the maize germination percentage (Fig. 2A). Therefore, variable-frequency ultrasonic can significantly improve the seed treatment efficiency.

One potential mechanism of action is that ultrasonic can break down the mechanical barriers to seed germination. The seed coat can then produce multiple pores and fissures that increase the water permeability of the surrounding environment of the seed. This makes it easier for water to enter the interior of the seed, which, in turn, increases water absorption [28]. Ultrasonic treatment also triggers physiological and metabolic processes in plants, helping to enhance seed germination [29]. During seed germination, ultrasonic of the appropriate intensity can activate a variety of hydrolytic enzymes within the seed. This degrades large molecules, such as amylose, proteins, and lipids, into small molecules, such as dextrin, glucose, small peptides, amino acids, and fatty acids, which facilitate rapid nutrient uptake in the seed., thereby increasing nutrient bioavailability [30]. The ACP activity of the US treatment significantly increased by 96.4%. More ACP in the seeds could help to break down proteins into small molecules, such as amino acids, small peptides, and enzymes, thereby improving nutrient use. Amino acids are required for plant growth, and enzymes can play a key role in the decomposition of other macromolecules in the seeds, making it easier for stored nutrients to be absorbed and used by the seeds [31]. Compared with CK, the α-amylase and β-amylase

activities of US were increased significantly by 73.8% and 49.1%. Amylose in the endosperm is broken down into glucose by amylase. Glucose not only serves as an energy source for seed germination but also supplies the growth of radicles and endosperms [32]. It can also regulate the synthesis of plant hormones such as GA and ABA [33]. Therefore, variable-frequency ultrasonic promotes the hydrolysis of macromolecules by increasing the activities of acid protease, α-amylase and β-amylase in the seeds, providing energy for seed germination.

Transcriptome analysis revealed that the significant enrichment of DEGs were associated with plant metabolism. US altered more metabolism-related pathways than CK (Fig. 4B). The results showed that the phenylpropanoid biosynthesis pathway was enriched for upregulated expression. Pathways could directly or indirectly regulate the physiological status of seeds by regulating the expression of target genes and modulating cellular physiological processes. This can affect the key processes of seed germination, radicle development, and root formation [34]. Pyruvate metabolism pathway-related gene expression regulates pyruvate content, which provides a substantial energy supply for seed germination and seedling growth [35]. The sesquiterpenoid and triterpenoid biosynthesis pathways mediate the defense response triggered by the *Verticillium dahlia* effector PevD1 [36]. Oxidative phosphorylation and phenylpropanoid biosynthesis may play important roles in embryo germination [37]. Secondary metabolites are produced during the secondary metabolic process, affecting the osmotic function of the seed coat and ultimately affecting seed germination [38]. The metabolism provides energy and secondary metabolites and is involved in defense and cellular physiological processes. Therefore, the regulation of seed germination by these metabolic processes has not been isolated as they act together to regulate seed germination and development.

TFs, primarily NAC, MYB, BHLH, WRKY, AP2, bZIP, and ARF, are involved in maize germination. The NAC family is extensively involved in plant growth and development, and maintains cell membrane integrity under abiotic stress [39]. The NAC TF family can interact with ABA to promote *Arabidopsis* seed germination and seedling growth [40]. However, their roles in maize require further investigation. The MYB gene family regulates primary and secondary metabolism, plant hormone signaling, seed germination, and response to seedling stress [41, 42]. The bHLH family not only significantly improves plant growth, development, and water use efficiency but also significantly increases seed germination [43, 44]. The WRKY gene family mediates seed germination and late growth under stress (biotic and abiotic stress) conditions [45].Under drought stress, some WRKY transcription factors are also able to mediate plant stomatal closure, thereby reducing cellular water loss [46]. The bZIP family is associated with proteins involved in secondary metabolism and hormone signaling, provides the necessary energy for seed germination, and enhances seed germination and coleoptile sheath elongation [47, 48]. The AP2 family is actively expressed in the early stage of seed germination and is involved in water absorption and ABA signal transduction in the early stage of seed germination [49]. ARFs are TFs that regulate auxin-responsive gene expression. The maize ARF gene is involved in seed development and germination [50]. Therefore, ultrasonic treatment may increase maize seed germination through the involvement of TFs such as the NAC, MYB, bHLH, WRKY, AP2, bZIP, and ARF families.

Seed germination is a key step in the life cycle of plants, which is precisely regulated by a variety of endogenous hormones [51]. Understanding the ultrasonic regulation of plant hormones is an effective way to improve seed germination percentage. Compared with CK, the ultrasonic treatment significantly increased the IAA content of the seeds (Fig. 5F). IAA can disrupt seed dormancy and promote root development [52]. Genes regulating growth hormone response proteins (Zm00001d016582, Zm00001d021062, Zm00001d014774, and Zm00001d038784) were upregulated and expressed after ultrasonication (Fig. 5A). Auxin response proteins can form complexes with their corresponding receptors to regulate gene expression. These proteins can activate or inhibit the transcription of target genes, thereby regulating plant growth and development [53]. The ABA content in the seeds treated with ultrasonic will decrease (Fig. 5G). ABA plays an inhibitory role in mediating seed germination [54]. Genes encoding ABA stress maturation proteins (Zm00001d016760 and Zm00001d025401) were also upregulated (Fig. 5B). Under drought conditions, ABA-stressed mature proteins in plants are expressed, which can protect the integrity of cell membranes and maintain the correct structure of proteins, thereby reducing membrane permeability and promoting plant growth [55]. ABA receptor families, that is, Zm00001d028793, Zm00001d002533, Zm00001d010445, Zm00001d053396, and Zm00001d016294, were upregulated and expressed in this experiment (Fig. 5B). In maize, the ABA receptor family (ZmPYL) act directly as receptors for ABA and interact with other signaling pathways mediated by JA, brassinosteroid (BR), and growth factors [56]. Therefore, ultrasonic treatment reduced the content of ABA. However, some genes related to stress tolerance were upregulated. Ultrasonic treatment significantly increased GA content in the seeds (Fig. 5H). GA can relieve seed dormancy, promote the synthesis of amylase and protease in the seed endosperm, and provide energy and nutrients for germination and growth [57]. This is consistent with the results of the present study. The results showed

that genes related to the regulation of GA were altered, of which the genes related to the regulation of gibberellin oxidase (Zm00001d038695, Zm00001d037724, and Zm00001d013725) were upregulated and expressed (Fig. 5C). Gibberellin oxidase, a key enzyme in gibberellin metabolism, controls gibberellin levels in plants and affects germination, flowering, and fruiting [58]. The SA content of the seeds also increased significantly after ultrasonic treatment (Fig. 5J). SA can control seed germination by regulating GA-induced α-amylase gene expression. Under salt stress, SA can reduce oxidative damage to cells through another signaling pathway, thereby promoting seed germination [59]. Two DEGs, that is, Zm00001d015217 and Zm00001d036164, related to SA were upregulated and play a role in defense (Fig. 5D) [60]. The ratio between germination-inhibiting ABA and germination-promoting GA is critical for the regulation of germination [61]. In this study, compared CK, the ABA/GA content of the ultrasonic treatment was significantly reduced (Fig. 5I). This had a positive effect on breaking seed dormancy and promoting germination. However, the optimal ABA/GA ratio to promote maize seed germination remains unclear.

Under conditions of abiotic stress (salt stress, drought stress and waterlogging stress), seed germination and seedling growth of plant are inhibited [62]. Studies have shown that under salt stress and drought stress, the germination percentage of plant seeds treated by ultrasonic is higher [63]. Under waterlogging stress, seed germination and seedling development become slower [64]. After ultrasonic treatment, the inhibition of seed germination and seedling growth caused by abiotic stress could be alleviated. The results of this study may contribute to future studies using ultrasonic treated seeds to improve seed performance under abiotic stress.

Therefore, variable-frequency ultrasonic promotes seed germination and adaptability to abiotic stress by affecting metabolism and genetic information processing, that is, ribosome, proteasome, and pyruvate metabolism, sesquiterpenoid, triterpenoid, and phenylpropanoid biosynthesis, and oxidative phosphorylation, transcription factors in NAC, MYB, bHLH, WRKY, AP2, bZIP and ARF families, as well as IAA, ABA, GA, and SA in plant hormones. This study suggests that physical treatments in the form of variable-frequency ultrasonic treatments have considerable potential to improve germination of maize seeds.

However, the diversity of seeds, seed quality, ultrasonic frequency, duration of ultrasonication, and other conditions indicate that appropriate ultrasonication conditions should be selected for the treatment of different seeds. Future studies will focus on selecting more maize varieties for ultrasonication, testing germination percentage, and selecting the most appropriate seeds. Improving seed germination is important for increasing crop yield, reducing agricultural costs, and improving plant tolerance.

Conclusion

This study found that variable-frequency ultrasonic treatment can greatly improve the germination percentage of maize seeds. Variable-frequency ultrasonic can significantly increase the acid protease and amylase content, providing more material basis for seed germination. The results of transcriptome analysis showed that ultrasound enhanced the metabolic processes during seed germination, and the contents of plant hormones IAA, ABA, GA and SA were significantly changed, and different TFs (NAC, MYB, bHLH, WRKY, AP2, bZIP, and ARF families) were also involved. In addition, ultrasonic treatment promotes seed germination and growth under abiotic stress conditions (salt, drought, and waterlogging). Overall, ultrasonic treatment has promising applications in enhancing the germination of maize seeds. However, owing to the diversity of seeds, it is necessary to use different ultrasonic parameters to treat the seeds according to their characteristics, optimize the ultrasonic conditions, and explore the regulatory mechanisms of related genes.

Abbreviations

US	Ultrasonic treatment for 40 s
CK	Non-ultrasonic treatment
ACP	Acidic proteinase
α-AL	α-amylase
β-AL	β-amylase
IAA	Auxin
ABA	Abscisic acid
GA	Gibberellin
SA	Salicylic acid
NaCl	Salt stress
PEG	Drought stress
ML	Waterlogging stress

Acknowledgements

We thank the Allwegene Technology Technology Co., Ltd. (Beijing, China) for providing guidance and technical assistance in the study.

Author contributions

M.G., M.K., Q.Y.H., J.X.H., W.H. and G.H.L. conceived and designed the experiments; M.G., M.K. and Q.Y.H. conducted most of the experiment, Z.S.Y, C.L., J.H., Y.W.J. and J.Q.S. provided technical assistance; M.G., M.K., Q.Y.H, G.H.L. and W.H. wrote the manuscript. All authors have read and approved the manuscript for publication.

Funding

This work was supported by Key Research and Development Program of China [2023YFD2303100] and Agricultural Science and Technology Innovation Program [CAAS-ZDRW202407].

Data availability

All raw sequence data has been deposited in the NCBI database under BioProject PRJNA1141261 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1141261). SRA numbers SRR30017285, SRR30017284, SRR30017283, SRR30017282, SRR30017281, SRR30017280 contain the RNA-seq reads used throughout this study.

Declarations

Ethics approval and consent to participate
Our study was conducted in full compliance with local regulations. This article did not involve any studies with human, animals, or endangered species. The collection of plant materials and the experimental procedures conducted in this study adhered to institutional, national and international guidelines and legal requirements.

Consent for publication
Not Applicable.

Competing interests
The authors declare no competing interests.

Received: 15 May 2024 / Accepted: 31 July 2024
Published online: 08 August 2024

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