CSN improves seed vigor of aged sunflower seeds by regulating the fatty acid, glycometabolism, and abscisic acid metabolism

Yutao Huang a, Shuyu Cai b, Xiaoli Ruan c, Jun Xu d, Dongdong Cao a,*

a Institute of Crop and Nuclear Technology Utilization, Zhejiang Academy of Agricultural Science, 310021 Hangzhou, China
b School of Architectural Engineering, Shaoxing University Yuanpei College, 312000 Shaoxing, China
c Zhejiang Nongke Seed Co. Ltd, 198 Shiqiao Road, Hangzhou 310021, China
d Bureau of Agriculture and Rural Affairs of Pinhu City, 500 Xinhuabei Road, Pinhu 314200, China

Graphical abstract

Article info

Article history:
Received 20 October 2020
Revised 3 January 2021
Accepted 29 January 2021
Available online 9 February 2021

Keywords:
Abscisic acid
Aged seeds
CSN
Fatty acid
Seed vigor
Sunflower

Abstract

Introduction: Sunflower seeds possess higher oil content than do cereal crop seeds. Storage of sunflower seeds is accompanied by loss of seed vigor and oxidation of storage and membrane lipids.

Objectives: This study first reported that compound sodium nitrophenolate (CSN), a new plant growth modulator, improved the germination and seedling emergence of aged sunflower seeds. The present study provides a future reference as to the potential applications of CSN and the regulation mechanism of exogenous substances in increasing aged crop seed vigor.

Methods: Phenotypic analysis was performed to investigate the effect of CSN on germination and seedling emergence from naturally- and artificially-aged sunflower seeds. The biochemical and enzyme activity analysis were conducted to test the CSN-induced effect on glycometabolism, fatty acid and abscisic acid metabolism. Meanwhile, gene expression analysis was carried out to detect the changes in the transcription level of sunflower seeds during early germination period after CSN treatment.

Results: CSN application significantly increased the germination rate and seedling emergence rate of sunflower seeds under natural and artificial aging. Biochemical analysis indicated that, CSN treatment significantly enhanced the sucrose and fructose contents in aged sunflower seeds during early germination.
Introduction

Sunflower (Helianthus annuus L.) originated in North America, is one of the vital oilseed crops globally [1]. According to the statistical data from the Food and Agriculture Organization of the United Nations (UNAFO) in 2015, the global sunflower planting area is approximately 25.4 million ha. Additionally, sunflower is also the third-largest oil crop in China, with the planting area of up to 1.15 million ha and the total output of 299 t, only second to oilseed rape and peanut [2]. Sunflower has come under the spotlight in recent years due to its high ornamental, edible, and oil values. Sunflower seed is rich in unsaturated fatty acids (UFAs), multiple vitamins and trace elements, which can suppress the cholesterol synthesis in human body. Currently, sunflower oil is the preferred cooking oil in developed countries [3]. The sunflower production in China shows an increasing trend on the whole, which increases from 278,000 tons to 2,899,700 tons from 1978 to 2016 [2].

Seeds are the foundation of agricultural production. The successful seed germination and seedling emergence are of paramount importance to the plant growth and yield formation of crops [4]. Seed germination is a complicated process controlled jointly by a variety of endogenous and exogenous factors [5]. Seed vigor stands for an integrative seed characteristic determining the rapid and neat seed emergence capacity in the complicated field environment [6]. As an important part in seed vigor, seed longevity is determined by seed genetic and physiological protection potentials, as well as the storage conditions [7]. High vigor seed is closely related to the high filed emergence and productivity, while low vigor seed always leads to a decrease of yields. The early seedling emergence from germinated seed is another critical development stage. Before the seedling obtains photosynthetic capacity, both seed germination and seedling emergence of aged sunflower seeds. Besides, further interpreting the molecular mechanisms during the germination and seedling emergence processes of aged seeds sheds more lights on the scientific research of sunflower.

Compound sodium nitrophenolate (CSN) is a novel artificially synthesized modulator for plant growth, which has been extensively used in agricultural production on various crops, including tomato [20], Chinese chive [21], cotton [22], and Rape [23]. CSN can promote cellular protoplasmic streaming, break seed dormancy, accelerate rooting and sprouting, improve fruit quality, and prevent flower and fruit drop [24,25]. However, so far, there are few studies on the role of CSN in seed vigor regulation, in particular for seed germination as well as seedling emergence of aged sunflower seeds. The present study found that CSN had the function on seed vigor improvement in naturally and artificially aged sunflower seeds by regulating fatty acid, glycometabolism, and abscisic acid metabolism. CSN application boosted the triacylglycerol hydrolysis, promoted the conversion of fatty acids to sugars, and decreased the abscisic acid content during imbibitions of aged sunflower seeds, and resulted in the improvement of germination and seedling emergence of aged sunflower seeds. The present research might provide a future reference as to the potential applications of CSN and the regulation mechanism of exogenous substances in increasing aged crop seed vigor.

Materials and methods

Experimental materials

The sunflower (Helianthus annuus L.) seeds were employed in this study. Hybrid sunflower seeds were produced from parental inbreds through synchronous hand cross-pollination in the experimental farm of Zhejiang Academy of Agricultural Science.
(Hangzhou, China). The filled sunflower seeds were harvested to store for 1, 6, 12, 18 and 24 months, respectively. The sunflower seeds were stored in the dry airtight container under ambient temperature.

Accelerated aging test

The accelerated aging test (AAT) was carried out according to the method of Missaoui and Hill [26] with small modifications. Sunflower seeds stored for 1 month were used for the AAT. Briefly, 100 seeds of sunflower were put in each seed aged box and incubated at 45 °C and 100% relative humidity for 48 h (the accelerated aging test condition were determined by preliminary experiments), and then those processed seeds were subjected to 2 days of drying under ambient temperature prior to germination tests.

Seed germination and seedling emergence tests

Seed germination was carried out after disinfection of 0.1% sodium hypochlorite solution for 15 min. Fifty seeds of sunflower were put in each rolled towels, and four duplicates were set. Rolled towels were immersed in 25 mg L⁻¹ CSN solution and purified water, respectively. Subsequently, the rolled towels were incubated at 25 °C in germination chamber under a diurnal cycle of 12 h of light and 12 h of darkness. The number of germinated seeds were counted every 12 h. Seeds samples were collected at diverse time points (0, 12, 24, 36, and 48 h) during imbibitions for further testing.

Fifty sunflower seeds were planted in soil within greenhouses at 25 °C and the 12 h light/dark cycle. At 7 days later, the seedling emergence rates, seedlings height, seedlings dry weight, and total chlorophyll content were determine based on experimental requirements.

CSN content measurement

High performance liquid chromatography-mass spectrometry (HPLC-MS) was performed to determine CSN content of sunflower seeds by the methods of Zhang et al [27]. With mobile phase of methanol-10 mM ammonium acetate solution (60:40, v/v), the mass spectrometry was operated in the positive ion mode of multiple reaction monitoring.

Quantification of various sugars

Total soluble sugar content in sunflower seeds was determined through anthrone-H₂SO₄ colorimetry by the method of Zhu et al [28]. The resorcinol method was used to measure sucrose level and the absorbance (OD) value at 480 nm was adopted for sucrose content estimation [29]. The fructose determination was performed with the method of Cai et al [30]. HPLC was performed to determine glucose content in sunflower seed by the method of Bailly [31].

ATP and energy charge analysis

ATP and energy charge were determined by HPLC, as described by Emami and Kempken [32] with minor modifications. Data of ATP and energy charge analysis were expressed as means of four replicate determinations.

Fatty acid content measurement

The fatty acid extraction was performed with the method of Zhou et al [18] with small modification. Sunflower seeds were ground into powders and used to extract fatty acids. In brief, after adding 2 ml n-hexane into the grinded sunflower seeds (50 mg in each tube), the sample was subjected to ultrasonic extraction (40 kHz) for 15 min, then standing for 3 h under ambient temperature. Then the solution was centrifuged at 10,000 rpm and 4 °C for 10 min. Afterwards, supernatants were collected, followed by 30 s of vortex oscillation after the addition of 3 ml methanolic potassium hydroxide solution (Me-OH, 0.4 M). Subsequently, upper liquid layer was transferred into the 5 ml bottle and diluted with n-hexane until 5 ml. The extract was added to a gas chromatograph-mass spectrometer (GC-MS) system using the 0.22 μm filter (organic phase). Afterwards, fatty acids were identified and quantified by the method of Yang et al [33].

Assay of fatty acid, glycometabolism, abscisic acid and gibberellin metabolism-related enzymes activity

The activities of triacylglycerol lipase (LIPG), glycerol-3-P dehydrogenase (GPDH), CoA-SH oxidase (ACK), phosphoenolpyruvate carboxykinase (PCK), invertase (INV), 9-cis-epoxy-carotenoid dioxygenase (NCED) activity, zeaxanthin epoxidase (ZEP), abscisic acid aldehyde oxidase (AAO), abscisic acid-8'-hydroxylases (ABA8ox), GA3-oxidase (GA3ox), GA20-oxidase (GA20ox), and GA2-oxidase (GA2ox) were detected with enzyme-linked immune kit (Milbio, Shanghai, China) according to the protocol of manufacturer. Briefly, sunflower seeds were extracted in 1 ml of extraction buffer and then centrifuged at 15,000 rpm for 15 min. Add 10 μl supernatant of enzyme extraction and 40 μl Sample Dilution in each testing sample well and after incubation at 37 °C for 30 min, removed the solution. Afterwards, washed five times by filling each well with 50 μl Wash Buffer. Then 50 μl conjugate reagent was added and repeated the incubation and wash process. Subsequently, 50 μl Chromogen Solution A and 50 μl Chromogen Solution B were added to each well for chromogenic reaction. The color change was measured spectrophotometrically at a wavelength of 450 nm with enzyme mark instrument. The activities of fatty acid and glycometabolism metabolism-related enzymes in seeds were then measured by comparing the O.D. of the samples to the standard curve.

Abscisic acid and gibberellin determination

ABA and GA were extracted from sunflower seeds in accordance with the method of Huang et al [34] The HPLC system equipped with an ultraviolet detector and reverse-phase (C18) column (6.0 mm × 120 mm, 5 mm particle size, Shim-Pack CLC-ODS) was used for identification and quantification of ABA and GA levels in the extracting solution. The mobile phase consisted of methanol/water (64:36, v/v) was ran at the 1.0 ml min⁻¹ flow rate. The ABA and GA analysis were performed with four biological replications.

Real-Time Quantitative PCR

The RNA extraction and reverse-transcription of each seeds sample was carried out using PrimeScript™ RT reagent Kit (Vazyme, Nanjing, China). The qPCR protocol was performed on a CFX96 Touch Real-Time PCR instrument (Biorad) with the ChamQ SYBR qPCR Master Mix (Vazyme, Nanjing, China). Primers applied in this study were listed in Supporting Information Table S1. 18srRNA was used as the internal control. Calculations of the fold change of expression (FC) were determined according to FC = EΔCt, where E corresponds to the mean value of the amplification efficacy of the gene, ΔCt is the difference between the mean Ct values of all the biological replicates between the two samples that are being compared. All data were expressed as the mean SD after normalization.

4
Statistical analyses

The Statistical Analysis System (SAS) software was used for data statistical analysis by one-way analysis of variance (ANOVA). The data was tested for normality and homoscedasticity before ANOVA. In addition, multiple comparisons were accomplished by the least significant difference of p < 0.05 (LSD<sub>0.05</sub>). The percentage data were subjected to arcsin transformation prior to statistical comparison according to \( y = \arcsin(\sqrt{x/100}) \).

Results

Natural aging remarkably inhibited sunflower seed germination and seedling emergence

After storage for 6, 12, 18 and 24 months, the germination and seedling emergence capacities of sunflower seeds were determined (Fig. 1). The result showed that the germination rates of sunflower seeds in long-term storage (18 and 24 months) were remarkably lower than those in short-term storage. At 7 days of germination time, the germination rates of sunflower seeds stored for 6 and 12 months were 95% and 85%, respectively, while those after storage for 18 months was only 53%. After storage for 24 months, 85% of sunflower seeds lost the germination ability (Fig. 1A–E).

Thereafter, the present study analyzed the effect of storage process on the seedling emergence of sunflower seeds. Consistently, the sunflower seedling emergence rate decreased with the increase in storage time. The seedling emergence rates of sunflower seeds stored for 6 and 12 months were 91% and 78%, respectively; while those after storage for 18 months was only 38%. After storage for 24 months, all the sunflower seeds lost the seedling emergence capacity (Fig. 1FG). Moreover, it should be pointed out that, the seedling emergence rate of sunflower seeds after storage for 6 months was lower than the germination rate, and a similar rule

---

**Fig. 1.** Natural aging remarkably suppressed sunflower seed germination and seedling emergence. (A–D) Typical images of naturally aged sunflower seeds in the imbibition process (48 h). Sunflower seeds after 6, 12, 18 and 24 months of storage were used for analysis. Scale bar, 10 mm. (E) The germination rates of different samples (A–D) at 84 h post-sowing. (F) The seedling emergence phenotypes for different samples (A–D) after germination (at 5 days post-germination). Scale bar, 600 mm. (G) The seedling emergence rates of different samples (A–D) at 5 days post-germination. (H) Typical images for sunflower seedling. Scale bar, 200 mm. (I) Quantitative analysis on the seedling height shown in (H). Four biological replicates each with 50 seeds for each treatment were set in seed germination and seedling emergence tests. The diverse lowercase(s) on top of the bars were indicative of significant differences (p < 0.01, LSD) across treatments.
was also observed in sunflower seeds after storage for 12, 18 and 24 months. Particularly, after storage for 24 months, the seedling emergence rate of sunflower seeds was 0%, although its germination rate reached 15%. We also investigated seedling height and confirmed the inhibiting effect of natural aged process on sunflower seedling growth (Fig. 1 HI). To sum up, the above results indicated that the natural aging process apparently reduced the sunflower seed vigor and seedling emergence capacity.

**CSN treatment promoted germination and seedling emergence of aged sunflower seeds**

In the preliminary experiment, several extensively used plant growth regulators in agricultural production were screened, and their effects on improving the post-storage germination and seedling emergence capacities of sunflower seeds were determined. According to the results, compound sodium nitrophenolate (CSN) application improved the germination and seedling emergence capacities of the naturally aged sunflower seeds (Fig. 1HI). To sum up, the above results indicated that, the natural aging process apparently reduced the sunflower seed vigor and seedling emergence capacity.

Exogenous CSN significantly enhanced the germination rate of sunflower seeds after storage for 18 months. At 7 days after germination, the germination rate of untreated sunflower seeds was only 55%, while those in CSN-treatment was as high as 79% (Fig. 2AB). Besides, CSN treatment also improved the seedling emergence of naturally aged sunflower seeds, for CSN-treated seeds, the seedling emergence rate was 68%, which doubled that of 34% for the untreated sunflower seeds (Fig. 2CD). The data on the effect of CSN on seedling height was consistent with the effect on seedling emergence (Fig. 2EF).

Given that CSN treatment promotes seed vigor of naturally aged sunflower seeds, the present study further analyzed the impact of CSN on the germination and seedling emergence of fresh sunflower seeds (storage for 1 month). The results indicated that CSN treatment had no significant effect on the germination and seedling emergence processes of fresh sunflower seeds (Supporting Information Fig. S1). Thus, it was suggested that the promotion effect of CSN on sunflower seed vigor was only reflected in the aged seeds, but not the fresh seeds.

To further confirm the regulatory effect of CSN on the vigor of aged sunflower seeds, we adopted the accelerated aging test (AAT) to obtain the artificial aged sunflower seeds. The germination rate and seedling emergence rate of AAT-treated sunflower seeds...
seeds were significantly lower than those of the non-aged seeds (Fig. 3). Consistent with the CSN effect on promoting germination of natural aged sunflower seeds, exogenous CSN also improved the germination and seedling emergence capacities of artificial aged sunflower seeds. The germination rate and seedling emergence rate of AAT + CSN treatment were 1.31 and 1.40 folds of those in AAT + H2O treatment, respectively (Fig. 3 BD). Exogenous CSN also increased the seedling characteristic of AAT-aged seeds, with the seedling height of CSN-treated AAT-aged seeds being 1.5-fold that of AAT-aged seeds without CSN treatment (Fig. 3 EF).

To further verify the relationship between CSN treatment and improved sunflower seed vigor, the CSN content in imbibitions sunflower seeds was measured (Supporting Information Table S2). The results showed that CSN treatment significantly increased the CSN content in AAT-aged sunflower seeds during imbibitions time. After clarifying the role of CSN in promoting the germination and seedling emergence of naturally and artificially aged sunflower seeds, only artificially aged seeds were used in subsequent experiments.

CSN treatment increased the contents of soluble sugars, ATP and energy charge in aged sunflower seeds during imbibitions time

The seed storage substances are the major sources of energy during early seed germination and seedling emergence, and sucrose, glucose and fructose are the main nutrients produced by the decomposition of storage substances. To further analyze the mechanism of CSN in promoting the germination and seedling emergence of aged sunflower seeds, this study determined the contents of soluble sugar, sucrose, glucose and fructose in sunflower seeds at the early germination stage (0–48 h) (Fig. 4). The results revealed that, CSN treatment remarkably improved the soluble sugar, sucrose and fructose contents in the AAT-treated sunflower seeds at the early germination stage, especially at 12 and 24 h after germination. While no significant difference in glucose content was detected between AAT + H2O and AAT + CSN treatments (Fig. 4). Furthermore, significant increased ATP content and energy charge were observed in AAT + CSN sunflower seeds as compared with AAT + H2O treatment (Supporting Information Table S3).
The above findings revealed that CSN accelerated aged sunflower seed germination and seedling emergence through elevating soluble sugar and ATP levels.

CSN increased several fatty acids contents in aged sunflower seeds during imbibitions

As is well-known, in the seed germination and early seedling emergence processes of oilseed crop, hydrolysis of triacylglycerol (oil) produced glycerol and fatty acids, while they are thus converted into diverse soluble sugars through gluconeogenesis [35,36]. For understanding the reasons responsible for the increased soluble sugar levels in AAT-aged sunflower seeds after CSN treatment, we measured the contents of several fatty acids during sunflower seed imbibitions.

As suggested GA-MS analysis, the AAT treatment evidently decreased the total fatty acid, unsaturated fatty acid and saturated fatty acid contents. It was worth noting that the content of total, saturated, and unsaturated fatty acids were significantly increased in AAT + CSN seeds compared with AAT + H2O seeds at 24 h and 48 h of imbibitions time (Fig. 5). Subsequently, we test the changes in the main fatty acid content in sunflower seeds, such as the stearic, palmitic, oleic, linoleic and linolenic acids (Supporting Information Fig. S3). The results showed that CSN treatment increased the contents of oleic and linoleic in aged sunflower seeds during imbibitions. By contrast, the palmitic content of AAT + CSN seeds was significant lower than those in AAT + H2O seeds. While no significant difference in stearic acid and linolenic acids contents were detected between AAT + CSN and AAT + H2O treatments.

CSN increased the activities of several key enzymes which are involved in fatty acid and glycometabolism metabolism in aged sunflower seeds during imbibitions time

Given that CSN application increased levels of soluble sugars and fatty acids during aged sunflower seeds imbibitions, the CSN function in activities of several critical enzymes related to triacylglycerol conversion into sugars and fatty acids was examined (Fig. 6). AAT treatment significantly lowered the activities of triacylglycerol lipase (LIPG), glycerol-3-P dehydrogenase (GPDH), CoA-SH oxidase (ACX), phosphoenolpyruvate carboxykinase (PCK) and invertase (INV) in sunflower seeds during early germination time. By contrast, remarkably up-regulated activities of triacylglycerol lipase, glycerol-3-P dehydrogenase, and PCK were detected in AAT + CSN seeds compared with AAT + H2O treatment at 12 h, 24 h, 36 h, and 48 h of imbibitions time. Moreover, CSN application also enhanced the CoA-SH oxidase activity in AAT-treated seeds at 12 h, 24 h, and 36 h of imbibitions time. These results were in consistent with the positive effect of CSN on contents of fatty acid and soluble sugar in AAT-treated sunflower seeds during imbibitions.

CSN increased the transcriptional levels of fatty acid and glycometabolism metabolism-related genes in aged sunflower seeds during imbibitions

The qPCR analysis revealed that the transcriptional levels of HaLIPG1, HaLIPG3, HaGPDH3, HaGPDH3, HaACX3, HaPK2, HaPK2, HaINV1, HaINV2, and HaINV3 were decreased in AAT-treatment seeds as compared with seeds without AAT treatment. By contrast, CNS
treatment significantly enhanced the transcriptional levels of \( \text{HaLIPG1} \), \( \text{HaLIPG3} \), \( \text{HaPGDH1} \), and \( \text{HaPGDH3} \). Similarly, the gene expressions of \( \text{HaACX3} \) and \( \text{HaPKC2} \) in AAT + CSN seeds were significantly higher than those in AAT + \( \text{H}_2\text{O} \). Besides, CSN application remarkably increased the transcriptional levels \( \text{HalNV1} \) and \( \text{HalNV3} \) in AAT-aged sunflower seeds (Fig. 7).

**CSN decreased abscisic acid content in aged sunflower seeds during imbibitions**

It was found that AAT treatment significantly enhanced the ABA content of sunflower seeds during early germination. CSN application significantly decreased the ABA content in AAT-aged sunflower seeds at 0–42 h of imbibitions time (Fig. 8A). Subsequently, we further test the effects of CSN on the activities of several key enzymes involved in the ABA metabolism, namely 9-cis-epoxycarotenoid dioxygenase (NCED), zeaxanthin epoxidase (ZEP), abscisic acid aldehyde oxidase (AAO), and ABA-8'-hydroxylases (ABA8ox). The enzyme activity analysis revealed that activities of NCED and AAO in AAT-aged sunflower seeds were significantly lower than those in imibed fresh seeds (Fig. 8BD). Besides, AAT treatment significantly decreased ABA8ox activity of sunflower seeds at 24 h, 36 h, and 48 h of imbibitions time (Fig. 8E). In contrast, CSN treatment markedly decreased NCED activity and increased ABA8ox activity in AAT-treated sunflower seeds at most of the time points (Fig. 8BE). While no significant difference in ZEP activity of sunflower seeds were observed among treatments during early imbibitions time (Fig. 8C).

For GA, it was significantly lowered by AAT treatment during sunflower seed imbibitions time (Supporting Information Fig. S4). AAT treatment remarkably decreased the activities of \( \text{GA3ox} \) and \( \text{GA2ox} \) of sunflower seeds at 24 h, 36 h, and 48 h of imbibitions time. While exogenous CNS made no significant effect on GA content and activities of \( \text{GA3ox} \), \( \text{GA2ox} \), and \( \text{GA2ox} \) in AAT-treatment seeds during early germination time.

**CSN regulated the transcriptional levels of abscisic acid metabolism-related genes in aged sunflower seeds during imbibitions**

The qPCR analysis revealed that the transcriptional levels of \( \text{HaNCED1}, \text{HaNCED2}, \text{HaNCED3}, \) and \( \text{HaAAO} \) were increased in AAT-treatment seeds as compared with seeds without AAT treatment (Fig. 8F). AAT treatment also down-regulated the transcriptional levels of \( \text{HaABA8ox1} \) and \( \text{HaABA8ox3} \) of sunflower seeds at 24–48 h of imbibitions time. It was worth noting that the effect of CNS application on ABA metabolism-related gene expression was opposite to that of AAT treatment. CNS treatment significantly decreased the expressions of \( \text{HaNCED1}, \text{HaNCED3}, \) and \( \text{HaAAO} \); by contrast, CNS significantly up-regulated the expressions of \( \text{HaABA8ox1} \) and \( \text{HaABA8ox3} \) in AAT-treatment sunflower seeds during imbibitions time. Such result conformed to the CNS impact on the activities of AAO, NCED, and ABA8ox in AAT-aged sunflower seeds in the imbibitions process.

**Discussion**

Seed germination is a complicated process under the control of various exogenous and endogenous factors [5]. In the present study, the germination and seedling emergence were significantly inhibited in both natural aged and artificially aged sunflower seeds. Typically, the blocked triacylglycerol conversion into soluble sugars, along with the elevated ABA content, might be the important reason for the decrease of germination and seedling emergence in aged sunflower seeds. The germination test and phenotypic analysis revealed that the plant growth regulator CNS effectively alleviated the inhibition of naturally aging and artificially aging on germination and early seedling emergence of sunflower seeds. That CNS contributes to improved germination and seedling emergence of aged sunflower seeds might be related with the metabolism of fatty acid, glycometabolism, and abscisic acid.
CSN positively affected germination and early seedling emergence of aged sunflower seeds

Seeds are the foundation of agricultural production. The successful seed germination and seedling emergence are of paramount importance to the plant growth and yield formation of crops [37]. The reduced seed vigor showed positive correlation with the storage period [38–40]. In traditional agriculture, crop seeds are susceptible to aging due to poor preservation environment and long preservation time, thus affecting the agricultural production. Consequently, enhancing the germination and seedling emergence capacities of the aged seeds is a worthy objective of concern in agricultural production.

Hydrated graphene ribbon application boost aged wheat seed germination by enhancing the catabolism of starch and triglycerides [41]. Mahakham et al [42] developed a nanopriming technology for boosting seed germination of aged rice seeds using phytosynthesized silver nanoparticles. Besides, ultrasonic treatment was proved had the function on germination improvement in tall fescue and Russian wildrye aged seeds [43]. Interestingly, a biomaterial was recently developed on the basis of trehalose and silk fibroin, which effectively boosts seed germination and alleviates soil salinity [44]. However, most of these studies focused germination rather than seedling emergence of the aged seeds, and suggested that there were divergent mechanisms underlying the effects improving germination of aged seeds.

Compound sodium nitrophenolate (CSN) is a highly efficient plant growth regulator first discovered by Asahi Kagaku Kogyo Co., Ltd (Japan) in the 1960s. Its effective components are sodium ortho-nitrophenolate, sodium para-nitrophenol and 5-nitroguaiacol sodium salt. In 1997, CSN was approved by the US Environmental Protection Agency into the American Green Food Project, and it was designated as the Green Food Project-recommended plant growth regulator by the Food and Agriculture Organization of the United Nations (FAO). Several studies have showed that CSN has a wide range of biological functions on plant growth and development regulation, such as flower differentiation, fruit development, senescence, and seed germination [20–23]. This work extended the actual applications of CSN, suggesting that CSN might alleviate the suppression of naturally and artificially aging on sunflower seed germination and seedling emergence.

Fig. 6. Positive impacts of CSN on the activities of several key enzymes related to fatty acid metabolism and glycometabolism in sunflower seeds during imbibition time. (A) Triacylglycerol lipase activity. (B) Glycerol-3-P dehydrogenase activity. (C) CoA-SH oxidase activity. (D) PCK activity. (E) Invertase activity. Without AAT: healthy seeds without accelerated aging test (AAT); AAT + H2O: AAT seeds with H2O; AAT + CSN: AAT seeds with compound sodium nitrophenolate (CSN) treatment. Four biological replicates for each treatment were set in enzymes activity assay. The asterisk (*) was indicative of significant differences (p < 0.01, LSD) across treatments. Exogenous CSN at 25 mg L−1 was employed.
CSN promoted the hydrolysis of triacylglycerol to sugars in aged sunflower seeds

As is well known, soluble sugars represent the major energy for plant seeds at the early germination stage, which mainly includes sucrose and fructose [12]. For oilseeds, such as soybean and sunflower, the catabolism of triglyceride plays an important role in providing energy for seed germination and seedling emergence. Triglyceride can be decomposed into fatty acid and glycerol under the catalysis of LIPG, and GPDH catalyzed the hydrolysis of glycerol to form CoA-SH [45]. It was proposed that the changes of different fatty acids content might be related to the regulation of CSN on germination ability of aged sunflower seeds.

Moreover, it was worth noting that CSN treatment significantly decreased palmitic content in sunflower seeds during imbibitions. While the contents of oleic and linoleic were enhanced by CNS treatment. The relationship between seed fatty acid content and seed vigor has been studied in soybeans and sweet peppers (such as endosperm and endosperm cap), thus inducing seed germination [55]. In contrast, high concentration of ABA induces seed dormancy and induce seed germination. It is shown that GA can activate the endogenous hydrolyase activity of seeds to promote the decomposition of stored substances and weaken barrier tissues (such as endosperm and endosperm cap), thus inducing seed germination [55]. In contrast, high concentration of ABA induces seed dormancy that suppresses seed germination [56,57]. Gibberellin (GA) and ABA metabolism is closely related to the seed aging process. Lin et al [58] reported that the natural aged process dramatically increased the ABA content and decreased the GA content in rice seeds. After storage, seeds with lower seed vigor had higher ABA content while lower GA content. However, whether the regulation of CSN on germination of aged-sunflower seeds is related to the GA and ABA metabolism has not been reported. In this study, AAT treatment significantly increased ABA content of sunflower seeds during imbibitions, closely associated with the remarkably increased activities of ABA biosynthesis enzymes (NCED and AAO) and decreased activity of inactivation enzyme ABA8ox.

Abscisic acid (ABA) and Gibberellin (GA) are two important phytohormones that play important roles during seed dormancy and germination [52–54]. For example, GA can break seed dormancy and induce seed germination. It is shown that GA can activate the endogenous hydrolyase activity of seeds to promote the decomposition of stored substances and weaken barrier tissues (such as endosperm and endosperm cap), thus inducing seed germination. In contrast, high concentration of ABA induces seed dormancy that suppresses seed germination. Gibberellin (GA) and ABA metabolism is closely related to the seed aging process. Lin et al [58] reported that the natural aged process dramatically increased the ABA content and decreased the GA content in rice seeds. After storage, seeds with lower seed vigor had higher ABA content while lower GA content. However, whether the regulation of CSN on germination of aged-sunflower seeds is related to the GA and ABA metabolism has not been reported. In this study, AAT treatment significantly increased ABA content of sunflower seeds during imbibitions, closely associated with the remarkably increased activities of ABA biosynthesis enzymes (NCED and AAO) and decreased activity of inactivation enzyme ABA8ox.

Exogenous CSN weakened the induction of ABA content by AAT, notably, only associated with the regulation of NCED and ABA8ox.

Moreover, it was worth noting that CSN treatment significantly decreased palmitic content in sunflower seeds during imbibitions. While the contents of oleic and linoleic were enhanced by CNS treatment. The relationship between seed fatty acid content and seed vigor has been studied in soybeans and sweet peppers (such as endosperm and endosperm cap), thus inducing seed germination. It is shown that GA can activate the endogenous hydrolyase activity of seeds to promote the decomposition of stored substances and weaken barrier tissues (such as endosperm and endosperm cap), thus inducing seed germination. In contrast, high concentration of ABA induces seed dormancy and induced seed germination. It is shown that GA can activate the endogenous hydrolyase activity of seeds to promote the decomposition of stored substances and weaken barrier tissues (such as endosperm and endosperm cap), thus inducing seed germination. It is shown that GA can activate the endogenous hydrolyase activity of seeds to promote the decomposition of stored substances and weaken barrier tissues (such as endosperm and endosperm cap), thus inducing seed germination. In contrast, high concentration of ABA induces seed dormancy and induced seed germination. It is shown that GA can activate the endogenous hydrolyase activity of seeds to promote the decomposition of stored substances and weaken barrier tissues (such as endosperm and endosperm cap), thus inducing seed germination.
enzymes. Besides, AAT treatment down-regulated the activities of GA biosynthesis enzymes (GA3ox and GA20ox), and thereby suppressed GA accumulation in sunflower seeds during imbibitions. However, CSN treatment made no significant effect on GA content in AAT-treated sunflower seeds during imbibitions time. It was proposed that GA metabolism might not play a crucial role in the regulation of CSN on sunflower seed germination.

Conclusions

To sum up, this study revealed that, the CSN application could improve the germination and seedling emergence capacities of naturally and artificially aged sunflower seeds. During the germination process of aged sunflower seeds, exogenous CSN enhanced the hydrolysis rate of triacylglycerol into fatty acids and glycerin. Meanwhile, it promoted the conversion of fatty acids and glycerin into sucrose and fructose. Moreover, CSN treatment reduced the ABA content by regulating the activities and genes expressions of NCED and ABA8ox in aged sunflower seeds (Fig. 9). On the one hand, the present study revealed that the fatty acid, glycometabolism, and abscisic acid metabolism are crucial to the germination and seedling emergence of aged sunflower seeds. On the other hand, it provided theoretic and practical basis for using CSN to enhance safety production for sunflower.

Compliance with ethics requirements

This article does not contain any studies with human or animal subjects.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
Buti M, Giordani T, Cattonaro F, Cossu RM, Pistelli L, Vukich M, et al. Temporal...

Zhou WG, Chen F, Zhao SH, Yang CQ, Meng YJ, Shuai HW, et al. DA-6 promotes...

Celus M, Salvia-Trujillo L, Kyomugasho C, Maes I, Loey AV, Grauwet T, et al. Ch...

Nonogaki H, Bassel GW, Bewley JD. Germination-still a mystery. Plant Sci...

Zhang Y, Zhang WL. Analysis of changes and trends in world sunflower...

Goepfert S, Poirier Y. TCA...

Dang XJ, Thi TGT, Dong GS, Wang H, Edzesi WM, Hong DL. Genetic diversity and association mapping of seed vigor in rice (Oryza sativa, L.). Planta 2014;239:1309–19.

Rajjou L, Duval M, Gallardo K, Catusse J, Bally J, Job C, et al. Seed germination and vigor. Annu. Rev. Plant 2012;63(3):507–33.

Nonogaki H, Bassel GW, Bewley JD. Germination-still a mystery. Plant Sci 2010;179:574–81.

Rajjou L, Duval M, Gallardo K, Catusse J, Bally J, Job C, et al. Seed germination and vigor. Annu. Rev. Plant. Biol 2012;63(3):507–33.

Wang L, Hu W, Zahoor R, Yang XN, Wang YH, Zhou ZG, et al. Cool temperature caused by late planting affects seed vigor via altering kernel biomass and antioxidant metabolism in cotton (Gossypium hirsutum L.). Field Crop Res 2019;236:145–54.

Dang XJ, Thi TGT, Dong GS, Wang H, Edzesi WM, Hong DL. Genetic diversity and association mapping of seed vigor in rice (Oryza sativa, L.). Planta 2014;239:1309–19.

Chen M, Thelen JJ. The plastid isoform of triose phosphate isomerase is required for the postgerminative transition from heterotrophic to autotrophic growth in Arabidopsis. Plant Cell 2010;22:77–90.

Theodoulou FL, Eastmond PJ. Seed storage oil catabolism: a story of give and take. Curr. Opin. Plant. Biol 2012:15:322–8.

Xiong YT, Ren Y, Li W, Wu FS, Yang WJ, Huang XL, et al. NF-YC12 is a key multifunctional regulator of accumulation of seed storage substances in rice. J. Exp. Bot 2018;70:15.

Goepfert S, Poirier Y. Oxidation in fatty acid degradation and beyond. Curr. Opin. Plant. Biol 2010;13(1):245–53.

Chen M, Thelen JJ. The plastid isoform of triose phosphate isomerase is required for the postgerminative transition from heterotrophic to autotrophic growth in Arabidopsis. Plant Cell 2010;22:77–90.

Acknowledgments

This study is supported by the Key Research and Development Program of Zhejiang Province (No. 2019C02004).

Author contributions

Huang Y designed and performed most of the experiments; Cai S performed the seed storage and germination test. Ruan X and Xu J contributed to the HPLC and qRT-PCR analysis. Cao D reviewed and edited the whole manuscript. All authors read and approved the final version of the manuscript.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jare.2021.01.019.

References

[1] Buti M, Giordani T, Cattonaro F, Cossu RM, Pistelli L, Vukich M, et al. Temporal dynamics in the evolution of the sunflower genome as revealed by sequencing and annotation of three large genomic regions. Theor. Appl. Genet 2011;123(3):779–91.

[2] Zhang Y, Zhang WL. Analysis of changes and trends in world sunflower production and trade structure. World Agr 2018;5:119–26.

[3] Celus M, Salvia-Trujillo L, Kyomugasho C, Maes I, Loey AV, Grauwet T, et al. Structurally modified pectin for targeted lipid antioxidant capacity in linseed/sunflower oil-in-water emulsions. Food Chem 2018;241:86–96.

[4] Nonogaki H, Bassel GW, Bewley JD. Germination-still a mystery. Plant Sci 2010;179:574–81.

[5] Rajjou L, Duval M, Gallardo K, Catusse J, Bally J, Job C, et al. Seed germination and vigor. Annu. Rev. Plant. Biol 2012;63(3):507–33.

[6] Wang L, Hu W, Zahoor R, Yang XN, Wang YH, Zhou ZG, et al. Cool temperature caused by late planting affects seed vigor via altering kernel biomass and antioxidant metabolism in cotton (Gossypium hirsutum L.). Field Crop Res 2019;236:145–54.

[7] Dang XJ, Thi TGT, Dong GS, Wang H, Edzesi WM, Hong DL. Genetic diversity and association mapping of seed vigor in rice (Oryza sativa, L.). Planta 2014;239:1309–19.
Zvinavashe AT, Lim E, Sun H, Marelli B. A bioinspired approach to engineer Cai Y, Shao L, Li X, Liu G, Chen S. Gibberellin stimulates regrowth after Eastmond PJ, Astley HM, Parsley K, Aubry S, Williams BP, Menard GN, et al. in hybrid rice seeds Penfield S, Clements S, Bailey KJ, Gilday AD, Leegood RC, Gray JE, et al. Expression An YQ, Lin L. Transcriptional regulatory programs underlying barley Quettier AL, Eastmond PJ. Storage oil hydrolysis during early seedling growth. Missaoui AM, Hill NS. Use of accelerated aging as a surrogate phenotyping approach to improve endophyte survival during storage of tall fescue seed. Field Crop Res 2015;183:43–5. Zhang SW, Gu ZB, Wang XM. Determination of compound sodium nitroprusside residues in fruit by ultra performance liquid chromatography tandem mass spectrometry. Chem Anal Meter 2015;24(1):68–70. Zhu L, Cao D, Hu Q, Guan Y, Hu W, Nawaz A, et al. Physiological changes and shSHP genes relative transcription in relation to the acquisition of seed germination during maturation of hybrid rice seed. J Sci Food Agr 2015;96:1–8. Shi H, Wang B, Yang P, Li Y, Miao F. Differences in sugar accumulation and mobilization between sequential and non-sequential senescence wheat cultivars under natural and drought conditions. PLoS ONE 2016;11:e0166155. Cai Y, Shao L, Li X, Liu G, Chen S. Gibberellin stimulates regrowth after defoliation of sheepegrass (Leymus chinensis) by regulating expression of fructan-related genes. J Plant Res 2016;129:935–44. Bailly C, Audigier C, Ladonne F, Wagner MH, Coste F, Corbineau F, et al. Changes in oligosaccharide content and antioxidant enzyme activities in developing bean seeds as related to acquisition of drying tolerance and seed quality. J Exp Bot 2010;52(357):701–8. Emami H, Kempken F. PRECOCIOUS1 (POCO1), a mitochondrial pentatricopeptide repeat protein affecting flowering time in Arabidopsis thaliana. Plant J 2019;100(2):265–78. Yang C, Iqbal N, Hu B, Zhang Q, Wu H, Liu X, et al. Targeted metabolomics analysis of fatty acids in soybean seeds using GC-MS to reveal the metabolic manipulation of shading in the intercropping system. Anal Methods-UK 2017;9:2144–52. Huang YT, Lin C, He F, Li Z, Guan YJ, Hu QJ, et al. Exogenous spermidine improves seed germination of sweet corn via involvement in phytohormone interactions, H2O2 and relevant gene expression. BMC Plant Biol 2017;17:1–16. Eastmond PJ, MONODEHYROASCORBATE REDUCTASE is required for seed storage oil hydrolysis and postgerminative growth in Arabidopsis. Plant Cell 2007;19:1376–87. Quettier AL, Eastmond PJ. Storage oil hydrolysis during early seedling growth. Plant Physiol Bioch 2009;47:485–90. Zhang KL, Yao LJ, Zhang Y, Baskin JM, Baskin CC, Xiong ZM, et al. A review of the seed biology of Paonia species (Paonieae), with particular reference to dormancy and germination. Planta 2019;249:291–303. Yin GK, Xin X, Xiao CS, Chen L, Zhang JM, Wu SH, et al. Activity levels and expression of antioxidant enzymes in the ascorbateglutathione cycle in artificially aged rice seed. Plant Physiol Bioch 2014;80:1–9. Yin X, He D, Gupta R, Yang P. Physiological and proteomic analyses on artificially aged Brassica napus seed. Front Plant Sci 2015;6:112. Fleming MB, Richards CM, Walters C. Decline in RNA integrity of dry-stored soybean seeds correlates with loss of germination potential. J Exp Bot 2017;68:2219–30.