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Metabolic and transcriptional regulation of phenolic conversion and tocopherol biosynthesis during germination of sesame (*Sesamum indicum* L.) seeds†

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This study aims to evaluate the changes in phenolic acids, lignans and tocopherols of sesame seeds during 0–6 days of germination by monitoring the activities of phenolic metabolism-related enzymes and the expression of key genes in the tocopherol synthesis pathway. Sesamol, which is the most active lignan antioxidant, greatly increased, and most of the phenolic acid contents increased to varying degrees after germination. Correspondingly, the related enzymes, including phenylalanine ammonia-lyase (PAL), cinnamate-4-hydroxylase (C4H), and 4-coumarate:coenzyme A ligase (4CL), were activated. Germination also promoted the conversion of γ-tocopherol to α-tocopherol with the expression of related genes changed. Additionally, there was a high correlation between the tocopherol content and the relative expression levels of key genes. The germination process also increased the bio-accessibility of lignans and tocopherols. Therefore, germination can be utilized to improve the nutritional value of sesame-related products.

1. Introduction

As an important oilseed crop and traditional food for humans, sesame (*Sesamum indicum* L.) has long been used in eastern countries.[1] Currently, sesame is widely used in making edible flour, salads, cakes, and oil. Sesame seeds contain lignans, tocopherols, phenolic acids, and other bioactive compounds, which have been reported to have many health benefits such as anti-oxidant, anti-cancer, and anti-inflammatory effects, and prevent cardiovascular diseases.[2]

Germination is an effective method for increasing the nutrition and activity of grains and seeds.[3] As previously reported, germination causes the degradation of carbohydrates and proteins in cell walls, increases the amounts of monosaccharides and free amino acids, and results in the continuous release of binding phenolic substances.[4,5] During the germination process, the endogenous enzymes in seeds are activated, resulting in significant changes of the physicochemical properties of the seeds, including the synthesis, hydrolysis, and methylation of active substances.[6] To the best of our knowledge, the studies on the germination of sesame thus far have only included characterization of fundamental substances, such as total phenolic content, free amino acids, fatty acids, and antioxidant activities.[5,7,8] Therefore, although germination is an important means to increase the nutrients in plants, the release and transformation of monomeric phenolic substances, lignans, and tocopherols as well as the mechanisms at the enzyme level and transcriptional level in sesame during germination remain unclear.

Sesamin and sesamolin are the two main lignans in sesame seeds that were found to be antioxidative and health-promoting. Sesamolin hydrolysis results in the production of sesamol during heating, and the antioxidant activity of sesamol was found to be higher than those of sesamin and sesamolin, which is the main reason for the strong antioxidant activity of sesame after roasting.[9] The formation of phenolic compounds is closely related to the regulation of metabolic enzymes. The phenylpropanoid pathway catalyzes phenylalanine into secondary metabolites, such as flavonoids, phenolic acids, lignans, and stilbenes, and it is an important pathway in secondary plant metabolism.[10] Because phenylalanine ammonia-lyase (PAL), cinnamate-4-hydroxylase (C4H), and 4-coumarate:coenzyme A ligase (4CL) are considered to be the key enzymes in phenylpropanoid metabolism, we speculate that they are closely related to the accumulation of phenolic compounds in sesame. Therefore, increasing the activity of the above

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enzymes is the key to increasing the content of phenolic compounds.

Tocopherols (Toc) are other important active compounds in sesame. They have many beneficial properties, such as anti-proliferation of cancer cells and anti-inflammatory activity.[11] Tocopherols mainly have three forms in sesame, α-Toc, γ-Toc, and δ-Toc. The differences between them are the number and position of methyl groups on the chromanol moiety. Because α-Toc has the highest *in vivo* antioxidant activity and the lowest content in sesame, increasing the α-Toc content through germination is crucial to increasing the biological activity of sesame seeds. The biosynthetic pathway of tocopherols presents several key regulatory enzymes (Fig. 4A). Homogentisate phytyltransferase (HPT) is a key enzyme that catalyzes the condensation of homogentisate (HGA) and phytyldiphosphate (PDP) during the first step of tocopherol biosynthesis. Tocopherol cyclase (TC) cyclizes both intermediates involved in the biosynthesis of δ- and γ-Toc. γ-Tocopherol methyltransferase (γ-TMT) catalyzes the final steps in the synthesis of tocopherols, and converts γ-Toc and δ-Toc to the α- and β-isoforms.[12] Thus, several studies examined the effect of germination on the content of tocopherols;[7,13] however, the evidence of whether tocopherol biosynthesis is regulated at the transcriptional level in seed germination is lacking.

In addition to noting the changes in the content of bioactive compounds, the premise that nutrient molecules play a beneficial role in the human body is that they are bio-accessible.[14] Bio-accessibility refers to the amount of a bioactive molecule or nutraceutical released from the food matrix in the digestive tract for absorption.[14] Because germination changes the sesame seed matrix, the concentration of the active components and the existing forms in the matrix also changes. Because lignans and tocopherols exhibit various pharmacological activities, it is necessary to determine the bio-accessibility of these antioxidant compounds during germination. However, to the best of our knowledge, there are no reports available describing the impact of the germination process on the bio-accessibility of lignans and tocopherols of sesame seeds.

Therefore, this study aimed to investigate the following during the germination of sesame: (1) the impact of specific phenolic compounds, the lignan content, and the activities of related enzymes; (2) the changes in the tocopherol composition and relative expression levels of key genes in tocopherol synthetic pathways; and (3) the bio-accessibility of lignans and tocopherols of sesame seeds.

2. Materials and methods

2.1 Chemicals and materials

Sesame seeds were purchased from Henan Academy of Agricultural Sciences (Zhengzhou, China). Sesamol, sesamolin, sesamin, α-tocopherol, γ-tocopherol, β-tocopherol, δ-tocopherol, chlorogenic acid, gallic acid, caffeic acid, ferulic acid, *p*-coumaric acid, rutin, quercetin, and apigenin standards were provided by Sigma-Aldrich (St Louis, MO, USA). High-performance liquid chromatography (HPLC)-grade acetonitrile and methanol were purchased from Tedia Company Inc. (Fairfield, OH, USA). Other reagents were analytical grade and obtained from Beijing Chemical Factory (Beijing, China). Enzyme-linked immunosorbent assay (ELISA) kits for PAL and C4H were purchased from Solarbio (Beijing, China). An ELISA kit for 4CL was obtained from Shanghai Jianglai Biotech (Shanghai, China). OminiPlant RNA Kit, Transcriptase Mixture, and MltraSYBR Mixture were purchased from Kangwei (Beijing, China).

2.2 Germination process

Sesame seeds were selected for germination and incubated in a growth chamber at 25 °C for 6 days without sunlight. The seeds were watered every 8 hours with de-ionized water. Seed samples were collected at seven time points (0 d, 1 d, 2 d, 3 d, 4 d, 5 d, 6 d). The collected samples were freeze-dried, milled through an 80-mesh sieve, and then stored at 4 °C until analysis.

2.3 HPLC analysis for lignans

Sesamin, sesamol, and sesamolin in sesame were quantitated according to a protocol from a previous study with some modifications using an HPLC system equipped with an ultraviolet (UV) detector (Model L-2400, Hitachi, Tokyo, Japan).[1] First, 0.5 g of sesame powder was weighed into 15 mL centrifuge tubes (three replicates per sample), and then extracted for 30 min with 5.0 mL of 80% methanol. The liquid was centrifuged at 2000*g* for 3 min at 25 °C. The liquid was transferred to another tube for re-extraction of the residue. The supernatants were combined, and the volume was adjusted to 10 mL with 80% methanol, and then filtered through a 0.45 μm organic phase membrane before HPLC analysis. The mobile phases were water (solvent A) and methanol (solvent B) with a gradient system: 0–15 min, 90% B; 25 min, 90% B; 26 min, 20% B. The flow rate was 1.0 mL min^{-1} (injection volume 20 μL) with a detection wavelength of 290 nm.

2.4 HPLC analysis for phenolic acids

The quantification of phenolic compounds was modified from previous methods using an HPLC system equipped with a UV detector and a C18 column (4.6 mm id × 250 mm length, 5 μm, Shiseido, Japan).[8] The standards, including *p*-coumaric, gallic, chlorogenic, caffeic, and ferulic acids, as well as apigenin, quercetin, and rutin, were dissolved in methanol for analysis. The powdered seeds (1.0 g solids) were ultrasonically extracted in 10 mL of 80% methanol for 1 h at room temperature. The extract was centrifuged at 3000*g* for 5 min at 4 °C before the supernatant was filtered through a 0.45 μm organic phase filter membrane for HPLC analysis. The two mobile phases were 0.1% acetic acid in water (eluent A) and 0.1% acetic acid in acetonitrile (eluent B) with the following gradient: 0–5 min, 5% B; 40 min, 80% B; 50 min, 80% B; 51 min, 5% B. The total running time was 60 min with a flow rate of 1.0 mL min^{-1} and the column temperature was maintained at

25 °C. The volume of injection was 20 μL and the detection of phenolic acids was monitored at 254 nm.

2.5 Determination of enzymatic activity related to phenolic metabolism

The activities of the three enzymes (PAL, C4H, and 4CL) were examined. The freeze-dried seeds that germinated at 0–6 days were ground in liquid nitrogen. The quantities of PAL and C4H were determined using an ELISA kit from Solarbio (Beijing, China), and the 4CL activity was measured using an ELISA kit from Shanghai Jianglai Biotech (Shanghai, China).

2.6 HPLC analysis for tocopherols

Tocopherols were detected using HPLC following a procedure described in a previous study that was modified.[15] First, 1.0 g of sesame powder was added to 20.0 mL of *n*-hexane for each sample. Samples were ultrasonically extracted for 30 min and then centrifuged at 2000*g* for 5 min. The supernatants were transferred to 50 mL centrifuge tubes, and the residue was re-extracted with 20.0 mL of *n*-hexane. All extracted solutions were combined and evaporated to dryness in a rotavac, redissolved in 5 mL of methanol, and then filtered through a 0.45 μm organic phase filter membrane.

The mobile phase consisted of 2% water (solvent A) and 98% methanol (solvent B) with equal gradient elution for 23 minutes, and a reverse phase C18 column (250 × 4.6 mm, 5 μm) was used; column temperature: 25 °C; injection volume: 20 μL; flow rate: 0.8 mL min^{-1}; and a fluorescence detector was used with an excitation wavelength of 294 nm and an emission wavelength of 340 nm.

2.7 Determination of expression levels of key genes in tocopherol synthesis

Total RNA from the germinating seeds (0, 3, 6 days) was extracted using the OminiPlant RNA Kit (Kangwei, Beijing, China) according to the manufacturer's instructions. HiFiScript gDNA Removal RT MasterMix (Kangwei, Beijing, China) was used to reverse to cDNA. RT-qPCR was performed on the cDNA using the Bio-Rad MyiQ iCycler qPCR instrument, and SYBR Green was used as the fluorophore in a qPCR supermix. The thermal cycle conditions were optimized as 10 min at 95 °C, followed by 45 cycles of amplification (15 s at 95 °C, and 60 s at 60 °C). For each sample, RT-qPCR was conducted with specific primers for HPPD, HPT, TC, and γ-TMT, and the primer sequences are listed in ESI Table S1.† Sesame *Actin* was selected as a reference gene. Ct values were processed with the $2^{-\Delta\Delta Ct}$ method for calculating the relative expression. The results are expressed as the mean ± standard deviation (SD).

2.8 Determination of bio-accessibility of sesame lignans and tocopherols

For the bio-accessibility of lignans and tocopherols, we simulated the human gastrointestinal tract (GIT) using a three-stage gastrointestinal model according to a previous study.[16] Duplicate samples (3.0 g) were weighed into glass beakers and mixed with 20 mL of simulated saliva fluid containing 0.03 g mL^{-1} mucin, which was preheated to 37 °C. The pH was adjusted to 6.8, and then, the liquid was incubated in a shaker for 10 min at 37 °C to simulate agitation in the mouth. To simulate the gastric phase, the samples produced by the oral phase were mixed with 20 mL of simulated gastric fluid containing 0.0032 g mL^{-1} pepsin in a 37 °C water bath. The pH was adjusted to 2.5, and then, the liquid was incubated for 2 h.[17] The pH of the solution from the stomach phase was subsequently adjusted to 7.0, and then, 3.5 mL of bile salt solution and 2.0 mL of simulated intestinal fluid were added to the samples with constant stirring. The pH was adjusted back to 7.0 after 3.5 mL of lipase solution was added, and the solution was then incubated for 2 h at 37 °C. After the completion of the intestinal phase, the samples were centrifuged for 60 min at 8000*g*. Next, 5 mL of the supernatant was extracted with 5 mL of methanol and then filtered through a 0.45 μm organic phase filter membrane before conducting HPLC to analyze sesamin, sesamol, and sesamolin according to the method described in section 2.3. To prepare the solutions for HPLC, 10 mL of the supernatant was extracted with 10 mL of *n*-hexane. All extracted solutions were combined and evaporated to dryness in a rotavac, redissolved in 5 mL of methanol, and then filtered through a 0.45 μm organic phase filter membrane to analyze the tocopherols according to the method described in section 2.6. The bio-accessibility (%) of lignans and tocopherols was calculated using the following expression:

$$\text{Bio-accessibility } (\%) = C_1/C_0 \times 100$$

where C_0 and C_1 are the amounts of lignans and tocopherols in the initial sesame and digested samples.

2.9 Statistical analysis

The results were statistically analyzed by one-way ANOVA using SPSS 17.0 (SPSS, Inc., Chicago, IL, USA), and were considered to be statistically significant when *P*-values were less than 0.05.

3 Results and discussion

3.1 The transformation of lignans during germination

The HPLC chromatograms shown in Fig. 1(A–C) compare the changes in lignan composition before and after germination. The lignan content of sesame seed samples during germination is shown in Fig. 1(D). Raw sesame seeds mainly contain sesamin and sesamolin. The sesamol content was dramatically increased during germination, from 0.05 mg g^{-1} to 5.70 mg g^{-1}, approximately 113-fold. However, interestingly, the amount of sesamolin and sesamin gradually decreased with the germination time and stabilized 5 days after germination. Total lignans decreased within 1–3 days of germination and then increased to levels not less than those of the raw seeds. Thus, the increase in the amount of sesamol balanced the decrease of sesamin and sesamolin and did not affect the total lignan content. It is generally known that sesamol is produced by the heat of hydrolysis of sesamolin.[9,18] Because the three lignans have a common molecular unit, it is likely that the

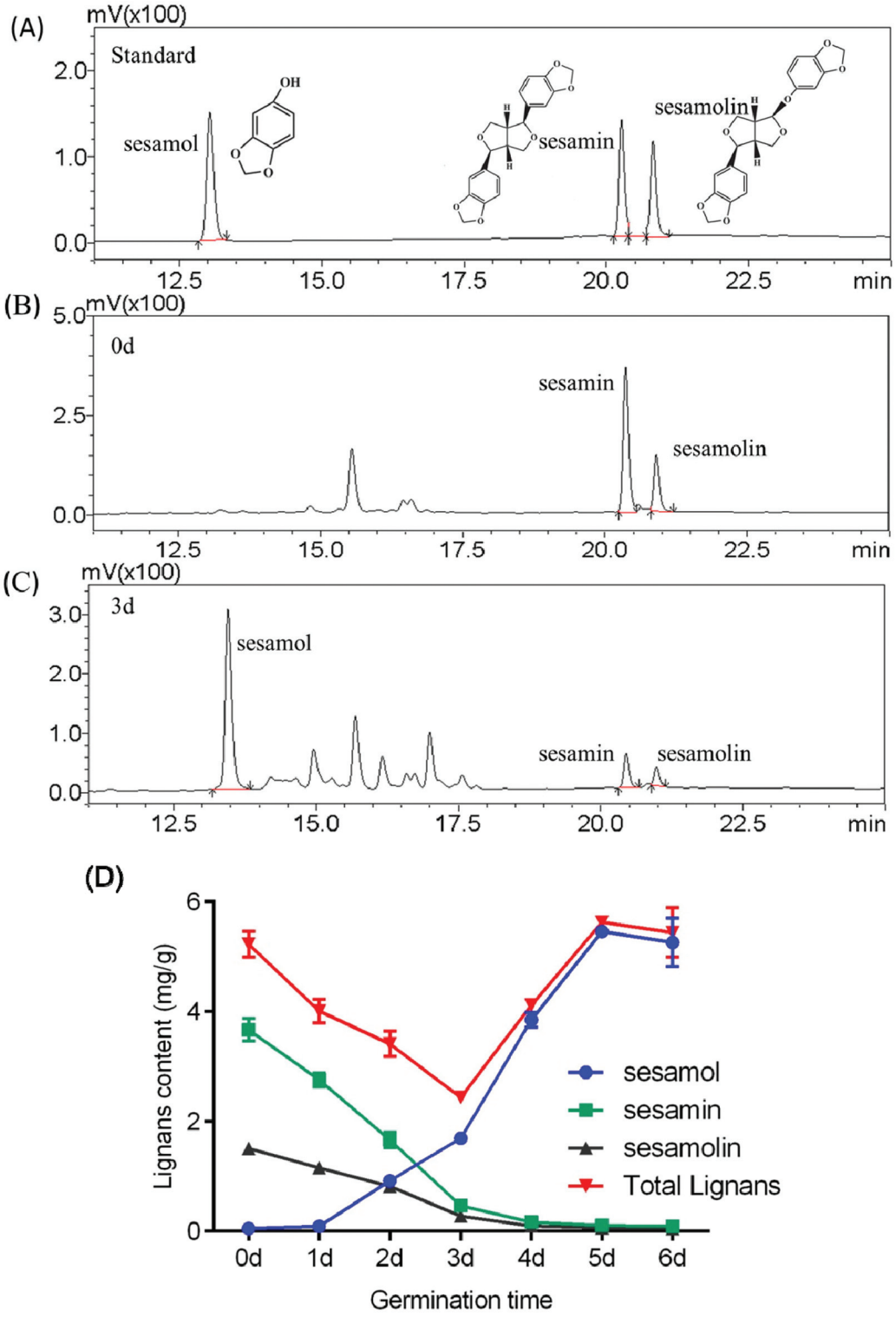

Fig. 1 HPLC chromatograms of a mixed standard solution of sesamol, sesamin, and sesamolin (A), 0-day (0d) germination of the sesame sample (B), and 3-day (3d) germination of the sesame sample (C). Changes in sesamol, sesamolin, sesamin, and total lignan contents of sesame during germination (0–6 days) (D).

increase in sesamol in germinated sesame seeds can be due to the activation of enzymes involved in the synthesis pathways of sesamol or the transformation from other lignans, especially the hydrolysis of sesamolin, but the specific mechanism remains to be studied. Sesamol is a powerful phenolic antioxidant that effectively inhibits lipid peroxidation and pro-

gression of atherosclerosis.[19,20] Due to the presence of the hydroxyl group, the ability to bind metal ions, and the hydrophilic and lipophilic nature of sesamol, it is more effective as a health-promoting substance than sesamolin and sesamin.[21] Additionally, the antioxidant activity of sesamol was found to be higher than those of sesamolin and sesamin.[18] Thus, this result showed that unroasted sesame seeds with a high sesamol content obtained *via* the germination process could be an important source of antioxidants.

3.2 Comparison of phenolic acids during germination

HPLC was used for the investigation of phenolic acids (*i.e.*, gallic, chlorogenic, caffeic, *p*-coumaric, and ferulic acids, as well as rutin, quercetin, and apigenin). The changes in the content of the main phenolic acids during germination are shown in Table 1. All of the phenolic acids increased to varying degrees after germination except for quercetin. Chlorogenic acid was only found in germinated seeds and reached 1.43 mg per 100 g at 5 days after germination. The maximum gallic acid level was achieved at 6 days of germination, reaching 7.55 mg per 100 g. Ferulic acid significantly increased at the initial germination stages, reached 22.7-fold at 3 days after germination, and then, its content gradually decreased but was still higher than that of ungerminated seeds. The level of caffeic acid increased from 0.29 to 9.06 mg per 100 g. Similar to caffeic acid, the content of *p*-coumaric acid, apigenin, and rutin changed with a steadily increasing trend, and the content of rutin showed a notable increase, which ranged from 0.14 to 520.11 mg per100 g. A significant decrease in quercetin occurred during germination, where 91.1% of its initial level was lost at 3 days after germination. The results we obtained are similar to those of a previous study, where it was reported that the content of rutin increased 31 times 9 days after the germination of buckwheat seeds, and the content of quercetin decreased from 15.2 mg g^{-1} (1-day sprouts) to 0 mg g^{-1} (7-day sprouts).[22] Thus, we speculated that quercetin decreased because it was used for the synthesis of rutin. Phenolic acids (more specifically, caffeic acid, *p*-coumaric acid, and rutin) are powerful antioxidants and can counteract oxidative stress.[23] Therefore, germinated sesame seeds can be used as a good source of antioxidants in human diets.

3.3 The effect on enzymatic activities related to the phenolic metabolism of germination

Fig. 2 shows the changes in PAL, C4H, and 4CL activities during the germination of sesame. PAL is the key enzyme in the phenylalanine pathway, and it has been reported that the activation of PAL leads to the synthesis of phenolic acids during the germination of rice.[24] Ren and Sun also found that the PAL activity is positively correlated with phenolic acid accumulation ($r^2 = 0.9761$, $p < 0.01$) during the germination of buckwheat, indicating that PAL is involved in the synthesis of phenolic compounds.[22] However, the effect of germination on enzymes related to the phenolic metabolism of sesame has not been reported. A continuous increase in PAL activity in sesame samples, which reached 2.45-fold that of raw seeds, was measured until 6 days after germination. PAL catalyzes the first step in the phenylalanine pathway, converting phenylalanine to *trans*-cinnamic acid.[25] Therefore, this result further confirmed that the synthesis of specific classes of hydroxycinnamic acids (*p*-coumaric acid, caffeic acid, ferulic acid, and chlorogenic acid) is caused by the activation of PAL. The activation of PAL is also the primary condition for the synthesis of phenolic acids.

There was no significant change in C4H activity within 3 days of germination in sesame, but the activity sharply

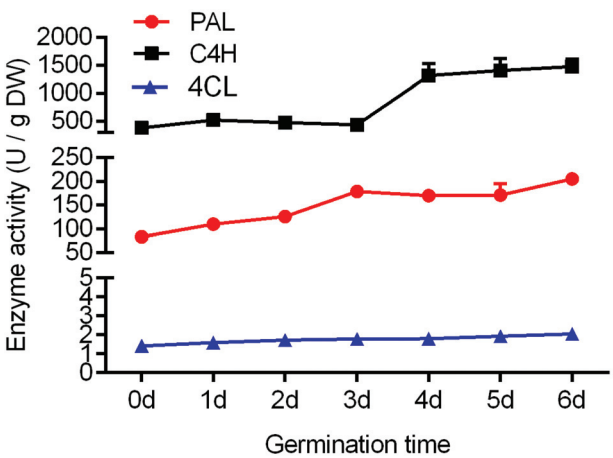

Fig. 2 Changes in PAL, C4H, and 4CL activities of sesame at different germination times (0–6 days).

Table 1 Changes in phenolic acid contents in sesame during germination

Germination times	Phenolic acids (mg per 100 g)							
	Chlorogenic acid	Gallic acid	Caffeic acid	*p*-Coumaric acid	Ferulic acid	Rutin	Quercetin	Apigenin
0 d	ND	1.68 ± 0.23f	0.29 ± 0.04f	0.22 ± 0.02e	0.72 ± 0.03f	0.14 ± 0.01f	2.73 ± 0.06a	2.58 ± 0.09d
1 d	0.02 ± 0.02f	1.13 ± 0.02e	0.09 ± 0.01g	0.18 ± 0.01e	1.70 ± 0.06e	2.52 ± 0.10f	1.33 ± 0.03b	0.81 ± 0.20g
2 d	0.16 ± 0.05e	2.13 ± 0.02d	0.60 ± 0.01e	0.43 ± 0.03e	8.79 ± 0.18d	57.16 ± 0.82e	0.97 ± 0.02c	1.27 ± 0.11f
3 d	0.64 ± 0.01d	3.67 ± 0.01c	2.54 ± 0.03d	0.38 ± 0.28d	17.12 ± 0.22a	212.16 ± 5.50d	0.62 ± 0.07e	2.39 ± 0.06e
4 d	1.06 ± 0.04c	5.36 ± 0.24b	5.50 ± 0.06c	13.37 ± 0.27c	14.18 ± 0.58b	364.12 ± 4.62c	0.24 ± 0.03f	2.85 ± 0.05c
5 d	1.43 ± 0.01a	7.52 ± 0.20a	9.06 ± 0.17a	24.72 ± 0.37a	9.04 ± 0.60d	520.11 ± 3.37a	1.01 ± 0.05c	5.47 ± 0.19a
6 d	1.14 ± 0.07b	7.55 ± 0.05a	6.43 ± 0.09b	16.17 ± 0.71b	11.91 ± 0.05c	444.89 ± 4.96b	0.89 ± 0.01d	3.92 ± 0.04b

increased on the fourth day. C4H functions aerobically and is dependent on NADPH, which catalyzes the second step of the phenylalanine pathway, and is also involved in the first oxidation reaction of this pathway. In response, *trans*-cinnamic acid is catalyzed to form *p*-coumaric acid. This is consistent with the significant increase in the *p*-coumarin content after germination that was observed in our study.

4CL activity steadily increased throughout the germination process, from 1.42 to 2.05 U per g DW. 4CL acts on the last step of the phenylalanine pathway, catalyzing the formation of the corresponding thioesters of various hydroxycinnamic acids, and mainly uses coumaric acid, caffeic acid, and ferulic acid as catalytic substrates to finally convert into secondary metabolites, such as flavonoids, lignans and other compounds.[26] This result provided a more accurate explanation for the accumulation of phenolic compounds such as phenolic acids and lignans during sesame seed germination.

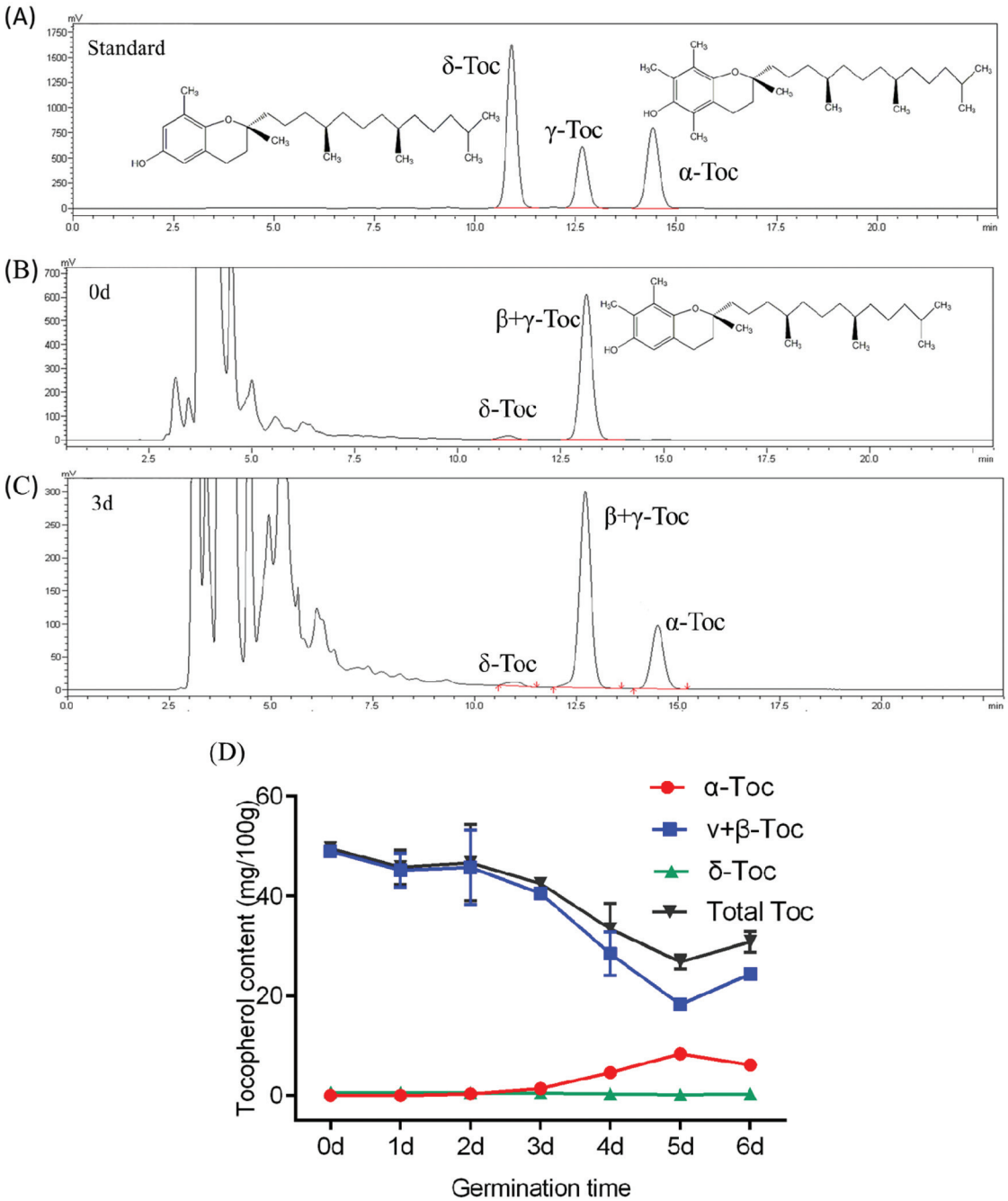

Fig. 3 HPLC chromatograms of a mixed standard solution of σ-tocopherol (σ-Toc), γ-tocopherol (γ-Toc), and α-tocopherol (α-Toc) (A), 0-day (0d) germination of the sesame sample (B), and 3-day (3d) germination of the sesame sample (C). Changes of σ-tocopherol, γ + β-tocopherol, α-tocopherol, and total tocopherol contents of sesame during germination (0–6 days) (D).

3.4 The transformation of tocopherols during germination

Tocopherols are considered to be effective antioxidants and growth promoters that act synergistically with polyphenols. In this study, the effect of germination on various forms of tocopherols was determined by HPLC. Because the amount of β-Toc is generally low in sesame, the separate peak of β-Toc was not isolated by this method. As shown in Fig. 3(D), the results indicated that the raw sesame seeds included significantly higher levels of γ-Toc than those of δ-Toc and α-Toc. α-Toc steadily increased from 0 to 8.35 mg per 100 g at 5 days after germination, whereas γ-Toc was reduced from 48.93 mg per 100 g to 18.28 mg per 100 g at the end of day 6 after germination, and δ-Toc exhibited a slight decrease. However, the total tocopherol content decreased after germination. These results are consistent with those from our previous study on flaxseed germination.[13] Typical chromatograms are shown in

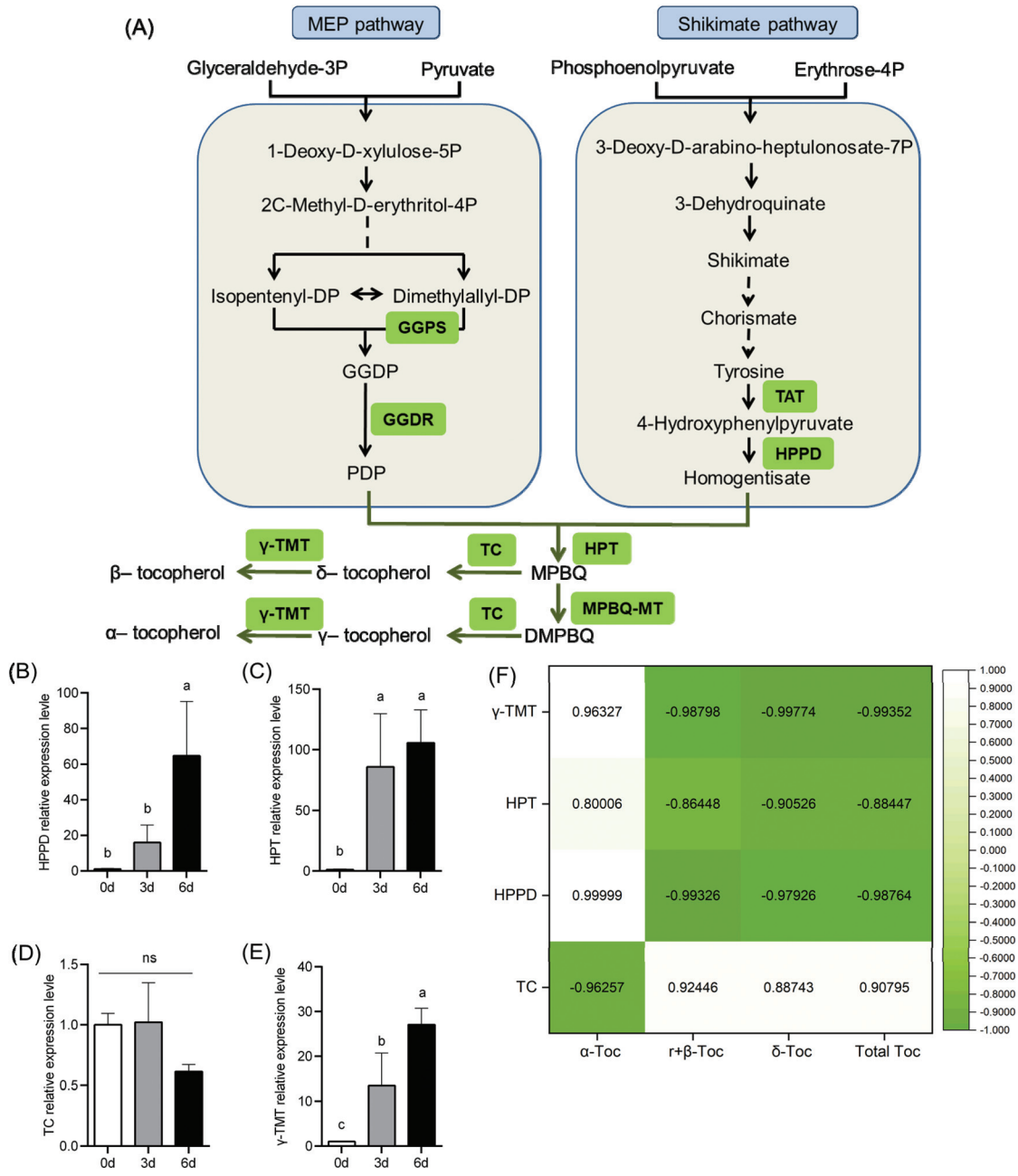

Fig. 4 Tocopherol biosynthetic pathway. MEP, methylerythritol 4-phosphate; GGPS, geranylgeranyl diphosphate synthase; GGDP, geranylgeranyldiphosphate; GGDR, geranylgeranyl diphosphate reductase; PDP, phytyldiphosphate; TAT, tyrosine aminotransferase; HPPD, *p*-hydroxyphenylpyruvate dioxygenase; HPT, homogentisate phytyltransferase; MPBQ-MT, 2-methyl-6-phytylbenzoquinol; TC, tocopherol cyclase; γ-TMT, γ-tocopherol methyltransferase (A). Relative expression of HPPD (B), HPT (C), TC (D), and γ-TMT (E). The correlation of the relative gene expression levels and tocopherol content during germination (F).

Fig. 3(A–C), which indicate that the composition of tocopherols changed during germination. α-Toc contains more methyl groups as compared to other tocopherols. Therefore, in our study, the increase in α-Toc during germination can be attributed to the methylation of γ-Toc. Because α-Toc is traditionally considered to be the most active and powerful biological antioxidant form of tocopherols in humans,[11,27] with the strongest biological activity and molar concentration of lipid-soluble antioxidants,[28] germination can be used to increase the tocopherol activity in sesame seeds.

3.5 Expression and correlation analysis of tocopherol biosynthesis genes

To investigate whether the tocopherol biosynthesis is regulated at the transcriptional level during germination in sesame, RT-qPCR was used to analyze the relative expression of key genes for tocopherol synthesis during three stages (0 days, 3 days, and 6 days) of germination. Because of nonspecific primers, only four genes were analyzed, namely HPPD, HPT, TC, and γ-TMT. As shown in Fig. 4(B–E), the relative expression levels of genes varied with the elongation of the germination time. It was observed that the expression of HPT drastically increased after germination, and was 85 and 105 times greater than that of the non-germinated group at 3 and 6 days after germination, respectively. The relative expression levels of HPPD and γ-TMT were also increased, and were 63 and 26 times higher than the initial level at 6 days after germination, respectively. As for TC, the expression levels remained relatively stable and there was no significant difference before or after germination. These results are closely related to the changes in the amount of tocopherols during germination. The correlations of the relative expression of genes and tocopherol components during germination were analyzed using Origin 2019b software, and the result is shown in Fig. 4(F). Once the germination began, *de novo* α-Toc synthesis occurred. Except for TC, α-Toc was positively correlated with the relative expression levels of the other three genes, especially HPPD and γ-TMT. γ-TMT is the key enzyme in the final step of the pathway of tocopherol synthesis, and it methylates δ-Toc and γ-Toc to β-Toc and α-Toc, respectively. Studies have shown that γ-TMT plays an important role in the synthesis of α-Toc.[29] Therefore, the high expression of γ-TMT resulted in the conversion of γ-Toc to α-Toc during germination. Similarly, because TC catalyzes the synthesis of γ-Toc, the decrease in the γ-Toc content was also highly correlated with the down-regulation of the TC expression level. Thus, there is a transcriptional regulation of tocopherol levels during sesame seed germination.

3.6 Bio-accessibility of sesame lignans and tocopherols

Biological molecules can be utilized by the human body as long as they are bio-accessible. The food matrix largely affects the absorption and release of nutrients in the digestive system.[30] Lignans and tocopherols are two types of representative active components in sesame. Therefore, we selected sesame seeds at 0, 3, and 6 days after germination to explore the effects of germination on the bio-accessibility of lignans and tocopherols. The results in Fig. 5(A) show that, because sesamol is almost undetectable in the ungerminated sesame seeds, there is no bio-accessibility. After germination, the bio-accessibility of sesamol reached 30%. Germination significantly increased the bio-accessibility of sesamin and sesamolin, and the bio-accessibility increased with the germination time. Fig. 5(B) shows the bio-accessibilities of tocopherols in sesame. Similar to sesamol, the raw seeds do not contain α-tocopherol, but the bio-accessibilities reached 2.95% at 6 days after germination. The amounts of γ-tocopherol and δ-tocopherol did not change much with germination before digestion, but the bio-accessibility revealed a significant increase after germination. In general, germination promotes the digestion and degradation of carbohydrates and proteins in cell walls, and increases the release and effective utilization of active components, especially of lignans. Thus, it is likely that the sesame seed cell walls may be disrupted and disorganized during germination, which assists in facilitating the release of lignans, and leads to higher bio-accessibility values. This result elucidates how increased nutrition may be obtained from sesame seeds, which will contribute to health benefits for the human body.

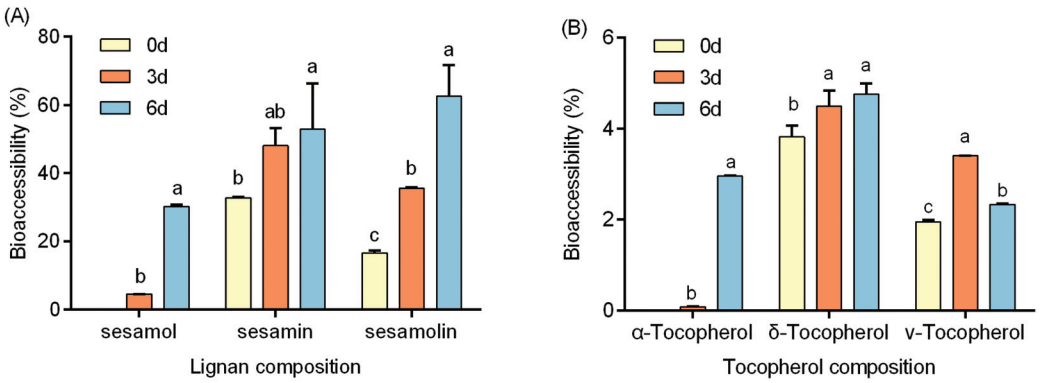

Fig. 5 Bio-accessibility of lignans (A) and tocopherols (B) in sesame at different germination times.

4. Conclusion

As shown in this study, the germination of sesame leads to significant changes in the composition of phenolic compounds, as well as the associated enzymes, including PAL, C4H, and 4CL. Germination promoted the conversion between lignan components, and this was measured during germination because the sesamol content significantly increased while those of the other two components decreased. Most phenolic acids increased with the activation of enzymes that participated in phenolic metabolism. We also explored the changes in the tocopherol composition, the relative expression levels of key genes involved in tocopherol synthesis, and the correlation of the gene expression levels and tocopherol content during germination. The amount of α-tocopherol and the expression levels of related genes increased after germination. Therefore, tocopherol synthesis may be regulated by transcription during germination. Thus, germinated sesame seeds are a satisfactory source of potent natural antioxidants. Additionally, germination increased the bio-accessibility of lignans and tocopherols. Our results can be beneficial for developing functional food with high sesamol and α-tocopherol contents, and provide a reference for the variation in the tocopherol content in sesame during germination and the development of sesame oil with higher antioxidant activity and bio-accessibility.

Abbreviations used

PAL	Phenylalanine ammonia-lyase
C4H	Cinnamate-4-hydroxylase
4CL	4-Coumarate:coenzyme A ligase
HPT	Homogentisate phytyltransferase
HGA	Homogentisate
γ-TMT	γ-Tocopherol methyltransferase
TC	Tocopherol cyclase
PDP	Phytyldiphosphate
MEP	Methylerythritol 4-phosphate
GGPS	Geranylgeranyl diphosphate synthase
GGDP	Geranylgeranyldiphosphate
GGDR	Geranylgeranyl diphosphate reductase
PDP	Phytyldiphosphate
TAT	Tyrosine aminotransferase
HPPD	*p*-Hydroxyphenylpyruvate dioxygenase
MPBQ-MT	2-Methyl-6-phytylbenzoquinol

Conflicts of interest

There are no conflicts to declare.

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