Changes in physio-biochemical metabolism, phenolics and antioxidant capacity during germination of different wheat varieties

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ABSTRACT

Changes in physio-biochemical metabolism, phenolics and antioxidant capacity during germination were studied in eight different wheat varieties. Results showed that germination enhanced sprout growth, and caused oxidative damage, but enhanced phenolics accumulation. Ferulic acid and *p*-coumaric acid were the main phenolic acids in wheat sprouts, and dihydroquercetin, quercetin and vitexin were the main flavonoids. The phenolic acid content of Jimai 44 was the highest on the 2th and 4th day of germination, and that of Bainong 307 was the highest on the 6th day. The flavonoid content of Hei jingang was the highest during whole germination. The enzymes activities of phenylalanine ammonia lyase (PAL), cinnamic acid 4-hydroxylase (C4H) and 4-coumarate coenzyme A ligase (4CL) were up-regulated. The activities of catalase, polyphenol oxidase and peroxidase were also activated. Antioxidant capacity of wheat sprouts was enhanced. The results provided new ideas for the production of naturally sourced phenolic rich foods.

1. Introduction

Wheat (*Triticum aestivum* L.) is one of the three traditional food crops in the world. Wheat is rich in nutrients such as starch, protein and minerals, as well as dietary fiber and bioactive substances. Wheat phenolic compounds and their antioxidant activities have been the focus of research in recent years (Baranzelli et al., 2023; Menga et al., 2023; Shamanin et al., 2022). Epidemiological studies have shown that a plant-based diet was associated with reducing oxidative stress-related diseases such as cancer and cardiovascular disease (Alvarez-Jubete, Wijngaard, Arendt, & Gallagher, 2010; Tomé-Sánchez et al., 2020). Wheat contains different phenolic compounds, mainly phenolic acids and flavonoids (Tomé-Sánchez et al., 2020). Phenolic acid, as an important secondary metabolite, contains phenolic hydroxyl and carboxylic groups, and has physiological effects such as anti-oxidation, anti-cancer and antibacterial (Hagos, Chandravanshi, Redi-Abshiro, & Yaya, 2023; Pham, Hatcher, & Barker, 2011). Flavonoids are important components in plants. It has been reported that flavonoids have health

benefits, such as anti-obesity, anti-inflammatory, anti-cardiovascular, anti-cancer and antioxidant activities (Ah-Reum et al., 2020). Studies have shown that wheat sprouts have the effects of enhancing human immunity, antioxidant, anticancer and reducing the risk of chronic diseases, and these health effects were closely related to the unique phenolic substances and their antioxidant properties (Yu & Beta, 2015). After germination, wheat grains could effectively promote the accumulation of phenolic substances and the improvement of antioxidant activity (Tomé-Sánchez et al., 2020). Pham et al. (2011) found that total phenolic acid content and antioxidant capacity of germinated Canadian wheat were higher than those of ungerminated wheat, and Zilic et al. (2014) studied the germination process of Serbian wheat seeds and found that total phenolic content, total free radical scavenging activity, and ferulic, *p*-coumaric, and caffeic acid content were increased in wheat. Tomé-Sánchez et al. (2020) found that germination time had a significant impact on the accumulation of phenolic substances in wheat sprouts. On the 7th day of germination, the content of phenolic acid and flavone in wheat reached the highest value, and the antioxidant capacity

was also enhanced. Similarly, Arashdeep, Hanuman, Savita, Baljit, and Antima (2021) showed that germination significantly increased the total phenolic acid and total flavonoid content of wheat, and sprouted wheat had higher antioxidant activity. Baranzelli et al. (2023) found that during wheat germination, apigenin exhibited a U-shaped behavior independent of variety, decreasing at 48 h of germination and then increasing at 72 h. In different studies, there are differences in the content and composition of phenolic substances in different wheat varieties during germination, and the number of varieties studied so far is limited.

Plant phenolic compounds are biosynthesised mainly through the phenylpropane metabolic pathway, PAL, C4H and 4CL are the key enzymes in the phenolic compound synthesis pathway, the synthesis and accumulation of phenolic substances are closely related to the gene expression of these enzymes (Vogt, 2010). Deaminated by PAL to form trans-cinnamic acid, which generates phenolic substances such as flavonoids, phenolic acids and anthocyanosides under the action of C4H and 4CL (Vogt, 2010). Arashdeep et al. (2021) found that phenolics and flavonoids were synthesized during wheat germination, and PAL was activated to convert phenolics into flavonoids, and different enzymes or cofactors were produced. Researchers found that PAL activity was enhanced during buckwheat grains germination, and the total phenolic content and its antioxidant activity increased (Ren & Sun, 2014). Similarly, (Wang, Ding et al., 2020) showed that PAL, C4H and 4CL activities increased and phenolic compounds accumulated during barley seed germination. Previous studies on phenolics in wheat mainly focused on raw seeds and tissue parts of different varieties, and lacked systematic studies on phenol content, phenolic acid composition and content, and phenolic compound synthases during wheat germination.

In this study, eight varieties of wheat, including Fu xilv, Zhongmai 175, Jimai 23, Jimai 22, Taikeheimai 1, Hei jingang, Bainong 307, and Jimai 44 were selected, and the physiological and biochemical changes, total phenolic content, phenolic components, the enzyme activities of PAL, C4H and 4CL in phenolic synthesis, and antioxidant capacity of wheat sprouts at different germination stages were studied. This study will provide scientific basis and technical support for the research and development of healthy cereal sprout food.

2. Materials and methods

2.1. Materials and chemicals

Eight wheat varieties, including Fu xilv, Zhongmai 175, Jimai 23, Jimai 22, Taikeheimai 1, Hei jingang, Bainong 307, and Jimai 44, were all planted in China and harvested in 2022. All wheat grains were stored at −20 °C before use.

Methanol, acetic acid, ethyl acetate, glycerol, sodium hypochlorite: Tianjin Fuyu Fine Chemical Co., LTD. Barium hydroxide, oxalic acid: Sinopharm Group Chemical Reagent Co., LTD. Trichloroacetic acid, L-ascorbic acid, thiobarbituric acid, mercaptoethanol: Shanghai McLean Biochemical Technology Co., LTD. ABTS, DPPH: Beijing Boaotoda Technology Co., LTD. Polyethylene glycol, pvpp, pvp: Beijing Solaibao Technology Co., LTD.

2.2. Experimental design

Wheat grains were sterilized with 0.5% NaClO solution at a ratio of 1:5 (*w/v*) for 15 min and then soaked in deionized water for 6 h. Wheat grains were uniformly placed in a small germinator with an automatic spraying system provided a 2 min mist every 1 h. The automatic germination machines (CB-A323B, Foshan Candy Electric Co., Ltd., Guangdong, China) were placed in a constant temperature incubator at 25 °C. The germinators were irradiated with LED white light for 12 h per day, The samples were collected on the 2th, 4th and 6th day, respectively. A portion of the wheat sprout samples were washed three times with deionized water and then wiped clean. The samples were collected into sample bags and stored in a refrigerator at −80 °C after rapid freezing with liquid nitrogen. The other portion of wheat sprouts were harvested and freeze-dried, were ground into powder with a grinder and then passed through a 40 mesh sieve and stored for later use.

2.3. Measurement indexes and methods

2.3.1. Determination of sprout/root length

Sprout/root length: the length of sprout/root of wheat sprouts was determined by randomly selecting 30 wheat sprouts.

2.3.2. Determination of respiration rate

The respiration rate was measured according to the method of Ma, Wang, Chen, Gu, and Yang (2018). The 20 mL of 0.05 mol·L^{-1} Ba(OH)$_2$ solution was added to a wide mouthed bottle. The rubber stopper was immediately tightened and the bottle was fully shaken for 2 min. After all the CO_2 in the bottle was absorbed, the small rubber stopper was pulled out and 2 drops of phenolphthalein were added. The burette was inserted into the hole and a standard oxalic acid solution was used for blank titration until the red color had just disappeared. The volume of the oxalic acid consumed was recorded as V_0 (mL). The 20 mL of Ba(OH)$_2$ solution was reintroduced while weighing about 5 g of wheat sprouts, noted as m (g). The wheat sprouts were put into a small basket and left to stand for 30 min, which was recorded as t (h). Two drops of phenolphthalein were dropped into the bottle and the stopper was immediately resealed. Blank titration with standard oxalic acid solution until the red color has just disappeared, and recorded the volume of oxalic acid consumed as Vs (mL). Respiratory rate was calculated using the Eq. (1).

$$\text{Respiration rate } [\text{mg } CO_2/(\text{g·h})] = (V_0 - Vs) \times 1/(m \times t) \tag{1}$$

2.3.3. Determination of oxidative damage during wheat germination

The content of malondialdehyde content (MDA) was determined using the method of Wang et al. (2023). Fresh wheat sprouts (1.0 g) was ground with 5 mL of 10% TCA. The homogenate was centrifuged at 10,000 ×*g* for 20 min at 4 °C, 2 mL of supernatant was mixed with 2 mL of 0.67% TBA, then the mixture was boiled for 30 min. After the mixture was centrifuged at 4000 ×*g* for 15 min at 4 °C, the absorbance values of the supernatants were determined at 450 nm, 532 nm and 600 nm, respectively.

The content of superoxide anion ($O_2^-\cdot$) was determined using the method of Ma et al. (2018). Fresh wheat sprouts (1.0 g) were mixed with 5.0 mL of extraction buffer (including 1 mmol·L^{-1} EDTA, 0.3% Triton X-100, 2% PVP) and ground at 4 °C. The homogenate was centrifuged at 12,000 *g* for 20 min at 4 °C. Then the 1.0 mL of supernatant was mixed with 1.0 mL of 50 mmol·L^{-1} phosphoric acid buffer with pH 7.8 and 1.0 mL of 1 mmol·L^{-1} hydroxylamine hydrochloride solution, and the mixture was stored at 25 °C for 1 h. Then the 1.0 mL of 17 mmol·L^{-1} *p*-aminobenzenesulfonic acid solution and 1.0 mL of 7 mmol·L^{-1} α-naphthylamine solution were added to the mixture, which was stored at 25 °C for 20 min, and the absorbance was measured at 530 nm.

The content of hydrogen peroxide (H_2O_2) was determined using the method of Ma et al. (2018). Fresh wheat sprouts (1.0 g) were mixed with 5.0 mL of precooled acetone and ground at 4 °C. The homogenate was centrifuged at 12,000 ×*g* for 20 min at 4 °C. Then the 1.0 mL of supernatant was mixed with 100 μmol·L^{-1} of H_2O_2-acetone, pre-cooled acetone, 10% titanium-tetrachlorohydrochloric acid solution and concentrated ammonia water. After the mixture was reacted for 5 min, it was centrifuged at 12,000 ×*g* for 15 min at 4 °C. Then the supernatant was discarded to leave a precipitate, and 3.0 mL of 2 mol·L^{-1} sulfuric acid was added to the precipitate. The absorbance was measured at 530 nm.

2.3.4. Extraction of free and bound phenolic

The content of free and bound phenolic was extracted according to

the method of Chen et al. (2017). Free phenolic extraction: wheat sprouts were freeze-dried, crushed and sifted through 40 mesh, the 1 g of wheat sprout powder was extracted with 80% methanol for 3 times (20 mL each time), then it was shaken at 200 rpm for 1 h on a shaker. After the mixture was centrifuged at 10,000 $\times$g for 15 min at 4 °C, the extracted liquid was combined and filtered, and evaporated to dry at 40 °C. Then the free phenolic extract was dissolved in 50% methanol at a constant volume.

Boud phenolic extraction: the residue of the extracted free phenolic was hydrolyzed with 20 mL of 2 mol·L^{-1} NaOH, and the mixture was shaken at 200 rpm for 4 h on a shaker. Then the pH of the hydrolysate was adjusted between 1.5 and 2.0 using 6 mol·L^{-1} HCl. The 15 mL of ethyl acetate was thoroughly mixed with the hydrolysate for 15 min. After the mixture was centrifuged at 10,000 $\times$g for 10 min at 4 °C, then the upper layer of ethyl acetate was taken, and the operation was repeated three times. The combined ethyl acetate layer was evaporated to dry by rotation at 40 °C, and dissolved with 50% methanol at a constant volume as the bound phenolic extract.

2.3.5. Determination of phenolic acid content

The 10 μL of the above extracts were respectively filtered by 0.22 μm organic filter membrane and analyzed by high performance liquid chromatography (Agilent 1260 Infinity II, USA) and diode array detector (DAD). The analytical column was 3.0*150 mm, 2.7 μm diameter, C18 column (Phenomenex, Torrance, California, USA). The conditions of the high performance liquid phase were as follows: the flow rate was 0.3 mL·min^{-1}, the mobile phase A solution was 0.1% acetic acid aqueous solution, and the B solvent was 0.1% acetic acid methanol solution. At a constant temperature of 35 °C and a flow rate of 0.3 mL·min^{-1}, the gradient elution procedure was as follows: 0–11 min, 9–14% B; 11–14 min, 14–15% B; 14–17 min, 15% B; 17–24 min, 15–16.5% B; 24–28 min, 16.5–19% B; 28–30 min, 19–25% B; 30–36 min, 25–26% B; 36–38 min, 26–28% B; 38–41 min, 28–35% B; 41–46 min, 35–40% B; 46–48 min, 40–48% B; 48–50 min, 9% B. The wavelength was measured at 280 nm.

2.3.6. Determination of flavonoid content

The 10 μL of the above extracts were respectively filtered by 0.22 μm organic filter membrane and analyzed by high performance liquid chromatography (Agilent 1260 Infinity II, USA) and diode array detector (DAD). The analytical column was 3.0*150 mm, 2.7 μm diameter, C18 column (Phenomenex, Torrance, California, USA). The conditions of the high performance liquid phase were as follows: the flow rate was 0.3 mL·min^{-1}, the mobile phase A solution was 0.1% acetic acid aqueous solution, and the solvent B was methanol solution. At a constant temperature of 35 °C and a flow rate of 0.3 mL·min^{-1}, the gradient elution procedure was as follows: 0–4 min, 10–35% B; 4–27 min, 35–65% B; 27–30 min, 65–10% B. The wavelength was measured at 280 nm.

2.3.7. Determination of PAL, C4H and 4CL activities during wheat germination

The extraction and determination of PAL was referred to the method of Han et al. (2017). Fresh wheat sprouts (1.0 g) were mixed with 0.1 mol·L^{-1} boric acid buffer (pH 8.8, containing 40 g·L^{-1} PVP, 2 mmol·L^{-1} EDTA, 5 mmol·L^{-1} β-mercaptoethanol). The mixture was ground into a homogenate at 4 °C. After centrifugation at 4 °C and 12,000 $\times$g for 30 min, the 200 μL of supernatant was combined with 3.0 mL of borax buffer reaction solution (50 mmol·L^{-1}, pH 8.8) and 0.5 mL of *L*-phenylalanine solution (20 mmol·L^{-1}) for 60 min at 37 °C. At the end of the reaction, 0.1 mL of hydrochloric acid solution (6 mol·L^{-1}) was added to terminate the reaction. One unit of PAL activity was equal to a change of 0.01 at 290 nm per min, and expressed as U·g^{-1} FW.

The extraction and determination of C4H was referred to the method of Han et al. (2017). Fresh wheat sprouts (1.0 g) were mixed with 0.1 mol·L^{-1} phosphoric acid buffer (pH 7.6, containing 0.25 mol·L^{-1} sucrose, 0.5 mmol·L^{-1} EDTA, 2 mmol·L^{-1} mercaptoethanol). The mixture was ground into homogenate at 4 °C. After centrifugation at 4 °C and

12,000 $\times$g for 30 min, the 0.5 mL of supernatant was combined with 3.0 mL of 0.1 mmol·L^{-1} phosphoric acid buffer, 0.2 mL of 50 mmol·L^{-1} cinnamic acid and 2 mL of 0.5 g·L^{-1} NADPH for 10 min at 30 °C. At the end of the reaction, 0.1 mL of 6 mol·L^{-1} hydrochloric acid solution was added to terminate the reaction, the supernatant was adjusted to pH 11 with NaOH. One unit of C4H activity was equal to a change of 0.01 at 290 nm per min, and expressed as U·g^{-1} FW.

The extraction and determination of 4CL was referred to the method of Han et al. (2017). Fresh wheat sprouts (1.0 g) were mixed with 50 mmol·L^{-1} Tris-HCl extraction buffer (pH 8.9, containing 15 mmol·L^{-1} mercaptoethanol and 10% glycerol, 4 mmol·L^{-1} MgCl$_2$, 5 mmol·L^{-1} Vc, 10 μmol·L^{-1} leupaprotinin, 1 mmol·L^{-1} PMSF, 0.15% *W/V* PVP). The mixture was ground into homogenate at 4 °C. After centrifugation at 4 °C and 12,000 $\times$g for 30 min, the 0.5 mL of supernatant was mixed with 2.0 mL of 5 mmol·L^{-1} MgCl$_2$, 0.5 mL of 5 mmol·L^{-1} ATP, 0.05 mL of 0.4 mmol·L^{-1} CoA and 0.05 mL of 0.6 mmol·L^{-1} *p*-coumaric acid. Then the mixture was reacted at 40 °C for 10 min. At the end of the reaction, 0.1 mL of 6 mol·L^{-1} hydrochloric acid solution was added to terminate the reaction. One unit of 4CL activity was equal to a change of 0.01 at 333 nm per min, and expressed as U·g^{-1} FW.

2.3.8. Determination of antioxidant enzyme activities during wheat germination

The extraction and determination of CAT refer to the methods of Ma et al. (2018). Fresh wheat sprouts (1.0 g) were mixed with 100 mmol·L^{-1} phosphate buffer (pH 7.5, containing 5 mmol·L^{-1} DTT, 5% PVP). Then the mixture was ground into homogenate at 4 °C and centrifuged at 4 °C and 12,000 $\times$g for 30 min. Then, the 100 μL of supernatant were mixed with 2.9 mL of 20 mmol·L^{-1} H$_2$O$_2$. The absorbance was measured at 240 nm. The reduction of light absorption value per gram of wheat sprout per minute by 0.01 was 1 unit of catalase activity, expressed as U·g^{-1} FW.

The extraction and determination of PPO refer to the method of Hystad, Martin, Graybosch, and Giroux (2015). Fresh wheat sprouts (1.0 g) were mixed with 100 mmol·L^{-1} acetoacetate sodium buffer (pH 5.5, containing 1 mmol·L^{-1} polyethylene glycol, 4% PVPP, 1% Triton X-100). Then the mixture was ground into homogenate at 4 °C. After centrifugation at 4 °C and 12,000 $\times$g for 30 min, the 0.5 mL of supernatant was mixed with 4.0 mL of 50 mmol·L^{-1} acetoacetate sodium acetate buffer solution and 1.0 mL of 50 mmol·L^{-1} collar resorcinol solution. Then the absorbance was measured at 420 nm. An increase of 1 per gram of light absorption per minute of wheat sprouts was 1 unit of peroxidase activity, expressed as U·g^{-1} FW.

The extraction and determination of POD refer to the method of Ma et al. (2018). 1 g of wheat sprouts were mixed with 100 mmol·L^{-1} acetoacetate sodium buffer (pH 5.5, containing 1 mmol·L^{-1} polyethylene glycol, 4% PVPP, 1% Triton X-100). Then the mixture was ground into homogenate at 4 °C. After centrifugation at 4 °C and 12,000 $\times$g for 30 min, the 0.5 mL of supernatant were mixed with 3.0 mL of 25 mmol·L^{-1} guaiacol solution and 200 μL of 0.5 mol·L^{-1} H$_2$O$_2$ solution. Then the absorbance was measured at 470 nm. An increase of 1 per gram of light absorption per minute of wheat sprouts was 1 unit of peroxidase activity, expressed as U·g^{-1} FW.

2.3.9. Determination of antioxidant capacity during wheat germination

The free radical scavenging ability of DPPH was slightly modified according to the method of Ma et al. (2018). 200 μL of free phenolic and bound phenolic extracts were added to 3.8 mL of DPPH· solution and placed in the dark at room temperature for 30 min, then the absorbance value was determined at 515 nm. The standard curve was formulated with Trolox, and the DPPH scavenging capacity was measured in μmol TE·g^{-1} DW.

The determination of free radical cation scavenging activity of ABTS was slightly modified according to the method of Ma et al. (2018). The extraction solution of free phenolic and bound phenolic was added to 3.0 mL of ABTS$^+$· solution. Then the mixed solution was placed in a dark

place at room temperature for 30 min, and the absorption value was determined at 734 nm. The standard curve was formulated with Trolox, and the ABTS scavenging capacity was measured in µmol TE·g^{-1} DW.

The determination of FRAP value was slightly modified according to Thaipong, Boonprakob, Crosby, Cisneros-Zevallos, and Byrne (2006). The 100 µL of free phenolic and bound phenolic extracts were added to 3.9 mL of FRAP solution, and reacted at 37 °C for 10 min. Then the absorption value was determined at 593 nm. FRAP values were expressed as micromolars of Trolox equivalent per ml of sample (µmol·TE mL^{-1}).

2.4. Data processing and statistical analysis

Three biological replicates were set up, and the results were expressed as means ± SD difference. SPASS 20.0 was used for statistical analysis of the data, and Duncan multiple comparisons were used between the mean values, and the significance level was $p < 0.05$.

3. Results and analysis

3.1. Effects of germination on wheat sprout/root length

As can be seen from Fig. 1a, the sprout length of eight wheat varieties (Fu xilv, Zhongmai 175, Jimai 23, Jimai 22, Taikeheimai 1, Hei jingang, Bainong 307, Jimai 44) increased significantly with the prolongation of germination time (p < 0.05). During the whole germination period, the sprouts of Fu xilv wheat were the longest. On the 4th day of germination, the sprout length of Fu xilv, Zhongmai 175, Jimai 23, Jimai 22, Taikeheimai 1, Hei jingang, Bainong 307, Jimai 44 were longer than those of the 2th day, which were 485%, 496%, 726%, 702%, 488%, 491%, 573% and 508%, respectively. The increasing range of late germination was less than that of early germination. The sprout length of Fu xilv, Zhongmai 175, Jimai 23, Jimai 22, Taikeheimai 1, Hei jingang, Bainong 307, Jimai 44 increased by 50%, 53%, 49%, 69%, 53%, 38%, 50% and 49%, respectively, on the 6th day of germination compared with the 4th day.

As can be seen from Fig. 1b, the root length of eight wheat varieties, Fu xilv, Zhongmai 175, Jimai 23, Jimai 22, Taikeheimai 1, Hei jingang, Bainong 307, and Jimai 44, increased significantly with the prolongation of germination time (p < 0.05). At the 4th day of germination, the root length of Fu xilv, Zhongmai 175, Jimai 23, Jimai 22, Taikeheimai 1, Hei jingang, Bainong 307, and Jimai 44 increased by 149%, 183%, 211%, 236%, 195%, 170%, 255% and 189%, respectively, compared with the 2th day. On the 6th day of germination, the root length of Fu xilv green was the shortest, and the root length of Jimai 22 was the longest.

3.2. Effects of germination on respiration rate of wheat sprouts

As can be seen from Fig. 2, with the extension of germination time, the respiration rate of wheat sprouts gradually increased. The respiratory rate of Jimai 44 was higher than that of other varieties under the same germination time. Compared with the 2th day of germination, the respiration rate of Fu xilv, Zhongmai 175, Jimai 23, Jimai 22, Taikeheimai 1, Hei jingang, Bainong 307 and Jimai 44 on the 4th day of germination increased by 106%, 337%, 73%, 144%, 76%, 131%, 244% and 129%, respectively. Compared with the 4th day of germination, the respiration rate of Fu xilv, Zhongmai 175, Jimai 23, Jimai 22, Taikeheimai 1, Hei jingang, Bainong 307 and Jimai 44 on the 6th day of germination increased by 177%, 263%, 191%, 143%, 160%, 273%, 76% and 102% respectively. The change trend of the rate was consistent with the growth of wheat sprouts, indicating that the extension of germination time promoted the growth of wheat sprouts, so the respiratory rate increased.
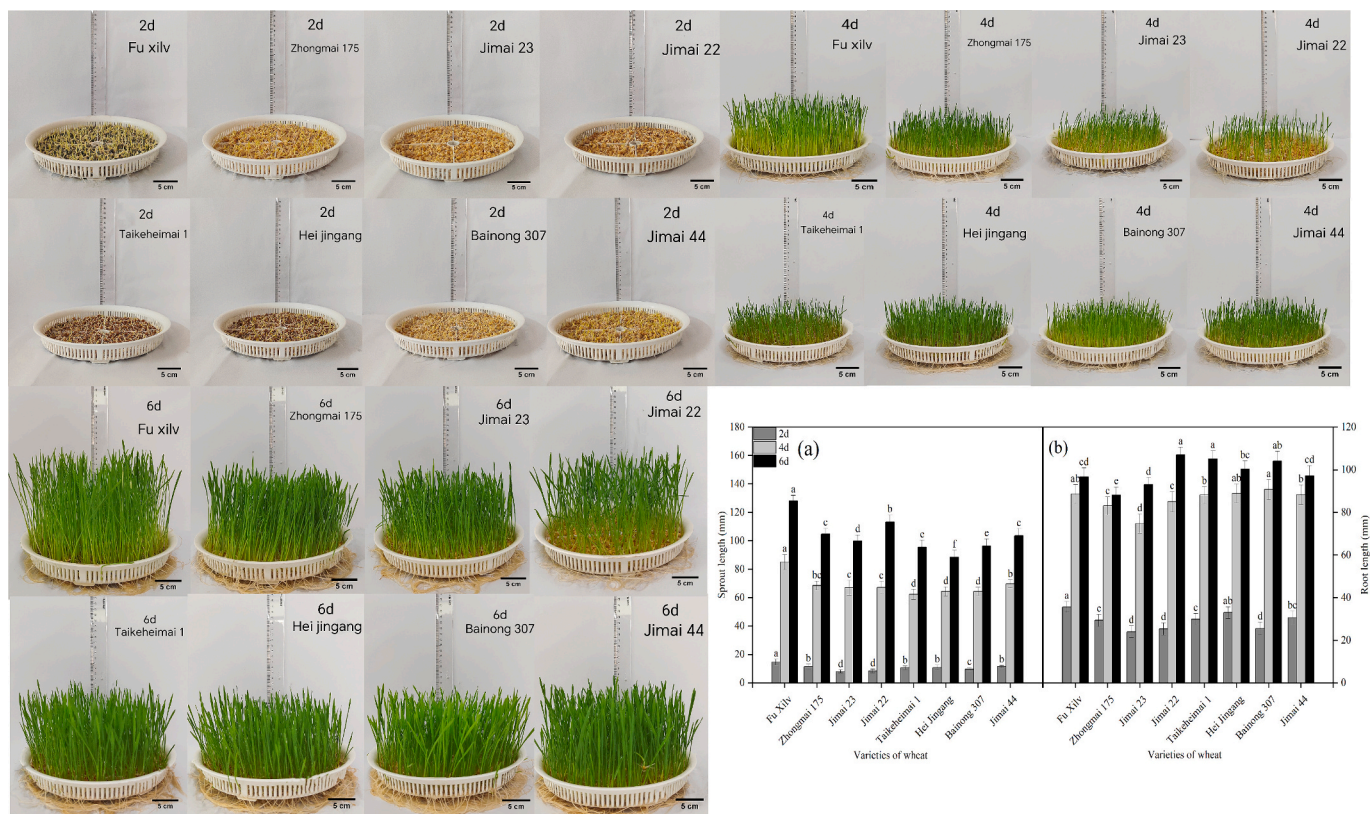

Fig. 1. Changes of sprout length (a)、root length (b) during germination of different wheat varieties. Different lowercase letters indicated that different wheat varieties had significant differences in the same germination stage ($p < 0.05$).
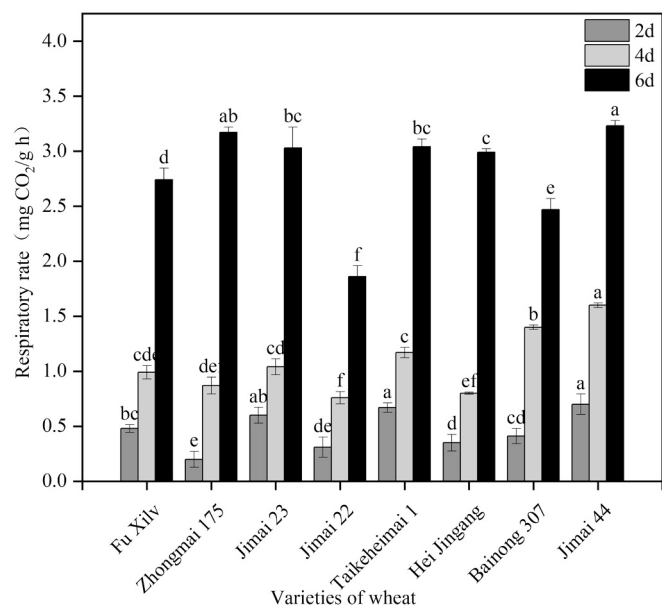
Fig. 2. Changes of respiration rate of different wheat varieties during germination.

Different lowercase letters indicated that different wheat varieties had significant differences in the same germination stage (p < 0.05).

3.3. Effects of germination on oxidative damage of wheat sprouts

As can be seen from Fig. 3a, the MDA content of Fu xilv, Zhongmai 175, Jimai 23, Hei jingang, Bainong 307, and Jimai 44 gradually increased with the prolongations of germination time, while the MDA content of Jimai 22 and Taikeheimai 1 increased first and then decreased. During the whole germination period, the content of MDA in Fu xilv was higher than that of other varieties, indicating that the membrane lipid peroxidation of Fu xilv cells was higher and the antioxidation ability was weaker. As can be seen from Fig. 3b, compared with the 2th day of germination, the $O_2^-\cdot$ content of Fu xilv, Zhongmai 175, Jimai 23, Jimai 22, Taikeheimai 1, Hei jingang, Bainong 307 and Jimai 44 on the 4th day of germination increased by 65%, 76%, 90%, 19%, 106%, 119%, 404% and 104%, respectively. As can be seen from Fig. 3c, compared with the 2th day of germination, the H_2O_2 content of Fu xilv, Zhongmai 175, Jimai 23, Jimai 22, Taikeheimai 1, Hei jingang, Bainong 307 and Jimai 44 on the 4th day of germination increased by 6%, 20%, 25%, 201%, 104%, 38%, 38%, respectively.

3.4. Effects of germination on phenolic acid synthesis of wheat sprouts

As can be seen from Fig. 4, eight phenolic acids were identified in wheat sprouts, including gallic acid, *4*-hydroxybenzoic acid, vanillic acid, caffeic acid, syringic acid, *p*-coumaric acid, ferulic acid and sinapic acid. Ferulic acid and *p*-coumaric acid were the main phenolic acids in wheat sprouts, and germination could significantly increase the contents of *p*-coumaric acid and ferulic acid in wheat sprouts. From the 2th day to the 4th day of germination, the *p*-coumaric acid content of Fu xilv, Zhongmai 175, Jimai 23, Jimai 22, Taikeheimai 1, Hei jingang, Bainong 307 and Jimai 44 sprouts increased by 187%, 114%, 199%, 235%, 240%, 201%, 92% and 171%, respectively. From the 2th day to the 4th day of sprouts, the ferulic acid content of Fu xilv, Zhongmai 175, Jimai 23, Jimai 22, Taikeheimai 1, Hei jingang, Bainong 307 and Jimai 44 sprouts increased by 198%, 73%, 129%, 149%, 96%, 59%, 131% and 80%, respectively.
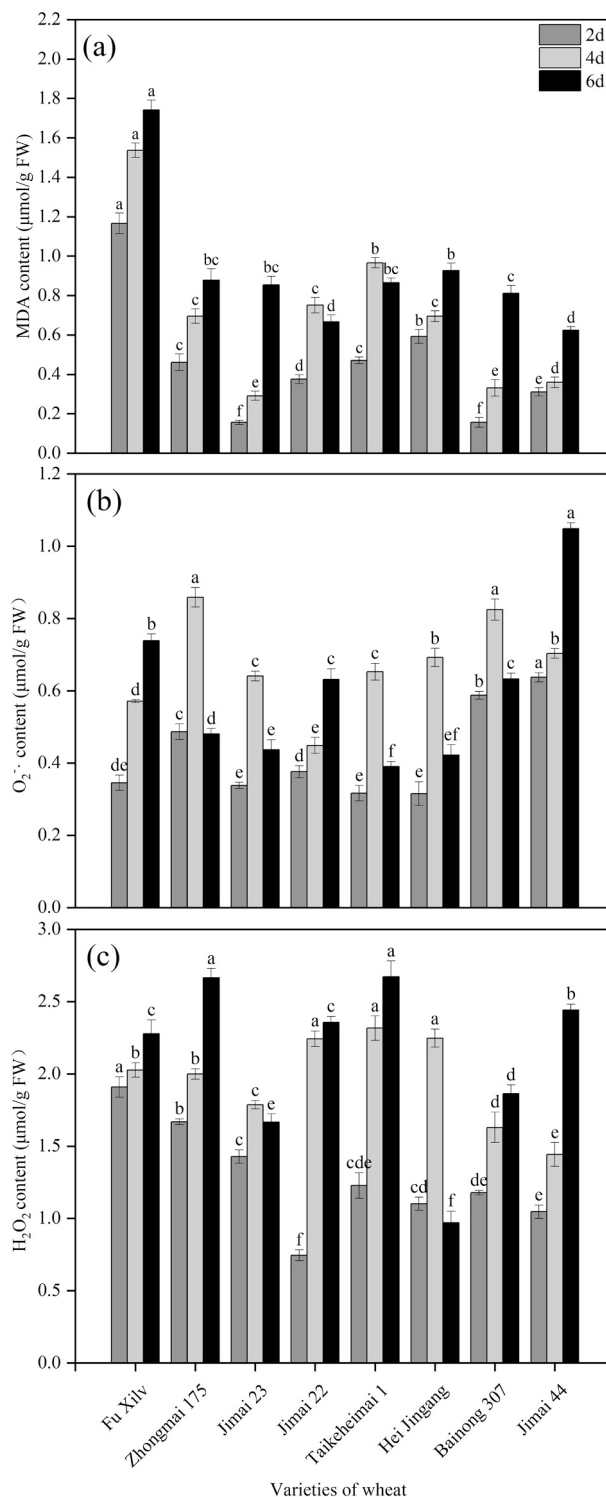

Fig. 3. Changes of malondialdehyde content (a), superoxide anion content (b) and hydrogen peroxide content (c) in different wheat varieties during germination. Different lowercase letters indicated that different wheat varieties had significant differences in the same germination stage (p < 0.05).

3.5. Effects of germination on flavonoid synthesis of wheat sprouts

As shown in Fig. 5, nine flavonoids were identified in wheat sprouts, including dihydroquercetin, vitexin, dihydrokaempferol, rutin, quercitrin, quercetin, naringenin, kaempferol and apigenin. Dihydroquercetin, quercitrin and vitexin were the main flavonoids in wheat sprouts.
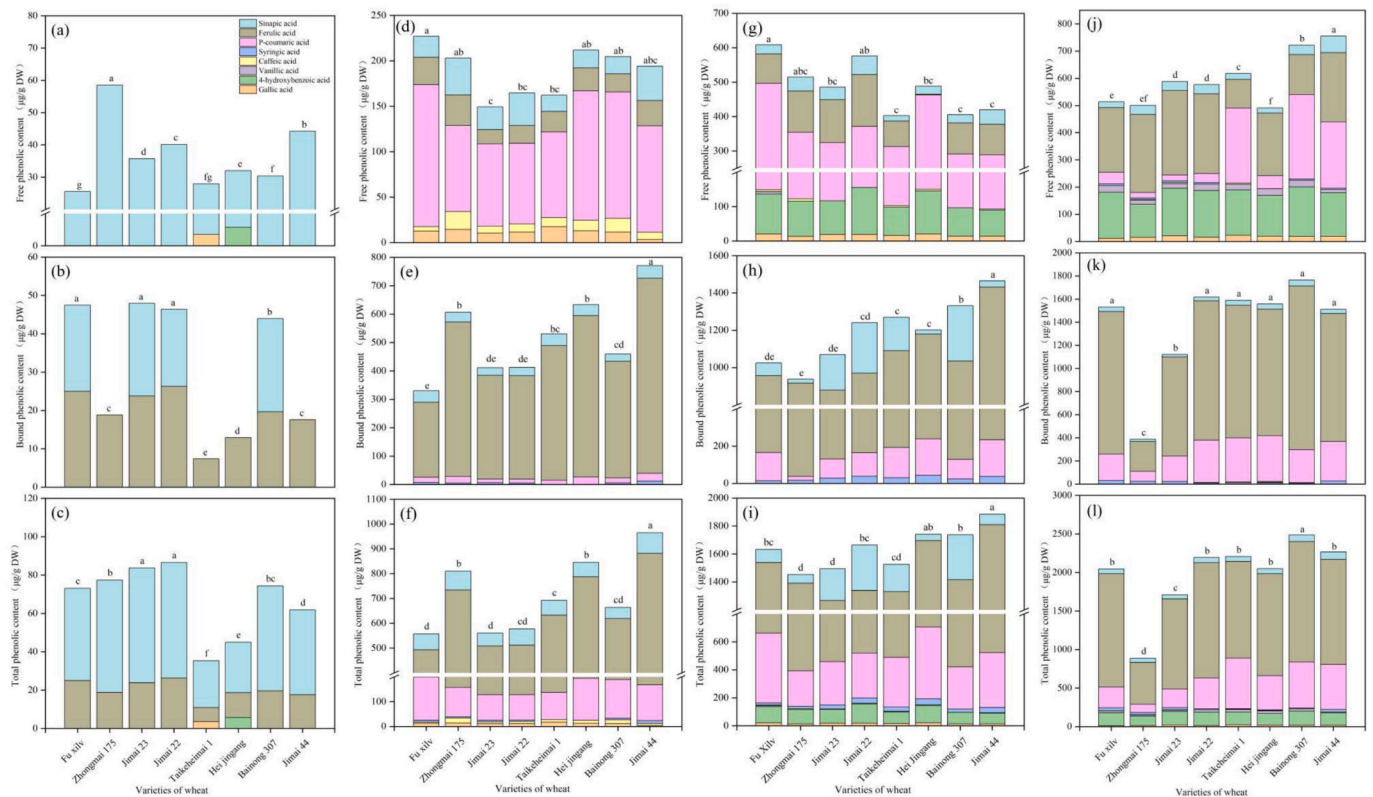
Fig. 4. Changes of free phenolic acids content (a), (d), (g) and (j), bound phenolic acids content (b), (e), (h) and (k) and total phenolic acids content (c), (f), (i) and (l) in different wheat varieties during germination. Wheat sprouts for 0d: (a)-(c); wheat sprouts for 2d: (d)-(f); wheat sprouts for 4d: (g)-(i); wheat sprouts for 6d: (j)-(l). Different lowercase letters indicated that different wheat varieties had significant differences in the same germination stage (p < 0.05).

Germination could significantly increase the content of dihydroquercetin in wheat sprouts. From the 2th day to the 4th day of sprouting, the dihydroquercetin content in Fu xilv, Zhongmai 175, Jimai 23, Jimai 22, Taikeheimai 1, Hei jingang, Bainong 307 and Jimai 44 sprouts increased by 523%, 424%, 456%, 499%, 746%, 546%, 485% and 645%, respectively. From the 2th day to the 4th day of sprouting, the quercitrin content of Fu xilv, Zhongmai 175, Jimai 23, Jimai 22, Taikeheimai 1, Hei jingang, Bainong 307 and Jimai 44 sprouts increased by 1550%, 189%, 424%, 164%, 49%, 85%, 600% and 2816%, respectively.

3.6. Effects of germination on PAL, C4H and 4CL activities of wheat sprouts

PAL is the first step enzyme in phenylpropane metabolic pathway and an important rate-limiting enzyme (Janczak-Pieniazek, Cichonski, Michalik, & Chrzanowski, 2023). As shown in Fig. 6a, with the prolonging of germination time, PAL activity of Fu xilv, Zhongmai 175, Jimai 23, Taikeheimai 1, Hei jingang and Bainong 307 showed a trend of first increasing and then decreasing, and reached the highest enzyme activity on the 4th day. PAL activity of Jimai 22 and Jimai 44 increased with the prolongation of germination time. On the 6th day of germination, PAL activity of Jimai 44 and Hei Jingang was significantly higher than that of other wheat varieties (*p* < 0.05). As shown in Fig. 6b, with the elongation of germination time, the C4H activity of Zhongmai 175, Jimai 22, Taikeheimai 1, Hei jingang and Jimai 44 showed an increasing trend, while the C4H activity of Fu xilv, Jimai 23 and Bainong 307 showed a first increasing trend and then a decreasing trend. On the 4th day of germination, the C4H activity of Fu xilv, Zhongmai 175, Jimai 23, Jimai 22, Taikeheimai 1, Hei jingang, Bainong 307 and Jimai 44 increased by 166%, 59%, 77%, 268%, 11%, 35%, 22% and 11%, respectively, compared with that on the 2th day of germination. As

shown in Fig. 6c, the 4CL activity of Fu xilv, Taikeheimai 1 and Jimai 44 continued to increase with the prolongating of germination time, while the 4CL activity of Zhongmai 175, Jimai 23, Jimai 22, Hei jingang and Bainong 307 showed a trend of first increasing and then decreasing.

3.7. Effects of germination on antioxidant enzyme activities of wheat sprouts

As shown in Fig. 7a, with the prolongation of germination time, the CAT activities of Fu xilv, Zhongmai 175, Taikeheimai 1, Hei Jingang and Bainong 307 showed a trend of first increasing and then decreasing, while the CAT activities of Jimai 23, Jimai 22 and Jimai 44 showed an increasing trend, and the increase of CAT activities in the early stage of germination was greater than that in the late stage of germination. As shown in Fig. 7b-c, the PPO and POD activities of eight wheat varieties were significantly increased with the prolongation of germination time. PPO activity of Jimai 22 on the 6th day of germination was significantly higher than that of other wheat varieties (*p* < 0.05). On the 4th day of germination, PPO activity of Fu xilv, Zhongmai 175, Jimai 23, Jimai 22, Taikeheimai 1, Hei Jingang, Bainong 307 and Jimai 44 increased by 8%, 64%, 33%, 136%, 24%, 80%, 25% and 127%, respectively, compared with the 2th day of germination. On the 4th day of germination, POD activity of Hei Jingang was significantly higher than that of other varieties, and the POD activity of Fu xilv was the lowest (p < 0.05).

3.8. Effects of germination on antioxidant capacity of wheat sprouts

As shown in Fig. 8, the antioxidant capacity of wheat sprouts increased with the prolongation of germination time, and the antioxidant activities of ABTS, DPPH and FRAP of both free and bound total phenolic were enhanced. On the 2th day of germination, the free DPPH antioxidant activities of Fu xilv, Zhongmai 175, Jimai 23, Jimai 22,
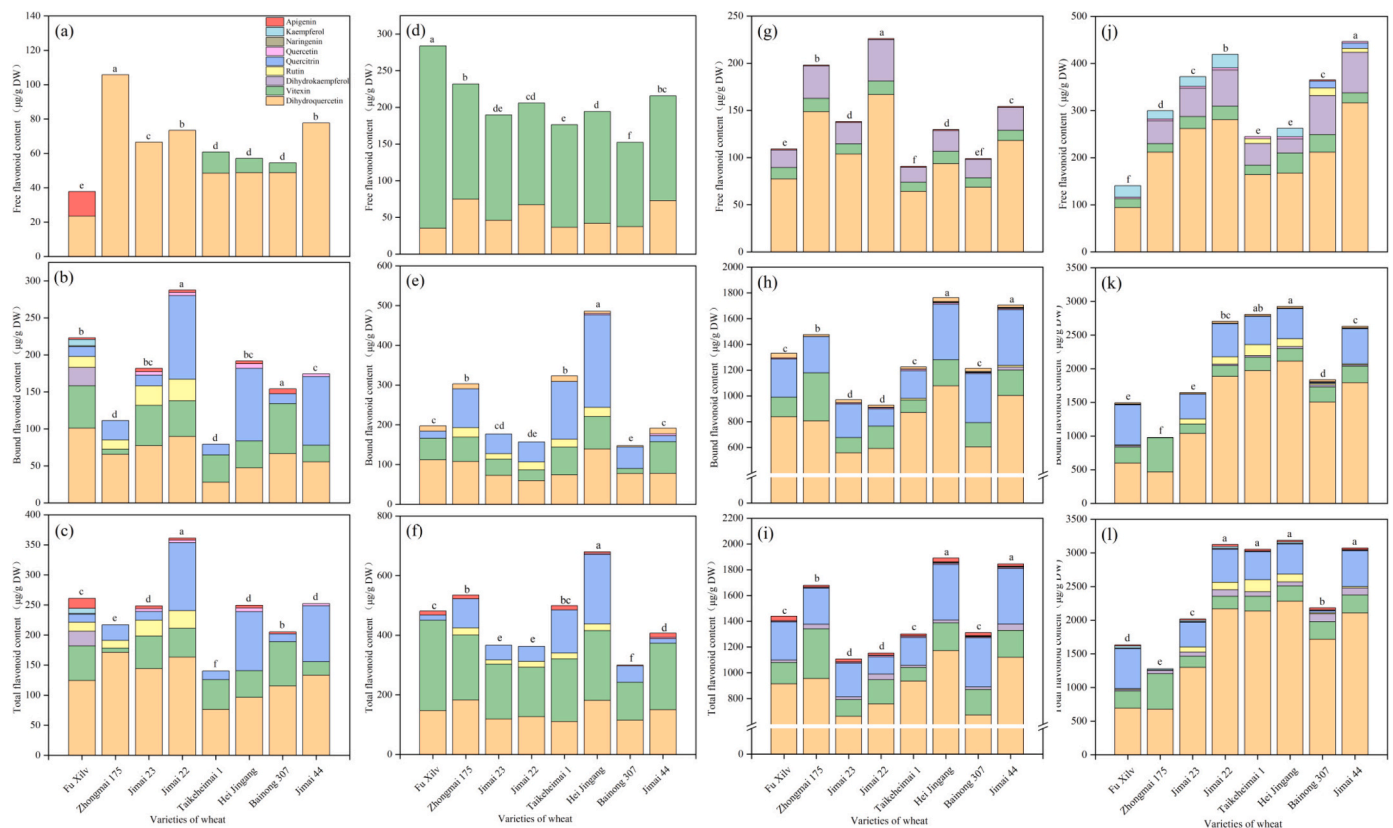
Fig. 5. Changes of free flavonoids content (a), (d), (g) and (j), bound flavonoids content (b), (e), (h) and (k) and total flavonoids content (c), (f), (i) and (l) in different wheat varieties during germination. Wheat sprouts for 0d: (a)-(c); wheat sprouts for 2d: (d)-(f); wheat sprouts for 4d: (g)-(i); wheat sprouts for 6d: (j)-(l). Different lowercase letters indicated that different wheat varieties had significant differences in the same germination stage (p < 0.05).

Taikeheimai 1, Hei Jingang, Bainong 307 and Jimai 44 were 2.45, 2.02, 1.76, 1.79, 1.56, 1.90, 2.01 and 2.54 μmol TE·g^{-1} DW, respectively (Fig. 8a). The free DPPH values of Fu xilv, Zhongmai 175, Jimai 23, Jimai 22, Taikeheimai 1, Hei Jingang, Bainong 307 and Jimai 44 on the 6th day of germination were 5.02, 6.19, 7.95, 8.62, 9.05, 7.10, 8.52 and 7.69 times of those on the 2th day, respectively. As shown in Fig. 8b, the bound DPPH value of Fu xilv, Zhongmai 175, Jimai 23, Jimai 22, Taikeheimai 1, Hei Jingang, Bainong 307 and Jimai 44 on the 6th day of germination was 5.79, 4.63, 9.61, 7.83, 7.50, 7.70, 14.22 and 5.25 times that of the 2th day, respectively. Among germinated seeds, the total DPPH value of Jimai 44 on the 6th day of germination increased by 148% compared with that of ungerminated seeds (Fig. 8c). Among the germinated seeds, the free DPPH value of Jimai 44 on the 6th day of germination increased by 212% compared with that on the 2th day of germination. The free ABTS values of Fu xilv, Zhongmai 175, Jimai 23, Jimai 22, Taikeheimai 1, Hei Jingang, Bainong 307 and Jimai 44 on the 2th day of germination were 24.82, 24.20, 21.47, 24.96, 21.33, 19.79, 22.59 and 23.36 μmol TE·g^{-1} DW, respectively (Fig. 8d). On the 6th day of germination, Jimai 44 showed higher free, bound and total ABTS free radical scavenging activities. As shown in Fig. 8h, the free FRAP values of Fu xilv, Zhongmai 175, Jimai 23, Jimai 22, Taikeheimai 1, Hei Jingang, Bainong 307 and Jimai 44 on the 6th day of germination were 15.81, 17.68, 19.51, 13.98, 17.34, 14.91, 16.78 and 17.44 μmol TE·mL^{-1}. The antioxidant activity of bound FRAP of Hei Jingang, Bainong 307 and Jimai 44 was higher than that of other germinated seeds, which was 2.76, 2.50 and 2.44 times higher on the 6th day of germination than on the 2th day of germination, respectively (Fig. 8g). On the 6th day of germination, the total antioxidant capacity of Jimai 44 was stronger, which was 107% higher than that on the 2th day of germination (Fig. 8i).

4. Discussion

In this study, the sprout length, and root length of eight wheat varieties, including Fu xilv, Zhongmai 175, Jimai 23, Jimai 22, Taikeheimai 1, Hei Jingang, Bainong 307 and Jimai 44, increased with the extension of germination time (Fig. 1). The growth of wheat sprouts were varied at different germination stages. In the three germination stages of this study (0–2 d, 2–4 d, 4–6 d), the growth of wheat sprouts showed a typical "slow-fast-slow" trend, with germination slowing down at 0–2 d, accelerating at 2–4 d, and slowing down at 4–6 d, which was consistent with Wang et al. (2023) research on the growth during pea germination. Respiration rate was an important physiological index of plant metabolism (Ikkonen, Shibaeva, Sherudilo, & Titov, 2020). Plant respiration can provide most of the energy for plant life activities, and also provide the raw material for the synthesis of other compounds in the body (Schmiege, Heskel, Fan, & Way, 2023). The respiration rate of wheat sprout was closely related to its growth condition. In this study, the respiration rate of wheat sprout increased with the prolongation of germination time. The respiratory rate of Jimai 44 was higher than that of other varieties under the same germination time (Fig. 2). MDA was the final product of membrane lipid peroxidation, and its content reflected the degree of membrane lipid peroxidation (Yusuf, Chand, Mishra, & Joshi, 2016). The increase of MDA content was due to the difficulty of water and nutrients absorption in plants, which affected the double-layer structure of protein and phospholipid in the plasma membrane system, thus reducing the stability of biofilm. At the same time, a large number of reactive oxygen species were produced in the cells, resulting in membrane lipid peroxidation and enhanced plasma membrane permeability (Li, Wang, Li, Zheng, & Jiang, 2013). The contents of MDA, O_2^-· and H_2O_2 in wheat sprouts basically increased with the extension of germination time, and the differences among
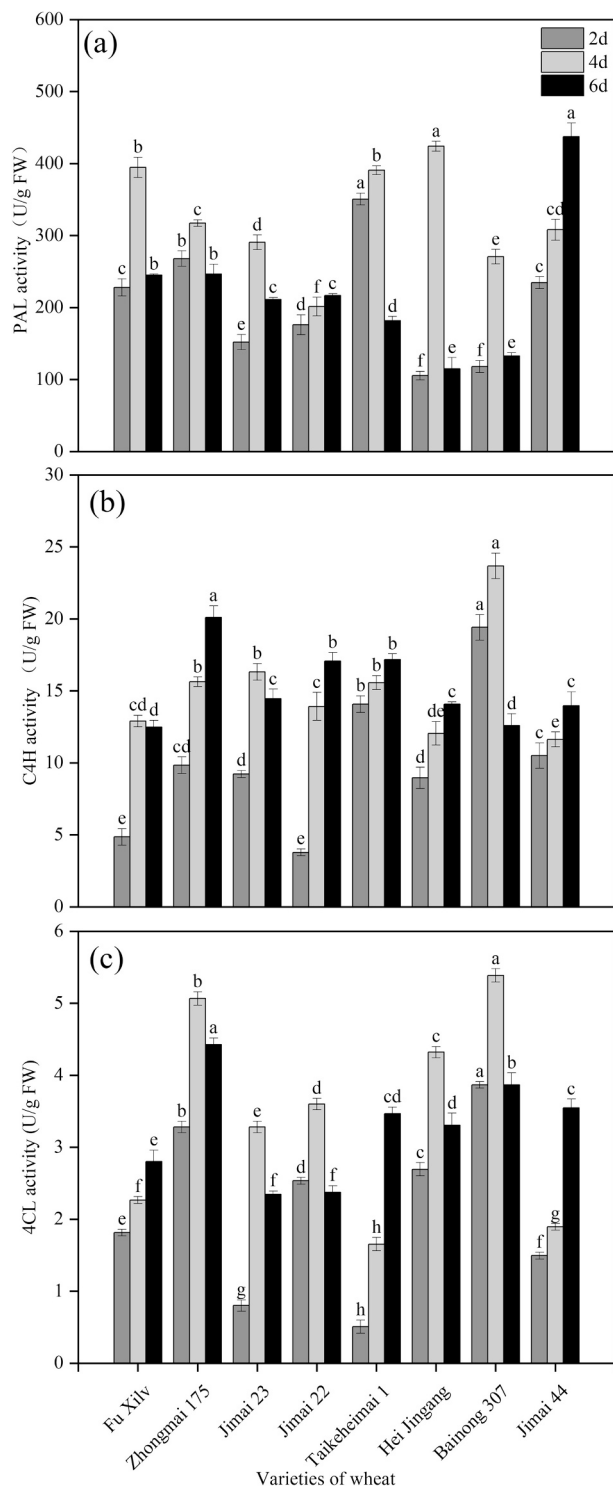
Fig. 6. Changes of PAL (a), C4H (b) and 4CL (c) in different wheat varieties during germination. Different lowercase letters indicated that different wheat varieties had significant differences in the same germination stage (p < 0.05).

Fig. 7. Changes of CAT (a), PPO (b) and POD (c) in different wheat varieties during germination. Different lowercase letters indicated that different wheat varieties had significant differences in the same germination stage (p < 0.05).

varieties were large (Fig. 3), indicating that lipid peroxidation was enhanced during the germination of wheat sprouts, which was similar to the change trend of sprouted soybean sprouts studied by Schneider et al. (2020).

Phenolic acids are secondary metabolites containing phenolic hydroxyl and carboxylic groups, which are widely distributed in higher plants, and have various biological activities such as antioxidant, antibacterial and anticancer (Li et al., 2013). Phenolic acids are the most
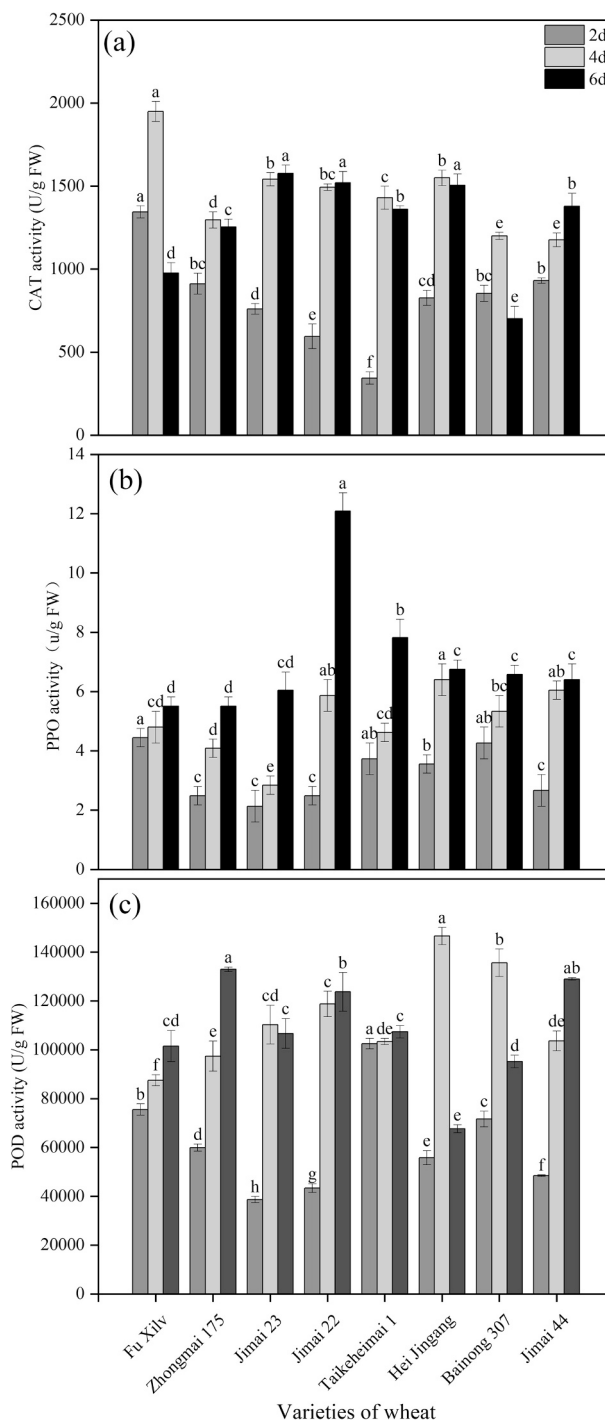
abundant phenolic compounds in wheat grains (Zhang, Wang, Yao, Yan, & He, 2012). Studies have shown that phenolic acids such as ferulic acid and *p*-coumaric acid were the most important binding phenolic substances in gramineous seeds (Chen, Yu, Wang, Gu, & Beta, 2016; Li, Li, Wang, & Li, 2022). In this study, ferulic acid was also found to be the highest content of phenolic acid in wheat grains and sprouts, and it mainly existed in the form of combination, this was consistent with Shamanin et al. (2022) who reported that ferulic acid was the major bound phenolic acid in purple wheat. The content of bound *p*-coumaric acid and ferulic acid in eight wheat varieties showed an increasing trend
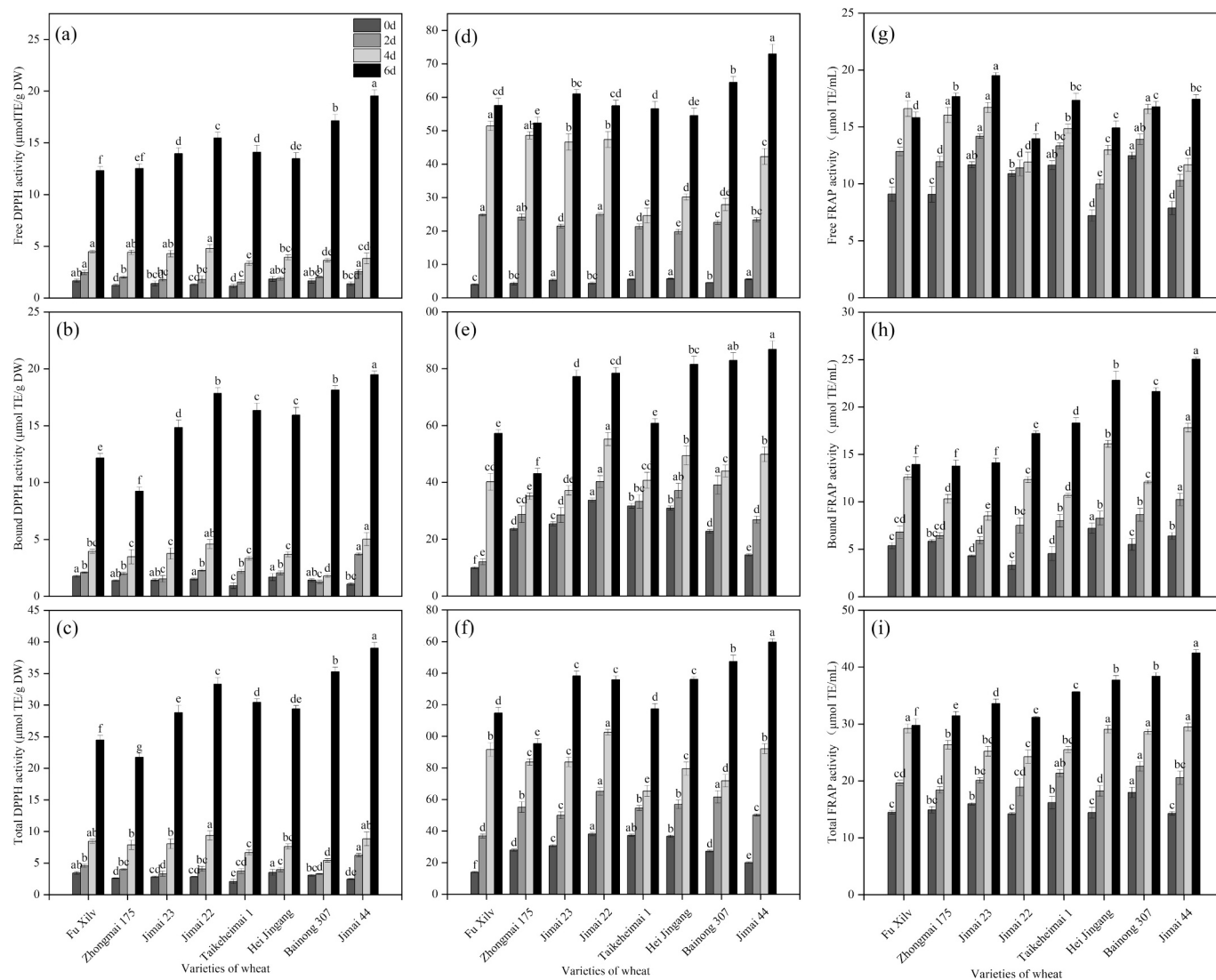
Fig. 8. Changes of antioxidant activities of free (a), bound (b), total (c) DPPH free radical, free (d), bound (e), total (f) ABTS free radical and free (g), bound (h), total (i) FRAP during germination of different wheat varieties. Different lowercase letters indicated that different wheat varieties had significant differences in the same germination stage (p < 0.05).

with the extension of germination time (Fig. 4). Flavonoids were another important secondary metabolites in wheat grains (Wang, Zhang, et al., 2020). During the germination process of wheat, the content of flavonoids were increased, which was consistent with the research results of Bae and Kim (2022). Dihydroquercetin was the highest content of flavonoids in wheat grains and sprouts. After two days of germination, the free dihydroquercetin content in eight varieties of wheat sprouts was lower than that in wheat grains, and the total dihydroquercetin content showed an increasing trend with the extension of germination time. Some studies have shown that flavonoids were mainly present in bound form (Liu, Qiu, & Beta, 2010), and it was also found in this study that at the 6th day of germination, the bound dihydroquercetin of Fu xilv, Zhongmai 175, Jimai 23, Jimai 22, Taikeheimai 1, Hei jingang, Bainong 307 and Jimai 44 accounted for 86%, 69%, 80%, 87%, 92%, 93%, 88% and 85% of the total dihydroquercetin, respectively (Fig. 5). Compared with wheat grain, three flavonoids, dihydrokaempferol, naringin and kaempferol were increased in germinated wheat.

The increase of phenolic substances content was related to the change of gene expression and activity of key enzymes in the synthesis pathway of phenolic substances, PAL, C4H and 4CL were key enzymes for the biosynthesis of phenolic content (Janczak-Pieniazek et al., 2023).

It was shown that genetic suppression of PAL, C4H and 4CL genes significantly reduced the content of phenolic compounds in the studied plants (Feduraev et al., 2020). PAL was the first step enzyme in the phenylpropane metabolic pathway and an important rate-limiting enzyme (Feduraev et al., 2020). It has been shown that treatment of plants with precursors for the synthesis of phenylpropanoid compounds, such as phenylalanine and tyrosine, significantly increased the content of various phenolics and enhanced the activity of phenylalanine deaminase (Feduraev et al., 2021). In this study, in the early stage of germination, the change trend of PAL was consistent with the change trend of total phenolic content of eight wheat varieties, all of which increased with the extension of germination time, which was the direct reason for the accumulation of phenolic substances (Fig. 6). This was consistent with Peng, Wang, Wang, Wang, and Bian (2023) who found that germination could activate PAL activity in buckwheat sprouts. The PAL enzyme activity of other wheat varieties was decreased in the late stage of germination except for Jimai 22 and Jimai 44, but phenolic substances continued to increase, which may be because the enzyme activity showed a dynamic change and the sampling time was limited. In addition, although the activity of PAL has decreased, but it was still higher than that at the early stage of germination, which also led to

continuous accumulation of phenolic substances and increased content. C4H is a key enzyme in the synthesis of flavonoids (Yun, Chen, Deng, & Yogo, 2007). In this study, the change trend of C4H was consistent with that of total phenolic content in eight varieties of wheat sprouts, all of which increased with the extension of germination time. The C4H enzyme activity of Fu xilv, Jimai 23 and Bainong 307 decreased at the late stage of germination, but was higher than that at the early stage of germination. During the germination process, the changes of 4CL varied greatly with different wheat varieties, and Fu xilv, Taikeheimai 1 and Jimai 44 showed an increasing trend during the whole germination process. C4H and 4CL activities were closely associated with phenolic accumulation in wheat, which was consistent with the report by Yin, Hu, Yang, Fang, and Yang (2023) that C4H and 4CL promoted the accumulation of phenolic substances.

A large number of studies have shown that plants remove reactive oxygen species (ROS) by producing antioxidant substances and activating antioxidant enzymes to protect their normal physiological metabolism (Rasheed, Ashraf, Arshad, Iqbal, & Hussain, 2020). During the process of wheat seed germination, a large number of enzymes were activated and some phenolic substances with antioxidant activity were synthesized. The accumulation of ROS was a major factor in crop losses, as ROS affect many cellular functions by damaging nucleic acids, oxidizing proteins and causing lipid peroxidation. Plant cells contain antioxidant enzymes such as CAT, PPO and POD (Ji, Wang, Lu, Ma, & Chen, 2022), which can eliminate these highly harmful ROS, thus inhibiting oxidative stress damage (Zhou, Chen, Zhao, Gao, & Liu, 2020). In this study, germination significantly activated the activities of CAT, PPO and POD in wheat sprouts of different varieties (Fig. 7), which was consistent with the study results of Abdel-Aty, Elsayed, Salah, Bassuiny, and Mohamed (2021), who found that antioxidant enzymes such as POD and CAT were also activated in large amounts during chia seed germination, phenolic substances and antioxidant activities were closely related to the activities of CAT, POD and PPO. In this study, DPPH and ABTS free radical scavenging system and FRAP reduction system were used to evaluate the antioxidant capacity of wheat sprouts. These three antioxidant activity evaluation methods are all based on electron transfer and involve the reduction of colored oxidants (Ngueumaleu et al., 2023). In this study, with the extension of germination time, the inhibition ability of wheat free and bound phenolic extracts on DPPH and ABTS free radical and the reduction power of iron ions were gradually enhanced (Fig. 8). Comparing the changes in the content and composition of phenolic compounds during wheat germination, it was possible that the content of active substances such as ferulic acid, *p*-coumaric acid, and dihydroquercetin in sprouted wheat has significantly increased, which led to the enhancement of antioxidant activity of wheat sprouts.

5. Conclusion

This effects of germination on the formation of phenolics and antioxidant capacity of eight varieties of wheat, including Fu xilv, Zhongmai 175, Jimai 23, Jimai 22, Taikeheimai 1, Hei Jingang, Bainong 307, and Jimai 44, were studied. The results showed that germination activated key enzymes in the synthesis of phenolics, promoted the synthesis of phenolics, especially ferulic acid, *p*-coumaric acid, dihydroquercetin, quercetin and vitexin, and also activated the activity of antioxidant enzymes, which significantly enhanced the antioxidant capacity of wheat sprouts. The results can provide a new idea for the deep processing of wheat and lay a theoretical foundation for the development of wheat products rich in phenols and other functional components.

CRediT authorship contribution statement

Wenxin Li: Writing – original draft, Formal analysis, Data curation. **Xiaoyong Liu:** Resources, Project administration, Methodology. **Yan Ma:** Writing – review & editing, Funding acquisition, Conceptualization.

Xianqing Huang: Project administration, Conceptualization. **Dan Hai:** Software. **Yongxia Cheng:** Software, Investigation. **Ge Bai:** Project administration. **Yinping Wang:** Methodology, Investigation. **Bei Zhang:** Methodology. **Mingwu Qiao:** Supervision. **Lianjun Song:** Supervision. **Ning Li:** Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial-linterests or personal relationships that could have appeared to influencethe work reported in this paper.

Data availability

Data will be made available on request.

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