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Research Article

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Red light enhances folate accumulation in wheat seedlings

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Abstract: Red, white, blue, green, and yellow lights were applied to investigate their effects on folate accumulation in wheat seedlings. The different lights, especially red light, significantly increased the total folate content. Total folate showed maximum accumulation under 30 μmol/(m^2·s) of red light, with an increase of 24% compared with the control (darkness). 5-Methyltetrahydrofolate (5-CH_3-THF) was the dominant folate component, and was significantly increased by red light irradiation. In addition, under red light, the folate content of leaves was higher and more sensitive to light than that of endosperm or roots. Red light up-regulated the expression of guanosine triphosphate (GTP) cyclohydrolase 1 (*GCH1*) and aminodeoxychorismate synthase (*ADCS*), enhanced the activity of GCH1 and ADCS, and increased the content of precursors of folate synthesis, including pterin and *p*-aminobenzoic acid (*p*ABA). Hence, the increased folate accumulation promoted by light could be attributed to the increased content of folate synthesis precursors, the activity of key enzymes, and related gene expression.

Key words: Wheat; Red light; Light intensity; Folate accumulation

1 Introduction

Wheat (*Triticum aestivum* L.) is an important grain in the world, with abundant yields and great potential for utilization. The nutritional and health benefits of whole wheat have gradually attracted the attention of researchers. In recent years, consumers have been looking for more whole-grain choices in the market. Germinated grains are a new whole-grain option which is becoming more mainstream. Studies have reported that germination not only significantly increases the contents of dietary fiber, phenolics, vitamin C, vitamin E, and folate (Liu T et al., 2017; Mwando et al., 2020), but also reduces the content of antinutrients, such as phytic acid (Marti et al., 2017). Germinated wheat can be milled into whole-wheat flour. Incorporating germinated whole-wheat flour into grain products meets the desire of health-conscious consumers for more whole-grain options, along with the added nutritional benefits (Chen et al., 2019). Germinated wheat has been promoted for traditional use as a fresh germinated grain and also as flour to be used in different foods including breakfast items, salads, soups, and baked products (Marti et al., 2017). The flour can also be supplemented with normal flour in the preparation of specialty breads (Dhillon et al., 2020).

Folate consists of a group of components including tetrahydrofolate (THF) and its derivatives. It is a water-soluble vitamin B particularly important for maintaining normal cell functions (Watanabe et al., 2017). Folate mediates a one-carbon (1C) transfer reaction to provide methyl for DNA modification and participates in numerous basic metabolic processes, including the biosynthesis of purine, thymidylic acid, amino acids (glycine, serine, and methionine), and pantothenic acid (vitamin B5) (Gorelova et al., 2017). Thus, folate is an essential vitamin for humans. Insufficient folate intake can cause megaloblastic anemia and fetal neural tube development defects. Low levels of folate intake are associated with Alzheimer's disease, cardiovascular diseases, and many cancers (Strobbe and van der Straeten, 2017). The United Nations Food and Agriculture Organization (FAO) and the World Health Organization (WHO) recommend that adults take folate at 400 μg/d to meet the body's normal life activities (Fu et al., 2021). Unlike plants and microorganisms, humans cannot synthesize folates de novo by themselves, and

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are dependent on their dietary intake of folates (Atta et al., 2016).

The basic skeleton of folate is composed of 2-amino-4-hydroxypteridine (pterin), *p*-aminobenzoic acid (*p*ABA), and glutamic acid (Atta et al., 2016). Pterin is synthesized from guanosine triphosphate (GTP) in the cytosol. GTP is converted to dihydropterin triphosphate under the catalysis of GTP cyclohydrolase 1 (GCH1) (Gorelova et al., 2019), followed by the formation of 6-hydroxymethyldihydropterin. *p*ABA is synthesized from chorismate in the plastid. Chorismate is converted to aminodeoxychorismate (ADC) via deoxidation by the catalysis of aminodeoxychorismate synthase (ADCS) and is then converted to *p*ABA (Koma et al., 2014). The conjunction of *p*ABA and pterin is catalyzed by hydroxymethyldihydropterin pyrophosphokinase-dihydropteroate synthase (HPPK-DHPS) in the mitochondria. Finally, folypolyglutamate synthetase (FPGS) sequentially adds glutamate residues to form polyglutamate THF (H_4F-Glun) in the final biosynthetic step (Gorelova et al., 2017). A more detailed diagram of the folate synthesis pathway is shown in Fig. S1.

Several methods have been applied to enhance the content of folate in plant food materials, including over-expression of multiple enzymes such as GCH1 and ADCS (de Lepeleire et al., 2018; Liang et al., 2019). However, the use and implementation of such techniques have raised public concerns (as well as suffering from strict regulations in many countries), and therefore, there is interest in seeking alternative approaches. One promising strategy is to increase the expression of related genes and enzymes involved in folate synthesis through the application of natural external stimuli (such as light) to promote the synthesis of precursors and folate during seed germination and seedling growth. Generally, germination is characterized by high folate accumulation (Boz, 2021). Previous reports have shown that *GCH1* expression is regulated by developmental signals (McIntosh et al., 2008). During plant development, the expression of *GCH1* becomes up-regulated in plant tissues when folate is needed to be synthesized for cell replication (McIntosh et al., 2008). During seed germination, rapid cell division increases the demand of 1C units for cell metabolism and nucleotide biosynthesis (Liu FY et al., 2017). Thus, the synthesis of folate in developing seedlings is accelerated. Seedlings are particularly sensitive to changes in environmental factors. Light not only provides radiation energy for plant photosynthesis, but also acts as a signal to regulate plant growth and metabolism. Reports have shown a positive effect of the radiation of red light-emitting diode (LED) light on the antioxidant capacity of lettuce plants (Samuolienė et al., 2012). In addition, light spectrums regulate some metabolic activities of plants by intervening in sugar and hormonal signals (Spalholz et al., 2020). Arif et al. (2016) found that the folate content of green pea seedlings was higher than that of etiolated pea seedlings. When the etiolated pea seedlings were exposed to light again, the content of folate increased significantly. Długosz-Grochowska et al. (2016) investigated the effect of LED supplemental light on the folate content of lamb's lettuce in the greenhouse, and found that plants supplemented with red and blue lights had a higher folate content than those without supplementary light. These studies indicated that light is involved in a certain stage of folate biosynthesis, and might be one of the regulatory factors at certain points in the folate synthesis pathway in plants. In addition, folate is one of the chromophores in cryptochrome photoreceptors (Samuolienė et al., 2012). This was our inspiration for investigating the influence of light on folate accumulation in wheat seedlings: is it possible that the application of different wavelengths of light could enhance the natural synthesis of dietary folate in wheat seedlings?

The main aim of this experiment was to find the best light spectrum and intensity for stimulating folate synthesis using different light spectrums (white, red, blue, green, and yellow) during wheat seedling growth. By measuring the folate distribution, folate precursor content, enzyme activity, and gene expression in folate biosynthesis, this study could reveal the mechanism by which light regulates folate accumulation in wheat seedlings. Because our method uses only LED light without genetic modification, it is a practical method for producing folate-abundant wheat seedlings and would be a valuable and cost-effective intervention to address the problem of folate deficiency worldwide.

2 Materials and methods

2.1 Materials and chemicals

Wheat seeds (*T. aestivum* L. cv. Huaimai 33) were kindly provided by the Huaiyin Agricultural Science

Research Institute, Xuhuai, Jiangsu, China. They were cultivated in Suqian in Jiangsu Province in Eastern China, a region with a subtropical monsoon climate characterized by a mean rainfall of 910 mm/year. After harvest in 2019, the wheat seeds were stored at −20 ℃ before use. The folate standards, including 10-formylfolic acid (10-CHO-FA), 5-CHO-THF, 5-methyl-THF (5-CH_3-THF), THF, and FA, were purchased from Sigma-Aldrich Chemical Corp. (Fairfield, OH, USA). *p*ABA and 6-hydroxymethylpterin were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Methanol and acetonitrile (liquid chromatograph-mass spectrometer (LC-MS) grade) were purchased from Fisher Scientific (Gail, Belgium). Rat serum was sourced from Soleibao Technology Co., Ltd. (Beijing, China), and stored in a refrigerator at −80 ℃ before use. Folate standard stock solutions were dissolved in solution (50:50 (volume ratio) methanol:distilled water, 0.1% (volume fraction) ascorbic acid, 20 mmol/L ammonium acetate; pH 6.2) at a concentration of 200 μg/mL, stored in a refrigerator at −80 ℃, and then diluted with distilled water to 5, 10, 50, 100, and 200 ng/mL for use. All other chemicals were analytical grade reagents purchased from Nanjing Sode Biotechnology Co., Ltd. (Nanjing, China).

2.2 Cultivation condition and treatments

Wheat seeds were rinsed in distilled water and disinfected with 1% (volume fraction) sodium hypochlorite for 15 min. Then they were washed with distilled water and soaked in distilled water at 25 ℃ for 6 h. Then the soaked seeds were evenly spread on a seedling tray in a germination machine (CB-A321, Foshan Shunde Jiahao Industrial Co., Ltd., Foshan, China) and germinated in an incubator (LB-300-II, Shanghai Longyue Instrument Equipment Co., Ltd., Shanghai, China) at 25 ℃. The seeds were sprayed with distilled water once for 2 min every hour during germination. The culture solution was changed once a day. After germination for 2 d in the dark at 25 ℃, the seeds were irradiated with LED white light, red light (peak at 655 nm), blue light (peak at 445 nm), green light (peak at 520 nm), and yellow light (peak at 595 nm) at an intensity of 30 μmol/(m^2·s) on the third day, for 4 d of continued cultivation at 25 ℃. The photoperiod was 16-h light and 8-h darkness. In the light intensity study, the light intensity was set to 0, 15, 30, 45, or 60 μmol/(m^2·s). Wheat seedlings cultivated in the dark served as the control. The seedlings under different treatments were collected at a fixed time for further analysis. The samples were freeze-dried, milled, and stored at −20 ℃ for a maximum of two weeks prior to further analysis. The major technical parameters of the light spectrums' energy distributions under LED are shown in Table S1.

2.3 Folate extraction and determination

Folate was extracted and the content was determined using the method of Riaz et al. (2019), with some modifications. Briefly, the sample was extracted with phosphate buffer (5 mmol/L, with 1% (volume fraction) sodium ascorbate and 0.2% (volume fraction) β-mercaptoethanol, pH 7.2) for 10 min in a boiling water bath. Following cooling and centrifugation, rat serum was added to the supernatant to deconjugate the polyglutamylated tails. After centrifugation, the supernatant was ultrafiltrated at 12 000*g* for 20 min on a 3 kDa ultra-filtration tube (Millipore, MA, USA). Finally, the resulting solution was subjected to separation using high-performance liquid chromatography (HPLC), followed by tandem mass spectrometry (MS/MS) detection on a SCIEX Triple Quad™ 5500 spectrometer (Foster City, CA, USA), using electron spray ionization (ESI). The experiments were performed in triplicate. A detailed description of the analytical procedures can be found in the supplementary methods.

2.4 Determination of pterin and *p*ABA

The determination of pterin and *p*ABA followed the method of Ramírez Rivera et al. (2016). The pterin was quantified relative to standard 6-hydroxymethylpterin. The content of *p*ABA represents total *p*ABA (i.e., free *p*ABA plus *p*ABA glucose ester). The experiments were performed in triplicate. A detailed description of the analytical procedures can be found in the supplementary methods.

2.5 GCH1 and ADCS activity assays

GCH1 activity assay was conducted according to the previous studies (McIntosh and Henry, 2008; McIntosh et al., 2008). One unit of the activity was defined as producing 1 nmol of 6-hydroxymethylpterin per 60 min of the reaction. ADCS activity was determined according to Basset et al. (2004). One unit of activity was defined as producing 1 nmol of *p*ABA per 60 min of the reaction. The experiments were

performed in triplicate. A detailed description of the analytical procedures can be found in the supplementary methods.

2.6 Gene expression analysis

Frozen wheat seedlings were ground to a fine powder in liquid nitrogen. An RNA extraction kit (TaKaRa, Nanjing, China; catalog No. 9769) was used for extracting the total RNA of the wheat seedlings. First-strand complementary DNA (cDNA) was synthesized with the reverse transcription-polymerase chain reaction (RT-PCR) Master Mix Kit (TaKaRa; catalog No. RR036A). Quantitative real-time polymerase chain reaction (qPCR) analysis was performed with the SYBR Premix ExTaq™ (TaKaRa; catalog No. RR420A) using the ABI 7500 PCR system (Applied Biosystems, Foster City, CA, USA). The primer sequences used in this study are shown in Table S2.

2.7 Statistical analysis

Experimental data are expressed as mean±standard deviation (SD), with n=3. SPSS 18.0 software (SPSS Inc., Chicago, IL, USA) was applied for significance testing. Data were analyzed by Duncan's multiple-range tests at P<0.05.

3 Results

3.1 Changes in folate content during the germination of wheat

The total folate content initially increased gradually, reached the maximum after 6 d of growth, and then decreased during growth without light irradiation (Fig. 1a). The total folate content of 6-d germinated wheat (230.99 μg/100 g dry weight (DW)) was 5.27 times that of ungerminated seeds (43.84 μg/100 g DW). 5-CHO-THF and 5-CH_3-THF were the predominant folate components, accounting for 52.05% and 26.54% of total folate content, respectively. After wheat germination, the proportions of THF and 5-CH_3-THF in the total folate increased, while those of 5-CHO-THF and 10-CHO-FA decreased (Fig. 1b). After 6 d of germination, 5-CH_3-THF and THF had become the predominant folates, accounting for 46.37% and 30.61% of total folate content, respectively. The 10-CHO-FA and FA contents were relatively low in the initial germination stage, and did not change drastically as germination progressed, indicating that germination increased the contents of mainly 5-CH_3-THF and THF in the wheat seeds.

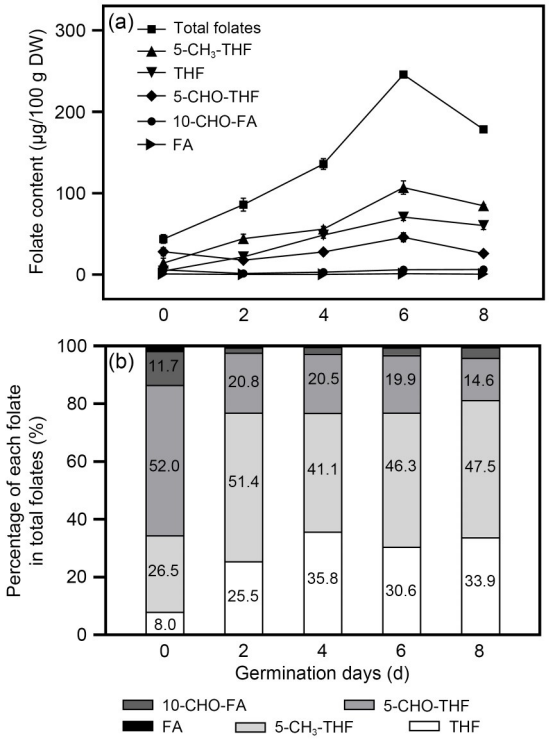

Fig. 1 Changes of folate content (a) and the proportion (b) during wheat germination. Wheat seeds were sprayed with distilled water once for 2 min every hour during germination. The culture solution was changed once a day. (a) Data are expressed as mean±standard deviation (SD), n=3. DW, dry weight; THF, tetrahydrofolate; FA, folic acid; 10-CHO-FA, 10-formyl-FA; 5-CHO-THF, 5-formyl-THF; 5-CH_3-THF, 5-methyl-THF.

3.2 Effects of light spectrums and intensity on folate content in wheat seedlings

The total folate content showed significant differences among wheat seedlings irradiated by different light spectrums, and ranged from 220.68 to 274.02 μg/100 g DW (Fig. 2a). The total folate content in wheat seedlings irradiated by red, blue, and yellow lights increased by 23.87%, 18.21%, and 17.45%, respectively, due mainly to the increase of 5-CH_3-THF content, compared with that in the dark. There was no significant difference in the total folate content under white and green lights compared with the control (darkness). In addition, according to LC-MS/MS analysis, THF, 5-CH_3-THF, and 5-CHO-THF accounted for 90% or more of the total folate content under

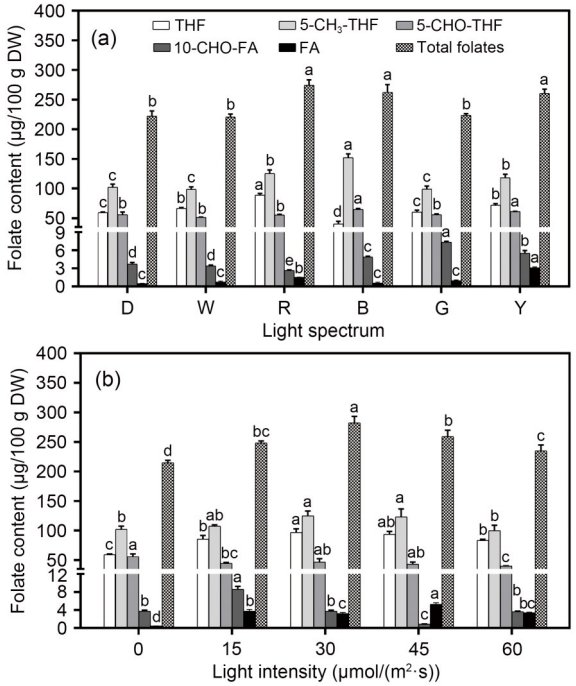

Fig. 2 Effects of light spectrums (a) and intensity (b) on the folate content of wheat seedlings. D, dark; W, white light; R, red light; B, blue light; G, green light; Y, yellow light. Wheat seedlings were grown in the dark for the first two days. Light irradiation started on the third day and lasted 4 d. The distance between the light-emitting diode (LED) light board and the seedling tray was 30 cm. The light intensity on the seedling tray was set by adjusting the current of the LED light board. In the study of light spectrums, the light intensity was uniformly set to 30 μmol/(m^2·s). In the study of light intensity, wheat seedlings were cultivated in red light. Wheat seedlings grown for 6 d were taken for study. Duplicate determinations were made on folate extractions from each replication. Data are expressed as mean± standard deviation (SD), *n*=3. Different lowercase letters indicate significant differences among the different treatments or intensities (*P*<0.05). THF, tetrahydrofolate; FA, folic acid; 10-CHO-FA, 10-formyl-FA; 5-CHO-THF, 5-formyl-THF; 5-CH$_3$-THF, 5-methyl-THF.

various light irradiations (Fig. 2a), indicating that they were the most abundant folate derivatives in the wheat seedlings. The content of THF under white, red, and yellow lights increased by 12.23%, 49.82%, and 22.37%, respectively, compared with the dark, while irradiation with blue light resulted in a 32.21% decrease. The content of 5-CH$_3$-THF increased by 49.55%, 23.97%, and 16.65% in the seedlings irradiated with red, blue, and yellow lights, respectively, compared with that in the dark. However, the content of 5-CH$_3$-THF did not change significantly under irradiation with white or green light. Blue and yellow lights increased the 5-CHO-THF content by 16.27% and 10.02%, respectively, compared with the dark, but the other light spectrums had no obvious effect on 5-CHO-THF content. The content of 10-CHO-FA in the seedlings irradiated by various light spectrums ranged from 2.66 to 7.36 μg/100 g DW, and the content of FA ranged from 0.36 to 3.10 μg/100 g DW. The contents of 10-CHO-FA and FA were relatively low in the wheat seedlings. In general, red, blue, and yellow light irradiations showed an advantage for folate synthesis. THF is the first folate synthesized in the folate biosynthetic pathway and also accounts for a high proportion of the total folate. The content of THF was highest under red light irradiation. Therefore, red light was well suited for further study on the effect of light on the pathway of folate synthesis.

With increasing light intensity (red light, 0–60 μmol/(m^2·s)), the total folate content and the content of each folate component, except 5-CHO-THF, first increased then decreased (Fig. 2b). However, the highest content of each folate occurred under different intensities. The total folate was highest at 30 μmol/(m^2·s) of intensity, an increase of 23.32% compared with that in the dark. Light irradiation decreased 5-CHO-THF content. The peak value of 10-CHO-FA content was observed at an intensity of 15 μmol/(m^2·s). The highest contents of THF and 5-CH$_3$-THF appeared at an intensity of 30 μmol/(m^2·s). These results indicated that 30 μmol/(m^2·s) of red light irradiation resulted in higher folate accumulation in wheat seedlings.

3.3 Distribution of folate in wheat seedlings under red light irradiation

The composition and proportion of folate in each part of the wheat seedlings are shown in Fig. 3. There were significant differences in the total folate content in each part of the seedlings: it was highest in the leaves, followed by the roots, and lowest in the endosperm. Among the folate components, the content of 5-CH$_3$-THF was highest in all parts of wheat seedlings, followed by THF and 5-CHO-THF, whereas the contents of 10-CHO-FA and FA were extremely low.

Following red light irradiation, total folate content in the leaves increased by 10.02% compared with the control (CK, darkness), caused mainly by the increased 5-CH$_3$-THF and THF content (Fig. 3b). In contrast to the leaves, the total folate content in the roots was reduced by 23.25%, due mainly to the

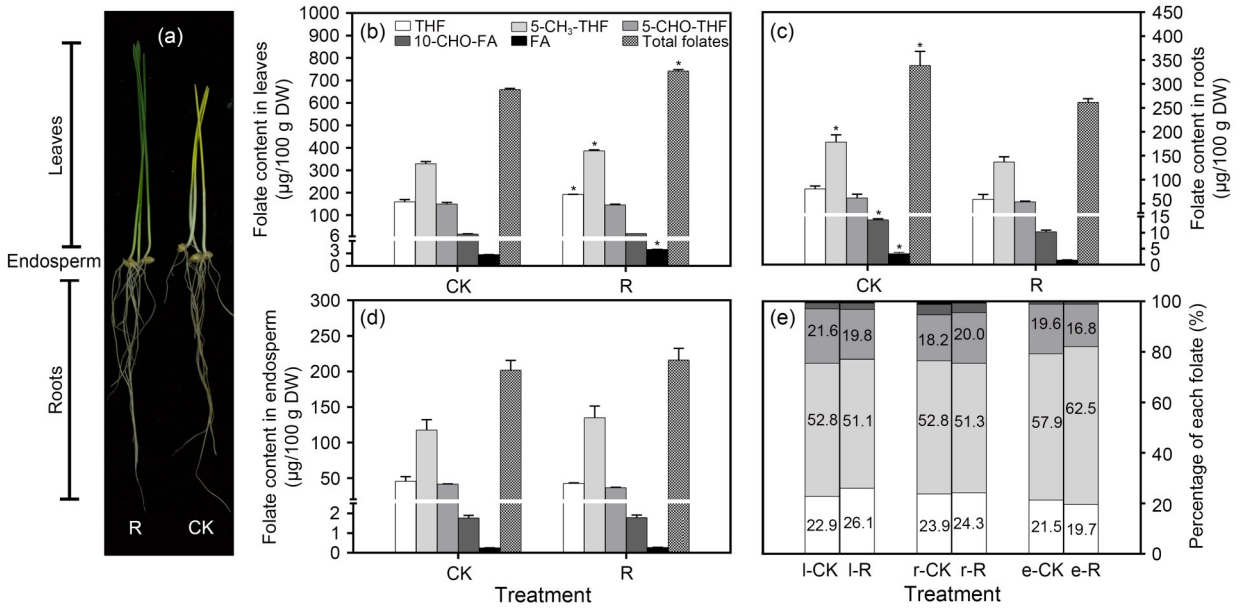

Fig. 3 Changes of folate content in the leaves (b), roots (c), and endosperm (d), and their proportion (e) in wheat seedlings (a) under red light irradiation. CK, control, wheat seedlings grown in the dark; R, wheat seedlings grown under red light irradiation of 30 μmol/(m^2·s). l, leaf; e, endosperm; r, root. Wheat seedlings were grown in darkness for the first 2 d. Light irradiation was started on the third day and lasted 4 d. Wheat seedlings grown for 6 d were taken for study. (b–d) Data are expressed as mean±standard deviation (SD), n=3. * Indicated a significant difference at P<0.05 between the treatment R and CK for each folate, and t-tests were used. THF, tetrahydrofolate; FA, folic acid; 10-CHO-FA, 10-formyl-FA; 5-CHO-THF, 5-formyl-THF; 5-CH_3-THF, 5-methyl-THF.

reduction of 5-CH_3-THF (Fig. 3c). The total folate content in the endosperm did not change significantly after red light irradiation (Fig. 3d).

The content of 5-CH_3-THF in each part of the wheat seedlings was highest, accounting for more than 50% of the total folate, followed by THF and 5-CHO-THF. The contents of 10-CHO-FA and FA were relatively low (Fig. 3e). In leaves, the contents of THF and 5-CH_3-THF increased significantly after red light irradiation. Moreover, the increase of THF content was significantly greater than that of 5-CH_3-THF, resulting in an increase in the proportion of THF in total folate. In endosperm, the proportion of 5-CHO-THF decreased, while that of 5-CH_3-THF increased. In roots, the proportions of 5-CH_3-THF and 5-CHO-THF showed the opposite trend.

3.4 Effects of red light irradiation on pterin and *p*ABA content, and GCH1 and ADCS activity in wheat seedlings

Red light had different effects on the content of folate precursors in each part of the wheat seedlings (Figs. 4a and 4b). After red light irradiation, the contents of pterin and *p*ABA in leaves were 111% and 81% higher, respectively, than those of the control. The content of pterin increased significantly in the roots and that of *p*ABA increased significantly in the endosperm. Leaves had a higher pterin and *p*ABA content, which was consistent with the high levels of folate in leaves.

The activity levels of GCH1 and ADCS in the leaves, endosperm, and roots of wheat seedlings were investigated (Figs. 4c and 4d). Red light increased GCH1 activity in leaves, endosperm, and roots by 71.35%, 38.86%, and 32.27%, respectively, compared with the control, while it increased ADCS activity only in the leaves and endosperm. Compared with endosperm, leaves and roots had higher levels of GCH1 and ADCS activity, which was consistent with the high content of folate precursors in those tissues. These results indicated that red light irradiation had a positive effect on GCH1 and ADCS activity.

3.5 Expression of relevant genes

In plants, *GCH1*, *ADCS*, *HPPK-DHPS*, and *FPGS* are the key genes in the folate biosynthesis pathway, and their expression is closely related to folate biosynthesis. Except for *FPGS*, the expression of these genes in wheat seedlings irradiated with red light was

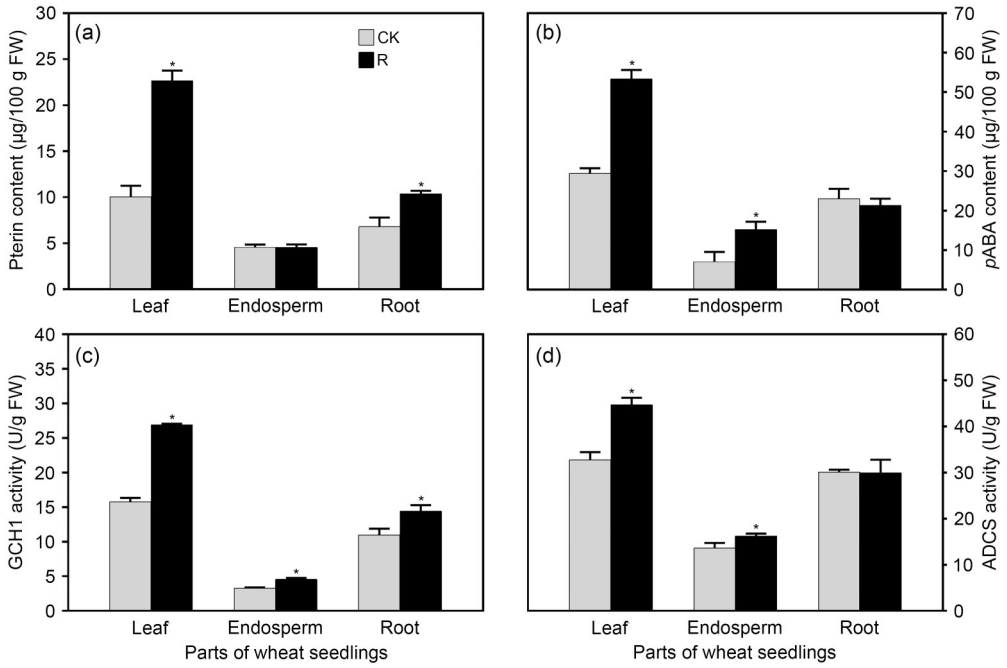

Fig. 4 Changes of precursor content (a, b) and the activity of key enzymes (c, d) in various parts of wheat seedlings under red light irradiation. Data are expressed as mean±standard deviation (SD), n=3. * Indicated a significant difference at P<0.05 between the treatment R and CK, and t-tests were used. CK, control, wheat seedlings grown in the dark; R, wheat seedlings grown under red light irradiation at an intensity of 30 μmol/(m^2·s), FW, fresh weight; GCH1, guanosine triphosphate (GTP) cyclohydrolase 1; ADCS, aminodeoxychorismate synthase; *p*ABA, *p*-aminobenzoic acid.

generally up-regulated, but the degree of up-regulation differed among the plant parts (Fig. 5). The expression of *GCH1* in wheat seedlings irradiated with red light was significantly up-regulated compared with the control (Fig. 5a). *GCH1* expression in roots was increased by 118.12% compared with the control. However, up-regulated expression of *ADCS* was observed only in roots compared with the control (Fig. 5b). The expression of *HPPK-DHPS* was significantly up-regulated by red light. In particular, the expression in roots increased by 97.79% compared with the control (Fig. 5c). Under red light irradiation, the expression of *FPGS* in wheat seedlings was significantly up-regulated except endosperm compared with the control (Fig. 5d). The expression of genes in each part of the wheat seedlings differed, indicating that the expression of genes involved in folate synthesis was spatially specific.

4 Discussion

In the present study, the folate content increased significantly during wheat germination, due mainly to an increase in 5-CH_3-THF (Fig. 1a). The increased folate content could be attributable to accelerated synthesis of folate because of increased demand for methyl groups (1C units) during germination (Liu FY et al., 2017). After seeds germinate, their endogenous proteases are activated and stored proteins are hydrolyzed, resulting in a gradual increase in the contents of peptides and free amino acids (Ma et al., 2018). Glutamic acid is one of the substrates of folate synthesis, and an increase of glutamic acid can directly promote folate synthesis. In addition, the high contents of phenylalanine, tyrosine, and tryptophan induce feedback inhibition on the activity of related enzymes, which promotes chorismate to synthesize more *p*ABA, thereby increasing folate synthesis (Watanabe et al., 2017). In this study, with the extension of the germination time, the folate content in wheat seedlings first increased and then decreased, and reached the maximum after 6 d of germination. In the later stages of germination, the nutrients in endosperm gradually become depleted, the life activity of the plants declines, and metabolic activity slows down. Hence, the demands for 1C units would be reduced in the wheat seedlings, leading to a decrease in folate synthesis (Lee et al., 2021).

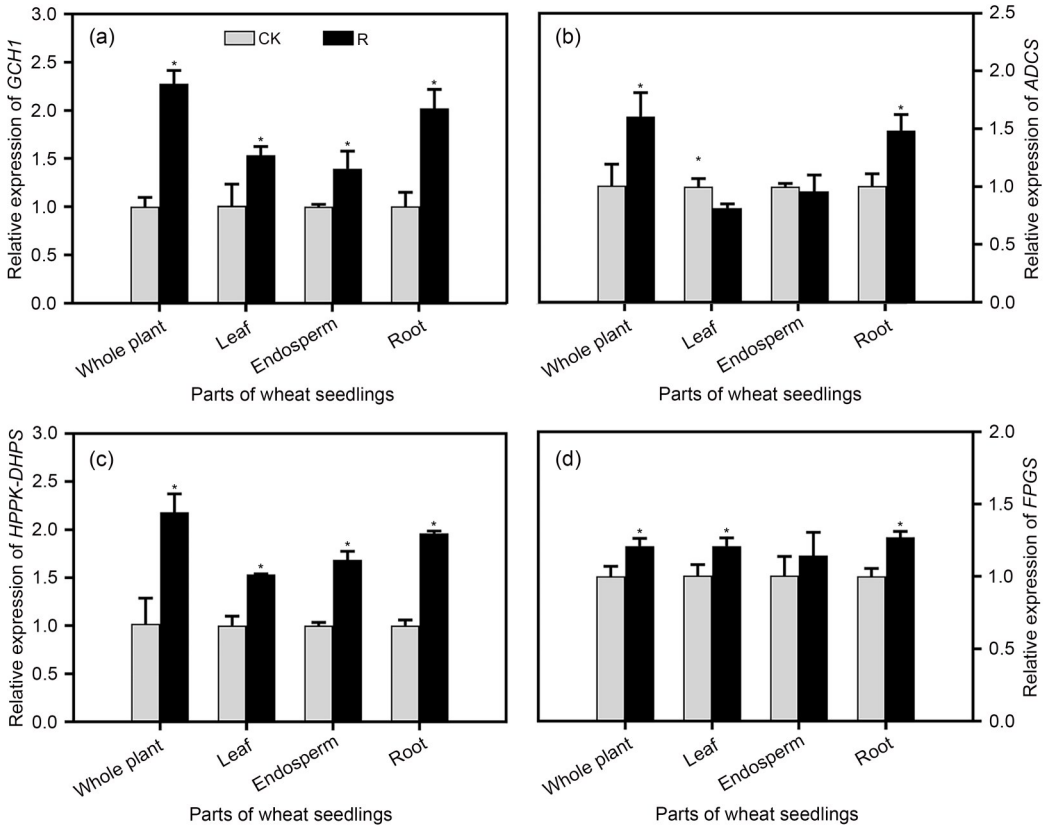

Fig. 5 Effects of red light irradiation on the expression of genes for key enzymes participating in folate biosynthesis of wheat seedlings. (a) Guanosine triphosphate (GTP) cyclohydrolase 1 (*GCH1*); (b) Aminodeoxychorismate synthase (*ADCS*); (c) Hydroxymethyldihydropterin pyrophosphokinase-dihydropteroate synthase (*HPPK-DHPS*); (d) Folypolyglutamate synthetase (*FPGS*). Data are expressed as mean±standard deviation (SD), n=3. * Indicated a significant difference at P<0.05 between the treatment R and CK, and t-tests were used. CK, control, wheat seedlings grown in the darks; R, wheat seedlings grown under red light irradiation of 30 μmol/(m^2·s).

Interestingly, the total folate content in the endosperm of germinated wheat also increased (Fig. 3d) compared with that of raw materials (Fig. 1a). This might be attributable to the consumption of nutrients in the endosperm during germination, leading to a reduction of dry weight and an increase in folate concentration. In addition, 5-CHO-THF was the most abundant form of folate in ungerminated wheat, whereas after germination, 5-CH_3-THF was the most abundant form (Fig. 1b). We concluded that the increase in total folate content of the wheat seedlings was due mainly to the increase in 5-CH_3-THF. Previous research indicated that 5-CH_3-THF was the most abundant folate in germinated wheat (Liu FY et al., 2017). 5-CH_3-THF is known to be the physiologically active form of folate for bioavailability in vivo, and can be used directly by organisms. Other forms of folate need to be converted into 5-CH_3-THF in the human body to participate in 1C metabolism (Golja et al., 2020). Therefore, it is more beneficial for the human body to uptake 5-CH_3-THF directly. During seed germination and seedling growth, the ratio of different forms of folate in wheat changes greatly, and 5-CH_3-THF becomes the main form (Fig. 2b). Hence, folate in germinated wheat is more conducive to its utilization in the human body.

During plant development, light is the energy source for photosynthesis and stimulates photoreceptors including phytochromes and cryptochromes to control many photomorphogenic reactions (Lee et al., 2014; Oh et al., 2020). Thus, light affects the biomass and synthesis of secondary metabolites. A previous study has shown that the light spectrum affects the synthesis of many compounds in plants (Taulavuori et al., 2018). However, there have been few studies on the effect of cultivation on the synthesis and accumulation of folate. Previous research found that folate content in green seedlings was higher than that in etiolated seedlings. However, the folate content of etiolated

pea seedlings after 24 h of light exposure was the same as that of pea seedlings grown under constant light (Gorelova et al., 2017). Długosz-Grochowska et al. (2016) investigated the effect of LED supplemental lighting in greenhouses on lamb's lettuce and found that supplementary lighting treatment induced a higher total folate content, and 5-CH_3-THF was the dominant form of folate. In the present study, we found that different light spectrums had a positive effect on folate synthesis, including red, blue, and yellow lights (Fig. 2a). The total folate content of wheat seedlings under red light irradiation (274.02 μg/100 g DW) was highest, due mainly to the increases of THF and 5-CH_3-THF. THF is the first folate synthesized in the folate biosynthetic pathway and also accounts for a high proportion of the total folate. The content of THF was highest under red light irradiation. Thus, red light had a greater impact on folate biosynthetic pathways than blue or yellow light. Light intensity significantly affected folate content in wheat seedlings. In our study, 30 μmol/(m^2·s) of red light showed superiority for folate synthesis and affected mainly the synthesis of 5-CH_3-THF (Fig. 2b). Higher intensity was not beneficial for folate synthesis. The reason might be that excessive absorption of monochromatic light causes disturbance and damage to the metabolic system, which interferes with the normal physiological metabolism of the plants and affects the pathway for folate synthesis. Therefore, in the early stages of seedling development, irradiation at an appropriate light intensity could achieve a balance in the metabolic system to maximize folate accumulation. Jabrin et al. (2003) suggested that each organ/tissue is autonomous for the synthesis of folate during germination and seedling development. There are phytochromes and cryptochromes in the leaves of wheat seedlings, and more 1C units and THF are required for photosynthesis and photorespiration (Yang et al., 2018). In addition, previous studies had found folate synthesis and accumulation in vigorous leaves and actively dividing tissues. Folate mediates 1C transfer reactions in cells. Many 1C transfer reactions are involved in cell division, so the demand for folate increases. Therefore, the folate content was higher in leaves than in endosperm and roots, and was more affected by red light (Fig. 3). This indicated that folate accumulated more in vigorous green leaves, and red light could participate in a certain stage of folate biosynthesis. Unexpectedly, red light irradiation significantly reduced the total folate content in the roots of wheat seedlings, caused mainly by the decrease of 5-CH_3-THF content (Fig. 3c). Red light irradiation stimulates photosynthesis and photorespiration in leaves (Yang et al., 2018), so that the demand for 1C units increases, which increases the demand for folate in the leaves. Therefore, we speculated that folate in the roots might have been transported to the leaves to support growth needs, and thus the folate content in the roots was reduced. There are abundant nutrients in the endosperm. During germination and seedling development, the endosperm provides mainly energy for the growth of seedlings, and undergoes no significant cell proliferation. Therefore, the folate content in the endosperm was lowest compared to those of leaves and roots, and red light did not significantly affect it (Fig. 3).

GCH1 and ADCS are the key enzymes in the two branches of the folate synthesis pathway. The activity of GCH1 and ADCS in wheat seedlings increased significantly after red light irradiation (Figs. 4c and 4d), which was consistent with the increases of pterin and *p*ABA (Figs. 4a and 4b). This indicated that red light irradiation stimulated GCH1 and ADCS to promote the synthesis of more pteridine and *p*ABA in the seedlings. There were high levels of GCH1 and ADCS activity in leaves and roots (Figs. 4c and 4d). Accordingly, the folate content in leaves and roots was higher than that in endosperm. Hence, the up-regulated GCH1 and ADCS activity positively contributed to folate synthesis (de la Garza et al., 2007). In addition, the expression and activity of HPPK-DHPS in leaves significantly increased after red light irradiation, indicating that red light also stimulated the synthesis of HPPK-DHPS. During organ differentiation, rapidly dividing tissues and photosynthetic leaves contain 3–5 times or more folate than other parts, and have high levels of *HPPK-DHPS* messenger RNA (mRNA) and protein (Arif et al., 2016). In the present study, the mRNA level of *HPPK-DHPS* was up-regulated by red light irradiation (Fig. 5c). The expression of other genes involved in folate synthesis, including *GCH1*, *ADCS*, and *FPGS*, was also stimulated by exposure to red light (Fig. 5). Therefore, both up-regulated expression of genes and increased activity of enzymes participating in folate biosynthesis were the key factors promoting folate accumulation in wheat seedlings under red light irradiation.

Red light irradiation is a safe and effective strategy to accumulate folate, especially 5-CH_3-THF, without genetic modification. In general, the recommended intake of folate for adults is 400 μg/d (Fu et al., 2021). In our research, 100 g of ungerminated wheat contained only 43.84 μg of folate, whereas 100 g of wheat seedlings irradiated by red light contained 274.02 μg of folate, representing 68.5% of the recommended daily allowance for adults. The germinated wheat can be milled into whole-wheat flour. Incorporating germinated whole-wheat flour into grain products meets the desire of health-conscious consumers for more whole-grain options, along with the added folate intake. Therefore, the use of red light irradiation to accumulate folate has important practical significance, and is a valuable and cost-effective intervention to solve the problem of folate deficiency.

5 Conclusions

Germination and light irradiation increased folate accumulation in wheat, and 30 μmol/(m^2·s) red light irradiation had the most positive effect. The accumulation of folate in wheat seedlings promoted by red light irradiation was mainly in the leaves, especially the increase of 5-CH_3-THF. In addition, gene expression related to the biosynthesis of folate was up-regulated, resulting in an increase in the activity of key enzymes and precursors of folate synthesis.

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Author contributions

Jianwei CHANG performed the experimental research and data analysis, and wrote and edited the manuscript. Zhenxin GU performed the experimental research and data analysis. Runqiang YANG contributed to the study design. Chong XIE, Pei WANG, and Yongbin HAN contributed to the study design, writing and editing of the manuscript. All authors have read and approved the final manuscript, and therefore, have full access to all the data in the study and take responsibility for the integrity and security of the data.

Compliance with ethics guidelines

Jianwei CHANG, Chong XIE, Pei WANG, Zhenxin GU, Yongbin HAN, and Runqiang YANG declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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Supplementary information

Supplementary methods; Tables S1 and S2; Fig. S1