The Crop Journal xxx (xxxx) xxx

Contents lists available at ScienceDirect

The Crop Journal

journal homepage: www.keaipublishing.com/en/journals/the-crop-journal/

Arginine promotes seed energy metabolism, increasing wheat seed germination at low temperature

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ARTICLE INFO

Article history:
Received 16 February 2024
Revised 26 April 2024
Accepted 24 May 2024
Available online xxxx

Keywords:
Low temperature
Seed germination
Arginine
Energy metabolism
Wheat

ABSTRACT

Low temperatures during germination inhibit seed growth, lead to small and weak seedlings, and significantly reduce the wheat yield. Alleviating the adverse effects of low temperature on wheat seed germination is highly important for achieving high and stable wheat yields. In this study, Tongmai 6 (insensitive) and Zhengmai 113 (sensitive), which have different low-temperature sensitivities during germination were treated with low temperature during germination. The transcriptome, metabolome and physiological data revealed that low temperature decreased the germination rate, downregulated the expression of a large number of genes involved in regulating glycometabolism, and inhibited carbon, nitrogen (especially amino acids) and energy metabolism in the seeds. Arginine content increased at low temperature, and its increase in the low-temperature-tolerant variety was significantly greater than that in the sensitive variety. Arginine priming experiment showed that treatment with an appropriate concentration of arginine improved the seed germination rate. The conversion of starch to soluble sugar significantly increased under exogenous arginine conditions, the content of key metabolites in energy metabolism increased, and the utilization of ATP in the seeds increased. Taken together, arginine priming increased seed germination at low temperature by relieving inhibition of seed carbon and nitrogen metabolism and improving seed energy metabolism.

1. Introduction

As crop production pays more attention to the rational allocation and efficient use of light and heat resources, “double late” technology (late harvest of corn and late sowing of wheat) has been widely applied in wheat production in China [1]. With the application of this technology and unfavorable weather conditions, such as continuous rain during wheat sowing, the wheat sowing date may be delayed [2]. A late sowing date reduces temperature during seed germination, resulting in a decreased germination rate, late emergence, weak seedlings, poor tillering, and torpid development, and also makes wheat more susceptible to spring cold in the later growth period [3,7,9]. All of these factors reduce wheat yield [4–6,8]. Ensuring normal germination of wheat seeds under low-temperature conditions is necessary for high and stable yield of late-sown wheat.

As essential substances for plant growth and development, amino acids regulate the life activities and stress resistance of plants [10–12]. Among the various amino acids, arginine (Arg) plays a key regulatory role in plant metabolism [13]. Arg serves as a nitrogen storage nutrient for reuse and participates in the regulation of multiple physiological mechanisms in plants, such as stress resistance [14,15]. Under low-temperature, drought, and salinity stress, the expression patterns of genes encoding enzymes associated with arginine synthesis and metabolism in wheat plants also change [16]. Arginine can increase the cold resistance of tea plants by activating specific cold regulation pathways [17]. In addition to stress resistance, arginine affects seed germination [18–20]. As the amino acid with the highest nitrogen proportion, Arg accounts for a large proportion of the amino acid composition of seed reserve proteins [21]. After seed germination starts, storage proteins are hydrolyzed into Arg, which is subsequently converted by a series of enzymes into other nitrogen-containing compounds for plant utilization [21]. Exogenous Arg can alleviate the harmful effects of drought stress on wheat seed germination [14]. Thus, Arg functions in the regulation of wheat seed germination.

The germination of seeds requires the decomposition of their own stored substances to supply the material base, and energy is required to convert these substances into components of new cells

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https://doi.org/10.1016/j.cj.2024.05.018

Please cite this article as: J. Li, Z. Li, Y. Tang et al., Arginine promotes seed energy metabolism, increasing wheat seed germination at low temperature, The Crop Journal, https://doi.org/10.1016/j.cj.2024.05.018

[22]. In the early stages of germination, an adequate supply of energy helps initiate the life cycle [23]. After the seed absorbs water, metabolic processes are activated, and the metabolic intensity increases, which requires a more adequate energy supply [24]. Insufficiency of energy substances hinders seed germination [25]. Energy metabolism is also critical for plant stress resistance [30]. The level of energy metabolism affects the heat resistance of rice [26]. Lipid-mediated energy metabolism regulates the response of wheat and *Arabidopsis* to high or low temperature stress [27,28]. Exogenous abscisic acid increases the low-temperature tolerance of wheat plants during the overwintering period by ensuring plant respiration and maintaining high ATP synthesis levels [29]. Thus, energy metabolism functions in the regulation of seed germination and plant stress resistance.

Amino acid metabolism provides energy to plant cells [31]. The substances generated from the decomposition of amino acids also enter the TCA cycle to participate in energy metabolism [32]. When energy is scarce, free amino acids can be degraded to simple carbon skeletons, which are integrated into the mitochondrial TCA cycle to generate respiratory energy [33]. Free branched-chain amino acids can be broken down by enzymes in the mitochondria and used as a source of energy during sugar shortages [34]. Higher amino acid accumulation helps to maintain energy status, which in turn preserves fruit quality [35]. As an important player in nitrogen metabolism and a precursor for the generation of NO, vigorous Arg metabolism may guarantee seed germination and seedling growth. High levels of free amino acids affect energy metabolism in germinated seeds and subsequent seedling establishment [36]. Arginine synthesis is critical for cell proliferation [31]. When the respiratory chain is inhibited, arginine supplementation alone is sufficient to promote rapid cell growth [37]. Arg metabolism significantly affects energy metabolism.

Both Arg and plant energy metabolism are involved in regulating seed germination and stress resistance of crops. However, whether Arg participates in the regulation of wheat seed germination under low-temperature stress and whether its effect on wheat seed germination is related to energy metabolism are unclear. We hypothesized that Arg relieves the inhibitory effect of low temperature on wheat seed germination by affecting energy metabolism.

The objectives of this study were to determine (1) whether Arg is involved in regulating the response of wheat seed germination to low-temperature stress and (2) whether the effect of Arg on seed germination at low temperature is related to energy metabolism. The experimental approach was to subject wheat cultivars with differing low-temperature tolerances to low temperature during seed germination with and without exogenous Arg priming, and measure germination, gene expression, and substance metabolism in the seeds.

2. Materials and methods

2.1. Experimental design

2.1.1. Experiment 1: Effect of low temperature on seed germination

Tongmai 6 (low-temperature resistant) and Zhengmai 113 (low-temperature sensitive) were tested. Seed germination experiments were performed in a light–temperature incubator. Petri dishes (9 cm diameter) with lids were used as containers, and 50 wheat seeds were placed in each dish. Double-circle qualitative filter paper (9 cm diameter) was spread in each dish, and water was added every day to maintain moisture. Two germination temperature treatments were used for each variety: low (12 °C/8 °C) and normal (22 °C/18 °C). A two-factor split-plot experiment was designed, with three replications for each treatment. The environment was kept dark for the first two days of germination; beginning on the third day after germination, the light conditions were set to 180 μmol m^{-2} s^{-1} (16 h) and 0 μmol m^{-2} s^{-1} (8 h).

2.1.2. Experiment 2: Seed priming

Zhengmai 113 was used as the experimental variety, and Arg was used as the priming solution at a concentration of 0.005 mmol L^{-1}. The seeds were soaked in equal volumes of initiating solution for 18 h in the dark at 25 °C and dried at 25 °C to constant weight, and germinated at low temperature for 7 d before being returned to a normal temperature. The other treatments were as in experiment 1. A two-factor split-plot experiment was designed, with three replications for each treatment.

2.2. Germination and growth

A 7-day germination cycle was used. If the length of the radical and coleoptile reached the full and half length of the seed, respectively, the seed was defined as germinated [38]. The number of germinations was recorded every day during the cycle, and the germination rate and germination potential were calculated on the seventh day. The number of roots and the length (cm) of the roots and buds were recorded.

2.3. Determination of soluble sugar and starch contents

The soluble sugar and starch contents were determined by the anthrone method [39]. The samples were extracted with 80% ethanol, and the extract was used for the determination of total soluble sugars. The sample residue was extracted with 9.2 mol L^{-1} perchloric acid, and the extract was used for the determination of starch. Colorimetry was performed at 620 nm with a microplate reader.

2.4. Enzyme activity measurement

Amylase activity was determined following Wang and Huang [39]. A 0.2 g fresh seed sample was ground, extracted at room temperature for 20 min, and centrifuged. The supernatant was used to determine he α-amylase activity. Then, 1 mL of the supernatant was diluted to 10 mL, after which total amylase activity was determined. The absorbance at 540 nm was measured using DNS (3,5-dinitrosalicylic acid) as a chromogenic reagent with a microplate reader. A maltose standard curve was prepared, and the α-amylase and total amylase activities were calculated based on the standard curve. The difference between the two was the difference in β-amylase activity.

The activities of glycometabolism-related enzymes were determined using kits from Solarbio (https://www.solarbio.com) and colorimetrically measured with a UV–vis spectrophotometer.

2.5. Transcriptome and metabolome measurements

Eukaryotic reference transcriptome and high-resolution nontargeted metabolomic measurements were performed by Personalbio (https://personalbio.bioon.com.cn), and energy metabolism was measured with Maiwei Metabolism (https://www.metware.cn).

2.5.1. Transcriptome measurement

The samples were sequenced to generate FASTQ raw data. HISAT2 (https://ccb.jhu.edu/software/hisat2/index.shtml), an updated version of TopHat2, was used to filter some reads with connectors and low quality in the raw data. High-quality sequences (clean data) were aligned to the reference genome of this species. The read count of each gene was statistically aligned using HTSeq, and the result was used as the original expression level of the gene. The expression level was normalized to FPKM

(fragments per kilobase per million fragments) value and used as the expression level of the gene. DESeq was used to idenfify differences in gene expression using the criteria $|\log_2 FoldChange| > 1$ and P value < 0.05. Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis was performed using clusterProfiler. During the analysis, the differentially expressed genes (DEGs) annotated by KEGG pathway analysis were used to calculate the gene list and the number of genes for each pathway, and the P value was subsequently calculated by the hypergeometric distribution method (the criterion for enrichment was a P value < 0.05). The KEGG pathways that were enriched in the DEGs were identified by comparison with the whole-genome background to determine the main biological functions of the DEGs.

2.5.2. Metabolome measurement

The samples were extracted at 4 °C. The supernatant was dried under vacuum, redissolved in 100 μL of acetonitrile aqueous solution and subjected to ultrahigh-performance liquid chromatography-tandem time-of-flight mass spectrometry (UHPLC-Q-TOF MS). Peak alignment, retention time correction, and peak area extraction were performed using XCMS software. Energy metabolizing substances were quantified by liquid chromatography tandem mass spectrometry (LC–MS/MS). A sample of 50 ± 2.5 mg was extracted with 70% methanol–water solution. After centrifugation, the supernatant was held at −20 °C for 30 min, and centrifuged at 4 °C. The supernatant was passed through a protein precipitation plate for subsequent measurement. The total ion chromatogram of the QC sample, principal component analysis (PCA) of the population sample, correlation of the QC samples, Hotelling's T2 test of the population sample, multivariate control chart of the QC samples, and relative standard deviation of the QC samples were used to assess data quality. Univariate statistical analysis, multidimensional statistical analysis, differentially abundant metabolite screening, correlation analysis of differentially abundant metabolites, and KEGG pathway analysis were performed.

2.6. Statistical analysis

Means were compared by analysis of variance (ANOVA) using IBM SPSS Statistics (with the LSD method) at the 5% probability level. Origin 2022 and Excel were used to construct charts.

3. Results

3.1. Differences in germination of different wheat genotypes under low-temperature treatment

At normal temperature, there was no significant difference in germination rate between Tongmai 6 and Zhengmai 113 (Fig. 1A). Low-temperature treatment inhibited the germination of the seeds of both genotypes, but the sensitivity of the two varieties to low temperature differed. Seven days after low-temperature treatment, the germination rate of Zhengmai 113 (70% on day 7) was lower than that of Tongmai 6 (94% on Day 7). Low temperature significantly reduced the germination index (Tongmai 6 decreased from 16.7 to 9.1, and Zhengmai 113 decreased from 16.5 to 5.4) (Fig. 1B), root length, shoot length and dry weight (Table S1) of the two varieties. The germination index and the length and dry weight of Zhengmai 113 roots under low-temperature treatment were lower than those under Tongmai 6, while there was no difference between the two under normal temperature. Thus, Tongmai 6 was more tolerant to low-temperature stress than was Zhengmai 113 during germination.

3.2. Transcriptome analysis of wheat seed germination at low temperature

Compared with the control treatment, 10,815 DEGs were detected in Tongmai 6 treated with low temperature, with 4667 upregulated genes and 6148 downregulated genes (Fig. 2A, F). A total of 17,478 DEGs were detected in Zhengmai 113, of which 6955 DEGs were upregulated and 10,523 were downregulated (Fig. 2B, F). Among these DEGs, the two varieties shared 6480 genes (Fig. 2C).

The DEGs were enriched in four major pathways: genetic information processing, metabolism, cellular processes and environmental information processing (Fig. 2D, E). The top five metabolic pathways were selected according to the degree of significance, and amino acid metabolism and glyoxylate and dicarboxylate metabolism (ko00630) were significantly enriched in both genotypes. The related amino acid metabolic pathways included alanine, aspartate and glutamate metabolism (ko00250) and Arg synthesis (ko00220) (Fig. 2D, E). Not only did the enrichment of the Arg synthesis pathway overlap between the two genotypes, but its P value was also among the top three.

A comparison of the enriched pathways and gene expression patterns of the DEGs in the two wheat genotypes at low temperature revealed that the expression of genes in glycolysis (EMP), the tricarboxylic acid cycle (TCA), and the starch and sucrose metabolism pathways significantly changed (Fig. 2G–I). Among the EMPs, most were downregulated in both varieties; a few genes, such as those regulating pyruvate kinase (PK), were upregulated at low temperature; genes regulating hexokinase (HK), phosphoglycerate dehydrogenase (GAPDH) and phosphoglycer ate mutase (PGAM) were downregulated in Zhengmai 113; and both up- and downregulated genes were found in Tongmai 6 (Fig. 2G). In the TCA cycle, except for the gene regulating ketoglutarate dehydrogenase (OGDH), which was upregulated and downregulated in Tongmai 6 but downregulated only in Zhengmai 113, the other genes were downregulated in both varieties (Fig. 2H). The invertase (INV) and sucrose synthase (SUS) genes that regulate sucrose hydrolysis were both downregulated in Zhengmai 113 but were up- and downregulated in Tongmai 6 (Fig. 2I). The expression of genes that regulate several enzymes that catalyze the conversion of substances between the three pathways also changed, including the genes regulating phosphoglucomutase and pyruvate dehydrogenase (PDH) (Fig. S1). In general, the number and magnitude of changes in genes in Zhengmai 113 were much greater than those in Tongmai 6.

3.3. Metabolomic analysis of wheat seed germination at low temperature

The metabolome identified 11,406 metabolites (Fig. 3A, B). Compared with those in the control treatment, 5310 metabolites were upregulated, and 6096 metabolites were downregulated in Tongmai 6 (Fig. 3A), while 4691 metabolites were upregulated and 6715 metabolites were downregulated in Zhengmai 113 (Fig. 3B) under low-temperature treatment. Five KEGG pathways were significantly enriched together, in which amino acid synthesis and starch and sucrose synthesis coincided with transcriptome enrichment. The Arg metabolic pathway and the TCA cycle were independently enriched in Tongmai 6 and Zhengmai 113, respectively (Fig. 3C).

Focusing on the glycometabolism pathways, the levels of eight substances, namely, glucose, *cis*-aconitic acid, α-ketoglutarate, malic acid, citric acid, glucose 1-phosphate (G1P), D-fructose, and sucrose, exhibited significant changes at low temperatures (Fig. 3D–F). The contents of α-ketoglutarate and malic acid significantly increased (Fig. 3E). The sucrose content decreased in Tong-

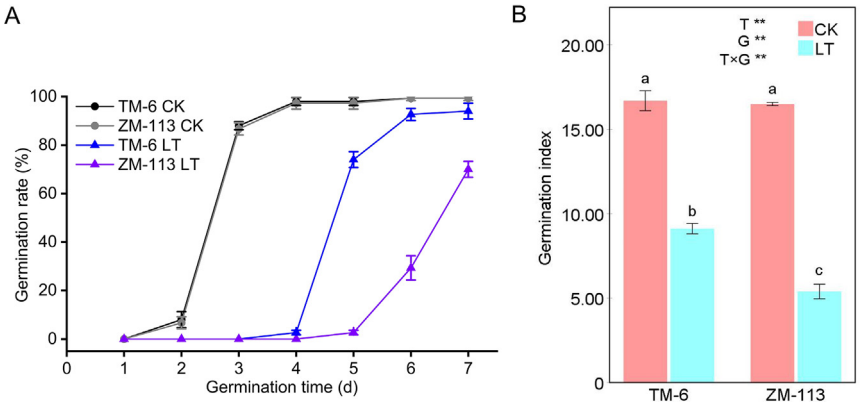

Fig. 1. Germination rate (A) and germination index (B) at low temperature. CK and LT represent the normal temperature treatment (as the control group) and low temperature treatment, respectively, on the 7th day after germination. The error bars represent the SDs ($n = 3$). Different letters ($P<0.05$) and asterisks (*, $P < 0.05$; **, $P < 0.01$) indicate significant differences by two-way ANOVA with the LSD test. TM-6, Tongmai 6; ZM113, Zhengmai 113; T, treatment; G, genotype.

mai 6 but increased in Zhengmai 113. The other substances all decreased, most of them significantly. These changes corresponded to the comprehensive changes in the expression of the enzyme-regulating genes in these pathways (Figs. 2G–I, 3D–F). We further focused on amino acids at different concentrations between normal temperature and low temperature, and 13 amino acids were detected. Those that differed under the interaction and different temperatures were Arg, proline, leucine and methionine (Fig. 3G). Among the significantly different amino acids, the content of Arg tended to increase at low temperature, and its content increased significantly in Tongmai 6 but changed weakly in Zhengmai 113 (Fig. 3G).

3.4. Effect of priming with arginine solution on seed germination at low temperature

Arg promoted germination at low temperature, and the promoting effect differed with concentration. Arg4 (0.005 mmol L^{-1}) had the most obvious effect on promoting germination (Fig. S2) and increased the germination rate (97.3% on day 7, 27.3% greater than that in LT) and germination index, and resulted in the longest root length and bud length of Zhengmai 113 (Table 1).

3.5. Effect of priming with arginine solution on seed energy metabolism at low temperature

The priming concentration was 0.005 mmol L^{-1}. The energy metabolism of sugars and amino acids and the enzyme activities of the priming group and the control group were measured on the 3rd and 5th days of germination.

A total of 68 substances associated with energy metabolism were detected. There were 22 substances that exhibited significant differences after 3 days of germination and 11 substances after 5 d of germination (marked with * in Fig. S3A, B). In terms of glycometabolism, arginine priming could ameliorate the effect of low temperature on the levels of several key substances, which was mainly reflected on the 3rd day of germination (Fig. S3A, red *). Compared with those in the control group, the levels of G1P, glucose, glucose 6-phosphate (G6P), fructose 6-phosphate (F6P), fructose 1,6-diphosphate, 3-phosphoglyceric acid, phosphoenolpyruvate (PEP), *cis*-aconitic acid and isocitric acid, increased, and the content of fumaric acid slightly decreased. The changes in substance contents were close to those under a normal temperature (Fig. S3A, 4A). After Arg priming, the activities of HK, phosphofructokinase (PFK) and PK, which are required for EMP; citrate synthase (CS), which is required for the TCA cycle; and PDH, which is the rate-limiting enzyme connecting the EMP and the TCA cycle, increased on the 3rd and 5th days of low-temperature germination (Fig. 4B–F). These results echoed the changes in the expression of enzyme-regulating genes and substances involved in glycometabolism mentioned above, indicating that Arg priming mitigates the adverse effects of low temperature on seed germination.

Arginine priming increased the soluble sugar content and decreased the starch content on the 3rd and 5th days of germination (Fig. 5A, B), and the activities of both α-amylase and β-amylase were significantly increased (Fig. 5C, D). On the 3rd day of germination, the ATP content significantly decreased, while the NAD^+ content significantly increased (Fig. 5E, F).

Arginine priming increased alanine, leucine, cystine and tyrosine contents during seed germination at low temperature, and these effects reached a significant level on the 3rd day of germination (Fig. 6A–D). Arginine priming also significantly increased the activities of nitrate reductase (NR) and glutamine synthetase (GS) (Fig. 6E, F).

4. Discussion

4.1. Arginine relieves the inhibitory effect of low temperature on wheat seed germination

Arginine is considered an organic nitrogen sink and nitrogen transformation mediator because of its high nitrogen-to-carbon ratio, and it is the main form of organic nitrogen storage and transport in plants [40]. Arginine also participates in the biosynthesis of proline and polyamines [41,42] and plays an important role in regulating plant responses to stress. Drought limits the rate of nitrogen accumulation and redistribution in wheat, which is related to the effect of drought on Arg synthesis and thus on polyamines synthesis [41]. The expression of genes related to Arg biosynthesis is downregulated under drought conditions, which leads to the obstruction of carbon metabolism in spikelets [43], and Arg pretreatment can ameliorate this adverse effect [14]. The function of differentially expressed genes under salt stress is also closely related to Arg metabolism [44]. The key genes involved in the cold resistance of rubber trees are NAGS (gene16028, gene33765), ArgC (gene2487) and ASS (gene6161), which are related to Arg biosynthesis[45]. Arginine is related to the biosynthesis of signaling molecules and plays a critical role in stress tolerance [46], its exogenous application can trigger a defensive response to adverse stress [42]. Higher Arg levels can improve wheat resistance to plumbum and cadmium stress [47], and Arg biosynthesis is highly

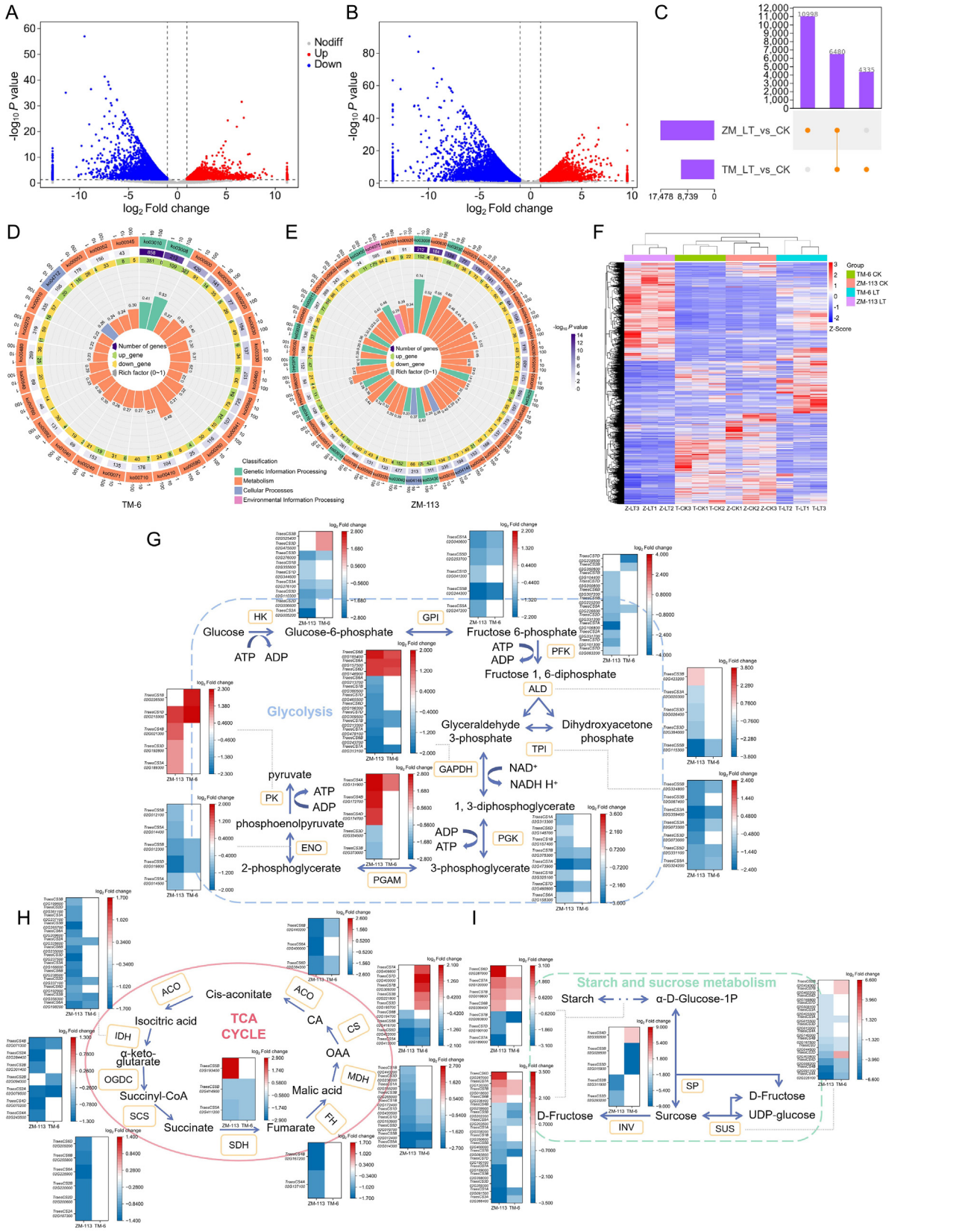

Fig. 2. Transcriptome analysis of wheat seeds under normal temperature (CK) and low temperature (LT) treatments. DEG statistical analysis (C) of Tongmai 6 (A) and Zhengmai 113 (B). Clustering heat map (F) of the DEGs and the KEGG enrichment pathways (D, E) associated with the two varieties. Heat map of the changes in the expression of genes that regulate enzymes involved in EMP (G), the TCA cycle (H) and starch-sucrose metabolism (I) at low temperatures. TM-6/TM/T, Tongmai 6; ZM113/ZM/Z, Zhengmai 113.

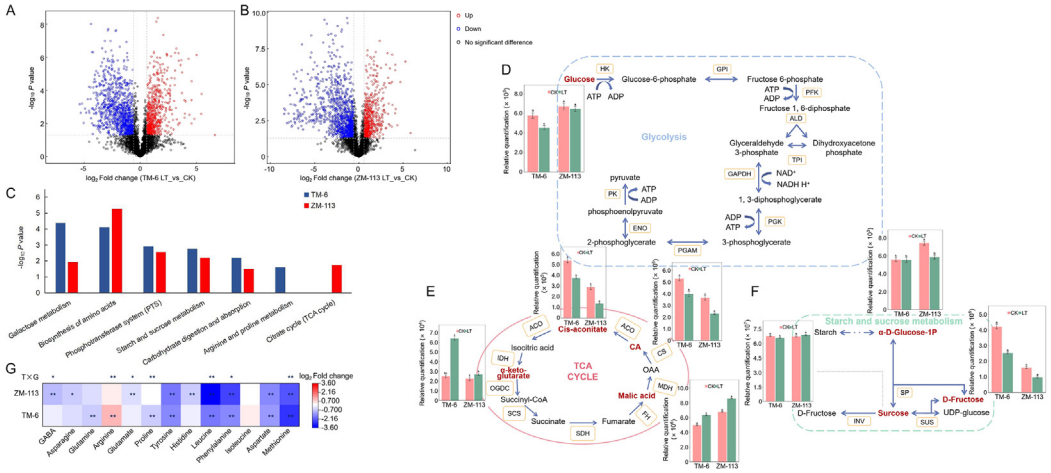

Fig. 3. Metabolomic analysis of different wheat seeds under normal temperature (CK) and low temperature (LT) treatments. Volcano map (A, B) and KEGG enrichment of differentially abundant metabolites (C). Changes in the contents of glycometabolic substances (D, E, F) and amino acids (G) at low temperature. The term "LT_vs_CK" refers to the use of CK as a reference for evaluating the changes in substances at LT. Different letters indicate differences between treatments of the same variety ($P < 0.05$). The error bars represent the SDs ($n = 4$). Asterisks (*, $P < 0.05$; **, $P < 0.01$) indicate differences by two-way ANOVA with the LSD test. T, Treatment; G, Genotype; TM-6, Tongmai 6; ZM113, Zhengmai 113.

Table 1
Germination conditions under arginine-priming.

Treatment	Maximum root length (cm)	Bud length (cm)	Germination rate (%)	Germination index
CK	7.82 ± 0.07 a	5.44 ± 0.28 a	99.33 ± 1.15 a	16.50 ± 0.08 a
LT	2.61 ± 0.22 c	1.01 ± 0.02 c	70.00 ± 4.00 b	5.39 ± 0.37 c
LT+A	4.83 ± 0.06 b	2.37 ± 0.15 b	97.33 ± 0.58 a	10.32 ± 0.09 b

CK, normal temperature (as the control group); LT, low temperature; LT+A, arginine priming in the low-temperature treatment. Different letters indicate significant differences between treatments ($P < 0.05$).

related to the antioxidant defense system [48]. Exogenous Arg treatment can significantly reduce the negative effects of salt stress on wheat growth and productivity [49] and can increase the plant height, tillering number, leaf number and flag leaf area of barley plants under drought stress [50].

Because the difference in the germination of different cold-resistant wheat varieties under low temperature increased beginning on the 3rd day of germination, the 3rd day was selected for analysis. Metabolome analysis revealed 13 amino acids with altered contents. Under low-temperature conditions, the levels of Arg and aspartic acid increased in Tongmai 6, which has better cold resistance, while the levels of other amino acids tended to decrease (Fig. 3G). Analysis of variance (ANOVA) revealed that Arg was significantly increased in Tongmai 6 but not in the sensitive variety Zhengmai 113, and the interaction effect between variety and temperature was significant. According to the metabolic pathway results, arginine metabolism was enriched only in Tongmai 6, and this pathway differed between the two varieties (Fig. 3C). We speculated that differences in Arg content was one of the reasons for the differences in cold resistance among the varieties and that an increased Arg content helps seeds withstand low temperature.

After exogenous Arg priming, the germination rate and germination index of the wheat seeds significantly increased at low temperature, and the root length and bud length also significantly increased (Fig. S2; Table 1). The findings that wheat seed germination and root and bud length increased at low temperature indicate that Arg can ameliorate the adverse effects of low temperature on wheat seed germination and increase cold resistance.

Amino acid levels affect the regulation of seed vigor and seed germination. Isopropylmalate synthase (IPMS) is the rate-limiting enzyme in leucine biosynthesis. OsIPMS1 gene mutants in rice exhibit decreased activity under various conditions, and the amino acid content in germinated seeds is reduced, while the exogenous application of amino acids largely improves the germination status of mutant seeds [51]. BCAAs could be used as alternative energy sources for Arabidopsis seed germination [52]. Consistent with these conclusions, in the present study, the Arg content of the different varieties differed at low temperature. After priming with exogenous arginine, not only did the contents and activities of key substances and key enzymes involved in energy metabolism increase, but the content of amino acids, including leucine, also increased, increasing the activity of the seeds and promoting seed germination at low temperature. These may also explain why Arg promotes wheat seed germination under low-temperature stress.

4.2. Energy metabolism mediates arginine priming-induced improvements in seed germination at low temperature

Cold resistance in wheat is associated with sucrose and amino acid biosynthetic pathways [53]. In the present study, at the transcriptome level, the starch and sucrose metabolism pathways were unique to Tongmai 6, while carbon fixation in photosynthetic organisms and fatty acid degradation were unique to Zhengmai 113 (Fig. 2D, E). At the metabolome level, the Arg metabolic pathway and the TCA cycle were independently enriched in Tongmai-6 and Zhengmai 113, respectively (Fig. 3C). Differences in the Arg content between the two varieties were detected (Fig. 3G). Low temperature limited the degradation of starch, resulting in a short supply of soluble sugars, and this effect was more obvious in Zhengmai 113 (Fig. S4). These findings are similar to the results of previous studies [54].

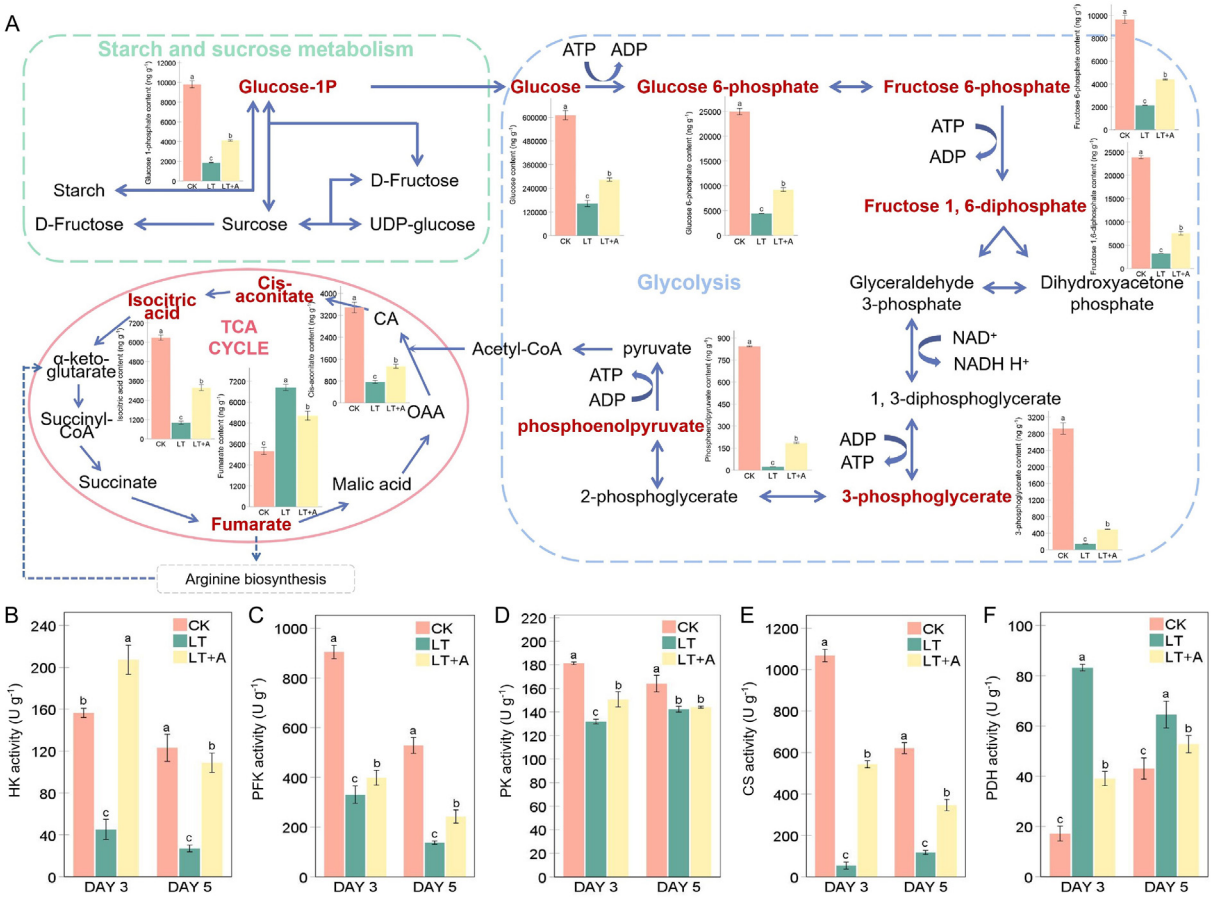

Fig. 4. Changes in substance content (A) and enzyme activity (B-F) during glycometabolism under arginine priming conditions. Different letters indicate differences between treatments (on the same day) by one-way ANOVA with the LSD test ($P < 0.05$). The error bars represent the SDs ($n = 3$). CK, LT and LT+Arg represent normal temperature, low temperature, and arginine priming under low-temperature treatment, respectively. DAY 3, the third day of seed germination; DAY 5, the fifth day of seed germination.

The genes differentially expressed in response to low temperature were associated with the EMP, TCA cycle, and starch and sucrose metabolism pathways. In Zhengmai 113, 45 genes regulating six key rate-limiting enzymes in the EMP and TCA cycles were altered, 41 of which were downregulated. In Tongmai 6, only 25 genes were changed, 16 of which were downregulated. Compared with that in Tongmai 6, the expression of more Zhengmai 113 genes changed, and the majority of these genes were downregulated (Fig. 2G–I). Among these genes, the genes regulating MDH, ACO, FH, and PDH play key roles in regulating TCA cycle efficiency to increase energy production during seed germination [55], and their downregulation inhibited the TCA cycle and reduced energy levels during seed germination. Downregulation of genes regulating CS, ACO, FH, SUS and INV significantly reduced the levels of intermediate metabolites such as citric acid, *cis*-aconitic acid, malic acid and D-fructose involved in glycometabolism, while the downregulation of genes regulating IDH and OGDC led to a change in the α-ketoglutarate content (Fig. 3D–F), which inhibited the TCA cycle and reduced the energy source [56,57], eventually inhibiting the cycle of energy metabolism. The blockage of energy metabolism leads to reduced seed vigor and slower germination [58,59]. The disruption of energy metabolism may be the key reason why Zhengmai 113 is cold sensitive and cannot germinate normally at low temperature.

Under energy starvation conditions, the contents of most free amino acids, especially key amino acids with high N/C ratios, increase [60,61]. These free amino acids are degraded to simple carbon skeletons by catabolic enzyme cascades, which are integrated into the mitochondrial TCA cycle to generate respiratory energy [33]. Arginine synthesis and metabolism are closely related to the TCA cycle. Both α-ketoglutarate and fumarate are critical for Arg metabolism and the TCA cycle, and Arg can affect the supply of carbohydrates [62]. Moreover, arginine indirectly participates in the pyruvate pathway and the Calvin cycle [63], which are closely related to energy metabolism. In the present study, both wheat genotypes showed increases in endogenous Arg content under low temperature, with a greater increase occurring in Tongmai 6 (Fig. 3G). There were fewer downregulated genes involved in glycometabolism in Tongmai 6, and the decreases in the contents of intermediate substances and soluble sugars were both lower than those in Zhengmai 113, suggesting that Tongmai 6 has a greater ability to access Arg to participate in energy production at low temperature than Zhengmai 113. The exogenous application of amino acids can restore the growth of rice plants with low seed vigor [51], and it can also promote photosynthesis and the formation of coenzymes, supporting the development of plants in the face of environmental stress [64,65]. As the amino acid with the highest nitrogen storage efficiency, the exogenous application of Arg can promote the absorption and utilization of nitrogen, phosphorus, and potassium in apple plants under low-nitrogen stress by changing their photosynthetic capacity and amino acid metabolism [66], and a relatively high content of this amino acid can also

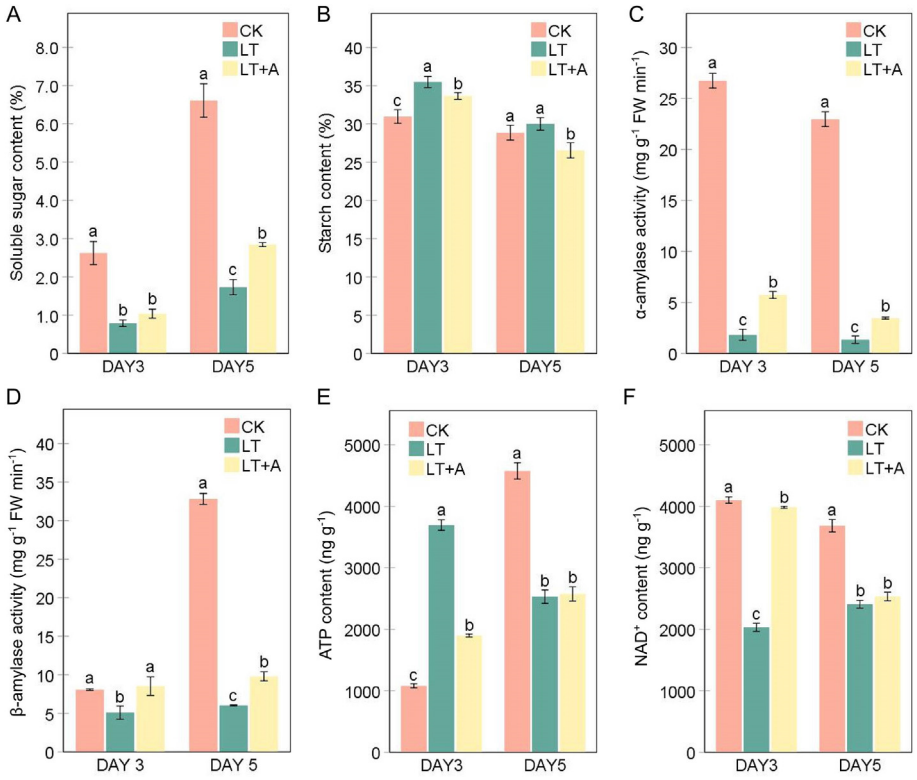

Fig. 5. Changes in soluble sugars (A), starch (B), amylase (C, D), ATP (E) and NAD+ (F) under arginine-priming conditions. Different letters indicate significant differences between treatments on the same day according to one-way ANOVA with the LSD test (P<0.05). The error bars represent the SDs (n = 3). CK, LT and LT+Arg represent normal temperature, low temperature, and arginine priming under low-temperature treatment, respectively. DAY 3, the third day of seed germination; DAY 5, the fifth day of seed germination.

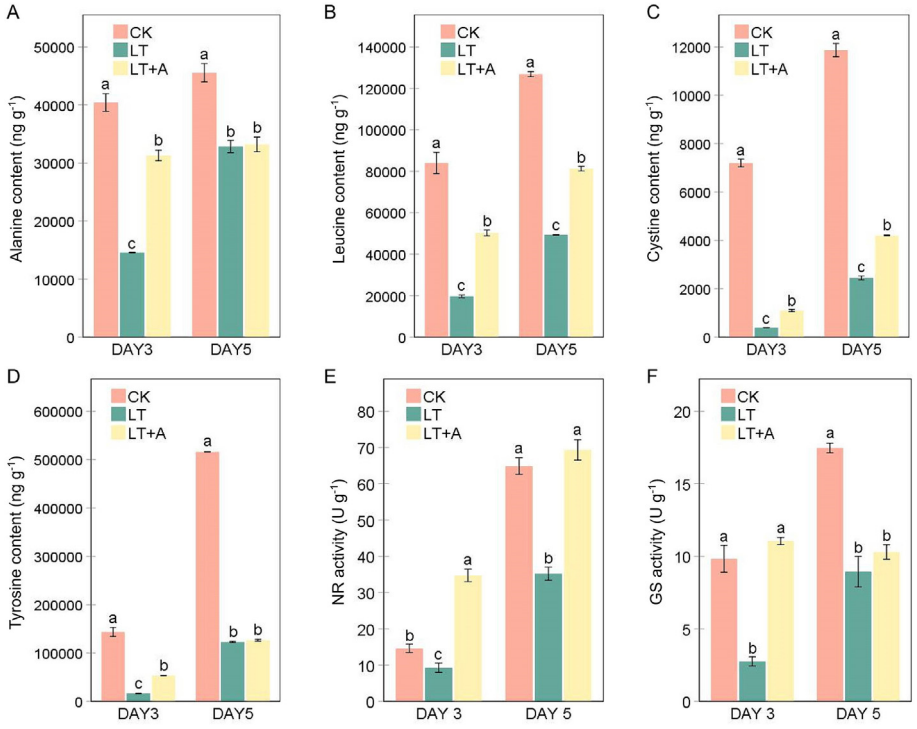

Fig. 6. Changes in amino acid content (A–D) and enzyme activity (E, F) under arginine-priming conditions. Different letters indicate differences between treatments on the same day according to one-way ANOVA with the LSD test (P<0.05). The error bars represent the SDs (n = 3). CK, LT and LT+Arg represent normal temperature, low temperature, and arginine priming under low-temperature treatment, respectively. DAY 3, the third day of seed germination; DAY 5, the fifth day of seed germination.

maintain osmotic balance and ensure the integrity of the membrane structure, thus maintaining the photosynthetic function of wheat plants under drought stress [67].

Exogenous Arg treatment increased the activities of the key rate-limiting enzymes HK, PFK and PK as well as those of CS and PDH, among which HK, PFK and PK are the key enzymes involved in ATP production through the EMP pathway [68]. Moreover, an increase in the activity of these enzymes can accelerate the production of ATP for seed utilization at low temperature. Increases in enzyme activity were accompanied by significant increases in the contents of G1P, G6P, F6P, fructose 1,6-diphosphate, 3-phosphoglycerate, PEP, *cis*-aconitic acid and isocitric acid, which are involved in glycometabolism (Fig. 4). The contents of these substances are close to normal temperature, providing sufficient substrates for glycometabolism at low temperature for an enhanced metabolism. Starch is the main storage form of carbohydrates in seeds, and it is degraded during germination to produce energy and metabolites [69]. Exogenous Arg treatment reduced the starch and ATP contents and increased the amylase activity and soluble sugar content in the seeds (Fig. 5), indicating that it accelerated the degradation of starch and the utilization of energy substances such as ATP. Adequate intracellular availability of NAD^+ helps plants obtain sufficient energy at low temperature [70]. After Arg treatment, the NAD^+ content in the seeds increased, which can contribute to energy acquisition. The alanine, leucine, cystine, and tyrosine contents as well as the activities of NR and GS increased after Arg treatment (Fig. 6), where leucine and tyrosine are the precursors for the synthesis of acetyl-CoA, and an increase in acetyl-CoA can accelerate the TCA cycle during seed germination [55]. Exogenous Arg promoted the cycle of carbon and nitrogen metabolism as well as the release and utilization of energy, which could restore the speed of seed germination to a certain extent and help the seeds fight cold.

The TCA cycle is the main pathway for energy-related metabolism in cells and the bridge for the mutual transformation and metabolism of several amino acids [71], while nitrogen accumulates in the form of amino acids (especially Arg) and amino acid derivatives, which can compensate for carbon deficiency [72,73]. In the present study, the changes in genes controlling enzymes related to glycometabolism were more significant in terms of both degree and quantity in Zhengmai 113 (Fig. 2 G–I), and the changes in regulatory genes of the enzymes (phosphoglucomutase and PDH) linking the EMP, TCA and starch and sucrose metabolism pathways in Zhengmai 113 were also greater than those in Tongmai 6 (Fig. S1). These findings indicate that low temperature inhibited the energy metabolism of the seeds during germination. Based on the changes in substances after the addition of exogenous Arg, the promoting effect of Arg on seed germination at low temperature occurred through increasing the contents of key substances and the activities of rate-limiting enzymes in the EMP and TCA pathways; accelerating the metabolism of starch, ATP and NAD^+; and increasing the content of amino acids. These changes can coordinate metabolic pathways and promote the flow and transfer of substances and thus the energy metabolism of carbon and nitrogen; that is, exogenous Arg can enhance energy metabolism so that seeds can accumulate more energy to address low-temperature stress and maintain seed germination vigor. This difference may be the result of exogenous Arg compensating for carbon deficiency and further balancing carbon and nitrogen metabolism.

5. Conclusions

Arg participates in the response of wheat seed germination to low-temperature stress. Priming with an appropriate concentration of Arg can restore carbon and nitrogen metabolism in seeds and promote energy metabolism, increasing seed resistance to low temperature and mitigating the adverse effects of low temperature on seed germination (Fig. S5).

CRediT authorship contribution statement

Jiayu Li: Conceptualization, Visualization, Writing – original draft, Writing – review & editing. **Zhiyuan Li:** Writing – review & editing. **Yangyang Tang:** Writing – review & editing. **Jianke Xiao:** Writing – review & editing. **Vinay Nangia:** Writing – review & editing. **Yang Liu:** Conceptualization, Funding acquisition, Supervision, Visualization, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This research was supported by the Key Research and Development Program of Shaanxi (2021NY-083) and the National Natural Science Foundation of China (31871567).

Appendix A. Supplementary data

Supplementary data for this article can be found online at https://doi.org/10.1016/j.cj.2024.05.018.

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