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Faba Bean Extracts Allelopathically Inhibited Seed Germination and Promoted Seedling Growth of Maize

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Abstract: Allelopathic interactions between crops in an intercropping system can directly affect crop yields. Faba beans may release allelochemicals to the cropping system. However, the allelopathic effects in the faba bean–maize relay intercropping system are still unclear. Maize seeds and seedlings were treated with a 50 mL of 100 g L^{-1} faba bean leaf extract (L1), 150 g L^{-1} faba bean leaf extract (L2), 100 g L^{-1} faba bean stem extract (S1), or 150 g L^{-1} faba bean stem extract (S2) and sterile water (CK) to study the allelopathic effects of faba bean extracts on maize seed germination and seedling growth. The α-amylase activities, antioxidant enzyme activities, phytohormones and allelochemical content in maize seeds were determined to evaluate the allelopathic effects of faba bean extracts on maize seed germination. The agronomic traits, photosynthetic parameters and nutrient absorption characteristics of maize seedlings were determined to explore the allelopathic effects of faba bean extracts on maize seedling growth. High-concentration (150 g L^{-1}) faba bean stem extracts released allelochemicals, such as 4-hydroxybenzoic acid, hydrocinnamic acid, trans-cinnamic acid, and benzoic acid. These allelochemicals entered the interior of maize seeds and increased the abscisic acid, salicylic acid and indole-3-acetic acid content in maize seeds but decreased the aminocyclopropane carboxylic acid in maize seeds. High-concentration (150 g L^{-1}) faba bean stem extracts increased the superoxide dismutase and peroxidase activity and decreased the α-amylase activity in maize seeds at germination (36 h). Faba bean extracts released nitrogen, potassium and phosphorus and increased nitrogen content, phosphorus content, potassium content and photosynthesis of maize seedling. In summary, faba bean extracts released allelochemicals that inhibited the germination of maize seeds but released nutrients and promoted the growth and development of maize seedlings. The research results provide a basis for improving the Faba bean–maize relay strip intercropping.

Keywords: faba bean; allelopathy; germination; physiology; phytohormone

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1. Introduction

Faba bean (*Vicia faba* L.)–maize (*Zea mays* L.) relay strip intercropping is a common cropping system in the lower reaches of the Yangtze river in China [1,2]. This system is an efficient management tool to control weeds, especially when no appropriate herbicide is available or where herbicides cannot be used, such as in organic farming systems [1]. Faba bean/maize intercropping systems increase the N_2 fixation of faba beans under intercropping due to interactions with micro-organisms [3]. Faba bean/maize intercropping including relay intercropping could increase the yield, biomass and nutrient accumulation of faba beans and maize [4–6]. Faba beans can fix up to 200 kg N ha^{-1}, while the incorporation of legume residues into the soil improves soil properties, such as organic matter content, bulk density, porosity and field capacity [7–9]. Faba beans are often used as a cover crop, mulch, or green manure after harvest [10–12]. However, faba beans may

release a large number of allelochemicals, such as benzoic, p-hydroxybenzoic, vanillic, adipic, succinic, malic, glycolic, and p-hydroxyphenylacetic acids, into the soil solution immediately after incorporation as green manure [10,13]. Allelochemicals released from crops could affect the germination, growth and yield of other crops or the same crop [14,15]. However, there are relatively few research reports on the effects of allelochemicals released from faba bean substances on maize growth.

Allelopathy refers to the phenomenon in which chemicals released by plants affect themselves or other populations [16]. Allelopathy plays an important role in agricultural production [16,17]. Allelochemicals mainly affect the seed germination, growth and yield of crops [14,15,18]. They mostly inhibit seed germination and the growth of crops [19]. Seed germination and seedling growth are the basis for ensuring crop yields [20]. However, there is limited research on the allelopathic effects of faba bean extract on maize seed germination and seedling growth.

In the present study, the activities of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and α-amylase, malondialdehyde (MDA), hormone and allelochemical content in maize seeds were determined, and transcriptome differences in maize seeds were analyzed to evaluate the allelopathic effects of faba bean leaf and stem extracts on maize seed germination. We investigated the effects of faba bean extracts on the agronomic traits, nutrient accumulation, photosynthesis and gene expression of maize seedlings to study the allelopathic effects of faba bean extracts on maize seedling growth. The results provide a basis to investigate the mechanism of the allelopathic effect on seed germination and seedling growth. The results are also important for the development of strategies to further improve faba bean–maize relay intercropping cultivation methods.

2. Material and Methods

2.1. Preparation of Faba Bean Stem and Leaf Extracts

Faba bean 'Tongcanxian 7' plants were provided by the Jiangsu Yanjiang Institute of Agricultural Sciences (Nantong, China). Faba bean plants planted on 30 October 2022 were selected during the harvest period on 18 May 2023. The plants were gently washed with tap water and then with deionised water, then air dried at approximately 25 °C for 24 h to dry the water used for cleaning plants, and divided into stems and leaves. The 100 g and 150 g fresh weight stems and leaves were cut into about 1 cm pieces, which were then soaked in 1 L sterile distilled water at 25 °C for 48 h [21,22]. The leaf extract and the stem extract were each filtered and stored at 4 °C until use.

2.2. Experiment 1: Experimental Design to Determine Allelopathic Effects of Faba Bean Extracts on Maize Seed Germination

The maize variety is 'Suyunuo 14', and the seeds were provided by the Jiangsu Yanjiang Institute of Agricultural Sciences (Nantong, China). Fifty maize seeds of similar size were placed in a germination box (19 cm length × 13 cm width × 12 cm height) lined with filter paper. Then, 50 mL of 100 g L^{-1} faba bean leaf extract (L1), 150 g L^{-1} faba bean leaf extract (L2), 100 g L^{-1} faba bean stem extract (S1), or 150 g L^{-1} faba bean stem extract (S2) was added. Sterile water was added in the control (CK) [23]. Each treatment contained 150 maize seeds and was replicated three times. The germination boxes were placed in an incubator at 20 °C for 7 d with 12 h of illumination (8000 lx) and 12 h of shading over 1 d. The maize seeds were sampled at 36 h to determine the activities of SOD, CAT, POD and α-amylase, the MDA content and allelochemical content.

2.2.1. Antioxidant Enzyme Activities and MDA Assay

The SOD, CAT and POD activities and MDA content in the maize seeds were measured by using assay kits (Keming, Suzhou, China) following the manufacturer's instructions [24,25].

2.2.2. α-Amylase Activity Assay

The activity of a-amylase was measured by using biochemical kits from Suzhou Comin Biotechnology Co., Ltd. (Suzhou, China; http://www.cominbio.com/, accessed on 20 June 2023). The samples were measured following the manufacturer's instructions [26].

2.2.3. Microstructure Observation of Maize Seeds

Maize seeds were sampled and sectioned into 2 mm thick slices using a clean razor blade. The slices were promptly immersed in 2.5% glutaraldehyde, prepared by diluting 25% glutaraldehyde in pH 7.2 phosphate buffer and fixed for 48 h at 4 °C. Following fixation, the samples were rinsed three times with phosphate buffer, each lasting 10 min. The samples were subsequently dehydrated in graded ethanol concentrations of 20%, 40%, 60%, 70%, 80%, 90%, 95%, and 100% and then infiltrated with propylene oxide and Spurr low-viscosity resin. Polymerization occurred in a 70 °C incubator for 12 h. The polymerized samples were cut into 1 μm semi-thin sections using an ultramicrotome (Leica Ultracut R Laika Company, Wetzlar, Germany) and a glass knife. The sections were stained with 0.5% methyl violet and examined under an optical microscope (DMLS, Leica, Wetzlar, Germany) equipped with a camera. Each sample was analyzed in triplicate.

2.2.4. Determination of Phytohormones Content in Maize Seeds

Maize seeds were collected and subjected to extraction using 1 mL of cold 50% acetonitrile (ACN) aqueous solution (vol/vol) at germination (36 h). The extraction process involved sonication at 4 °C for a duration of 3 min, followed by centrifugation at 15 rpm and 4 °C for 30 min. Subsequent to centrifugation for 10 min at 12,000 rpm and 4 °C, the supernatant was carefully transferred to a clean plastic microtube. All samples underwent purification and equilibration prior to analysis. The resultant extracts were analyzed with a UPLC–Orbitrap-MS system, comprising a Vanquish UPLC and a Q Exactive hybrid Q–Orbitrap mass spectrometer. The analytical method employed the following parameters: For UPLC, a Waters ACQUITY UPLC HSS T3 column (1.8 μm, 2.1 mm × 50 mm) was maintained at 40 °C with a flow rate of 0.3 mL/min and an injection volume of 2 μL. The solvent system consisted of water (0.1% acetic acid) and acetonitrile (0.1% acetic acid) with the gradient program set to 90:10 (v/v) at 0 min, sustained at 90:10 (v/v) until 1 min, transitioning to 10:90 (v/v) at 5 min, maintaining this ratio until 7 min, and reverting to 90:10 (v/v) by 7.1 min, which was held until 9 min. High-resolution mass spectrometry (HRMS) data were acquired using the Q Exactive mass spectrometer equipped with a heated electrospray ionization (ESI) source (Thermo Fisher Scientific, Waltham, MA, USA), following selected ion monitoring (SIM) acquisition methods. The parameters for the ESI source were optimized as follows: spray voltage at 3.0 kV, sheath gas pressure at 40 arb, auxiliary gas pressure at 10 arb, sweep gas pressure at 0 arb, capillary temperature at 320 °C, and auxiliary gas heater temperature at 350 °C [27].

2.2.5. Detection of Allelochemicals in Faba Bean Stem Extracts and Maize Seeds

Samples were treated with 2 mL of 4 M aqueous NaOH. The mixed solution was hydrolyzed at 40 °C for 2 h in a gas bath with shaking and protection from light. The pH value was adjusted to 2 by adding 4 M aqueous HCl. The mixture was shaken with 2 mL of n-hexane at room temperature for 20 min to remove the n-hexane layer. Ethyl acetate (2 × 2 mL) was used to extract the aqueous layer, and the mixed extracts were concentrated to nearly dry on a rotary evaporator at 35 °C under reduced pressure. Before analysis, the residue was dissolved in 200 μL of 50% methanol/water and transferred to insert-equipped vials. The sample extracts were subjected to analysis using a UPLC–Orbitrap mass spectrometry system (Thermo Fisher Scientific, Waltham, MA, USA), specifically a Vanquish UPLC coupled with a Q Exactive mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). The analytical parameters for the UPLC were as follows: A Waters ACQUITY UPLC HSS T3 column (1.8 μm, 2.1 mm × 50 mm) was maintained at a temperature of 40 °C, with a flow rate of 0.3 mL/min and an injection volume of 2 μL. The solvent system

consisted of water with 0.1% acetic acid and acetonitrile with 0.1% acetic acid, employing a gradient program that started at 90:10 (v/v) at 0 min, remained at 90:10 (v/v) until 2.0 min, transitioned to 40:60 (v/v) at 6.0 min, maintained this ratio until 8.0 min, and then returned to 90:10 (v/v) at 8.1 min, sustaining this composition until 12.0 min. High-resolution mass spectrometry (HRMS) data were collected using the Q Exactive hybrid Q–Orbitrap mass spectrometer equipped with a heated electrospray ionization (ESI) source (Thermo Fisher Scientific), utilizing full MS acquisition methods. The ESI source parameters were configured as follows: spray voltage set to −2.8 kV, sheath gas pressure at 40 arb, auxiliary gas pressure at 10 arb, sweep gas pressure at 0 arb, capillary temperature at 320 °C, and auxiliary gas heater temperature at 350 °C. Data acquisition was performed on the Q Exactive using Xcalibur version 4.1 (Thermo Scientific, Waltham, MA, USA) and subsequently processed with TraceFinder™ version 4.1 Clinical (Thermo Scientific, Waltham, MA, USA). The experiment was quantified using the external standard method, and fitting curves were prepared using standard samples of different concentrations. The standard products mainly come from Sigma company (Sigma Chemical Co., St. Louis, MO, USA). The quantified results were exported in Excel format [28].

2.3. Experiment 2: Experimental Design to Determine Allelopathic Effects of Faba Bean Extracts on Maize Seedling Growth

The allelopathic effect of faba bean extract on maize seedling growth was studied by using sand cultivation experiment. First, 20 germinating maize seeds were sown in each box (19 cm length × 13 cm width × 12 cm height) placed with 300 g sand passed through a 50–80 mesh. Then, 50 mL of 100 g L^{-1} faba bean leaf extract (L1), 150 g L^{-1} faba bean leaf extract (L2), 100 g L^{-1} faba bean stem extract (S1), or 150 g L^{-1} faba bean stem extract (S2) with sterile water (CK) was added. When maize seedlings grew for 21 days, they were collected to measure the SPAD value, plant height and fresh weight. Photosynthetic traits and nutrient contents of maize seedlings were determined at growth 21 d (Figure 1).

Figure 1. Maize seedlings in control (**CK**) and (**L2**) treatment (150 g L^{-1} faba bean leaf extract) at 21 d.

2.3.1. Determination of Nutrient Contents in Plants and Faba Bean Extracts

At the 21-day mark of their development, maize seedlings were collected from the incubation chambers. The collected specimens underwent an initial drying phase at a temperature of 105 degrees Celsius for a duration of 30 min, followed by a secondary drying process to achieve a stable weight at 80 degrees Celsius. Once the samples reached a constant weight, they were pulverized into a fine powder to facilitate the quantification of nitrogen, phosphorus and potassium levels. The nitrogen concentration in the maize seedlings was ascertained through the micro-Kjeldahl digestion technique; the phosphorus levels were determined by colorimetric analysis employing the vanado-molybdate procedure; and the potassium levels were assessed via flame photometry [29]. In the case of faba bean extracts, the nitrogen content was quantified using the potassium persulfate oxidation method coupled with ultraviolet spectrophotometry. The phosphorus content within the faba bean extracts was evaluated by the potassium persulfate oxidation-molybdenum blue colorimetric assay. Lastly, the potassium content in the faba bean extracts was analyzed using inductively coupled plasma mass spectrometry (ICP-MS).

2.3.2. Determination of Photosynthetic Traits

At the 21-day growth stage, a suite of photosynthetic parameters was quantified employing a Li-6800 portable photosynthesis system, a device manufactured by LI-COR Inc., Lincoln, NE, USA. The parameters assessed included the rate of photosynthesis (Pn), the rate of transpiration (Tr), stomatal conductance (Gs), and the concentration of CO_2 within the intercellular spaces (Ci) [6]. The leaf chamber uses a red–blue light source (1 cm × 3 cm). The light intensity is set to 1500 μmol·m^{-2}·s^{-1}. The CO_2 concentration is set to 400 μmol·mol^{-1}. Photosynthetic parameters were measured after 21 d seedling growth.

2.3.3. RNA Extraction and Sequencing

Maize seeds undergoing germination from the L2 treatment group and the control group (CK) were collected at 36 h post-application of the extract. After a growth period of 21 days, the maize samples from both the L2 treatment and the control group were harvested. Total RNA was isolated from these samples using the RNAprep Pure Plant Kit (catalog number DP432, Tiangen, Beijing, China). RNA purification, reverse transcription, library construction and sequencing were performed at Shanghai Majorbio Bio-pharm Biotechnology Co., Ltd. (Shanghai, China) according to the manufacturer's instructions (Illumina, San Diego, CA, USA)

2.3.4. Transcriptome Data Analysis

Quality control procedures were implemented to evaluate the suitability of raw sequencing reads for further analytical processes. The transcript abundance for each unique gene was quantified employing the fragments per kilobase of transcript per million mapped reads (FPKM) approach. Genes were classified as differentially expressed when comparing the L2 and CK samples based on stringent criteria: a fold change of at least 2.0 and a p-value less than 0.05. To elucidate the molecular roles, cellular locations, biological processes and metabolic pathways associated with the differentially expressed genes (DEGs), a significant enrichment analysis of Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways was conducted. The GO and KEGG enrichment analyses were conducted using the Goatool (https://geneontology.org/, accessed on 12 September 2023) [30]. In total, 45,634,375 and 45,922,781 raw reads were obtained from the CK and S2 maize seed treatments at germination (36 h), respectively. From the CK and S2 treatment raw reads, 44,886,758 and 45,157,799 clean reads, respectively, were retained for further analysis, of which 86.38% and 87.63%, respectively, were mapped. At maize seedling growth for 21 d, 61,267,373 and 59,404,195 raw reads were obtained from CK and L2, respectively. In total, 60,248,475 and 58,299,177 clean reads were obtained for CK and L2, respectively, and 89.37% and 88.03% of the clean reads were successfully mapped to the reference genome.

2.4. Statistical Analysis

The experiments were conducted in a completely randomized design. Each treatment has 3 replicates, and each replicate is sampled for measurement. All data are presented as means. SPSS version 19.0 software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis including Fisher's test.

3. Results

3.1. Effects of Faba Bean Extracts on the Allelochemicals and Phytohormones Content in Maize Seeds

Allelochemicals, such as 4-hydroxybenzoic acid, trans-ferulic acid, hydrocinnamic acid, benzoic acid, vanillin, gallic acid, trans-cinnamic acid, vanillic acid, protocatechualdehyde, salicylic acid, sinapic acid, caffeic acid, 3,4-dihydroxybenzoic acid, and syringic acid, were detected in faba bean stem extracts (Table 1). Among them, the content of hydrocinnamic acid, benzoic acid,4-hydroxybenzoic acid, trans-cinnamic acid, protocatechualdehyde, salicylic acid, 3,4-dihydroxybenzoic acid in maize seeds treated by faba bean stem extracts was higher than these in CK. Faba bean stem extracts increased the abscisic acid, salicylic acid and indole-3-acetic acid content in maize seeds at germination (36 h) (Figure 2A,E,I) but decreased aminocyclopropane carboxylic acid in maize seeds at germination (36 h) (Figure 2B).

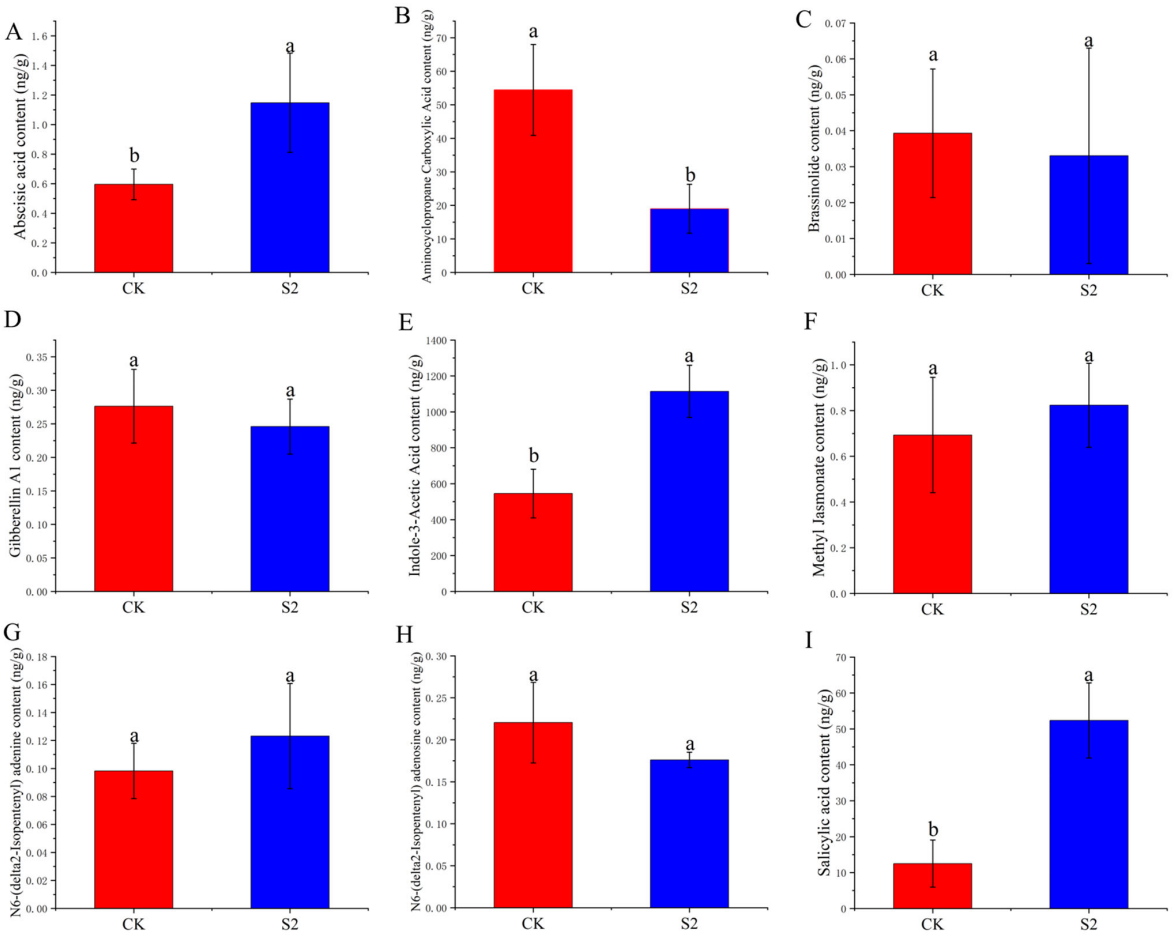

Figure 2. The phytohormones content in maize seeds treated by faba bean extracts. (**A**) Abscisic acid, (**B**) aminocyclopropane carboxylic acid, (**C**) brassinolide, (**D**) gibberellin A1, (**E**) indole-3-acetic acid, (**F**) methyl jasmonate, (**G**) N6-(delta2-Isopentenyl) adenine, (**H**) N6-(delta2-Isopentenyl) adenosine, (**I**) salicylic acid. S2 maize seeds treated by 150 g L^{-1} of faba bean stem extracts; CK, maize seeds treated by sterile water. Lowercase letters above the bar indicate significant differences ($p < 0.05$).

Table 1. Allelochemicals in faba bean stem extracts and maize seeds.

Allelochemicals	Faba Bean Stem Extracts	Maize Seeds	
		CK (ng/g)	S2 (ng/g)
Trans-Ferulic acid	+	277,268.10 a	268,762.80 a
4-Hydroxybenzoic acid	+	1023.00 b	1237.30 a
Hydrocinnamic acid	+	3.60 b	25.80 a
Trans-Cinnamic acid	+	193.3 b	263.00 a
Vanillic acid	+	2302.10 a	2375.90 a
Vanillin	+	2485.70 a	2292.70 a
Gallic acid	+	29.20 a	31.00 a
Benzoic acid	+	1163.30 b	1393.70 a
Protocatechualdehyde	+	32.40 b	67.50 a
3,4-Dihydroxybenzoic acid	+	264.50 b	358.20 a
Caffeic acid	+	2406.40 a	2054.90 a
Syringic acid	+	6228.80 a	6730.10 a
Sinapic Acid	+	33,547.80 a	34,293.70 a
Salicylic acid	+	247.60 b	396.20 a

S2 maize seeds treated by 150 g L^{-1} of faba bean stem extracts; CK, maize seeds treated by sterile water. Data were shown as means (n = 3), and different lowercase letters in the same column indicate significant difference ($p < 0.05$). Detected (+).

3.2. Effects of Faba Bean Extracts on the Germination Rate and α-Amylase Activity, SOD, CAT, POD, and MDA in Maize Seeds

Compared with CK, faba bean extracts decreased the germination rate by 10.24–1.81% (Figure 3A). There was no significant difference during L1, L2, S1, and S2. Faba bean extracts increased the SOD activity and POD activity in maize seeds during germination for 36 h (Figure 3B,C). Faba bean extracts also increased the CAT activity in maize seeds during germination at 36 h (Figure 3E) and increased the MDA content in maize seeds treated at germination (36 h) (Figure 3F). However, faba bean extracts decreased the α-amylase activity in maize seeds during germination for 36 h (Figure 3D).

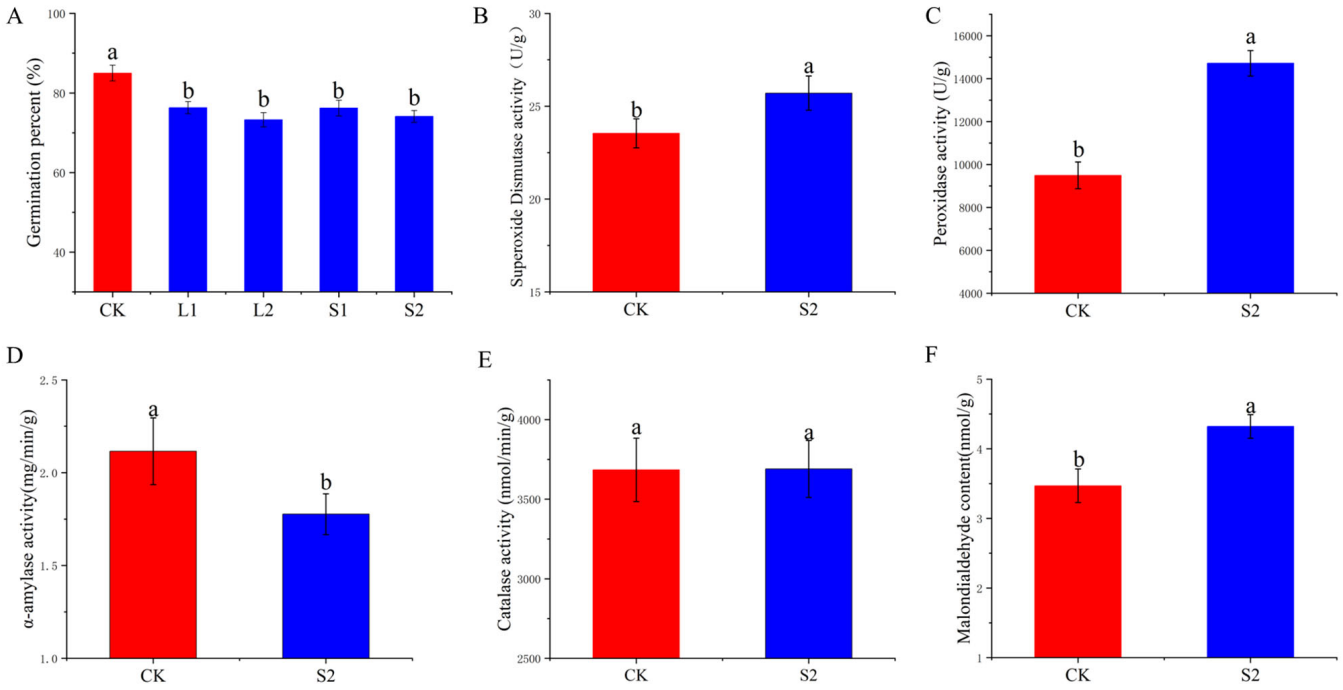

Figure 3. The germination rate, α-amylase activity, SOD, CAT, POD activity and MDA content in maize seeds treated by faba bean extracts. (**A**) Germination rate of maize seeds treated by faba bean

extracts, (**B**) SOD activity, (**C**) CAT activity, (**D**) α-amylase, (**E**) CAT activity, (**F**) MDA content. L1, maize seeds treated by 100 g L^{-1} faba bean leaf extracts; L2, maize seeds treated by 150 g L^{-1} faba bean leaf extracts; S1, maize seeds treated by 100 g L^{-1} faba bean stem extracts; S2 maize seeds treated by 150 g L^{-1} faba bean stem extracts; CK, maize seeds treated by sterile water. Lowercase letters above the bar indicate significant differences ($p < 0.05$).

3.3. Effects of Faba Bean Extracts on Microstructure of Maize Seeds

Figure 4A,B show the morphology of maize seed germination at 36 h. The CK had faster germination of caryopsis and larger volume compared with L2 (Figure 4A,B). Figure 4C–F show the amyloplast degradation. The amyloplasts were degraded in the endosperm cells, and the endosperm structure was relatively loose at 36 h after germination (Figure 4C,E). The degradation degree of amyloplasts in the maize endosperm treated with 150 g L^{-1} faba bean stem extracts was lower than those treated with water (Figure 4D,E).

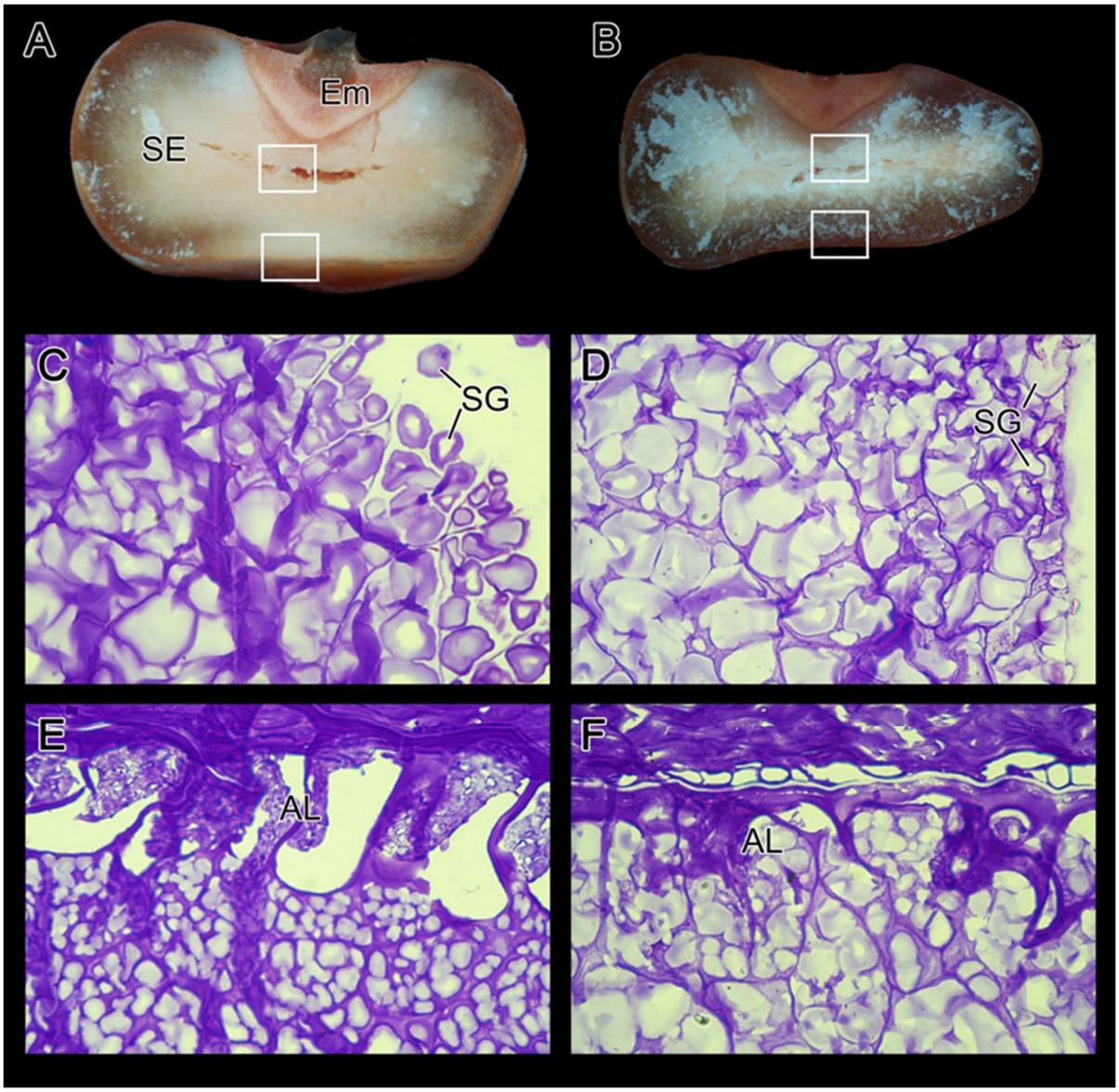

Figure 4. Maize seed structure and amylolytic degradation in maize endosperm cells. (**A**,**C**,**E**) CK, (**B**,**D**,**F**) maize seeds treated by faba bean stem extracts (S2); AL, aleurone layer; Em, embryo; SG, starch granules; SE, starch endosperm; (**C**,**D**) structure in the central position of the endosperm; (**E**,**F**) structure in the surface powder layer.

3.4. Effects of Faba Bean Extracts on Photosynthesis, SPAD Value, Plant Height, and Fresh Weight of Maize Seedlings

The SPAD value, plant height, and fresh weight of maize seedlings are presented in Table 2. When the maize seedlings grew for 21 d, the treatments except L2 had no significant difference, and the SPAD value of L2 was 7.89% higher than CK at 21 d. The plant height of L1, L2 and S2 was higher than CK when maize seedlings grew for 21 d. The fresh weight of maize seedlings treated with faba bean extracts was higher than CK when maize seedlings grew for 21 d. The fresh weight of L2 was 8.53% higher than that of L1 when maize seedlings grew for 21 d. The fresh weight of L2 was higher than S2 when maize seedlings grew for 21 d.

Table 2. The impact of faba bean extracts treatments on photosynthetic parameters and seedling growth of maize.

Treatment	Tr mmol m^{-2} s^{-1}	Pn μmol m^{-2} s^{-1}	Ci μmol mol^{-1}	Gs mmol m^{-2} s^{-1}	SPAD	Plant Height (cm)	Fresh Weight (g)
L1	1.19 a	9.74 ab	110.96 b	55.32 b	29.76 b	19.33 a	1.29 b
L2	1.23 a	10.00 a	133.99 a	66.13 a	31.16 a	19.44 a	1.4 a
S1	1.00 b	9.23 b	112.41 b	54.99 b	28.89 b	17.65 b	1.24 b
S2	1.21 a	9.35 b	127.37 a	55.93 b	29.98 b	18.78 a	1.26 b
CK	0.89 c	6.28 c	106.65 b	42.02 c	28.88 b	17.44 b	1.13 c

L1, maize seedlings treated by 100 g L^{-1} faba bean leaf extracts; L2, maize seedlings treated by 150 g L^{-1} faba bean leaf extracts; S1, maize seedlings treated by 100 g L^{-1} faba bean stem extracts; S2 maize seedlings treated by 150 g L^{-1} faba bean stem extracts; CK, maize seeds treated by sterile water. Data were shown as means (n = 3), and different lowercase letters in the same column indicate significant difference ($p < 0.05$).

3.5. Effects of Faba Bean Extracts on Nutrient Absorption of Maize Seedlings

The nitrogen, potassium and phosphorus were detected in faba bean extracts (Figure 5D,E,F). The nitrogen and phosphorus contents in faba bean leaf extracts were higher than those in stem extracts. The nitrogen, phosphorus and potassium contents in maize seedlings treated with faba bean extracts are shown in Figure 5. The nitrogen and phosphorus contents were significantly higher in L2 than in the other treatments, significantly higher in L2 than in L1. The nitrogen, potassium and phosphorus contents of CK were lower than in the other treatments (Figure 5D,E,F).

3.6. Transcriptome Comparison Results

The Venn diagrams presented in Figure 6A,B represent the sample-specific and gene overlap among CK, S2, and L2. At germination after 36 h, 587 and 724 specific genes were expressed in CK and S2, respectively, and a total of 23,153 overlap genes (i.e., genes expressed in both CK and S2) were expressed at this stage. At growth after 21 d, 1869 and 693 specific genes were expressed in CK and L2, respectively, and the number of overlap genes was 20,440.

To verify the effect of faba bean extracts on the antioxidant enzyme activity and α-amylase activity in maize seeds, several related genes were selected, as listed in Table 3. Several genes with Gene id from Zm00001d038652 to Zm00001d028815 in Table 3 were related to antioxidant enzyme activity. As seen in the heat map of DEGs in Figure 6C, most of the genes related to antioxidant enzymes (Zm00001d028815, Zm00001d013629, Zm00001d029279, Zm00001d047830, Zm00001d038652) were upregulated. The genes with Gene ids of Zm00001d039394 and Zm00001d043411 related to gibberellin were downregulated (Figure 6C). To confirm the effect of faba bean extracts on the photosynthesis and nutrient absorption of maize seedlings, several genes were selected, as listed in Table 3. Several genes with Gene ids from ZemaCp052 to Zm00001d046928 in Table 3 were related to photosynthesis and were upregulated (Figure 6D). The gene with a Gene id of Zm00001d040125 related to phosphorus absorb was upregulated (Figure 6D). The genes with Gene ids Zm00001d038412, Zm00001d034782 and Zm00001d025831 related to nitrogen

absorb were upregulated (Figure 6D). The gene with a Gene id of Zm00001d033068 related to potassium absorption was upregulated (Figure 6D).

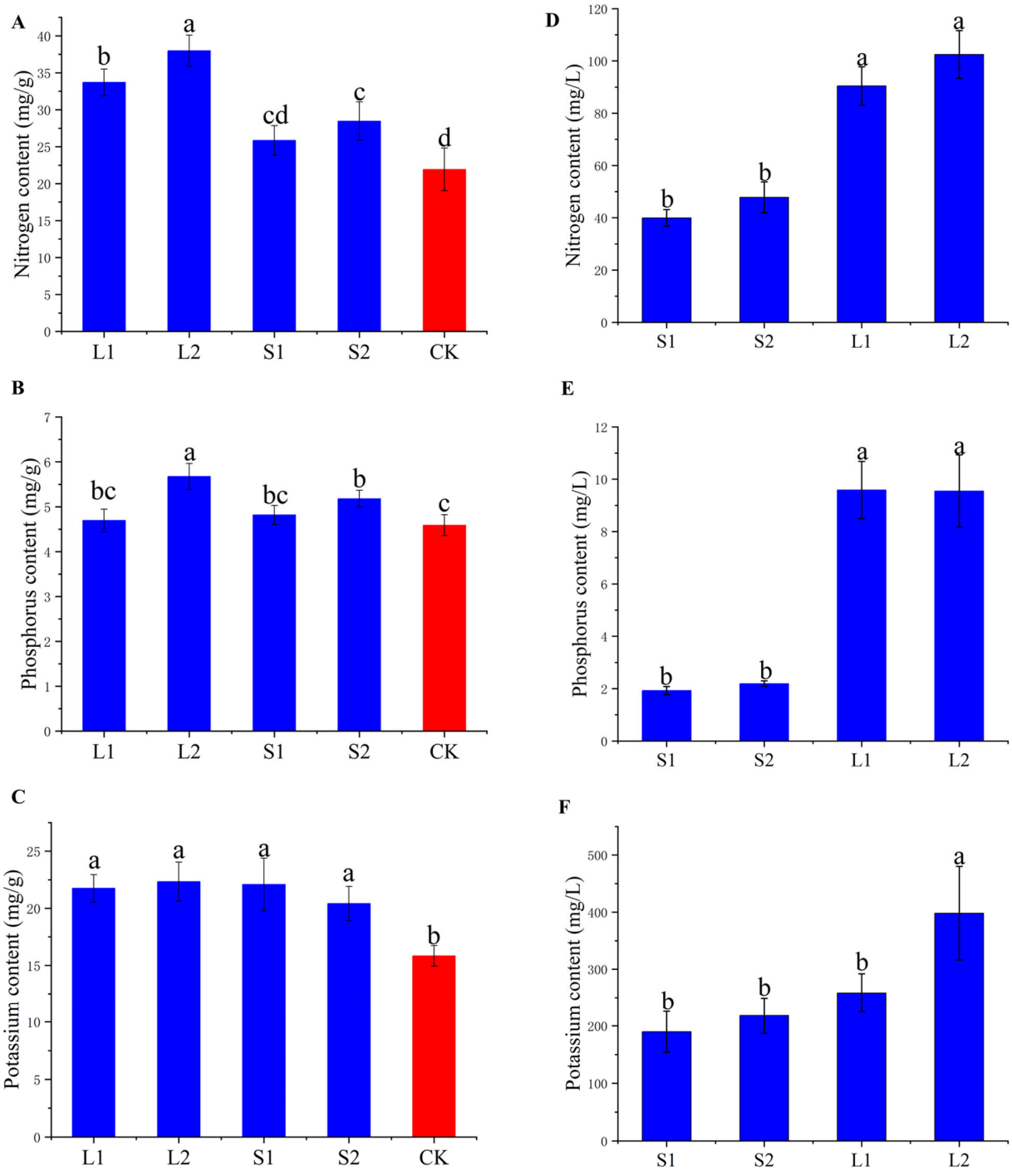

Figure 5. Nitrogen, phosphorus and potassium content in maize seedling and faba bean extracts. (**A**) Nitrogen content in maize seedling, (**B**) phosphorus content in maize seedling, (**C**) potassium content in maize seedling. (**D**) Nitrogen content in faba bean extracts, (**E**) pohosphorus content in faba bean extracts, (**F**) potassium content in faba bean extracts. L1, maize seedlings treated by 100 g L^{-1} faba bean leaf extracts; L2, maize seedlings treated by 150 g L^{-1} faba bean leaf extracts; S1, maize seedlings treated by 100 g L^{-1} faba bean stem extracts; S2 maize seedlings treated by 150 g L^{-1} faba bean stem extracts; CK, maize seeds treated by sterile water. Lowercase letters above the bar indicate significant differences ($p < 0.05$).

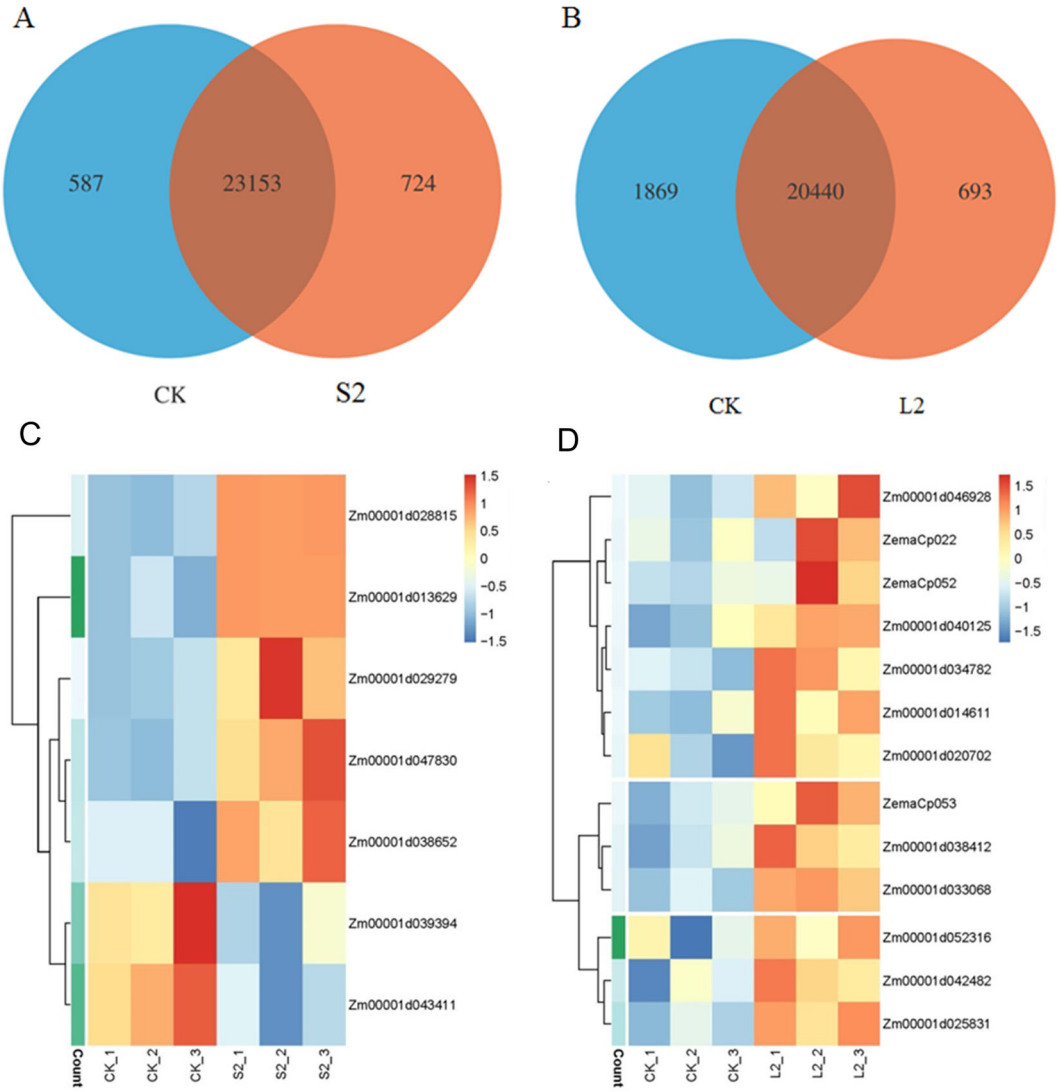

Figure 6. Venn diagrams, heat map of genes and GO enrichment analysis of differentially expressed genes. (**A**) Venn diagrams of CK and L2 at germination 36 h, (**B**) Venn diagrams of CK and L2 at growth 21 d. Numbers in a single-shaded region indicate sample-specific genes, while those in a double-shaded region show the overlap genes. (**C**) Heat map of genes related to antioxidant enzymes, gibberellin in CK and S2, (**D**) heat map of genes photosynthesis and nutrient absorption in CK and L2.

Table 3. Some related genes enriched in the KEGG pathway.

Treatment	Gene_id	Gene Description
S2/CK	Zm00001d038652	Thioredoxin H4
	Zm00001d013629	cytochrome P450 family 87 subfamily A polypeptide 2
	Zm00001d047830	kaurenoic acid oxidase2
	Zm00001d038652	Thioredoxin H4
	Zm00001d029279	Peroxidase 64
	Zm00001d028815	Pathogenesis-related protein 10
	Zm00001d039394	gibberellin 2-oxidase13
	Zm00001d043411	gibberellin 2-oxidase3

Table 3. *Cont.*

Treatment	Gene_id	Gene Description
L2/CK	ZemaCp052	petB
	ZemaCp053	petD
	Zm00001d052316	Ferritin-1%2C chloroplastic
	ZemaCp022	psaB
	Zm00001d042482	bHLH-transcription factor 132
	Zm00001d046928	histidine kinase5
	Zm00001d014611	Phosphate import ATP-binding protein pstB 1
	Zm00001d020702	Mitochondrial phosphate carrier protein 2 mitochondrial
	Zm00001d040125	phosphate transporter 1
	Zm00001d038412	Ammonium transporter 2
	Zm00001d034782	Ammonium transporter 2
	Zm00001d025831	ammonium transporter 1
	Zm00001d033068	Potassium transporter 5

S2 maize seeds treated by 150 g L^{-1} faba bean stem extracts; L2, maize seedlings treated by 150 g L^{-1} faba bean leaf extracts; Gene description was annotated from the KOG (https://ftp.ncbi.nih.gov/pub/COG/, accessed on 12 September 2023) database.

4. Discussion

4.1. Allelopathic Effects of Faba Bean Extracts on Maize Seed Germination

Benzoic, p-hydroxybenzoic, vanillic, adipic, succinic, malic, glycolic, and p-hydroxyphenylacetic acids were detected in the root exudates of faba beans [10]. Various common allelochemicals were also detected in this study (Table 1). However, there are more allelochemicals in the faba bean extract than these, and the types and contents of allelochemicals in faba bean extract still need to be further studied for widespread application in agricultural production worldwide.

Allelochemicals' most pronounced effects are the inhibition of seed germination and growth of neighboring plants [19]. In the present study, faba bean stem extracts increased the content of allelochemicals, including hydrocinnamic acid, 4-hydroxybenzoic acid, protocatechualdehyde,trans-cinnamic acid, benzoic acid, 3,4-dihydroxybenzoic acid and salicylic acid in maize seeds. The 4-hydroxybenzoic, hydrocinnamic acid, trans-cinnamic acid, protocatechualdehyde could inhibit seed germination [31–33]. The allelochemicals released from faba bean extracts enter the interior of the seed and inhibit maize seed germination.

Reactive oxygen species (ROS) is an important signal molecule that plays a critical role in seed germination [34]. Excessive ROS accumulates in the cell. This condition affects the cellular structure and inhibits seed germination [35,36]. The GO enrichment analysis results showed that genes related to the ROS metabolic process (Zm00001d029279) were upregulated (Table 3, Figure 6C). Genes related to the response to toxic substances were expressed differently. This showed that faba bean extracts are poisonous to maize seed germination. The faba bean extracts increased the SOD, POD and CAT activity levels upon maize seed germination at 36 h. Maize seeds increased the antioxidant enzyme activity in response to the effects of allelochemicals on seed germination.

During seed germination, α-amylase decomposes starch into small-molecule sugars such as dextrin and maltose, which can be absorbed and utilized by cells to provide energy and nutrients for seed germination [37,38]. In this study, faba bean extracts decreased the α-amylase activity in maize seeds during germination for 12 h to 48 h (Figure 2D). The degradation degree of amyloplasts in the maize endosperm treated with 150 g L^{-1} faba bean stem extracts was lower than that in maize endosperms treated with water (Figure 4). Allelochemicals released from faba bean extracts could inhibit amylase activity and reduce the conversion and utilization efficiency of starch in seeds, thereby inhibiting plant seed germination.

Phytohormones regulate seed germination [39]. In this study, high-concentration (150 g L^{-1}) faba bean stem extracts increased the abscisic acid (ABA), salicylic acid and indole-3-acetic acid content and decreased aminocyclopropane carboxylic acid in maize

seeds at germination (36 h). Abscisic acid (ABA) negatively regulates seed germination [40,41]. Indole-3-acetic acid, salicylic acid, and aminocyclopropane carboxylic acid could promote seed germination [42–44]. The results of this experiment showed an inhibitory effect, which may be due to the stronger effect of abscisic acid than salicylic acid, indole-3-acetic acid in this experiment. ABA levels have been observed as a response to exposure to environmental stresses [45]. In this study, the abscisic acid content in maize seeds was significantly increased by high-concentration faba bean stem extracts. This also indirectly shows that the faba bean extract has an inhibitory effect on seed germination. The myrigalone interferes with gibberellin metabolism and affects the ratio of GA to ABA, thereby inhibiting *Lepidium sativum* seed germination [46]. The high-concentration faba bean stem extracts increased the concentration of abscisic acid and had no significant effect on the concentration of gibberellins. This showed that high-concentration faba bean stem extracts also affect the balance between ABA and GA. The GO enrichment analysis results showed that genes related to the gibberellin biosynthetic process, amylase activity, regulation of hormone levels, hormone metabolic process and response to stimulus were expressed differently (Figure 6B). The allelochemicals released from faba bean extracts have a toxic effect on seeds and affect gibberellin synthesis, thereby inhibiting seed germination. The allelochemicals released from faba bean extracts into seeds affect gene expression related to seed germination, thus affecting ABA synthesis and reducing α-amylase activity, thereby inhibiting seed germination (Figure 7).

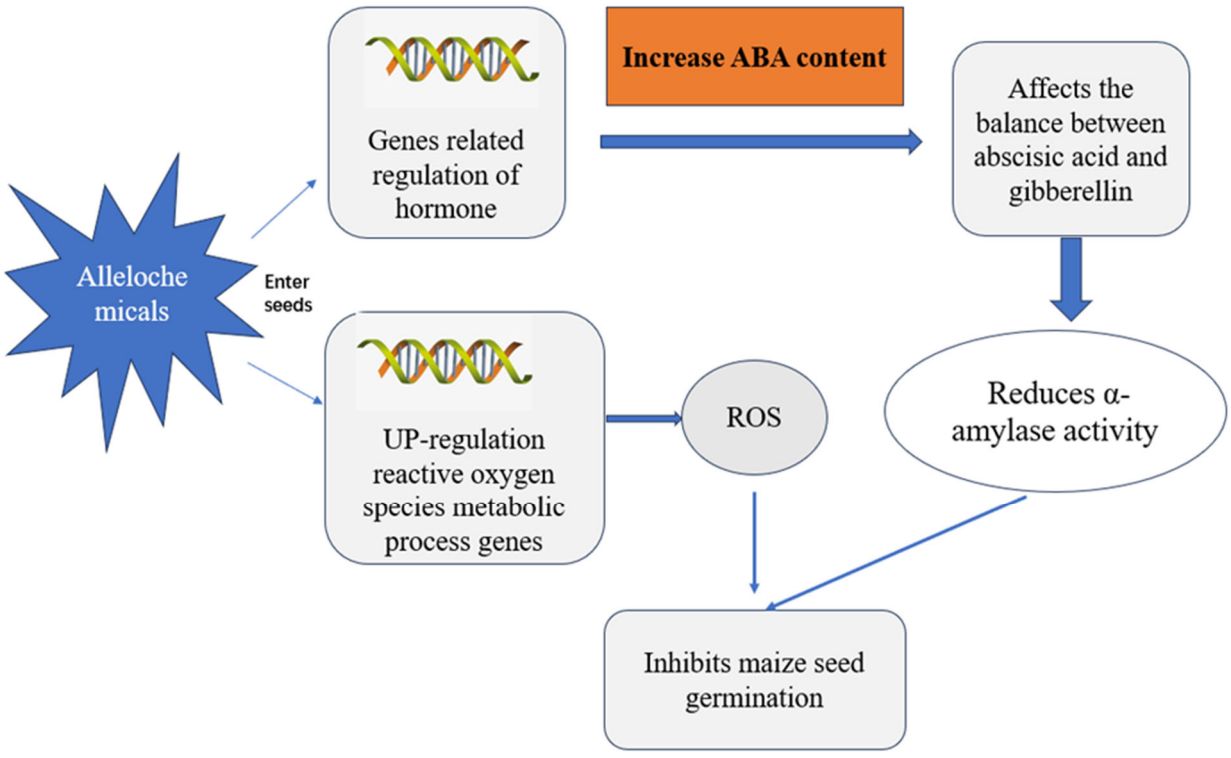

Figure 7. Proposed model of faba bean extracts inhibited germination of maize seeds.

4.2. Allelopathic Effects of Faba Bean Extracts on Maize Seedling Growth

Many allelochemicals affect nutrient absorption in plants [47]. Allelochemicals can inhibit the activities of ATPase involved in the absorption and transport of ions at the cell plasma membrane, which suppresses the cellular absorption of ions [48]. However, the faba bean extracts increased nitrogen, phosphorus and potassium contents in maize seedlings in this study. The nitrogen, potassium and phosphorus were detected in faba bean extracts. The main reason for improving the absorption of nutrient elements in maize seedlings may be the release of nutrients from faba bean extracts. Moreover, the released nutrients

have a greater promoting effect on the absorption of nutrients by maize seedlings than the inhibitory effect of allelochemical release from faba bean extracts. Several genes related to ammonium, phosphate and potassium transporters were upregulated (Figure 6D). The faba bean extracts may release nitrogen, phosphorus and potassium nutrients, which promotes the absorption of the nutrient elements of maize seedlings and improves the growth and development of the seedlings.

Most studies have shown that allelochemicals have an inhibitory effect on photosynthesis [47]. However, faba bean extracts promoted the photosynthesis of maize seedlings in this study (Table 3). The faba bean extracts increased nitrogen, phosphorus and potassium contents in maize seedlings. A best-fit positive linear relationship existed between the leaf chlorophyll and leaf nitrogen content [49]. The nutrients released from faba bean extracts may promote the development of chloroplasts, thereby enhancing the photosynthesis of maize and promoting the growth and development of maize seedlings. Several photosynthesis-related genes were also upregulated (Figure 6D).

This study found that faba bean extracts could promote the growth of maize seedlings. The fresh weight of maize seedlings treated with faba bean extracts was higher than those of CK at seedling growth after 14 and 21 d (Table 2). In the present study, faba bean extracts inhibited maize seed germination but promoted maize seedling growth. This condition may be because the seeds consume the stored nutrients first during the germination period and the endosperm provides the germination energy. The seedlings mainly absorb the nutrients needed for growth from the outside through the root system and accumulate substances through photosynthesis. The nitrogen, potassium and phosphorus were detected in faba bean extracts. Faba bean extracts released nutrients to promote maize seedling growth.

In this study, we detected common allelochemicals in faba bean extracts (Table 1). However, the number of allelochemicals in faba bean extracts is higher than these compounds. The isolation and identification of allelochemicals in faba bean stalks are yet to be studied. Although the faba bean extract inhibited the germination of maize seeds, the extract stimulated the growth and development of maize seedlings. Faba bean–maize relay strip intercropping is a common cropping system in China, where the growing season is too short for double cropping. Faba beans can be used as green manure to reduce the use of chemical fertilizers. In production, increasing the sowing amount could help in coping with the inhibitory allelopathic effect of faba beans on maize seed germination, improving the utilization of faba bean green manure to alleviate the inhibitory effect on seed germination

5. Conclusion

Faba bean extracts release allelochemicals, such as 4-hydroxybenzoic acid, trans-ferulic acid, hydrocinnamic acid, gallic acid, vanillic acid, trans-cinnamic acid, vanillin, benzoic acid, protocatechualdehyde, caffeic acid, 3,4-dihydroxybenzoic acid, salicylic acid, syringic acid and sinapic acid. Allelochemicals entered the interior of maize seeds and increased the abscisic acid content and antioxidant enzyme activity and decreased the α-amylase activity in maize seeds, ultimately leading to an inhibitory effect on maize seed germination.

Faba bean extracts release nutrients and promote nitrogen, phosphorus and potassium absorption of maize seedlings, while promoting photosynthesis and the fresh weight of maize seedlings. The research results provide a basis for improving faba bean–maize relay strip intercropping.

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