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The effect of exogenous gibberellin and its synthesis inhibitor treatments for morphological and physiological characteristics of Tartary buckwheat

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Gibberellin (GA_3) is an important plant hormone involved in many physiological and developmental processes in plants. However, the physiological mechanism of GA_3 on the regulation yield and grain shell thickness of Tartary buckwheat is still unclear. In this study, the thick-shelled cultivar "Jinqiao 2" and thin-shelled cultivar "Miku 18" were used to study the effects of different concentrations (0, 50, and 100 mg L^{-1}) of exogenous GA_3 and chlorocholine chloride (CCC, GA_3 synthesis inhibitor) on the cellulose content, amylase, and sucrose synthase (SS) activity in grain shell and the yield of Tartary buckwheat. The application of exogenous GA_3 can improve the cellulose content and the activity of amylase and SS in the grain shell of the two Tartary buckwheat varieties. It can also increase the main stem node number, main stem branch number, grains per plant, and yield. Compared with the control treatment (CK, 0 mg L^{-1}), the 100 mg/L exogenous GA_3 treatment increased the number of grains per plant, grain weight per plant, 1000-grain weight, and yield of Jinqiao 2 by 20.1%, 41.9%, 13%, and 34.7%, respectively. These items of Miku 18 were increased by 26%, 15.2%, 10.2%, and 23.8%. The application of CCC reduced the activity of amylase and SS and cellulose content in grain shell. In addition, it decreased the main stem node number, main stem branch number, grains per plant, and yield of Tartary buckwheat. In summary, exogenous GA_3 treatment not only improved the yield of Tartary buckwheat but also increased the thickness of grain shell by enhancing the activity of amylase and SS and promoting the synthesis and accumulation of cellulose. The results can provide theoretical references for clarifying the physiological mechanism of the difference in shell thickness between Tartary buckwheat varieties.

Keywords Tartary buckwheat, Gibberellin, Cellulose content, Grain shell, Yield

Tartary buckwheat (*Fagopyrum tataricum* Gaertn) is an important cereal crop in China[1,2]. It is rich in protein and flavonoids and has outstanding nutritional and health value[3,4]. According to the thickness of grain shell, Tartary buckwheat can be divided into two types: thick- and thin-shelled Tartary buckwheat. The shell rate of thick-shelled Tartary buckwheat is more than 20%, and the shell is thick and tough without cracking. The shell rate of thin-shelled Tartary buckwheat is less than 20%, and the shell is thin and easy to crack[5]. The yield of thick-shelled Tartary buckwheat is high, but its shelling process is challenging, which seriously affects the processing quality. Thin-shelled Tartary buckwheat has an easy shelling process, but its yield is low[6]. At present, the research on Tartary buckwheat shells mainly focuses on the separation of shells and the improvement in shell characteristics[7]. Few reports are available on the reasons for the difference in shell thickness between Tartary buckwheat varieties. The main components of buckwheat shell are lignin and cellulose, with contents of 34.8% and 36.5%, respectively[8]. The study of Wu[9] further showed that the difference in shell composition between thick- and thin-shelled Tartary buckwheat was mainly due to the difference in cellulose content[9]. Therefore, the level of cellulose content is an important reason for the difference in shell thickness between Tartary buckwheat varieties[10].

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Sucrose synthase (SS) is a glycosyltransferase. Its main function is to provide substrate uridine diphosphate glucose (UDPG) and indirect substrate adenosine diphosphate glucose for the biosynthesis of polysaccharides such as cellulose, callose, and starch. It plays a key regulatory role in the distribution of carbon sources in plants[11,12]. Sucrose phosphate synthase (SPS) is a glycosyltransferase that regulates the carbon partitioning between cellulose and sucrose and starch synthesis in plants[13]. Studies have shown that SS and SPS play an important role in UDPG supply to cellulose biosynthesis[14]. Notably, α-amylase and β-amylase are important enzymes involved in the storage of starch degradation, and they can affect the activity of SS by degrading starch[15]. Meanwhile, the activity of SS affects the formation of cellulose in the shell[16]. Therefore, the activity of α-amylase and β-amylase is closely related to the fiber content in the shell.

Gibberellin (GA) is an important plant hormone involved in many physiological and developmental processes in plants, including cell elongation, photomorphogenesis, flowering, and seed development[17]. GAs have many types, with GA_3 being the most widely used. Studies have shown that GA_3 can affect the carbohydrate metabolism of higher plants[18]. GA_3 promotes plant growth and yield by inducing the production of α-amylase, protease, and other hydrolytic enzymes to decompose the storage in the grain[19]. Zhao[20] showed that exogenous GA_3 treatment could increase the shell thickness of walnut. Lu[21] found that spraying appropriate concentration of exogenous GA_3 on walnut can increase the thickness of walnut shell and the mechanical strength of shell by increasing the cellulose content in walnut peel, which can affect the formation of walnut shell. Therefore, we hypothesized that exogenous GA_3 may have a certain regulatory effect on the thickness of grain shell by affecting the cellulose content in Tartary buckwheat shell. However, studies relevant to this hypothesis are lacking. In the present work, thick- and thin-shelled Tartary buckwheat cultivar, namely, Jinqiao 2 and Miku 18, were used as test materials. They were treated with different GA_3 and chlorocholine chloride (CCC, GA_3 synthesis inhibitor) concentrations, and the effects on the amylase activity, sucrose synthase, cellulose content, and yield of Tartary buckwheat were analyzed. The results can provide theoretical references for clarifying the physiological mechanism of the difference in shell thickness between Tartary buckwheat varieties and the high-yield cultivation of Tartary buckwheat.

Materials and methods

Plant materials and treatment

The thick-shelled Tartary buckwheat cultivar Jinqiao 2 (high-yield and the male parent to breed Miku 18) and thin-shelled Tartary buckwheat cultivar Miku 18 (This cultivar obtained by cross breeding with Jinqiao 2 as male parent and Xiaomiqiao, a thin-shelled and low-yield Tartary buckwheat variety, as female parent) were provided by the School of Life Science of Guizhou Normal University, China (26° 35′ N, 106° 43′ E), and complied with relevant institutional, national, and international guidelines and legislation. We have obtained the permission to collect seeds. The experiment was conducted during the growing season of Tartary buckwheat (March–June) from 2021 to 2022 at Xiaba's Cultivation Experimental Station of Guizhou Normal University, Guiyang City, Guizhou Province, China (1250 m, 106.9493° E, 26.7309° N). The soil used was yellow loam, and the nutrient contents of the shallow tillage layer (0–20 cm) at the test site were as follows: 23.43 mg kg^{-1} available phosphorus, 26.98 mg kg^{-1} available potassium, 11.68 mg kg^{-1} ammonium nitrogen, 22.18 mg kg^{-1} nitrate nitrogen, and 33.09 g kg^{-1} organic matter. Soil nutrient contents were determined using a multichannel intelligent soil nutrient meter (OK-V24, China).

The experiment was conducted using a single-factor randomized block design with three replicates. Grains were sown on March 15, 2021 and March 13, 2022, and each test plot area was 10 m^2 (5 m × 2 m). The row spacing and seeding were 33 cm and 3.75 g m^{-2}, respectively, and approximately 90–100 reserved plants were available for each square meter. Exogenous GA_3 and CCC were sprayed at the budding stage of Tartary buckwheat (May 2, 2021 and May 1, 2022). The entire Tartary buckwheat plant was sprayed with an appropriate amount to form water droplets on the leaves. The spraying was conducted continuously for 3 days to ensure effectiveness. According to the results of previous studies, the spraying concentration of GA_3 and CCC were 50 and 100 mg L^{-1}, and the same amount of water was sprayed as the control treatment (CK, 0 mg L^{-1}). Each treatment was planted in one plot. In accordance with the local optimal dosage, 600 kg ha^{-1} (N: P_2O_5: K_2O = 15: 15: 15) compound fertilizer was applied as the base fertilizer at one time[2]. No fertilizer was applied during the entire growth period. Tartary buckwheat grains were harvested on June5, 2021 and June 3, 2022, when 75% of the grains had matured. Normal agricultural practices were implemented.

Sample preparation

Plants with uniform growth and without diseases and insect pests were selected from the plots of each treatment. Five days after GA_3 and CCC spraying, about 2000 flowers (per plot, located on the top 1–3 nodes of the main stem) that boomed on the same day were marked on the calyx with a brush dipped in black ink. After 5 days, marked flowers were sampled for the first time and every 5 days until maturation. In each plot, 200 labeled grains were collected every time and dehulled with tweezers. The shells of 50 grains were cut into small pieces with scissors, mixed well, frozen in liquid nitrogen for 30 s and stored in a − 80 °C refrigerator. The shells of the remaining 150 grains were dried to constant weight in a 60 °C oven and then crushed through a 40-mesh sieve.

Measurement

Determination of α-amylase and β-amylase activity in shell

The α-amylase activity was determined according to the methods of McCleary and Sheehan[22] and He and Hu[23]. A 1.0 g fresh sample of Tartary buckwheat grain shell was weighed, added with 2.0 g NaCl, 0.04 g $CaCl_2$, and 0.04 g sodium azide, diluted to 20 mL, extracted at room temperature for 30 min, and then centrifuged to obtain the supernatant for later use. The mixture of 0.2 mL BPNPG7 and α-glucosidase was used as the substrate, which

was accurately reacted with 0.2 mL of enzyme extraction diluent for 10 min at 40 °C, and the absorbance was measured at a wavelength of 410 nm. The experiment was repeated three times.

The β-amylase activity was determined following the methods of McCleary and Codd[24] and He and Hu[23]. A 1.0 g fresh sample of Tartary buckwheat grain shell was weighed and extracted with 10.0 mL of extraction buffer at room temperature for 1 h. The supernatant was centrifuged and used for the determination of β-amylase activity. The mixture of 0.2 mL PNP β-G_3 and β-glucanase was used as the substrate, which was accurately reacted with 0.2 mL of enzyme extraction diluent at 40 °C for 10 min, and the absorbance was measured at 410 nm. The experiment was repeated three times.

Determination of sucrose synthase activity in shell

The SS and SPS activities were determined in accordance with the method of Chopra et al.[25]. A 0.5 g fresh sample of fresh Tartary buckwheat shell was ground evenly with 3 mL of Hepes–NaOH extraction buffer and centrifuged at 10,000 r/min for 10 min. The supernatant was used as the enzyme solution to be tested. The reaction mixture comprised 50 μL of Hepes–NaOH buffer (pH 7.5), 20 μL of 0.05 mol L^{-1} $MgCl_2$, 20 μL of 0.1 mol L^{-1} uridine diphosphate glucose, 20 μL of 0.1 mol/L fructose, and 50μL of crude enzyme extract. The control was replaced with the inactivated enzyme solution. The abovementioned mixture was bathed in water at 30 °C for 0.5 h, and 0.2 mL of 2 mol L^{-1} NaOH solution was added to terminate the reaction. The mixture was bathed in boiling water for 10 min. After cooling, 1.5 mL of 30% HCl and 0.5 mL of 0.1% resorcinol were added, and the mixture was bathed in water at 80 °C for 10 min. The optical density value at 480 nm was recorded. The experiment was repeated three times.

Determination of cellulose content in shell

The content of cellulose in the grain shell was determined following the method of Li[26]. A 0.2 g dry sample of Tartary buckwheat grain shell was weighed, added with 60 mL of 60% H_2SO_4, and digested for 30 min. The digested cellulose solution was transferred into a 100 mL volumetric flask and diluted with 60% H_2SO_4. After shaking well, it was filtered in another beaker with a Buchner funnel. The above mentioned filtrate was collected, and 5 mL of it was placed in a cold water bath. It was shaken well, and 2 mL was transferred in a stopper tube. Then, 0.5 mL of 2% anthrone reagent was added to it, followed by 5 mL of concentrated sulfuric acid. The mixture was shaken well, allowed to stand for 12 min, and the absorbance was measured at a wavelength of 620 nm.

Determination of agronomic characteristics and yield

According to the method of Zhou et al.[27], the plant height, main stem branch number, main stem node number, grain number per plant, grain weight per plant, and 1000-grain weight of Tartary buckwheat at the maturity stage were determined. In the middle of each treatment plot, the grains on all Tartary buckwheat plants in 1 m^2 (not sampled during the experimental process, excluding border plants) were randomly selected and used to determine the yield after air drying[28].

Statistical analysis

Data were processed using Microsoft Excel 2020 and SPSS20.0. Origin 2021 was used to plot the data. One-way ANOVA was performed, and means were compared using the least significant difference at the 0.05 probability level. The results for 2021 and 2022 were similar. Therefore, the data were presented as the average across the two study years, and the data for 2021and 2022 were deposited as Supplementary Data.

Results

Effects of exogenous gibberellin on amylase activity in the grain shell

With the advancement of growth, the activities of α-amylase and β-amylase in the grain shell of Jinqiao 2 and Miku 18 increased first and then decreased, and the activities peaked at 15 days after marking the flowers (Fig. 1). The activities of α-amylase and β-amylase in the grain shell increased gradually with the rise in the concentration of GA_3 and were highest under the 100 mg L^{-1} treatment. The activities decreased gradually with the increase in the concentration of CCC and were lowest under the 100 mg L^{-1} treatment. Compared with CK treatment, GA_3 treatment increased the α-amylase activity in the grain shell of Jinqiao 2 and Miku 18 by average of 22.80% and 41.68%, respectively. The activity of β-amylase of Jinqiao 2 and Miku 18 was increased by average of 24.52% and 20.52%, respectively. Compared with CK treatment, CCC treatment decreased the α-amylase activity of Jinqiao 2 and Miku 18 by average of 18.36% and 24.86%, respectively. The activity of β-amylase of Jinqiao 2 and Miku 18 was increased by average of 12.86% and 8.17%, respectively. The activities of α-amylase and β-amylase in the grain shell of Jinqiao 2 were higher than those of Miku 18.

With the advancement of growth, the activities of SS and SPS in the grain shell of Jinqiao 2 and Miku 18 increased first and then decreased (Fig. 2). The activity of SS reached the maximum at 10 days after marking the flowers, and the activity of SPS reached the maximum at 15 days after marking the flowers. The activity of SS in the grain shell increased gradually with the increase in the concentration of GA_3 and was highest under the 100 mg L^{-1} treatment, whereas the activity of SPS increased first and then decreased and was highest under the 50 mg L^{-1} treatment. With the increase in CCC concentration, the activities of SS and SPS decreased continuously and were lowest under the 100 mg L^{-1} treatment. Compared with CK treatment, GA_3 treatment increased the SS activity of Jinqiao 2 and Miku 18 by average of 11.10% and 11.21%, respectively. The activity of SPS of Jinqiao 2 and Miku 18 was increased by average of 9.02% and 10.42%, respectively. Compared with CK treatment, CCC treatment decreased the SS activity of Jinqiao 2 and Miku 18 by average of 6.74% and 12.33%, respectively. The SPS activity of Jinqiao 2 and Miku 18 was decreased by average of 9.14% and 8.61%, respectively. The activities of SS and SPS in the grain shell of Jinqiao 2 were higher than those of Miku 18.

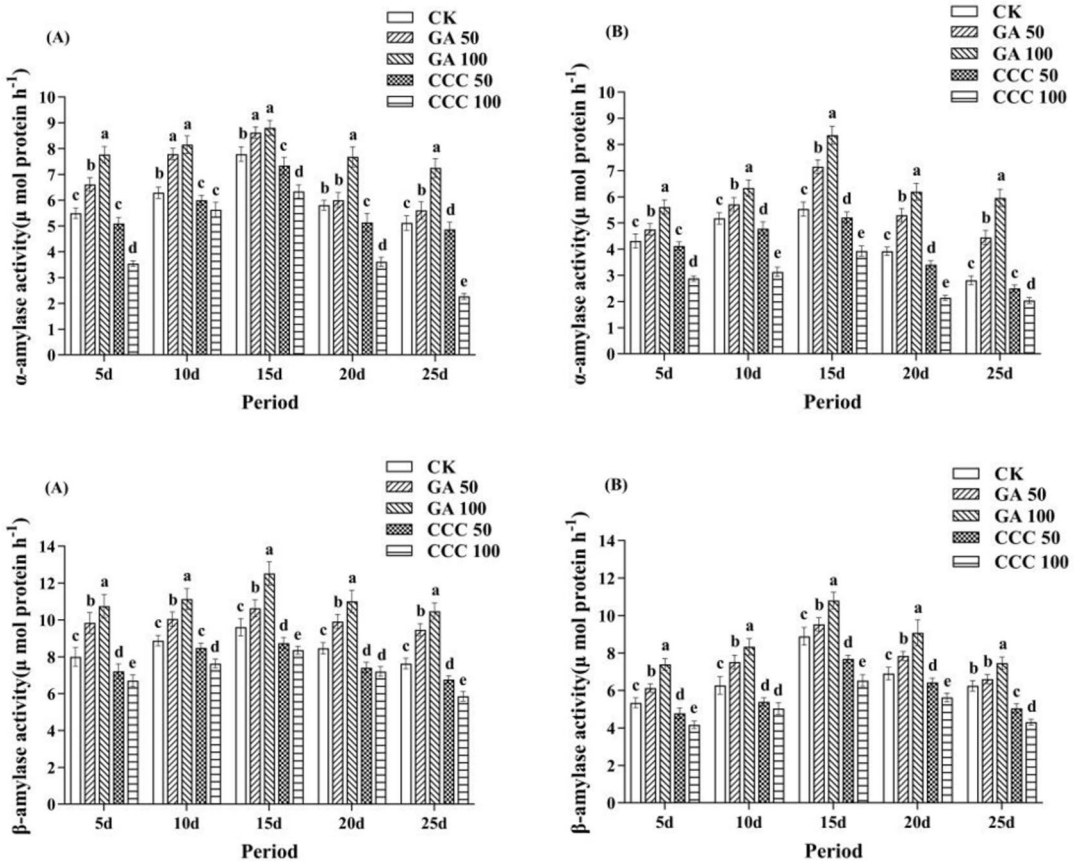

Fig. 1. Effects of exogenous gibberellin and chlormequat chloride on amylase activity in the grain shell at different growth period of Tartary buckwheat. (**A**) represent Jinqiao 2. (**B**) represent Miku 18. 5d, 10d, 15d, 20d, and 25d represent 5 days, 10 days, 15 days, 20 days, and 25 days after marking the flowers, respectively. CK represent the application of gibberellic acid or chlormequat chloride was 0 mg L^{-1}. G_{50} represent the application of gibberellic acid were 50 mg L^{-1}. G_{100} represent the application of gibberellic acid were 100 mg L^{-1}. C_{50} represent the application of chlormequat chloride were 50 mg L^{-1}. C_{100} represent the application of chlormequat chloride were100 mg L^{-1}. Error bars are standard deviation (n = 6). Small letter in the same column means significant difference at $p < 0.05$.3.2. Effects of exogenous gibberellin on sucrose synthase activity in the grain shell.

The cellulose content in the grain shell of Jinqiao 2 and Miku 18 increased continuously with the advancement of growth period, and it reached the maximum at 25 days after marking the flowers (Fig. 3). The cellulose content in the grain shell of Jinqiao 2 and Miku 18 increased continuously with the rise in GA_3 concentration, and the contents were significantly higher under the 100 mg L^{-1} treatment than under the two other treatments. The cellulose content in the grain shell of Jinqiao 2 and Miku 18 decreased continuously with the increase in CCC concentration, and the contents were significantly lower under the 100 mg L^{-1} treatment than those under the two other treatments. Compared with CK treatment, GA_3 treatment increased the cellulose content in the grain shell of Jinqiao 2 and Miku 18 by average of 16.56% and 13.98%, respectively, whereas CCC treatment decreased the cellulose content by average of 16.33% and 13.52%, respectively. The cellulose content in the grain shell of Jinqiao 2 was higher than that of Miku 18.

Compared with CK treatment, GA_3 treatment increased the plant height, number of main stem nodes, the number of main stem branches, and the diameter of main stem of Jinqiao 2 by average of 7.36%, 7.59%, 4.14%, and 15.16%, respectively (Table 1). Those of Miku 18 were increased by 10.54%, 11.63%, 6.81%, and 9.58%, respectively. Compared with CK treatment, CCC treatment decreased the plant height and the number of main stem branches of Jinqiao 2 by average of 29.33% and 16.22%, respectively. Those of Miku 18 were decreased by average of 23.43% and 6.04%. Compared with CK treatment, CCC treatment increased number of main stem nodes and the diameter of main stem of Jinqiao 2 by average of 3.82% and 43.79% respectively, and those of Miku 18 were increased by average of 8.92% and 32.39%.The plant height of Jinqiao 2 were higher than those of Miku 18, while the number of main stem nodes, the number of main stem branches and main stem diameter were lower than those of Miku 18.

The number of grains per plant, grain weight per plant, 1000-grain weight, and yield of Jinqiao 2 and Miku 18 were increased treated with GA_3 treatment, and were significantly higher under the 100 mg L^{-1} treatment than the other two treatments (Table 2). The grains per plant number of Jinqiao 2 and Miku 18 under CCC50 were significantly higher than those under the other two treatments. Compared with CK treatment, GA_3 treatment

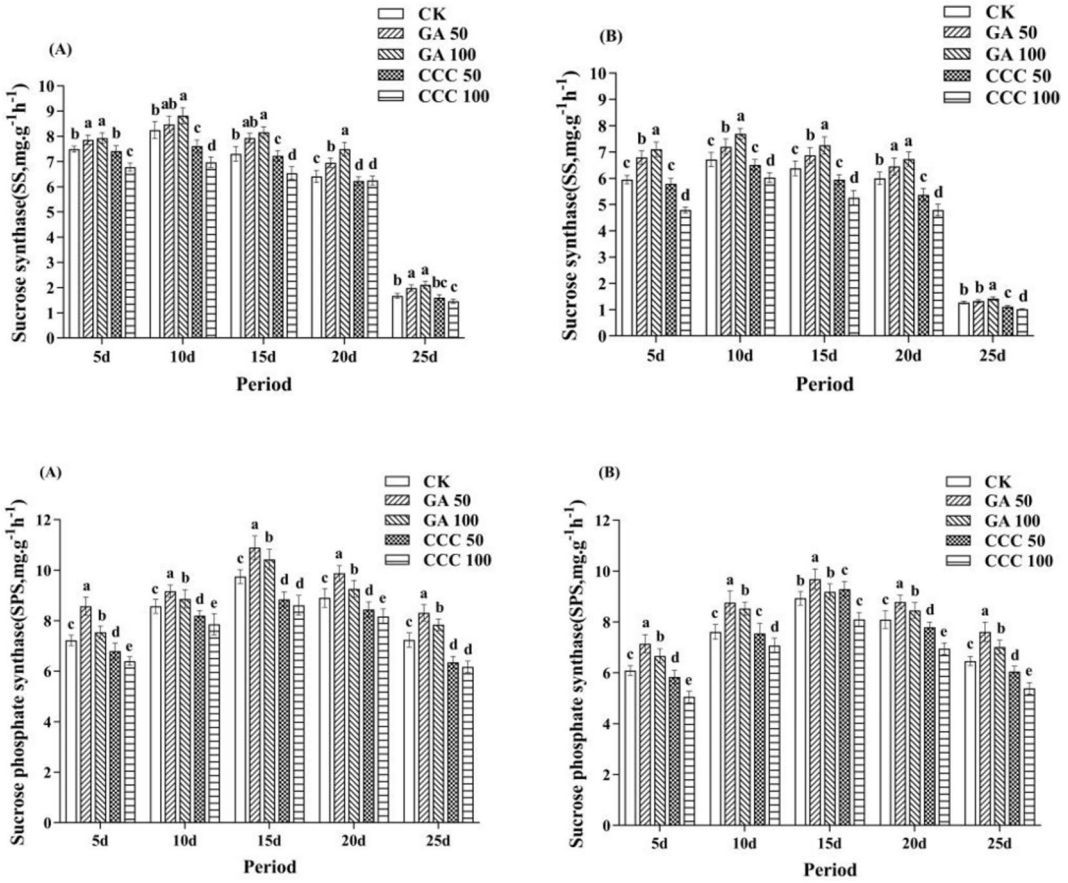

Fig. 2. Effects of exogenous gibberellin and chlormequat chloride on sucrose synthase activity in the grain shell at different growth period of Tartary buckwheat. (**A**) represent Jinqiao 2. (**B**) represent Miku 18. 5d, 10d, 15d, 20d, and 25d represent 5 days, 10 days, 15 days, 20 days, and 25 days after marking the flowers, respectively. CK represent the application of gibberellic acid or chlormequat chloride was 0 mg L^{-1}. G_{50} represent the application of gibberellic acid were 50 mg L^{-1}. G_{100} represent the application of gibberellic acid were 100 mg L^{-1}. C_{50} represent the application of chlormequat chloride were 50 mg L^{-1}. C_{100} represent the application of chlormequat chloride were100 mg L^{-1}. Error bars are standard deviation (n = 6). Small letter in the same column means significant difference at $p < 0.05.3.3$. Effects of exogenous gibberellin on cellulose content in the grain shell.

increased the grain number per plant, grain weight per plant, 1000-grain weight, and yield of Jinqiao 2 by average of 25.98%, 28.47%, 8.52%, and 28.71%, respectively. Those of Miku 18 were increased by average of 32.09%, 11.86%, 7.20%, and 18.45%, respectively. Compared with CK treatment, CCC treatment decreased grain weight per plant, 1000-grain weight, and yield of Jinqiao 2 by average of 7.00%, 7.75%, and 7.43%, respectively. Those of Miku 18 were decreased by average of 6.23%, 13.68%, and 17.48%, respectively. The grain number per plant, grain weight per plant, 1000-grain weight, and yield of Jinqiao 2 were higher than those of Miku 18.

Effects of exogenous gibberellin on cellulose content in the grain shell of Tartary buckwheat

SS and SPS are key enzymes regulating cellulose synthesis. They catalyze the transfer and decomposition of sugar groups to sugar and glucose and provide a large number of substrates for cellulose synthesis[29,30]. Studies have shown that the activity of SS determines the increase or decrease in cellulose synthesis[31,32]. SPS can regulate the sucrose cycle in cells, and its activity increases with the rise in cellulose synthesis rate in secondary wall deposition[33,34]. Tan et al.[35] found that exogenous GA_3 treatment could enhance the activity of SS and SPS in longan fruit. The results of this experiment showed that, compared with the CK treatment, the activities of SS and SPS in Tartary buckwheat grain shells increased to a certain extent after spraying different concentrations of exogenous GA_3. Meanwhile, under the treatment of CCC, the activities of SS and SPS in Tartary buckwheat shells decreased to a certain extent, which further confirmed the promoting effect of exogenous GA_3 on SS and SPS activities. This deduction may be related to the fact that GA_3 can increase the strength of crop sinks, which promotes the enhancement in SS and SPS activities[36].

Notably, α-amylase and β-amylase are important enzymes involved in starch degradation. Studies have shown that GA_3 can regulate the synthesis of α-amylase and the expression of β-amylase gene and promote the hydrolysis of starch. This induction process is affected by the concentration of GA_3[37]. In this study, spraying exogenous GA_3 can improve the activity of α-amylase and β-amylase in the grain shell of Tartary buckwheat, while spraying CCC

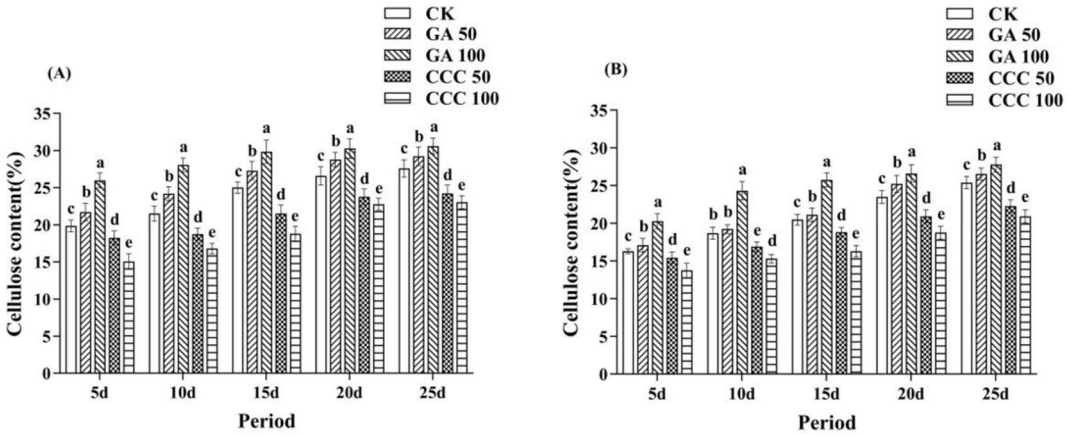

Fig. 3. Effects of exogenous gibberellin and chlormequat chloride on cellulose content in the grain shell at different growth period of Tartary buckwheat. (**A**) represent Jinqiao 2. (**B**) represent Miku 18. 5d, 10d, 15d, 20d, and 25d represent 5 days, 10 days, 15 days, 20 days, and 25 days after marking the flowers, respectively. CK represent the application of gibberellic acid or chlormequat chloride was 0 mg L^{-1}. G_{50} represent the application of gibberellic acid were 50 mg L^{-1}. G_{100} represent the application of gibberellic acid were 100 mg L^{-1}. C_{50} represent the application of chlormequat chloride were 50 mg L^{-1}. C_{100} represent the application of chlormequat chloride were100 mg L^{-1}. Error bars are standard deviation (n = 6). Small letter in the same column means significant difference at $p < 0.05$.3.4. Effects of exogenous gibberellin on agronomic characteristic.

Cultivar	Concentration (mg L^{-1})	Plant height (cm)	Node number of main stem	Number of branches of main stem	Main stem diameter (mm)
Jinqiao 2	CK	117.51 ± 7.63b	11.00 ± 0.34c	11.84 ± 0.47b	6.76 ± 0.27d
	$GA_3$50	118.88 ± 6.34b	12.17 ± 0.28a	12.33 ± 0.33a	7.95 ± 0.24c
	$GA_3$100	133.44 ± 6.32a	11.50 ± 0.60b	12.33 ± 0.58a	7.62 ± 0.24c
	CCC50	88.31 ± 4.86c	11.67 ± 0.35b	10.34 ± 0.46c	9.18 ± 0.25b
	CCC100	77.77 ± 4.55d	11.17 ± 0.43bc	9.50 ± 0.53d	10.26 ± 0.37a
Miku 18	CK	95.15 ± 6.73b	12.17 ± 0.75d	12.33 ± 0.66bc	8.09 ± 0.27d
	$GA_3$50	104.36 ± 4.86a	13.84 ± 0.81a	12.67 ± 0.83b	9.07 ± 0.50b
	$GA_3$100	105.99 ± 3.21a	13.33 ± 0.46b	13.67 ± 0.59a	8.66 ± 0.29c
	CCC50	78.16 ± 4.02c	13.84 ± 0.49a	12.00 ± 0.45c	10.59 ± 0.61a
	CCC100	67.56 ± 2.29d	12.67 ± 0.45c	11.17 ± 0.25d	10.83 ± 0.44a

Table 1. Effects of gibberellic acid and chlormequat chloride on agronomic characters of Tartary buckwheat. CK represent the application of gibberellic acid or chlormequat chloride was 0 mg L^{-1}. G_{50} represent the application of gibberellic acid were 50 mg L^{-1}. G_{100} represent the application of gibberellic acid were 100 mg L^{-1}. C_{50} represent the application of chlormequat chloride were 50 mg L^{-1}. C_{100} represent the application of chlormequat chloride were100 mg L^{-1}. Error bars are standard deviation (n = 6). Small letter in the same column means significant difference at $p < 0.05$.3.5. Effects of exogenous gibberellin on the yield.

can reduce the activity of α-amylase and β-amylase. Therefore, an appropriate concentration of exogenous GA_3 can improve the activity of α-amylase and β-amylase in the grain shell of Tartary buckwheat, which promotes the hydrolysis of starch in the grain shell.

With the advancement of growth period, the activities of α-amylase and β-amylase in the grain shell showed a downward trend after 15 days of marked flowers, which may be related to the inhibition of α-amylase and β-amylase activities by the continuous increase in cellulose in the grain shell during the same period[38]. The results of this experiment showed that, compared with the CK treatment, spraying exogenous GA_3 could significantly increase the cellulose content in the grain shell, while spraying CCC could significantly inhibit the accumulation of cellulose in the grain shell (Fig. 3). The reason may be that spraying exogenous GA_3 can promote the hydrolysis of starch by advancing the increase in α-amylase and β-amylase activity in the shell, which raises the sucrose content of the grain shell. The increase in sucrose content makes the catalytic reaction substrate involved in sucrose synthase sufficient. Consequently, this condition increases the cellulose content, which improves the shell thickness to a certain extent.

The content of cellulose in the buckwheat grain shell is rich, which is one of the main components of the buckwheat grain shell. The thickness of buckwheat grain shell varies greatly among different buckwheat varieties[8]. The results of this experiment showed that the content of cellulose in the shell of Jinqiao 2 was significantly

Cultivar	Concentration (mg L^{-1})	Grain number per plant	Grain weight per plant (g)	1000-grain weight (g)	Yield (kg ha^{-1})
Jinqiao 2	CK	479.82 ± 14.63d	8.71 ± 0.29d	17.49 ± 0.73c	962.51 ± 30.71c
	$GA_3$50	576.45 ± 19.96b	10.06 ± 0.66b	18.19 ± 0.49b	1181.09 ± 37.56b
	$GA_3$100	632.50 ± 22.26a	12.32 ± 0.59a	19.77 ± 0.69a	1296.63 ± 47.47a
	CCC50	530.87 ± 18.33c	8.53 ± 0.36c	16.82 ± 0.47d	936.85 ± 27.86d
	CCC100	478.94 ± 15.44d	7.67 ± 0.25e	15.45 ± 0.67e	845.11 ± 22.89d
Miku 18	CK	403.49 ± 13.45d	6.66 ± 0.16c	13.96 ± 0.35c	663.58 ± 21.22c
	$GA_3$50	508.28 ± 15.32b	7.23 ± 0.23b	14.54 ± 0.40b	750.33 ± 30.21b
	$GA_3$100	557.62 ± 13.15a	7.67 ± 0.31a	15.39 ± 0.27a	821.73 ± 29.65a
	CCC50	460.16 ± 19.69c	6.53 ± 0.17c	12.48 ± 0.48d	573.92 ± 20.08d
	CCC100	344.76 ± 15.49e	5.96 ± 0.20d	11.62 ± 0.16e	521.25 ± 22.56e

Table 2. Effects of gibberellic acid and chlormequat chloride on yield of Tartary buckwheat. CK represent the application of gibberellic acid or chlormequat chloride was 0 mg L^{-1}. G_{50} represent the application of gibberellic acid were 50 mg L^{-1}. G_{100} represent the application of gibberellic acid were 100 mg L^{-1}. C_{50} represent the application of chlormequat chloride were 50 mg L^{-1}. C_{100} represent the application of chlormequat chloride were100 mg L^{-1}. Error bars are standard deviation (n = 6). Small letter in the same column means significant difference at $p < 0.05$.4. Discussion.

higher than that of Miku 18. This result may be related to the higher activity of the key enzyme in the synthesis of cellulose in the grain shell of Jinqiao 2 than that of Miku 18. The difference in the content of cellulose in the grain shell is an important reason for the distinction in the thickness of the shell between the varieties of Tartary buckwheat[19].

Effects of exogenous gibberellin on the yield of Tartary buckwheat

The agronomic traits of plants can reflect the growth and development of individual plants. Feng et al.[39] found that the plant height, stem diameter, node number of main stem, and branch number of main stem were important indexes of buckwheat morphology, which reflected the growth status of buckwheat. Studies have shown that the yield of Tartary buckwheat is closely related to its agronomic traits, and the branch number of main stem plays a major role in the yield[40]. Grain weight and grain number are important components of crop yield and significantly affect it[41]. Fang et al.[42] found that the yield of common wheat was significantly positively correlated with grain number per plant and 1000-grain weight. Zhou et al.[1] found that the composition of Tartary buckwheat yield was mainly reflected in the grain weight per plant and the number of plants per unit area. In the case of a certain number of plants per unit area, grain weight per plant is an important factor affecting the yield of Tartary buckwheat. The study of Guo et al.[43] showed that spraying appropriate GA_3 could increase the yield of Tartary buckwheat. In the present study, compared with the CK treatment, spraying exogenous GA_3 increased the number of grains per plant, grain weight per plant, 1000-grain weight, and yield of Tartary buckwheat[43]. By contrast, after spraying CCC, agronomic trait-related indicators and yield were inhibited. This finding is consistent with the above mentioned research results. This consistency may be related to exogenous GA_3 treatment, which improves the photosynthetic performance of leaves. This enhancement promotes the production of more photosynthetic products. Consequently, this phenomenon provides more nutrients for grains, which increases the number of grains per plant, grain weight, and yield[44].

Conclusions

The difference in cellulose content between Tartary buckwheat varieties is an important physiological reason for the distinction in shell thickness between varieties. Spraying exogenous GA_3 can increase the sucrose content of the grain shell by promoting the rise in α-amylase and β-amylase activity in the grain shell and facilitating starch hydrolysis. The increase in sucrose content makes the catalytic reaction substrate involved in sucrose synthase sufficient, which in turn increases the cellulose content and shell thickness to a certain extent. GA_3 treatment increased the yield of Tartary buckwheat by improving the number of main stem nodes, the number of main stem branches, grains per plant, and 1000-grain weight. Exogenous GA_3 treatment not only increased the yield of Tartary buckwheat but also improved the shell thickness of Tartary buckwheat. Although CCC treatment reduced the thickness of the shell, it also decreased the yield of Tartary buckwheat. Therefore, in actual production, exogenous GA_3 or CCC treatment can be reasonably selected according to the processing purpose of Tartary buckwheat.

Data availability

The data used and analysed during the current study are available from the corresponding author on reasonable request.

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Author contributions

Q.Y. and K.F.H. designed the project and drafted the manuscript; Q.Y. and J.G.T. conducted the experiments and analyzed the data; X.Y.H. contributed to sample collection. Q.Y., K.F.H., and X.Y.H. revised the manuscript. All authors have read and agreed to the published version of the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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