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Effects of exogenous 2,4-epibrassinolide and its inhibitors on the seed setting and yield of Tartary buckwheat

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Abstract: The aim of this study was to evaluate the effects of exogenous 2,4-epibrassinolide (EBR) on the yield of Tartary buckwheat (*Fagopyrum tataricum* (L.) Gaertn.). A 2-year field experiment was conducted on Tartary buckwheat (cv. Jinqiao 2) with different concentrations (0, 0.1, 0.5, 1.0, and 2.0 mg/L) of EBR and brassinolide (BRZ, inhibitor of brassinolide synthesis). The seed setting rate, agronomic traits, and yield initially increased and then decreased with an increase in the EBR application rate. The seed setting rate, agronomic traits, and yield decreased gradually with an increase in BRZ concentration, and yield was the lowest at 2.0 mg/L. The appropriate application of exogenous EBR could promote the increase of Tartary buckwheat yield. Compared with 0 mg/L (control), the 0.1, 0.5, and 1.0 mg/L treatments increased yield by 13.53, 32.73, and 7.08%, respectively, while the high-concentration treatment (2.0 mg/L) decreased by 4.13%. In conclusion, the appropriate concentration of EBR treatment (0.5 mg/L) delayed the senescence of Tartary buckwheat by increasing its root activity and the activity of antioxidant enzymes in leaves. Simultaneously, it increased the chlorophyll content of Tartary buckwheat leaves, enhanced photosynthesis, increased nonstructural carbohydrate content, and augmented the "source," increasing the seed setting rate and yield of Tartary buckwheat. This concentration is recommended for use in the production of Tartary buckwheat.

Keywords: rhizosphere soil nutrients; pollen viability; concentration-dependent; grain weight

Tartary buckwheat (*Fagopyrum tataricum* (L.) Gaertn.) belongs to the *Fagopyrum* genus of the family Polygonaceae. Tartary buckwheat in China is mostly distributed in the Yunnan-Guizhou-Sichuan area in the southwest and the high-altitude areas in the north (Tang et al. 2024). Tartary buckwheat is rich in nutrients and flavonoids, such as rutin, quercetin, D-chiral inositol, and active polysaccharides, which are of considerable significance for reducing human blood lipids and cholesterol, and thus, preventing cardiovascular and cerebrovascular diseases. In addition, Tartary buckwheat contains selenium, which is scarce in other food crops. Selenium is beneficial for cancer prevention. Tartary buckwheat is an important medicinal and edible crop (Fabjan et al. 2003) with broad market prospects. However, the yield per unit area of Tartary buckwheat in China is relatively low, seriously restricting the development of the Tartary buckwheat industry. Improving yield per unit area has become an urgent scientific problem that must be solved in Tartary buckwheat production (Zhou et al. 2023a).

Crop yield is closely related to flowering and fruiting, and seed setting rate is an important indicator for measuring crop yield (Cheng et al. 2023). The level of pollen viability is the key to whether plants

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can form fruits and seeds, and it plays an essential role in the fruiting of plants (Rao et al. 1992). The primary energy storage form of crops is non-structural carbohydrate (NSC). NSC in the various organs of crops is an important substance involved in plant life metabolism. The accumulation, transport, and distribution of NSC are important factors that affect crop yield; as an important energy substance, NSC is closely related to crop seed setting (Cao et al. 2020). The leaf is the most important source organ for the photosynthesis, respiration, and transpiration of crops. Leaf senescence can directly lead to crop yield reduction (Zhou et al. 2023b). The activities of antioxidant enzymes, such as superoxide dismutase (SOD) and peroxidase (POD), are commonly used to judge the senescence process of plants. Panda and Sarkar (2012) determined that the activities of SOD and other enzymes gradually decreased with the advancement of the senescence process. The yield of crops is closely related to seed setting rate, NSC content and transport, and plant senescence.

Brassinosteroids (BRs) are sterol phytohormones that play an important role in regulating plant cell expansion, morphogenesis, leaf senescence, and yield formation (Hu et al. 2000). In addition, BRs can regulate the formation of flowers and fruits (Vukašinović et al. 2021). 2,4-epibrassinolide (EBR) is a synthetic analogue of brassinolide that exhibits high activity. Brassinozole (BRZ) is a specific BR biosynthesis inhibitor that inhibits the synthesis of BRs by preventing the hydroxylation of 6-deoxteasterone into 6-deoxcathasterone in the C-6 oxidation pathway (Asami et al. 2001). It provides a new way to understand the physiological role of brassinolide comprehensively. Mohammadi et al. (2019) found that the application of EBR increased yield by enhancing the photosynthesis of kidney beans. Otie et al. (2021) reported that EBR promoted seed setting by increasing plant leaves' antioxidant capacity and photosynthetic pigment content. Zhang et al. (2022) determined that an appropriate concentration of exogenous EBR promoted the filling of Tartary buckwheat by delaying senescence and enhancing the utilisation efficiency of the light source, and thus, increasing the grain weight and yield of Tartary buckwheat. Zhang et al. (2019) reported that exogenous EBR improved the antioxidant capacity of rice panicles, reduced spikelet degradation, promoted rice seed setting, and increased rice yield. The studies above have shown that EBR can increase yield by promoting seed setting. Therefore, the current study hypothesised that brassinolide may increase the yield of Tartary buckwheat by promoting seed setting, delaying senescence, and increasing NSC content. However, relevant research on this hypothesis is lacking. In the present study, the Tartary buckwheat cultivar Jinqiao 2 was used as the experimental material, and different concentrations of exogenous EBR and BRZ treatments were established to study their effects on the seed setting and yield of Tartary buckwheat. The results can provide a new agronomic method for the high-yield cultivation of Tartary buckwheat.

MATERIAL AND METHODS

Plant materials and growth. Jinqiao 2, a high-yield Tartary buckwheat cultivar, was provided by the Buckwheat Industry Technology Research Center of Guizhou Normal University. EBR and BRZ were purchased from Shanghai McLean Biochemical Technology Co., Ltd. (https://www.macklin.cn), with an analytical purity of 98%. The single-factor randomised block design was used in the experiment with three replicates, and the plot area was 10 m^2 (2 m × 5 m). Grains were sown on August 13, 2022, and March 11, 2023, at the experimental base of the Buckwheat Industry Technology Research Center in Xiaba Town, Guiyang City, Guizhou Province, China (1 250 m a.s.l., 106.95°E, 26.73°N). The area had a subtropical plateau monsoon humid climate. The annual average temperature was 14.95 °C, and the annual average precipitation was 1 319.05 mm. The soil used was xanthic ferralsols with 20.56 mg/kg available phosphorus, 24.36 mg/kg available potassium, 7.99 mg/kg ammonium nitrogen, and 1.62% organic carbon (soil nutrient analyser determination, model OK-Q3).

Strip-seeding sowing was adopted. The row spacing, seeding rate, and basic seedlings were 0.33 m, 3.65 g/m^2, and 90–100 plants/m^2, respectively. By the local optimal dosage, 600 kg/ha of a compound fertiliser was applied as base fertiliser (nitrogen:phosphorus:potassium = 15:15:15) to each plot simultaneously (Zhang et al. 2023a). No fertiliser was applied throughout the entire growth period. By the method of Zhang et al. (2022), different concentrations of EBR (0.1, 0.5, 1.0, and 2.0 mg/L) and BRZ (0.1, 0.5, 1.0, and 2.0 mg/L) were established, and the same amount of distilled water was sprayed as the control treatment (CK). The buds of Tartary buckwheat were sprayed at 8 a.m. during the early budding stage (September 29, 2022, and May 5, 2023), with no rain for 2 days after

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spraying. The buds were thoroughly moist, and water droplets were formed. The buds were sprayed once every 2 days for three times. Grains were harvested on November 17, 2022, and June 21, 2023, when 70% of the grains had matured. Other cultivation measures were the same as those of local high-yield cultivation management. The monthly average temperatures from August to November in 2022 and from March to June in 2023 were 18.8 °C and 18.4 °C, the monthly average mean rainfall was 24.1 mm and 168.8 mm, and the monthly average sunshine hours were 155.4 h and 120.2 h, respectively. The weather station is about 4.2 km away from the trial site.

Sample preparation. During the flowering, grain-filling and mature stages of Tartary buckwheat, 10 plants with the same growth vigour were selected from each treatment plot, and the root was cut off. After being cleaned with running water, the root was carefully dried with absorbent paper to measure root activity. Simultaneously, the leaves on 1–3 nodes at the top of the main stem were collected. Half of the leaves were frozen in liquid nitrogen for 1 min and stored in a refrigerator at –70 °C to determine antioxidant enzyme activity. The remaining half of the leaves were placed in an oven at 105 °C for 10 min and dried to constant weight at 60 °C to determine soluble sugar and starch contents. During the full-bloom stage of Tartary buckwheat, the open flowers on the top 1–3 plant nodes were cut with scissors, stored in ice bags, and brought back to the laboratory to determine pollen viability. During the flowering, grain-filling, and mature stages of Tartary buckwheat, 10 plants with the same growth vigour were selected from each treatment plot. Soil that was loosely bound to the root was shaken. Conversely, rhizosphere soil was collected to determine rhizosphere soil nutrients.

Determination of agronomic traits and yield. Plant height, stem diameter, 1 000-grain weight, and yield were measured in accordance with the methods of Zhou et al. (2023a) and Zhang et al. (2024).

Determination of seed setting rate. In accordance with the method of Guo et al. (2021), 10 plants with consistent growth vigour were randomly selected from each plot during the mature period to investigate the seed setting rate.

$$\text{Grain set rate (\%)} = \text{number of mature grains/number of total flowers} \times 100\%$$

Determination of pollen rate. The pollen viability of the flowers on the top 1–3 nodes of the Tartary buckwheat plants during the full-bloom stage was determined by the method of Li (2000). The pollen grains were stained in 0.5% triphenyl tetrazolium chloride for 15 min and then transferred to a microscope for observation. Pollen grains that were dyed a red colour were considered viable, while others were considered nonviable. Pollen viability was determined by the ratio of viable pollen number over the total pollen number.

Determination of root activity and rhizosphere soil nutrients. The contents of ammonium nitrogen, available phosphorus, and available potassium in rhizosphere soil were determined using a soil nutrient rapid analyser (OK-Q3, Zhengzhou, China) by the method described by Huang et al. (2024). Root activity was determined through the 2,3,5-triphenyl tetrazolium chloride method (Li 2000). In brief, 0.5 g of the root tip-sample was weighed and placed in a glass test tube. Then, 5 mL of 0.4% TTC solution and 5 mL of pH 7.0 phosphate buffers were added until the root was wholly immersed in the solution. The root was stored in darkness and heated at 37 °C for 1 h. After 15 min, the solution was added dropwise, and then 20 mL of methanol was added. The root was placed in an incubator at 30–40 °C until the root tissue completely turned white. The sample was colorimised at 485 nm.

Determination of antioxidant enzyme activity. Superoxide dismutase activity was determined by the nitrogen blue tetrazole (NBT) method (Li 2000, Huang et al. 2024). In brief, 0.5 g of the sample was accurately weighted to prepare SOD crude extract. Phosphoric acid buffer solution, Met solution, NBT solution, EDTA-Na_2 solution, riboflavin, SOD enzyme crude solution, and distilled water were successively added into a 5 mL test tube in a certain proportion and placed under light for 20 min. SOD activity was then calculated by colourimetry at 560 nm.

Peroxidase activity was determined by the guaiacol method (Li 2000, Huang et al. 2024). In brief, 0.5 g of the sample was weighed and extracted with phosphoric acid buffer. Phosphoric acid buffer, H_2O_2, and guaiacol were added and colourimetrised at 470 nm. The enzyme activity unit was 0.01 change of A470 per minute.

Determination of NSC content. The soluble sugar and starch content was determined by the method of Liu et al. (2020). A dry sample (0.1 g) was combined with 10 mL ethanol and incubated in a 15 mL centrifuge tube at 80 °C for 0.5 h and then centrifuged at 2 000 *r*/min for 20 min after cooling to room temperature. The supernatant was transferred to a 100 mL volumetric flask, and the extraction was

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Table 1. Effect of different concentrations of 2,4-epibrassinolide and brassinozole treatments on agronomic traits

Treatment	Concentration (mg/L)	Plant height (cm)	Stem diameter (cm)	1 000-grain weight (g)	Yield (kg/ha)
2,4-Epibrassinolide (EBR)	CK (0)	105 ± 5.76^{cd}	5.48 ± 0.37^{b}	19.06 ± 0.24^{b}	1 766 ± 39.10^{d}
	0.1	119 ± 2.14^{b}	5.56 ± 0.22^{b}	19.47 ± 0.35^{ab}	2 005 ± 41.54^{b}
	0.5	140 ± 4.64^{a}	6.10 ± 0.28^{a}	19.80 ± 0.24^{a}	2 344 ± 38.48^{a}
	1.0	117 ± 4.71^{b}	5.74 ± 0.88^{ab}	18.95 ± 0.32^{c}	1 891 ± 38.10^{c}
	2.0	109 ± 5.77^{c}	5.77 ± 0.68^{ab}	18.51 ± 0.56^{d}	1 693 ± 34.98^{e}
Brassinozole (BRZ)	0.1	104 ± 4.91^{cd}	5.37 ± 0.34^{bc}	18.88 ± 0.14^{cd}	1 600 ± 49.34^{f}
	0.5	101 ± 5.39^{d}	5.44 ± 0.42^{b}	18.36 ± 0.30^{e}	1 430 ± 28.22^{g}
	1.0	98 ± 7.48^{de}	4.93 ± 0.34^{c}	18.02 ± 0.24^{f}	1 388 ± 31.95^{g}
	2.0	96 ± 7.71^{e}	4.87 ± 0.57^{c}	17.58 ± 0.51^{g}	1 316 ± 29.11^{h}

Data are presented as mean ± standard error of the mean. Different small letter in the same column means significant difference at $P < 0.05$

repeated three times. The three supernatants were pooled in a flask and combined with distilled water to a final volume of 100 mL. An aliquot of the extract was used to measure soluble sugars with an anthrone reagent. To determine starch content, the residue after centrifugation in the tube was added to 2 mL of distilled water and then shaken in a boiling water bath for 15 min. After the addition of 2 µL perchloric acid (9.36 mol/L), the tube was immersed in an ice bath for 15 min to completely digest the starch into glucose. After centrifugation, the supernatant of the extract was collected in a 100 mL volumetric flask, and the extraction was repeated by placing the residue in 2 mL 4.68 mol/L perchloric acid for 15 min a second time. The supernatants were pooled and made to 100 mL with distilled water. The colourimetric assay measured optical density at 620 nm in a spectrophotometer (UV-1800; Shimadzu, Japan). Glucose released in the extraction was estimated with anthrone reagent and converted to a starch value by multiplying by 0.9.

The NSC accumulation was calculated as the sum of the starch and soluble sugar contents (Liu et al. 2020).

Determination of chlorophyll content. The chlorophyll *a*, chlorophyll *b*, and total chlorophyll contents of the leaves were measured using Li's method (2000). In brief, 0.2 g of fresh Tartary buckwheat leaves were weighed, grind into homogenate, added 10 mL ethanol was to continue grinding, stood for 3–5 min, and filtered the extract to a 25 mL brown volumetric flask. The light absorption value at 665, 649, and 470 nm was measured after shaking the mixture (Li 2000). The total chlorophyll content was calculated as the sum of the chlorophyll *a* and *b* contents.

Table 2. Effects of exogenous 2,4-epibrassinolide and brassinozole on the seed setting rate

Treatment	Concentration (mg/L)	Seed setting rate (%)	Pollen viability (%)
2,4-Epibrassinolide (EBR)	CK (0)	26.31 ± 0.25^{c}	89.90 ± 0.18^{c}
	0.1	29.24 ± 0.50^{b}	93.77 ± 1.38^{b}
	0.5	30.33 ± 0.61^{a}	98.28 ± 0.20^{a}
	1.0	29.10 ± 0.46^{b}	93.88 ± 0.21^{b}
	2.0	26.43 ± 0.43^{c}	89.21 ± 0.95^{c}
Brassinozole (BRZ)	0.1	24.16 ± 0.64^{d}	87.32 ± 0.23^{d}
	0.5	22.67 ± 0.38^{e}	85.71 ± 0.41^{e}
	1.0	20.36 ± 0.83^{f}	83.62 ± 0.29^{f}
	2.0	18.66 ± 0.45^{g}	82.02 ± 0.23^{g}

Data are presented as mean ± standard error of the mean. Different small letter in the same column means significant difference at $P < 0.05$

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Statistical analysis. Data were processed using Office 2019 (Microsoft Corp., Redmond, USA) and SPSS 26.0 (IBM Corp., Armonk, USA). One-way ANOVA was performed, and means were compared using the least significant difference at the 0.05 probability level. The 2022 and 2023 results were similar, so the data were presented as the average of the two years. The 2022 and 2023 data were deposited as supplementary data.

Table 3. Effects of exogenous 2,4-epibrassinolide and brassinozole on the rhizosphere soil nutrients and root activity

Item	Treatment	Concentration (mg/L)	Period		
			flower	grain-filling	mature
Ammonium nitrogen (mg/kg)	2,4-epibrassinolide (EBR)	CK (0)	11.76 ± 0.34^{ef}	14.09 ± 0.38^{e}	10.90 ± 0.35^{e}
		0.1	16.04 ± 0.48^{c}	17.00 ± 0.37^{c}	12.58 ± 0.17^{c}
		0.5	18.83 ± 0.31^{a}	20.71 ± 0.44^{a}	16.06 ± 0.25^{a}
		1.0	18.01 ± 0.20^{b}	18.27 ± 0.42^{b}	15.29 ± 0.32^{b}
		2.0	14.37 ± 0.39^{d}	16.05 ± 0.27^{d}	11.68 ± 0.37^{d}
	brassinozole (BRZ)	0.1	11.92 ± 0.31^{e}	12.36 ± 0.47^{f}	10.39 ± 0.40^{f}
		0.5	11.49 ± 0.23^{f}	12.09 ± 0.30^{fg}	9.69 ± 0.43^{g}
		1.0	10.56 ± 0.27^{g}	11.57 ± 0.37^{g}	8.64 ± 0.32^{h}
		2.0	9.71 ± 0.28^{h}	10.32 ± 0.58^{h}	8.05 ± 0.28^{i}
Available phosphorus (mg/kg)	2,4-epibrassinolide	CK (0)	22.84 ± 2.79^{e}	32.68 ± 3.24^{d}	25.10 ± 2.14^{e}
		0.1	38.21 ± 3.10^{c}	41.97 ± 3.12^{c}	40.81 ± 3.06^{c}
		0.5	60.31 ± 4.27^{a}	68.79 ± 4.91^{a}	64.63 ± 3.90^{a}
		1.0	45.29 ± 3.37^{b}	52.67 ± 6.73^{b}	51.19 ± 3.38^{b}
		2.0	30.74 ± 2.71^{d}	34.43 ± 2.49^{d}	36.26 ± 3.04^{d}
	brassinozole	0.1	22.17 ± 1.78^{ef}	29.19 ± 4.37^{de}	25.71 ± 4.15^{e}
		0.5	19.84 ± 3.19^{f}	26.59 ± 3.25^{e}	20.47 ± 1.71^{f}
		1.0	18.43 ± 2.14^{fg}	24.48 ± 3.52^{ef}	17.82 ± 2.10^{f}
		2.0	16.50 ± 1.62^{g}	21.89 ± 3.31^{f}	17.00 ± 2.35^{f}
Available potassium (mg/kg)	2,4-epibrassinolide	CK (0)	7.27 ± 0.60^{de}	8.94 ± 0.74^{d}	5.93 ± 0.67^{c}
		0.1	9.49 ± 1.29^{c}	10.61 ± 1.17^{c}	6.60 ± 0.97^{c}
		0.5	15.62 ± 1.12^{a}	18.99 ± 0.70^{a}	9.30 ± 0.67^{a}
		1.0	12.75 ± 1.23^{b}	16.06 ± 1.41^{b}	8.40 ± 0.59^{b}
		2.0	8.11 ± 1.10^{d}	9.00 ± 0.85^{d}	6.10 ± 0.87^{c}
	brassinozole	0.1	7.17 ± 0.59^{de}	8.00 ± 1.05^{e}	4.96 ± 0.33^{d}
		0.5	6.76 ± 0.85^{e}	7.08 ± 0.91^{ef}	4.61 ± 0.45^{de}
		1.0	6.11 ± 0.78^{ef}	6.49 ± 1.12^{f}	3.99 ± 0.57^{e}
		2.0	5.40 ± 0.43^{f}	5.63 ± 0.44^{g}	3.61 ± 0.31^{ef}
Root activity (μg/(g/h))	2,4-epibrassinolide	CK (0)	142.41 ± 8.43^{d}	196.76 ± 12.37^{c}	122.70 ± 10.61^{c}
		0.1	176.17 ± 12.87^{c}	262.35 ± 13.01^{b}	131.91 ± 11.33^{bc}
		0.5	236.89 ± 11.98^{a}	308.81 ± 13.77^{a}	165.46 ± 13.03^{a}
		1.0	213.44 ± 12.19^{b}	278.09 ± 12.11^{b}	146.95 ± 9.02^{b}
		2.0	173.07 ± 10.40^{c}	264.26 ± 14.14^{b}	139.10 ± 13.14^{b}
	brassinozole	0.1	135.30 ± 11.58^{de}	191.04 ± 15.99^{c}	119.80 ± 12.86^{c}
		0.5	121.72 ± 11.11^{e}	168.51 ± 13.46^{d}	103.48 ± 11.74^{d}
		1.0	113.34 ± 12.69^{f}	152.46 ± 10.95^{de}	95.11 ± 12.60^{de}
		2.0	100.88 ± 9.53^{g}	136.07 ± 12.87^{e}	82.12 ± 10.55^{e}

Data are presented as mean ± standard error of the mean. Different small letter in the same column means significant difference at $P < 0.05$

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Table 4. Effects of exogenous 2,4-epibrassinolide and brassinozole on the activity of antioxidant enzymes

Item	Treatment	Concentration (mg/L)	Period		
			flower	grain-filling	mature
Superoxide dismutase activity (U/(g/h))	2, 4-epibrassinolide (EBR)	CK (0)	288.91 ± 11.10^{c}	367.15 ± 13.11^{c}	116.64 ± 15.62^{de}
		0.1	307.15 ± 13.41^{b}	383.08 ± 10.45^{c}	234.30 ± 32.68^{b}
		0.5	338.58 ± 13.64^{a}	447.60 ± 13.49^{a}	265.56 ± 10.40^{a}
		1.0	320.57 ± 10.92^{b}	418.84 ± 14.40^{b}	229.64 ± 14.84^{b}
		2.0	278.08 ± 10.99^{c}	384.29 ± 10.95^{c}	199.76 ± 13.84^{c}
	brassinozole (BRZ)	0.1	260.39 ± 10.30^{d}	327.36 ± 14.23^{d}	131.61 ± 12.32^{d}
		0.5	241.73 ± 10.57^{e}	312.31 ± 14.71^{d}	100.60 ± 5.15^{e}
		1.0	227.75 ± 11.94^{e}	283.16 ± 12.56^{e}	86.52 ± 9.58^{f}
		2.0	191.14 ± 12.15^{f}	244.52 ± 16.01^{f}	66.99 ± 8.94^{g}
Peroxidase activity (U/(g/h))	2, 4-epibrassinolide	CK (0)	1.44 ± 0.11^{cd}	3.18 ± 0.30^{c}	1.56 ± 0.13^{d}
		0.1	1.80 ± 0.14^{b}	6.69 ± 0.38^{a}	1.87 ± 0.11^{b}
		0.5	2.00 ± 0.10^{a}	7.09 ± 0.41^{a}	2.05 ± 0.14^{a}
		1.0	1.85 ± 0.13^{b}	6.67 ± 0.30^{a}	1.93 ± 0.14^{ab}
		2.0	1.49 ± 0.12^{c}	5.37 ± 0.70^{b}	1.76 ± 0.10^{c}
	brassinozole	0.1	1.38 ± 0.10^{cd}	2.95 ± 0.21^{c}	1.44 ± 0.12^{de}
		0.5	1.30 ± 0.10^{d}	2.80 ± 0.16^{c}	1.35 ± 0.13^{e}
		1.0	1.22 ± 0.09^{de}	2.33 ± 0.12^{d}	1.23 ± 0.10^{f}
		2.0	1.10 ± 0.07^{e}	2.11 ± 0.16^{d}	1.08 ± 0.12^{g}

Data are presented as mean ± standard error of the mean. Different small letter in the same column means significant difference at $P < 0.05$

RESULTS

Agronomic traits and yield. Compared with the control, the 0.1, 0.5, and 1.0 mg/L EBR treatments could increase the yield by 13.53, 32.73, and 7.08%, respectively, while the 2.0 mg/L treatment reduced yield by 4.13% (Table 1). With an increase in the BRZ application rate, plant height, stem diameter, 1 000-grain weight, and yield of Tartary buckwheat gradually decreased, and all the treatments reached the lowest value at 2.0 mg/L.

Seed setting rate. With an increase in the EBR application rate, Tartary buckwheat's seed setting rate and pollen viability initially increased and then decreased, reaching the maximum at 0.5 mg/L (Table 2). The 0.5 mg/L treatment increased the seed setting rate and pollen viability by 4.02% and 8.38%, compared with the control. The application of BRZ reduced the seed setting rate and pollen viability.

Rhizosphere soil nutrients. The contents of ammonium nitrogen, available phosphorus, and available potassium in rhizosphere soil and the root activity initially increased and then decreased with an increase in exogenous EBR concentration, reaching the maximum under the 0.5 mg/L treatment (Table 3). The application of BRZ reduced the nutrient content of rhizosphere soil and root activity.

Antioxidant enzyme activity. The antioxidant enzyme activity initially increased and then decreased with an increase in exogenous EBR application concentration, reaching the maximum under the 0.5 mg/L treatment (Table 4). Compared with the control, the 0.5 mg/L treatment increased SOD activity by an average of 55.59% and POD activity by an average of 64.42%. BRZ treatment reduced the activity of antioxidant enzymes.

NSC content. With an increase in exogenous EBR application concentration, the NSC content of leaves initially increased and then decreased, reaching the maximum under the 0.5 mg/L treatment (Table 5). Compared with the control, the 0.5 mg/L treatment increased NSC content by an average of 56.66%. With an increase in BRZ application concentration, the NSC content of leaves decreased gradually.

Chlorophyll content. With an increase in EBR concentration, the chlorophyll *a*, chlorophyll *b*, and total chlorophyll contents initially increased and then decreased, and the content reached the maximum un-

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Table 5. Effects of exogenous 2,4-epibrassinolide and brassinozole on the non-structural carbohydrate content of leaves

Item	Treatment	Concentration (mg/L)	Period		
			flower	grain-filling	mature
Soluble sugar content (mg/g, DW)	2, 4-epibrassinolide (EBR)	CK (0)	10.23 ± 1.02^{d}	14.13 ± 1.34^{d}	8.09 ± 0.55^{e}
		0.1	12.21 ± 1.04^{c}	17.46 ± 1.03^{c}	12.04 ± 0.87^{c}
		0.5	19.00 ± 1.37^{a}	24.91 ± 1.53^{a}	16.70 ± 0.97^{a}
		1.0	15.15 ± 0.95^{b}	21.56 ± 2.30^{b}	14.28 ± 1.04^{b}
		2.0	13.75 ± 1.59^{b}	16.49 ± 0.95^{c}	10.64 ± 0.96^{d}
	brassinozole (BRZ)	0.1	9.90 ± 0.95^{d}	13.96 ± 0.80^{d}	8.08 ± 0.85^{e}
		0.5	8.98 ± 0.96^{d}	12.05 ± 0.86^{e}	6.39 ± 0.50^{f}
		1.0	6.41 ± 1.10^{e}	9.64 ± 0.91^{f}	5.85 ± 0.84^{f}
		2.0	4.53 ± 0.84^{f}	8.62 ± 0.67^{f}	3.85 ± 0.73^{g}
Starch content (mg/g, DW)	2, 4-epibrassinolide	CK (0)	6.52 ± 0.06^{e}	4.13 ± 0.09^{c}	3.09 ± 0.08^{de}
		0.1	6.90 ± 0.08^{c}	4.36 ± 0.05^{b}	3.34 ± 0.02^{c}
		0.5	7.24 ± 0.05^{a}	4.84 ± 0.11^{a}	3.70 ± 0.03^{a}
		1.0	7.00 ± 0.07^{b}	4.44 ± 0.02^{b}	3.41 ± 0.03^{b}
		2.0	6.75 ± 0.14^{d}	4.12 ± 0.06^{c}	3.13 ± 0.06^{d}
	brassinozole	0.1	6.22 ± 0.04^{f}	3.99 ± 0.11^{d}	3.05 ± 0.07^{e}
		0.5	6.04 ± 0.08^{g}	3.87 ± 0.10^{e}	2.86 ± 0.06^{f}
		1.0	5.84 ± 0.07^{h}	3.57 ± 0.06^{f}	2.63 ± 0.04^{g}
		2.0	5.53 ± 0.05^{i}	3.42 ± 0.05^{g}	2.34 ± 0.05^{h}
Non-structural carbohydrate (NSC) (mg/g, DW)	2, 4-epibrassinolide	CK (0)	16.75 ± 0.97^{d}	18.26 ± 1.32^{d}	11.17 ± 0.60^{e}
		0.1	19.11 ± 1.10^{c}	21.81 ± 1.00^{c}	15.38 ± 0.88^{c}
		0.5	26.24 ± 1.33^{a}	29.75 ± 1.59^{a}	20.40 ± 0.96^{a}
		1.0	22.14 ± 0.95^{b}	26.00 ± 2.29^{b}	17.69 ± 1.05^{b}
		2.0	20.50 ± 1.67^{c}	20.61 ± 1.00^{c}	13.76 ± 0.97^{d}
	brassinozole	0.1	16.11 ± 0.97^{de}	17.95 ± 0.79^{d}	11.12 ± 0.89^{e}
		0.5	15.02 ± 1.02^{e}	15.91 ± 0.93^{e}	9.25 ± 0.52^{f}
		1.0	12.25 ± 1.14^{f}	13.21 ± 0.88^{f}	8.48 ± 0.88^{f}
		2.0	10.05 ± 0.87^{g}	12.04 ± 0.70^{f}	6.19 ± 0.75^{g}

Data are presented as mean ± standard error of the mean. Different small letter in the same column means significant difference at $P < 0.05$; DW – dry weight

der the 0.5 mg/L treatment (Table 6). Compared with the control, the 0.5 mg/L EBR treatment increased chlorophyll *a*, chlorophyll *b*, and total chlorophyll contents by an average of 20.64, 32.77, and 25.67%, respectively. Chlorophyll content decreased with an increase in BRZ concentration rate.

DISCUSSION

Antioxidant enzymes, such as SOD, POD, and catalase (CAT), can remove the damage of free radicals that accumulate in cells to biofilms during plant senescence to delay plant senescence (Zhang et al. 2024). Studies have shown that root activity can affect the senescence of leaves, and thus, delaying leaf senescence is possible by increasing root activity (Muchow and Carberry 1989). Chlorophyll is the primary substance for photosynthesis and plays an important role in preventing oxidative damage. Chlorophyll degradation is the most significant sign of leaf senescence (Hauenstein et al. 2016). Studies have shown that EBR treatment delays leaf senescence by enhancing the activity of antioxidant enzymes and increasing chlorophyll content in crops (Lu et al. 2019). Wang et al. (2020) determined that EBR delayed the senescence of kiwifruit by reducing the respiration rate, maintaining the integrity of the mitochondrial membrane and increasing the

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Table 6. Effects of exogenous 2,4-epibrassinolide and brassinozole on the chlorophyll content

Item	Treatment	Concentration (mg/L)	Period		
			flower	grain-filling	mature
Chlorophyll *a* (mg/g)	2, 4-epibrassinolide (EBR)	CK (0)	2.27 ± 0.19^{bc}	2.85 ± 0.21^{c}	1.86 ± 0.20^{c}
		0.1	2.47 ± 0.18^{b}	2.99 ± 0.15^{b}	1.99 ± 0.19^{b}
		0.5	2.78 ± 0.12^{a}	3.21 ± 0.20^{a}	2.36 ± 0.10^{a}
		1.0	2.47 ± 0.17^{b}	3.13 ± 0.20^{ab}	2.19 ± 0.12^{ab}
		2.0	2.36 ± 0.11^{b}	2.94 ± 0.15^{bc}	1.97 ± 0.09^{c}
	brassinozole (BRZ)	0.1	2.10 ± 0.21^{c}	2.47 ± 0.11^{d}	1.51 ± 0.14^{d}
		0.5	1.97 ± 0.16^{cd}	2.30 ± 0.12^{de}	1.41 ± 0.15^{de}
		1.0	1.82 ± 0.13^{d}	2.20 ± 0.15^{e}	1.22 ± 0.23^{e}
		2.0	1.69 ± 0.19^{e}	2.01 ± 0.19^{f}	1.09 ± 0.14^{f}
Chlorophyll *b* (mg/g)	2, 4-epibrassinolide	CK (0)	1.63 ± 0.19^{c}	1.87 ± 0.07^{c}	1.58 ± 0.19^{c}
		0.1	1.93 ± 0.19^{b}	2.06 ± 0.17^{b}	1.86 ± 0.13^{ab}
		0.5	2.31 ± 0.19^{a}	2.36 ± 0.16^{a}	2.06 ± 0.27^{a}
		1.0	2.00 ± 0.19^{b}	2.26 ± 0.12^{a}	1.76 ± 0.20^{b}
		2.0	1.65 ± 0.13^{c}	2.01 ± 0.21^{bc}	1.61 ± 0.15^{c}
	brassinozole	0.1	1.47 ± 0.19^{cd}	1.73 ± 0.12^{d}	1.45 ± 0.16^{d}
		0.5	1.32 ± 0.16^{d}	1.49 ± 0.11^{e}	1.38 ± 0.16^{de}
		1.0	1.19 ± 0.15^{e}	1.31 ± 0.12^{f}	1.31 ± 0.18^{e}
		2.0	1.07 ± 0.14^{f}	1.19 ± 0.11^{f}	1.06 ± 0.12^{f}
Chlorophyll (*a* + *b*) (mg/g)	2, 4-epibrassinolide	CK (0)	3.90 ± 0.26^{c}	4.72 ± 0.21^{c}	3.44 ± 0.24^{d}
		0.1	4.40 ± 0.31^{b}	5.05 ± 0.07^{b}	3.85 ± 0.24^{bc}
		0.5	5.09 ± 0.22^{a}	5.57 ± 0.21^{a}	4.42 ± 0.29^{a}
		1.0	4.47 ± 0.24^{b}	5.39 ± 0.16^{a}	3.96 ± 0.29^{b}
		2.0	4.01 ± 0.12^{c}	4.95 ± 0.15^{b}	3.58 ± 0.15^{c}
	brassinozole	0.1	3.57 ± 0.29^{d}	4.20 ± 0.08^{d}	2.96 ± 0.13^{e}
		0.5	3.29 ± 0.25^{de}	3.79 ± 0.11^{e}	2.79 ± 0.21^{ef}
		1.0	3.00 ± 0.04^{e}	3.50 ± 0.19^{f}	2.53 ± 0.31^{f}
		2.0	2.77 ± 0.20^{f}	3.20 ± 0.23^{g}	2.15 ± 0.23^{g}

Data are presented as mean ± standard error of the mean. Different small letter in the same column means significant difference at $P < 0.05$

activity of antioxidant enzymes. Zhu et al. (2023) demonstrated that EBR could delay the degradation of chlorophyll and its derivatives by reducing the activity of enzymes related to the chlorophyll degradation pathway and decrease the accumulation of jasmonic acid and abscisic acid in leaves, delaying the senescence of Chinese cabbage leaves. The current study showed that an appropriate concentration of EBR could increase the SOD and POD activities and the chlorophyll content of leaves, indicating that an appropriate EBR treatment could delay the senescence of Tartary buckwheat functional leaves. This finding may be attributed to the application of EBR enhancing the activity of antioxidant enzymes and, consequently, the effect of antioxidant enzymes on scavenging free radicals in cells, reducing the damage of free radicals to organelles, such as chloroplasts, maintaining the chlorophyll content at a high level, and ultimately delaying the senescence of Tartary buckwheat leaves. Nazir et al. (2021) showed that EBR treatment could increase the root activity of tomatoes. In the present study, EBR treatment increased the root activity of Tartary buckwheat, and this finding was consistent with the above results, which could be attributed to exogenous EBR being able to control the cell cycle process of the root meristem and play a regulatory role during differentiation. EBR treatment can accelerate the proliferation and differentiation of the root meris-

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tem and promote root cell elongation and lateral root growth, increasing root activity (Heyman et al. 2013) and further indicating that appropriate EBR treatment can delay the senescence of Tartary buckwheat.

Seed setting rate is an important index for measuring crop yield (Guo et al. 2021). Studies have shown that EBR is involved in plant flowering regulation, pollen development, and other life processes (Kutschera and Wang 2012), affecting the development of floral organs. EBR exhibits some characteristics of auxin and gibberellin, which can promote vegetative growth, flower bud differentiation and growth, and plant development (Gagné et al. 2006). During the reproductive growth stage, the appropriate concentration of EBR treatment can promote the increase of grain number and weight per plant, increasing the seed setting rate (Zhang et al. 2023b). The current study found that the appropriate concentration of EBR treatment increased the seed setting rate and pollen viability of Tartary buckwheat, which might be due to the EBR treatment in this study being able to delay the senescence of Tartary buckwheat leaves, augment the "source," further increase the NSC content of Tartary buckwheat leaves, and realise transport from "source" to "sink," promoting flower bud formation and improving seed setting (Liang et al. 2023). The results of the BRZ treatment also confirmed this conclusion.

Chen et al. (2022) determined that the application of EBR could increase rice yield. Fang et al. (2020) found that EBR treatment increased wheat yield. The current study showed that the appropriate EBR treatment significantly increased Tartary buckwheat's grain weight and yield, and the effect of the 0.5 mg/L treatment was the best. This result might be due to the appropriate concentration of EBR treatment increasing the chlorophyll content of Tartary buckwheat leaves, delaying leaf senescence, and increasing dry matter accumulation. It might also be due to EBR increasing the root activity of Tartary buckwheat and promoting the absorption of rhizosphere soil nutrients, thus promoting the growth of the aboveground part, further augmenting its "source," increasing NSC content, and promoting NSC transport to increase seed setting rate and grain weight, and ultimately, increase yield. The results of the EBR inhibitor BRZ treatment further confirmed the view above. In the current study, the yield of Tartary buckwheat began to decrease with the further increase of EBR treatment concentration. This finding is consistent with the results of Gao et al. (2017), and it may be related to the concentration-dependent regulation of EBR on the growth and development of Tartary buckwheat. In this study, our findings showed that the appropriate concentration of exogenous EBR treatment (0.5 mg/L) can promote the seed setting rate and increase the yield of Tartary buckwheat, which can be recommended for use in the production of Tartary buckwheat.

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