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Research Article

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Posted Date: November 17th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11573138/v1

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Additional Declarations: No competing interests reported.

Exogenous brassinolide at different concentrations modulates growth development and physiological indices in *Capsicum annuum* L. pepper

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Abstract: Brassinolide (BR), as an emerging plant growth regulator, has shown significant effectiveness in promoting plant growth, enhancing stress resistance, and improving crop quality. This study used the seeds of the pepper variety 'Changxian Tianxia' as experimental materials to investigate the effects of different concentrations of brassinolide on the growth and development of chili peppers. Five treatment groups were set up with brassinolide concentrations of 0 $mg \cdot L^{-1}$ (CK), 0.1 $mg \cdot L^{-1}$, 0.2 $mg \cdot L^{-1}$, 0.3 $mg \cdot L^{-1}$, and 0.4 $mg \cdot L^{-1}$. Each treatment group had three replicates, and the growth and physiological indicators of the plants were measured to explore the effects of brassinolide on the growth and development of peppers. The experimental results showed that brassinolide can significantly enhance the growth vitality of pepper plants, effectively increase chlorophyll content, enhance peroxidase (POD) activity, and reduce malondialdehyde content; Among them, brassinolide at a concentration of 0.3 $mg \cdot L^{-1}$ has the most outstanding effect in promoting pepper growth. This study explores the mechanism by which different concentrations of brassinolide affect the growth and development of peppers, providing a scientific basis for high-yield and

high-quality cultivation of peppers and promoting the application of brassinolide in agricultural production.

Keywords: Peppers (*Capsicum annuum* L.), brassinolide, mass concentration, seed treatment, seedling growth and development

1 Introduction

Peppers (*Capsicum annuum* L.) belongs to the Solanaceae family and is an annual herbaceous plant. Its global planting area has exceeded 5 million hectares, forming a characteristic industrial belt in southwestern China (such as Guizhou and Sichuan) and Southeast Asian countries(Li et al. 2023). This crop is rich in capsaicin, vitamin C, and carotenoids, and is not only an important spice, but also has potential for development in the fields of functional foods and medicine(Iqbal et al. 2023). As an important economic crop widely cultivated worldwide, it occupies a key position in agricultural production due to its rich nutritional value and wide consumer demand(Barboza et al. 2022). With the continuous development of modern agriculture, how to effectively improve the yield and quality of peppers has become a research hotspot (Dludla et al. 2023).

Brassinolide is a new type of plant growth regulator. As a substance that can promote the growth and development of crops, it can be widely used in rice, wheat, potatoes, vegetables, strawberries, fruits, cotton, hemp, flowers and other crops(Lu et al. 2024). It can enhance the cold and drought resistance of crops, reduce the impact of crop diseases and insect pests, pesticide, fertilizer, and freezing damage, and also improve crop quality and yield(Zhao et al. 2024). It is an environment-friendly regulator in line with the development of new green agriculture. It is also regarded as the sixth category of plant hormones, with low toxicity and high efficiency(Cheon et al. 2013;Hu et al. 2016;Mu et al. 2022).

Numerous studies have shown that lower concentrations of brassinolide can promote plant growth and enhance plant resistance(Divi et al. 2009). In terms of rice, it has an improving effect on the emergence rate, seedling success rate, root growth,

dry matter quality of seedlings, and seedling strength index of rice straw substrate dry cultivation (Bajguz et al. 2009;Nakashita et al. 2003); 0.01% brassinolide water solution can significantly increase yield and enhance the cold resistance of rice; When cotton seeds are soaked with brassinolide at an appropriate concentration (0.01-0.02 $mg \cdot L^{-1}$), the germination potential and root activity are significantly enhanced. When the field spray dose is 0.02-0.04 $mg \cdot L^{-1}$, the growth of cotton plant height, fruit branches and vegetative branches is promoted (Chu et al. 2016); Spraying 0.01% soluble brassinolide solution three times during the bud stage, initial flowering stage, and peak flowering stage can promote cotton nutrition and reproductive growth, achieving a 14.38% increase in seed cotton yield (Liao et al. 2017;Sun et al. 2019). Soaking peanuts in 0.1 $mg \cdot L^{-1}$ brassinolide can increase germination rate by 62.7%, and BR treatment of stems and leaves during seedling stage can promote growth indicators such as seedling height and root length (Meng et al. 2020); Under drought stress, the application of brassinolide to maize seedlings can increase biomass, enhance root vitality, photosynthetic pigment content, and photosynthetic performance, increase antioxidant enzyme activity and proline content, reduce MDA content, and thus improve drought resistance (Sun et al. 2025). In the Hoagland nutrient solution cultivation system in the greenhouse, spraying 28 high brassinolide at a concentration of 0.05 $mg \cdot L^{-1}$ on 5-leaf tobacco seedlings can increase seedling strength index, root total length, surface area, and volume, and improve net photosynthetic rate. However, when the concentration exceeds 0.4 $mg \cdot L^{-1}$, it will have an inhibitory effect (Liu et al. 2023).

China is a large agricultural country, and due to the large-scale use of pesticides and fertilizers, the space for increasing crop yields is becoming smaller and smaller. The application of brassinolide can improve the utilization of water resources, fertilizers, and light by crops, and obtain high returns at low cost (Della et al. 2022;Kour et al. 2021). Research on brassinolide has been continuously deepening both domestically and internationally, with foreign studies focusing on revealing its molecular mechanism for promoting pepper growth and development (Ahammed et al.

2020). It has been found that brassinolide can regulate hormone balance in peppers, promote the synthesis and signal transduction of hormones such as auxin and cytokinin (Shen et al. 2023), affect cell division, elongation, and differentiation, and thus promote pepper growth and development(Xu et al. 2025). Domestic research extensively involves the application effects of brassinolide on various crops, such as foliar spraying of appropriate concentrations of brassinolide during the flowering period of alfalfa(Yuan et al. 2016), which can significantly improve multiple indicators of its growth stage and seed yield; In the cultivation of Danshen in Sichuan Province(Jiang et al. 2023), brassinolide also has important effects on its growth and development, yield, and effective components. These studies provide important references for the application of brassinolide in pepper cultivation.

This study used pepper seeds as experimental materials, based on the low and efficient growth promoting characteristics of brassinolide. The germination of pepper seeds and the growth and development process of seedlings were studied to explore its effects on chili seed germination and seedling growth and development. The aim was to screen for the optimal concentration of brassinolide suitable for chili growth, providing a theoretical basis for cultivating robust pepper seedlings and a reference for other crop production. Providing ideas and methods for the study of brassinolide in the growth and development of chili peppers can help to gain a deeper understanding of its mechanism of action in plant growth, promote its widespread application in agricultural production, and help improve the economic benefits of pepper cultivation.

1. Materials and Methods

Plant materials and treatments

The experiment was conducted in the Biology Laboratory of Huaibei Normal University from February to May 2025. The experimental materials were purchased from the Chinese market, and the chili variety was' ChangxianTianxia '

Select pepper seeds of the same variety to ensure their purity and vitality. The

brassinolide used in the experiment is a commercially available product with clear active ingredients. Select plump and pest free chili seeds, rinse them with clean water, and soak them in warm water for 6-8 hours to promote seed germination. Remove the soaked seeds and drain them for later use. The experiment set up 5 groups of treatments (T1 (CK), T2, T3, T4, T5). The content of brassinolide in T1 (CK) group was 0 mg; the content of drug in T2 group was 0.1 mg; the content of drug in T3 group was 0.2 mg; the content of drug in T4 group was 0.3 mg; and the content of drug in T5 group was 0.4 mg. After weighing the drug, dissolve it thoroughly in 1000 g of clean water and stir evenly, and implement corresponding treatments for each group. Take 15 culture dishes and lay 2 layers of moist filter paper inside. Place 20 seeds evenly in each dish, with 3 replicates per group.

Determination of the physiological index

During the seed germination period (1-7 days), record the number of germinated seeds daily (based on the criterion of the embryo root breaking through the seed coat) to calculate the seed germination rate and vigor. When the chili seedlings grow to a specific stage (such as the three leaf and one heart stage), observe the indicators of "seedling height, stem thickness, and leaf number" based on the investigation method of chili agronomic traits. The chlorophyll content in leaves was detected using a reagent kit combined with UV visible spectrophotometry; The activity of peroxidase (POD) was determined according to Zhang Zhiliang's method (Zhang et al.1990); The content of malondialdehyde (MDA) was determined using the thiobarbituric acid (TBA) method (Liu et al.2010).

Statistical analysis

The data are presented as the means of replications (plants), data were analyzed with IBM SPSS 25.0 and Excel 2010. All statistically significant differences were tested at the $P \leq 5\%$ level(Liu et al. 2024;Liu et al. 2025).

Results

The effect of different treatments on the germination of chili seeds

From Table 1 and Figure 1A, it can be seen that the average germination rate of the control group (T1, 0 mg . L^{-1}) was 61.5%, while the T2-T5 group treated with brassinolide showed a significant increase in germination rate, indicating that brassinolide has a promoting effect on pepper seed germination. There are differences in the effects of different concentrations, with T4 (0.3 mg . L^{-1}) having the highest average germination rate of 93.5% and an average germination number of 18.7; The average germination rate of T3 (0.2 mg . L^{-1}) was 91.5%, which also showed excellent performance. From this, it can be concluded that appropriate concentrations (0.1-0.4 mg . L^{-1}) of brassinolide can effectively promote the germination of chili tissue culture materials, and a concentration of 0.3 mg . L^{-1} has the most prominent effect on increasing germination number and germination rate, significantly better than the control group without brassinolide application (T1).

The effect of different treatments on the main growth indicators of chili peppers

During the growth process of chili peppers, the differences in agronomic traits between the treatment groups with different concentrations of brassinolide gradually became apparent. According to Table 2, in terms of seedling height, the treatment group showed T4>T5>T3>T2>T1 (CK), with T4 significantly higher than the other treatments. This indicates that under the action of this concentration of brassinolide, the height growth advantage of chili seedlings is extremely prominent. As the control group, T1 had the lowest seedling height, indicating that the growth of chili seedlings was limited when brassinolide was not applied. There were significant differences ($P<0.05$) among the other treatments, reflecting that different concentrations of brassinolide have a regulatory effect on the height growth of pepper seedlings, and appropriate concentrations can promote seedling height growth; The stem thickness shows T4>T3=T2>T5=T1 (CK). The T4 treatment group had the largest stem thickness, indicating that this concentration of brassinolide has the best promoting effect on the thickening and growth of chili stems. T3 and T2 have the same stem thickness and are significantly larger than T1 and T5, indicating that the concentration of brassinolide in these two treatment groups can effectively promote stem thickness

172 growth, but the effect is slightly inferior to T4. The maximum leaf length is shown as
173 T4>T3>T2>T5>T1 (CK), and there is a significant difference between the groups
174 ($P<0.05$); The maximum leaf width showed T4>T3>T5>T2>T1 (CK), and there were
175 significant differences among the other treatments ($P<0.05$); In terms of leaf number,
176 only the T4 and T3 treatment groups had 6 leaves, significantly higher than the other
177 treatment groups ($P<0.05$).

178 This indicates that spraying different concentrations of brassinolide has different
179 effects on agronomic traits during the growth stage of chili peppers. Within the
180 experimental concentration range, specific concentrations of brassinolide have a
181 positive effect on promoting the growth of chili plants. Among them, the T4 treatment
182 group showed the best performance in multiple indicators, which can provide
183 reference for optimizing the concentration of brassinolide application in the future. By
184 precisely regulating the concentration of brassinolide, it is expected to cultivate
185 pepper plants with better growth and agronomic traits, thereby improving pepper yield
186 and quality. At the same time, it also reminds us to strictly control the concentration
187 of brassinolide in practical applications to avoid affecting the growth of chili peppers
188 due to improper concentration.

189 **Changes in stem length of chili peppers**

190 Figure 1B shows the growth of chili seedlings treated with different
191 concentrations of brassinolide. Table 3 shows the changes in stem length of chili
192 peppers treated with different concentrations of brassinolide. According to Table 3,
193 under treatment with different concentrations of brassinolide (BR), the growth length
194 of chili seedlings was higher than that of the control group, and there was no
195 significant change within 20 days. During the growth stage of chili peppers, the
196 application of brassinolide can promote stem growth of chili seedlings, and different
197 concentrations of brassinolide have varying effects on the length of chili stem growth.
198 Among the five concentrations set, T4 treatment with a mass concentration of 0.3 $mg \cdot L^{-1}$
199 had the best effect on stem growth of chili seedlings. After 30 and 40 days of
200 application, the increase in stem length was significantly higher than that of the

control group. When the mass concentration exceeds 0.3 $mg \cdot L^{-1}$, the increase in stem height tends to decrease.

The effect of changes in the concentration of brassinolide on the relative content of chlorophyll

Figure 2 shows the relative chlorophyll content of chili seedlings under the variation of brassinolide concentration. By analyzing the bar chart data, it can be concluded that different concentrations of brassinolide have varying effects on the chlorophyll content of chili leaves. Among them, the T4 treatment group had the highest relative chlorophyll content, significantly higher than the other groups. The overall trend is that within a certain concentration range, as the concentration of brassinolide increases, the relative content of chlorophyll in pepper leaves shows an initial increase followed by a decrease. This indicates that a suitable concentration of brassinolide (0.3 $mg \cdot L^{-1}$) can promote chlorophyll synthesis in pepper leaves, while the promotion effect weakens when the concentration is too high or too low.

The effect of changes in the concentration of brassinolide on physiological and biochemical indicators

Table 4 shows the peroxidase activity and malondialdehyde content under different concentrations of brassinolide treatment. Peroxidase is an oxidoreductase widely present in animals, plants, and microorganisms. It is the core enzyme of the plant antioxidant system, and its increased activity helps to clear hydrogen peroxide and maintain cellular redox balance. From the perspective of growth rate, the T4 group showed the largest increase, reaching 7.47%, significantly higher than other groups, indicating that this concentration of brassinolide has the best effect on enhancing POD activity. The T3 and T5 groups showed the same increase of 3.80%, while the T2 group showed an increase of 3.33%. Although these three groups did not show the same increase as the T4 group, they also demonstrated a certain promoting effect. In summary, brassinolide has a promoting effect on the increase of POD activity, and the concentration corresponding to T4 has the best effect.

Malondialdehyde is a membrane lipid peroxidation

A decrease in the content of the product usually means a reduction in cell membrane damage. Based on the data, it is speculated that brassinolide treatment may enhance plant stress resistance mechanisms and reduce membrane lipid peroxidation, with T3 treatment being the most effective in reducing malondialdehyde content. Compared with T1 (CK), the content of malondialdehyde showed a decreasing trend with changes in treatment, with T3 showing the largest decrease and T5 the smallest. Indicating that different treatments (changes in the concentration of brassinolide) have inhibitory effects on membrane lipid peroxidation, and some treatments have significant inhibitory effects.

Correlation analysis between physiological and biochemical indicators and growth indicators under different concentrations of brassinolide treatment

According to the data analysis results in Table 5, the following conclusions can be drawn: chlorophyll content is significantly positively correlated with the seedling height, stem thickness, and maximum root length of chili peppers, indicating that chili growth can increase chlorophyll content; POD activity is positively correlated with seed germination, but significantly negatively correlated with seedling height, indicating that moderate POD activity is beneficial for seed germination but excessive POD activity may inhibit seedling growth; The MDA content is significantly negatively correlated with seed germination and growth indicators, confirming that membrane lipid peroxidation can inhibit the growth and development of chili peppers. Overall, T4 treatment has the most significant effect in reducing MDA content and can effectively alleviate membrane lipid peroxidation damage.

Discussion

Brassinolide is a highly efficient and broad-spectrum plant growth regulator with auxin like functions. Under suitable cultivation conditions, it can significantly affect the growth and development process of plants (Nolan et al. 2017; Zhang et al. 2021). In this study on chili peppers, brassinolide showed multiple promoting effects on the

growth and development of plants. Photosynthesis is crucial for the growth and development of plants. Brassinolide can effectively enhance the efficiency of photosynthesis in plants by promoting the synthesis of photosynthetic pigments (Yuan et al. 2017). Research has found that the application of brassinolide can maintain the integrity of pepper leaf cell structure, stabilize its morphology and function, enhance leaf antioxidant capacity, delay leaf aging, prolong green retention time (Anjum et al. 2011), and ultimately increase leaf chlorophyll content and enhance photosynthesis (Zhang et al.2008).In addition, this experiment also revealed a concentration dependent dual regulatory effect of brassinolide on pepper growth, namely low concentration promotion and high concentration inhibition, which is consistent with the conclusion of Qin Xiao et al.'s study on tobacco seedlings(Qin et al.2023). Overall, exogenous brassinolide has a positive regulatory effect on the growth of chili peppers. However, in practical applications, the concentration and method of brassinolide application need to be optimized and adjusted based on specific planting conditions such as soil fertility and climate environment to achieve the best regulatory effect on chili pepper growth and development.

Peroxidase is an important protective enzyme in plants. Its activity changes can sensitively reflect the physiological state and stress resistance of plants. Brassinolide can promote the synthesis of peroxidase in plant cells by activating the expression of related genes (Bajguz et al.2010). In cellular metabolism, harmful substances such as superoxide anions and hydrogen peroxide are constantly produced, and their accumulation can damage cells. The increased peroxidase can timely remove harmful substances (Yu et al.2020), effectively delay cell aging, maintain normal physiological functions of cells, and create favorable internal conditions for plant growth and development. The plant cell membrane is an important structure for maintaining life metabolism. When plants encounter adversity such as low temperature, high salt, the cell membrane undergoes membrane peroxidation, and malondialdehyde is one of the products of membrane peroxidation. The content of malondialdehyde is negatively correlated with the degree of membrane peroxidation.

Excessive content can damage the structure and function of cell membranes, leading to cell damage. Brassinolide can regulate the fluidity and stability of cell membranes, enhancing their resistance to adversity(Li et al.2022).

This study found that different concentrations of brassinolide have a significant impact on the growth of peppers. A suitable concentration (such as 0.3 $mg \cdot L^{-1}$) can promote the growth of chili plants. From the appearance, it can significantly increase plant height, stem thickness, and leaf number, making the plants more robust. However, excessive concentrations of brassinolide (such as 0.4 $mg \cdot L^{-1}$), although superior to the control group in certain growth indicators, may have adverse effects on the physiological balance of plants, leading to certain growth inhibition. In actual agricultural production, the concentration of brassinolide should be reasonably selected according to the variety, growth environment, and growth stage of chili peppers, in order to fully exert its role in promoting growth and improve the yield and quality of peppers.

Acknowledgements

This work was supported by the Open Project of National Engineering Research Center of Tree breeding and Ecological restoration (LMYZKY2023001), supported by 2024 National College Student Innovation and Entrepreneurship Training Program Project (202410373052), 2023 Anhui Province College Students Innovation and Entrepreneurship Training Program (S202310373010S), supported by 2019 Huaibei Normal University's newly introduced doctoral teachers' research startup fund （03106059）, and supported by 2023 Anhui Province Watermelon and Melon Biological Breeding Engineering Center Open Project Fund （AHXTKF2023003）.

Author Contribution

Chun Liu was responsible for experimental design and manuscript writing, Xibao Xu, Jinjin Li and Xingwang Zhang implemented the experimental content, and corrected and revised the paper.

Data availability

All relevant data can be found within the manuscript and its supporting materials.

Declaration

Ethics approval

The plant material used in this study (chili seeds) is a traditional local variety widely planted in southern China. The collection of plant samples is carried out in accordance with local and national guidelines and regulations. The plant materials studied were collected from cultivated sources with the consent of local farmers/owners and do not involve endangered or protected species. Therefore, this study does not require specific licenses or collection permits. All methods comply with relevant institutions, national and international plant guidelines research.

Consent to participate

Not applicable.

Consent to publish

Not applicable.

Competing interests

The authors declare that there is no conflict of interest.

References Cited

Ahammed G.J., Li X., Liu A., Chen S. 2020. Brassinosteroids in plant tolerance to abiotic stress. J. Plant Growth Regul. 39:1451-1464. https://doi.org/10.1007/s00344-020-10098-0.

Anjum SA, et al. 2011. Brassinolide application improves the drought tolerance in maize through modulation of enzymatic antioxidants and leaf gas exchange. J Agron Crop Sci. 197:177-185. https://doi.org/10.1111/j.1439-037X.2010.00459.x.

Bajguz A., Hayat S. 2009. Effects of brassinosteroids on the plant responses to environmental stresses. Plant Physiology and Biochemistry. 47(1):1-8. https://doi.org/10.1016/j.plaphy.2008.10.002.

340 Bajguz A. 2010. An enhancing effect of exogenous brassinolide on the growth and
341 antioxidant activity in Chlorella vulgaris cultures under heavy metals stress.
342 Environ Exp Bot. 68:175 – 179. https://doi.org/10.1016/j.envexpbot.2009.11.003.

343 Barboza GE, García CC, Bianchetti LB, Romero MV, Scaldaferro M. 2022.
344 Monograph of wild and cultivated chili peppers (Capsicum L., Solanaceae).
345 PhytoKeys. https://doi.org/10.3897/phytokeys.200.71667. PMID: 36762372;
346 PMCID: PMC9881532.

347 Cheon J, Fujioka S, Dilkes BP, Choe S. 2013. Brassinosteroids regulate plant growth
348 through distinct signaling pathways in Selaginella and Arabidopsis. PLoS One.
349 8(12):e81938. https://doi.org/10.1371/journal.pone.0081938. PMID: 24349155;
350 PMCID: PMC3862569.

351 Chu SH, Li L, Zhu WD. 2016. The effect of 0.01% brassinolide water solutio-n on
352 rice growth, yield, and quality [J]. Hubei Agricultural Science, 55 (24): 6445-6447.

353 Della R.F., Piacentini D., Fattorini L., Girardi N., Bellanima D., Falasca G., Altamura
354 M.M., Betti C. 2022. Brassinosteroids mitigate cadmium effects in arabidopsis root
355 system without any cooperation with nitric oxide. Int. J. Mol. Sci. 23:825.
356 https://doi.org/10.3390/ijms23020825.

357 Divi U. K., Krishna P. 2009. Brassinosteroid: a biotechnological target for enhancing
358 crop yi-eld and stress tolerance. New Biotechnology. 26(3-4):131-136.
359 https://doi.org/10.1016/j.nbt.2009.07.006.

360 Dludla PV, Cirilli I, Marcheggiani F, Silvestri S, Orlando P, Muvhulawa N,
361 Moetlediwa MT, Nkambule BB, Mazibuko-Mbeje SE, Hlengwa N, Hanser S,
362 Ndwandwe D, Marnewick JL, Basson AK, Tiano L. 2023. Bioactive Properties,
363 Bioavailability Profiles, and Clinical Evidence of the Potential Benefits of Black
364 Pepper (Piper nigrum) and Red Pepper (Capsicum annum) against Diverse
365 Metabolic Complications. Molecules. 28(18):6569. https://doi.org/
366 10.3390/molecules28186569. PMID: 37764345; PMCID: PMC10534530.

Hu Y, Xia S, Su Y, Wang H, Luo W, Su S, Xiao L. 2016. Brassinolide Increases Potato Root Growth In Vitro in a Dose-Dependent Way and Alleviates Salinity Stress. Biomed Res Int. 2016:8231873. https://doi.org/10.1155/2016/8231873. Epub 2016 Oct 10. PMID: 27803931; PMCID: PMC5075642.

Iqbal B, Hussain F, Khan MS, Iqbal T, Shah W, Ali B, Al Syaad KM, Ercisli S. 2023. Physiology of gamma-aminobutyric acid treated Capsicum annuum L. (Sweet pepper) under induced drought stress. PLoS One. 18(8):e0289900. https://doi.org/10.1371/journal.pone.0289900. PMID: 37590216; PMCID: PMC10434925.

Li GC, Xu H, Liu ZB, et al. 2023. The Spread of Chili in the World [J]. Journal of Tropical Crops, 44 (07): 1307-1316.

Li M, Zhang Y, Xu X, Chen Y, Chu J, Yao X. 2022. The combined treatments of brassinolide and zeaxanthin better alleviate oxidative damage and improve hypocotyl length, biomass, and the quality of radish sprouts stored at low temperature. Food Chem X. 15:100394. https://doi.org/10.1016/j.fochx.2022.100394. PMID: 36211765; PMCID: PMC9532720.

Li C, Fu S, Li F, et al. 2021. The effect of high brassinolide on the growth and development of tobacco seedlings [J]. Shandong Agricultural Science, 53 (8): 107-111.

Liao S, Tan XM, Li MY, et al. 2017. The effect of different brassinolide treatments on rice seedling growth in straw substrate [J]. Journal of Jiangxi Agricultural University, 39 (5): 851-858.

Liu C, Guo Y, Li H. et al. 2024. Establishment of Shoot-tip Regeneration System of Watermelon[J].HortScience,59(9):1419-1421.https://doi.org/10.21273/HORTSCI18094-24.

Liu, C., & Li, J. 2025. Transcriptomic Profiling of the Salt Tolerance Response in

Roots of the Punica granatum ‘Fuanyihao’. J. Amer. Soc. Hort. Sci., 150(1), 1-9. https://doi.org/10.21273/JASHS05446-24.

Liu JY. 2010. Experimental Course in Plant Physiology [M]. Beijing: Higher Education Press, 72-75.

Liu X., Zhu Q.W., Liu W.T., Zhang J. 2023. Exogenous brassinosteroid enhances zinc tolerance by activating the phenylpropanoid biosynthesis pathway in citrullus lanatus L. Plant Signal. Behav.18:2186640. https://doi.org/10.1080/15592324.2023.2186640.

Lu Y, Wang B, Zhang M, Yang W, Wu M, Ye J, Ye S, Zhu G. 2024. Exogenous Brassinolide Ameliorates the Adverse Effects of Gamma Radiation Stress and Increases the Survival Rate of Rice Seedlings by Modulating Antioxidant Metabolism. Int J Mol Sci. 25(21):11523. https://doi.org/ 10.3390/ijms252111523. PMID: 39519076; PMCID: PMC11546918.

Jiang YY, Su JH, Zhang ZZ, et al. 2023. The effect of brassinolide on the growth, development, and quality of Salvia miltiorrhiza [J]. Modern Agricultural Science and Technology, (12): 67-73.

Kour J., Kohli S.K., Khanna K., Bakshi P., Sharma P., Singh A.D., Ibrahim M., Devi K., Sharma N., Ohri P., et al. 2021. Brassinosteroid signaling, crosstalk and, physiological functions in plants under heavy metal stress. Front. Plant Sci.12:608061. https://doi.org/10.3389/fpls.2021.608061.

Meng L, Du MW, Li YB, et al. 2020. The effect of several plant growth promoters on cotton growth and the application of defoliant ripening agents [J]. China Cotton, 47 (11): 16-21.

Mu DW, Feng NJ, Zheng DF, Zhou H, Liu L, Chen GJ, Mu B. 2022. Physiological mechanism of exogenous brassinolide alleviating salt stress injury in rice seedlings. Sci Rep. 12(1):20439.https://doi.org/10.1038/s41598-022-24747-9. Nakashita H., Yasuda M., Nitta T., et al. 2003. Brassinosteroid functions in a broad range of

421 disease resistance in tobacco and rice. The Plant Journal. 33(5):887-898.
422 https://doi..org/10.1046/j.1365-313x.2003.01675.x.

423 Nolan T, Chen JN, Yin YH. 2017. Cross-talk of brassinosteroid signaling in
424 controlling growth and stress responses[J]. Biochemical Journal,
425 474(16):2641-2661.

426 Qin X, Wang ZF, Tang XM, et al. 2023. The effect of brassinolide on the growth and
427 development of tobacco seedlings. Journal of Hunan University of Arts and
428 Sciences (Natural Science Edition), 35 (02): 67-72.

429 Shen XM, Wang N, Ren SW, et al. 2023. Introduction of Propionyl Brassinolide and
430 Its Application in Regulating Pepper Growth and Yield Increase [J]. Shandong
431 Chemical Industry, 52 (12): 75-77,81.

432 Sun X, Jiang XY, Yao CT, et al. 2019. Application of three different structures of
433 brassinolide in cotton [J]. Chinese Journal of Agronomy, 35 (11): 121-126.

434 Sun SA, He FL, Yao XF, et al. 2019. Brassinolide can enhance the drought resistance
435 of maize seedlings [J]. Chinese Journal of Plant Physiology, 55 (6): 829-836.

436 Sun S, Zhao X, Shi Z, He F, Qi G, Li X, Niu Y, Zhou W. 2025. Exogenous
437 24-Epibrassinolide Improves Low-Temperature Tolerance of Maize Seedlings by
438 Influencing Sugar Signaling and Metabolism. Int J Mol Sci.
439 26(2):585.https://doi.org/10.3390/ijms26020585. PMID: 39859301; PMCID:
440 PMC11765667.

441 Xu QS, Yan YL, Wang HF, et al. 2025. The effect of applying brassinolide in
442 advance on the growth, development, and yield of early rice [J]. Journal of Nuclear
443 Agriculture,39 (4): 817-826.

444 Yu J, Mou Y, Luo ZL, et al. 2020. Study on the effect of brassinolide on the growth,
445 development and quality of Panax notoginseng [J]. Chinese Herbal Medicine, 43
446 (7): 1541-1546.

447 Yuan Z. 2016. The effect of foliar spraying of brassinolide on the growth,

development, and seed yield of alfalfa [J]. Journal of Grassland Science, 24 (04): 879-885.

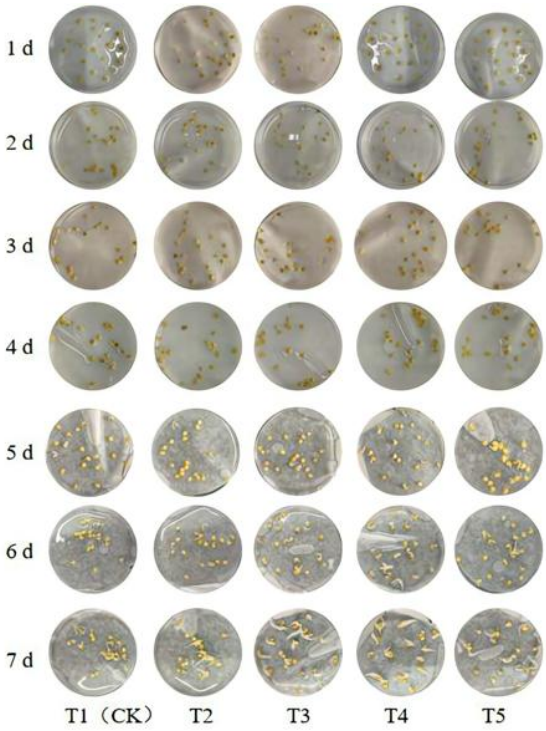
Yuan XY, Zhang LG, Huang L, Yang HJ, Zhong YT, Ning N, Wen YY, Dong SQ, Song XE, Wang HF, Guo PY. 2017. Spraying Brassinolide improves Sigma Broad tolerance in foxtail millet (Setaria italica L.) through modulation of antioxidant activity and photosynthetic capacity. Sci Rep. 7(1):11232.https://doi.org/10.1038/s41598-017-11867-w.PMID:28894251;PMCID: PMC5593917.

Zhang ZL.1990. Guidelines for Plant Physiology Experiments [M]. Beijing: Higher Education Press, 154-155.

Zhang H, Sun X, Dai M. 2021. Improving crop drought resistance with plant growth regulators and rhizobacteria: Mechanisms, applications, and perspectives. Plant Commun.3(1):100228.https://doi.org/10.1016/j.xplc.2021.100228.

Zhang MC, et al. 2008. Brassinolide alleviated the adverse effect of water deficits on photosynthesis and the antioxidant of soybean (Glycine max L.) Plant Growth Regul. 56:257–264. https://doi.org/10.1007/s10725-008-9305-4.

Zhao X, He F, Qi G, Sun S, Shi Z, Niu Y, Wu Z. 2024. Transcriptomic and Physiological Studies Unveil that Brassinolide Maintains the Balance of Maize's Multiple Metabolisms under Low-Temperature Stress. Int J Mol Sci. 25(17):9396. https://doi.org/10.3390/ijms25179396.



(A)



(B)

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478 **Fig.1 The effects of different concentrations of Brassinolide on Chili Pepper growth. (A)**
479 **Germination of pepper seeds under different mass concentration BR treatments, (B) Growth**
480 **of pepper seedlings under different mass concentrations of brassinolide treatment**

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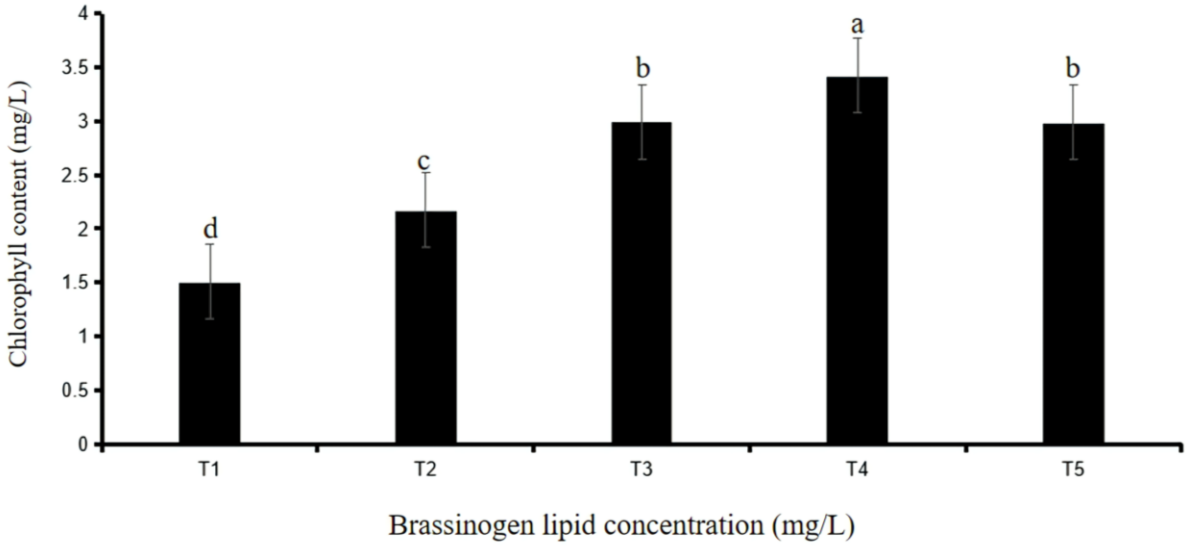


Fig.2 Relative content of chlorophyll in tissue cultured seedlings under different mass concentration BR treatments

505 **Table 1 Germination numbers of chili seeds under different concentration treatments**

Treatments	The number of germinated seeds in the first group	The number of germinated seeds in the second group	The number of germinated seeds in the third group	Average germination number	Average germination rate
T1(CK)	13/20	12/20	12/20	12.3	61.5%
T2	19/20	19/20	14/20	17.3	86.5%
T3	19/20	19/20	17/20	18.3	91.5%
T4	19/20	18/20	19/20	18.7	93.5%
T5	15/20	18/20	20/20	17.7	88.5%

506 Note：The content of brassinolide in T1 (CK) group was 0 mg; the content of drug in T2 group
507 was 0.1 mg; the content of drug in T3 group was 0.2 mg; the content of drug in T4 group was 0.3
508 mg; and the content of drug in T5 group was 0.4 mg. After weighing the drug, dissolve it
509 thoroughly in 1000 g of clean water and stir evenly, and implement corresponding treatments for
510 each group. Take 15 culture dishes and lay 2 layers of moist filter paper inside. Place 20 seeds
511 evenly in each dish, with 3 replicates per group.
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Table 2 Main agronomic traits of chili pepper after 20 days of growth under different concentrations of treatment

Treatments	seedling height/cm	stem diameter/mm	Maximum leaf length/cm	Maximum leaf width/cm	Maximum Root Length/cm	Maximum number of blades
T1（CK）	4.40±0.26^{e}	1.6±0.06^{c}	2.23±0.15^{d}	1.37±0.12^{e}	5.17±0.12^{c}	4
T2	4.73±0.21^{d}	1.9±0.10^{b}	2.63±0.11^{c}	1.57±0.06^{d}	6.57±0.05^{b}	5
T3	5.47±0.21^{c}	1.9±0.06^{b}	2.77±0.06^{b}	1.73±0.15^{b}	5.67±0.10^{c}	6
T4	6.53±0.15^{a}	2.2±0.06^{a}	3.27±0.13^{a}	2.0±0.16^{a}	7.13±0.11^{a}	6
T5	6.23±0.21^{b}	1.6±0.06^{c}	2.57±0.12^{c}	1.67±0.15^{c}	4.37±0.10^{d}	4

Note: Different letters in the same column indicate significant differences between treatments ($P<0.05$).

543 **Table 3 Changes in stem length of chili seedlings under different concentration treatments**

Treatments	Stem growth length (cm)		
	20 d	30 d	40 d
T1（CK）	4.4[a]	6.9[a]	8.1[a]
T2	4.7[a]	7.1[b]	9.0[b]
T3	5.4[b]	7.8[c]	10.3[c]
T4	6.5[c]	8.8[d]	11.0[d]
T5	6.2[c]	8.4[d]	10.3[c]

544 Note: Different letters in the same column indicate significant differences between treatments
545 ($P<0.05$).
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564 **Table 4 Peroxidase activity and malondialdehyde content under different concentrations of**
565 **treatment**

Treatments	POD activity	MDA content(umol/L FW)
T1(CK)	77.52^{c}	0.3998^{a}
T2	80.10^{b}	0.0953^{c}
T3	80.46^{b}	0.0623^{d}
T4	83.31^{a}	0.0853^{c}
T5	80.46^{b}	0.1842^{b}

566 Note: Different letters in the same column indicate significant differences between treatments
567 ($P<0.05$).
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Table 5 Correlation analysis between physiological and biochemical indicators and other factors under different concentration treatments

Dimension	M±SD	Seed germination rate	seedling height	stem diameter	Maximum Root Length
Chlorophyll content	2.62±0.734	0.884**	0.92**	0.78*	0.85*
POD activity	80.37±1.93	0.675*	-0.88**	-0.65	-0.72
MDA content	0.165±0.129	-0.965**	0.95**	0.82*	0.89*

Note: * At the 0.05 level (double tail), the correlation is significant; At the 0.01 level (double tail), the correlation is significant. M±SD represents the sample mean plus or minus the standard deviation.