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EFFECTS OF BRASSINOLIDE ON THE PHYSIOLOGICAL CHARACTERISTICS OF MAIZE (*ZEA MAYS* L.) CULTIVARS UNDER SALT STRESS

Biao Ji, Jie Li[1], Yina Xia and Zan Li*

School of Agronomy and Horticulture, Jiangsu Vocational College of Agriculture and Forestry, Jurong, 212400, China

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Abstract

Effects of brassinolide on the physiological characteristics of maize under salt stress were investigated. Effect of exogenous application of brassinolide on the antioxidant system, endogenous hormone levels, sodium and potassium ion contents of the plants were measured in a salt-tolerant maize cultivar, Xianyu 335, and a salt-sensitive maize cultivar, Dongnong 253. Brassinolide induced better alleviation of physiological damage in different salt-sensitive maize cultivars under salt stress treatment. Exogenous administration of brassinolide was found to increase the antioxidant enzyme activity, modulate the endogenous hormone levels, and promote potassium and sodium excretion by the plants. The effect was more significant in salt-sensitive maize cultivars.

Introduction

Salt stress inhibits crop yields and affects plant growth and development. More than 800 million hectares of land worldwide are affected by salinization (Liang *et al.* 2018, Feng *et al.* 2019). Salt stress is considered one of the serious threats that restrict crop production in arid and semi-arid regions (Rahat *et al.* 2011, Kordrostami *et al.* 2016). Maize (*Zea mays*) is an important food and economic crop widely cultivated worldwide. However, its tolerance to salt stress is poor. Besides the breeding of salt-tolerant corn varieties, this problem can be solved through the use of suitable exogenous additives to improve the salt tolerance of corn plants.

Salt stress reduces the stability of the plant cell membrane and inhibits the photosynthetic rate and the activity of antioxidant enzymes. At the same time, salt stress causes an imbalance in ion absorption by plants, especially sodium and potassium ions, inducing a toxic ion effect. This, in turn, leads to the accumulation of reactive oxygen species (ROS) such as superoxide and hydrogen peroxide, causing oxidative damage (Yang and Guo 2018). In addition, salt stress can significantly reduce the accumulation of plant biomass, causing serious effects on growth and development, and then affecting crop yield (Romero-Aranda *et al.* 2001). Many agronomic or physiological methods have been used to mitigate the adverse effects of salt stress on plants and induce salt tolerance. The use of plant growth regulators has been identified as an effective approach to this end (Shahzad *et al.* 2017). Brassinosteroids (BRs), a newly developed category of plant hormones, play a major role in regulating plant growth and development. Brassinolide (BL) is a highly active synthetic analog to BRs and is crucial in the regulation of salt tolerance in plants (Kim *et al.* 2009). Exogenous BL showed a better promoting effect on the germination and seedling growth of seeds under salt stress, while it was closely associated with the accumulation of auxin (Ashraf *et al.* 2010, Liu *et al.* 2014). Brassinolide mitigated the damage caused by salt stress by improving the accumulation of zeatin riboside (ZR, a plant cytokinin), enhancing the activity of antioxidant

*Author for correspondence: <174429926@qq.com >. [1]Nanjing Jiahua Agricultural Development Co. Ltd., Jurong, 212400, China

enzymes, and regulating the ratio of Na^+/K^+ ions. Brassinolide has been applied in the cultivation of rice and tomatoes with satisfactory outcomes (Wu *et al.* 2017, Su *et al.* 2020).

Maize is often subjected to salt stress because of the increasing salinization of arable land, which results in reduced yield and quality of maize. This present study was aimed to investigate the effect of exogenous BL on the salt tolerance of maize seedlings. Xianyu-335 (a salt-tolerant corn variety) and Dongnong-253 (a salt-sensitive variety) used in the experiment. The effects of exogenous BL on the antioxidant enzyme systems, endogenous hormone levels, and Na^+/K^+ ion contents of the corn seedlings under salt stress were systematically analyzed to elucidate the mechanism by which BL affected the salt tolerance of corn from a physiological perspective.

Materials and Methods

The Xianyu-335 and Dongnong-253 seeds were provided by the College of Agriculture at Northeast Agricultural University. Brassinolide was purchased from Shanghai Canspec Scientific and Technology Company. Full Xianyu-335 and Dongnong-253 seeds of equal sizes were selected, treated with 75% ethanol, soaked in sterile water for 24 hrs, and sowed in vermiculite pots for germination in an illumination incubator at 25 and 16℃ in a 16/8 hrs cycle. After the seedlings were unearthed for 10 days, they were transplanted into 1/2 Hoagland nutrient solution for hydroponic treatment. The solution was replaced every 3 days, and the temperature and light cycle remained unchanged for 6 days. Thereafter, the seedlings were treated with salt (NaCl; 150 mmol/l) to simulate salt stress. Each seedling was then treated with 5 ml of BL (0.15 mg/l) applied through foliar spraying. The seedlings were divided into three groups: the CK group (no treatment), the NaCl group (treated only with salt), and the NaCl + BL group (treated with salt and BL foliar spray). The leaf blades were collected for analysis 0, 6, 12, 24, and 48 hrs after treatment.

The superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) activities and malondialdehyde (MDA) content of the plants were analyzed using a reagent test kit manufactured by Suzhou Comin Biotechnology Company (Suzhou, China) following the manufacturer's protocols.

The indole-3-acetic acid (IAA), gibberellin (GA), zeatin riboside (ZR), and abscisic acid (ABA) levels of the plants were analyzed using an enzyme-linked immunosorbent assay performed using a reagent test kit provided by Shanghai Mlbio Company (Shanghai, China) following the manufacturer's protocols. The Na^+/K^+ ion content of the corn plants was analyzed using an inductively coupled plasma optical emission spectrometer (Optima 5300DV, Perkin-Elmer, Massachusetts, USA).

Each test was performed at least three times ($n \geq 10$). Data were presented as means ± standard errors of mean. GraphPad Prism 5 was used for data significance tests and mapping.

Results and Discussion

Figure 1 presents the physiological indices related to the antioxidant systems of the corn seedlings exposed to different treatments. Malondialdehyde (MDA) is the final product of membrane lipid peroxidation and reflects the levels of oxidative damage in plant cells to a certain extent. The MDA contents of the Xianyu-335 seedlings treated with NaCl alone increased gradually over time, whereas the Xianyu-335 seedlings treated with BL exhibited significantly lower MDA accumulation. After 24 hrs of treatment, the MDA content of the Xianyu-335 seedlings treated with BL was 40.17% which is lower than that of the Xianyu-335 seedlings treated with NaCl alone. In the Dongnong-253 seedlings, BL treatment significantly inhibited MDA accumulation. After 24 hrs of treatment, the Dongnong-253 seedlings treated with BL showed 64.84% less MDA content than those treated with NaCl alone.

Superoxide dismutase (SOD), Peroxidase (POD), and Catalase (CAT) are antioxidant enzymes that play active roles in scavenging free radicals and ROS generated by salt stress. Under salt stress, SOD and POD activities first increased and then decreased in both maze varieties. In the Xianyu-335 seedlings treated with NaCl alone, the SOD activity peaked to 87.25 U/g 12 hrs after treatment; in those treated with BL, SOD activity peaked to 114.88 U/g 24 hrs after treatment. The

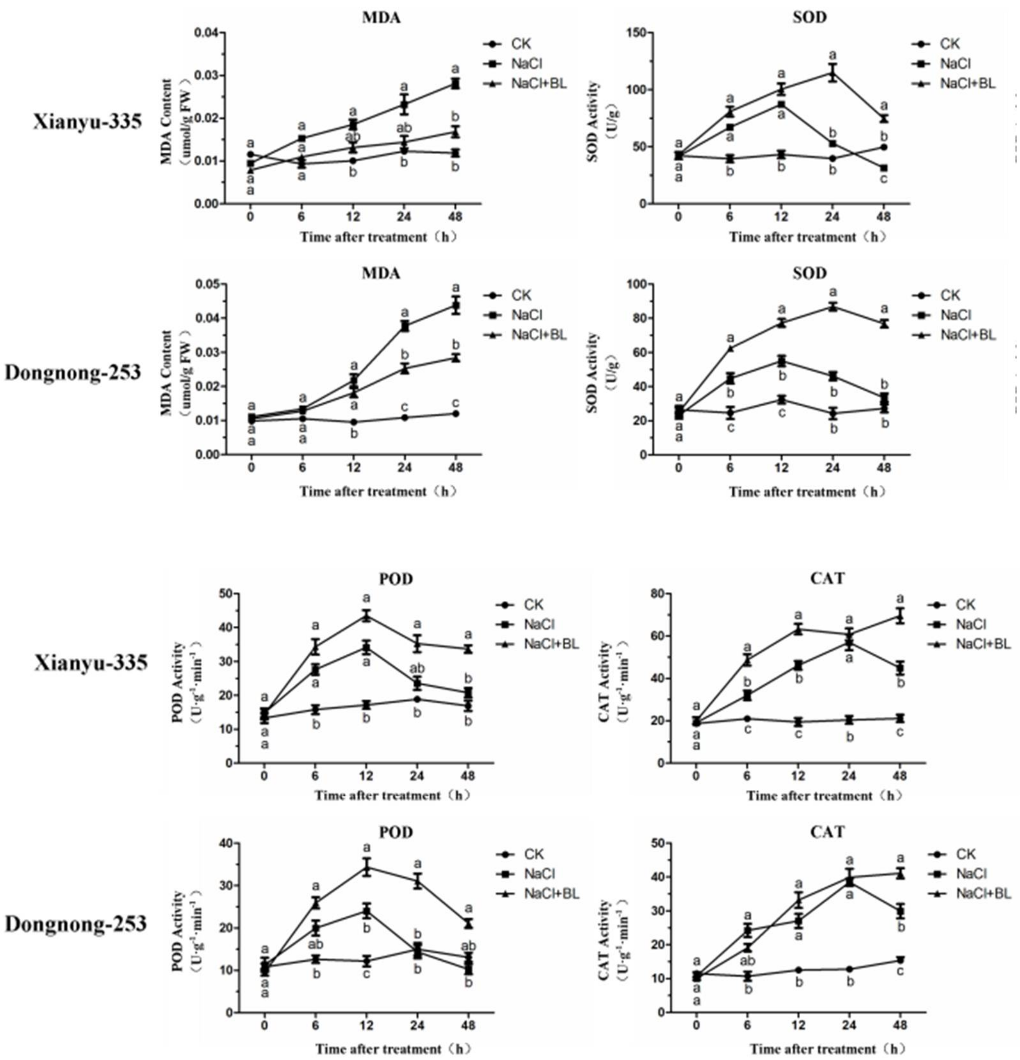

Fig. 1. Effects of exogenous BL on the antioxidant system of different salt-sensitive maize seedlings under salt stress. Different lowercase letters indicate significant differences ($P < 0.05$) in values between different treatment groups at the same time point.

SOD activity in the Dongning-253 seedlings followed a similar trend to that in the Xianyu-335 seedlings. The seedlings treated with BL had higher SOD activity levels. Similar findings were obtained in the POD activity analysis. Further, 24 hrs after treatment with salt, the Xianyu-335 seedlings treated with BL showed 49.55% higher POD activity than those treated with NaCl alone. The Dongnong-253 seedlings treated with BL also showed 116.14% higher POD activity than those treated with NaCl alone. Exogenous BL improved the CAT activity of both maize varieties, but the improvement was greater in the Xianyu-335 seedlings than Dongnong-253 seedlings. The Xianyu-335 seedlings treated with BL showed higher CAT activity than those treated with NaCl alone. Specifically, the peak CAT activity of the seedlings treated with BL (48 hrs after treatment) was 69.57 $U \cdot g^{-1} \cdot min^{-1}$. The Dongnong-253 seedlings treated with BL showed significantly (37.33%) higher CAT activity than those treated with NaCl alone 48 hrs after treatment.

The IAA, GA, ZR, and ABA contents of the seedlings exposed to different treatments were analyzed to explore the effect of exogenous BL on the endogenous hormone levels of the Xianyu-335 and Dongnong-253 corn seedlings under salt stress (Fig. 2). The IAA content of the Xianyu-335 seedlings decreased gradually over time under salt stress. The Xianyu-335 seedlings treated with BL showed nonsignificantly higher IAA content than those treated with NaCl alone. The IAA content of the Dongnong-253 seedlings treated with NaCl decreased significantly over time. The Dongnong-253 seedlings treated with BL showed significantly higher IAA content than those treated with NaCl alone 12, 24, and 48 h after treatment, with a peak IAA content of 223 ng/g (12 hrs after treatment).

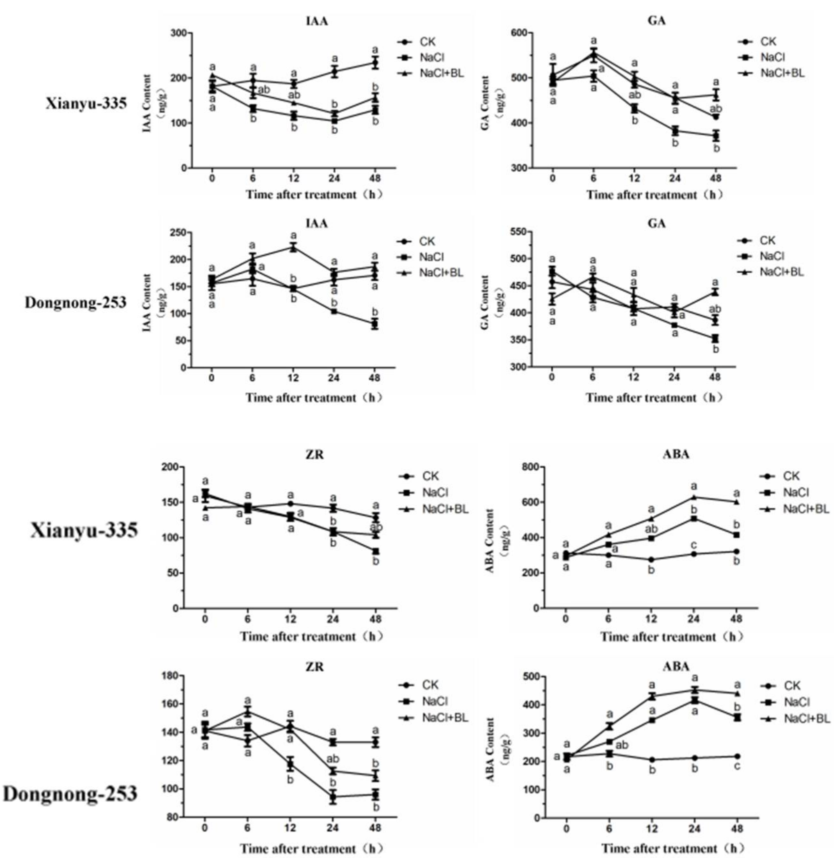

Fig. 2. Effects of exogenous BL on the endogenous hormone contents in different salt-sensitive maize seedlings under salt stress. Different lowercase letters indicate significant differences ($P < 0.05$) in values between different treatment groups at the same time point.

The GA contents of the seedlings of both the varieties which were left untreated decreased considerably over time. The GA content of the seedings treated with NaCl decreased over time. The Xianyu-335 and Dongnong-253 seedlings treated with BL showed 24.39 and 24.50% higher GA content, respectively, than those treated with NaCl alone 48 hrs after treatment. The ZR contents of the seedlings of both the varieties treated with NaCl decreased over time. Although BL treatment improved the ZR contents of the seedlings to a certain extent, but the improvement was nonsignificant. The ABA content of both varieties increased over time after treatment. The ABA content of the Xianyu-335 seedlings was higher than that of the Dongnong-253 seedlings. The ABA contents of the seedlings of both the varieties treated with NaCl alone peaked 24 hrs after treatment. Exogenous BL treatment profoundly improved their ABA contents. The ABA contents of the Xianyu-355 and Dongnong-253 seedlings treated with BL peaked to 629.33 and 452.67 ng/g, respectively, 24 hrs after treatment.

Results are presented in Fig. 3 showed that under salt stress, the Na^+ contents of both the varieties increased over time; 48 hrs after treatment. The Na^+ content of the Xianyu-335 and Dongnong-253 seedlings was 9.22 and 10.37 mg/g, respectively. Brassinolide treatment inhibited Na^+ accumulation considerably, particularly in the Dongnong-253 seedlings. The K^+ contents of both the varieties treated with NaCl alone first increased and then decreased. After 48 hrs, the K^+ content of the Xianyu-335 and Dongnong-253 seedlings was 7.27 and 6.99 mg/g, respectively. Brassinolide treatment increased K^+ accumulation considerably. The Xianyu-253 seedlings treated with BL exhibited higher K^+ content than those treated with NaCl alone after 12, 24, and 48 hrs, peak at 12 hrs after treatment. The Dongnong-253 seedlings treated with BL showed higher K^+ content compared with those treated with NaCl alone and those left untreated 24 and 48 hrs after treatment, peaking at 24 hrs after treatment.

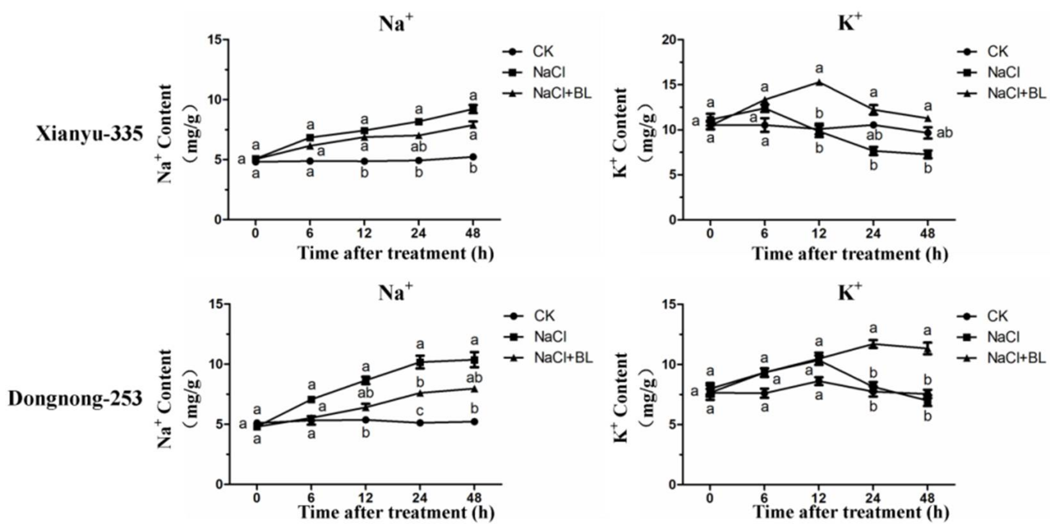

Fig. 3. Effects of exogenous BL on the endogenous Na^+/K^+ ion contents in different salt-sensitive maize seedlings under salt stress. Different lowercase letters indicate significant differences ($P < 0.05$) in values between different treatment groups at the same time point.

Soil salinization negatively affects corn growth, yield, and quality. BL treatment is effective in enhancing salt tolerance in plants (Zhu 2016, Tanveer *et al.* 2018). BRs significantly mitigate the physiological damage caused by salt stress and improve the photosynthetic properties of corn;

however, no studies have compared the effects of BRs on corn varieties differing in salt tolerance (Wei *et al.* 2018). In the present study the effects of exogenous BL treatment on salt-tolerant Xianyu-335 and salt-sensitive Dongnong-253 corn were compared. Results of the study might serve as a reference in the use of BL for increasing salt tolerance in corn.

Salt stress affects plants by inducing osmotic stress, ion toxicity, and oxidative damage. The production and accumulation of ROS in plants under salt stress can increase significantly, causing peroxidation of the plasma membrane and producing MDA. Meanwhile, plants have antioxidant systems that scavenge ROS and contain antioxidant enzymes such as SOD, POD, and CAT (Fariduddin *et al*. 2013). López-Gómez *et al.* (2016) reported that the administration of BL helps in reducing the level of plant lipid peroxidation, thereby reducing the negative effects exerted by oxidative stress. In the present study, salt stress simulated using NaCl significantly increased MDA accumulation in both the maize varieties. Exogenous BL treatment substantially inhibited MDA accumulation in both the varieties, mitigating oxidative damage induced by salt stress. In pea (*Pisum sativum*), mint (*Mentha canadensis*), and rice, exogenous BL similarly significantly inhibited MDA accumulation in plants under salt stress (Sharma *et al*. 2013, Shahid *et al*. 2014, Çoban and Baydar 2016). Brassinolide treatment effectively increased the SOD, POD, and CAT activities in the Xianyu-335 and Dongnong-253 seedlings, with the increase in the overall enzymatic activity of the Dongnong-253 seedlings being greater than that of the Xianyu-335 seedlings. These findings reflected the active role of BL in regulating the antioxidant systems of salt-sensitive corn varieties. Results of Bajguz's study indicated that exogenous BL increased the SOD and CAT activities of chickpeas (*Cicer arietinum*) under salt stress by 75 and 52%, respectively (Bajguz and Hayat 2009). Under extreme conditions such as salt stress, the exogenous application of BL can trigger the antioxidant defense system of the plant, which in turn reduces the negative effects due to oxidative stress (Wu *et al.* 2017). The reason why BL improves antioxidant enzyme activities in plants is the promotion of antioxidant enzyme gene expression. This view has been preliminarily confirmed in Arabidopsis, but the detailed mechanism in maize requires further exploration (Goda *et al.* 2002).

Plant hormones serve as crucial growth regulators and play a key role in plants' responses to salt stress. Compared with the seedlings left untreated, those subjected to salt stress had lower IAA, GA, and ZR contents in the present study. IAA promotes plant growth (Luo *et al.* 2018), and GA promotes cell division and increases plants' tolerance to abiotic stress (Binenbaum *et al.* 2018), and ZR delays leaf senescence and promotes growth (García-Fortea *et al.* 2020). The decrease in the IAA, GR, and ZR contents of the seedlings treated with NaCl indicated that the seedlings' growth was inhibited considerably by salt stress, and BL treatment improved the levels of all three hormones in the seedlings, particularly in the Dongnong-253 seedlings. Brassinolide upregulates the levels of hormones that promote plant growth, enabling plants to resist salt stress. ABA signaling pathways are crucial to regulating the responses of plants to external abiotic stress (Chen *et al.* 2020). The present investigation revealed that NaCl treatment promoted ABA accumulation in both corn varieties, and BL administration significantly increased ABA accumulation. This indicated that ABA pathways might play a key role in the mechanisms by which BL regulated the responses of plants to salt stress.

Ionic toxicity is a major hazard to plants that is induced by salt stress and usually caused by the accumulation of excess Na^+ ions in plant cells (Dai *et al.* 2018). The study further showed that, NaCl treatment led to a gradual increase in Na^+ content and a decrease in K^+ content in both corn varieties. BL treatment inhibited Na^+ accumulation considerably, particularly in the Dongnong-253 seedlings, and reduced the K^+ content of the seedlings treated with BL compared with the seedlings treated with NaCl alone. Exogenous BL treatment promoted K^+ absorption and Na^+ expulsion, alleviating the cytotoxicity induced by Na^+ ions. This was consistent with the findings reported by Azhar *et al*. (2017), which revealed that BL reduced K^+ efflux by passing through depolarization-activated K^+ channels, thereby alleviating salt toxicity in plants.

In the present study, exogenous BL treatment improved the antioxidant properties, endogenous stress–resistance hormone levels, and Na^+/K^+ ion ratios of two corn varieties differing in salt tolerance under salt stress. This supported BL's potential applications in increasing the salt tolerance of corn plants, especially salt-sensitive varieties.

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