EVALUATING THE EFFECT OF BRASINOLIDE ON THE SALINITY TOLERANCE OF SPRING ONION (Allium fistulosum L.) UNDER SALINE IRRIGATION CONDITIONS

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Abstract

Spring onions are an essential spice in Vietnamese food preparation for its favor and aroma. Tra Vinh, one of the coastal provinces, is greatly affected by climate change and saline intrusion. Hence, this study was conducted to access the effects of Brasinoline (Br) on the growth of Spring onion under normal conditions and salt stress events. The experiment embraced four Br concentrations (0 ppm, 0.05 ppm, 0.1 ppm, and 0.15 ppm) under both fresh water and 3‰ saltwater irrigation conditions, distributed according to the 2-factor Randomized Completely Block Design (RCBD). Preliminary results, with concentrations of Br 0.1 ppm and 0.15 ppm, show a high increase in leaf length, number of leaves/brush, number of shoots, stem diaphragm and plant green weight. Under 3‰ salt water irrigation conditions, Br concentration of 0.15 ppm showed supporting effectively the growth of Spring onions equivalent to that in fresh water.

Keywords: Brassinoline, growth and development, salinity, spring onions.

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ĐÁNH GIÁ ẢNH HƯỞNG CỦA BRASINOLIDE LÊN KHẢ NĂNG CHỊU MẶN CỦA HÀNH ĂN LÁ (Allium fistulosum L.) TRONG ĐIỀU KIỆN TƯỚI MẶN

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Tóm tắt

Hành ăn lá là một loại rau gia vị không thể thiếu trong trong chế biến các món ăn của người Việt Nam do hương vị của nó mang lại. Trà Vinh là một trong những tỉnh ven biển chịu tác động lớn bởi biến đổi khí hậu và xâm nhập mn. Vì vậy, nghiên cứu này được thực hiên đề đánh giá ảnh hưởng của Brasinoline (Br) lên khả năng sinh trưởng của cây hành ăn lá trong điều kiện thường và trong điều kiện stress mặn. Thí nghiệm được thực hiện với 4 nồng độ Br là 0 ppm, 0,05 ppm, 0,1 ppm và 0,15 ppm trong điều kiện tưới nước ngọt và nước mặn 3‰ và được bố thí theo kiểu khối hoàn toàn ngẫu nhiên (RCBD) 2 nhân tố. Kết quả bước đầu cho thấy, khi tưới nước ngọt, với nồng độ 0,1 ppm và 0,15 ppm cho kết quả cao về chiều dài lá, số lá, số tép/bụi, hoành thân và trọng lượng cây. Trong điều kiện tưới nước mặn 3‰ thì nồng độ Brasinoline 0,15 ppm cho thấy khả năng hỗ trợ sự sinh trưởng của cây hành tương đương với khi chỉ canh tác hoàn toàn bằng nước ngọt.

Từ khóa: Brasinoline, độ mặn, hành lá, sự sinh trưởng và phát triển.

1. Introduction

Tra Vinh has a total cultivated land area of more than 141,000 hectares, of which vegetables of all kinds account for over 10% of the area, and green onions are a popular vegetable grown in Tra Vinh. However, in the dry seasons, saltwater intrusion occurs due to climate change, which more and more and directly affects agricultural production, causing harm by reducing crop productivity and quality (Hiep, 2019). Climate change is greatly concerned globally, with the lack of fresh water for farming as well as saltwater intrusion becoming increasingly serious. In farming, using salt water for irrigation affects all major processes such as growth, yield, photosynthesis, protein synthesis and lipid metabolism of plants (El-Mashad & Mohamed., 2012). Salt stress is one of the most severe abiotic stresses, significantly reducing crop productivity. Under salt stress conditions, there is an increase in the concentration of free radicals (ROS) such as superoxide and hydrogen peroxide, which disrupt cellular enzymatic activity. Salt stress also disturbs the stability of cell membranes, leading to reduced plant biomass (Izahrani et al., 2019).

Using the hormone 24-Epibrassinolide has proven to be an effective method to improve salt stress. The 24-Epibrassinolide is an active by-product from the biosynthesis of Brassinolide (Br), which has shown resistance to salt stress significant in plants (Tanveer et al., 2018). Br is a growth hormone and can function effectively in plants as an immunogen when applied at the appropriate concentration and at the right stage of plant development. This substance is involved in plant responses to environmental stressors. Applying Br to crops such as cereals, potatoes, vegetables, fruits, mushrooms and ornamental plants, helps improve crop productivity (Bhardwaj et al., 2006). On strawberries, Br increased fruit yield from 9% to 34% (Salazar, 2006). Brassinosteroid increases total leaf area by 150-180%, the number of leaves, petiole length, dry weight of leaves, petioles and stems. It also significantly increases the total number of flowers and inflorescences per plant compared to with plants not treated with Br (Pipattanawong et al., 1996). It was found to ameliorate the adverse effects of salt stress and thereby increase crop yield. The opposite relationship exists between Na⁺ and K⁺ ion content during salt stress and brassinolide administration may have promoted osmotic adjustment, a lower Na^+/K^+ ratio being a good indication of the ability salt tolerance of plants (Das & Shukla, 2011). Thus, Br opens a new approach to plant resistance to environmental risks (Bajguz & Hayat., 2009).

However, no research has so far been conducted on the effects of Br on the growth of Spring onions under saline irrigation conditions in Tra Vinh. Therefore, to cope with the adverse effects of climate change as well as current saltwater intrusion, research on Br application to Spring onions was conducted with the goal of evaluating its effects on the growth of Spring onions and found the appropriate concentration of Brassinolide for cultivation under conditions of using saline water for irrigation.

2. Materials and methods

2.1. Materials and experimental design

The research was conducted from February 2022 to June 2022 at the Horticulture Experimental and Research camp, Tra Vinh University. The experiment was arranged in a completely randomized block design (RCBD) with 2 factors including 4 Br concentrations (0 ppm, 0.05 ppm, 0.1 ppm and 0.15 ppm) and 2 salt concentrations of 0 ‰ and 3‰. The experiment was carried out in a black PE pot with a pot mouth diameter of 23 cm. Each treatment was repeated 3 times, each repetition consisted of 3 pots, each pot planted 3 onion bushes. Water with salt concentrations of 0‰ and 3‰ in the form of NaCl was watered every day in the morning, Br was sprayed to wet the leaves every 7 days with corresponding concentrations in the experiment.

All data were collected every 7 days. Leaf length: measured from the ground to the top of the highest leaf of the Spring onion; Number of leaves/brush: count all green leaves over 50%; Stem diaphragm: 1 stem accurately marked for measurement and use for later data collection, measured around 1 stem. Fresh weight of leaves and stems: total weight of 3 replicated at harvest.

Table	1.	Exp	oerimental	design
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NaCl	Brassinolide concentration (B)				
concentration	0 ppm	0.05	0.1	0.15	
(A)	0 ppm	ppm	ppm	ppm	
0‰	EP1	EP3	EP4	EP5	
3‰	EP2	EP6	EP7	EP8	



a) Spring onion seedling



b) Experimental design c) Collect data

Figure 1. Materials and Experimental design

2.2. Statistical analysis: The data were analyzed by 2-factor ANOVA analysis of variance to find the difference in the effect of Br on the growth of onion plants under freshwater and 3‰ saline irrigation conditions at the significance level $p \le 1$ % via SPSS v. 20.0. At the same time, the DUNCAN test method was used to compare the average values.

3. Results and discussion

3.1. Effect of Br on number of leaves/brush of Spring onion under fresh water and salty watering conditions

From table 2, it shows that the number of leaves

on the treatment started to differ from 14 days after planting, at this stage the plants started to produce new leaves and there was no statistical difference between EP1, EP2, EP4, EP5, EP6. The treatment with the highest number of leaves/brush was EP3 with 12.5 leaves statistically different from the remaining treatments. In particular, the number of leaves in EP1 (11.2 leaves) and EP8 (11.3 leaves) did not differ at a statistically significant level. At 21 days after planting, the number of leaves of the experimental treatments increased, the highest number of leaves in EP1 (12.9 leaves), EP3 (12.5 leaves) and EP4 (12.6 leaves) did not differ but different from the remaining treatments. The effect of Br on the growth of leaves was clearly evident 28 days after planting and 35 days after planting. At 28 days after planting, the number of leaves/brush in EP1 (13.5 leaves), EP3 (13.5 leaves), EP4 (12.8 leaves) and EP8 (12.8 leaves) was not statistically different 1% significance. The treatment with the lowest number of leaves was EP2 and EP7 which had 11.3 leaves. The number of leaves/brush of Spring onion 35 days after planting had a statistically significant difference in all EPs, in which EP1 (16.5 leaves) was the highest, followed by EP8 (14.7 leaves), EP5 (14.2 leaves) and the lowest is EP2 (12.7 leaves). Two-factor ANOVA analysis also showed a statistically significant interaction effect of Br and irrigation water conditions (salt water and fresh water irrigation) on the number of leaves/brush of Spring onion at different stages (Table 2).

	Days after planting				
Treatment	14	21	28	35	
EP1	11.2b±0.2	12.9a±0.7	13.5a±0.4	16.5a±0.5	
EP2	10.2b±0.2	10.5e±0.7	11.3c±0.4	12.7d±0.4	
EP3	12.5a±0.5	12.5ab±0.1	13.5a±0.4	13.2cd±0.7	
EP4	10.8b±0.7	12.6a±0.2	12.8ab±0.6	13.1cd±0.6	
EP5	10.4b±0.8	10.7e±0.1	11.9bc±0.6	14.2bc±0.1	
EP6	10.7b±0.6	11.7bcd±0.1	12.3bc±0.8	13.5cd±0.6	
EP7	9.4c±0.8	10.7de±0.9	11.3c±0.5	13.1cd±0.2	
EP8	11.3ab±0.9	11.4cde±0.9	12.8ab±0.9	14.7b±1.1	
F (A x B)	**	**	**	**	
CV%	15.1	14.4	10.3	15.2	

 Table 2. Interaction effect between Brassinolide and irrigation conditions on number of leaves/brush of Spring onion

The results of this study show that vegetable plants with essential oils are susceptible to salt stress conditions, so they will decrease in height as well as number of branches, number of leaves and root weight. (Meriem et al., 2014). Studies have shown that salt stress causes osmosis in plants to be affected, disturbing water balance and water transport ultimately affecting various physiological and biochemical processes. Thereby, it reduced plant growth because the energy produced in plants was redirected to maintain water balance instead of providing nutrients for plant growth (Sarker & Oba., 2020). However, in mature leaves, brassinosteroids (BR) stimulated cell expansion, increased leaf index, promoted starch accumulation, enhanced CO2 release through the tricarboxylic acid cycle, and boosted biomass production. The growth promotion by BR may rely on the enhanced supply and utilization of carbohydrates and energy, stimulating both anabolic and catabolic pathways (Schröder et al., 2014). Consequently, BR can help increase the number of leaves in plants.

3.2. Effect of Br on leaf length of Spring onion under fresh and salty watering conditions

When young leaves began to appear, there was

a significant difference in leaf length between EPs 21 days after planting, the highest leaf length is in EP4 (27.5 cm) followed by EP1 (25.9 cm).), EP5 (25.7 cm) was the lowest and EP2 (21.7 leaves). At 28 days after planting, EP4 (29.1 cm) had the highest leaf length and was significantly different from the remaining EPs. EP1 (26.1 cm), EP3 (25.8 cm), EP5 (25.1 cm), EP8 (25.1 cm) had no statistically significant difference. The EPs with low leaf length are EP2 (22.9 cm), EP6 (23.4 cm) and EP7 (23.6 cm). Leaf length continued to increase on day 35 after planting and there was a statistically significant difference compared to other EPs. By 42 days after planting, the leaf length reached a maximum of 34.8 cm in EP4, a difference at the 1% significance level compared to the treatments EP1 (31.8 cm), EP3 (30.8 cm), EP5 (31.1 cm), EP8 (32.8 cm), the treatment with the shortest leaf length was EP2 reaching 28.1 cm and EP7 reaching 28.2 cm (Table 3).

Similarly, a statistically significant interaction effect between Brassinolide and irrigation water conditions [F (A x B)] on leaf length was shown in the two-factor ANOVA analysis (Table 3). This indicates that there was a change in the influence of irrigation water conditions on leaf length parameters depending on the Br concentration used.

Treatment	Days after planting				
Treatment	21	28	35	42	
EP1	25.9b±1.0	26.4b±0.8	28.9a±1.7	31.8b±1.0	
EP2	21.7f±0.3	22.9c±0.9	24.3cd±0.3	28.1d±0.2	
EP3	22.2ef±1.1	25.8b±0.9	25.7b±0.5	30.8bc±1.0	
EP4	27.5a±0.4	29.1a±0.9	29.3a±0.6	34.8a±0.5	
EP5	25.7b±1.3	25.1b±1.0	25.5bc±1.0	31.1bc±0.7	
EP6	25.1bc±0.6	23.4c±1.0	24.7bcd±0.4	28.9cd±0.3	
EP7	23.2de±1.1	23.6c±1.1	24.2d±0.7	28.2d±0.5	
EP8	23.9cd±0.7	25.1b±1.1	25.6b±0.6	32.8b±0.3	
F (A x B)	**	**	**	**	
CV%	14.0	14.3	13.3	14.5	

 Table 3. Interaction effect between Brassinolide and irrigation conditions on leaf length of Spring onion (unit: cm)

From the results of leaf length through the measurements, it shows that when using irrigation water with increased salinity, leaf length will decrease (Kalhoro, 2016). Under conditions of using fresh water and spraying with Br at a concentration of 0.1 ppm (EP4), the longest leaf length was achieved. This may indicate that Br has an effect on increasing leaf length. At the same time, the data also shows that, during cultivation, when using irrigation water with a salinity concentration of 3‰, when spraying Br with a concentration of 0.15 ppm (EP8), leaf length does not differ significantly compared to when completely using fresh water for irrigation (EP1). This result can be explained by the fact that Br can reduce the adverse effects of salt stress on leaf length and biomass (Sharma et al., 2013). By doing so, BR influences the rate of cell division, stimulating cell elongation, which is found to be accompanied by the generation of protons and hyperpolarization of the cell membrane (Zurek et al., 2014). Thus, Br plays a pivotal role in promotion of cell expansion, cell elongation, cell division, and vascular differentiation, and provides protection against various abiotic and biotic stresses (Mohammad et al., 2017).

3.3. Effect of Br on the number of shoots of Spring onion under fresh and salt water irrigation conditions

The results of the number of shoots collected from 14 to 35 days after planting of all treatments increased significantly and there was no increase in data collected after 35 days of planting. Table 4 shows that, after 35 days of planting, the number of shoots in EP1 increased from 3.4 to 7.6 shoots. At the same time, the remaining treatments also had a continuously increasing number of shoots. However, EP2 (saline irrigation) and EP6 (saline irrigation combined with 0.05 ppm Br addition) at 35 days after planting reduced the average number of shoots of Spring onions. This result is due to the negative influence of salt water causing some shoots to die, thus leading to a sharp decrease in the number of shoots in these EPs. Sodium ions compete with essential nutrients such as potassium (K^+), calcium (Ca^{2+}), and magnesium (Mg²⁺) for uptake sites in plant roots. Excessive Na⁺ and Cl⁻ ions created an ionic imbalance within the soil and the plant, disrupting the normal uptake of other essential minerals. This imbalance can lead to nutrient deficiencies and affect overall plant health and productivity (Grattan et. al., 1999).

Tuestan	Days after planting				
Treatment	14	21	28	35	
EP1	3.4bc±0.2	4.5ab±0.5	6.4a±0.1	7.6ab±0.5	
EP2	3.1c±0.2	4.1c±0.4	5.5bc±0.2	4.2d±0.2	
EP3	4.0a±0.1	4.8a±0.3	6.4a±0.3	6.5c±0.7	
EP4	3.6b±0.2	4.5ab±0.2	5.7bc±0.7	6.1c±0.7	
EP5	3.2bc±0.3	4.8a±0.1	6.4a±0.2	8.1a±0.9	
EP6	3,2bc±0,1	4,7a±0,1	6,5a±0,2	5,8c±0.2	
EP7	3.3c±0.3	4.1c±0.2	5.4c±0.7	6.5c±0.8	
EP8	3.4bc±0.1	4.2c±0.3	6.1ab±0.1	6.7bc±0.5	
F (A x B)	**	**	**	**	
CV%	14.1	11.5	13.1	20.5	

 Table 4. Interaction effect between Brassinolde and irrigation water conditions on the number of shoots of Spring onion.

Specifically, the number of shoots of the secondary treatments recorded 14 days after planting was from 3 to 4 shoots. Later on, this number changed and most clearly demonstrated the impact of Br on increasing shoots in the treatment using Br is 6 or more shoots. The highest number of shoots on day 35 was in EP5 using fresh water irrigation combined with spraying 0.15 ppm Br (8.1 shoots), EP 1 irrigated completely with fresh water (7.6 shoots). Statistical difference significant compared to EP3 (6.5 shoots), EP7 (6.5 shoot), EP6 (5.8 shoots) and EP2 (4.2 shoots). However, the saline irrigation treatment using Br at a concentration of 0.15 ppm (EP8) produced a shoot number of 6.7 shoots, which was not statistically different from EP1 (Table 4).

The interaction effect between Br and watering conditions on the number of onion shoots was also shown in the statistical analysis (Table 4). This can show that applying Br adds the ability to help plants withstand saline conditions, specifically 3‰ salinity because BR helps plants withstand saline conditions by minimizing the negative effects of it affects the physiological and biochemical processes in plants (Ashraf et al., 2010). Br improves photosynthetic efficiency by increasing chlorophyll content and stabilizing the photosynthetic apparatus. This leads to better light capture and energy utilization, which supports shoot growth even under salt stress (Fariduddin et al., 2014). And, BR treatment alleviated the negative effects of salinity on shoot growth by enhancing antioxidant enzyme activities and improving photosynthetic efficiency (Ali et al., 2008).

3.4. Effects of Br on the diaphragm of Spring onion under fresh and salt water irrigation conditions

Researched results showed that there were statistically significant differences between treatments across data collection times. When using fresh water for irrigation over time, it showed continuous growth and increases faster than when using 3‰ salt water. Later, the growth-stimulating effect of Br became more and more evident when Br was continuously added every 7 days. This stimulation was further shown through the diaphragm size of the treatments 35 days after planting, when using fresh water for irrigation supplemented with Br specifically at EP3 (2.4 cm), EP4 (2.3 cm) and EP5 (2.7 cm) are statistically significantly different from EP1 (2.1 cm). Accumulation of stress-reducing substances in plants over time when continuously using Br in combination with fertilizer will improve crop quality and yield (Bhardwaj et al., 2006).

The value of diaphragm when using 0.15 ppm Br is 2.4 cm (EP8) higher than EP1 which is 2.1 cm. This result could suggest that Br has a very good effect in helping plants tolerate salty conditions and supporting the growth and development of Spring onion plants.

Treatment	Days after planting			
Ireatment	21	28	35	42
EP1	1.8ab±0.25	1.7bc±0.21	2.0b±0.05	2.1c±0.03
EP2	1.1b±0.06	1.3d±0.05	1.7c±0.06	1.8c±0.06
EP3	1.9a±0.23	2.1a±0.05	2.3a±0.02	2.4b±0.12
EP4	1.7ab±0.16	2.0ab±0.05	2.4a±0.12	2.3b±0.08
EP5	1.7ab±0.06	1.8bc±0.08	1.9b±0.17	2.7a±0.16
EP6	1.3ab±0.03	1.4d±0.03	$1.6c\pm 0.07$	1.9c±0.09
EP7	1.4ab±0.56	1.6c±0.09	1.6c±0.02	2.0c±0.15
EP8	1.3ab±0.07	1.8bc±0.10	2.3a±0.07	2.4b±0.02
F (A x B)	**	**	**	**
CV%	1.9	1.4	16.4	12.4

 Table 5. Interaction effect between Brassinolde and irrigation water conditions on the change in stem diaphragm of Spring onion (unit: cm)

3.5. Effects of Br on the green weight of Spring onion under fresh water and salt water irrigation conditions

The average weight of Spring onions of 3 repetitions of EP1 (using fresh water for irrigation) reached 86.4 g, higher than that when using salt water (EP2), which was 73.3 g. On the other hand, the results in table 6 also show that Br has a positive effect on plant productivity. Spraving The average weight of Spring onion brushes in 3 repetitions of EP1 (using fresh water for irrigation) reached 86.4 g, higher than when using salt water (EP2), which was 73.3 g. On the other hand, the results in table 6 also show that Br has a positive effect on plant productivity. Spraying Br with a concentration of 0.15 ppm (EP4) gave the highest Spring onion weight of 105.6. However, plant weight was lower in EPs treated with lower Br concentrations (0.05 and 0.1 ppm concentrations) and in EP1 (Table 6). This result is because changing the endogenous Br concentration will change the quality of the plant (Vriet et al., 2012).

In general, treatments using fresh water and using fresh water supplemented with Br at different

concentrations all resulted in higher plant weights than when using salt water for irrigation (Table 6). However, in the salt water irrigation combined with Br spraying at different concentrations showed that the average weight of Spring onion was higher than EP2 (salted irrigation but no Br addition). In particular, the treatment using salt water combined with spraying 0.15 ppm Br (EP8) had an average plant weight of 84.3 g that was significantly higher than other salt water treatments, but not different with statistically significant difference compared to green weight in the treatment using fresh water for irrigation (EP1), which was 83.3 g.

The results of 2-factor ANOVA analysis showed a statistically significant interaction effect between Br and irrigation water conditions on the average weight of onion plants at harvest time. This indicates that the effect of salinity on Spring onion yield varies with the Br concentration used (Table 6). The results of this study are completely consistent with research on *Trifolium alexandrinum L* when spraying Brassinolide improved growth and yield when grown on saline soil (Daur & Tatar., 2013).

Treatment		Brassinolide (B)				
(A)	0 ppm	0,05 ppm	0,1 ppm	opm 0,15 ppm A	Average	
NaCl 0‰ (fresh water)	83.3c±1.5	80.0d±1.0	90.0b±1.1	105.6a±1.1	86.4a±0.2	
NaCl 3‰	68.3e±0.5	70.6e±1.0	70.0e±2.0	84.3c±3.0	73.3b±0.2	
Average	69.1d±2.0	75.5c±5.5	82.1b±5.3	92.8a±3.8		

Table 6. Effects of Br and irrigation water conditions
on the average green weight of Spring onion at harvest time (unit: g)

Note: Different letters in the same column represent statistically significant differences at the 1% significance level, CV: coefficient of variation; A. NaCl concentration,; B: B r concentration.

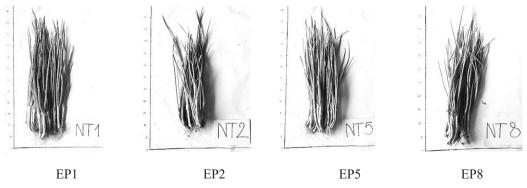


Figure 2. Harvest Spring onions in EPs

4. Conclusion

Saltwater exerts detrimental effects on various growth and productivity parameters of Spring onion plants, including leaf count, leaf length, shoot/ brush, stem diaphragm, and average green weight. However, employing freshwater irrigation alongside supplemental application of Br at a concentration of 0.15 ppm (EP5) demonstrates optimal growth and vield outcomes. Furthermore, when Br is applied at the same concentration (0.15 ppm) alongside irrigation with 3‰ saline water (EP8), yields comparable to those achieved with freshwater irrigation (EP1) are attained. Given Br's beneficial impact on stimulating Spring onion growth and vield, coupled with its ability to mitigate the adverse effects of salt stress in Spring onion cultivation, its incorporation into cultivation practices is warranted. Nonetheless, further investigation is necessary to comprehensively understand the response of Spring onions to prevailing saline conditions.

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