

Effect of Salt Stress on Growth and Mineral Composition of Two Types of Amaranthus (Tolerant Vs. Sensitive) as Alleviated by Foliar Spray with Brassinolide

¹Tartil M. Emam, ¹A.M. Hosni, ¹M. Hewidy and ²Ahmed Ismail

¹Horticulture Department, Faculty of Agriculture, Ain Shams University, Cairo, Egypt

²Horticulture Department, Faculty of Agriculture, Damanhour University, Damanhour, Egypt

Abstract: This study was carried out in the greenhouse of Al-Kanater Al-Khairia Horticulture Research Station during the period of 2021 to 2022 in a pot experiment. The current study was conducted to assess the beneficial effect of Brassinolide (BR) as a plant hormone at 100 ppm on two Amaranthus species (green and red) grown under four levels of salinity stress (control, 2000, 4000 and 7000 ppm) to alleviate salt stress injury. The experiment was a factorial one in a complete randomized design with three replications. Results collected on plant height, number of leaves, chlorophyll (SPAD), fresh and dry weights and leaf mineral content, showed that salinity stress decreased plant growth parameters i.e., plant height, number of leaves/plants, both fresh and dry weights, SPAD reading and leaf mineral content in the both used Amaranthus species. However, the red species was more tolerant than the green one. Also, BR spraying enhanced plant growth and mineral content under salinity stress. It could be concluded that the Brassinolide spraying at 10 ppm could be utilized as a low-cost and environment-friendly effective strategy to mitigate the negative effects of salinity stress on the growth and the quality of Amaranthus.

Key words: Amaranthus • Salinity • Brassinolide • Growth • Mineral composition • Salt stress • Salt tolerant • Salt sensitive • Abiotic stress

INTRODUCTION

Amaranthus genus is a widely spread annual plant in the tropical and subtropical regions. It belongs to Amaranthaceae and has about 70 species in various types, such as cosmopolitan weeds or cultivated plants. In addition, it can be used as a consumed vegetable or ornamental plant [1]. The red Amaranthus (*Amaranthus cruentus*) is considered as a summer annual ornamental plant or popular vegetable with pharmaceutical properties in many countries, it contains many nutrients such as protein, carbohydrate, vitamin A, minerals and many antioxidants [2].

Salinity is a major environmental limiting crop production factor [3]. This phenomenon is particularly expressed in arid and semi-arid regions due to high evaporation and low precipitation.

A group of steroidal hormones known as Brassinosteroids (BRSs) control plant growth at various stages of its life cycle. BRS performs a variety of crucial roles under biotic and abiotic stresses. Under stressful

conditions, BRS regulates a variety of physiological and biochemical processes in plants. Vikram *et al.* [4] observed that BR treatment alleviated the detrimental effects of salt stress by enhancing turfgrass quality and increasing shoot growth and clipping yield in perennial ryegrass (*Lolium perenne*). In a recent study, Bakshi and Sharma [5] reported that Brs overcame the salt stress effect on pea, bean and cucumber plants and enhanced growth attributes and leaf chlorophyll content.

Qin *et al.* [6] reported that leaf chlorophyll content decreased significantly under NaCl stress relative to the control. Talaat and Shawky [7] observed that BR application maintain the ionic balance in plant cell by regulating the uptake of different ions like Na, K, Ca, Mg, N and P. Also, BR enhances the uptake of all ions except Na. Vikram *et al.* [4] found as well that the content of Na was sharply induced by salt stress in the shoot and roots. When exogenous BR was applied, the Na content decreased significantly [8]. The K⁺ content increased in the shoots and roots due to salt stress and exogenous BR

application. Spraying BR at 3 mg/L improved vegetative growth, total chlorophyll (SPAD) and leaf chemical composition when begonia plants [9].

Accordingly, this study aimed to investigate the effect of foliar application with BR on the growth and mineral composition of two *Amaranthus* species to alleviate salt stress under different salinity levels.

MATERIALS AND METHODS

During each of the seasons 2021 and 2022, a pot experiment was conducted in the greenhouse of Horticulture Research Station AlKanater Alkhairia, Kaluobia governorate. Transplants of two different species, green *Amaranthus* (*A. Hybocondriacus*) and red *Amaranthus* (*A. cruentus*) were planted in 25 cm pots with sand and peat moss 2:1 (v/v) medium when they were 6 weeks old on 15th May. The NaCl concentrations were control, 2000, 4000 and 7000 ppm. Half of the plants were treated during salt treatments with foliar application of 100 ppm of Barasinolide (BR) and the other were not treated. Control plants were supplied with tap water. After 60 days from transplanting, three randomly selected plants were used to record plant height from the plant base to the shoot tip, number of leaves was calculated / plant (all the leaves from the tip to the base) and both fresh weight and dry weight of plant. For the determination of chlorophyll content, a SPAD-502 chlorophyll meter (Konica Minolta, Tokyo, Jaban) was used. For estimating mineral composition, the leaves were dried for 24h in oven at 70°C and grounded in a mill and digested with HNO₃

(65%), HClO₄ (70%) and H₂SO₄. An atomic absorption spectrophotometer device was used to read the absorbance to determine Fe, Zn and Mn. Nitrogen content (N %) was determined in dried leaves using the micro-Kjeldahl method, as described by Pregl [10]. Phosphorus (P) content (%) was estimated using the method described by King [11]. Potassium (K) content (%) was determined according to the methods described by Cottenie *et al.* [12]. The experiment was a factorial one in a Complete Randomized Design with three replications. Each replicate was 10 pots all in all a total of 160 pots. Statistical analysis: all obtained data were statistically analyzed as the method of Gomez and Gomez [13].

RESULTS AND DISCUSSION

Plant Height: Results in Table (1) revealed that both salinity stress and Brassinolide (BR) treatment had significant effects on plant height of the two *Amaranthus* genotypes in the two tested seasons. The highest plant height values were recorded in the control treatment of green *Amaranthus* followed by the lowest which used a concentration of NaCl (2000 ppm). On the other side, the shortest plants were recorded in plants exposed to 7000 ppm of NaCl without exogenous application of BR in both green and red *Amaranthus* genotypes in both tested years. Our results agree with those reported by Amalia and Rachmawati [14] who found that salinity stress caused plants to experience osmotic stress which disrupts the water balance resulted in` water loss and gradually reduce cell division and cell elongation. This condition

Table 1: Effect of foliar application with Brassinolide (BR) on plant height (cm) of *Amaranthus hypochondriacus* (green *Amaranthus*) and *Amaranthus cruentus* (Red *Amaranthus*) under different salinity levels during 2021 and 2022 seasons.

Var	Salinity (ppm)	Brassinolide (BR)	Plant height (cm)	
			First season	Second season
Green	Control		32.56a	34.00 a
			30.44 ab	30.56 ab
	2000	Not treated	29.56 b	30.11 b
		Treated	23.78 cde	23.56 cde
	4000	Not treated	25.67 c	26.33 c
		Treated	20.78 f	20.22 f
7000	Not treated	23.89 cd	22.56 cd	
	Treated			
Red	Control		23.67 a	26.33 cde
			23.22 cdef	24.11 cdef
	2000	Not treated	21.89 def	22.89 def
		Treated	21 ef	21.33 ef
	4000	Not treated	24.44 cd	23.67 cd
		Treated	21.89 def	22.56 def
7000	Not treated	24.00 cd	24.67 cd	
	Treated			

Values in the same column followed by the same letter (s) are not statistically different according to Duncan's multiple range tests.

Table 2: Effect of foliar application with Brassinolide (BR) on a number of leaves/plants of *Amaranthus hypochondriacus* (green Amaranthus) and *Amaranthus cruentus* (Red Amaranthus) under different salinity levels during 2021 and 2022 seasons.

Var	Salinity (ppm)	Brassinolide (BR)	Number of leaves / plants	
			First season	Second season
Green	Control		28.11 a	29.44 a
	2000	Not treated	24.33 ab	25.11 abc
		Treated	23.33 abc	23.11 bc
	4000	Not treated	14.89 de	14.00 e
		Treated	16.44 de	16.78 de
	7000	Not treated	11.78 e	12.44 e
		Treated	14.67 de	16.44 e
	Red	Control		23.22 abc
2000		Not treated	24.00 abc	25.67 abc
		Treated	26.00 abc	26.56 ab
4000		Not treated	22.11 bc	22.00 bc
		Treated	21.56 bc	25.78 abc
7000		Not treated	19.00 cd	21.33 cd
		Treated	24.11 ab	24.56 bc

Values in the same column followed by the same letter (s) are not statistically different according to Duncan's multiple range tests.

Table 3: Effect of foliar application with Brassinolide (BR) on chlorophyll (SPAD unite) of *Amaranthus hypochondriacus* (green Amaranthus) and *Amaranthus cruentus* (Red Amaranthus) under different salinity levels during 2021 and 2022 seasons

Var	Salinity (ppm)	Brassinolide (BR)	Chlorophyll (SPAD unite)	
			First season	Second season
Green	Control		33.14 a	34.5 a
	2000	Not treated	28.2b c	28.02 bcde
		Treated	29.67 b	30.5 b
	4000	Not treated	25.18 cde	25.09 def
		Treated	28.2 bc	29.42 bc
	7000	Not treated	16.02 i	16.12 i
		Treated	21.67 fg	22.61 fg
	Red	Control		27.78 bc
2000		Not treated	20.43 gh	20.24 gh
		Treated	24.32 def	25.00 ef
4000		Not treated	17.92 hi	16.96 hi
		Treated	22.47 efg	22.6 fg
7000		Not treated	20.34 gh	17.50 hi
		Treated	25.89 cd	26.98 cde

Values in the same column followed by the same letter (s) are not statistically different according to Duncan's multiple range tests.

results in a reduction in the CO₂ fixation process so that the rate of photosynthesis decreases. This affects the translocation of photosynthates from source to sink and carbohydrate metabolism in leaves, causing a reduction in overall plant growth.

Number of Leaves/Plants: Results in Table 2 showed that the number of red and green Amaranthus leaves were significantly decreased with NaCl treatments as compared to control. (Refer to Table 2 for details) Results here are similar to those obtained by Bellache *et al.* [15] Moreover, plants sprayed with BR showed a higher number of leaves than those without BR treatment. The results agree with those found by Shahbaz and Ashraf [16] who mentioned

that exogenous application of BR as foliar spray increased the growth of wheat plants grown under saline stress also, Ahanger *et al.* [17] reported that BR treatment helped plants to alleviate the deleterious effects of salinity

Chlorophyll Readings (SPAD Units): Salinity stress had a significant negative effect on chlorophyll content as (SPAD units) of the two tested *Amaranthus* species in both seasons (Table 3). Results also revealed that the chlorophyll reading was positively enhanced by BR treatment which mitigated the harmful effect of the salinity stress treatments. The results obtained here are in the same direction as Vazquez *et al.* [18] and Bakshi and Sharma [5].

Table 4: Effect of foliar application with Brassinolide (BR) on the fresh and dry weights of *Amaranthus hypochondriacus* (green Amaranthus) and *Amaranthus cruentus* (Red Amaranthus) under different salinity levels during 2021 and 2022 seasons.

Var	Salinity (ppm)	Brassinolide (BR)	Fresh weight (g)		Dry weight (g)	
			First season	Second season	First season	Second season
Green	Control		15.78 d	17.28 d	7.78 b	13.02 b
	2000	Not treated	11.67 e	12.39 e	7.78 b	9.77 b
		Treated	11.44 e	13.17 e	8.44 b	10.33 b
	4000	Not treated	9.22 ef	9.17 ef	5.89 bc	6.39 bc
		Treated	8.67 ef	10.28 ef	5.89 bc	7.11 bc
	7000	Not treated	5.17 g	6.28 g	3.89 c	3.5 c
		Treated	6.22 fg	5.78 fg	2.44 bc	3.99 bc
	Red	Control		37.10 a	38.28 a	15.44 a
2000		Not treated	34.78 ab	34.44 abc	13.00 a	12.40 c
		Treated	36.33 a	36.28 ab	14.23 a	14.50 bc
4000		Not treated	33.00 ab	36.83 ab	13.11 a	14.78 bc
		Treated	35.45 ab	38.28 a	14.25 a	15.94 b
7000		Not treated	34.11 ab	31.11 bc	14.22 a	14.25 ab
		Treated	34.33 ab	33.61 c	14.78 a	16.67 a

Values in the same column followed by the same letter (s) are not statistically different according to Duncan's multiple range tests.

Table 5: Effect of foliar application with Brassinolide on nitrogen, phosphorus and potassium percentages of *Amaranthus hypochondriacus* (green Amaranthus) and *Amaranthus cruentus* (Red Amaranthus) under different salinity levels during 2021 and 2022 seasons

Var	Salinity (ppm)	Brassinolide (BR)	N %		P%		K%	
			1 st season	2 nd season	1 st season	2 nd season	1 st season	2 nd season
Green	Control		1.55 ab	1.88 ab	0.42 a	0.63 a	4.86 a	4.83 a
	2000	Not treated	1.59 ab	1.58 ab	0.24 cde	0.45 cde	3.71 abc	2.18 abc
		Treated	1.61 ab	1.66 ab	0.28 bcde	0.49 bcde	3.42 bed	2.15 bed
	4000	Not treated	1.92 a	1.82 a	0.34 abc	0.55 abc	3.51 bcd	2.35 bcd
		Treated	1.05 ab	1.11 ab	0.32 abcd	0.53 abcd	3.83 ab	1.46 ab
	7000	Not treated	1.76 ab	1.76 ab	0.31 abcd	0.52 abcd	3.78 abc	2.25 abc
		Treated	1.51 ab	1.5 ab	0.24 cde	0.45 cde	3.51 bcd	1.96 bcd
	Red	Control		1.90 a	2.31 a	0.39 ab	0.60 ab	4.12 ab
2000		Not treated	1.28 ab	1.29 bc	0.2 de	0.41 de	2.41 de	1.70 de
		Treated	0.88 b	1.06 c	0.21 cde	0.42 cde	2.11 e	1.36 e
4000		Not treated	1.03 ab	1.21 c	0.15 e	0.36 e	1.75 e	1.39 e
		Treated	1.16 ab	1.29 bc	0.21 cde	0.42 cde	1.63 e	1.96 e
7000		Not treated	1.66 ab	1.86 ab	0.27 bcde	0.48 bcde	2.57 cde	2.17 cde
		Treated	1.14 ab	1.11 c	0.25 cde	0.46 cde	2.33 de	1.64 de

Values in the same column followed by the same letter (s) are not statistically different according to Duncan's multiple range tests.

Table 6: Effect of foliar application with Brassinolide on iron, zinc and manganese (ppm) content of *Amaranthus hypochondriacus* (green Amaranthus) and *Amaranthus cruentus* (Red Amaranthus) under different salinity levels during 2021 and 2022 seasons.

Var	Salinity (ppm)	Brasinolide (BR)	Fe (ppm)		Zn (ppm)		Mn (ppm)	
			1 st season	2 nd season	1 st season	2 nd season	1 st season	2 nd season
Green	Control		46.38 a	44.47 a	112.37 a	107.73 a	45 ab	53.1 ab
	2000 ppm	Not treated	16.41 ab	17.04 ab	69.23 a	76.37 a	37.98 ab	43.97 ab
		Treated	15.51 ab	15.54 ab	85.07 a	86 a	38.4 ab	42.5 ab
	4000 ppm	Not treated	21.19 ab	19.72 ab	98.75 a	99.53 a	53.85 ab	44.8 ab
		Treated	11.72 b	12.75 b	90.92 a	93.13 a	39.13 ab	40.93 ab
	7000 ppm	Not treated	15.23 b	15.23 b	90.68 a	89.83 a	36.58 ab	38.38 ab
		Treated	15.59 ab	14.93 ab	89.38 a	88.93 a	36.92 ab	38.72 ab
	Red	Control		16.20 ab	20.02 ab	84.85 a	88.62 a	41.95 ab
2000 ppm		Not treated	21.50 ab	21.56 ab	70.98 a	70.1 a	85.35 a	88.05 a
		Treated	40.07 ab	31.12 ab	96.52 a	89.37 a	39.72 ab	42.42 ab
4000 ppm		Not treated	40.81 ab	48.33 ab	88.92 a	91.8 a	53.43 ab	56.13 ab
		Treated	39.91 ab	33.46 ab	90.67 a	81.67 a	39.42 ab	42.12 ab
7000 ppm		Not treated	18.81 ab	20.31 ab	81.77 a	83.92 a	32.03 b	34.73 b
		Treated	24.29 ab	26.47 ab	92.55 a	102.3 a	41.12 ab	43.82 ab

Values in the same column followed by the same letter (s) are not statistically different according to Duncan's multiple range tests.

Table 7: Effect of foliar application with Brassinolide (BR) Sodium (%) of *Amaranthus hypochondriacus* (green Amaranthus) and *Amaranthus cruentus* (Red Amaranthus) under different salinity levels during 2021 and 2022 seasons.

Var	Salinity (ppm)	Brassinolide (BR)	Na%	
			Second season	First season
Green	Control		1.27 d	2.38 d
	2000	Not treated	1.43 d	2.63 d
		Treated	1.72 cd	2.64 cd
	4000	Not treated	2.13 abcd	2.76 abcd
		Treated	1.89 bcd	2.90 bcd
	7000	Not treated	2.9 abc	5.54 abc
Treated		3.31 a	7.61 a	
Red	Control		0.93 d	1.81 d
	2000	Not treated	1.89 bcd	1.78 d
		Treated	1.21 d	2.14 bcd
	4000	Not treated	1.92 bcd	2.31 bcd
		Treated	1.45 d	2.10 abcd
	7000	Not treated	3.05 ab	3.36 a
Treated		2.23 abcd	2.68 ab	

Values in the same column followed by the same letter (s) are not statistically different according to Duncan's multiple range tests.

Fresh and Dry Weights / Plant: The results presented in Table (4) showed clearly that the highest significant values of fresh weight were obtained from the control, plants of red Amaranthus in both seasons, BR treatment enhanced the fresh and dry weights of plants in green Amaranthus particularly under the highest salinity level (7000 ppm)

Similarly, results shown in Table (4) indicated also that all used salinity treatments significantly decreased dry weight of both red and green Amaranthus than control in the first season. The reduction in plant dry weight of green Amaranthus was greater than red Amaranthus at all salinity levels Whereas, the red type was more tolerant than the green one. A similar result was reported by Vazquez *et al.* [18] and Gandonou *et al.* [19] on Amaranthus plant.

Leaf Mineral Content: Data in Tables (5) and (6) revealed that increasing salinity stress led to a reduction in N, P, K, Fe, Mn and Zn content in both growing seasons. In most cases BR enhanced the accumulation of N, P, K, Fe, Mn, Zn and Na particularly under the highest salinity level (7000 ppm) in both seasons, BR spray was the most effective treatment in mitigating the severe stress impacts. The results obtained in this research study are in agreement with those obtained by Talaat and Shawky [7] on wheat plants and Wu *et al.* [21] on *Malus hupehensis* plants.

Sodium Content (%): From the results shown in Table (7) it was clear that Sodium (Na) content increased in green Amaranthus as plants were exposed to the different salinity stress levels. The lowest Na content was recorded

in control plants in the two Amaranthus species, On the other hand, the highest values were detected in plants exposed to the highest salinity level (7000 ppm) which sprayed or not with BR, such results here were similar to those reported by Wu *et al.* [21].

REFERENCES

1. Siswanti, D.U. and N.A. Khairunnisa, 2021. The effect of biofertilizer and salinity stress on *Amaranthus tricolor L.* Growth and Total Leaf Chlorophyll content. Bio Web of Conferences, 33.02004.
2. Sarker, U., M.A. Iqbal, M.N. Hossain, S. Oba, S. Ercisli, C.C. Muresan and R.A.A. Marc, 2022. Colorant Pigments, Nutrients, Bioactive Components and Antiradical Potential of Danta Leaves (*Amaranthus Lividus*). Antioxidants, 11: 1206.
3. Maggio, A., D. Pascale, M. Fagnano and G. Barbieri, 2011. Saline agriculture in Mediterranean environments. Italian Journal of Agronomy, 6: 36-43.
4. Vikram S. Sharma, J. Pooja and A. Sharma, 2022. Role of Brassinosteroids in plants responses to salinity stress: A review. Journal of Applied and Natural Science, 14(2): 582-599.
5. Bakshi, A. and K. Sharma, 2023. Impact of Brassinosteroids on salt stress tolerance in horticulture crops. The pharma Innovation Journal 12(6): 3184-3190.
6. Qin, L., S. Guo, W. Ai, Y. Tang, Q. Cheng and G. Chen, 2013. Effect of salt stress on growth and physiology in amaranth and lettuce: Implications for bioregenerative life support system. Advances in Space Research, 51: 476-482.

7. Talaat, N.B. and B.T. Shawky, 2013. Epibrassinolide alleviates salt-induced inhibition of productivity by increasing nutrients and compatible solutes accumulation and enhancing antioxidant in wheat (*Triticum aestivum L.*) Acta Physiol Plant, 35: 729-740.
8. Multu, S.S., H. Atesoglu, C. Selim and S. Tokgoz, 2017. Effect of 24-epibrassinolide application on cool-season turfgrass growth and quality under salt stress. Grassland, 63: 61-65.
9. Eltarawy, A.M. and N.M. Tolba, 2023. Improving *Begonia semperflorenes* quality by foliar application with silicon nanoparticles and brassinolide. Journal of Plant Production, 14(4): 177-182.
10. Pregl, P., 1945. Quantitative Organic Microanalysis. 4th Ed., Churchill Publ. Co., London, pp: 200.
11. King, E.J., 1951. Micro-Analysis in Medical Biochemistry. 2nd Ed., Churchill Publishing Co., London, pp: 260.
12. Cottenie, A., M. Verloo, L. Kiekens, G. Velghe and R. Camerlynck, 1982. Chemical Analysis of Plant and Soil. Laboratory of Analytical and Agrochemistry, State Univ. Ghent. Belgium., pp: 100-129.
13. Gomez, A.A. and K.A. Gomez, 1984. Statistical procedures for agricultural research: Second Edition. A Wiley-Interscience Publication., 6: 690.
14. Amalia, D.R. and D. Rachmawati, 2023. Morphophysiological responses of red amaranthus (*Amaranthus tricolor L.*) to osmopriming treatment to overcoming salinity stress. IOP Conf. Series: Earth and Environmental Science, 10: 1088.
15. Bellache, M., L. Allal Benfekih, N. Torres-Pagan, R. Mir, M. Verdeguer, O. Vicente and M. Boscaiu, 2022. Effects of Four-week Exposure to salt treatments on germination and growth of two *Amaranthus* Species. Soil System, 6: 57.
16. Shahbaz, M. and M. Ashraf, 2007. Influence of exogenous application of BRassinosteroids on growth and mineral nutrients of wheat (*Triticum aestivum L.*) under saline conditions. Pakistan Journal of Botany, 39: 513-522.
17. Ahanger, M.A., R.A. Mir, M.N. Alyemeni and P. Ahmed, 2020. Combined effects of Brassinosteroid and kinetin Mitigates salinity stress in tomato through the modulation of antioxidant and osmolyte metabolism. Plant physiology and Biochemistry, 147: 31-42.
18. Vazquez, M.N., Y.R. Guerrero, W.T. Noval, L.M. Gonzalez and M.A.T. Zullo, 2019. Advances on exogenous application of brassinosteroids and their analogies to enhance plant tolerance to salinity: A review. Australian Journal of Crop science 13(1): 115-121.
19. Gandonou, C.B., H. Projinoto, S.A. Zznklan, A.D. Wouyou, S. Lutts, D.H. Montcho, F.A. Komlan and A.C.G. Mensah, 2018. Effects of salinity stress on growth in relation to gas exchange parameters and water status in *Amaranthus cruentus*. International Journal of Plant Physiology and Biochemistry, 10(3): 19-27.
20. Su, Q., X. Zheng; Y. Tian and C. Wang, 2020. Exogenous brassinolide alleviates salt stress in *Malus hupehensis Rehd.* by regulating the transcription of nhx-type na^+ (k^+)/ h^+ *antiporters*. front. Plant Sci., 11: 38.
21. Wu, W., Q. Zhang, E.H. Ervin, Z. Yang and X. Zhang, 2017. Physiological mechanism of enhancing salt stress tolerance of perennial ryegrass by 24-epibrassinolide. Front Plant Sci., 8: 1-11.