

Vegetative and Seminiferous Propagation

Physiological potential of pepper seeds hydroprimed and primed with 24-epibrassinolide and subjected to salt stress1

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ABSTRACT

The 24-epibrassinolide (24-EpiBL), in association with the physiological conditioning of seeds, is recognized for providing several advantages in seeds, among which the most important one is the tolerance to abiotic stresses. Thus, the objective of this work was to evaluate the vigor of pepper seeds primed with 24-EpiBL and then subjected to salt stress. Three lots of pepper seeds of the cultivar Airetama Biquinho Amarela were used. The research was divided into three steps: the first for initial characterization of seed lots upon germination and vigor tests; the second for defining the water absorption process and drying of primed seeds; and the third for the priming of seeds with water (hydropriming) and 24-EpiBL (10^{-8} M), as well as priming with 24-EpiBL (10^{-8} M) of seeds subjected to the salt stress with the following osmotic potentials: 0.0, -0.2, and -0.4 MPa. A mitigation of deleterious effects of salt stress was found in seeds hydro-primed and primed with 24-EpiBL. The use of pepper seeds hydro-primed and primed with 24-EpiBL (10⁻⁸ M) is a viable alternative for decreasing injuries, morphological and biochemical changes, and growth and development limitations caused by salt stress.

Keywords: brassinosteroid; *Capsicum chinense* Jacq.; *Priming*; abiotic stress; vigor tests.

INTRODUCTION

Pepper plants are affected by salt stress conditions, which are extremely unfavorable for the establishment of this crop, as salt stress decreases the physiological potential, resulting in several injuries during phases of the germination process and problems for seedling establishment in the field (Sá *et al.*, 2019). Pepper seeds are generally known by presenting slow and irregular germination due to some type of physiological dormancy, which is commonly connected with the impermeability of the seed coat or pericarp to water and oxygen. These structures may also present chemical inhibitors, as coumarin or para-sorbic acid. The seeds present low physiological potential and combined with the saline soils commonly found in arid and semiarid regions have resulted in low yields. Current studies using seeds and/or seedlings have been conducted with the main objective of finding appropriate management practices that enable the use of low-quality water without negatively affecting the development and yield of crops (Oliveira *et al.*, 2015; Pereira *et al.*, 2020).

Pre-germination treatments decrease the period between sowing and emergence of seedlings and increase tolerance of seeds to environmental adverse conditions. Thus, limitations in seed performance can be mitigated by using a new approach known as seed priming (Silva *et al.*, 2015)*.*

This technique aims to enhance the performance of seeds in laboratory and mainly in the field. The procedure

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consists of a controlled addition of water to the seeds, focused on the activation of processes that precede germination, without allowing the protrusion of the primary root; phases I and II of the absorption process are closely monitored (Batista *et al.*, 2015).

Studies combining seed priming with the use of plant growth regulators have been conducted for improving the advantages of this technique. The 24-epibrassinolide (24-EpiBL) stands out among the several regulators used; it belongs to the brassinosteroid group and has been shown to improve seed germination performance and increase tolerance to abiotic stresses (Shahid *et al.*, 2011).

Therefore, studies and auxiliary techniques are focused on favoring germination and performance of crop species at their initial stages and providing higher tolerance to stress conditions. Thus, the objective of the present study was to evaluate the physiological potential of pepper seeds primed with 24-EpiBL and then subjected to salt stress.

MATERIAL AND METHODS

The study was conducted at the Laboratory of Plant Propagation of the Engineering and Agricultural Sciences Campus of the Federal University of Alagoas (CECA/UFAL) from 2019 to 2021. Three lots of pepper seeds of the cultivar Airetama Biquinho Amarela (2019 crop season) provided by the Isla Sementes company were used. The seed lots were stored during the experimental period by packing them in aluminum bags and keeping them at 10 °C. The research was divided into the following steps: 1) Initial identification of the physiological potential of the seed lots through germination and vigor tests (first count, germination speed index, seedling length, and accelerated aging with a saturated salt solution); 2) water absorption process and drying of primed seeds; and 3) physiological potential after salt stress.

Step 1: Physiological potential

Water content

Water content was determined by the oven method at 105 ± 3 °C for 24 hours, according to procedures described in the Rules for Seed Analysis (MAPA, 2009), using two subsamples of 50 seeds; the results were expressed in percentage (wet basis)

Germination test

The test was conducted with four replications of

50 seeds, which were sown on filter paper previously moistened with a 0.2% potassium nitrate $(KNO₃)$ solution equivalent to 2.5 times the weight of the dry substrate, which was placed inside transparent acrylic boxes $(11 \times 11 \times 3.5$ cm). The boxes were covered with plastic bags to prevent water loss and kept in a germination chamber set to 30 °C; pre-tests were carried out to determine this temperature. An eight-hour photoperiod was used. The seedlings were evaluated 14 days after sowing and the results were expressed as percentage of normal seedlings in each lot (MAPA, 2009).

First germination counting

The evaluation was carried out together with the germination test, by counting the number of normal seedlings 7 days after sowing (MAPA, 2009).

Germination speed index (GSI)

GSI was calculated based on the daily counts of the number of normal seedlings in the germination test, following the formula proposed by Maguire (1962).

Seedling length

Four replications of 25 seeds were used; they were sown on two paper towel sheets previously moistened with distilled water diluted with 0.2% KNO₃ to overcome dormancy. Rolls were formed, wrapped in plastic bags to prevent water loss, and kept in a germination chamber under 30 °C for 14 days. Subsequently, the seedling lengths were measured using a ruler (mm) and then the measurements from each replication were summed and divided by the number of seedlings. The results were expressed in cm seedling-1 (Rocha *et al.*, 2020).

Accelerated aging with saturated salt solution

A single layer of seeds was distributed on a wire mesh screen attached to an acrylic box (Gerbox) containing 40 mL of saturated salt solution, obtained by diluting 40 g of NaCl per 100 mL of water. The boxes were covered to obtain a relative humidity of 76% and maintained in a biological oxygen demand (BOD) incubator at 41 °C for 72 hours (Torres, 2005). Four subsamples of 50 seeds were placed to germinate over this period, following the method described for the germination test; a single evaluation was carried out 7 days after sowing, calculating the percentage of normal seedlings.

Step 2: Water absorption process and seed drying and priming

Water absorption process

All seed lots were subjected to preliminary evaluations to determine the water absorption curve and identify the moisture content required to reach phase II of water absorption. The curve of water absorption by the seeds was obtained by weighing four 200-seed replications, firstly at every two hours and then at longer intervals after the results stabilized. The samples were moistened with three sheets of paper towel moistened with distilled water at a volume equivalent to 2.5 times the dry weight of the paper towel; the water content was indirectly calculated based on the initial water content of the seeds and their wet weight at different intervals. Weighing of the seeds was carried out until 50% of the seeds had primary root emergence; the results were expressed in percentages.

Procedure for drying of primed seeds

After priming, the seeds were dried until they reached their initial moisture content. The procedure was started by removing excess water from the seed coat using paper towels. Drying was conducted at room temperature and air humidity (approximately 29 °C and 55%, respectively) for 24 hours.

Priming with 24-EpiBL (10-8 M) and hydropriming (water).

The seeds were primed using an aqueous solution of 24-EpiBL (10-8 M) (Silva *et al.*, 2015) until they reached phase II of the triphasic pattern of absorption. The time for the seeds to reach the triphasic pattern of absorption was established based on preliminary evaluations of the water absorption process in the seeds. Then, germination and vigor tests were carried out.

Step 3: Salt stress with NaCl

After priming with 24-EpiBL, hydropriming, and drying the seed lots, vigor and germination tests were conducted to evaluate them under salt stress conditions induced by NaCl at potentials of 0.0 (control), -0.2, and -0.4 MPa. The salt solutions were prepared using sodium chloride (NaCl) and distilled water, adjusting the osmotic potentials through the Van't Hoff equation.

Statistical analysis

The experiment was conducted in a completely randomized design with four replications for each seed lot. The results were subjected to analysis of variance, in a 4×3 factorial arrangement, consisted of four treatments (control, hydropriming, priming with 24-EpiBL at 10-8 M, priming with 24 -EpiBL + a NaCl concentration) and three seed lots. The means were compared by the Tukey's test ($p < 0.05$).

RESULTS AND DISCUSSION

The physiological potential of three lots of pepper seeds of the cultivar Airetama Biquinho Amarela is shown in Table 1. The results showed a similarity in water contents among seed lots, which is positive. According to Guedes *et al.* (2011), high variations in moisture among seed lots can compromise the results, making tests for analysis of physiological potential unfeasible. Obtaining consistent data in this parameter is essential for the success of the procedures, as high water contents can favor or hinder the performance of seeds during the tests.

Considering the data obtained for final percentage, germination speed index (GSI), and seedling length, the three evaluated seed lots were not significantly different, denoting a high initial quality and uniformity of seeds. However, according to data obtained in the first count test, seeds from lots 2 and 3 were the most vigorous, as the seeds from lot 3 were not significantly different from the seeds from lot 1, which had the lowest physiological quality (Table 1). High sensitivity in the first germination count test, showing differences among seed lots, was also found by Torres *et al.* (2012).

The data obtained in the accelerated aging test with saturated salt solution denoted a high variation in physiological quality among seed lots; seeds from lot 3 were the most vigorous (Table 1). Accelerated aging with saturated salt solution is an alternative accelerated aging method that has been recommended as one of the most appropriate for small seeds, as is the case of vegetable seeds. In the present study, a ranking was found among seed lots, with lot 2 presenting the seeds with lower physiological quality, i.e., lower vigor. The first count test, GSI, and seedling length of this lot indicated a high physiological quality. The evaluation of initial physiological potential is important, as the use of seed lots with different physiological potentials enables to assess the efficacy of application methods for seed priming.

The water absorption process showed that seeds of the three lots had the same time/pattern for primary root emergence, which occurred after 48 hours of absorption, with a small variation in water content percentages (16.5%) to 16.8%) (Figure 1).

	TA	G	PC			AASS (%)
Lots		$\frac{0}{0}$		GSI	SL (cm)	
	8.4	89 a	75 b	6.3 a	3.5a	72 _b
2	8.4	97 a	87 a	7.5 a	4.1 a	57 c
3	8.2	92 a	76 ab	6.6 a	3.8a	86 a
$CV(\%)$		5.9	7.7	9.1	10.6	4.7

Table 1: Water content (TA), germination (G), first count (PC), germination speed index (GSI), seedling length (SL), and accelerated aging with saturated salt solution (AASS) in three lots of pepper seeds of the cultivar Airetama Biquinho Amarela

Means followed by the same letter in the columns are not significantly different from each other by the Tukey's test ($p < 0.05$).

Considering the evaluation periods, the 24-hour period was considered the safest for seed priming, as the water content reached after this time was similar to that reached after primary root emergence.

Pepper is propagated by seeds that can be directly or indirectly sown. After water enters the internal tissues until it reaches the embryo, a reorganization and metabolic reactivation occur, leading to cell multiplication. Thus, monitoring the water absorption curve in seeds is essential to determine the optimal timing for priming, as it is associated with the elucidation of the seed germination process (Marthandan *et al.*, 2020).

Pre-germination treatments have been applied as an alternative for improving physiological quality of seeds, reducing the germination period, and ensuring the uniformity of seed lots. These conditions are essential requirements for a successful seedling production (Masetto *et al.*, 2013).

The germination test showed no significant difference among the treatments (Table 2). These results may be attributed to the high percentages of initial seed germination in all lots, which were above the minimum required for commercialization, i.e., approximately 80%, according to the Normative Instruction no. 42 of September 17, 2019. The responses of high-vigor seeds to priming are, in general, limited, as found for the seed lots evaluated in the present study. However, the first count test showed differences in seed physiological quality, with significant differences for the seed lots 1 and 2 (Table 3).

The promising effects of priming that favor variables related to germination speed are the main benefit of this technique (Arif *et al.*, 2014). However, the different

Table 2: Germination (%) of three pepper seed lots (cultivar Airetama Biquinho Amarela) not subjected to priming (control) and subjected to hydropriming and priming with 24-EpiBL (10⁻⁸ M)

Means followed by the same letter in the rows are not significantly different from each other by the Tukey's test ($p < 0.05$).

responses of cultivars and seed lots to priming treatments may vary according to the species.

expansion development.

Delian *et al.* (2018) found no positive effects of hydropriming on germination and vigor of tomato seeds compared to the control. However, Kikuti & Marcos-Filho (2009) found significant effects of priming on germination percentage of cauliflower seeds. Similar results were found for flax seeds primed with 24 -EpiBL $(10^{-8}$ M), which presented significant increases in germination percentage, shoot and root lengths, fresh and dry weights, and anthocyanin contents (Aghaee & Rahmani, 2020). Similarly, carrot seeds had showed important effects of osmotic conditioning with polyethylene glycol in the first germination count test (Lopes *et al.*, 2011). The use of 24-EpiBL also increased plant growth for pea plants (Xiong *et al.*, 2016).

The seed lots exhibited similar GSI in the first count test; lots 1 and 2 stood out (Table 4). Araújo *et al.* (2011) evaluated priming of *Cucumis anguria* seeds and found similar results, with a positive effect of hydropriming on seed vigor.

The results showed that hydropriming has beneficial effects on GSI, which is favorable for the establishment of plants in the field. The seedling lengths found showed significant growth only for seeds from lot 1 treated with 24-EpiBL, whereas the other seed lots presented no significant differences from the control treatment (Table 5).

Several studies have found that seedling length is highly sensitive to stratifying seed lots into different vigor levels. This ranking, according to Fridman & Savaldi-Goldstein (2013), may be affected by the action of 24-EpiBL on cell

Regarding the accelerated aging test with saturated salt solution, the treatments with hydropriming and priming with 24-EpiBL stood out compared to the control treatment (Table 6).

The accelerated aging test simulates an environment with unfavorable conditions of air temperature and humidity, enabling the selection of seed lots that probably fit to less favorable environments. Nascimento & Lima (2008) evaluated osmoprimed eggplant seeds and found that the germination percentage was favored when subjected to germination at low temperatures (15 and 20 °C). These results corroborate those found in the aging test in the present study, as germination at low temperatures and accelerated aging are vigor tests that subject seeds to unfavorable conditions.

Brassinolides are recognized by ensuring higher resistance to seedlings against adverse stresses, as proven by a recent study carried out with lettuce seeds primed with 24-EpiBL $(10^{-6} M)$, in which the highest percentage of normal seedlings was obtained under unfavorable conditions (evaluated through accelerated aging test with saturated salt solution) (Rocha *et al.*, 2020).

The third step of this study consisted of evaluating hydroprimed seeds and primed seeds (with 24-EpiBL) under different salt stress levels. In the germination test, significant results were found only for seed lot 2 at level -0.2 MPa, with treatments with hydroprimed and primed seeds showing superior results (Table 7). The level of -0.4 MPa resulted in superior results for seed lots 1 and 3 when compared to the control treatment.

Table 3: First germination count (%) in three lots of pepper seeds (cultivar Airetama Biquinho Amarela) not subjected to priming (control) and subjected to hydropriming and priming with 24 -EpiBL (10^{-8} M)

Lots	Control	Hydropriming	Priming with 24-EpiBL
	63 b	88 a	83 a
$\overline{2}$	74 b	84 a	87 a
	82 a	83 a	89 a
CV(%)		9.0	

Means followed by same letter in the rows are not significantly different from each other by the Tukey's test ($p < 0.05$).

Lots	Control	Hydropriming	Priming with 24-EpiBL
	6.3 _b	8.5 a	8.1 a
	7.2 _b	8.3 a	8.5 a
	7.6a	7.8 a	8.7 a
CV(%)		8.2	

Table 4: Germination speed index (GSI) in three lots of pepper seeds (cultivar Airetama Biquinho Amarela) not subjected to priming (control) and subjected to hydropriming and priming with 24-EpiBL (10-8 M)

Means followed by the same letter in the rows are not significantly different from each other by the Tukey's test ($p < 0.05$).

Table 5: Seedling length (cm) for three lots of pepper seeds (cultivar Airetama Biquinho Amarela) not subjected to priming (control) and subjected to hydropriming and priming with 24 -EpiBL (10^{-8} M)

Lots	Control	Hydropriming	Priming with 24-EpiBL	
	4.7 _b	4.6 _b	6.3a	
$\overline{2}$	4.8a	5.1 a	5.0a	
3	4.7a	5.3 a	5.2a	
CV(%)		11.5		

Means followed by the same letter in the rows are not significantly different from each other by the Tukey's test ($p < 0.05$).

Table 6: Accelerated aging with saturated salt solution (%) in three lots of pepper seeds (cultivar Airetama Biquinho Amarela) not subjected to priming (control) and subjected to hydropriming and priming with 24-EpiBL (10⁻⁸ M)

Lots	Control	Hydropriming	Priming with 24-EpiBL	
	36 _b	92 a	90 a	
2	68 b	84 a	87 a	
3	36 _b	84 a	89 a	
CV(%)		6.2		

Means followed by the same letter in the rows are not significantly different from each other by the Tukey's test ($p < 0.05$).

Table 7: Germination (%) of three lots of pepper seeds (cultivar Airetama Biquinho Amarela) not subjected to priming (control) and subjected to hydropriming and priming with 24-EpiBL (10⁻⁸ M) (24-EpiBL), subsequently subjected to two salt stress conditions (-0.2 and -0.4 MPa)

Lots	Control	HP	24 -EpiBL	Control	HP	24 -EpiBL
		-0.2 MPa $-$			-0.4 MPa	
	91 a	97 a	98 a	48 _b	91 a	93 a
$\overline{2}$	87 b	96 a	96 a	81 a	88 a	89 a
3	93 a	97 a	96 a	76 b	96 a	94 a
$CV(\%)$		4.4			10.2	

Means followed by the same letter in the rows are not significantly different from each other by the Tukey's test ($p < 0.05$).

Salinity can hinder germination through the toxic effects of sodium and chloride ions on embryo viability; these ions can disrupt enzyme and macromolecule structures, damage cellular organelles and the plasma membrane, and interrupt respiration, photosynthesis, and protein synthesis (Daszkowska-Golec, 2011). Additionally, substrates moistened with salt solutions tend to decrease germination, as these solutions inhibit or limit the absorption of water by the seeds (Hannachi & Van Labeke, 2018).

High evapotranspiration occurs in environments with high temperatures and low relative air humidity, causing water losses; consequently, salt is concentrated around roots, affecting their water absorption capacity. Several studies on agricultural crops have shown the harmful effects of salinity, which affects seed germination and seedling emergence (Thiam *et al.*, 2013; Sarker *et al.,* 2014; Dkhil, *et al.*, 2014; Soliman & El-Shaieny, 2014; Abdel-Haleem & El-Shaieny, 2015; Zavariyan, *et al.*, 2015).

The results of the first germination count test showed that both treatments were efficient in mitigating the harmful effects of salts. All seed lots showed this trend, except for lot 3 under the salt level of -0.2 MPa, which presented no difference among treatments (Table 8).

The comparison between the results of the first germination count and the germination test showed that the results of the first count were the most affected in the control treatment. The first counting and GSI showed significant results for the tested treatments (Table 9), although the control treatment had severe effects. The evaluation of this parameter is necessary because the faster the establishment of the stand of seedlings, the shorter the time that the crop will be exposed to abiotic stresses.

Lopes *et al.* (2014) evaluated broccoli seeds and found that germination percentages and GSI of seed lots subjected to more negative osmotic potentials were significantly affected. Similar results were found for other vegetables, indicating that limitations in seed germination speed and percentage increases as the osmotic potential decreases and becomes more negative due to increased salt concentration in the germination medium (Pereira *et al.*, 2020). Considering pepper seeds, the treatments with primed and hydroprimed seeds were efficient in mitigating negative effects of salt stress.

Studies have indicated that using 24-EpiBL can reestablish the growth of seedlings under salt stress, performing an important role in plant metabolism. In addition to proven benefits on plant growth and development, it triggers antioxidant activities and catalyzes different growth regulators, including abscisic acid, gibberellin, and salicylic acid in plants under salt stress (Wu *et al.*, 2017; Tanveer *et al.*, 2018).

Table 8: First germination count (%) in three lots of pepper seeds (cultivar Airetama Biquinho Amarela) not subjected to priming (control) and subjected to hydropriming and priming with 24-EpiBL (10-8 M) (24-EpiBL), subsequently subjected to two salt stress conditions (-0.2 and -0.4 MPa)

Means followed by the the same letter in the rows are not significantly different from each other by the Tukey's test ($p < 0.05$).

Table 9: Germination speed index for three lots of pepper seeds (cultivar Airetama Biquinho Amarela) not subjected to priming (control) and subjected to hydropriming and priming with 24-EpiBL (10-8 M) (24-EpiBL), subsequently subjected to two salt stress conditions (-0.2 and -0.4 MPa)

Means followed by the same letter in the rows are not significantly different from each other by the Tukey's test ($p < 0.05$).

.-0.2 and -0.4 MPa)							
	Control	HP	24 -EpiBL	Control	HP	24 -EpiBL	
Lots		-0.2 MPa $-$			-0.4 MPa		
	3.6 _b	4.7a	4.4a	2.2 _b	3.2a	3.7a	
2	4.7a	4.6a	4.2a	2.6 _b	3.5a	3.7a	
3	3.2 _b	4.8a	4.9a	3.2a	3.6a	3.2a	
$CV(\%)$		7.7			9.7		

Table 10: Seedling length (cm) for three lots of pepper seeds (cultivar Airetama Biquinho Amarela) not subjected to priming (control) and subjected to hydropriming and priming with 24-EpiBL (10⁻⁸ M) (24-EpiBL), subsequently subjected to two salt stress conditions $(1, 0.13 \text{ m})^3$

Means followed by the same letter in the rows are not significantly different from each other by the Tukey's test ($p < 0.05$).

Table 11: Accelerated aging with saturated salt solution (%) in three lots of pepper seeds (cultivar Airetama Biquinho Amarela) not subjected to priming (control) and subjected to hydropriming and priming with 24-EpiBL (10⁻⁸ M) (24-EpiBL), subsequently subjected to two salt stress conditions (-0.2 and -0.4 MPa)

Lots	Control	HP	24 -EpiBL	Control	HP	24 -EpiBL
		-0.2 MPa $-$			-0.4 MPa \cdot	
	87 a	93 a	90 a	41 _b	60 a	61 a
2	77 _b	91 a	89ab	39 _b	49ab	59 a
3	73 _b	88 a	91 a	31c	45 _b	64 a
$CV(\%)$	8.4				12.6	

Means followed by the same letter in the rows are not significantly different from each other by the Tukey's test ($p < 0.05$).

The results of seedling length (Table 10) showed that lot 2 in the treatment with -0.2 MPa and lot 3 in the treatment with -0.4 MPa were not significantly affected; however, the other lots and treatments showed promising results for mitigating salt stress, mainly in treatments containing hydroprimed seeds and those primed with 24-EpiBL.

This benefit in growth and development of seedlings from seeds subjected to priming is due to the reactivation of metabolic processes, which occurs after induction of protein synthesis and finishes with a favorable metabolic balance, improving seedling growth (Holbig *et al.,* 2011).

Considering the osmotic restraining caused by NaCl concentration, the seeds probably increased metabolic activity for osmotic adjustment, which resulted in a satisfactory water absorption and germination, even under adverse conditions, as also found by Sá *et al.* (2019). Tomato seeds primed with 24-EpiBL (10⁻⁶ M) and subsequently subjected to salt stress conditions had increased in radicle length (Maia-Júnior *et al.*, 2021).

The seeds were subjected to stress in the accelerated aging test with saturated salt solution, which showed that seeds from lot 1 in the control treatment presented no significant differences from those osmoprimed with water and/or 24-EpiBL, differently from lots 2 and 3, whose seeds

presented lower results in the control treatment (Table 11).

The germination of seeds from lot 3 was severely hindered when the seeds were subjected to -0.4 MPa; this negative effect was mitigated when the seeds were hydroprimed with water and 24-EpiBL. The treatment with 24-EpiBL stood out for presenting better germination results. Fazlali *et al.* (2013) highlighted that the initial quality of seed lots can be delayed or impaired by several abiotic stresses and that germination is one of the main affected parameters.

Serna *et al.* (2015) found a significant action of Brassinolides with promising results in reducing salinity effects on lettuce plants subjected to salt stress conditions. Salt stress causes degenerative changes in seed metabolism, stimulating disruption and loss of integrity of the cell membrane system (Amirinejad *et al.*, 2017). Salt concentrations do not prevent seed germination, but increase germination time by delaying the beginning of the process (Thiam *et al.*, 2013).

CONCLUSION

The use of pepper seeds hydro-primed and primed with 24-EpiBL $(10^{-8}$ M) is a viable alternative for decreasing injuries, morphological and biochemical changes, and growth and development limitations caused by salt stress.

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