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COMBINED EFFECT OF SALT STRESS AND ASLYCILIC ACID ON GERMINATION OF TOMATO *LYCOPERSICUM ESCULUNTUM* UNDER CONTROL CONDITIONS

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Abstract

Description of the subject: Soil Salinity is a severe obstacle to seed germination and early seedling growth; any boost treatment that improves Salt resistance during these critical developmental stages will be of major interest.

Objective This study precise the influence of Salicylic acid (AS) on seed germination in presence of $MgCl_2$ (SS).

Methods : A hydroponic experiment was conducted on two tomato varieties, namely Saint-Pierre and Semillas de Alta Selection, to assess the percentage and kinetics of germination, as well as the length and fresh weight of seedlings (hypocotyls and cotyledons), in response to different treatments. Six treatments were applied, including a control group, concentrations of 50 mM and 100 mM $MgCl_2$, and combinations of $MgCl_2$ (50 mM or 100 mM) with salicylic acid (0.25 mM or 0.50 mM).

Results: The results of the study demonstrate that the application of salicylic acid (SA) promotes germination under saline stress conditions, and the physiological processes related to salt tolerance were investigated. The effect of SA on salt tolerance during germination reveals that the application of SA (SA + $MgCl_2$) significantly increases the reduced germination and growth rates (in terms of hypocotyl and cotyledon length) caused by saline stress ($p \leq 0.05$). The application of SA to soils with different levels of salinity improves seedling growth. SA increases the seed germination percentage and enhances seedling elongation in both studied cultivars. Increasing the dose of SA has negligible effects on most measured parameters; however, irrigation with a 0.25 mM concentration of SA in the presence of $MgCl_2$ significantly improves germination and fresh weight of seedlings, indicating more effective management of saline stress .

Conclusion: The study found that using AS to regulate physiological mechanisms in tomatoes could help to minimize the negative impact of Salt stress. To conclude, AS can be used as a booster agent for improvement of Salinity resistance at the germination stage.

Keywords: Salinity; Salicylic acid; Germination; Resistance; Tolerance.

EFFET COMBINÉ DU STRESS SALIN ET DE L'ACIDE SLYCILIQUE SUR LA GERMINATION DE LA TOMATE *LYCOPERSICUM ESCULUNTUM* DANS LES CONDITIONS CONTROLÉES

Résumé

Description du sujet : La Salinité du sol est un obstacle sévère à la germination des graines et à la croissance précoce des semis ; tout traitement de stimulation qui améliore la résistance au sel pendant ces étapes critiques du développement sera d'un intérêt majeur.

Objectifs : Cette étude a pour objectif d’étudier l’effet de l’hormopriming des graines de Tomate *Lycopersicum esculuntum* sur la germination et la croissance en conditions de stress salin.

L’effet de l'acide salicylique (AS) sur la germination des graines en présence de $MgCl^2$ (SS)

Méthodes : Une expérience hydroponique a été réalisée sur deux variétés de tomates, à savoir Saint-Pierre et Semillas de Alta Sélection, afin d'évaluer le pourcentage et la cinétique de germination, la longueur et le poids frais des semis (hypocotyles et coléoptiles) en réponse à différents traitements. Six traitements ont été appliqués, comprenant un groupe témoin, des concentrations de 50 mM et 100 mM de $MgCl^2$, et des combinaisons de $MgCl^2$ (50 mM ou 100 mM) avec de l'acide salicylique (0,25 mM ou 0,50 mM).

Résultats : Les résultats de l'étude démontrent que l'application d'acide salicylique (AS) favorise la germination dans des conditions de stress salin, et les processus physiologiques liés à la tolérance au sel ont été investigués. L'effet de l'AS sur la tolérance au sel pendant la germination révèle que l'application d'AS (AS + $MgCl^2$) induit une augmentation significative ($p \leq 0{,}05$) des taux réduits de germination et de croissance (en termes de longueur des hypocotyles et des coléoptiles) provoqués par le stress salin. L'application d'AS sur des sols présentant différents niveaux de salinité améliore la croissance des plantules. L'AS augmente le pourcentage de germination des graines et favorise l'allongement des plantules dans les deux cultivars étudiés. Une augmentation de la dose d'AS a un effet négligeable sur la plupart des paramètres mesurés ; en revanche, l'irrigation avec une solution d'AS à une concentration de 0,25 mM en présence de $MgCl^2$ améliore significativement la germination et le poids frais des plantules, indiquant une gestion plus efficace du stress salin.

Conclusion : L'utilisation de l'acide salicylique (AS) dans la régulation des mécanismes physiologiques des graines de tomate s'est avérée bénéfique pour atténuer les effets néfastes du stress salin et minimiser son impact négatif. L'application de l'AS a montré des effets stimulants, améliorant la résistance à la salinité lors de la phase de germination. Ces effets positifs peuvent être attribués à l'implication de l'acide salicylique dans divers stades du développement de la germination, de la croissance, etc. Cette étude constitue une contribution à la compréhension des effets de l'hormopriming par l'acide salicylique sur la physiologie de la germination, la croissance et la tolérance.

Mots clés: Salinité ; Acide Salicylique ; Germination ; Résistance ; Tolérance.

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INTRODUCTION

Plants primarily through their roots absorb water, which is subsequently carried to shoots for various processes. Tomato (*Solanum Lycopersicum*) is one of the world's most popular fruits and plays a significant role in human nutrition [1]. Abiotic stressors such as Salt, low temperature, drought, and cold have harmed plant development and quality in recent years, reducing agricultural productivity and food yields severely [2]. The improvement of tomato Salt stress resistance has received a lot of attention in recent years [3]. In arid and semi-arid areas, Salinity is one of the most important elements affecting plant growth and metabolism, leading to significant damage and a loss of yield [4]. A billion hectares are under threat, including 3.2 million in Algeria [5]. When plants are exposed to Salinity, their morphological and physiological activity changes [6] similarly [7] found that, Salt stress severely inhibits seed germination, lateral root formation, and biomass production. Plants suffer from hyperosmotic stress, oxidative stress, ionic imbalance, Na^+ toxicity, and even mortality as a result of high Salinity [8]. At early growth stages, such as germination and seedling establishment, most agricultural species are vulnerable to Salinity stress [9]. Plant survival is likely the most important sign of Saline tolerance during germination and emergence, whereas yield and growth reduction may be considered the final tolerance criterion afterward [9]. Seed absorbs water before germination, causing the seed embryo to expand and elongate. The process of seed germination is accomplished after the radicle has grown out of the covering seed layers [10]. Numerous scientific studies have focused on investigating the influence of plant hormones on seed germination processes across various plant species [10]. Given the increasing significance of mitigating salt stress for improved agricultural performance, salicylic acid (AS), a phytohormone, has emerged as a promising molecule capable of regulating antioxidant defense systems, transpiration rates, stomatal movement, and photosynthetic rates to reduce plant susceptibility to environmental stimuli [11]. Previous research has provided evidence supporting the role of salicylic acid (AS) in enhancing plant salt tolerance [12]. Several studies have demonstrated the beneficial effects of applying AS to plants, which help protect them from oxidative damage caused by drought stress [13]. However, many aspects of the underlying molecular mechanisms, such as the association between salicylic acid and dehydration-responsive element-binding proteins (DREBs), which are transcriptional activators capable of upregulating the expression of cold tolerance genes, remain poorly understood in peach fruit [14]. Furthermore, while salicylic acid is known to play a role in germination under stressful conditions, its precise functions and underlying physiological mechanisms have yet to be fully elucidated [15]. Despite the extensive exploration of the positive effects of AS on salt tolerance in numerous crops, including bean [16] and tomato [17], there is still a need for further research in this area. The purpose of this study was to investigate the impact of salt stress and the application of salt ($MgCl_2$), salicylic acid (AS), and a combined treatment of $MgCl^2$/AS on germination characteristics of two tomato cultivars. Specifically, the study aimed to assess the effect of different concentrations of $MgCl_2$ (0, 50, 100 mM) and two levels of salicylic acid (0.25, 0.5 mM) on seed germination and enhance salt tolerance in tomato seedlings.

MATERIALS AND METHODS

1. Plant material

This study was conducted at the Biotechnology of Crop Production Laboratory at Blida1 University (latitude: 36°30'36.38'N, longitude: 2°52'26.70'E) under controlled conditions. Two tomato cultivars, Saint-Pierre and Semillas de Alta Seleccion, were selected for the experiment. The seeds of Saint-Pierre were produced in 2015, while those of Semillas de Alta Seleccion were produced in 2019; the availability of both varieties facilitates the work. The aim of this study was to investigate the effects of salinity and salicylic acid on the germination of Saint-Pierre and Semillas de Alta Seleccion tomato cultivars.

2. Seeds Germination Test

The seeds were initially soaked in distilled water for 2 minutes and then disinfected with sodium hypochlorite (10%) for 5 minutes. They were subsequently rinsed with distilled water to remove any residual chlorine and to ensure aseptic conditions during the germination process. Only healthy seeds were selected for the germination tests. The seeds were placed on three filter papers in 10 cm Petri dishes, with four replicates of 10 seeds each. The seeds were irrigated with different concentrations of $MgCl_2$ (50 mM, 100 mM) and salicylic acid (0.25 mM, 0.50 mM), either individually or in combination.

The Petri dishes were kept in a growth chamber at a temperature of 25°C in complete darkness for 8 days. Germinated seeds, defined as those with a radical that had emerged and elongated by at least 2 mm, were counted daily. The choice of the plant, cultivars, and the concentrations of salicylic acid and $MgCl_2$ were based on specific experimental objectives.

3. Mechanism of Salicylic Acid for Salt Tolerance

To investigate the physiological mechanism of salicylic acid for salt tolerance, the seeds were subjected to different treatments: Control: Distilled water (C); Stressed 1: 50 mM $MgCl_2$ (S1); Stressed 2: 100 mM $MgCl_2$ (S2); Stressed 1 + 0.25 mM SA (S1+SA1); Stressed 1 + 0.50 mM SA (S1+SA2); Stressed 2 + 0.25 mM SA (S2+SA1); Stressed 2 + 0.50 mM SA (S1+AS2) The seeds were germinated under these treatments for 8 days, and the seedlings were subsequently used for further analysis. The specific concentrations of $MgCl^2$ and salicylic acid were chosen based on their potential to induce salt stress and their known effects on plant physiology.

Table 1: Description of treatments used (mM/L)

	Control	Stress1	Stress2	Stress1+SA1	Stress1+SA2	Stress2+SA1	Stress2+SA2
$MgCl_2$ (1)	-	50	-	50	50	-	-
$MgCl_2$ (2)	-	-	100	-	-	100	100
Salicylic acid(1)	-	-	-	0,25	-	0,25	-
Salicylic acid (2)	-	-	-	-	0,5	-	0,5

4. Plant analysis

The various experimental produced estimates of the following traits:

- ***Total germination percentage (GP %);*** According to Benazzouk, the germination rate is the percentage of seeds germinated compared to the total number of seeds sown, Measure on the eighth day using the formula [18]. $GP\ \% = \frac{\text{total number of germinated seeds}}{\text{total seed}} \times 100$

- ***Germination Kinetics;*** Correspond to the variations over time in the rate of seed germination of the test plant; it graphically represents the percentage of germination as a function of time it gives a precise vision of the evolution of germination of a seed placed in controlled conditions.

- ***Determination of hypocotyls and coleoptiles length;*** Seeds were measured on day 8 with a Digimizer software Version 4.0.0.0 Copyright© 2005-2011 MedCalcSoftware. Four independent biological repeats were measured.

- ***Fresh weight of seedlings ;*** Seeds were measured on day 8 with a weighting scale. The seedlings were appropriately organized to keep track of different types or treatments. Then they were placed on clean, dry paper towel on the weighing scale to prevent direct contact between the seedlings and the scale. Zero the scale: press the "Tare" to set the scale to zero with the paper towel. Carefully place one seedling on the paper towel and record the weight shown on the scale. Take note of the weight

5. Statistical analysis

All statistical analyses were conducted using STATGRAPHICS-CENTURION XVI (version 16.1.18) ANOVA ($p< 0.05$). using the software and significantly different means were separated by Fisher's LSD test at the probability threshold. (LSD) test at the 5 % probability level.

RESULTS AND DISCUSSION

1.Total germination percentage

As anticipated, the germination percentages of the two selected cultivars displayed contrasting outcomes under control conditions (Fig. 1). The final germination percentage of Saint-Pierre (Fig. 1A) was 63.3%, while Semillas de Alta Seleccion exhibited a higher germination percentage of 100% (Fig. 1B). Salinity significantly reduced the germination percentage in both cultivars. The presence of $MgCl_2$ at concentrations of 50 mM (SS1) and 100 mM (SS2) resulted in a notable decrease in germination percentage. In Saint-Pierre, the germination percentage dropped to 40% in SS1 and 10% in SS2. Similarly, in Semillas de Alta Seleccion, the germination percentages were 70% in SS1 and 40% in SS2. Several studies have reported similar effects of Salt stress in different plant species [10]. For instance, Orlovsky et *al.* [19] demonstrated that the inhibition of seed germination under Salt stress was correlated with the restriction of ethylene synthesis during imbibition. The harmful effects of sodium and chloride ions on embryo viability can influence seed germination [20].

Interestingly, the combination of AS and Salinity exhibited a positive impact on germination, with a significant effect. Upon comparing the germination percentages of different Salinity treatments with and without AS, it was observed that the germination percentage increased as AS concentrations decreased. The highest germination percentage was achieved at 0.25 mM of AS under different concentrations of $MgCl_2$ (50 mM, 100 mM), reaching 43.3% and 25% in Saint-Pierre, respectively. AS contributed to plant development under Salt-stressed conditions. Miao et *al.* [21] reported similar findings, stating additional AS enhanced carbohydrate accumulation by improving leaf photosynthetic capacity and root growth, leading to increased water and nutrient absorption. The statistical analysis revealed that SS2 and SS2+AS2 belong to the same homogeneous group, as well as SS1+AS1 with SS1+AS2 in Saint-Pierre. In Semillas de Alta Seleccion, SS1 and SS1+AS2 were grouped together, along with SS2 and SS2+AS2 in (B). The results of the study confirm that high soil concentrations of AS can induce abiotic stress in plants, leading to reduced tolerance to subsequent abiotic stresses. Consequently, plants exhibit a diminished capacity to withstand Salt stress in response to elevated levels of AS in the soil [22].

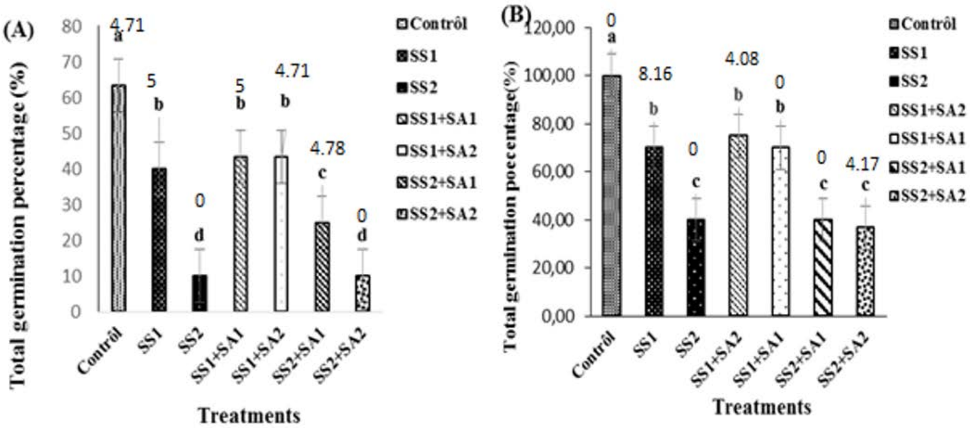

Figure 1: Germination percentage of seeds of two tomato genotypes

(**A**) Saint-Pierre (B) Semillas de altaseleccion sown on filter paper imbibed with water (control), SS1 (50 Mm of $MgCl_2$), SS2 (100 mM of $MgCl_2$), SS1+SA1(50 mM of $MgCl_2$+0.25 mM of SA), SS1+SA2 (50 mM of $MgCl_2$+0.50 mM of SA), SS2+SA1 (100 mM of $MgCl_2$+0.25mM of SA), SS2+SA2 (100mM of $MgCl_2$+ 0.50mM of SA) Values are means of four replicates. Each replicate consists of a batch of 10 seeds.

2.Germination kinetics

Germination kinetics is a germination curve that describes the germination sequence of tomato seed batches that have been evaluated under certain conditions. It most commonly shows the progression of cumulative germination percentages over time. This kinetics are derived from the cumulative rates of germinated seeds, that is, the variation in germination rates as a function of time expressed in days under all treatment circumstances. Figure 2 shows the germination kinetics of the two tomato cultivars' seeds for 8 days at the two Salinity levels (50, 100, mM) with 0.25 and 0.50 mM of Salicylic acid. The germination kinetics under control treatment, seeds start to germinate after 4 days of incubation in (A) and 3 days in (B) to reach a maximum of 67.5% 97.5% of GP after 7 days for (A) and (B) respectively.

Germination occurs slowly under the combined Salinity/ Salicylic acid treatment. Seeds start to show on the 6th day of incubation. Germinated seed levels in the SS1+AS1, SS1+AS2, and SS2+AS2 treatments do not increase those reported in the seeds of Saline treatments SAS and SS2. Except for the SAS1+AS1treatment (50 mM of $MgCl_2$ + 0.25 mM) of AS, which shows a clear improvement in germination rate after 4 days of incubation compared to the ASme Saline treatment + 0.50 mM of AS. The ASme note has been registered in (B). The result show that there is no a signicant difference between the control and seeds under stress. The ASme result was reported by Cherifa, & Zohra [23]. For the two sources tested, germination of stressed and non-stressed seeds began on the third day of cultivation. After 7 days of incubation, the germination of seeds has been influenced by the presence of Salicylic acid in the Saline treatment (Fig. 2). The seeds have higher germination rates under the influence of the AS in particular at the level of treatments SS1+AS1, SS2+AS1, respectively compared to natural Saline treatments devoid of Salicylic acid, namely SS1 and SS2.

Salicylic acid at 0.5 mm has no marked effect on the Saline waters tested, and where the treatment enriched with 100m $MgCl_2$ of Mg partially inhibits seed germination compared to the treatment enriched with 50 mM of $MgCl_2$. Thus, not all seeds have an identical capacity to tolerate Salinity; the decrease in germination rate of seeds subjected to Saline stress is due to an osmotic dormancy process developed under these stress conditions. Mokhtar et *al.* [24] found that the presence of Salicylic acid in the Saline conditions studied affects tomato seed germination. The tomato, in instance, has greater germination rates as a result of the AS effect and At 0.5 Mm, Salicylic acid has no noticeable impact on the Saline waters tested.

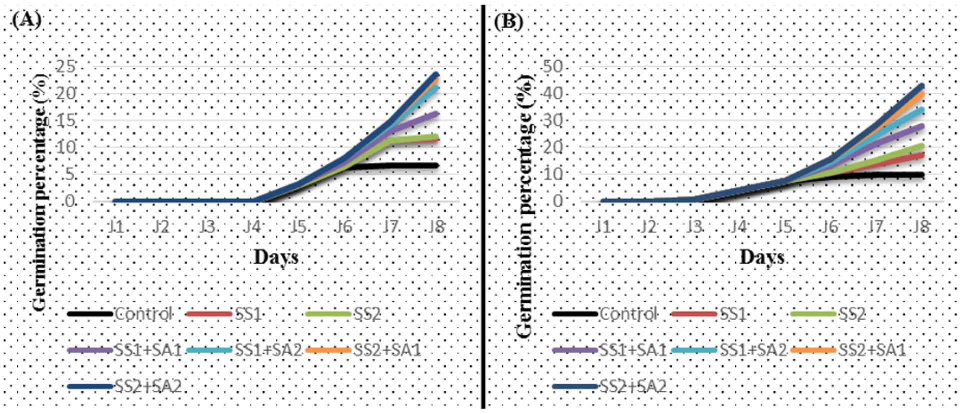

Figure 2: Germination Kinetics of seeds of two-tomato genotypes

(A) Saint-Pierre (B) Semillas de altaseleccion imbibed with water (control), SS1 (50 Mm of $MgCl_2$), SS2 (100 mM of $MgCl_2$), SS1+SA1(50 mM of $MgCl_2$+0.25 mM of SA), SS1+SA2 (50 mM of $MgCl_2$+0.50 mM of SA), SS2+SA1 (100 mM of $MgCl_2$+0.25mM of SA), SS2+SA2 (100mM of $MgCl_2$+ 0.50mM of SA) after 8 days of incubation at 25°C

3. Hypocotyls and Coleoptiles length

There were no significant differences in the length of hypocotyls and coleoptiles between the two varieties under control conditions. However, both varieties (A) and (B) exhibited a significant increase in hypocotyl length with 7.32 cm and 8.79 cm, and coleoptile length with 1.70 cm and 1.90 cm, respectively. Salinity had a detrimental effect on hypocotyl and coleoptile elongation in both genotypes compared to the control. In variety (A), the length decreased to 4.27 cm and 1 cm for hypocotyls, and 0.30 cm and 0.10 cm for coleoptiles at 50 mM and 100 mM $MgCl_2$, respectively. Similarly, in variety (B), the length decreased to 6.11 cm and 2.3 cm for hypocotyls, and 0.39 cm and 0.19 cm for coleoptiles at the same respective concentrations (Fig. 3). Wheat seedling development and elongation are also negatively affected by Salinity [9]. Other studies have reported reductions in radicle, plumule, and seedling growth at different Salinity levels, with higher Saline levels causing a greater decline in plumule and seedling development [25]. The addition of AS to the Salinity treatment significantly increased hypocotyl length, especially at a concentration of 0.25 mM, in both 50 mM and 100 mM $MgCl_2$ treatments. Hypocotyl length increased to 5.50 cm and 5.32 cm in variety (A), and 6.06 cm and 5.65 cm in variety (B) compared to SS at 50 mM and 100 mM $MgCl_2$, respectively. Seeds that were primed with AS exhibited longer radicles, plumules, and seedlings compared to non-primed seeds, with no significant differences observed based on AS concentration [25]. Another study suggested that AS enhances seed germination by inducing thermogenesis and stimulating gibberellic acid biosynthesis [26]. AS priming of wheat seeds under Salt stress resulted in more vigorous and larger seedlings, along with increased chlorophyll, dissolved sugars, and protein content [27]. Under Salinity, root and shoot length, as well as fresh and dry weight, were reduced, but pre-treatment with AS enhanced these parameters [25]. Although the exact mechanism by which AS promotes root and shoot growth in certain plants is unknown, it is believed to involve the regulation of cell elongation and division in conjunction with other chemicals such as auxin [28]. These findings confirm the positive effect of AS on hypocotyl length under Salt stress. Additionally, the addition of AS to SS2 promoted radical growth, particularly at a concentration of AS1, in both varieties. Increasing AS concentration to 0.50 mM did not show significant differences compared to seeds irrigated with 0.25 mM of AS. Statistical analysis indicates that SS1+AS1 and SS1+AS2 belong to the same homogeneous group across all studied parameters. Increased AS concentrations led to increased production of ABA, which inhibited seed germination [10].

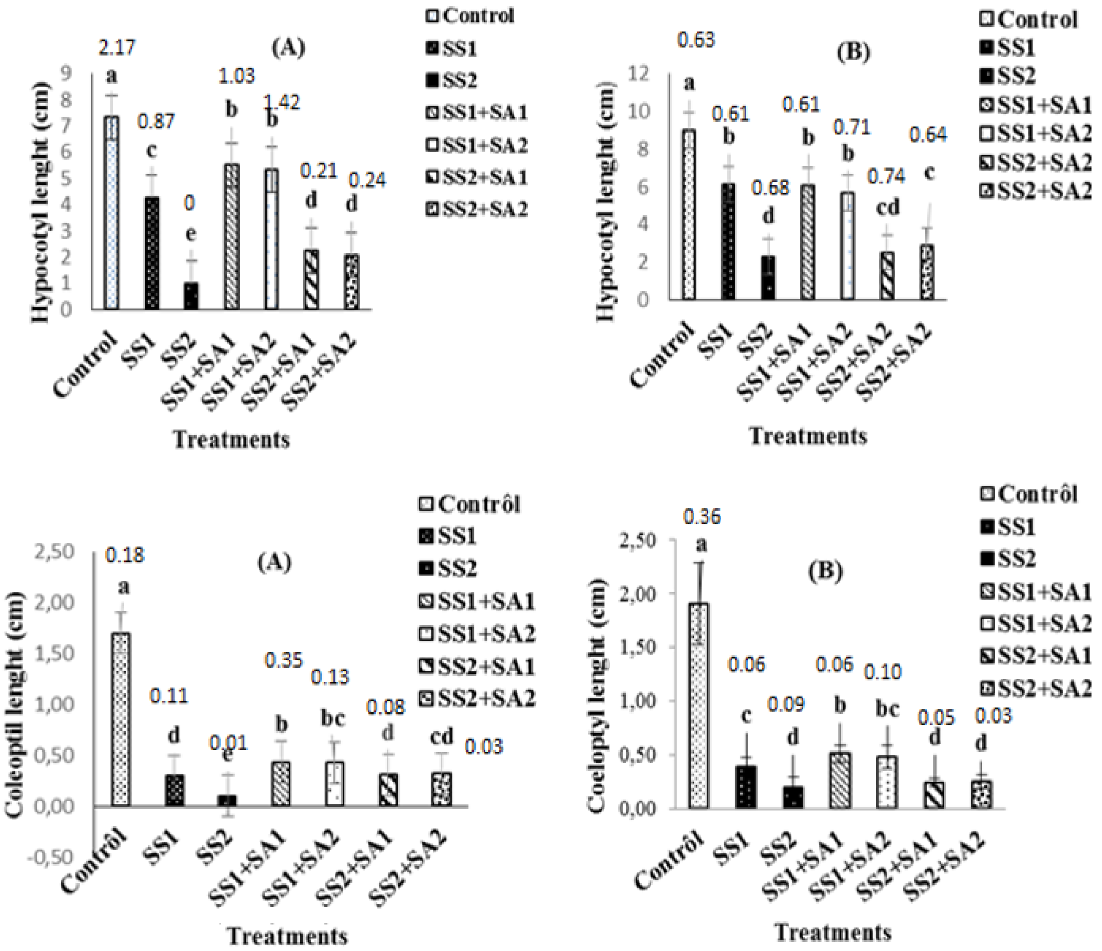

Figure 3: Effect of different treatments Hypocotyls and Coleoptiles length

water (control),SS1 (50 Mm of $MgCl_2$), SS2 (100 mM of $MgCl_2$), SS1+SA1(50 mM of $MgCl_2$+0.25 mM of SA), SS1+SA2 (50 mM of $MgCl_2$+0.50 mM of SA), SS2+SA1 (100 mM of $MgCl_2$+0.25mM of SA), SS2+SA2 (100mM of $MgCl_2$+ 0.50mM of SA) on the hypocotyls and coleoptile length (cm) of two tomato cultivars (A) Saint-Pierre and (B) Semillas de altaseleccion during 8 days in controlled condition.

4. Fresh weight of seedling (g)

The fresh weight of the seedlings decreased significantly, as Salinity increased compared to the control. Both tomato cultivars exhibited a significant decrease in fresh weight when grown under different concentrations of $MgCl_2$, indicating that Salinity had a detrimental effect on the growth performance of tomato plants. The results showed a significant decline in growth performance with increasing $MgCl_2$ concentration, indicating the harmful impact of Salt stress on growth. However, statistical analysis revealed that the application of AS improved the fresh weight compared to the corresponding Salinity treatment, particularly with the application of 0.25 mM of AS. Figure 4 further supports these findings and demonstrates that the Control treatment and SS1+AS1 treatment belong to the same homogeneous group (Fig. 4). The treatment with 0.50 mM of AS did not significantly affect fresh weight compared to the treatment with 0.25 mM of AS.

These results are consistent with previous studies by Moghaddam et *al.* [25] and Baghizadeh & Hajmohammadrezaei [29], which reported that drought stress led to a reduction in fresh and dry weight of roots and shoots in okra seedlings, while Salicylic acid increased the fresh and dry weight. Similar studies have shown that AS can have an active effect on the growth of Salt-stressed plants by acting as signaling compounds [30]. Furthermore, Khalil et *al.* [31] found that Salicylic acid, alone or in combination with kinetin or calcium, mitigates the toxic effects of nickel and/or lead stress and promotes various growth parameters in bean plants. Additionally, Corina Vlot et *al.* [32] reported that AS influences seed germination, seedling establishment, cell development, respiration, stomatal closure, senescence-associated gene expression, abiotic stress responses, thermotolerance, nodulation in legumes, and fruit yield.

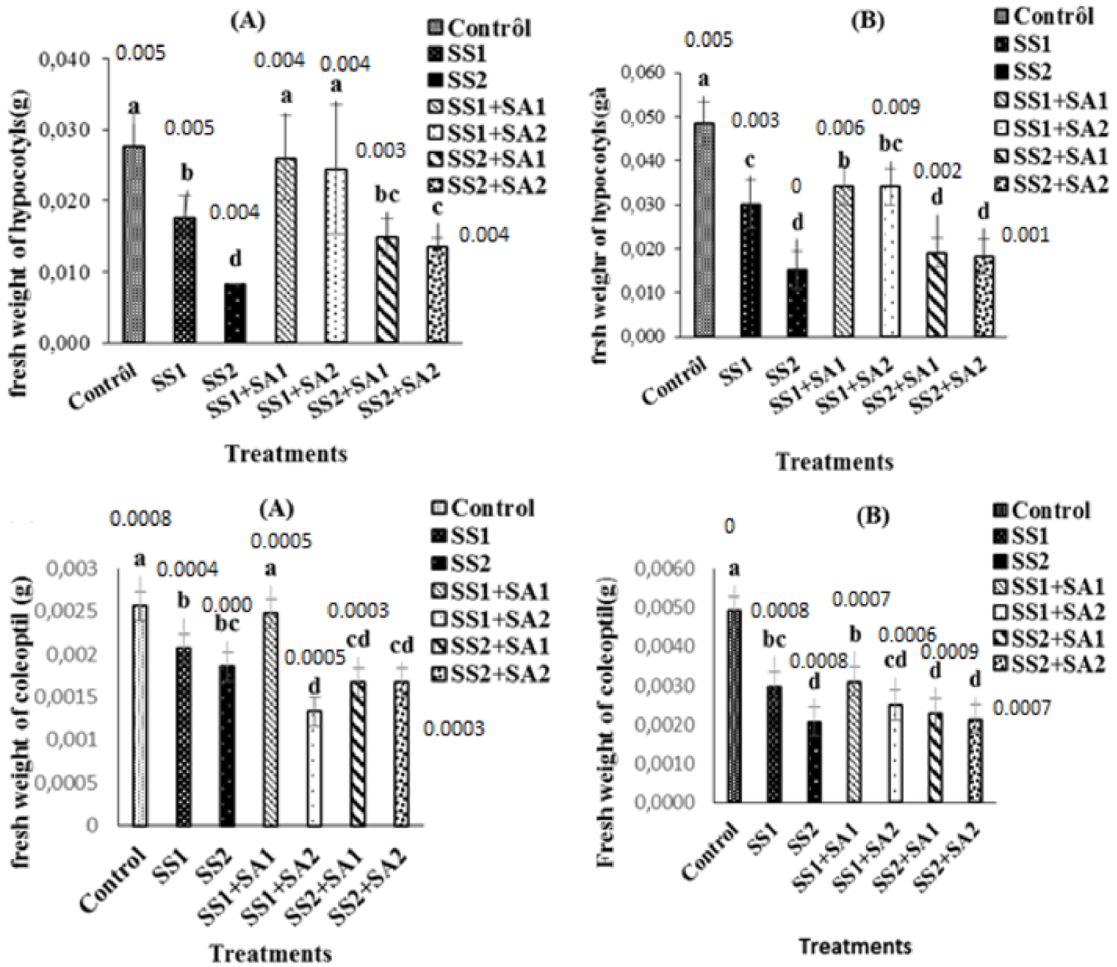

Figure 4: Fresh weights of hypocotyls and coleoptile (g) of two tomato cultivars (A) Saint-Pierre and (B) Semillas de altaseleccion grown in different treatment

water (control), SS1 (50 Mm of $MgCl_2$), SS2 (100 mM of $MgCl_2$), SS1+SA1(50 mM of $MgCl_2$+0.25 mM of SA), SS1+SA2 (50 mM of $MgCl_2$+0.50 mM of SA), SS2+SA1 (100 mM of $MgCl_2$+0.25mM of SA), SS2+SA2 (100mM of $MgCl_2$+ 0.50mM of SA).

CONCLUSION

The findings of this study demonstrate that Salt stress negatively affects seed germination characteristics in tomatoes. However, the exogenous application of AS mitigates the inhibitory effects of Salt stress and enhances germination under various stress levels. The results indicate that lower concentrations of AS have more favorable impact on tomato germination compared to higher concentrations. Specifically, the 0.25 mM AS concentration significantly improved germination percentage and germination compared to both the 0.25 mM and 0.50 mM concentrations. This suggests that a lower AS concentration can accelerate overall germination and enhance seedling establishment. Consequently, planted seeds will be less susceptible to soil-borne pests and diseases and yield higher biomass, especially in regions where irrigation water resources have high salinity levels at the time of planting.

Based on the findings, lower AS concentrations, particularly 0.25 mM, are recommended for managing low to high Salinity stress conditions. The presence of AS in the medium minimized the detrimental effects caused by $MgCl^2$ on certain studied parameters.

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