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Effects of Using Salicylic Acid, Potassium Chloride, and a Mixture of Salicylic Acid and Potassium Chloride Treatments on Salt-stressed Cucumis Sativus CV. Malaysia Timun2 Germination

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Abstract

Objective: In constructing a liquid enhancer, 300 mM NaCl was primed for 72 hours on sterile MTi2 seeds.
Methods: After that, deionized water was used as a control, and SA alone (salicylic acid) (0.25, 0.5, 0.75, 1 mM) and only KCl (Potassium chloride) (10, 20, 30, 40, and 50 mM) were applied. As previously stated, germination parameters were computed. Then, the appropriate ratio of KCl to SA was combined, and its efficacy as a germination activator on the Salt-stressed MTi2 seeds was examined. The data analysis software used was SPSS Windows version 22.
Results: To find the significant difference between treatments, data are first subjected to a two-way ANOVA with $p \leq 0.05$ confidence level. For purpose of comparing means, DMRT is next applied at a $p \leq 0.05$. According to the results, the best concentrations for boosting the germination and early growth of MTi2 seedlings in comparison to the control treatment were found to be 20–30 mM KCl and 0.5–0.75 mM SA. Furthermore, MTi2 seedling germination and early growth were more than 1x higher when the best concentrations of KCl (20-30 mM) and SA (0.5-0.75 mM) were combined.
Conclusion: Salicylic acid (SA) and low levels of KCl applied to salt-stressed MTi2 seeds can help reduce the negative effects of salinity stress and enhance the percentage, rate, vigour, length, and biomass of the seedlings that germinate. As the conclusion, salt-stressed MTi2 seeds can benefit from an enhancer that increases germination at low concentrations of KCl and SA.

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Introduction

Salinity affects the pH and availability of nutrients in soils, such as manganese, iron, and phosphorus [11]. Though it came from South Asia, cucumbers may now be found on most continents. Saline groundwater in semi-arid regions makes this plant a valuable crop for greenhouses. Consequently, further research is required to determine how salt affects this plant's ability to germinate [12].

It was determined that the cucumber plant was a glycophyte, meaning that it was somewhat susceptible to salinity. Previous research has demonstrated that salicylic acid (SA) plays a significant role in regulating how plants respond to a variety of abiotic stressors, including salt and water stress [13, 14]. Shakirova [15]; and Srivastava [16] discovered that it is crucial to the plant species' defense system against stress. Many application techniques were employed to protection different plant species in response to abiotic-stresses like salt, including soaking seeds in salicylic acid, Spraying or irrigating with SA solution after adding SA to the hydroponic solution [17, 18].

This study was conducted on the Malaysia cucumber cv. MTi2 which is the best known and most popular cucumber cultivars among the locals. Cucumber (Cucumis sativus) belongs to the gourd, family Cucurbitaceae. It is a widely cultivated, creeping vine that bears cylindrical, fruits that are used as culinary vegetables [19]. The cucumber originated from South Asia, but is currently found on most continents. This plant is an important greenhouse crop in semi- arid areas with saline ground water.

The present study aims to develop a liquid enhancer that will help salt-stressed *Cucumber sativus* cv. MTi2 seeds germinate more efficiently.

Materials and Methodology

Seed Materials and Sterilization

Cucumber (Cucumber sativus cv. MTi2) seeds were purchased from MARDI (Malaysia Agriculture Research, and Development Institute, Serdang, Selangor). Sterilization of seeds was done using the technique revealed by Panuccio [20] with a few slight modifications. The cucumber seeds that were chosen were mature, healthy, and of uniform size. They were then surface sterilized for 20 minutes using 5% sodium hypochlorite. The seeds were subsequently cleaned three times using distilled water, that had been sterilized to prevent fungal infections.

Experimental Site

The research project was carried out from June 2015 to October 2016 at the Tissue Culture Laboratory, Department of Biology, University of Putra Malaysia.

Designing an experiment to improve the germination of MTi2 seeds salt-stressed

In this research, salt-stressed MTi2 seedlings were treated with SA (salicylic acid), KCl (potassium chloride), or a combination of both. To do this, initially, the experiment was carried out with SA to determine the optimum concentration of SA that promotes the germination of MTi2 seedlings under salt stress. Second, to determine the optimum KCl concentration that promotes the germination of salt-stressed MTi2 seeds, MTi2 seeds that have been exposed to salt will be germinated in a range of KCl concentrations. Finally, the optimum ratio of SA to KCl will be combined to

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determine the ratio that optimizes the germination of MTi2 seeds salt-stressed **[21]**.

Salted Solutions Preparation

Five different concentrations of KCl, which are 10, 20, 30, 40, and 50mM were prepared and deionized water as a control. Four salicylic acid concentrations of 0.25, 0.50, 0.75, and 1 mM were prepared and deionized water was used as a control [21].

Halopriming of MTi2 seeds in NaCl

After being sterilized with MTi2, the seeds were soaked in 50 ml of 300 mM NaCl, and incubated for 72 hrs at 25 °C, in a growth incubator, according to the technique by Afzal [22]; and Elouaer [23].

MTi2 seeds Salt-stressed treated with KCl, SA, and a combination of SA and KCl

Improvement of salt-stressed MTi2 seed germination using SA:

Seven sterilized MTi2 seeds were placed on two layers of Whatman filter paper No. 1 in separate sterilized Petri dishes (9 cm diameter) containing 5 ml of different concentrations SA (0.25, 0.5, 0.75, 1 mM) and deionized water as a control. All treatments were kept in the growth chamber at 25 ± 1°C and will be monitored daily until 8 days. Measurements were made on seed vigor, germination rate, germination percentage, and early seedling growth **[21]**.

Germination parameters were calculated as follows:

$$\mathbf{GP\%} = \frac{\mathbf{NGS}}{\mathbf{TNSS}} \times \mathbf{100} \quad [24, 25]$$

$$\mathbf{GR} = \frac{\mathbf{NGS}}{\mathbf{D1}} + \cdots . + \cdots . + \frac{\mathbf{NGS}}{\mathbf{DL}} \quad [26]$$

$$\mathbf{SV} = \frac{\mathbf{(LH+LR)\times GP\%}}{\mathbf{100}} \quad [27]$$

Where: GP% is the Germination percentage, NGS is the Number of germinated seeds, TNSS is the Total number of seed sown., GR is the Germination rate, D1 is the Day of the first count, DL is the Day of the last count., SV is the Seed Vigor, LH is the Length of hypocotyl, and LR is the length of radical.

Improvement of salt-stressed MTi2 seed germination using KCl

Seven sterilized MTi2 seeds were placed in two layers of Whatman filter paper No. 1 in separate sterilized Petri dishes (9 cm diameter) containing 5 ml of different concentrations of KCl (10, 20, 30, 40, 50 mM) and as a control, deionized water. All treatment was kept in the growth chamber at 25 ± 1°C and will be monitored daily until 8 days. Measurements were made on seed vigor, germination rate, germination percentage, and early seedling growth as described in subtitle 2.3.i

Three seedlings from nine replicates of each treatment were selected randomly. The length of seedlings, radicals, and hypocotyls were measured on day 8. For biomass measurement, samples were placed in the oven at 60°C for several days until constant weight was obtained.

Improvement of salt-stressed MTi2 seed germination using a SA and KCl mixture

Seven sterilized MTi2 seeds were placed on two layers of Whatman filter paper No. 1 in separate sterilized Petri dishes (9 cm diameter) comprising the optimal ratio of KCl to SA, as shown in Table 1.

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Table 1. *Salt-stressed MTi2 seeds germinate using a combination of SA and KCl*

Treatment	Concentration (mM)
Control	**Deionized water**
SA (Salicylic acid) + KCl (Potassium chloride)	0.50 SA + 20KCl
SA (Salicylic acid) + KCl (Potassium chloride)	0.75 SA + 20KCl
SA (Salicylic acid) + KCl (Potassium chloride)	0.50 SA + 30KCl
SA (Salicylic acid) + KCl (Potassium chloride)	0.75 SA + 30KCl

All treatment was stored in the growing chamber at 25 ± 1°C and will be monitored daily until 8 days. Measurements were made on seed vigor, germination rate, germination percentage, and early seedling growth as described in subtitle 2.3.i.

Data Analysis

SPSS Windows Version 22 was used to conduct the statistical analysis. To investigate the differences in germination response across salt treatments, At a confidence level of p=0.05, a two-way analysis of variance (ANOVA) was conducted. Tukey HSD was then used at p=0.05 for mean comparison. To determine the significance difference utilizing SA, KCl, and a combination of SA and KCl, one-way ANOVA was performed at a confidence level of p=0.05. Tukey HSD was then used at p=0.05 for mean analysis.

Results

Treatment of MTi2 Seeds Salt-Stressed with SA:

The percentage and rate of germination, and Seed Vigor:

Results found that SA (salicylic acid) significantly increased the percentage and rate of germination of salt-stress MTi2 seeds. **Table 2** shows that the percentage of germination is significantly higher in all concentrations of SA than in deionized water.

The highest percentage of germination in 0.75 mM SA. Salicylic acid in all concentrations also increased the speed of germination of salt-stressed MTi2 seeds. However, the highest rate of germination was found in 0.5 mM SA.

The vigor of salt-stressed MTi2 seeds germinated in SA also increased. The vigor of seed is more than 2x higher in SA compared with deionized water. The Vigor of the seed increased 6X higher in 0.5 mM SA.

Table 2. *Salt-stressed MTi2 seeds germination percentage, germination rate, and seed vigor at different salicylic acid concentrations*

Conc. (mM)	GP (%)	GR	SV
0	37.1a±7.3	6.0a ±1. 4	2.91a±0.7
0.25	54.3ab±13.1	8.5ab ±2. 2	6.33ab±2.0
0.50	77.1b±7.3	13.2b ±1. 4	12.41c±1.7
0.75	80.0b±3.5	12.6b ±0. 2	10.87bc±0.7
1	60.0ab±8.3	10.4ab±1.5	5.24a±0.9

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The mean is used, and five replicates are used to calculate the standard errors of measurement. Significant variations between the means are indicated by superscripts with distinct letters inside the means of each column (a–b) (Tukey HSD test, $p<0.05$, Appendix 10).

Impact of Salinity on MTi2 Seedlings Grown in Early Stages:

Under salt stress, MTi2 seedlings grow much faster when salicylic acid is applied at all concentrations (ANOVA, $p<0.05$) as shown in **Table 3**. The highest length of hypocotyls, radical, seedling length, and biomass was found in 0.50 mM SA, which is 2x higher than in the control.

Table 3. *Salicylic acid concentration-dependent measurements of the hypocotyl, radical, seedling, and biomass of salt-stressed MTi2 seeds*

Conc. (mM)	HL (cm)	RL (cm)	SL (cm)	Biomass (g)
0	$4.1^{a}\pm0.4$	$3.4^{a}\pm0.3$	$7.5^{a}\pm0.6$	$0.011^{a}\pm0.0021$
0.25	$6.0^{ab}\pm0.6$	$4.7^{ab}\pm0.9$	$10.7^{ab}\pm1.2$	$0.016^{ab}\pm0.0024$
0.5	$7.6^{b}\pm0.2$	$8.4^{c}\pm0.7$	$16^{c}\pm0.7$	$0.022^{b}\pm0.0016$
0.75	$6.7^{b}\pm0.8$	$6.9^{bc}\pm0.\ 3$	$13.6^{bc}\pm0.\ 6$	$0.020^{b}\pm0.0013$
1	$3.8^{a}\pm0.\ 5$	$4.8^{ab}\pm0.\ 6$	$8.6^{a}\pm0.\ 7$	$0.014^{ab}\pm0.0021$

The mean is used, and five replicates are used to calculate the standard errors of measurement. Significant differences between the means are shown by superscripts with distinct letters inside the means of each column (a-c) (Tukey HSD test, $p<0.05$, Appendix 13-16).

Application of KCl to MTi2 Seeds Under Salt-Stress:

The percentage and rate of germination, and Seed Vigor:

The results found that KCl significantly increased the germination of salt-stressed MTi2 seeds (ANOVA, $p<0.05$). The optimum KCl concentration is 20-30 mM which has given more than 2x higher percentage and rate of germination, and Seed Vigor compared with the control as presented in Table 4.

Table 4. *Salinity-stressed MTi2 seeds treated in KCl: germination percentage, germination rate, and seed vigor*

Conc. (mM)	GP (%)	GR	SV
0	$37.1^{a}\pm7.3$	$6^{a}\pm1.4$	$2.91^{a}\pm0.7$
10	$51.4^{ab}\pm10.7$	$8.4^{ab}\pm1.8$	$4.50^{a}\pm1.2$
20	$74.3^{b}\pm11.4$	$12.4^{b}\pm1.8$	$10.1^{b}\pm1.8$
30	$82.9^{b}\pm7.0$	$14.2^{b}\pm1.1$	$10.4^{b}\pm1.0$
40	$65.7^{ab}\pm5.7$	$10.2^{ab}\pm0.6$	$6.6^{ab}\pm0.5$
50	$62.9^{ab}\pm7.3$	$9.40^{ab}\pm1.1$	$5.2^{a}\pm0.7$

The mean is used, and five replicates are used to calculate the standard errors of measurement. Significant differences between the means are shown by superscripts with distinct letters inside the means of each column (a-c) (Tukey HSD test, $p<0.05$, Appendix 17-19).

Impact of KCl on the Initial Growth of Salt-Stressed MTi2 Seedlings

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Table 5 shows that KCl significantly enhances the growth of salt-stressed MTi2 seedlings (ANOVA, $p<0.05$). KCl increased significantly in the length of hypocotyls compared with radicals. The seedling biomass also increased. This study found that the highest seedling length and biomass were found in 20 mM KCl.

Table 5. *Salt-stressed MTi2 seedlings in varying KCl concentrations: hypocotyl, radical, seedling length, and biomass*

Conc. (mM)	HL (cm)	RL (cm)	SL (cm)	Biomass (g)
0	4.1^{a}±0.4	3.4^{a}±0.3	7.5^{a}±0.6	0.011^{a}±0.0021
10	4.7^{ab}±0.7	3.9^{ab}±0.6	8.6^{a}±1	0.013^{ab}±0.0025
20	8.8^{d}±0.6	4.6^{ab}±0.3	13.3^{c}±0.5	0.021^{b}±0.001
30	7.2^{cd}±0.7	5.5^{b}±0.6	12.7^{bc}±0.9	0.018^{ab}±0.0032
40	6.9^{bcd}±0.0.5	3.3^{a}±0.3	10.2^{ab}±0.4	0.018^{ab}±0.0012
50	5.3^{abc}±0.5	2.9^{a}±0.3	8.2^{a}±0.3	0.013^{ab}±0.0018

The mean is used, and five replicates are used to calculate the standard errors of measurement. Significant differences between the means are shown by superscripts with distinct letters inside the means of each column (a-c) (Tukey HSD test, $p<0.05$, Appendix 20-23).

Combination of Salicylic Acid and KCl for the Treatment of Salt-Stressed MTi2 Seeds

The percentage and rate of germination, and Seed Vigor

Table 6 shows that the mixture of SA and KCl significantly increased the percentage and rate of germination of salt-stressed MTi2 seeds (ANOVA, $p<0.05$). The percentage and rate of germination of MTi2 seeds under salt stress treated with a mixture of SA and KCl increased more than 2x higher than deionized water (control). A mixture of 0.75 mM SA, and 30 mM KCl gave the highest germination percentage and germination rates as presented in Table 6. The mixture of SA and KCl also increased the vigor of salt-stressed MTi2 seeds more than 4x higher than deionized water (control). A combination of 0.50 mM SA and 20 mM KCl gave the highest seed vigor.

Table 6. *Salicylic acid and KCl used to treat salt-stressed MTi2 seeds resulted in positive germination percentages, rates, and vigor of seeds*

Conc. (mM)	GP (%)	GR	SV
0	37.1^{a}±7.3	6.0^{a}±1.4	2.91^{a}±0.7
0.50 SA + 20KCl	77.1^{b}±8.6	13.5^{b}±1.5	15.4^{b}±1.8
0.75 SA + 20KCl	80.0^{b}±5.7	14.1^{b}±0.9	13.5^{b}±1.1
0.50 SA + 30KCl	82.9^{b}±8.3	14.3^{b}±1.2	13.1^{b}±1.6
0.75 SA +30 KCl	94.3^{b}±7.8	16.2^{b}±0.4	13.4^{b}±0.4

The mean is used, and five replicates are used to calculate the standard errors of measurement. Significant differences between the means are shown by superscripts with distinct letters inside the means of each column (a-c) (Tukey HSD test, $p<0.05$, Appendix 24-26).

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Impact of Salicylic Acid and KCl Combination on Salt Stressed MTi2 Seedlings' Early Seedling Growth:

The combination of SA and KCl increased the early growth of salt-stressed MTi2 seedlings. **Table 7** shows that seedling length is more than 2x higher than control. The mixture also increased the length of hypocotyl, radical, and biomass. The highest seedling growth was found in the mixture of 0.50 mM SA and 20 mM KCl.

Table 7. *Salt-stressed MTi2 seedlings in varying SA and KCl concentrations: hypocotyl, radical, seedling length, and biomass*

Conc. (mM)	HL (cm)	RL (cm)	SL (cm)	Biomass (g)
0	$4.1^{a}\pm0.4$	$3.4^{a}\pm0.3$	$7.5^{a}\pm0.6$	$0.011^{a}\pm0.002$
0.50 SA + 20 KCl	$13.1^{d}\pm0.3$	$6.8^{c}\pm0.3$	$19.9^{d}\pm0.4$	$0.024^{c}\pm0.0004$
0.75 SA + 20 KCl	$10.3^{bc}\pm0.5$	$6.6^{bc}\pm0.5$	$16.9^{c}\pm0.5$	$0.022^{bc}\pm0.0009$
0.50 SA + 30 KCl	$10.7^{c}\pm0.6$	$5.0^{ab}\pm0.3$	$15.7^{bc}\pm0.6$	$0.020^{bc}\pm0.001$
0.75 SA + 30 KCl	$8.6^{b}\pm0.5$	$5.7^{bc}\pm0.5$	$14.3^{b}\pm0.6$	$0.019^{b}\pm0.0005$

The mean is used, and five replicates are used to calculate the standard errors of measurement. Significant differences between the means are shown by superscripts with distinct letters inside the means of each column (a-c) (Tukey HSD test, $p<0.05$, Appendix 27-30).

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Discussion

This experiment was carried out to increase the germination of salt-stressed MTi2 seeds by using SA, KCl, and a mixture of SA and KCl. The application of SA alone and KCl alone increased all germination parameters compared with the control. The ideal concentration for SA is 0.5 mM, and 0.75 mMA SA, while for KCl it is 20 and 30 mM of KCl. Moreover, the mixture of SA and KCl gave higher germination parameters compared with the control and SA alone and KCl alone.

Salicylic acid (SA) is a plant-produced phenolic compound and a plant hormone that acts as the main factor in the induction of plant defense against a multiple of biotic and abiotic stresses through morphological, physiological, and biochemical mechanisms **[17, 28, 29]**. **War [30]** reported that SA induced higher activity of peroxidase (POD), polyphenol oxidase (PPO), and amounts of total phenols, H_2O_2, and protein content in chickpea plants. The results suggest that SA at low concentrations could be used for the induction of a plant defensive system that would enable the plant to withstand various biotic and abiotic stresses.

The application of SA under saline conditions caused higher oxygen uptake, greater α-amylase activity, and the efficient mobilization of nutrients from the cotyledons to the embryonic axis and the higher contents of soluble sugar, protein, and free amino acids [31].

High salinity delayed germination but the inhibitory effect was reduced by low concentrations of SA [32]. Under high salinity, osmotic and ionic stresses may induce this stress and then may generate secondary effects such as oxidative stress [33]. SA promotes germination by reducing osmotic damage [32]. Other than that, the SA's exogenous application may induce the metabolic consumption of soluble sugars to form new cell constituents as a mechanism to enhance growth [17].

SA treatments activate a greater accumulation of free amino acids, including proline in the two organs (root and shoot) of the stressed plant. This compound is important as it is involved in osmotic adjustment in the presence of sodium ions. Proline (osmoregulant) is also involved in reducing the injurious effects of salinity and speeding up the repair processes following stress [17]. Proline may also contribute to the development of the antistress reaction [34].

El-Tayeb [17] found that seeds that are exposed to salt will accumulate ion content such as Na+ in the shoot and root. The accumulation of the ions causes a decrease in important nutrients of the plant tissue, for example, K, Ca, and P content. The application of SA reduces the Na+ content, thus indicating SA as the decreasing factor of toxicity absorption. Low content of toxicity elements in plant tissue results in low membrane injury, high water content, and dry matter production. ATP content in the shoot and root of the stressed seed increased, thus it reflects the high content of phosphorus after SA was applied.

Previous studies by Pareek [35]; Hussein [36]; and Mutlu [37] found that applying SA effectively improved the synthesis of soluble proteins, consequently enhancing plant adaptation to stress. Thus, increasing SA to stimulate protein accumulation in salt-stressed plants could lead to better resistance to salinity stress. Studies by El-Tayeb [17]; Deef [38]; and Ashraf [39] have shown that SA plays an influential role in improving crop resistance to salt stress, particularly at the seedling stage Several studies have indicated that the negative impact of salt stress on plants could be reduced by exogenous application of SA [35, 40, 39, 41, 42]. Bahrani [43]; and Baninasab [31] reported that lower doses of SA raised the seed germinates in cucumber, while extremely high concentrations reduced the germination percentage. Similarly, Farahbakhsh [44] in a study of Foeniculum vulgare reported that 0.5 mM of SA in measured traits was more effective than other levels.

Similar results were shown by Naz [45] who studied seed germination and seedling growth of Pisum sativum and found that the adverse effects of salinity were eliminated by applying potassium through seed priming with KCl, which can be a beneficial technique for the activation of germination of maize seeds, leading to safe seedling stands under salt stress during germination, which were enhanced using this priming process [46]. According to Leigh [47]; and Wang [48], the role of potassium is to offer the appropriate ionic environment for metabolic processes in the cytoplasm and therefore act as a regulator of numerous processes such as growth regulation. Both K and Cl are the major ions involved in the neutralization of charges and as the most important inorganic osmotically active ions in plant cells and tissues [49, 50]. K and Cl play an important role in the opening and closing of stomata [51, 52].

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K is a plant nutrient that plays a particular role as a surviving mechanism of crop plants under unfavorable stress conditions. The larger K requirement of plants under different abiotic stresses appears to be related to the inhibitory role of K against ROS production. NaCl treatments decreased the K nutrition of plants and suggested that K deficiency at the cellular level might be a contributory factor to salt-induced oxidative stress and related cell damage. Improving the K nutrition of plants under salt stress could be a remarkable strategy for minimizing oxidative cell damage, at least in part by reducing ROS formation [53].

Conclusion

Salinity stress can seriously affect the germination characteristics of MTi2 seeds. Thus, another aim of the current study is to identify an effective therapy that will boost the germination of MTi2 seeds that have been salt-stressed. According to the results, the best concentrations for boosting the germination and early growth of MTi2 seedlings in comparison to the control treatment were found to be 0.5-0.75 mM SA and 20-30 mM KCl. Furthermore, MTi2 seedling germination and early growth were more than 1x higher when the best concentrations of KCl (20-30 mM) and SA (0.5-0.75 mM) were combined. Salicylic acid (SA) and low levels of KCl applied to salt-stressed MTi2 seeds can help reduce the negative effects of salinity stress and enhance the percentage, rate, vigor, length, and biomass of the seedlings that germinate. Where maximum germination percentage (94.3%) was recorded in 0.75mM SA and 30mMKCl, the highest germination rate shows in0.75 mM SA and 30 mM KCl (14.2), the highest value of seed vigor shows in 0.5 mM SA and 20 mM KCl mM (15.4) and the highest length of seedlings shows in 0.5 mM SA and 20 mM KCl mM (19.9cm). Finally, the highest value of biomass was observed at 0.5 mM SA and 20 mM KCl mM (0.0244g).

Salicylic acid (SA) and a low concentration of KCl applied to salt-stressed MTi2 seeds can contribute to mitigating the negative effects of salinity stress and enhance seed vigor, germination rate, percentage, dry weight, and seedling length.

Conflicts of Interest

The authors hereby declare no conflicts of interest.

Authors Contribution:

All authors contributed in the experiments, analysis and preparation of this manuscript.

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