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# Contribution of QuitoMax® to the hormonal and enzymatic metabolism in tomato under saline stress

## Contribuição do QuitoMax® no metabolismo hormonal e enzimático em tomate sob estresse salino

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### ABSTRACT

Salinity stress severely restricts plant nutrition and hinders biochemical and physiological processes crucial for growth. In several crop systems bioactive products which confer growth promotion, are applied as a sustainable alternative for contributing to food security. The aim of this work was to evaluate the biochemical contribution of QuitoMax® to hormonal and enzymatic metabolism in tomato under saline stress. Three treatments were applied: saline without QuitoMax®, nonsaline + QuitoMax® and saline + QuitoMax®. A tolerant (Amalia) and a susceptible (Claudia) tomato variety were used as experimental models. The normalized difference vegetation index (NDVI) was measured as a morphological variable, and peroxidase (POD), glutamine synthetase (GS) and nitrate reductase (NR) enzyme activities were determined. Gibberellic (GA) and abscisic acid (ABA) concentrations were also determined. Due to the effects of QuitoMax®, the plants maintained high NDVI values even under saline conditions. A decrease in POD and GS activity and an increase in NR activity were also found. The GA concentration in the leaves was higher in the tolerant variety when QuitoMax® was applied than in the saline treatment but lower in the susceptible variety. The opposite behavior was found when the ABA concentration was quantified. This study demonstrates the protective action of QuitoMax® under salinity stress on tomato crops in both tolerant and susceptible varieties. In crux, QuitoMax® can be opted as a shotgun approach to tackle salinity in tomato.

**Index terms:** Peroxidase activity; glutamino synthetase; nitrato reductase; ABA; GA.

### RESUMO

O estresse salino é um dos fatores abióticos que mais limita a nutrição das plantas. Também limita o desempenho bioquímico e fisiológico que regula o crescimento em diversos sistemas de cultivo de produtos bioativos que conferem promoção de crescimento, são aplicados como alternativa sustentável para contribuir para a segurança alimentar. O objetivo do trabalho foi avaliar a contribuição bioquímica do QuitoMax® no metabolismo hormonal e enzimático do tomate sob estresse salino. Foram aplicados três tratamentos: solução salina sem QuitoMax®, QuitoMax® sem solução salina e solução salina com QuitoMax®. Uma variedade de tomate tolerante (Amalia) e uma suscetível (Claudia) foram utilizadas como modelos experimentais. O índice de vegetação por diferença normalizada (NDVI) foi medido como variável morfológica, e as atividades das enzimas peroxidase (POD), glutamina sintetase (GS) e nitrato redutase (NR) foram determinadas. As concentrações de ácido giberélico (GA) e abscísico (AB) também foram determinadas. Devido aos efeitos do QuitoMax®, as plantas mantiveram valores elevados de NDVI mesmo em condições salinas (T3). Também foram encontradas diminuição na atividade de POD e GS e aumento na atividade de NR. A concentração de GA nas folhas foi maior na variedade tolerante quando aplicado QuitoMax® do que no tratamento salino, mas menor na variedade suscetível. O comportamento oposto foi encontrado quando a concentração de ABA foi quantificada. Este estudo demonstrou a ação protetora do QuitoMax® sob estresse salino em culturas de tomate em variedades tolerantes e suscetíveis. Em suma, QuitoMax® pode ser escolhido como uma abordagem agressiva para combater a salinidade no tomate.

**Termos para indexação:** Atividade peroxidase; glutamino sintetase; nitrato redutase; ABA; GA.

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## Introduction

Tomato (*Solanum lycopersicum* L.) is, worldwide, one of the most important crops due to its extension, demand and uses in the food industry. It can be consumed fresh or processed in various products, such as tomato juices, sauces, ketchup, pulp and paste, and in dried form (Ji et al., 2023; Al-Roshdi et al., 2023).

Worldwide, tomato production covers over 5 million hectares of cultivated area, and more than 182 million tons of tomato are produced globally (Caruso et al., 2022). The organic tomato production trend has also been very successful, with a view to protecting the soil and offering fruits with lower fertilizer concentrations, mainly nitrogenous fruits (Adekiya et al., 2022).

Soil salinity causes serious negative effects on plants (Jiménez-Mejía et al., 2022). Its effects can be morphological, physiological and biochemical, such as the water regime, photosynthesis, and enzymatic metabolism, which largely affect fruit yield (Waqas et al., 2019; Bacha et al., 2017).

Tomato plants growth and fruit yield are affected by abiotic and biotic factors such as salinity (Falcón Rodríguez et al., 2021). When tomato is grown in saline soils, the yield decreases since it is a glycophyte species that is moderately susceptible to salts. Tomato plants exhibit various alterations from physiological to even acoustic signals under abiotic stress (Waqas, Van Der Straeten, D., & Geilfus, 2023). Particularly, salinity stress has harmful impacts on tomatoes, such as ionic imbalance, oxidative stress, nutrient deprivation, hormonal imbalance, and photosynthesis limitation. All of these eventually lead to decreased growth of tomato plants under salinity (Ors et al., 2021). The greatest sensitivity of this crop to salinity occurs when the electrical conductivity of the saturation extract ($EC_{se}$) of the soil exceeds 2.5 d $Sm^{-1}$ (Alam et al., 2021). However, utilizing PGR proves to be an effective strategy for managing salt stress (Ullah, Bano, & Khan, 2021).

Removing total salinity is practically impossible under field conditions. Therefore, it is necessary to use alternative methods that reduce the negative effect caused by the accumulation of salts in the soil (Galford et al., 2018). One of these alternatives is the use of development promoters under stress conditions, such as QuitoMax®.

QuitoMax® is a chitosan-based liquid formulation. This product has shown stimulating effects on plant growth, including grain and tuber species (Falcón Rodríguez et al., 2021). Its use stimulates initial seed germination, flowering and fruit filling (Reyes-Pérez et al., 2020). QuitoMax® has also been used in tomato and has caused an acceleration of plant metabolism and an increase of 15% in yield under saline soil (Avila-Amador et al., 2022).

This product was validated as a growth-yield promoter and for plant protection of crops of agricultural interest (Terry Alfonso et al., 2017). For example, in beans (*Phaseolus vulgaris*), corn (*Zea mays*), potato (*Solanum tuberosum*), tobacco (*Nicotiana tabacum*) and tomato, González et al. (2021) reported an increase in biomass and yield of approximately 17% under field conditions but not under salinity. Some of the most recent reports of its use for saline stress mitigation were developed by Avila-Amador et al. (2022) but were based on morphological and agronomical indicators. In this sense, our hypothesis is that QuitoMax® minimizes the effects of soil salinity and promotes biochemical activity in tomato plants. The experiment aimed to evaluate the contribution of QuitoMax® to salinity effect mitigation based on the NDVI, the peroxidase, nitrate reductase and glutamine synthetase enzyme activities, and the hormonal ABA-GA concentration at an electrical conductivity (EC) of 6 dS $m^{-1}$.

## Material and Methods

### Seedbed establishment for seedling production

The experiment was established in the seedling production greenhouse of the Tecnológico Nacional de México Campus Valle del Yaqui during January- March, 2023, under semicontrolled experimental conditions. The experimental area presented the following climatic conditions: an average temperature of 21°C and an RH of 37%. These variables remained constant during the crop cycle of both tomato varieties.

### Treatments and experimental design

The treatments were the following: T1: saline soil at an EC of 6.0 dS $m^{-1}$ (saline stress condition according to Tola et al. (2023) without QuitoMax®, T2: nonsaline soil whit the application of QuitoMax® at a dose of 300 mg $L^{-1}$; and T3: saline soil with an EC of 6.0 dS $m^{-1}$ with QuitoMax® at a dose of 300 mg $L^{-1}$ (Table 1). These treatments were applied to both varieties Amalia and Claudia, which are classified as tolerant and susceptible to salinity, respectively. The Amalia variety was obtained from the commercial varieties Campbell-28 and INCA-3. This variety has a biological cycle of 125 days. It is considered tolerant to salinity, drought and high temperatures. Its potential yield can reach 64 t $ha^{-1}$. The Claudia variety was obtained from the crossing of the commercial varieties Amalia and HC 3880. The Claudia variety has a biological cycle of 130 days and a potential yield of 55 t $ha^{-1}$ (Álvarez et al., 2008).

**Table 1:** Treatments used in the experiment.

| Treatments | EC (dS $m^{-1}$) | Dosage of QuitoMax® (mg $L^{-1}$) |
|---|---|---|
| T1 | 6.0 | 0 |
| T2 | 0.9 | 300 |
| T3 | 6.0 | 300 |

EC: Electric conductivity.

In this experiment the varieties were not considered as a source of variation. Treatments were distributed following a completely randomized experimental design, with five repetitions per treatment. The Quitomax® application was done to the plants foliarly at 25 days after germination and the second application at 10 days after flowering phenophase (45 days after germination).

### Substrate preparation and seeding

The seeds were placed in 128-cavity polypropylene trays (66.4 cm long x 33.5 cm wide and 7.7 cm high). The sowing was performed to a depth of one cm. A *SUMCHINE*-type inert substrate was used, which was moistened to 95%

of field capacity with common water with an electrical conductivity of 0.22 dS $m^{-1}$. The seeds used were disinfected with sodium hypochlorite for 30 seconds and then washed with distilled water.

Seeds of Amalia and Claudia varieties were used for the trials where the biostimulant (QuitoMax®) was subsequently applied as recommended by Terry Alfonso et al. (2017). Fertilization, irrigation and monitoring of possible pests or diseases to the seedlings were performed according to demand throughout the experiment. Plastic pots of 4.63 kg of capacity (wide: 21 cm and height: 19.5 cm) were used to conduct the experiment and were filled with a soil classified as Vertisol (Verhulst et al., 2011) with a 49% of clay (Table 2). This soil classification corresponds to the *Soil Taxonomy* classification methodology (Deckers et al., 2002). Such classification also correlates with the methodology proposed by the *Wolrd Reference Base (WRB)* (Nachtergaele et al., 2000).

To determine the chemical variables (Table 2), 537 g of soil was placed, and 300 mL of distilled water was added. This mixture was stirred for two min until a uniform pasty fluid-like saturated paste was achieved (Deng et al., 2020).

Chemical analyses were performed at the Instituto de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP) laboratory in Obregón city, Sonora, Mexico, based on the official Mexican standard NOM-138-SEMARNAT/SS-2003 (Norma Oficial Mexicana, 2005). The physical and chemical characteristics of the soil used in the experiment are presented in Table 2, showing mainly a Ca-Mg-K-Na saturation of 42. The $K^+$ content was about 49 $mmol_c$ $dm^{-3}$ in average. The organic matter content is low, which is typical of the soils of the region (Peñuelas-Rubio et al., 2022).

For the pots corresponding to the treatments without salt content, the soil was washed with distilled water until the electrical conductivity was reduced to 0.9 dS $m^{-1}$ (Beroisa, Kloster & Iturri, 2023). Four washes were performed, and a portable digital multiparameter meter was used to monitor the EC (se).

To raise an ECse of 6 dS $m^{-1}$ in the pots that had the saline treatment, a saline solution was prepared at an electrical conductivity of 1.19 dS $m^{-1,}$ which was added to complete an electric conductivity of the saturation extract of 6 dS $m^{-1}$ (García & Medina, 2003).

Each pot contained 4,63 kg of soil. They were irrigated to obtain 95% of the water retem capacity (WRC). Subsequently, the electrical conductivity ($EC_{se}$) of the saturation extract, pH, and temperature were determined. These parameters were measured at weekly intervals using a portable digital multiparameter (COMBO, USA).

The moisture content of the soil was determined by taking a composite sample of 109.7 g of moist soil, which was subsequently weighed on a semianalytical balance (*Scout-Pro)* and dried at room temperature for 72 hours to obtain a constant weight. The water content value (83.55% of the WRC) was obtained by gravimetry from the difference in weight. This information was corroborated with the use of a tensiometer.

## Transplanting, biofertilizer application and irrigation

Transplanting was carried out in pots 28 days after plant germination. Fertilization was applied to all treatments at a rate of 450 kg $ha^{-1}$ (0.002 kg per pot). The fertilizer used was TRIPLE-19, containing 19% nitrogen, 19% phosphorus and 19% potassium. It also contain the microelements iron, manganese, zinc, copper and molybdenum at rates of 1000, 500, 200, 110 and 70 ppm (mg $L^{-1}$). The same fertilization scheme was repeated 45 days after germination, according to the indications of the technological package for this crop under greenhouse conditions (Agrinova, 2022).

For irrigation applications in the pots, a soil moisture-measuring device (IRROMETER) was used. The device was located at a depth of 15 cm. The irrigation was applied up to a water retempot capacity (WRC) of 42%. An amount of 0.350 L per plant was applied at an irrigation interval of seven days. Other cultural attention (training, hilling and pruning) were carried out according to the technical instructions for the crop (Esquivel-Ayala et al., 2021).

**Table 2:** Physical and chemical characteristics of the Vertisol gleyco soil used in the experiment.

| Physical characteristics | | | | | | |
|---|---|---|---|---|---|---|
| ECse (dS $m^{-1}$) | | pH ($H_2O$) | Sand | Silt | Clay | OM |
| 4.81 | | 7.65 | 13.87 | 36.69 | 49.44 | 0.5 |
| Cation content ($mmol_c$ $dm^{-3}$) | | | | | | |
| N | P | $K^+$ | $Ca^{2+}$ | $Mg^{2+}$ | Ca-Mg-K-Na saturation | |
| 13.87 | 36.69 | 49.44 | 2.18 | 4.26 | 42 | |

*ECse: Electric conductivity of the saturation extract; pH: potential of hydrogen (in water); N, P, $K^+$, $Ca^{2+}$, $Mg^{2+}$: Nitrogen, Phosphorous, Potassium, Calcium and Magnesium. OM: Organic matter.

### Variables evaluated, Normalized difference vegetation index (NDVI)

The normalized difference vegetation index (NDVI) was measured with a portable sensor (GreenSeckeer) between 08:00 and 09:00 h in the flowering phenophase (45 days after emergence). In each treatment, 20 measurements were taken at a height of 0.60 m from the seedling canopy, according to the sensor reference (Govaerts & Verhulst, 2010). This variable was evaluated to compare the NDVI value in each treatment; -1<NDVI>1. The interpretation of NDVI can contribute to the rapid and directed diagnosis of crop nutrient conditions (especially nitrogen) and the possible incidence of stress. NDVI values close to 1 represent a better nutritional status of the plants (Inman, Khosla, R., & Mayfied, 2005).

### Peroxidase, nitrate reductase and glutamine synthetase enzyme activity

Peroxidase enzyme activity (POD) was measured according to the method described by Maehly and Chance (1954). The enzyme extract was obtained from a 0.25 g sample of leaf fragments sampled, at 10:00 h, from 15 leaves (three leaves per plant at each repetition) in each treatment, which were homogenized in a mortar with 5 mL of Tris-HCl buffer pH 7.4. The homogenate was centrifuged at 12,900 rpm for 15 min, and the supernatant was used to determine POD activity. The oxidative substrate was composed of guaiacol (2-methoxyphenol, catechol monomethyl ether, pyrocatechol monomethyl ether) (0.5 mL) and 30% hydrogen peroxide (0.1 mL). The POD extract (0.1 mL) was mixed with the oxidative substrate (0.25 mL) to measure the absorbance at 470 nm. This reaction was used to define enzyme activity units (EAU), where one unit was the 0.01 change in absorbance reading over one minute and is reported per gram of fresh weight.

For nitrate reductase (NR) enzyme activity, the methodology of Jaworsky (1971) was used. The samples were collected at 10:00 h. Each sample was composed by fragments of 3 $cm^2$ from the central part of three leaves per plant at each repetition. The sample was macerated during five minutes in a porcelain mortal at 0 °C. The homogenate was filtered and centrifuged at 30,000g for 20 minutes to obtain the enzymatic extract. A final volume of 2 mL of the reagents, 0.8 mL of phosphate buffer (100 mM $KH_2PO_4/KH_2PO_4$, pH 7.5), 0.2 mL of 100 mM $KNO_3$, 0.2 mL of 10 mM cysteine, and 0.2 mL of 2 mM NADH were added and mixed with 0.6 mL of enzyme extract. NR activity was stopped by adding 0.1 mL of 1 M zinc acetate after 30 min of incubation of the samples in the dark at 30 °C. Subsequently, to remove the precipitate formed after the addition of zinc acetate, the samples were centrifuged at 12,000 rpm for 15 min. NR activity data were expressed as µmol $NO_2^-$ g $FW^{-1}$ $h^{-1}$.

Glutamine synthetase (GS) activity was measured using the methodology proposed by O'Neal and Joy (1973). For this purpose, 0.5 g of fresh plant material composed by a sample of 15 leaf fragments: (three fragments per plant in each treatment) was homogenized with 5 mL of 0.2 M HEPES buffer, pH 7.9. The homogenate was centrifuged at 16,000 rpm for 20 min, and the supernatant obtained was used to measure GS activity. Enzyme activity was determined by the method described by Slawky and Rodier (1988). The extracted material was centrifuged at 11,000 rpm for 10 min. Then, from the resulting supernatant, a 50 µL sample was taken to quantify inorganic phosphorus (Pi) from the enzymatic use of ATP, which was determined by the vanadomolybdophosphoric colorimetric method (Hogue, Wilcow, G., & Cantliffe, 1970) at a wavelength of 430 nm and contrasted with a standard curve of $KH_2PO_4$ (5-100 µM) and was expressed as GS $s^{-1}$ $g^{-1}$ FW.

### Gibberellic acid and abscisic acid concentrations

For the determination of gibberellic acid (GA) and abscisic acid (ABA) by HPLC (expressed in ng $g^{-1}$ DW) at 30 d after germination, the methodology of Ortiz et al. (2001) and Verma, Azad and Singh (2022) was used. Leaf tissue (10 g fresh weight) were collected at 10:00 h was used and immediately frozen in liquid nitrogen. Frozen leaf tissue was freeze-dried for 48 h and ground, and extraction was performed in deionized distilled water with an extraction ratio of 1:40 (dry weight: mL water) at 4 °C (Wang et al., 2016).

### Statistical analysis

For the data procedure, the theoretical assumptions of normality and homogeneity of variance were tested (Kolmogorov, 1933). Subsequently, analyses of variance were of simple classification based on linear fixed-effect models developed for each variable (Fisher, 1935). When there were differences between treatments, Tukey's multiple comparison of means multiple range test was used for the 5% significance level (Tukey, 1960). The statistical indicators, standard error, coefficient of variation and coefficient of determination without adjustment were determined. From the means of the treatments, in each variety, the correlation networks were built between the variables NDVI, activity of the enzymes POD, GS, NR and ABA and GA concentration. For all analyses, the professional statistical software STATISTICA, version 14.2 for Windows, was used (Statsof, 2014).

## Results and Discussion

When NDVI was evaluated in tomato plants of both cultivars, significant differences were found between the treatments evaluated in both cultivars (Figure 1). In both cultivars, the behavior for NDVI was similar, with superiority for T2. The lowest values were obtained when tomato plants were subjected to a stress of 6.0 dS $m^{-1}$ in T1. The application of this biostimulant promotes better plant nutrition by considering the NDVI values.

At the same time, its presence helps the plant to recover from stress conditions by comparing T1 and T3, which shows a difference of 0.13 units in the cultivar Amalia and 0.17 units in Claudia in favor of the biostimulant (Figure 1).

Higher NDVI values (-1<NDVI>1) represent better plant nutritional status (Bian et al., 2021; Inman, Khosla, R., & Mayfied, 2005). The results obtained for this indicator reveal the positive influence of the application of the biostimulant QuitoMax® on tomato crops under salinity stress conditions. It favors the adequate nutrition and development of tomato plants regardless of the susceptibility of the variety to salt stress.

Zhang et al. (2011) demonstrated that NDVI is related to biomass, leaf area, plant cover, nitrogen and chlorophyll content in plants. It has also been observed that a plant in a good physiological state will reflect more near infrared waves due to the presence of chlorophyll, while a plant in a poor state will reflect fewer near infrared waves due to the absence of chlorophyll (Earth Observing System - EOS, 2020). This fact reveals the importance of the results found when evaluating NDVI by applying QuitoMax® under salt stress conditions.

Other authors have highlighted the importance of this indicator by reflecting that the green seeker calculates the normalized difference in the vegetative index using red and near-infrared light. It is based on the simple principle that plant chlorophyll absorbs red light as a source of energy during photosynthesis (Duhan et al., 2017), which reinforces the positive results obtained with QuitoMax® application under salt stress conditions.

## Activity of peroxidase (POD), glutamine synthetase (GS) and nitrate reductase (NR) enzymes in the flowering phenophase

Significant differences between treatments in both cultivars and in all enzymes evaluated were obtained when enzyme activity was evaluated. Regardless of the enzyme activity quantified, the highest values were always observed in the saline treatment without QuitoMax® application.

This result shows that salt stress activates all these enzymes in both tomato cultivars. Similarly, the lowest values were obtained in the nonsaline treatment, and intermediate values were obtained in the saline treatment with the application of the biostimulant (Figure 2). Although in all cases the values showed a similar pattern of behavior, the differences between treatments for NR content were lower or more stable among all treatments for both cultivars.

When the biostimulant was not applied under saline conditions, less enzyme synthesis was necessary in Amalia than in Claudia. When it was applied, levels decreased in both varieties, with lower accumulation of POD and GS in Amalia and higher accumulation of NR in Claudia (Figure 2). For T2, lower levels were always found in Claudia but were more marked in NR.

These results coincide with those reported by other authors who found that under saline conditions, the activity of these antioxidant enzymes, including SOD (superoxide dismutase), CAT (catalase) and POD (peroxidase), increases. The tolerant genotype also presented a higher antioxidant enzyme activity than the susceptible genotype (Gharsallah et al., 2016). The increased activity of antioxidants correlates with scavenging excessive reactive oxygen species production (Wagas et al., 2021). Based on the present results it is possible to infer that QM application strengthens the oxidative metabolism of tomato plants under salinity.

Other authors referring to the effects of the application of polysaccharides derived from halophytic plants (Bouteraa et al., 2022) and chitosan on tomato under saline conditions have reported an increase in enzyme activity (Balusamy et al., 2022; Mondal, Puteh, & Dafader, 2016).

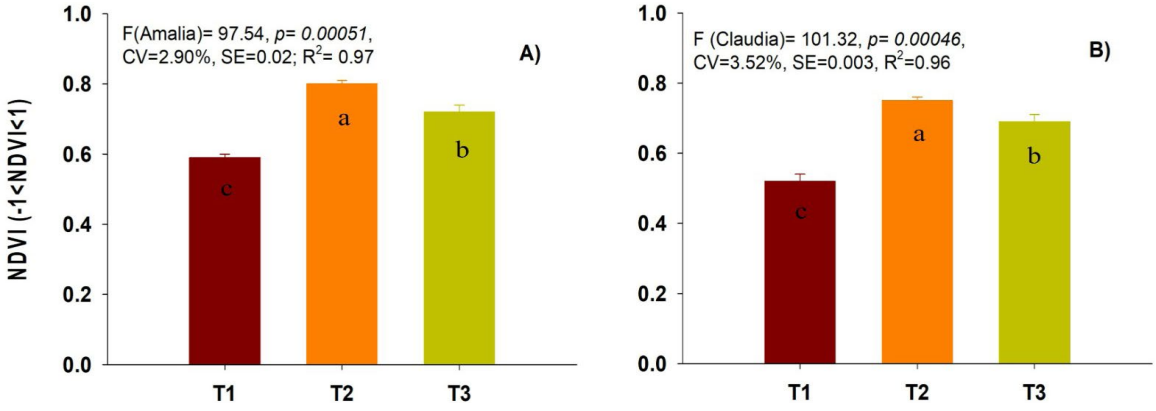


**Figure 1:** Effect of QuitoMax® application on the normalized difference vegetation index (NDVI) in Amalia (A) and Claudia (B) tomato varieties subjected to three treatments: T1: Salinity without QuitoMax®; T2: Nonsalinity + QuitoMax®; T3: Salinity + QuitoMax®. Different letters in the rectangular bars indicate significant differences by Tukey for p<0.01. F: Fisher's F value, p: probability, CV: coefficient of variation, SE: standard error, $R^2$: Coeficient of determination without adjustment.

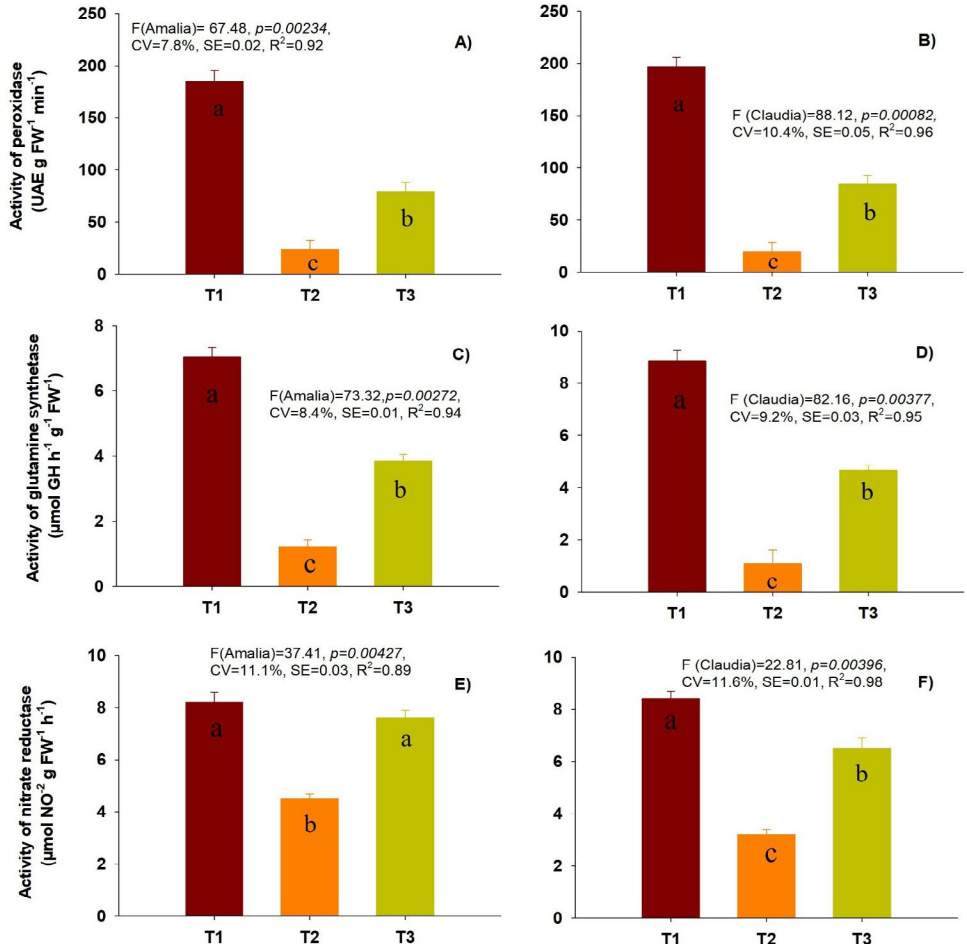


**Figure 2:** Peroxidase (A, B), glutamine synthetase (C, D) and nitrate reductase (E, F) enzyme activity in Amalia (A, C, E) and Claudia (B, D, F) tomato varieties subjected to three treatments: T1: salinity without QuitoMax®; T2: no salinity + QuitoMax®; T3: salinity + QuitoMax®. The rectangular bars represent the standard error of the treatments. UAE: unit of enzyme activity). Different letters in the rectangular bars indicate significant differences by Tukey for p<0.01. F: Fisher's F value, p: probability, CV: coefficient of variation, SE: standard error, $R^2$: Coeficient of determination without adjustment.

These findings coincide with our results if we compare them with those observed between the saline and nonsaline treatments (Zuzunaga-Rosas et al., 2022). In this case, we observed that the accumulation levels are regulated by QuitoMax®, probably adjusted to the needs of the plants under this stress condition, since other mechanisms may have been activated by the effect of the biostimulant.

Regarding the effects of QuitoMax® on tomato plants without the presence of stress, Rodríguez et al. (2020) reported that the mode of action through which QuitoMax® promotes growth and development in plants is not well defined. However, it is known that they activate responses such as the stimulation of enzymes related to primary metabolism (nitrate reductase). This coincides with what was previously stated by Falcón Rodríguez et al. (2015), who reported that chitosan, under nonsaline conditions, stimulates enzyme activity in leaves. Mondal et al. (2016) found the same effect under saline stress conditions.

## Concentrations of gibberellic acid (GA) and abscisic acid (ABA)

When evaluating GA concentration, significant differences were found between treatments in the Amalia variety, with higher accumulations in the saline treatments. Its value in the nonsaline treatment was significantly lower (Figure 3A). From the results of this study, QuitoMax® application in the saline treatment did not affect the concentration of GA found in the leaves of the Amalia variety (Figure 3A).

Significant differences were found in the Claudia variety (Figure 3B), and the highest values were found in the saline treatment (T1). These values were significantly lower in the treatments with biostimulant application regardless of the salinity levels in the medium (Figure 3B). This indicates that the application of QuitoMax® on tomato plants did not stimulate the GA concentration in a variety susceptible to salinity.

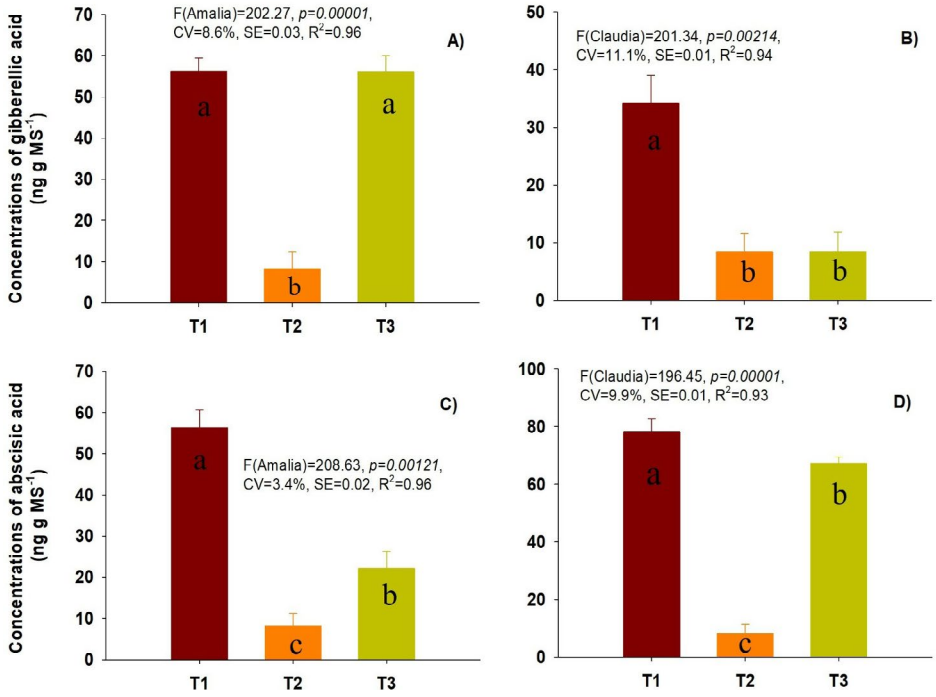


**Figure 3:** Concentrations of gibberellic acid (A, B) and abscisic acid (C, D) in tomato varieties Amalia (A, C) and Claudia (B, D) subjected to three treatments: T1: Saline without QuitoMax®; T2: Nonsaline + QuitoMax®; T3: Saline + QuitoMax®. Different letters in the rectangular bars indicate significant differences by Tukey for p<0.01. F: Fisher's F value, p: probability, CV: coefficient of variation, SE: standard error, R2: Coeficient of determination without adjustment.

The differences in the results observed among varieties in this indicator could be related to the intervarietal response to salt stress found among tomato varieties (Ávila-Amador et al., 2022). It is possible that the physiological mechanisms are not activated depending on the levels of tolerance to salinity stress due to the differences in the mechanism response among varieties.

When evaluating the ABA content, similar behavior was observed in both varieties (Figure 3C and 3D), and significant differences were found between treatments in the Amalia variety. The highest values were obtained when analyzing the samples of plants subjected to salt stress without applying QuitoMax® and the lowest in the nonsaline treatment (T2), with intermediate values when applying the biostimulant under salinity stress conditions (Figure 3C).

ABA is important in many physiological processes in plants. This hormone is necessary for the regulation of several events during the last stage of seminal development and is crucial for the response to environmental stress (drought, salinity and cold). It also controls plant growth and inhibits root elongation (Pilet & Chanson, 1981), which means that there is a negative correlation between growth and endogenous ABA contained in plants (Pilet & Saugy, 1987). Additionally, this plant hormone plays a central role in cell signaling between the roots and the aerial part of the plant during drought stress; it also participates in the regulation of growth and $g_s$ (Davies, Kudoyarova, & Hartung, 2005).

Several authors also refer to the activity of cytokinins and catalase, which act as antioxidants, such as catalase (ROS degradation factor). They also prevent the presence of other compounds that hinder the normal development of plants subjected to water stress. This is the case for cytokinins that counteract the negative effect of ABA in the leaves produced by the plant under this type of stress (Yang, Kloepper, & Ryu, 2008).

Curá et al. (2017) found an association between the decrease in ABA content and the lower perception of water stress by the plant, which resulted in lower lipid peroxidation and higher carbon, nitrogen, chlorophyll and relative water content at the leaf level, as well as higher biomass production. In another study under simulated drought stress conditions, Zhang et al. (2021) related increased plant height and increased root dry mass, aerial dry mass and relative water content to decreased ABA levels and increased antioxidant enzyme activity.

ABA is the main hormone involved in stomatal closure, ion homeostasis, the expression of stress response genes and other metabolic changes. Self-regulatory capacities in plants are collectively responsible for acclimation, evasion, or detoxification of stressors (Isah, 2019).

Oligosaccharides have been reported to promote an antitranspirant effect through stomatal closure by the direct action of increased ABA levels in stomatal chaperone cells, leading to improved water use in the plant (Mondal, Puteh, & Dafader, 2016; Dzung, 2011).

There was a positive correlation between ABA and GS, between ABA and POD, and between GS and POD. Between ABA and NDVI, the correlation was negative in the Amalia variety (tolerant) (Figure 4A).

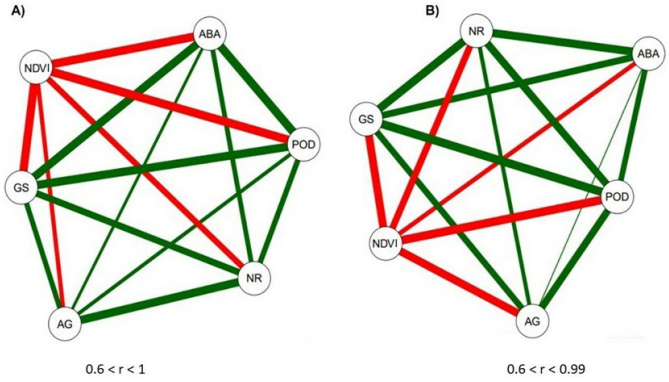


**Figure 4:** Network of correlations obtained by purchasing two tomato varieties [Amalia (A) and Claudia (B)] subjected to salt stress and considering the variables normalized difference vegetation index (NDVI), peroxidase (POD), glutamine synthetase (GS) and nitrate reductase (NR) enzyme activity, and concentrations of gibberellic acid (AG) and abscisic acid (ABA). Green and red traces represent positive and negative correlations, respectively. The thicker lines are the correlation closer to the value 1.

This NDVI variable correlated negatively with the rest of the variables evaluated in both cultivars. In the susceptible variety (Claudia) (Figure 4B), the highest positive correlation was between the GS and POD variables. On the other hand, the greatest negative correlation in this variety was between NDVI and POD.

The results obtained show that under stress conditions, the concentration of ABA increases. This hormone can decrease when development promoters such as QuitoMax® are applied. As explained before, due to the effect of the biostimulant, the POD activity decreased, showing less severe saline stress in the treatments.

The study of enzyme activity and hormonal relations shows the efficiency of the biochemical mechanisms activated in plants in response to stress conditions. These mechanisms are translated into morphological characteristics such as NDVI that verify a good nutritional status and a better adaptive response to the saline stress condition as the one imposed in the present study.

## Conclusions

The biochemical and physiological activities of tomato is benefited by the use of QuitoMax® in both tolerant and susceptible varieties under saline conditions. The main contribution of QuitoMax® application in plants under this stressing condition is to the maintenance of high NDVI and major plant health seen by means of NR activity.

## Author Contributions

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## References

Adekiya, A. O. et al. (2022). Organic and in-organic fertilizers effects on the performance of tomato (*Solanum lycopersicum*) and cucumber (*Cucumis sativus*) grown on soilless medium. *Scientific Reports*, 12(1):12212.

Agrinova. *Foligreen 19-19-19*, 2022. Available in: <https://agri-nova.com/product/fertilizantes-foliares/foligreen-19-19-19/>.

Alam, M. S. et al. (2021). Early growth stage characterization and the biochemical responses for salinity stress in tomato. *Plants*, 10(4):712.

Al-Roshdi, M. R. et al. (2023). Artificial microRNA-mediated resistance against Oman strain of tomato yellow leaf curl virus. *Frontiers in Plant Science*, 14:1164921.

Álvarez, M. et al. (2008). Claudia, Mercy y Mayle, tres nuevas variedades de tomate para el consumo fresco. *Cultivos tropicales*, 29(1):43-44.

Avila-Amador, C. et al. (2022). Respuesta del cultivo del tomate (*Solanum lycopersicum* L.) a la aplicación de QuitoMax® en condiciones de salinidad. *Research, Society and Development*, 11(12):e10111233870.

Bacha, H. et al. (2017). Impact of salt stress on morpho-physiological and biochemical parameters of *Solanum lycopersicum* cv. Microtom leaves. *South African Journal of Botany*, 108:364-369.

Balusamy, S. R. et al. (2022). Chitosan, chitosan nanoparticles and modified chitosan biomaterials, a potential tool to combat salinity stress in plants. *Carbohydrate Polymers*, 284:119189.

Beroisa, C., Kloster, N., & Iturri, L. A. (2023). Medición de cationes intercambiables en suelos afectados por sales de la región semiárida pampeana. *Ciencia del Suelo*, 41(1):123-129.

Bian, L. et al. (2021). Spatiotemporal changes of soil salinization in the yellow river delta of China from 2015 to 2019. *Sustainability,* 13(2):822.

Bouteraa, M. T. et al. (2022). Bio-stimulating effect of natural polysaccharides from *Lobularia maritima* on durum wheat Seedlings: Improved plant growth, salt stress tolerance by modulating biochemical responses and ion homeostasis. *Plants*, 11(15):1991.

Caruso, A. G. et al. (2022). Tomato brown rugose fruit virus: A pathogen that is changing the tomato production worldwide. *Annals of Applied Biology*, 181(3):258-274.

Curá, J. A. et al. (2017). Inoculation with *Azospirillum* sp. and *Herbaspirillum* sp. bacteria increases the tolerance of Maize to drought stress. *Microorganisms*, 5(3):41.

Davies, W. J., Kudoyarova, G., & Hartung, W. (2005). Long-distance ABA signaling and its relation to other signaling pathways in the detection of soil drying and the mediation of the plant's response to drought. *Journal of Plant Growth Regulation*, 24(4):285-295.

Deckers, J. A. et al. (2002). *World reference base for soil resources*. Encyclopedia of soil science, Marcel Dekker, p. 1446-1451.

Deng, X. et al. (2020). A method of electrical conductivity compensation in a low-cost soil moisture sensing measurement based on capacitance. *Measurement*, 150:107052.

Duhan, J. S. et al. (2017). Nanotechnology: The new perspective in precision agriculture. *Biotechnology Reports*, 15:11-23.

Dzung, N. A. (2011). Enhancing crop production with chitosan and its derivatives. In Se-Kwon Kim (Editor). *Chitin, chitosan, oligosaccharides and their derivatives: biological activities and applications*. ed. Taylor & Francis, Boca Raton, (p.619-631).

Earth Observing System - EOS. (2020). *NDVI: Índice de vegetación de diferencia normalizada*. Available in: <https://eos.com/ndvi/es/>.

Esquivel-Ayala, B. A. et al. (2021). El impacto de *Engytatus varians* (DISTANT) (HEMIPTER: MIRIDAE) en tomate dentro de jaulas en invernadero. *Revista Fitotecnia Mexicana*, 44(3):409-409.

Falcón Rodríguez, A. B. et al. (2015). Nuevos productos naturales para la agricultura: Las oligosacarinas*. Cultivos Tropicales*, 36:111-129.

Falcón Rodríguez, A. B. et al. (2021). Oligosacarinas como bioestimulantes para la agricultura cubana. *Anales de la Academia de Ciencias de Cuba*, 11(1):852.

Fisher, R. A. (1935). *Statistical methods for research workers*. Springer, New York.

Galford, G. L. et al. (2018). Cuban land use and conservation, from rainforests to coral reefs. *Bulletin of Marine Science*, *94*(2):171-191.

García, M., & Medina, E. (2003). Crecimiento y acumulación de prolina en dos genotipos de caña de azúcar sometidos a salinización con cloruro de sodio. *Revista de la Facultad de Agronomía*, 20(2):168-179.

Gharsallah, C. et al. (2016). Effect of salt stress on ion concentration, proline content, antioxidant enzyme activities and gene expression in tomato cultivars. *AoB Plants*, *8*, plw055.

González, G. L. et al. (2021). Efecto del tratamiento de semillas con QuitoMax® en el rendimiento y calidad de plántulas de tomate variedades ESEN y L-43. *Terra Latinoamericana*, 39:1-6. e803.

Govaerts, B., & Verhulst, N. (2010). *The normalized difference vegetation index (NDVI) Greenseeker (TM) handheld sensor: toward the integrated evaluation of crop management. Part A: concepts and case studies*: Mexico, D.F. CIMMYT. p.13.

Hogue, E., Wilcow, G., & Cantliffe, D. J. (1970). Effect of soil P on phosphate fraction in tomato leaves. *Journal of the American Society for Horticultural Science*, 95:174-176.

Inman, D., Khosla, R., & Mayfied, T. (2005). On-the-go active remote sensing for efficient crop nitrogen management. *Sensor Review*, 25:209-214.

Isah, T. (2019). Stress and defense responses in plant secondary metabolite production. *Biological Research*, 52:39.

Jaworsky, E. G. (1971). Nitrate reductase assay in intact plants tissues. *Biochemical and Biophysical Research Communications*, 43:1274-1279.

Ji, X. et al. (2023). Emerging *Alternaria* and *Fusarium* mycotoxins in tomatoes and derived tomato products from the China market: Occurrence, methods of determination, and risk evaluation. *Food Control*, 145:109464.

Jiménez-Mejía, R. et al. (2022). Teamwork to survive in hostile soils: use of plant growth-promoting bacteria to ameliorate soil salinity stress in crops. *Microorganisms*, 10(1):150.

Kolmogorov, A. (1933). *Basic concepts of probability theory*. Berlin: Julius Springer; 62p.

Maehly, A. C., & Chance, B. (1954). The assay of catalases and peroxidases. *Methods of biochemical análisis*, 1:357-424.

Mondal, M. M. A., Puteh, A. B., & Dafader, N. C. (2016). Foliar application of chitosan improved morpho-physiological attributes and yield in summer tomato (*Solanum lycopersicum*). *Pakistan Journal of Agricultural Sciences*, 53(2):339-344.

Nachtergaele, F. O. et al. (2000). New developments in soil classification: world reference base for soil resources. *Geoderma*, 96(4):345-357.

Norma Oficial Mexicana NOM-138-SEMARNAT/SS-2003. (2005). *Límites máximos permisibles de hidrocarburos en suelos y las especificaciones para su caracterización y remediación*. p. 1-21. Available in: <http://legismex.mty.itesm.mx/normas/ecol/semarnat138ss-05.pdf>.

O'neal, D., & Joy, K. W. (1973). Localization of glutamine synthetase in chloroplasts. *Nature New Biology*, 246(150):61-62.

Ors, S. et al. (2021). Interactive effects of salinity and drought stress on photosynthetic characteristics and physiology of tomato (*Lycopersicon esculentum* L.) seedlings. *South African Journal of Botany*, 137:335-339.

Ortiz, L. Y. et al. (2001). Determinación del ácido abscísico en papa (*Solanum* sp.) como respuesta a bajas temperaturas. *Agronomía Colombiana*, 18(1-3):31-38.

Peñuelas-Rubio, O. et al. (2022). Warming reduces the root density and wheat colonization by arbuscular mycorrhizal fungi in the Yaqui Valley, Mexico. *Agronomía Colombiana*, 40(3):440-446.

Pilet, P. E., & Chanson, A. (1981). Effect of abscisic acid on maize root growth: A critical examination. *Plant Science Letters*, 21(2):99-106.

Pilet, P. E., & Saugy, M. (1987). Effect on root growth of endogenous and applied IAA and ABA: A critical reexamination. *Plant Physiology*, *83*(1):33-38.

Reyes-Pérez, J. J. et al. (2020). Aplicación de quitosano incrementa la emergencia, crecimiento y rendimiento del cultivo de tomate (*Solanum lycopersicum* L.) en condiciones de invernadero. *Biotecnia*, *22*(3):156-163.

Rodríguez, A. B. F. et al. (2020). Oligosacarinas como bioestimulantes para la agricultura cubana. *Anales de la Academia de Ciencias de Cuba*, *11*(1):852.

Slawky, G., & Rodier, M. (1988). Biosynthetically active glutamine synthetase in the marine diatom *Phaeodactylum tricornutum*: optimization of the forward–reaction assay. *Marine Biology*, *97*:269-274.

Statsoft. *Satistica 13.3*. StatSoft Incorporation Version 13.3. 2014. Available in: <https://statistica.software.informer.com/13.3/>.

Terry Alfonso, E. et al. (2017). Respuesta agronómica del cultivo de tomate al bioproducto QuitoMax®. *Cultivos Tropicales*, *38*(1):147-154.

Tola, E. et al. (2023). Impact of water salinity levels on the spectral behavior and yield of tomatoes in hydroponics. *Journal of King Saud University-Science*, 35(2):102515.

Tukey, J. W. (1960). A survey of sampling from contaminated distributions. In I. Olkin. *Contribution to probability and statistics: essays in honor to harold hotelling*. Redwood City: Stanford University Press. (pp. 448-485).

Ullah, A., Bano, A., & Khan, N. (2021). Climate change and salinity effects on crops and chemical communication between plants and plant growth-promoting microorganisms under stress. *Frontiers in Sustainable Food Systems*, *5*:618092.

Verhulst, N. et al. (2011). Soil quality as affected by tillage-residue management in a wheat-maize irrigated bed planting system. *Plant and Soil*, *340*:453-466.

Verma, P., Azad, C. S., & Singh, P. K. (2022). Quantitative analysis of ABA and SA in rice (*Oryza sativa* L.) grown under drought stress. *Journal of Climate Change*, *8*(2):1-6.

Wang, X. et al. (2016). Effects of exogenous abscisic acid on the expression of citrus fruit ripening-related genes and fruit ripening. *Scientia Horticulturae*, *201*:175-183.

Waqas, M., Van Der Straeten, D., & Geilfus, C. M. (2023). Plants' cry'for help through acoustic signals. *Trends in Plant Science*, *28*(9):984-986.

Waqas, M. et al. (2021). Synergistic consequences of salinity and potassium deficiency in quinoa: Linking with stomatal patterning, ionic relations and oxidative metabolism. *Plant Physiology and Biochemistry*, *159*:17-27.

Waqas, M. et al. (2019). Soil drenching of paclobutrazol: an efficient way to improve quinoa performance under salinity. *Physiologia plantarum*, *165*(2):219-231.

Yang, J., Kloepper, J. W., & Ryu, C. (2008). Rhizosphere bacteria help plants tolerate abiotic stress. *Trends in Plant Science*, *14*(1):1-4.

Zhang, T. T. et al. (2011). Using hyperspectral vegetation indices as a proxy to monitor soil salinity. *Ecological Indicators*, *11*(6):1552-1562.

Zhang, G. et al. (2021). Exogenous application of chitosan alleviate salinity stress in lettuce (*Lactuca sativa* L.). *Horticulturae*, *7*(10):342.

Zuzunaga-Rosas, J. et al. (2022). Effect of a biostimulant based on polyphenols and glycine betaine on tomato plants' responses to salt stress. *Agronomy*, *12*(9):2142.