Effect of Foliar Application of Oligo-chitosan on physiological characteristics of Fragaria × ananassa under drought stress

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Abstract
Background: There are many factors that affect plant growth and agricultural productivity, including soil salinity. There is evidence that chitosan (CTS) can increase plant growth and resilience to abiotic stress. However, it is still unclear if the software. Salt stress on Fragaria can be alleviated by the addition of exogenous CTS. Methods: Because of this,Exogenous CTS foliar application to Fragaria plants was studied in this study. In a sodium chloride solution of 100 mM. Exogenous CTS increased Fragaria growth, according to the study's findings.increases in leaf chlorophyll a, proline, and the dry weight of shoots and roots peroxide and catalase activities, as well as reduced membrane permeability. Result: Compared to untreated plants, lipid peroxidation in response to salt stress. In addition,The addition of exogenous CTS to Fragaria resulted in an increase in K+ accumulation, but this effect was not statistically significant. compared to plants treated with NaCl alone. Conclution The effect on the K+/Na+ ratio As a result of these findings,CTS may be able to counteract the negative effects of salt stress on plant growth and development, according to the research.biomass by altering intracellular ion concentration, osmotic control, and improving Fragaria leaf antioxidant enzyme activity.

Keywords: antioxidant enzymes; chitosan; Fragaria; proline; salinity; soluble sugars

1. Introduction

Ten to twenty-five percent of agricultural crops can suffer from salt stress (Zaman, Shahid, and Heng 2018). It is estimated that about 20,000 square kilometers of farmland are being affected by salinity every year, which is restricting agricultural productivity .(Deinlein et al. 2014)(Ke et al. 2016) Soil can be salted through the use of a solvent or by natural processes. Soil salinity is a result of rock weathering and seawater flooding. High-salt irrigation water, excessive chemical fertilization, and poor soil management all contribute to the expansion of saline–alkaline land. Arid regions (e.g., Sahara in North Africa, Saudi Arabia, and large parts of Iran & Iraq), as well as California in the United States and South Africa (and most of Australia), experience higher evapotranspiration rates than the leaching fraction due to high temperatures and uneven rainfall (Sohaib et al. 2020).

Salinized agricultural land must be improved to increase the output of salt-tolerant plants and saline–alkali land. An iontoxic, hyperosmotic,
nutritionally imbalanced and oxidatively damaged body can be caused by salt stress. Salinity affects sessile plants in a variety of ways. Detoxification, ionization, and osmotication are the three mechanisms by which plants deal with salt stress(Yang and Guo 2018). Plants experience osmotic stress as a result of salt stress. Cell turgor is maintained by the plant using a variety of substances including proline, glycine betaine, soluble sugar, soluble protein, sodium (Na+), and potassium (K+) ions. Nonselective cation channels, K+ transporters, and Cl transporters allow Cl and na+ to enter roots. Nutritional deficiencies in plants can be caused by high concentrations of salts such as sodium (Na+) and chloride (Cl+). Plants rely on Na+ and K+ channels to keep their cytosolic Na+/K+ ratio stable (Assaha et al. 2017). ROS buildup in plants under salt stress leads to oxidative stress and toxicity. These electron transport chains and enzymes generate ROS sources such as O2, H2O2, and hydroxyl radical (_OH) (Lv, Chen, and Wang 2019). Salt stress-induced ROS damage can be counteracted in part by ascorbic acid (AsA), glutathione (GSH), and carotenoids (J. Li et al. 2015)(Del Río and López-Huertas 2016). However, despite the fact that plants employ these tactics, they struggle to survive in a salty climate. The biopolymer chitosan is produced by deacetylating crustacean chitin (poly[1,4]-2-amino-2 deoxy-D-glucose; CTS). (Betchem, Johnson, and Wang 2019) The amino/acetamido group, as well as the primary and secondary hydroxyl groups, enhance its affinity for ions and pollutants. In addition to being non-toxic, CTS is biodegradable, non-toxic to the environment, renewable, and inexpensive(Abdulshaheed and Alsaedi 2021). Plant growth and resistance to abiotic stress have improved with the use of CTS, which was discovered by Rouget in 1859. Increases water use efficiency, mineral nutrient uptake as well as chlorophyll content and photosynthesis are all facilitated by CTS (reduces oxidative stress). There are a number of environmental stresses that can be alleviated by adding exogenous CTS to the system. By

doi.org/10.31838/hiv23.01.57 Received: 13.09.22, Revised: 17.10.22, Accepted: 10.12.22

manipulating defense gene expression and osmotic regulation substances, exogenous CTS enhances plant resistance to both natural and man-made stressors. Raw Fragaria is eaten in salads. Fragaria production and cultivation has increased as a result of its marketability, flavor, and health benefits (Azarmi-Atajan and Sayyari-Zohan 2020). According to the FAO, Fragaria was grown on 243.97 million hectares and weighed 16.31 million tonnes worldwide in 2019, up 0.54 percent and 2.3 percent from the year before. Fragaria is largely grown in China, the United States, and India. China, the US, and India. Salinity determines how sensitive Fragaria is to salt. Salad seed germination, leaf number, photosynthetic rate, and cell growth are all slowed by sulfur dioxide (ROS), a salt-induced byproduct. A lot of research has been done on the impact of salt on Fragaria growth and production (Garrido et al. 2014), but there is no information on the impact of CTS. Exogenous CTS was used in this study to see if it could reduce the negative effects of salinity on Fragaria growth and physiology. Salt-grown Fragaria was found to be affected by the addition of exogenous CTS to osmolyte accumulation, antioxidant biosynthesis, and antioxidant enzyme activity.

2. Methodology

Plant Treatments and Substances: During the months of November 2020 and January 2021, an experiment was conducted with the test material. In the growth chamber, Fragaria were grown for 12 hours under 22°C cool white fluorescent lamps in urethane cubes (2.3,2.3,2.7 cm3) (Figure 1A). Jurong's Agricultural Engineering and Technology Center in Jurong selected uniform seedlings and moved them to a cultivation room 21 days after seeding (DAS). (Joshi et al. 2017) The plants were grown in a deep-flow hydroponic system with a nutrient solution with an EC of 1.5 0.2 and pH of 6.9 0.2. The nutrient solution was supplied with oxygen by air pumps. LED bulbs illuminated the plants in the greenhouse (Figure 1B). The light's photon flux density was 200 10 moles per square centimeter per second during its 16-hour photoperiod1. The daily humidity ranged from 65 to 5%, while the overnight range was between 25 and 20% lower. In order to help the Fragaria seedlings adjust to their new environment, they were moved into the cultivation room at 28 days after seeding (DAS). CTS, NaCl, NaCl + CTS, and NaCl + CTS were the four treatment groups for plants grown in nutrient solutions with water sprayed on their leaves. The hydroponically grown Fragaria was sprayed with CTS or water for five days beginning on 28 DAS. Each Fragaria leaf was sprayed with 30 mL of CTS or water solution on both the adaxial and abaxial surfaces. After the 5-day CTS induction treatment, the nutrient solution in groups (3) and (4) was supplemented with NaCl (4). Each treatment was repeated three times with the help of six plants. Plant samples were taken at 28 and 49 DAS for further investigation.

Analysis of Plant Growth: Using the 28 and 49 DAS samples, the total leaf area, fresh shoot and root weight (FW), and dry shoot and root weight (DWR) were all calculated for each plant (DW). An area meter for leaf area (Li-3100) was used to determine the total leaf surface (Li-Cor, Lincoln, NE, United States). At 80°C, shoots and roots can be dried in order to obtain dry whey (DW). Porra et al. tested fresh Fragaria leaves extracted in N,N-dimethylformamide for Chl content spectrophotometrically. The Ohtake et al. (Porra, Thompson, and Kriedemann 1989) method was used to estimate the relative growth rate (RGR), net assimilation rate (NAR), and leaf area ratio (LAR) (LAR).

Estimation of Electrolyte Leakage and Leaf Relative Water Content (RWC): Researchers Yamasaki and Dillenburg [42] assert that the equation.Ahmad et al. examined electrolyte leakage in their research (EL). Thirteen-millimeter leaf disks from each treatment group were submerged in eionized water to get an accurate reading on the ECa level. The ECb values of the leaf disks were calculated after the treatments had been incubated for 25 minutes at temperatures between 50 and 60 degrees. ECc was determined by boiling the leaf disks for ten minutes at 100 C. The following formula was used to determine the EL:

$$RGR = (1/W)\,(DW/Dt) = [\ln(W2) - \ln(W1)]/\,(t2 - t1)$$

$$EL\ (\%) = (ECb - ECa)/ECc\ \_\ 100$$

The Potassium and Sodium Contents of Fragaria Leaves were determined: Zhang et al. (G. Zhang et al. 2020) developed a method for determining Fragaria leaf potassium and sodium content using inductively coupled plasma optical emission spectrometry (Thermo Fisher Scientific).

Analysis of Proline, Glucose, and Ascorbic Acid Contents: Using the method described by Bates et al., the proline content of the leaf samples was determined. The anthrone–sulfuric acid method was used to determine the leaves' soluble sugar content (G. Zhang et al. 2020). In order to measure the ascorbic acid content (AsA) in leaves, the method of Kampfenkel et al. was used.

Enzyme Assays: Homogenized fresh leaf samples were centrifuged at 10,000 g for 15 minutes at 4 °C in phosphate buffer saline (50 mM, pH 7.8). SOD (EC: 1.15.1.1), POD (EC: 1.11.1.7), and catalase (EC: 1.11.1.6) activities were determined in the supernatants [51]. The Bradford method (Hammond and Kruger 1988) was used to determine the protein content. Units per milligram of protein were used to measure enzyme activity.

Statistical Analysis

Mean standard errors (SEs) of the three replications for each treatment are presented. The one-way analysis of variance with Tukey's HSD test was used for the statistical analysis (SPSS v. 18.0, IBM Inc.,

Chicago, IL, USA). The significance of p values less than 0.05 was deemed.

3. Results

Leaf Fragaria Growth and Biomass Increased with Exogenous CTS in NaCl Stress Environments: Leavening, FW, and DW were all significantly lower in NaCl-stressed Fragaria shoots than in the control plants (Table 1). The NaCl group's total leaf area decreased by 67.3 percent; the shoot FW decreased by 60.3 percent; the root FW decreased by 73.8 percent; and the shoot DW decreased by 66.5 percent. (For more information, see Table 1.) Exogenous CTS (100 mg/L) alleviated Fragaria growth inhibition due to salinity stress (Figure 2A). There was a 141.2 percent increase in the NaCl + CTS group's total leaf area, shoot FW, the root FW, the shoot DW, and the root DW compared to the NaCl group (Table 1). The NaCl + CTS group's total leaf area, shoot FW, root FW, shoot DW, and root DW were all lower than the control group's by 21.2 percent each (Table 1). There was no discernible difference between the CTS and control groups in any of the aforementioned growth parameters (Table 1). Chl a, Chl b, and total chloride concentrations were 14.4% lower in the NaCl group than in the control group (Table 1). Compared to NaCl, the NaCl + CTS group had a 10% and 8% higher Chl a and total Chl than NaCl (Table 1). In the NaCl + CTS group, the concentrations of Chl b and total chloride were 17.3% lower than in the control group (Table 1). Furthermore, plants that had only received exogenous CTS had significantly higher levels of Chl a than plants in the non-treated group, which only received endogenous CTS (Table 1). The RGR's NAR and LAR components are broken down for plant growth analysis purposes. The total DW and total leaf area described in the previous text were used to estimate the growth analysis parameters for each group, and the effects of morphological and physiological traits on plant biomass were examined. The lowest DW was found in the NaCl group, which had a lower RGR than the control group (Figure 2B, Table 1). Adding 100 mg/L of exogenous CTS had no effect on the NaCl group's RGR (Figure 2B). The same was true for the NAR pattern, with the lowest NAR being found in the NaCl sample. Additionally, there was a negligible difference in NAR between the NaCl + CTS and NaCl groups (Figure 2C). Between the two groups, there was no statistically significant difference in LAR (Figure 2D). Analysis of growth parameters showed no significant differences between the CTS and control populations.

Leaf Potassium and Sodium Contents Affect by NaCl and CTS on the RWC and EL: The NaCl group's RWC was 15.9% lower than the control group's (Table 2). CTS reversed the decline in leaf RWC caused by salt. Leaves' CTS increased. Leaf RWC was 15.7% greater in the NaCl + CTS group than in the NaCl group (Table 2). Leaf EL was 160.9 percent greater in the NaCl group than in the control group (Table 2). It was also 21.2 percent lower in the NaCl + CTS group than in the NaCl group (Table 2). A decrease in sodium accumulation of 46.0% was observed between the exogenous CTS and control groups, whereas sodium accumulation was higher in the NaCl group when salad plants were treated solely with exogenous CTS (Table 2). When compared to the control group, the NaCl group's K+/Na+ ratio decreased significantly, but the NaCl and NaCl + CTS groups saw no change. The potassium and sodium levels, as well as the K+/Na+ ratio, were nearly identical in both groups of participants (Table 2).

Results of NaCl and CTS on Proline Content, MDA Content, O2 Generation, H2 o2, Soluble Sugar Content, and AsA Content in the Fragaria Leaves: In the NaCl group, there was a 2.1-fold increase in proline concentration compared to the control group, indicating that salinity stress triggered proline biosynthesis (Figure 3A). The NaCl + CTS group had 66.5 percent more proline than the NaCl group (Figure 3A). An indicator of ROS-induced lipid peroxidation-induced membrane damage is MDA [44]. The NaCl group had MDA levels 1276.6 percent higher than the control group (Figure 3B). The NaCl + CTS group had a 14.3% lower MDA content than the NaCl group, which was also lower (Figure 3B). In Fragaria cells, ROS production was induced by salad. ROS like H2O2 and O2 were produced when Fragaria plants were treated with NaCl. The concentrations of H2O2 and O2 in the NaCl group were three and one-and-a-half times higher than in the control group (Figure 3C, D). H2O2 and O2 production were slowed by the addition of exogenous CTS, which inhibited plants exposed to NaCl (Figure 3C, D). The sugar soluble content was significantly different between the NaCl group and the control group in this experiment (Figure 3E). 40.8 percent more soluble sugar was found in NaCl+CTS participants than in NaCl only participants (Figure 3E). Proline, MDA, H2O2, the rate of superoxide radical production, soluble sugar, or AsA concentrations in the CTS and control groups did not differ significantly (Figure 3)

Antioxidant Enzyme Activity as Affected by NaCl and CTS in the Leaves: The antioxidant enzyme activity of Fragaria leaves was tested using NaCl and CTS. Different treatments impacted SOD, POD, and CAT differently. SOD activity was similar among the groups (Figure 4A). Instead, the NaCl group's POD and CAT activity was 43.3% lower and 181.9% higher than the control groups, respectively (Figure 4B, C). The CTS + NaCl group had significantly higher POD and CAT activities than the NaCl group (Figure 4B, C). Activities of SOD, POD, and CAT were similar in the CTS and control groups (Figure 3).

4. Discussion

It has been shown that salt stress has a negative impact on plant growth and productivity. According

to this research, salinity inhibited Fragaria plant growth and biomass as well as total leaf area, fresh weight, and dry weight (Table 1). Studies have shown that all three foods have similar effects (Latef and Chaoxing 2011). In agriculture, it's used a lot because it helps control plant growth and development and makes plants more resistant to environmental stressors. Chitin is a substance that is utilized by CTS. Adding CTS to ajowan (Mahdavi and Rahimi 2013), maize (Turk 2019), and wheat (Hoffmann and Poorter 2002) can alleviate salt stress. Exogenous application of CTS to Fragaria plants grown in saline water resulted in increased growth (Table 1, Figure 2). Growth analysis can be used to study plant growth (Rady et al. 2018). RGR is boosted by increasing plant biomass. The lower RGR of Fragaria grown in NaCl is in line with its lower biomass accumulation during the growth stage. The NAR (mean leaf growth) in the NaCl group was significantly lower than in the control group (Figure 2). LAR remained stable despite changes in its surrounding environment (Figure 2). NAR and not LAR reduced Fragaria RGR under salt stress, according to these findings. Figure 2B shows that when CTS was applied exogenously to reduce salt stress, Fragaria RGR was increased. NaCl + CTS and NaCl differed only slightly in NAR, as was to be expected (Figure 2). NaCl + CTS's RGR was boosted by NAR and LAR. Growth and photosynthesis can be predicted by leaf chlorophyll levels (Zou et al. 2018). Oxidative stress damages chloroplast membranes, resulting in chlorophyll loss. The Chl content in the NaCl group was significantly lower than in the control group (Sen et al. 2020). Chl pigment biosynthesis and degradation are slowed down in saline environments (Negrão, Schmöckel, and Tester 2017). Salad chlorophyll and total chlorophyll concentrations increased following saline CTS application (Table 1). Adding exogenous polysaccharides to salt-stressed wheat seedling leaves increased Chl a concentrations (Zou et al. 2018). Photosynthesis is triggered by Chl an, which absorbs light and begins the process. A buildup of excess chloroplast salt damages pigment–protein complexes. Chl a degradation in salt stressed Fragaria may have been prevented by CTS, which increased photosynthesis and Chl a level. When compared to the Na+CTS and Na+CTS groups, the levels of Chl b were nearly identical. Chl b can be converted to Chl an in the presence of salt. It is possible to compare a plant's water status to the maximum capacity of a turgid leaf using Leaf RWC. (Suriya-arunroj et al. 2004) The RWC of Fragaria leaves was reduced by salinity, but it was improved by CTS (Table 1). Adding CTS to salt-stressed creeping bentgrass increased leaf RWC and water utilization efficiency. This aids plants suffering from water stress due to salinity. To get the current results, it's possible that NaCl + CTS Fragaria manipulated water supply and transpiration in the leaves. CK and CTS Fragaria leaf Na+ content was indistinguishable (Table 1). Salt stress increased Na+ in Fragaria leaves but decreased K+ (Table 1). K+ levels were lower in saline-exposed salad leaves compared to those in the control group (Table 1). We saw a similar response to polysaccharides in salt-exposed wheat seedlings. Saline-exposed plants have organelles and cytoplasm poisoned by sodium. Chloroplastic Na+, like cytosolic Na+, is toxic to cells (Assaha et al. 2017). Na+ and K+ homeostasis can be skewed by excess Na+, resulting in an imbalanced ratio. In salt-stressed plants, Na+ competes with K+ for uptake, resulting in K+ deficiency. Photosynthesis and enzyme activity are aided by it (G. Zhang et al. 2020). (Cuin et al. 2008) Plants must have high K+/Na+ ratios in their shoots to be tolerant of salt. The K+/Na+ ratio decreased as Na+ accumulated in leaves under saline conditions. However, the K+/Na+ ratio of NaCl + CTS plants was higher than that of NaCl plants, and this was not statistically significant. The use of exogenous CTS may help plants grow by regulating their nutritional balance and decreasing the toxic effects of ion toxicity. As a result of their effect on Na+ efflux and storage gene expression, polysaccharides enhance wheat's salt tolerance. Photosynthetic tissues are prevented from absorbing Na+ by CTS, which upregulates AsHKT1 and Na+/H+ exchanger genes. Na+ levels in shoots and roots were not assessed in this study, despite the lower Na+ content in leaves in the NaCl + CTS group at harvest. Research on Na+ gene expression that hasn't been completed. Learn about Fragaria salt tolerance by examining its Na+ accumulation patterns in the shoots and roots, as well as the expression of the Na+ transport gene. A plant's defense mechanism is osmotic regulation. To regulate osmotic pressure, osmolytes are stored in the cytosol and other organelles (Zhao et al. 2019). Saline osmotic adjustment necessitates a substantial amount of proline, as evidenced by the high concentration of this amino acid in NaCl (Figure 3). Gene expression and cell structure stability are protected by proline in the presence of high salt concentrations (Zou et al. 2015). Fragaria leaves exposed to salt were protected by exogenous CTS, which maintained cell osmosis balance and protected the plant's photosystem. Proline production is increased by N metabolism (Da Rocha et al. 2012). A study found that adding exogenous CTS boosted the metabolism of wheat's N (X. Zhang et al. 2017). If N metabolism is altered, CTS may increase Fragaria proline levels. NaCl was more effective than exogenous CTS in increasing Fragaria soluble sugar capacity. More sugar was soluble in Fragaria when treated with exogenous CTS than when treated with just NaCl (Figure 3). NaCl stress may be mitigated by an increase in the solubility of sugars, which are critical for photosynthesis and ROS scavenging in leafy greens like Fragaria. As a result of salt stress, plants produce _OH, O2, and H2O2. [Stress-salt]. Oxidation and degradation of proteins and nucleic acids can lead to ROS-induced cell damage. Membrane lipid peroxidation, which is triggered by ROS (J.-T. Li et al. 2011), results in MDA

as a byproduct (Wu et al. 2017). (Wu et al. 2017) Oxidative damage to the cell membrane caused by ROS, which decreased its integrity and stability in the NaCl-treated group, compared to the control group (Figure 3–; Table 1). O2, H2O2, MDA, and EL were all lower in the CTS + NaCl group than in the control group (Figure 3; Table 1). In salt, CTS regulates membrane stability and reduces oxidative damage. It has been demonstrated that adding exogenous CTS to NaCl + CTS Fragaria leaves increases chlorophyll and decreases MDA levels, both of which are markers of salt-induced chlorophyll loss (Figure 3; Table 1). Adding exogenous CTS to peppers under salt stress reduced ROS and lipid peroxidation (Turk 2019). SOD, POD, and CAT, as well as proline and AsA, are found in plants. hydrogen peroxide and oxygen are produced by SOD's degradation of O2. This can be done by POD, CAT or ascorbate peroxidase (Kusvuran et al. 2020). The NaCl group had significantly higher levels of O2, H2O2, and MDA than the control group (Figure 3). It was found that NaCl group CAT and POD activities were different from those of the control group (Figure 4). ITEM NO. 4 Exogenous CTS decreased the levels of O2, H2O2, and MDA while increasing the activities of POD and CAT (Figure 3) and Figure 4). Figures 3B–D and 4 show examples of this concept in action. Research on maize (Zou et al. 2015) suggests that increasing POD and CAT activities in Fragaria leaves with CTS can reduce salt-induced damage to the cell membrane. It appears that POD and CAT scavenge ROS more effectively than SOD alone, as evidenced by the lack of effect of NaCl on SOD activity (Figure 4). No gene expression patterns for SOD, CAT, or POD were examined in this study. Oxidative damage is prevented by both proline and AsA. Figure 3A shows that proline concentrations in Fragaria leaves were significantly higher, indicating that Fragaria may accumulate proline to scavenge ROS, reduce oxidative damage, and shield cell membranes from salt stress. CTS + NaCl and NaCl groups had similar levels of AsA (Figure 3F), suggesting that AsA isn't critical for Fragaria antioxidant capacity in salt. The AsA content in plants treated with NaCl was unaffected by exogenous oligo-alginate, according to a recent study (Salachna et al. 2018).

5. Conclusion

Supplements that are not found in nature There are numerous agricultural products in which CTS is a safe and inexpensive polysaccharide. In a study on *Fragaria* under salt stress, exogenous CTS was found to be detrimental. Exogenous CTS increased biomass and growth in salt-stressed plants. Proline and soluble sugar accumulations, as well as the activity of peroxidase and catalase, were increased by CTS application. In plants treated with NaCl, the CTS decreased sodium accumulation while increasing potassium accumulation. These findings could help *Fragaria* growers in salty climates improve their methods of production. Despite this, CTS' ability to reduce salt damage remains a mystery. Analysis of Na+/K+ transporter genes and possible signaling pathways in *Fragaria* is warranted in order to better understand its CTS-regulated salt tolerance. The biopolymer may also chelate some of the Na+ in the root/lower tissue to prevent it from reaching photosynthetic cells, depending on how CTS is applied.

Compliance with Ethical Standards

Conflicts of interest The authors declare that they have no conflict of interest.Ethical approval Ethical approval for this research was obtained from the Al-Qadisiyah University Local Committee.

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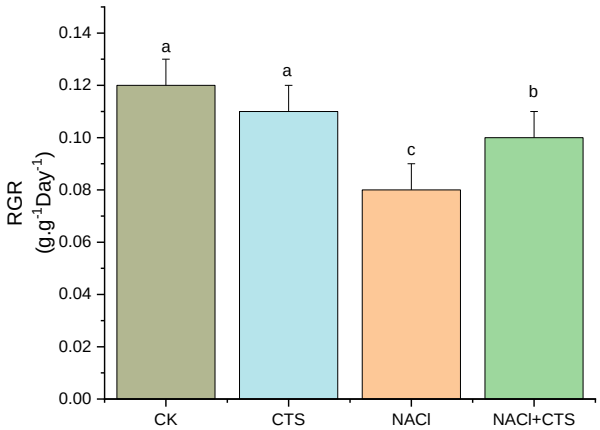

Figure 1. Studies on the effects of chitosan on ***Fragaria*** *plants under salt stress: (RGR) changes in plant morphology growth analysis parameters.*

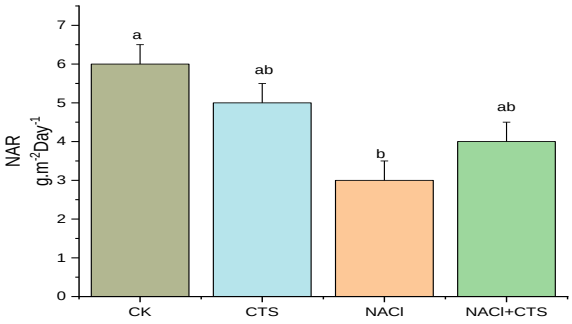

Figure 2. Studies on the effects of chitosan on Fragaria plants under salt stress: (NAR) changes in plant morphology growth analysis parameters.

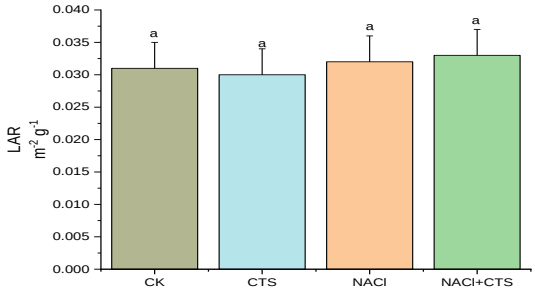

*Figure 3. Studies on the effects of chitosan on **Fragaria** plants under salt stress: changes in plant morphology and (LAR) growth analysis parameters.*

Table 2. A study on the effects of chitosan (CTS) on Fragaria plants' leaf RWC, EL and the potassium and sodium concentrations in their leaves.

Treatments	PWC (%)	EL (%)	Potassium Mg. g-1 DW	Sodium Mg. g-1 DW	Na+\K+ Ratio
(%)	73.8±1.3a	17.1±1.0c	75.19±1.07c	1.01±0.03c	68.14±3.76a
(%)	80.0±2.4a	14.3±2.4c	71.1±0.09c	1.05±0.06c	70.35±5.98a
	65.7±0.4c	42.0±4.6a	54.97±1.55c	24.18±1.23a	2.34±0.06b
Mg.g-1 DW	70.0±0.3b	36.1±1.0b	66.18±1.54b	14.1±1.54b	6.11±0.41b
Mg. g-1 DW					