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The Role of Chitosan in Improving the Cold Stress Tolerance in Strawberry varieties

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Abstract

Cold stress is considered one of the main factors that influence strawberry production in an open field. This study aimed to estimate the effects of three different concentrations of chitosan (COS) solution used by foliar spraying on the vegetative growth and yield parameters of three strawberry varieties. Moreover, the impact of foliar spraying of COS solutions on the induction of biochemical changes linked to cold stress was assessed. In this study, three different concentrations of COS solution (250, 500, and 1000 ppm) were applied to three strawberry varieties that genetically different by foliar spraying, compared with the untreated control (0 ppm COS). The results showed that the COS solution led to significant and non-significant increases in the vegetative and fruit growth parameters, depending on the concentration of COS and strawberry variety. On the other hand, it was shown that foliar application of strawberry varieties with COS solution under chilling stress causes changes in gene expression in strawberry varieties, either up-regulated or down-regulated. Furthermore, novel proteins related to cold tolerance, including pathogen-related proteins (PRPs), were identified in three strawberry varieties. For instance, new polypeptides of MWs (53 and 44 kDa) and (53; 44; and 8 kDa) were scored in Fortuna variety that was sprayed with 250 and 500 ppm COS respectively, compared with the corresponding control. In addition, one subunit of 29 kDa was detected in Florid and Festival varieties treated with 500 and 1000 ppm COS solutions. Consequently, these proteins could be used as biomarkers related to cold tolerance. Therefore, foliar spraying of COS can be used in strawberry breeding programs to protect them from cold stress.

Keywords: Abiotic stress, *Fragaria* x *ananassa* Duch, fruit yield, gene expression, vegetative growth.

1. Introduction

Strawberry (*Fragaria* × *ananassa* Duch.) belongs to the *Rosaceae* family; it is an herbaceous plant. The strawberry plant is considered to be an economically significant crop yield in Egypt and worldwide (Tan *et al*., 2003). It is considered to be one of the richest resources of bioactive chemical substances, e.g. vitamins, anthocyanins, flavonoids, carotenoids, and phenolics, all of which have marked antioxidant properties. In addition, phenolic substances like flavonoids, carotenoids, and anthocyanins found in strawberry plants have important anticancer activities (Zhang *et al.*, 2008; Hossain *et al.*, 2016; Rahman *et al.*, 2018). Recently, the production of strawberries in Egypt has been impacted by climate change. Temperature is considered a crucial environmental factor affecting strawberry plant growth under short-day conditions. In temperate regions, strawberry plant freezing injury is a main factor in decreasing the quantity and quality of crop yield (Roussos *et al*., 2020; Li *et al*., 2021; Han *et al*., 2023).

One of several natural substances that have demonstrated effectiveness against diseases in strawberries and different crops is chitosan (COS), a biopolymer chemically produced from crustaceans and soluble in organic acids (Malerba and Cerana, 2018). COS is considered ecologically sound for agricultural applications due to its easy environmental degradation and non-toxicity to people. Plant defense was elicited by COS and its derivatives, so it was applied as a natural compound to manage diseases before and after harvest (El Ghaouth *et al.*, 1991). Numerous studies have found that strawberry fruit treated with chitosan improves storage stability and increased anthocyanin content (Malerba and Cerana, 2018; El Ghaouth *et al.*, 1991). COS was commonly used as a coating agent for various fruits, to protect against post-harvest losses due to microbial diseases (Sakif *et al.*, 2016). The use of chitosan as a foliar spray has been reported previously by many investigators to increase vegetative growth, yield, and biochemical contents in plants (El-Miniawy *et al.*, 2013; Mukta *et al.*, 2017). Transcriptomic analysis revealed that COS stimulates the expression of genes in a variety of physiological processes, involving photosynthesis, the plant immune system, systemic acquired resistance, and hormone metabolism. Additionally, it affected the expression of the heat-shock protein (HSP) and the re-programming of protein metabolism with an increase in storage proteins (Landi *et al.*, 2017). However, there is little information available regarding the use of chitosan in plant growth, crop productivity, and quality. Therefore, the current

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investigation aims to estimate the effects of three different doses of COS solution by foliar spraying on the vegetative growth and fruit yield parameters of three strawberry varieties. Besides, the impact of foliar spraying of COS on the induction of biochemical changes with reference to cold stress.

2. Materials and Methods

2.1. Plant materials

The current study was conducted out in the South of Tahrir, Beheira Governorate, during the 2022/23 season (latitude: 30° 31' 48.3312", and longitude 30° 31' 48.3312" and altitude N 30° 31.8055'). Planting strawberry runners of three varieties (Fortuna, Festival, and Florida) were done in four lines, 30 cm apart, on terraces 120 cm wide, and the distance between plants was 30 cm. The experimental unit contained 40 plants. Thirty-day-old strawberry plug plants were transplanted in the field during the winter season. The chitosan treatment was applied in cold conditions, with temperatures ranging from 18-22/5-13°C daylight/night from mid-December, 2022 until the end of January 2023 and 50–60% humidity. All agricultural practices, including irrigation, fertilization, and pesticides, were performed as recommended by the Ministry of Agriculture, Egypt.

2.2. Chemical analysis of soil (pre-experiment)

Chemical analysis was done at Land Resources Evaluation and Mapping, National Research Centre, Dokki, Giza, Egypt. The results showed that the soil was suitable for growing strawberry plants (Table 1).

Table 1. Determination of soil chemical properties of strawberry.

Parameters	Experimental soil
pH	7.79*
EC dSm^{-1}	0.67**
EC ppm	428.8
Soluble cations and anions	meq/L
Ca^{++}	2.0
Mg^{++}	1.2
K^{+}	0.3
Na^{+}	2.8
$CO_3^{=}$	-
HCO_3^{-}	1.1
Cl^{-}	3.7
$SO_4^{=}$	1.5
Macro- and micro-elements	ppm
N	175.6
P	8.04
K	202.8
Fe	18.5
Mn	13.2
Zn	2.2
Cu	2.5

EC is electrical conductivity. * Determined in 1:2.5 soil suspension, ** measured in 1:5 soil extraction.

2.3. Chilling treatments

Practical-grade chitosan (COS) biopolymer (poly-ß-1,4-D-glucosamine) available in powder form was purchased from Bioworld company, Egypt. It was commercially prepared by the alkaline deacetylation of chitin obtained from shrimp shells (*Pandalus borealis*). The degree of de-acetylation was ≥ 75-85%, and viscosity was 200-800 cP. Three different concentrations, 250, 500, and 1000 ppm of COS solution were prepared by measuring the required amount of product, and diluting with distilled water and pH adjusted to 6.5 by adding drops of 0.1 M NaOH, or 0.1 M HCL (Benhamou *et al*., 1998). Freshly prepared COS solutions were sprayed on strawberry plants in each experimental unit before the flowering stage by spraying up to run off level for five successive times with an interval of 10 days between each. Control plants were sprayed with an equal volume of sterile water amended with an equal volume of 0.1 N HCl and NaOH to adjust pH at 6.5 (without chitosan).

2.4. Data collection

To determine the impact of spraying with COS on the plant's growth of three strawberry varieties, observations were recorded on randomly selected ten guarded plants for the number of leaves per plant, plant height (cm), root length (cm), leaf area (cm^2), shoot fresh and dry shoot weights (g), and root fresh and dry weights (g). Fruit yield characteristics were also estimated by repeated collection at intervals 1^{st}, 2^{nd}, and 3^{rd} harvests according to the flowering growth cycles of strawberry plants during the growing season according to the prevailing temperatures for number of fruits/plant, and weight of fruits/plant (g/plant).

2.5. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (*SDS-PAGE)*

2.5.1. Extraction of total protein

Protein was extracted and purified from ground samples using sodium dodecyl sulfate (SDS) extraction followed by trichloroacetic acid (TCA)-acetone precipitation, according to (Zheng *et al.*, 2007).

2.6. Electrophoresis on PAGE

SDS-PAGE was performed according to Laemmli, (1970) as modified by Studier, (1973) by using 12.5% SDS gel for total protein profiling. After that, the electrophoresis gel was stained with Coomassie Brilliant Blue dye G-250 and then was destained to visualize the protein bands.

2.7. Statistical analysis

The field experiment was laid out in a randomized complete block design (RCBD) with three replicates. Observations were recorded on randomly selected 10 guarded plants from each plot. The obtained data was statistically analysed using a two-way ANOVA (Snedecor and Cochran, 1980) to compare the treatments and varieties for all traits. Tukey's test was applied to compare significant differences between treatment means at $p<0.05$. The analysis was performed using 'GraphPad- prism' software (version 9.3.1, www.graphpad.com).

2.8. Cluster analysis

A matrix for SDS-PAGE was generated by scoring reproducible bands as 1 for their presence and as 0 for their absence across treated and untreated strawberry varieties. Genetic similarity coefficients were computed according to (Nei and Li, 1979). A dendrogram based on

Jaccard similarity coefficients was constructed by using the unweighted pair group method of arithmetic averages (UPGMA) (Sneath and Sokal, 1973), employing sequential, agglomerative hierarchic, and non-overlapping clustering (SAHN). All the computations were carried out using the PAST software (Ryan *et al.*, 1995). Correlation coefficients were calculated using similarity coefficients obtained from SDS-PAGE analysis.

3. Results

3.1. Effect of COS on vegetative growth parameters of strawberry varieties

The ANOVA analysis showed positive effects on vegetative growth parameters **(**number of leaves per plant**,** plant height, root length, shoot fresh and dry weight, and root fresh and dry weight) between treatments for each strawberry variety separately and the corresponding control (Figs. 1, 2, 3, and 4). It was observed that strawberry var. Festival treated with 500 ppm COS led to an increase in the number of leaves/plant, shoot fresh and dry weight, and root fresh and dry weight compared with the control. However, the plants sprayed with 1000 ppm COS solution caused a non-statistically significant increase in the plant height and leaf area (Figures 1, 2, 3, and 4). For the Fortuna variety, the application of 250 ppm COS led to an increase in root length, while the dose of 500 ppm COS induced positive changes in the number of leaves/plant and root fresh and dry weights. Moreover, the plants treated with 1000 ppm COS led to a significant increase in leaf area at $p<0.05$, compared with the untreated control. However, the dose of 1000 ppm COS caused a non-significant variation in the plant height (Figures 1, 2, 3, and 4). Finally, it was observed that the foliar spraying of 500 ppm COS solution on strawberry plants var. Florida caused an increase in the plant height, the root length, the number of leaves, and shoot fresh and dry weight, while the dose of 1000 ppm COS solution had positive effects on root fresh and dry weight (Figures 1, 2, 3, and 4). On the other hand, there were significant differences among three strawberry varieties sprayed with COS and the controls. For example, significant variations were recorded between strawberry var. Fortuna (500 ppm COS) and Florida (500 ppm COS). Also, Florida (500 ppm COS) and Fortuna (1000 ppm COS). Two strawberry varieties, Fortuna and Florida treated with 500 ppm COS had highly significant differences in the number of leaves, compared with Fortuna (250 ppm COS) and Festival (500 ppm COS), respectively (Figure 3). It is clear that 1000 ppm COS of var. Florida caused a substantial impact in the number of leaves, compared with var. Festival (250 and 1000 ppm COS) at $p<0.05$. In contrast, strawberry var. Florida (250 and 500 ppm COS) resulted in a significant increase in the number of leaves compared with Festival (1000 ppm COS). The latter significantly increased the number of leaves, compared with the Fortuna variety (1000 ppm COS) (Figure 3).

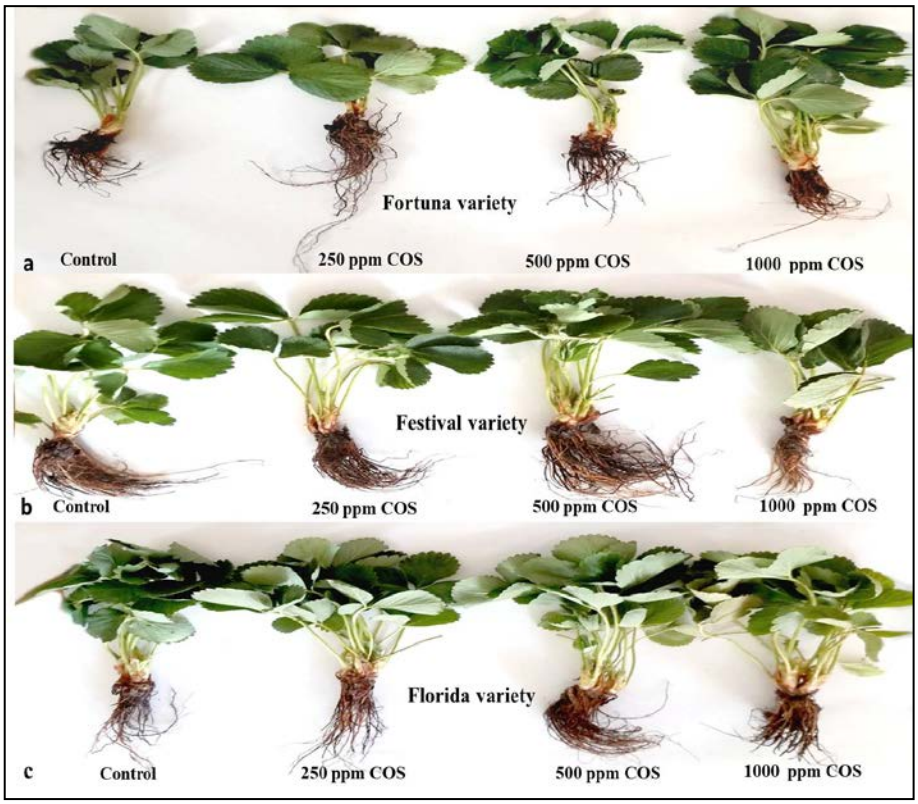

Figure 1. Showing variability in vegetative growth parameters of three different strawberry (**a**: Fortuna, **b**: Festival, and **c**: Florida) varieties, treated with three different concentrations of COS solution under cold-stress conditions, compared with untreated control. After 60 days of treatment with COS. Error bars represent cm.

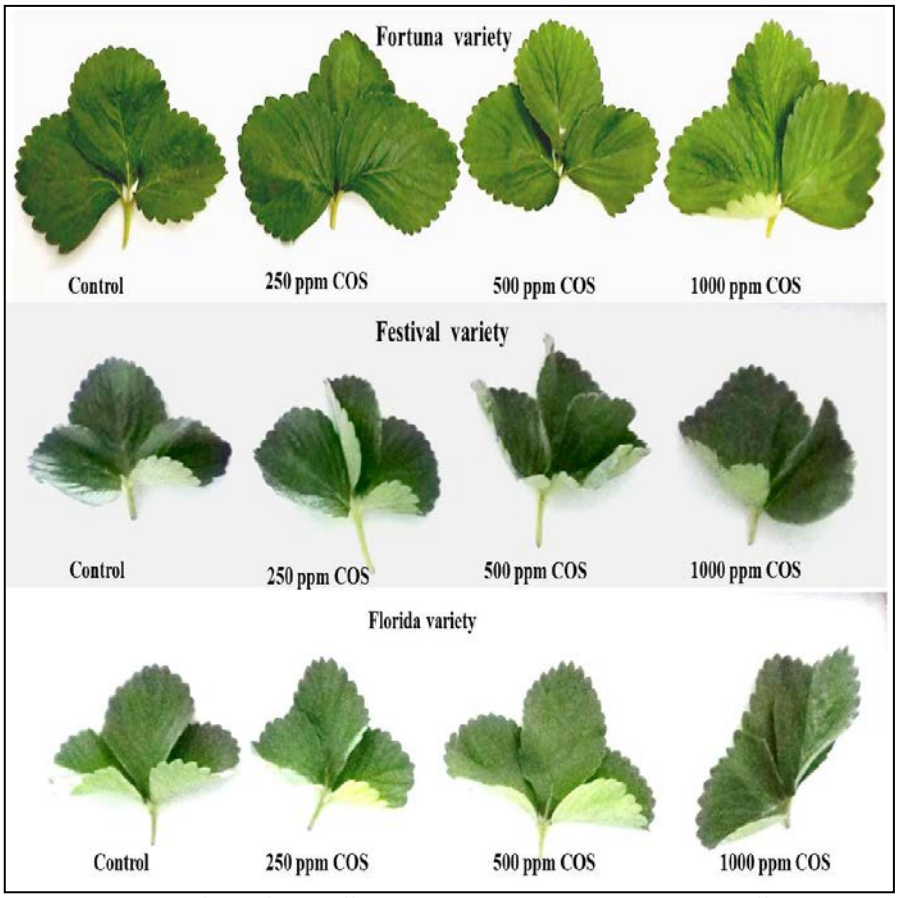

Figure 2. Showing variability in leaf area of three different strawberry varieties, treated with three different concentrations of COS solution under cold-stress conditions, compared with un-treated control. After 60 days of treatment with COS. Error bars represent cm.

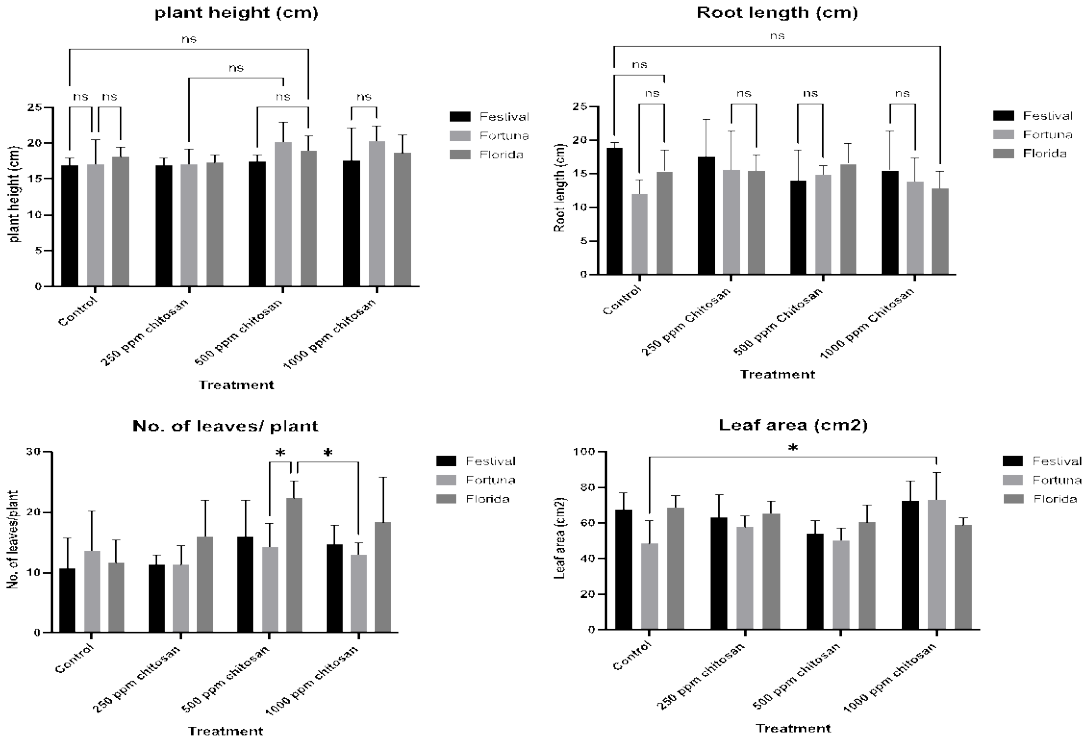

Figure 3. Bar plots show morphological characterization (plant height, root length, No. of leaves/plant, and leaf area) of three different strawberry varieties, treated with three different concentrations of COS solution under cold-stress conditions, compared with un-treated control. The asterisk means significant differences among treatments and genotypes according to range, $p<0.05$, and ns: non-significant.

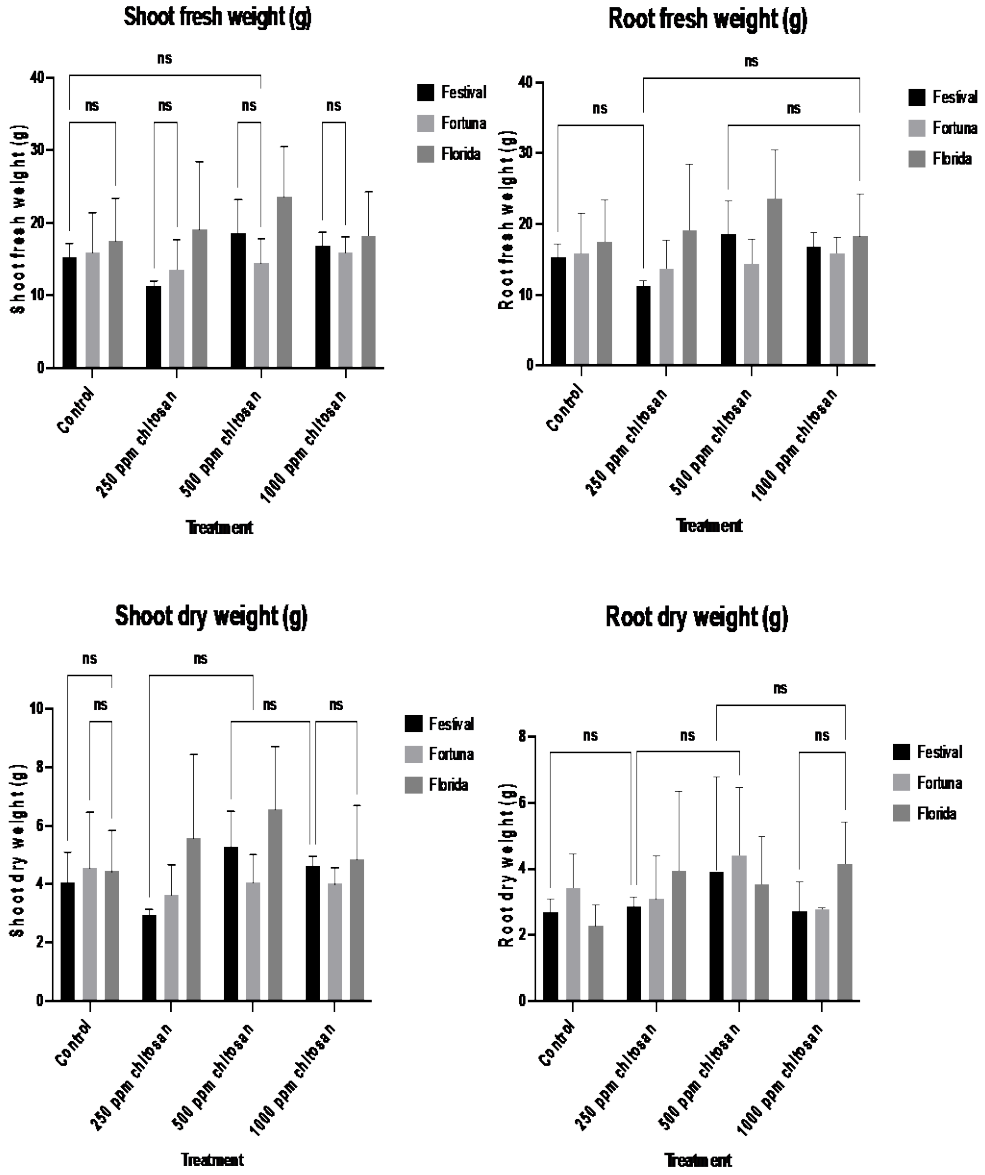

Figure 4. Bar plots show morphological characterization (shoot and root fresh weight and shoot and root dry weight) of three different strawberry varieties, treated with three different concentrations of COS solution under cold-stress conditions, compared with un-treated control. ns: non-significant.

3.2. Effect of COS on reproductive growth parameters of strawberry varieties

3.2.1. Number of fruits in the 1st, 2nd, and 3rd harvests

The strawberry var. Festival treated with 500 ppm COS solution had a highly significant increase in the number of fruits in the 1st harvest, compared with the control. Furthermore, the foliar spraying of 500 ppm COS caused positive effects on the number of fruits in the 2nd and 3rd harvests. For the Fortuna variety, it was shown that a dose of 250 ppm COS solution improved the number of fruits in three harvest times. However, the foliar application of 500 ppm COS of strawberry var. Florida grown in the field caused a significant increase in 1st harvest yield under cold stress conditions, compared with the control at $p<0.05$ (Figure 5). Also, plants treated with 500 and 1000 ppm COS improved the number of fruits in (the 1st and 2nd) and the 3rd harvests, respectively, compared with un-treated plants. On the other side, there were significant variations in the number of fruits in three harvests, between treatments and control of three different strawberry varieties. In the 1st harvest, control var. Florida positively and significantly influenced the number of fruits, compared with the control vars. Fortuna and Festival. Besides, the strawberry plants of Florida treated with different concentrations of COS solution (500 and 1000 ppm) induced significant effects compared with the control vars. Fortuna and Festival. Also, Festival (500 and 1000 ppm COS) led to significant changes in fruit yield, compared with Florida and Fortuna controls, respectively (Figure 5). For the 2nd harvest, results of the study showed that var. Festival sprayed with 500 ppm COS caused a highly significant increase in the fruit numbers, compared with the control plants. Besides, var. Fortuna (500 ppm COS) showed statistically significant differences in fruits number, compared with the control. It was observed that

var. Fortuna (250 and 500 ppm COS) led to highly significant changes in the number of fruits, compared with Festival var. treated with 250 ppm COS. There were substantial variations between 500 ppm COS of the Florida variety and control var. Fortuna as well as Fortuna (250 and 500 ppm COS) and Festival (1000 ppm COS) (Figure 5). In the 3rd harvest, it was clear that both Fortuna and Florida controls recorded highly significant differences in the number of strawberry fruits, compared with Festival control. On the contrary, there were no significant variations between each strawberry variety separately and controls (Figure 5).

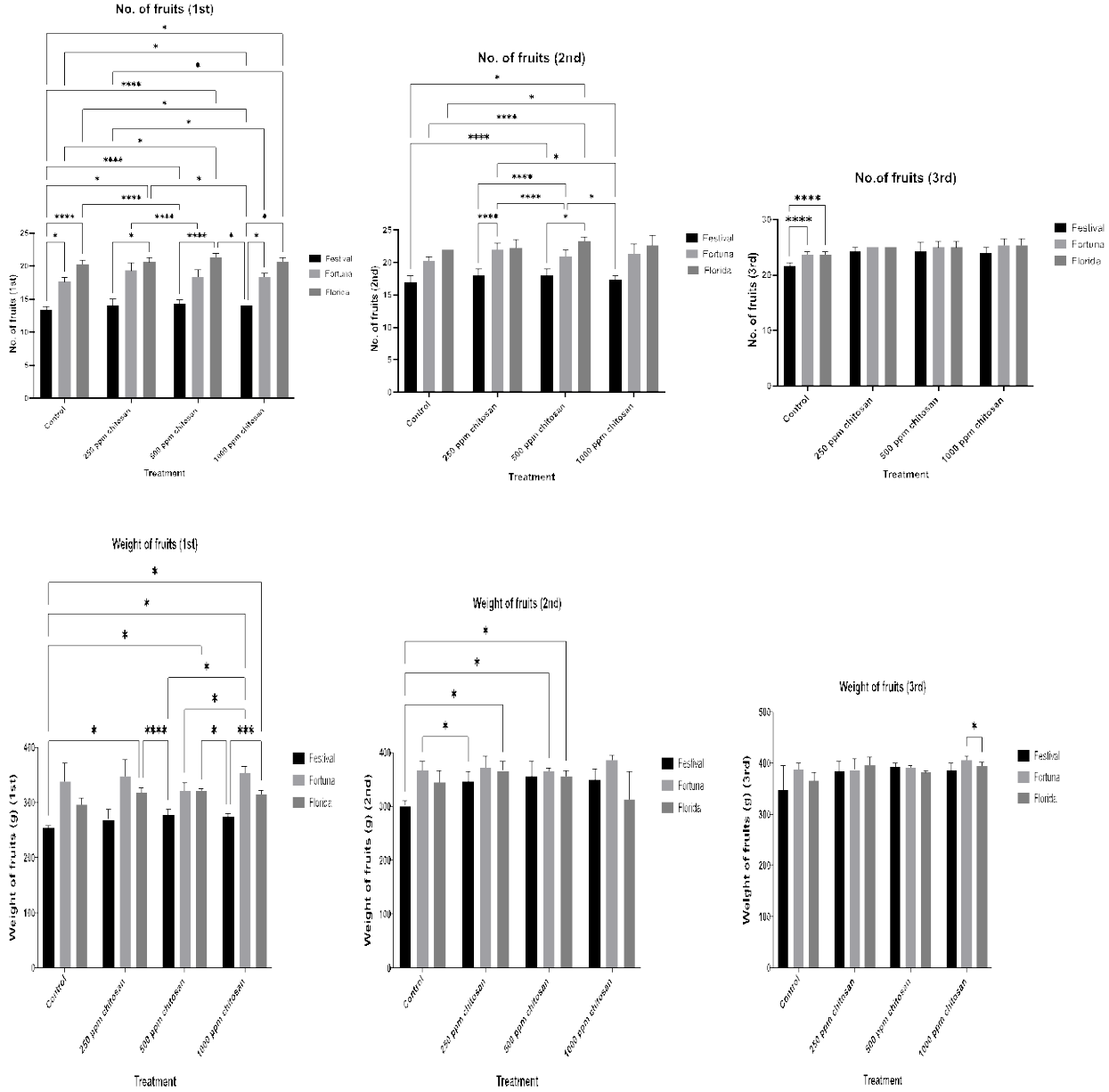

Figure 5. Charts show fruit yield (number and weight of fruits in 1st, 2nd, and 3rd harvests) of three different strawberry varieties, treated with three different concentrations of COS solution under cold-stress conditions, compared with untreated control. The asterisk means significant differences among treatments and genotypes according to range, $p<0.05$.

3.2.2. Weight of fruits in 1st, 2nd, and 3rd harvests

Regarding the results of the weight of fruits of three strawberry varieties in three harvests, it was found that foliar application of 500 ppm COS led to an increase in the fresh biomass in var. Festival through three harvests, compared with the untreated control. Moreover, the foliar spraying of 1000 ppm COS of strawberry var. Fortuna cultivated in the field induced a significant ($p<0.05$) increase in the weight of fruits under cold stress conditions in the 1st harvest and a non-significant increase in the 2nd, and 3rd harvests, compared with un-treated plants. Furthermore, strawberry var. Florida sprayed with 250 and 500 ppm COS solutions had positive effects on fruit yield in (the 2nd and 3rd) and the 1st harvests, respectively (Figure 5). On the other hand, it was clear that strawberry var. Florida treated with three different concentrations of COS solution (250, 500, and 1000 ppm) caused a significant increase in weight of fruits, compared with the control Festival (Figure 5). Moreover, Fortuna var. sprayed with 1000 ppm COS recorded a remarkable increment in fruit yield in the 1st harvest, compared with Festival

(control and 500 ppm COS). It was shown that 250 and 1000 ppm COS solution significantly increased fruits weigh in strawberry var. Florida, compared with 500 and 1000 ppm COS of var. Festival, respectively at $p<0.05$. Moreover, strawberry var. Florida treated with 500 ppm COS solution had a positive effect on the weight of the fruit, compared with 1000 ppm Festival variety. For 2nd harvest, it was found that vars. Florida (250 and 500 ppm COS) and Fortuna (500 ppm COS) stimulate a significant increase in yield, compared with the control Festival (Figure 5). According to the 3rd harvest, it was observed that 1000 ppm COS of var. Fortuna scored a statistically significant increase in fruit weight compared with 1000 ppm COS of Florida (Figure 5).

3.3. Gene expression of strawberry plants treated with COS by SDS-PAGE

SDS-PAGE revealed the differences in protein banding patterns of three varieties of strawberry (Florida, Festival, and Fortuna), treated with the three different concentrations of COS (250, 500, 1000 ppm, and un-treated control) (Figure 6). The electrophoregrams were estimated depending on the number of subunits and molecular weights of polypeptides (MWs) (kDa). A total of 26 polypeptides were recorded, ranging from 8 to 120 kDa; 18 bands out of 26 were monomorphic (69.23%). However, eight were polymorphic (30.77% polymorphism). The application of foliar spraying with COS under cold stress led to inducing changes in gene expression in strawberry varieties that were either up-regulated or down-regulated. The highest content of proteins was found in Fortuna variety treated with 500 ppm COS (25 subunits). In contrast, the lowest content of proteins was recoded in Florida variety sprayed with 250 ppm COS (19 polypeptides) (Figure 6). On the other hand, new proteins were induced in three strawberry varieties treated with COS under cold stress. For example, the Fortuna variety sprayed with 250 and 500 ppm COS recorded novel polypeptides of MWs (53 and 44 kDa) and (53; 44; and 8 kDa), respectively, compared with the corresponding control. Interestingly, Florid and Festival varieties sprayed with 500 and 1000 ppm COS induced only one band of 29 kDa (Figure 6).

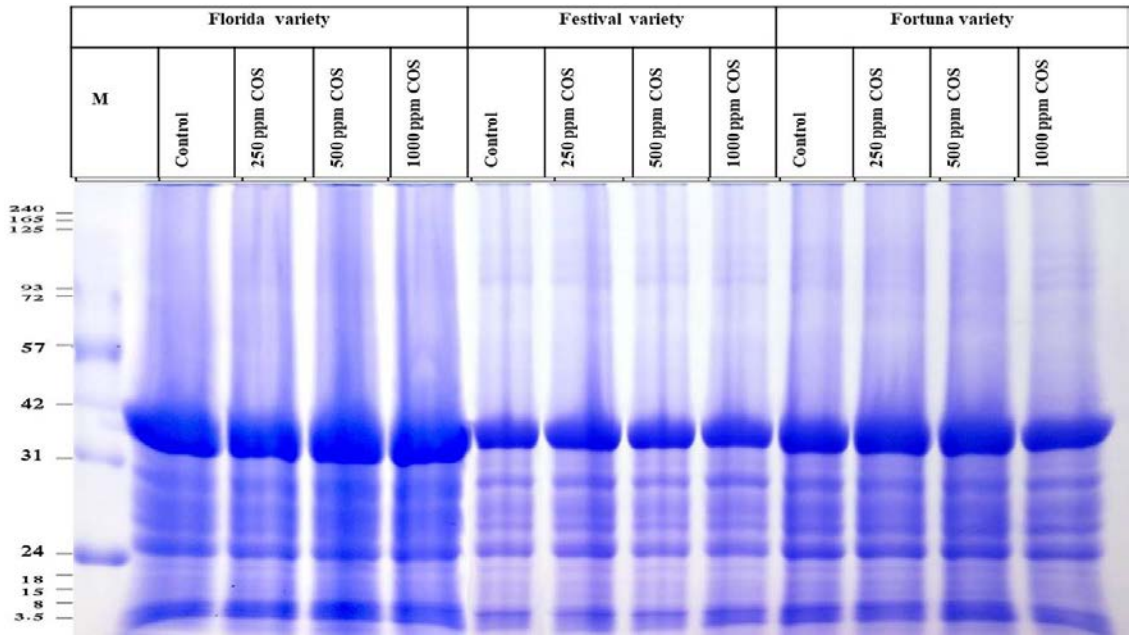

Figure 6. SDS-PAGE banding patterns of three strawberry varieties (Florida, Festival, and Fortuna) sprayed with three different concentrations of chitosan (COS) compared with the control. Lane M: Protein ladder.

3.4. Cluster analysis

The genetic similarity values among three strawberry varieties (Florida, Festival, and Fortuna) COS-treated ranged from 0.73 to 0.96 (Figure 7). The highest genetic similarity was 0.96, found between (Fortuna; 250 ppm) and (Fortuna; 500 ppm), while the lowest genetic similarity was 0.73, recorded between (Fortuna; 500 ppm) and (Festival; 1000 ppm) (Figure 7). A dendrogram showed two different clusters. The first cluster (I): composed of two groups. The first group (a) has COS-treated and untreated plants of Florida variety. However, the second group (b) included treated and untreated plants of Festival variety. Finally, the second cluster (II): contained the Fortuna variety treated with three different concentrations of COS and control, which conferred a higher genetic distance between the two clusters Fortuna and (Florida and Festival) (Figure 8).

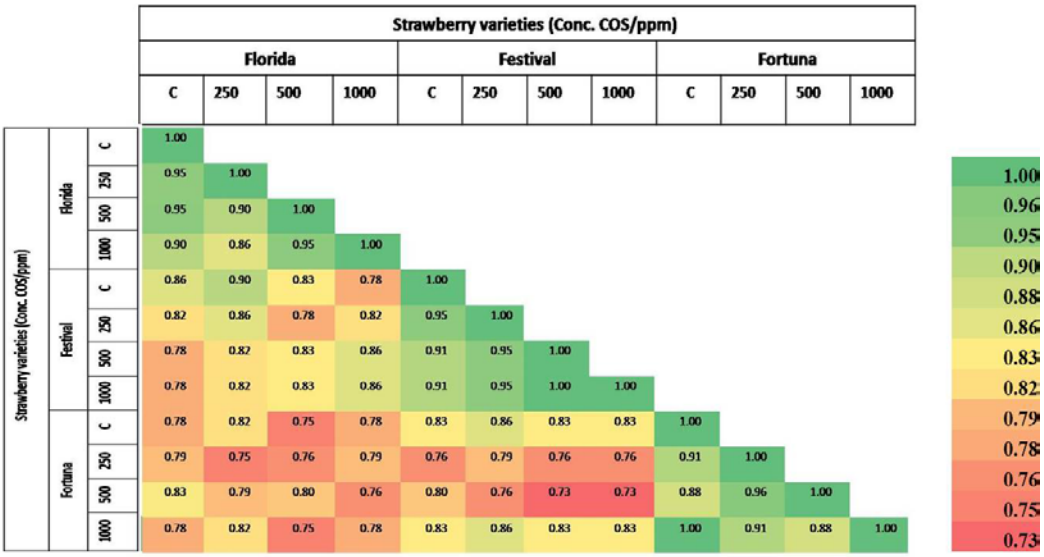

Figure 7. Heatmap was determined using the Jaccard index of three strawberry varieties (Florida, Festival, and Fortuna) sprayed with three different concentrations of chitosan (COS), compared with the control (C).

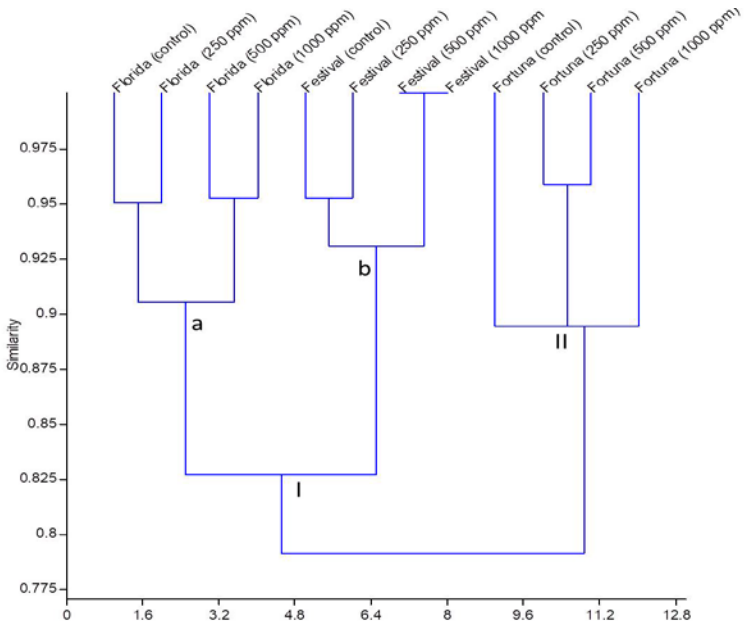

Figure 8. Dendrogram of three strawberry varieties (Florida, Festival, and Fortuna) sprayed with three different concentrations of chitosan (COS), compared with the control.

4. Discussion

Chilling is considered a very serious threat as a result of its harmful effects on the growth of different crops in the field. Also, climate change can impact quantity and quality of the vegetable and fruit yields in Egypt and worldwide, consequently affecting the accessibility of food in the future (Eissa *et al.*, 2013; Hasanuzzaman *et al.*, 2020). One of these climatic changes' manifestations is cold stress, which has a severe impact on the productivity of various crops, such as strawberry plants (Roussos *et al.*, 2020; Li *et al.*, 2021; Han *et al.*, 2023). A low temperature can affect biochemical and physiological operations in plants, causing different symptoms such as necrosis, chlorosis, and wilting (Ruelland and Zachowski, 2010). In this study, the ANOVA analysis showed positive effects in vegetative growth parameters (number of leaves/plant, leaf area, plant height, root length, shoot fresh and dry weight, root fresh, and dry weight) and reproductive growth (number of fruits and weight of fresh biomass) between COS treatments for each strawberry variety separately and the healthy control and among the tested strawberry varieties. Our findings showed that foliar application of COS solution to strawberry plants in an open field improved the plant growth, which depends on the concentration and variety. Similar results were observed by Pongprayoon *et al.*, (2022) who found that COS-treated plants induce beneficial responses in plants against abiotic

stresses. Thus, COS influence depends on concentration, plant species, structure, and the growth stage of the plant.

In the current investigation, the lowest vegetative and fruit growth parameters were recorded in the COS-untreated control. However, foliar spraying of 500 ppm COS solution on strawberry var. Festival led to improvements in vegetative and reproductive growth, while a dose of 1000 ppm had positive changes in plant height and leaf area. Moreover, a remarkable increment in both vegetative growth and yield of strawberry var. Fortuna depends on COS dose. For example, a dose of 250 ppm COS positively influenced root length, and the number of fruits in three harvests, whilst a dose of 500 ppm COS solution increased the number of leaves/plant and root fresh and dry weight, and the number of fruits in the 3rd harvest. Nevertheless, 1000 ppm COS induced remarkable changes in plant height, leaf area, and weight of fruits in the 1st, 2nd, and 3rd harvests. However, strawberry var. Florida treated with a dose of 500 ppm COS caused an increase in plant height, root length, no. of leaves, shoot fresh and dry weight, no. of fruits in the 1st, and 2nd harvests, weight of fruit in the 1st harvest, and root fresh, dry weight and the number of fruits in the 3rd harvest. These results agree with Pichyangkura and Chadchawan, (2015) and Rahman *et al*., (2018) who found that foliar spraying with different doses of COS enhanced the growth of strawberry plants compared with untreated control. However, a dose of 500 ppm recorded the highest growth parameters and improved resistance to diseases by mechanisms involving induced systemic resistance. The positive effect of COS on vegetative growth may be attributed to an increase in the uptake of water and nutrient elements by altering the osmotic pressure of the cell and decreasing the aggregation of harmful free radicals through increasing antioxidants, e.g. glutamine synthetase, nitrate reductase, and protease. It encourages the production of large quantities of phenolics, flavonoids, carotenoids, and anthocyanins. Besides, COS enhanced the transportation of nitrogen to leaves. Consequently, COS increased photosynthesis, which enhanced the growth and development of plants. In addition, COS has several characterization including being less expensive, safe for human health, and environment-friendly, abundantly available, and biodegradable (Guan *et al.*, 2009; Mondal *et al.*, 2012). Wang *et al.*, (2021) indicated that COS nanoparticles increase the efficiency of *Musa* sp. plants in the tolerance of chilling by decreasing the accumulation of reactive oxygen species (ROS) and the induction of non-enzymatic antioxidants, such as phenolic compounds, and increasing antioxidant enzyme activities. Besides, it causes the accumulation of osmoprotectants, such as amino acids, soluble carbohydrates, and proline. Therefore, it aids in enhancing the plants' tolerance to cold stress.

In this context, it was shown that foliar application of strawberry plants with COS solution under chilling stress stimulates changes in gene expression in strawberry varieties, either up-regulated or down-regulated. Furthermore, novel proteins belonging to pathogen-related proteins (PRPs) were induced in three strawberry varieties treated with COS under abiotic stress. For example, new polypeptides of MWs (53 and 44 kDa) and (53; 44; and 8 kDa) were scored in the Fortuna variety sprayed with 250 and 500 ppm COS, respectively compared with the corresponding control. Also, one subunit of 29 kDa was detected in Florid and Festival varieties treated with 500 and 1000 ppm COS solutions. These results agree with Lukoševičiūtė, (2014) who observed a significant increase in the content of 18 kDa protein in the shoots of two strawberry varieties during the freezing stress. Vítámvás and Prášil, (2008) found that one of the characteristics of acclimatization to cold stress is an increase in the concentration of soluble proteins and carbohydrates, which significantly reduces the damage that freezing causes to plant tissues. Besides, low temperatures cause an increase in total soluble proteins involving lipocalins and dehydrins that accumulate in plasma membranes when plants are exposed to freezing. Ouellet and Charron, (2013) indicated that these proteins play an important role in protecting cell structures from chilling damage and decreasing oxidative stress development. Kuwabara and Imai, (2009) found that some PRPs were induced under cold stress conditions, such as ß-1,3-glucanases, which were shown to be low temperature-induced and were cryoprotective activity similar to other extracellular PRPs (Hincha *et al.*, 1997). The evidence on the role of PRPs in cold tolerance in strawberry plants is little. Gharechahi *et al.*, (2014) mentioned that PRPs other than the PR-1 class are linked to the chilling stress of plants, and it was supposed that they could be included as components of the stress-regulated signal transduction pathway. Pihakaski-Maunsbach *et al.*, (2001) suggested that low temperatures interact with other environmental cues in plants. Interestingly, numerous studies have found a strong correlation between chilly signals and defensive reactions. There were a variety of PRPs, including -1,3-glucanases, endochitinases, and thaumatin-like proteins, that built up in winter rye during cold stress. These proteins play the main role in freezing tolerance (Griffith and Yaish, 2004). How cold stress stimulates their accumulation is still understood. The synthesis of PRPs under cold stress conditions ensures an appropriate strategy of defense against infection with pathogens that multiply during cold seasons. All this information shows the existence of a variety of signaling interactions between pathogens and cold responses. Van Loon *et al.*, (2006) mentioned that senescence, injury, or cold stress all cause the induction of several defense-related proteins, some of which have antifreeze functions. Numerous defense-related proteins are found in floral tissues on a constitutive basis, and a sizable number of PR-like proteins in pollen, fruits, and vegetables. Besides, these proteins play major roles in plant life, whether in defense or not.

Therefore, the exogenous application of COS led to the induction of novel polypeptides in all tested strawberry varieties. These new proteins were up-regulated compared with the corresponding control.

5. Conclusions

Chilling injury is considered one of the most important environmental stresses that affect the growth and productivity of strawberry crops in the world. In this study, it was observed that foliar spraying of three strawberry varieties with different concentrations of COS solution (250, 500, and 1000 ppm) in an open field, resulted in a significant increase in leaf area, and number of leaves of var. Fortuna. Furthermore, there was a substantial increment in the number of fruits in vars. Festival, Fortuna,

and Florida, compared with the un-treated control. On the other hand, COS had positive effects in vegetative growth parameters for the tested strawberry varieties **(**number of leaves/plant**,** leaf area, plant height, root length, shoot fresh and dry weight, root fresh, and dry weight) and reproductive growth (weight of fresh biomass) between COS treatments for each strawberry variety separately and the corresponding control. Therefore, the foliar application of COS solution decreases the negative effects of cold stress and improves the strawberry plants' tolerance to chilling by increasing the content of total soluble proteins.

Competing interests

The authors declare that they have no competing interests.

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