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RESEARCH PAPER

# Antioxidant, lipid peroxidation and cell membrane stability influence yield in *Cucumis sativus* L. by chitosan application under different sowing times

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**Key Message:** Chitosan foliar application enhances yield with booting thermos-tolerance ability by improving enzymatic and non-enzymatic antioxidant systems in cucumber genotypes under high temperature.

**Abstract:** Heat stress in a major obstacle in cucumber production in the Punjab province of Pakistan. To save cucumber production from heat stress, a study was designed to evaluate chitosan on cucumber genotypes for yield enhancement under three sowing dates. Four cucumber genotypes, two tolerant (L3466 and Desi-cucumber) and two susceptible (Suyo Long and Poinsett), were screened in a previous experiment under field conditions during 2016. The 1st sowing date was 15$^{th}$ March, followed by 1$^{st}$ April, and 15$^{th}$ April, respectively with four replications. Chitosan level (200 ppm) was applied as foliar spray at 30 days post-sow and then applied two times after a week interval. During present research, the highest summer temperature was 47.5 °C recorded in May, 48°C in June, and 46.1°C in July. Chitosan improved the enzymatic antioxidants and osmolytes in enhancing the ability of cucumber to tolerate heat stress. Enhancement in biochemical characteristics (Superoxide dismutase, peroxidase, catalase, and protein content) were observed in chitosan treated plants as compared with non-treated plants. It was revealed that yield per plant was highest in L3466 (2.12 kg) and Desi-cucumber (2.10 kg) with chitosan application on the 1$^{st}$ sowing date, while yield per plant was reduced in Suyo Long (0.31 kg) and Poinsett (0.29 kg) on the third sowing date with chitosan application. However, in 3$^{rd}$ sowing date Suyo Long and Poinsett could not survive at the fruiting stage without the chitosan application. L3466 and Desi-cucumber could be a benefit to farmers with chitosan application and breeders for further studies for heat stress tolerance. 

**Keywords:** Biochemical, Chitosan, Cucumber, Heat stress, Sowing dates

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## Introduction

Presently, agriculture is facing heat stress as a global drastic problem (Jha et al., 2014; Liu et al., 2019). Heat stress results from plants being exposed to gradually increasing temperature for a limited time (Faralli et al., 2015). Disturbance in normal biological processes, leaf area reduction, chlorosis, and increased transpiration rate are the foremost signals of heat stress (Ruelland & Zachowski, 2010). Genes responsible for photosynthesis in the chloroplasts are repressed after a heat stress event, ultimately leading to damage to cell membranes as a result of lipid peroxidation which are indicators of heat stress (Yoshida et al., 2012; He et al., 2019). This lipid peroxidation leads to oxidative stress (Liu & Huang, 2000; Kipp & Boyle, 2013). In response, plants develop defensive mechanisms with the production of lipid peroxidation (MDA) and activation of SOD, CAT, POD started in leaves and roots (Gommers, 2020). Superoxide dismutase (SOD) transcript was revealed by the exposure of various heat shock treatments (Kumar et al., 2013). High temperature leads to reduced plant biomass (Greer & Sicard, 2008). Heat stress is a chief cause of unwanted alterations in growth, development, physiology, and yield attributes of plants (Smith, 2019). In many species, heat stress normally appears very similar to drought stress (McKersie & Lesheim, 2013). Marketable produce of major crops is reduced under fluctuating temperatures and rain intensity with disturbed patterns which is a serious threat to the economy of a country (Shakoor et al., 2011). Chitosan was reported firstly as a sterilizer for plants. The main role of chitosan is to combat certain biotic and abiotic stresses (Katiyar et al., 2015). It is a plant growth enhancer and its functions resemble with plant growth promoters (Mondal et al., 2012). Chitosan has been used as a natural seed treatment of fruit and vegetables to enhance shelf-life and quality (Cui et al., 2020; Mohamed et al., 2020; Shah & Hashmi, 2020). Chitosan applications directly affect the gene regulation mechanism, effectively

boosting the defense mechanism of plants. Chitosan was formally used as a natural seed treatment, but foliar application was reported to be more efficacious than seed treatment (Janmohammadi et al., 2014).

Cucumber (*Cucumis sativus* L.) is an important horticultural crop that ensures food security as well as enhances income of farming communities. Cucumber is considered the oldest cultivated vegetable (Wehner & Guner, 2004). Cucumber has potential to be a valuable crop in Pakistan. Thermotolerance ability has been increased in cucumber by grafting on Momordica sp. (Xu et al., 2018). Previously, screening of genotypes was conducted under high temperature conditions (Kaur et al., 2016). Malondialdehyde content of cucumber rises under high temperature exposure (Zhang et al., 2012). However, high temperature above threshold levels deteriorates cucumber yield and fruit quality (Zhao et al., 2011). Currently, there is a need to mitigate heat stress in cucumber (Ding et al., 2016). This study aimed to evaluate morpho-physiological and biochemical attributes for heat tolerance in cucumber genotypes subjected to heat stress conditions and determine if yield increases in cucumber after chitosan foliar application under a controlled, high temperature environment.

## Materials and Methods

Desi cucumber (heat tolerant variety) and Poinsett (heat susceptible variety) selected from Experiment I were previously screened out by Ali et al. (2019), were sown in the vegetable research area of University of Agriculture, Faisalabad, during two consecutive years 2015-16. One month after sowing, chitosan (200 ppm) was applied as a foliar application. Chitosan was dissolved in water by adding 0.1 molar $C_2H_4O_2$ (at room temperature, twelve hours with continuous stirring). The first foliar spray was given thirty days after emergence while the second week after the first and the third again a week after the second application.

**Cell membrane thermo-stability**

It was measured indirectly by determining electrolyte leakage (EL) (%). To calculate the electrolyte leakage, leaf cells assessment of the cell membrane stability (CMS) was done by the method of Farkhondeh et al. (2012) with a few alterations. Leaf samples were taken, after washing 0.3 g of leaf samples with deionized water, these were placed in tubes which had 15 mL of deionized water and incubated for two hours at 25 °C, electrical conductivity of the solution ($L_1$) was determined. Samples were then autoclaved at 120 ºC for twenty minutes and the final conductivity ($L_2$) was calculated after equilibration at 25°C. Leaf electrolyte leakage (EL) was measured by the following formula:

$$EL\,\% = \frac{L1}{L2}\,x\,100$$

**Protein (mg/ml)**

Bradford method was applied for the determination of soluble proteins by Kruger (2009). First of all, 2 mL of Bradford reagent taken in a micro centrifuge tube and 50 µL of the sample added into it. Then Bradford reagent was taken as a blank. After that at a wavelength of 595 nm absorbance was detected. Finally, various levels of bovine serum albumin (BSA) were set and protein contents were found by standard curve methods.

**Antioxidant enzymes**

Antioxidant activities were measured by cucumber fresh leaves (0.5 g). Initially, these ice-cooled leaves were put in the grinder. Secondly, 5 mL of fifty mM cooled phosphate buffer which were having pH of 7.8. In the next step it was mixed evenly. Centrifuge at 15000 g at a cool temperature of 4°C for 25 minutes. The activities of the following enzymes were determined.

**Superoxide dismutase (SOD) (µmg$^{-1}$ Protein)**

SOD quantity was found by using various steps. In the beginning, reaction mixture with quantity of three mL with 50 mM of nitroblue tetrazolium, 1.3 mM riboflavin, 13 mM methionine, 75 mM EDTA, 50 mM phosphate buffer solution having pH of 7.8 along with 30 mL of enzyme extract was taken. In the second step, light of (fifteen fluorescent lamps) at seventy-eight mmol $m^{-2}$ $s^{-1}$ for 15 minutes was radiated on the test tubes for absorption. In the third step, by varying the amount of absorbance was detected with a wavelength of 560 nm by the use of a spectrophotometer (Hitachi-650, Japan). It was well thought-out that one unit of SOD activity was described as, the amount of enzyme that controlled 15 % of NBT photo decline.

**Catalase (CAT) (µmg$^{-1}$ Protein)**

Activities of catalase were calculated by Chance and Maehly (1955) method with a few changes in the procedure. Initially, there was catalase reaction mixture (3 ml) having 15 mM phosphate buffer (pH 7.0), 5.9 mM hydrogen peroxide and 0.1 ml of enzyme extract was irradiated with 240 nm of wavelength while, absorbance in reaction mixture was detected with 29 twenty seconds intervals. In the second step, catalase activity was detected as an absorbance change of 0.01 units per minute.

**Peroxidase (POD) (µmg$^{-1}$ Protein)**

Activities of POD were also calculated by Chance and Maehly (1955) method with a few changes in the procedure. A 3 mL POD reaction mixture was actually comprised of 50 mM phosphate buffer (pH 5.0), 20 mM guaiacol, 40 mM $H_2O_2$ along with 0.1 mL of enzyme extract and was irradiated. Its

absorbance with 470 nm wavelength was detected with 30 seconds intervals with 0.01 units per minute.

**Lipid peroxidation (MDA) (nmol $g^{-1}$ FW)**

MDA was calculated by applying the method of Cakmak & Horst (1991). In the first step, One-gram fresh tissue along with 5 mL of 1% of TCA was grounded in the presence of cold conditions and after that it was centrifuged at 15,000 rpm for 10 minutes. In the second step, in a test tube, a mixture of three mL TCA along with 0.5% thiobarbituric acid in 20% TCA and 0.5 mL of supernatant was poured into it. In the third step, this mixture was then incubated at 58°C in a shaking water bath for 50 minutes. Finally, the test tubes having mixture were kept on ice and optical densities measured at 532 and 600 nm.

**Glycine betaine (GB) (µmol $g^{-1}$ FW)**

Glycine betaine in cucumber leaves was determined by the procedure of Grieve & Grattan (1983). In this way, leaf extraction of selected cucumber genotypes was filled in a 20 mL test tube with chopped pieces of 0.5 g leaves along with 5 mL of toluene and poured with water mixture (0.05% toluene). In the second step, test tubes were placed in the shaker for 24 hours at 25°C. In the third step after filtration 0.5 mL of leaf extract was mixed with 1 mL of 2N HCl solution then and 0.1 mL of $KI_3$ solution (having 7.5 g iodine and ten g KI in 100 mL of 1 N HCl) was mixed and again placed in shaker in ice-cold water bath for 90 minutes. In the fourth step, 2 mL of ice-cooled water was poured into it and placed in the shaker along with 10 mL of 1, 2-dichloroethane (chilled at minus 10 ºC). In the fifth step, by passing a continuous stream of air for 1 to two minutes, mixture was separated into two distinct layers. In the seventh step the upper aqueous layer was discarding off, and optical density of organic layer was detected with 365 nm of irradiations.

**Proline (Pr) (µmol $g^{-1}$ FW)**

Proline contents of cucumber leaves were measured by using ninhydrin acidic method (Deng et al., 2011) with small variation. In the first step, after heating, 30 leaf samples and five mL three percent (w/v) aqueous $C_7H_6O_6S$ was homogenized and boiled at 100°C for 10 minutes. In the second step, this homogenous mixture was cooled to room temperature and after that centrifuged at 4,000 g for 10 minutes. In the third step, two mL supernatant was poured into a test tube and supplement with two mL glacial $CH_3COOH$ and 3 mL 2.5% (w/v) acid ninhydrin. In the fourth step the reaction solution was boiled at 100°C for 40 minutes. In the fifth step, the reaction was placed on ice. In the sixth and final step, the mixture was extracted with 5 mL toluene, and the absorbance of radiation was detected with 520 nm wavelengths by spectrophotometer (T6 New Century, Purkinje General).

**Yield attributes**

The number of fruit per plant was counted on five selected plants for each genotype, subjected to chitosan and control treatment, separately in order to calculate yield per plant in each sowing date. The average was calculated for each replication and means were calculated for each genotype in both treatments, separately. Fruit diameter (cm) was measured with Vernier caliper then the average was computed for each replication and means were calculated. Fruit length (cm) was measured using a Vernier caliper and the average was calculated for each replication. The fruit weight (g) was measured on a per plant basis for each genotype in each sowing date and in both chitosan treated and non-treated plants to measure yield per plant.

**Experimental design and statistical analysis**

It was a three factor factorial (different genotypes, chitosan application and sowing dates) experiment with four replications under Randomized Complete Block Design (RCBD). Analysis of variance technique was employed by Fisher's analysis and significance among treatment of means were compared by using Tuckey Highest Significant Difference Test (HSD) at $P \leq 0.05$ with STATISTIX 8.1.

## Results

**Biochemical analysis**

**Superoxide dismutase**

It was revealed that the second sowing date produced the highest SOD activity, followed by the first sowing. Desi-cucumber gave the highest SOD activity which was not significantly different from L3466 in the second sowing date. In the third sowing date, Poinsett had the lowest level of SOD activity followed by Suyo Long but SOD activity was not statistically significant. There was no interaction between genotypes and chitosan treatment (Fig. 1).

**Peroxidase**

Desi-cucumber (heat tolerant genotype) gave the highest level of POD activity but was not statistically significantly different from L3466, while Poinsett showed the lowest level of POD activity and was not statistically significantly different to Suyo Long. It was revealed that the second sowing date produced the highest POD activity, followed by the first sowing and then the third sowing, respectively, irrespective of genotype. Significant interaction was seen between genotype and sowing date. In the second sowing date, Desi-cucumber gave the highest POD activity which was not significantly different to L3466. In the

third sowing date, Poinsett had the lowest level of POD activity, followed by Suyo Long but they were not statistically significantly different (Fig. 2).

**Catalase**

It was revealed that CAT activity was maximum in the second sowing date followed by the first and then the third sowing. L3466 had maximum CAT activity in the first sowing date with foliar application of chitosan, while minimum catalase activity was observed in Poinsett without chitosan application in the third sowing date. Suyo Long and Poinsett also exhibited an increased CAT activity with chitosan application. Significant interaction of chitosan application and sowing date was observed (Fig. 3).

**Protein**

It was revealed that protein contents were maximum in the first sowing date followed by the second sowing date and the third sowing date, respectively. L3466 had the highest protein content in the first sowing date with chitosan foliar application while minimum protein content were observed in Suyo Long without chitosan application in the third sowing date. Suyo Long and Poinsett also exhibited increased protein content with chitosan application compared with no chitosan application (Fig. 4).

**Glycine betaine**

It was revealed that GB content was highest in the second sowing date followed by the first and the third. L3466 exhibited the highest GB content in the first sowing date with foliar application of chitosan while the lowest GB content were observed in Poinsett without chitosan application in the third sowing date. Suyo Long and Poinsett exhibited an increased GB content with chitosan application (Fig. 5).

**Proline**

Maximum proline (Pr) content was observed in L3466 which was not significantly different compared to Desi-cucumber which had an enhanced level of Pr content with the application of chitosan irrespective of sowing dates. Suyo Long showed low Pr content without chitosan treatment which was not significantly different from Poinsett irrespective of sowing date. The third showing date produced the lowest level of Pr content as compared with the 1st and the 2nd sowing time across all genotypes (Fig. 6).

**Lipid peroxidation**

Lipid peroxidation was measured indirectly by determining MDA content. In the third sowing date, Desi-cucumber possessed the highest MDA contents followed by Suyo Long but there was no statistical difference between these levels. Lower levels of MDA content were measured in L3466, followed by Desi-cucumber in the first sowing date, both were not statistically significantly different. Suyo Long and Poinsett also exhibited decreased MDA contents with the application of chitosan in each sowing date. The third showing date revealed the highest MDA contents as compared with the first and the second sowing date (Fig. 7). Cell membrane thermos-stability; Desi-cucumber had the lowest leaf EL (31%) and was not significantly different compared with L3466 (33%) during first sowing dates. The highest EL measurement was noticed in non-treated Suyo Long (77%) and Poinsett (79%) during the third sowing date. Suyo Long revealed a maximum value for EL but both Suyo Long and Poinsett were not significantly different in their response to chitosan application. The third showing date revealed the highest EL as compared with the first and the second sowing date (Fig. 8).

**Yield related attributes**

**Fruit set percentage**

Desi-cucumber had maximum fruit set percentage in the first sowing date, followed by L3466 but there was no statistical difference between these levels, while there was no fruit set on Suyo Long and Poinsett in the third sowing date when no chitosan was applied at temperatures that exceeded 47.5°C. Both Suyo Long and However, Poinsett produced fruits with the application of chitosan in the third sowing date. The first sowing date revealed the highest fruit set percentage as compared with the third and the second sowing date irrespective of genotypes (Fig. 9).

**Fruit length**

It was revealed that chitosan treated plants had a marked increase in fruit length with Desi-cucumber possessing the greatest fruit length in the first sowing date, followed by L3466. Overall, tolerant genotypes performed better irrespective of sowing dates. Suyo Long and Poinsett also exhibited markedly enhanced fruit length with chitosan application (Fig. 10).

**Fruit diameter**

Desi-cucumber had the highest fruit diameter in the first sowing date, followed by L3466. Overall, tolerant genotypes performed better irrespective of sowing dates. Suyo Long and Poinsett also exhibited markedly enhanced fruit diameter with chitosan application (Fig. 11).

**Fruit weight**

All three factors (genotypes, chitosan application and sowing dates) influenced fruit weight. Desi-cucumber gave maximum

average fruit weight in the first sowing date; however, there was not significant different when compared to L3466. Overall, tolerant genotypes performed better irrespective of sowing dates. Also, Suyo Long and Poinsett exhibited markedly enhanced fruit weight with chitosan application (Fig. 12).

**Yield per plant**

On the first sowing date, Desi-cucumber provided the highest yield per plant (2127 g) followed by L3466 (2103 g). Overall, tolerant genotypes performed better irrespective of sowing dates. Suyo Long and Poinsett also had enhanced yield per plant with chitosan application. In the third sowing date, both Suyo Long and Poinsett did not survive to produce fruit under extreme conditions of heat (47.5 °C). It was revealed marked increase in fruit yield with chitosan foliar spray. However, Suyo Long (316 g) and Poinsett (290 g) yielded few fruit at 47.5°C in the third sowing date and heat susceptible cucumber genotypes could not survive without foliar spray of chitosan (Table 1).

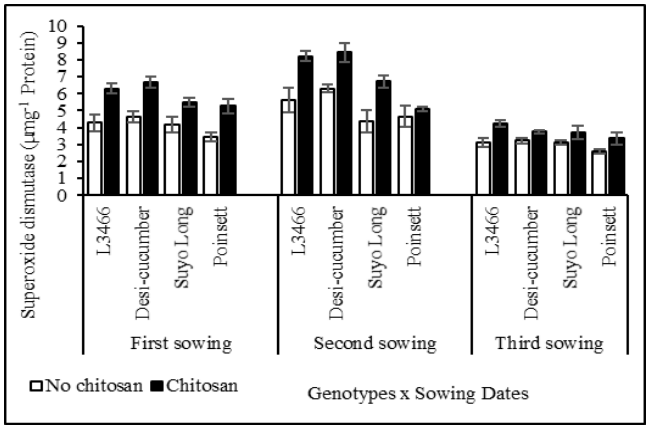


**Fig. 1** Superoxide dismutase in selected cucumber genotypes influenced by chitosan under different sowing dates

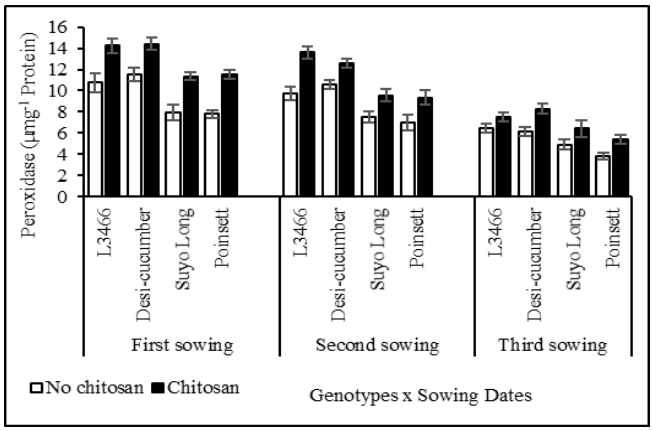


**Fig. 2** Peroxidase contents in selected cucumber genotypes influenced by chitosan under different sowing dates

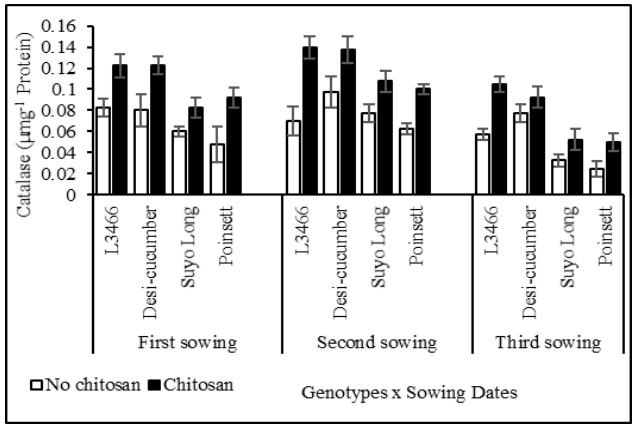


**Fig. 3** Catalase contents in selected cucumber genotypes influenced by chitosan under different sowing dates

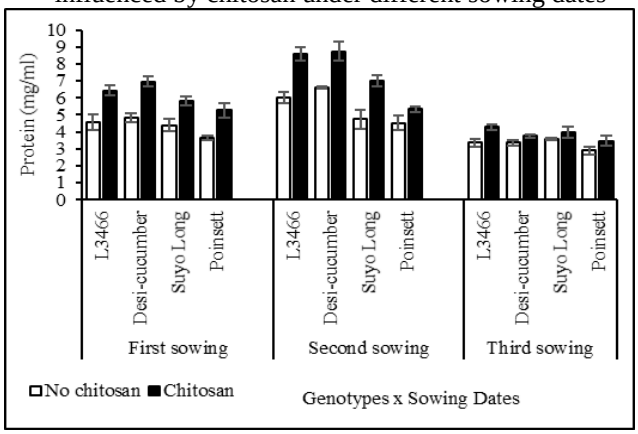


**Fig. 4** Protein concentration in selected cucumber genotypes influenced by chitosan under different sowing dates

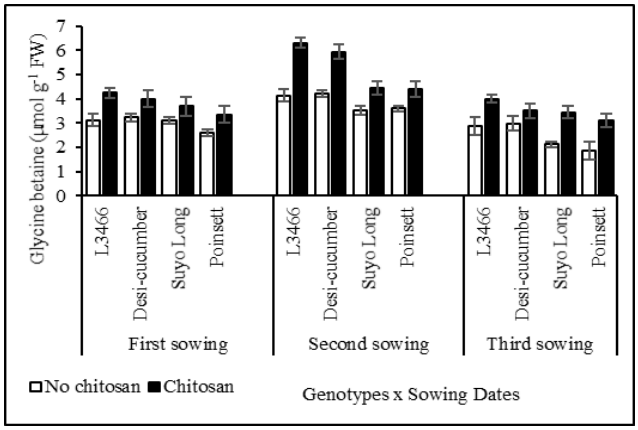


**Fig. 5** Glycine betaine concentration in selected cucumber genotypes influenced by chitosan under different sowing dates

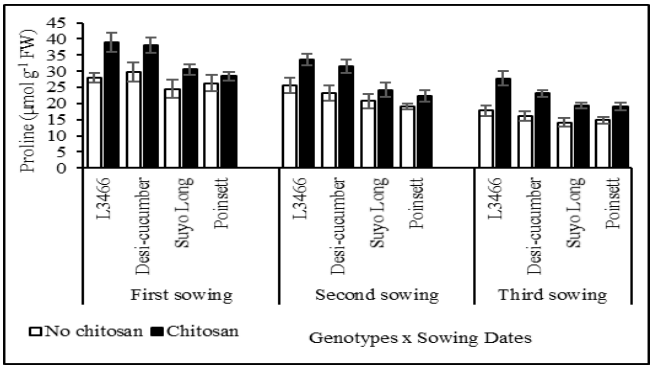


**Fig. 6** Protein level in selected cucumber genotypes Influenced by chitosan under different sowing dates

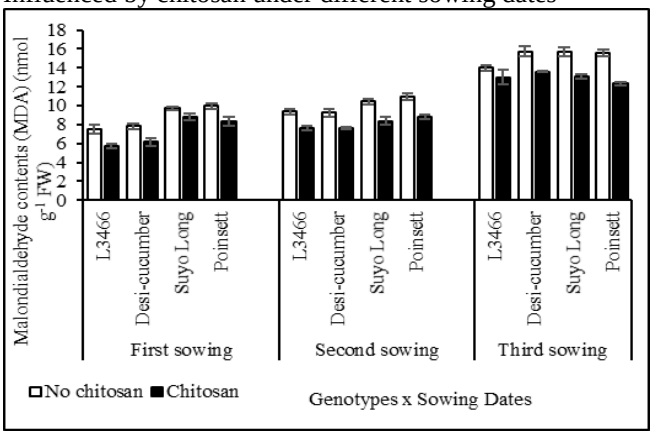


**Fig. 7** Malondialdehyde contents in selected cucumber genotypes influenced by chitosan under different sowing dates

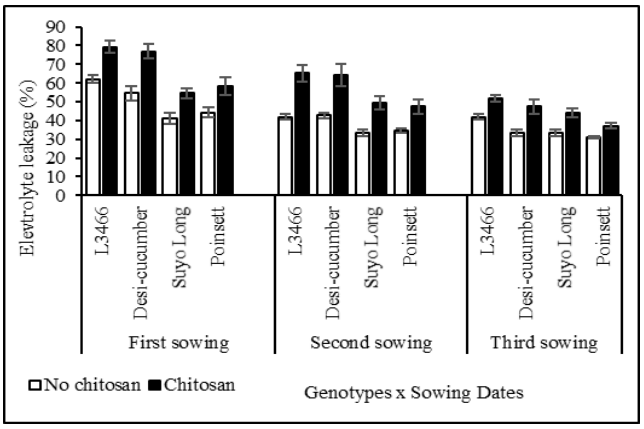


**Fig. 8** Electrolyte leakage in selected cucumber genotypes influenced by chitosan under different sowing dates

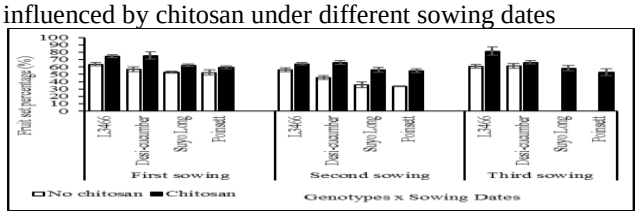


**Fig. 9** Fruit set percentage in selected cucumber genotypes influenced by chitosan under different sowing dates

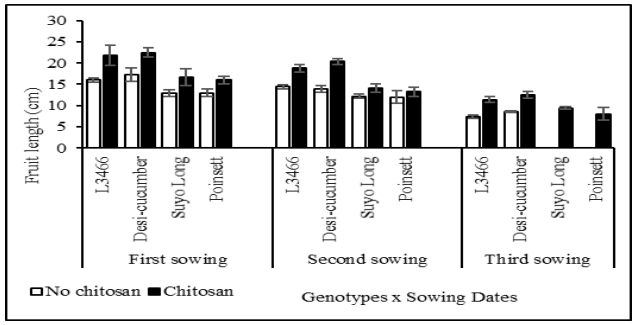


**Fig. 10** Fruit length in selected cucumber genotypes influenced by chitosan under different sowing dates

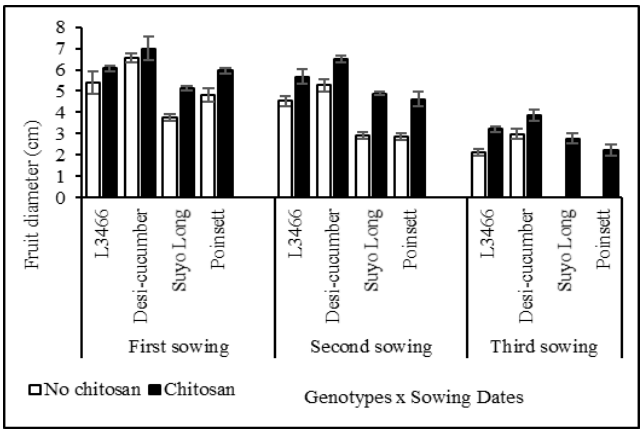


**Fig. 11** Fruit diameter in selected cucumber genotypes influenced by chitosan under different sowing dates

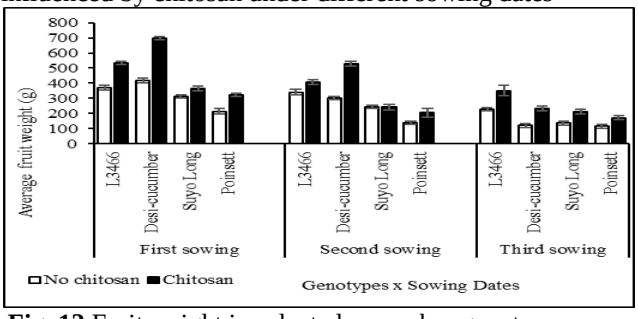


**Fig. 12** Fruit weight in selected cucumber genotypes influenced by chitosan under different sowing dates

## Discussion

Proper functioning metabolic activities depend on maintaining water relations. Recently, the use of antitranspirants has proven an effective technique to mitigate the effect of heat stress in horticultural crops (Dash et al., 2020; Tonhati et al., 2020). Previous research findings suggested that leaf area, chlorophyll content, temperature of leaf surface are major factors for carbohydrate synthesis during photosynthesis. Those cultivars with more intact leaves had more net photosynthetic rate (A). This might be due to larger leaf area to capture more sunlight for photosynthesis, consequently increasing carbon assimilates formation for enhanced growth and development (Amjad et al., 2001; Ekwu & Nwokwu, 2012).

**Table 1** Average yield (g) of selected cucumber genotypes influenced by chitosan application under different sowing times

| CHT x G | Sowing dates | | | CHT x G mean |
|---|---|---|---|---|
| | $SD_1$ | $SD_2$ | $SD_3$ | |
| No CHT x G1 | 1600 | 987 | 392 | 993[b] |
| CHT x G1 | 2127 | 1190 | 821 | 1379[a] |
| No CHT x G2 | 1532 | 735 | 425 | 897[bc] |
| CHT x G2 | 2103 | 1244 | 851 | 1399[a] |
| No CHT x G3 | 997 | 334 | Mortality | 444[e] |
| CHT x G3 | 1311 | 617 | 316 | 748[cd] |
| No CHT x G4 | 741 | 247 | Mortality | 329[e] |
| CHT x G4 | 1171 | 567 | 290 | 676[d] |

CHT = Chitosan; G = Genotype; SD = Sowing date; G1 = L3466; G2 = Desi-cucumber; G3 = Suyo Long; G4 = Poinsett. Means having similar letter(s) in in a column are statistically non-significant (with $P$ <0.05)

In one study, it was seen that genotypes with less leaf surface area had a higher temperature also had more chlorophyll content which in turn revealed better performance in photosynthetic rate. Such conclusions were reinforced by Shaheen et al. (2016) who observed that genotypes with a low leaf surface temperature and higher leaf SPAD value would have a higher photosynthetic rate. During the present examination, it was observed that heat tolerant genotypes had higher chlorophyll content (SPAD value) than susceptible genotypes in all sowing dates. This result was also confirmed by Hussain et al. (2016), and might be due to alterations in microscopic structures of okra genotypes when observed in different sowing times. Microscopic alterations have more influence in susceptible genotypes than tolerant genotypes which might be due to ability of tolerant genotypes to resist such transformations during heat stress (Balouchi, 2010). Outcomes of Reda and Mandoura (2011) described that elevated temperature decreases chlorophyll synthesis in plants. Leaf surface temperature also describes physiological life in plants; it directly affects A and WUE and in due course controls all growth stages. Increase in leaf surface temperature reduces photosynthesis by stimulating photorespiration (Salvucci et al., 2001). Rubisco function is reduced at moderately elevated leaf surface temperature resulting in reduced photosynthetic rates (Schrader et al., 2004). Tolerant genotypes exhibited low electrolyte leakage at the 1st sowing date. Conversely, heat susceptible genotypes revealed maximum cell membrane thermos-stability (EL) at same sowing date. It was observed that EL was higher in heat susceptible genotypes than heat tolerant genotypes. Camejo et al. (2005) found similar responses in tomato genotypes under heat stress which is most likely due to the fact that chemical bonds became loose in cell membranes as molecular kinetic energy (KE) was enhanced (Savchenko et al., 2002). Enhanced EL revealed a signal of decreased membrane thermos-stability which is a key for heat stress measurement found in barley plants (Wahid & Shabbir, 2005). The first sowing date showed superior performance because of fast growth regarding seedling length and number of leaves. This might be due to optimum growing conditions for plants to uptake water and nutrients etc. Similar results have previously been reported by Muhammad et al. (2001); Chattopadhyay et al. (2011); Dash et al. (2013). Genotypes having a greater number of leaves in the first sowing date could maintain normal photosynthesis producing more carbon assimilation, resulting in enhanced growth rate which was observed by Shaheen et al. (2016). It was observed that in both heat tolerant and heat susceptible (Suyo Long and Poinsett) genotypes produced high number of antioxidants such as superoxidase, peroxidase, and catalase regardless of sowing date with chitosan foliar application compared with plants that were not treated with chitosan. Previous findings of Nguyen et al. (2020) revealed that nanochitosan mixed with calcium chloride improved antioxidant in strawberry and reduced the MDA content which are in line with the current results. The present study revealed that protein content was also improved by the application of chitosan; this might be due to increased enzymatic function in leaves. It is reported that chitosan could be effectively employed as an ideal natural antioxidant by scavenging superoxide anion and hydroxyl radical (Park et al., 2003: Garcia et al., 2014: Soleymani & Tehar, 2015). Protein analysis was done to evaluate thermos-tolerance ability of cucumber plants by grafting onto Momordica rootstocks (Xu et al., 2018) as structural analysis proteins need emphasis for potential thermosensors in plants (Vu et al., 2019). At different temperatures (35 to 40°C), a number of genotypes showed enhanced growth response compared to other genotypes under high temperature regimes (Ali et al., 2016).

Significantly higher yield was revealed in chitosan treated plants as compared with non-treated plants regardless of sowing times and genotype. Past reports confirmed that chitosan enhanced growth and yield components in lentils under drought stress (Janmohammad et al., 2014). Similar conclusions were found in previous research where exogenously applied yeast and chitosan significantly improved vegetative growth i.e. shoot length, number of leaves per plant, fresh and dry mass of leaves, and stem mass of plants along with yield and quality of cucumber. It is reported that an exogenously applied chitosan concentration of 4 ml $L^{-1}$ showed the maximum vegetative growth, yield, and quality of cucumber plants (Shehata et al., 2012). Similarly, an experiment with chitosan application was carried out during two successive summer seasons to study the influence of

nitrogen and foliar application of chitosan on growth, yield, and biochemical attributes of fruits of summer squash grown in sandy soil. It was found that spraying plants with chitosan significantly increased plant growth characters, particularly yield (Ibraheim et al., 2015). Physiological processes in plants are influenced significantly by slight increases in temperature. Present research revealed that leaf surface temperature of heat susceptible genotypes was high and that led to high transpiration rate which reduced turgor and osmotic potential of leaves. Higher water loss in heat susceptible genotypes was due to high transpiration rate through the stomata which caused photosynthetic rate to decrease under heat stress (Ali et al., 2019). In this research, heat susceptible genotypes showed a decrease in photosynthetic rate during heat stress and this was probably because of increased stomatal conductance. During heat stress, an enhanced carbon dioxide ($CO_2$) assimilation rate in heat tolerant genotypes was observed compared to susceptible genotypes; this was due to efficient photosynthetic apparatus in tolerant genotypes which was validated in previous research reports. Higher stomatal conductance of water and $CO_2$ was observed in heat susceptible tomato genotypes (Camejo et al., 2005). Chitosan induces activation of the jasmonic acid (JA) pathways which plays a vital role in temperature stress in plants by biosynthesis of JA. Foliar application of chitosan or JA induced transcriptional activation by genes encoding phenylalanine ammonia lyase and protease inhibitors (Farmer & Ryan, 1990; Doares et al., 1995). Enhancement of water use efficiency in plants was observed by activation of the JA pathways which have similar functions to abscisic acid (ABA) (Sembdner & Parthier, 1993). With increase in level of ABA, closure of stomata accelerated, reducing loss of water by low transpiration rate (Mishra et al., 2006). Therefore, organizing the ABA signaling pathway decreased extra water consumption by plants (Grill & Ziegler, 1998). Foliar chitosan spray solutions were much more effective in reduction of stomatal apertures (Lee et al., 1999). Chitosan sprayed on tomato leaves produced the ability to inhibit light-induced opening of the stomata by inducing $H_2O_2$ in guard cells around the stomata. Reduction in stomatal aperture strongly suggests that chitosan is highly antitranspirant (El-Tantawy et al., 2009). Our results of high yield potential in heat tolerant genotypes under heat stress conditions are in line with Sita et al. (2017).

## Conclusion

It can be concluded from the results that chitosan enhanced the heat tolerance potential in cucumber plants under field conditions. Cucumber heat tolerant genotypes (L3466 and Desi-cucumber) responded more efficiently to chitosan as compared to heat susceptible genotypes (Suyo Long and Poinsett) for attributes studied during the first sowing date. Moreover, it could be suggested that chitosan induced heat resistance to a certain extent of temperature stress (44°C) and became less effective under extreme high temperature (above 47°C).

**Author Contribution Statement:** Mujahid Ali, conducted the research work and draw sketch of the article, Chaudhary Muhammad Ayyub, supervised the research work, Emily Silverman and Zahoor Hussain reviewed the article, Shahid Iqbal guided about statistical analysis, while Bilal Akram and Saqib Ayyub checked grammatical errors and corrected reference style etc.

**Conflict of Interest:** The authors declare that they have no conflict of interest

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## References

Ali, M., Ayyub, C. M., Amjad, M., & Ahmad, R. (2019). Evaluation of thermo-tolerance potential in cucumber genotypes under heat stress. *Pakistan Journal of Agricultural Sciences*, *56*(1), 53-61.

Ali, S., Khan, S. U., Gurmani, A. R., Khan, A., Khan, S. M., Farid, A., Khan, I., & Zainab, R. (2016). Agronomic and physiological response of wheat (*Triticum aestivum* L.) genotypes under terminal heat stress condition. *Journal of Biosciences*, *8*, 1-7.

Amjad, M., Anjum, M. A., & Hussain, S. (2001). Effect of different sowing dates and various doses of fertilizers on juvenility and productivity of okra. *Pakistan Journal of Agricultural Sciences*, *38*(2), 29-32.

Balouchi, H. R. (2010). Screening wheat parents of mapping population for heat and drought tolerance, detection of wheat genetic variation. *International Journal of Biology and Life Sciences*, *6*, 56-66.

Cakmak, I., & Horst, W. J. (1991). Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (*Glycine max*). *Physiologia Plantarum*, *83*(3), 463-468.

Camejo, D., Rodríguez, P., Morales, M. A., Dell'Amico, J. M., Torrecillas, A., & Alarcón, J. J. (2005). High temperature effects on photosynthetic activity of two tomato cultivars with different heat susceptibility. *Journal of Plant Physiology*, *162*, 281-289.

Chance, B., & Maehly, A. (1955). Assay of catalase and peroxidase. *Methods in Enzymology*, *2*, 764-817.

Chattopadhyay, A., Dutta, S., & Chatterjee, S. (2011). Seed yield and quality of okra as influenced by sowing dates. *African Journal of Biotechnology*, *10*(28), 5461-5467.

Cui, K., Shu, C., Zhao, H., Fan, X., Cao, J., & Jiang, W. (2020). Preharvest chitosan oligochitosan and salicylic acid treatments enhance phenol metabolism and maintain the postharvest quality of apricots (*Prunus armeniaca* L.). *Scientia Horticulturae*, *267*, 109334.

Dash, P. K., Chase, C. A., Agehara, S., & Zotarelli, L. (2020). Heat stress mitigation effects of kaolin and s-abscisic acid during the establishment of strawberry plug transplants. *Scientia Horticulturae*, *267*, 1-10.

Dash, P. K., Rabbani, M. G., & Mondal, M. F. (2013). Effect of variety and planting date on the growth and yield of okra. *International Journal of Biosciences*, *3*(9), 123-131.

Deng, Y., Chen, S., Chen, F., Cheng, X., & Zhang, F. (2011). The embryo rescue derived intergeneric hybrid between chrysanthemum and *Ajania przewalskii* shows enhanced cold tolerance. *Plant Cell Reports*, *30*(12), 2177-2186.

Ding, X., Jiang, Y., Hao, T., Jin, H., Zhang, H., He, L., Zhou, Q., Huang, D., Hui, D., & Yu, J. (2016). Effects of heat shock on photosynthetic properties, antioxidant enzyme activity, and downy mildew of cucumber (*Cucumis sativus* L.). *PLoS ONE*, *11*(4), 1-15.

Doares, S. H., Syrovets, T., Weiler, E. W. & Ryan, C. A. (1995). Oligogalacturonides and chitosan activate plant defensive genes through the octadecanoid pathway. *Proceedings of the National Academy of Sciences of the United States of America*, *92*, 4095-4098.

Ekwu, L. G., & Nwokwu, G. N. (2012). Effect of plant spacing and planting date on the growth and yield of okra (*Abelmoschus esculentus* L.) in Abakaliki. *International Journal of Agriculture and Rural Development*, *5*, 1041-1048.

El-Tantawy, E. M. (2009). Behavior of tomato plants as affected by spraying with chitosan and aminofort as natural stimulator substances under application of soil organic amendments. *Pakistan Journal of Biological Sciences*, *12*(17), 1164.

Faralli, M., Lektemur, C., Rosellini, D., & Gürel, F. (2015). Effects of heat shock and salinity on barley growth and stress-related gene transcription. *Biologia Plantarum*, *59*(3), 537-546.

Farkhondeh, R., Nabizadeh, E., & Jalilnezhad, N. (2012). Effect of salinity stress on proline content, membrane stability and water relations in two sugar beet cultivars. *International Journal of AgriScience 2*(5), 385-392.

Farmer, E. E., & Ryan, C. A. (1990). Interplant communication: airborne methyl jasmonate induces synthesis of proteinase inhibitors in plant leaves. *Proceedings of the National Academy of Sciences of the United States of America*, *87*, 7713-7716.

Garcia, M., Silva, Y., & Casariego, A. (2014). Development of a mayonnaise with chitosan as natural antioxidant. *Emirates Journal of Food and Agriculture*, *26*, 835-837.

Greer, D. H., & Sicard, S. M. (2009). The net carbon balance in relation to growth and biomass accumulation of grapevines (*Vitis vinifera* cv. Semillon) grown in a controlled environment. *Functional Plant Biology*, *36*(7), 645-653.

Grieve, C. M., & Grattan, S. R. (1983). Rapid assay for the determination of water-soluble quaternary ammonium compounds. *Plant and Soil*, *70*(2), 303-307.

Gommers, C. (2020). Keep cool and open up: Temperature-induced stomatal opening. *Plant Physiology*, *181*, 1188-1189

Grill, E., & Ziegler, H. (1998). A plants dilemma. *Science*, *282*, 252-253.

He, J., Jiang, Z., Gao, L., You, C., Ma, X., Wang, X., Xu, X., Mo, B., Chen, X., & Liu, L. (2019). Genome-wide transcript and small RNA profiling reveals transcriptomic responses to heat stress. *Plant Physiology*, *181*(2), 609-629.

Hussain, R., Ayyub, C. M., Amjad, M., Waraich, E. A., Shaheen, M. R., Mustafa, Z., Shah, S. Z. H., Shahid, A., Haider, M. W., Faiz, H., & Raza, A. (2016). Evaluation of heat tolerance potential of okra genotypes in field conditions at different sowing dates. *Transylvanian Review*, *12*, 3416-3427.

Ibraheim, S. K. A., & Mohsen, A. A. M. (2015). Effect of chitosan and nitrogen rates on growth and productivity of summer squash plants. *Middle East Journal of Agriculture Research*, *4*, 673- 681.

Janmohammadi, M., Mostafavi, H., Kazemi, H., Mahdavinia, G. R., & Sabaghnia, N. (2014). Effect of chitosan application on the performance of lentil genotypes under rainfed conditions. *Acta Technologica Agriculturae*, *4*, 86-90.

Jha, U. C., Bohra, A., & Singh, N. P. (2014). Heat stress in crop plants: its nature, impacts and integrated breeding strategies to improve heat tolerance. *Plant Breeding*, *133*(6), 679-701.

Katiyar, D., Hemantaranjan, A., & Singh, B. (2015). Chitosan as a promising natural compound to enhance potential physiological responses in plant: a review. *Indian Journal of Plant Physiology*, *20*(1), 1-9.

Kaur, N., Dhaliwal, M. S., Jindal, S., & Singh, P. (2016). Evaluation of hot pepper (*Capsicum annuum* L.) genotypes for heat tolerance during reproductive phase. *International Journal of Bio-resource and Stress Management*, *7*(1), 126-129.

Kipp, E., & Boyle, M. (2013). The effects of heat stress on reactive oxygen species production and chlorophyll concentration in *Arabidopsis thaliana*. *Research in Plant Sciences*, *1*(2), 20-3.

Kruger, N. J. (2009). The bradford method for protein quantitation. In the protein protocols handbook (pp. 17-24). Humana Press, Totowa, New Jersey, United States.

Kumar, R. R., Sharma, S. K., Goswami, S., Singh, K., Gadpayle, K. A., Singh, G. P., Pathak, H., & Rai, R. D. (2013). Transcript profiling and biochemical characterization of mitochondrial superoxide dismutase (mtSOD) in wheat (*Triticum aestivum*) under different

exogenous stresses. *Australian Journal of Crop Science*, *7*, 414–424.

Lee, S., Choi, H., Suh, S., Doo, I. S., Oh, K. Y., Choi, E. J., Schroeder-Taylor, A. T., Low, P. S., & Lee, Y. (1999). Oligogalaturonic acid and chitosan reduce stomatal aperture by inducing the evolution of reactive oxygen species from guard cells of tomato and *Commelina communis*. *Plant Physiology*, *121*(1), 147-152.

Liu, G. T., Jiang, J. F., Liu, X. N., Jiang, J. Z., Sun, L., Duan, W., Li, R. M., Wang, Y., Lecourieux, D., Liu, G. H., & Li, S. H. (2019). New insights into the heat responses of grape leaves via combined phosphoproteomic and acetylproteomic analyses. *Horticulture Research*, *6*(1), 1-15.

Liu, X., & Huang, B. (2000). Heat stress injury in relation to membrane lipid peroxidation in creeping bentgrass. *Crop Science*, *40*(2), 503-510.

McKersie, B. D., & Lesheim, Y., (2013). Stress and stress coping in cultivated plants. *Springer Science & Business Media*.

Mishra, G., Zhang, W., Deng, F., Zhao, J., & Wang, X. (2006). A bifurcating pathway directs abscisic acid effects on stomatal closure and opening in Arabidopsis. *Science, 312*, 264-266.

Mohamed, C., Etienne, T. V., & Yannick, K. N. G. (2020). Use of bioactive chitosan and *Lippia multiflora* essential oil as coatings for maize and sorghum seeds protection. *EurAsian Journal of BioSciences, 14*(1), 27-34.

Mondal, M. M. A., Malek, M. A., Puteh, A. B., Ismail, M. R., Ashrafuzzaman, M., & Naher, L. (2012). Effect of foliar application of chitosan on growth and yield in okra. *Australian Journal of Crop Science, 6*(5), 918-921.

Muhammad, A. M., Anjum, A., & Ahmed, A. (2001). Impact of planting geometry on growth, yield and quality of Bhendi (*Abelmoschus esculentus* L. Moerch). *International Journal of Agriculture and Biosciences, 3*(2), 345-349.

Nguyen, V. T., Nguyen, D. H., & Nguyen, H. V. (2020). Combination effects of calcium chloride and nano-chitosan on the postharvest quality of strawberry (*Fragaria x ananassa* Duch.). *Postharvest Biology and Technology, 162*, 1-8.

Park, P. J., Je, J. U., & Kim, S. K. (2003). Free radical scavenging of chitooliosaccharides by electron spin resonance spectrometry. *Journal of Agriculture and Food Chemistry*, *51*, 4624-4627.

Reda, F., & Mandoura, H. M. H. (2011). Response of enzymes activities, photosynthetic pigments, proline to low or high temperature stressed wheat plant (*Triticuma aestivum* L.) in the presence or absence of exogenous proline or cysteine. *International Journal of Academic Research, 3*(4), 108-115.

Ruelland, E., & Zachowski, A. (2010). How plants sense temperature. *Environmental and Experimental Botany, 69*(3), 225-232.

Salvucci, M. E., Osteryoung, K. W., Crafts-Brandner, S. J., & Vierling, E. (2001). Exceptional sensitivity of rubisco activase to thermal denaturation *in vitro* and *in vivo*. *Plant Physiology, 127*(3), 1053-1064.

Savchenko, G., Klyuchareva, E., Abramchik, L., & Serdyuchenko, E. (2002). Effect of periodic heat shock on the inner membrane system of etioplasts. *Russian Journal of Plant Physiology, 49*(3), 349-359.

Schrader, S. M., Wise, R. R., Wacholtz, W. F., Ort, D. R., & Sharkey, T. D. (2004). Thylakoid membrane responses to moderately high temperature in Pima cotton. *Plant Cell & Environment*, *27*(6), 725-35.

Sembdner, G., & Parthier, B. (1993). The biochemistry and the physiological and molecular actions of jasmonates. *Annual Review of Plant Physiology and Plant Molecular Biology*, *44*(1), 569-589.

Shah, S., & Hashmi, M. S. (2020). Chitosan–*Aloe vera* gel coating delays postharvest decay of mango fruit. *Horticulture, Environment, and Biotechnology, 61*, 279-289.

Shaheen, M. R., Ayyub, C. M., Amjad, M., & Waraich, E. A. (2016). Morpho-physiological evaluation of tomato genotypes under high temperature stress conditions. *Journal of Science of Food and Agriculture, 96*(8), 2698-2704.

Shakoor, U., Saboor, A., Ali, I., & Mohsin, A. Q. (2011). Impact of climate change on agriculture: Empirical evidence from arid region. *Pakistan Journal of Agricultural Sciences, 48*(4), 327-333.

Shehata, S. A., Fawzy, Z. F., & El-Ramady, H. R. (2012). Response of cucumber plants to foliar application of chitosan and yeast under greenhouse conditions. *Australian Journal of Basic and Applied Sciences*, *6*(4), 63-71.

Smith, L. M. (2019). The heat is on: Maize pollen development after a heat wave. *Plant Physiology, 181*(2), 387-388.

Sita, K., Sehgal, A., Kumar, J., Kumar, S., Singh, S., Siddique, K. H., & Nayyar, H. (2017). Identification of high temperature tolerant lentil (*Lens culinaris* medik.) genotypes through leaf and pollen traits. *Frontier in Plant Science, 8*, 1-27.

Soleymani, F., & Taheri, H. (2015). Effects of gibberellic acid, methyl jasmonate and chitosan on antioxidant enzyme activity in peppermint (*Mentha piperita* L.). *International Journal of Advances in Chemical Engineering and Biological Sciences, 2*(2), 135-137.

Tonhati, R., Mello, S. C., Momesso, P., & Pedroso, R. M. (2020). L-proline alleviates heat stress of tomato plants grown under protected environment. *Scientia Horticulturae, 268*, 109370.

Vu, D. L., Gevaert, K., & De Smet, I. (2019). Feeling the heat: Searching for plant thermosensors. *Trends in Plant Science*, *24*(3), 210-219.

Wahid, A., & Shabbir, A. (2005). Induction of heat stress tolerance in barley seedlings by presowing seed treatment with glycine betaine. *Plant Growth Regulators*, *46*, 133-141.

Wehner, T. C., & Guner, N. (2004). Growth stage, flowering pattern, yield and harvest date prediction of four types of cucumber tested at 10 planting dates. Proc. xxvi IHC. Advances in vegetable breeding (Eds.) J. D McCreight and E. J. Ryder Acta. Horticulturae, 637, ISHS.

Xu, Y., Yuan, Y., Du, N., Wang, Y., Shu, S., Sun, J., & Guo, S. (2018). Proteomic analysis of heat stress resistance of cucumber leaves when grafted onto Momordica rootstock. *Horticulture Research*, *5*(1), 1-18.

Yoshida, Y., Umeno, A., & Shichiri, M. (2012). Lipid peroxidation biomarkers for evaluating oxidative stress and assessing antioxidant capacity *in vivo*. *Journal of Clinical Biochemistry and Nutrition*, *52*(1), 9-16.

Zhang, J., Li, D. M., Gao, Y., Yu, B., Xia, C. X., & Bai, J. G. (2012). Pretreatment with 5-aminolevulinic acid mitigates heat stress of cucumber leaves. *Biologia Plantarum*, *56*(4), 780-784.

Zhao, X., Nishimura, Y., Fukumoto, Y., & Li, J. (2011). Effect of high temperature on active oxygen species, senescence and photosynthetic properties in cucumber leaves. *Environmental and Experimental Botany*, *70*(2), 212-216.