



# Evaluation of physiological traits and flowering in *Cucumis sativus* L. by foliar application of chitosan at three sowing dates grown under hot environment

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## Abstract

Heat stress is a major issue in tropical and subtropical regions of the world where vegetable crops are grown. Utilizing genetic diversity, two tolerant (L3466 and Desi-cucumber) and two susceptible (Suyo Long and Poinsett) genotypes were grown under field conditions in three sowing times (15<sup>th</sup> March, 1<sup>st</sup> April, and 15<sup>th</sup> April). Chitosan (CHT) (200 ppm) was exogenously applied on foliage at 30 days' post sow (DPS) and then twice more in one-week intervals. The peak temperature recorded in May was 47.8°C, 48.0°C in June, and 46.1°C in July, respectively. There was a significant difference in means of sowing dates, the first sowing date had the highest leaf turgor potential. Both Suyo Long (0.106 MPa) and Poinsett (0.126 MPa) responded more efficiently to CHT application in the first sowing date compared with non-treated CHT plants. In the first sowing date, the heat-tolerant genotypes treated with CHT had lower leaf water loss; Desi-cucumber had the lowest water loss with a transpiration rate of 2.97 mmol m<sup>-2</sup> s<sup>-1</sup> followed by L3466, 3.07 mmol m<sup>-2</sup> s<sup>-1</sup>, respectively. During the third sowing date, non-treated Poinsett had the highest transpiration rate of 4.38 mmol m<sup>-2</sup> s<sup>-1</sup> followed by Suyo Long with 4.18 mmol m<sup>-2</sup> s<sup>-1</sup>. Heat-sensitive genotypes had higher transpiration rates, lost more water at high temperatures which led to wilting of plants in the 3<sup>rd</sup> sowing date. Similarly, Desi-cucumber (10.54) had the most female flowers, at par with L3466 (10.19) with CHT application during the first sowing date. So, Desi-cucumber (6.63) exhibited the highest number of fruits by L3466 (6.40) with CHT treated plants, while Suyo Long (5.78) and Poinsett (5.53) showed significantly low in the number regarding CHT treated plants. For cucumber production, CHT treatment proved helpful to increase the yield potential grown under hot environments. © 2021 Department of Agricultural Sciences, AIOU

**Keywords:** Chitosan, *Cucumis sativus*, Heat stress, Photosynthesis, Water potential

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## Introduction

Heat stress causes excessive water loss in horticultural crops due to improper metabolic activity resulting in inability to maintain water potential and water use efficiency. So, antitranspirants are necessary for plants to cope with the loss of water (Tonhati et al., 2020). High temperature increases the loss of water by increasing the temperature of the soil (Prasad et al., 2008). In general, heat stress injury weakens the cell membrane, cause osmotic stress, and production of reactive oxygen species (ROS) with various biochemical changes (Xu et al., 2021), these biochemical changes leads to complications in photosystem II (PS-II) (Goraya et al., 2017) and in response plants develop defensive mechanisms (Gommers, 2020) biosynthesis of stilbenes, an enzyme, was observed as a result of abiotic stress (Ogneva et al., 2021). Kesici et al. (2013) stated that high temperature deleteriously affects photosynthesis and respiration (Sun & Guo, 2016). Heat

stress causes chlorophyll degradation which leads to the generation of ROS which also injures the mitochondria and disrupts their functions (Ali et al., 2020a). These ROS injure the photosynthetic systems and photosystem II (PS-II) (Ivanov et al., 2017). Hot temperature stress increases the liquidity of the thylakoid membrane and alters the sunshine harvest of complicated of photosystem-II (Sun & Guo, 2016).

Heat tolerance has been shown to enhance economic yield (Wahid et al., 2007) by adopting defensive mechanisms through altering gene expression which leads to transcript accumulation, and finally, heat stress proteins (HSPs) are generated (Rasul et al., 2017; Liang et al., 2021). HSPs contribute to the stress tolerance of plants. HSPs try to alleviate the damage caused by temperature stress (Merret et al., 2017). Heat stress seriously affected plant growth (reduction of leaf areas, stem elongation, and the number of nodes), development, and flowering in cherry tomatoes (Nduwimana & Wei, 2017). Rykaczewska

(2013) revealed that resistant cultivars of potatoes can produce a high amount of HSPs as a defensive mechanism. Romaisa et al., (2015) proposed a study to evaluate the combined response of water shortage and elevated temperature on growth and quality of carrot and found a remarkable decrease in morphological/growth parameters and alteration in photosynthetic pigment. Helyes et al. (2015) noted similar findings in eggplant. Gisbert-Mullor et al. (2021) used grafting methods to alleviate heat stress in chili plants.

Under drought conditions, cucumber plant biomass (dry matter contents), percentage leaf relative water content (RWC), soil-plant analysis development (SPAD) value, and net photosynthetic rate (Pn) were enhanced, while superoxide radicals ( $O_2^-$ ), percentage of electrolyte leakage (EL), and malondialdehyde (MDA) content decreased by functions of vital antioxidant enzymes. It has been seen that heat-resistant cultivars have enhanced  $CO_2$  assimilation than heat susceptible cultivars (Goraya et al., 2017). Cucumber seedlings exposed to heat shock had substantially reduced photosynthesis, efficient photochemical reactions, enhanced stomatal conductance, and transpiration rate. It was demonstrated that cucumber probably has a complex physiological mechanism to fight instant heat shock and prevent the spread of downy mildew disease caused by *Pseudoperonospora cubensis* (Ding et al., 2016).

Exogenous coating of seeds with chitosan has been proved to be effective in improving the growth, quality as well as post-harvest life of fruit and vegetables (Mohamed et al., 2020; Shah & Hashmi, 2020; Cui et al., 2020). The highest okra fruit production was achieved at the early stages of growth with the application of 100 or 125 ppm chitosan (Mondal et al., 2012). Heat stress ultimately produces water deficiency due to increased transpiration (Giri et al., 2017). Chitosan has strong anti-transpiring properties which help to conserve water resources used in agriculture. Exogenously applied chitosan in pepper induced closure of stomata, resulting in decreased transpiration (Bittelli et al., 2001). In another experiment, celak, *Thymus daenensis*, was subjected to drought stress and subsequent chitosan application where chitosan was proven to stimulate osmotic adjustment through proline accumulation and reduction of lipid peroxidation which provides stability of cell membranes in leaves (Bistgani et al., 2017). Further, low and medium-molecular-weight chitosan polymers were found to be more effective at increasing yield in Freesia than high-molecular-weight chitosan (Salachna & Zawadzinska, 2014). A study was conducted in Northwest Iran where lentils (*Lens culinaris*), seeds were soaked in a chitosan solution; this resulted in enhanced yield compared to non-treated plants under semi-arid regions (Janmohammadi et al., 2014). *Triticum aestivum* seeds were positively impacted by chitosan seed coating treatment showing improved growth attributes e.g. emergence, the fresh mass of seedling, length of roots, and impact of antioxidants activity (SOD, POD, CAT) and chlorophyll level as compared to the control treatment. Under drought stress, chitosan seed coating protected the wheat seedlings from damage and improved growth and

yield up to 13.6% compared to the control treatment (Zeng & Luo, 2012).

Exogenously applied chitosan comparatively reduced transpiration in the bean (Iriti et al., 2009). Choudhary et al. (2017) revealed that nano-chitosan blended with zinc and copper not only improved the vigor of the plant but also defended against pathogen attacks. Previous studies revealed a decrease of MDA levels in strawberries due to nano-chitosan particles observed by Nguyen et al. (2020) as described by Ali et al. (2020a) in cucumber when production of enzymatic and non-enzymatic antioxidants for coping cucumber against heat stress by foliar application of chitosan.

## Materials and Methods

L3466 and Desi-cucumber (heat-tolerant genotypes) along with Suyo Long and Poinsett (heat-sensitive genotypes) (Ali et al., 2020a) were directly grown under field conditions. The first, second, and third sowing was carried out during 15<sup>th</sup> March, 1<sup>st</sup> April, and 15<sup>th</sup> April, respectively. The study was conducted with four replications. An optimal dose of chitosan (200 ppm) (Ali et al., 2020b) was applied as a foliar spray after 30 DPS and again twice in one-week intervals. Chitosan was mixed with water by dissolving 0.1 molar  $C_2H_4O_2$  (at 25°C for 24 Hrs.) to improve solubility in water. Initially, chitosan foliar application was applied at 30 DPS, while the second application was performed at 37 DPS and the third application was conducted at 44 DPS. The detail of the daily mean temperature is given in Table 1. Two weeks after the chitosan treatment application, water relation and physiological attributes were observed.

### Measurement of water relations

#### Leaf water potential ( $\Psi_w$ ) (-MPa)

Fully expanded leaves were selected randomly. The tip of the growth point of third to fourth young leaves was cut with a razor. The initial reading of water potential was recorded with a pressure chamber (M1000, PMS Instrument Company, Albany, USA) before sunrise. Then, leaves were kept in the gasket of the pressure chamber (M615, USA) to measure leaf water potential.

#### Leaf osmotic potential ( $\Psi\pi$ ) (-MPa)

The leaf samples collected for water potential were kept in separate plastic bags and frozen (-20°C) for seven days before being thawed at room temperature for cell sap extraction using a disposable syringe. The first osmotic chamber was standardized with distilled water. Then ten  $\mu$ L of extracted sap was placed on an osmometer (Vapro-5520, Wescor Inc. USA) with the aid of a plastic syringe to measure osmotic potential ( $\Psi\pi$ ) in leaves.

**Table 1** Meteorological condition for Faisalabad, Pakistan during 2016

Month	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Record high °C	26.6	30.8	37	44	47.8	48	46.1	42	41.1	40	36.1	29.2
(°F)	79.9	87.4	99	111	117.5	118	115	108	106	104	97	84.6
Average high °C	19.4	22.2	27.4	34.2	39.7	41	37.7	36.5	36.6	33.9	28.2	22.1
(°F)	66.9	72	81.3	93.6	103.5	105.8	99.9	97.7	97.9	93	82.8	71.8
Average low °C	4.8	7.6	12.6	18.3	24.1	27.6	27.9	27.2	24.5	17.7	10.4	6.1
(°F)	40.6	45.7	54.7	64.9	75.4	81.7	82.2	81	76.1	63.9	50.7	43
Record low °C	-4	-2	1	7	13	17	19	18.6	15.6	9	2	-1.3
(°F)	25	28	34	45	55	63	66	65.5	60.1	48	36	29.7
Average precipitation mm	16	18	23	14	9	29	96	97	20	5	2	8
(inches)	0.63	0.71	0.91	0.55	0.35	1.14	3.78	3.82	0.79	0.2	0.08	0.31

(Meteorology Cell of Agronomy Department, University of Agriculture, Faisalabad, Pakistan, 2016)

### Leaf turgor potential ( $\Psi_p$ ) (MPa)

When water potential ( $\Psi_w$ ) and osmotic potential ( $\Psi_\pi$ ) potential of selected four genotypes of cucumber was determined leaf turgor potential was measured by the equation:

$$\Psi_p = \Psi_w - \Psi_\pi$$

### Relative water content (RWC) (%)

Plant leaves of selected cucumber genotypes were washed with distilled water and then blotted with tissue paper. After measuring the fresh mass of leaves (FM) the leaves were dipped in distilled water for twenty-four hours and their turgid mass (TM) was measured. After that, samples were dried in an oven at 72°C and dry mass (DM) was calculated by using an electrical weighing balance (Bosch AE-160, Germany). Finally, using a technique reported by Barrs and Weatherly, (1962) the average leaf RWC in selected genotypes was measured using the following formula:

$$RWC (\%) = \frac{FM - DM}{TM - DM} \times 100$$

### Physiological traits

Different physiological traits e.g. photosynthetic rate, transpiration rate, stomatal conductance, and sub-stomatal CO<sub>2</sub> were measured from healthy, young, and fully developed leaves. Data of the above mentioned physiological traits were recorded with a portable Infra-Red Gas Analyzer (IRGA) (LCi- SD, ADC Bio-scientific UK) from 10.00 a.m. to 12.00 p.m. IRGA chamber was Provided with atmospheric pressure (ATM) of 99.9

kilopascal, the molar flow of air per 403.3 mmol m<sup>-2</sup> s<sup>-1</sup>, PAR up to 1711 pmol m<sup>-2</sup> s<sup>-1</sup>, VP of 6.0 to 8.9 millibar, CO<sub>2</sub> 352 pmol mol<sup>-1</sup>, and ATM was around 22.4 to 27.9°C.

### Water use efficiency (WUE) (pmol CO<sub>2</sub> mmol<sup>-1</sup> H<sub>2</sub>O)

It was calculated as the ratio between photosynthetic (A) and transpiration rate (E) as given below:

$$WUE = \frac{A}{E}$$

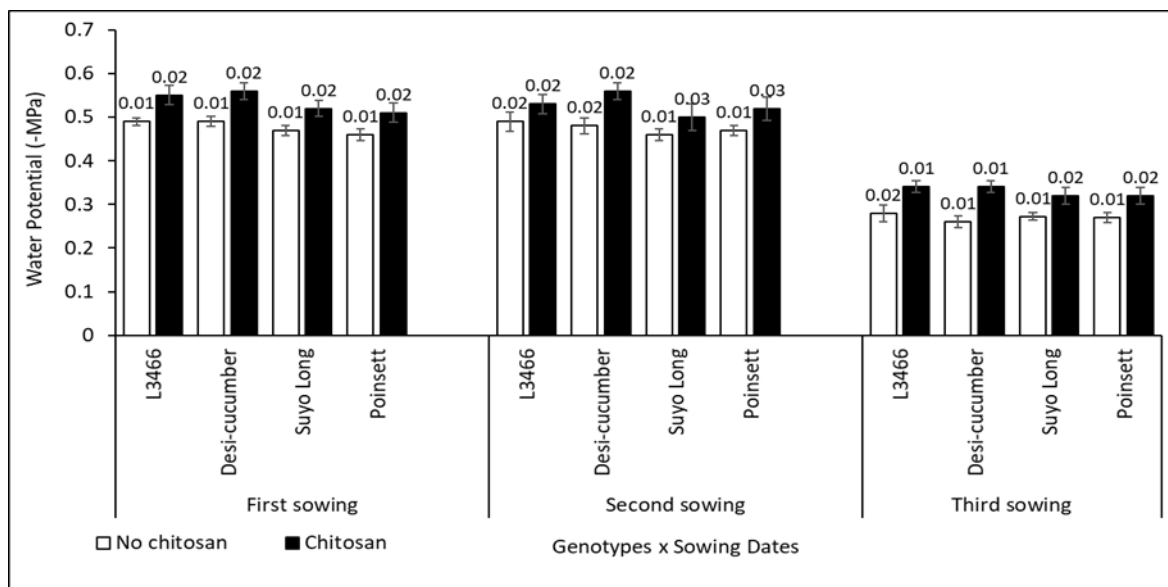
### Statistical analysis

The experiment was laid out under Randomized Complete Block Design (RCBD) with three-factor factorial and means were compared by using Tukey Honestly significant different test (HSD) at  $P \leq 0.05$  (Steel et al., 1997).

## Results

### Leaf water potential

With CHT application, all genotypes had more water potential than without application of CHT in all sowing times. During the first sowing date, Desi-cucumber (0.550 -MPa) had the highest leaf water potential with the application of chitosan. While Suvo Long (0.272 -MPa) and Poinsett (0.272 -MPa) also exhibited the lowest transpiration rates with chitosan (CHT) application in the third sowing date. The water potential of Desi-cucumber was at par with L3466. Suvo Long and Poinsett showed more responses to the CHT application (Fig. 1).

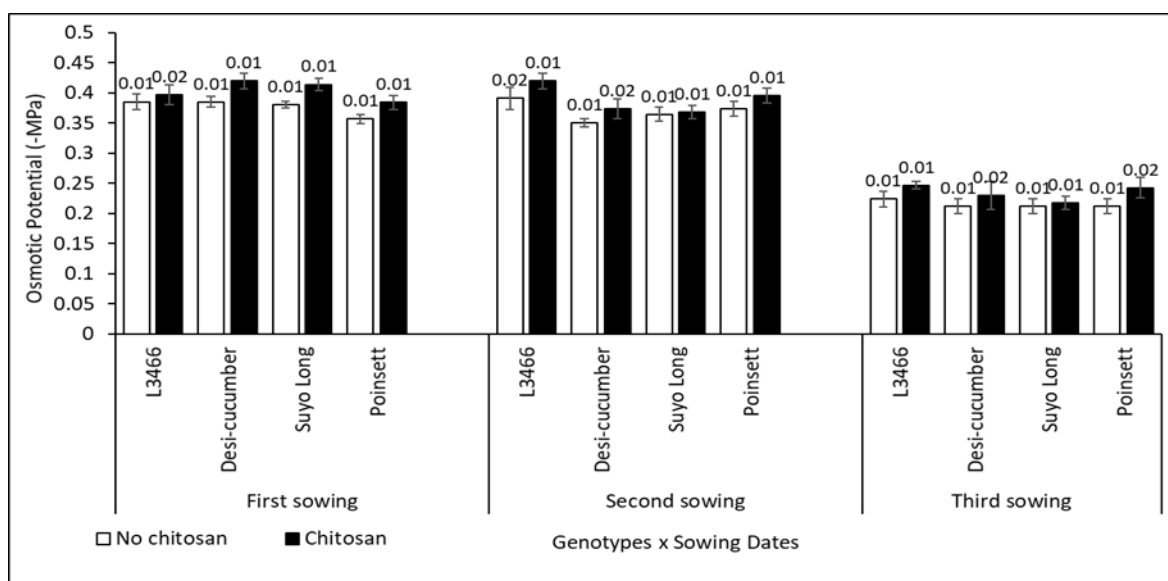


**Fig. 1** Influence of chitosan on leaf water potential of selected cucumber genotypes grown under different sowing dates

**Leaf osmotic potential**

L3466 had higher leaf osmotic potential was comparatively similar to Desi-cucumber, irrespective of sowing dates. A significant variation was revealed between genotype and sowing time interaction. The highest level of osmotic potential was observed in L3466 (0.420 -MPa) during the 1<sup>st</sup> April sowing followed by at 15<sup>th</sup> March (0.397 -MPa)

and at 15<sup>th</sup> April (0.354 -MPa), respectively when CHT was applied. The lowest level of osmotic potential was seen in Suyo Long (0.212 -MPa) and Poinsett (0.212 -MPa) at 15<sup>th</sup> April without CHT application. However, both Suyo Long (0.218 -MPa) and Poinsett (0.424 -MPa) exhibited an increased leaf osmotic potential to CHT application (Fig. 2).

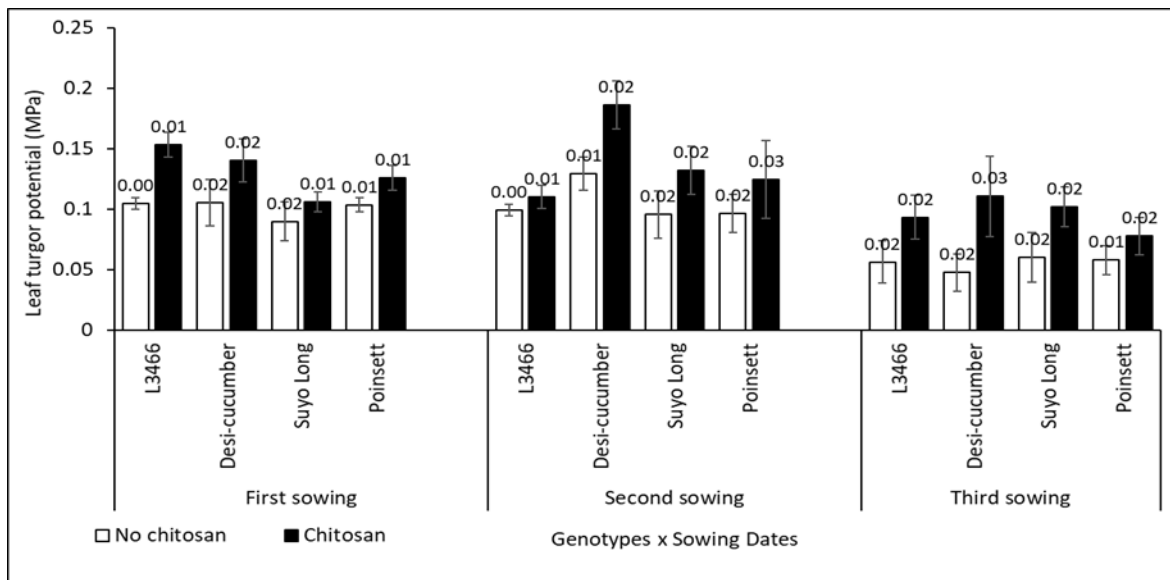


**Fig. 2** Influence of chitosan on osmotic potential of selected cucumber genotypes grown under different sowing dates

**Leaf turgor potential**

With CHT application, L3466 revealed the highest (0.153 MPa) leaf turgor potential, which was not significantly different when compared with Desi-cucumber (0.140 MPa), irrespective of sowing dates. There was a significant

difference in means of sowing dates, the first sowing date (15<sup>th</sup> March) had the highest leaf turgor potential. Both Suyo Long (0.106 MPa) and Poinsett (0.126 MPa) responded more efficiently to CHT application in the first sowing date compared with no CHT application (Fig. 3).

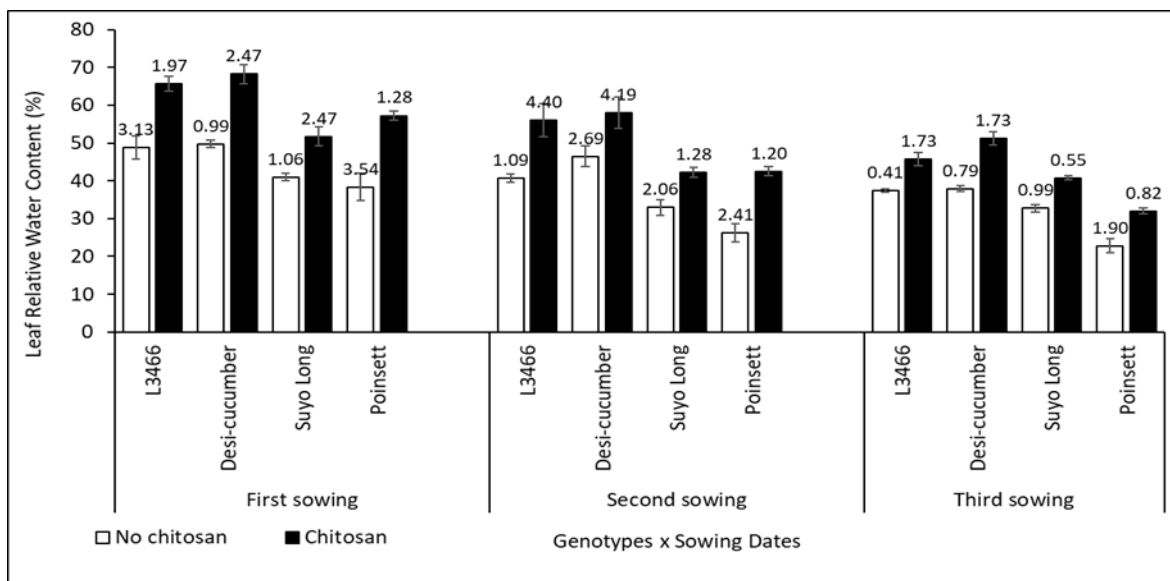


**Fig. 3** Influence of chitosan on leaf turgor potential of selected cucumber genotypes grown under different sowing dates

**Leaf relative water content**

It was observed that Desi-cucumber (68.3%), followed by L3466 (65.8%), had the maximum relative water contents at 15<sup>th</sup> March sowing with CHT treatment, while the

lowest value in Suyo Long (22.8%), followed by Suyo Lo Poinsett (32.8%), when CHT was not applied in the third sowing date (15<sup>th</sup> April). All genotypes L3466, Desi-cucumber, Suyo Long, and Poinsett had significantly more relative water content with CHT application (Fig. 4).

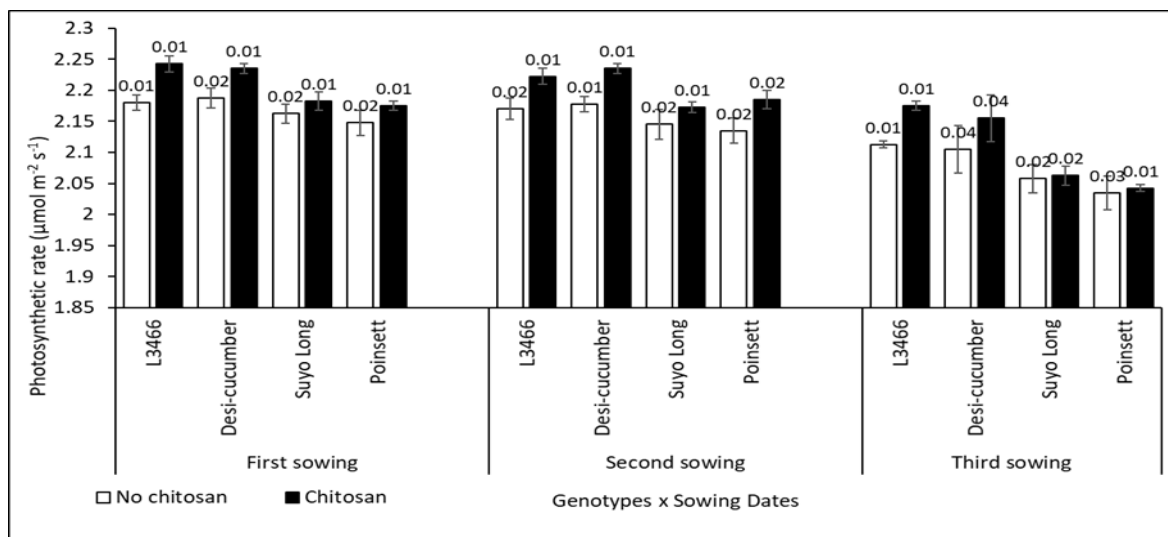


**Fig. 4** Influence of chitosan on relative water content of selected cucumber genotypes grown under different sowing dates

**Photosynthetic rate**

In the first sowing date, L3466 ( $2.24 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) and Desi-cucumber ( $2.24 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) revealed the highest rate of photosynthesis with CHT application. Both Suyo Long ( $2.18 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) and Poinsett ( $2.18 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) revealed efficient response to CHT as compared to no CHT

application in the first sowing date. L3466 and Desi-cucumber were more efficient in terms of photosynthetic rate than Suyo long and Poinsett. Suyo Long and Poinsett had more photosynthetic rate, however, genotypes did not differ in the photosynthetic rate at the first and the second sowing date but it was substantially decreased at the third sowing date (Fig. 5).

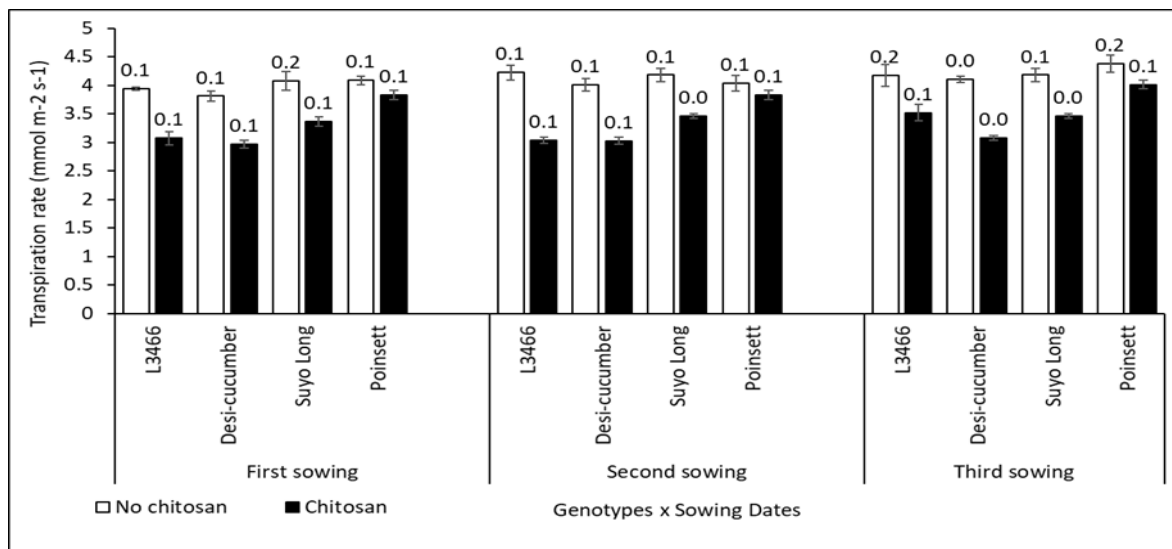


**Fig. 5** Influence of chitosan on photosynthetic rate of selected cucumber genotypes grown under different sowing dates

**Transpiration rate**

Desi-cucumber (3.07 mmol m<sup>-2</sup> s<sup>-1</sup>) had the lowest transpiration rate and was not significantly different compared to L3466 (2.97 mmol m<sup>-2</sup> s<sup>-1</sup>) in the first sowing date. Suoyo Long (3.37 mmol m<sup>-2</sup> s<sup>-1</sup>) and Poinsett (3.83 mmol m<sup>-2</sup> s<sup>-1</sup>) also had reduced transpiration rates with

CHT application on the first sowing date. Poinsett (4.17 mmol m<sup>-2</sup> s<sup>-1</sup>) and Suoyo Long (4.15 mmol m<sup>-2</sup> s<sup>-1</sup>) gave the highest level with no CHT application in the third sowing date. With an increase in the interval of sowing time transpiration rate of plants was enhanced irrespective of the genotypes (Fig. 6).

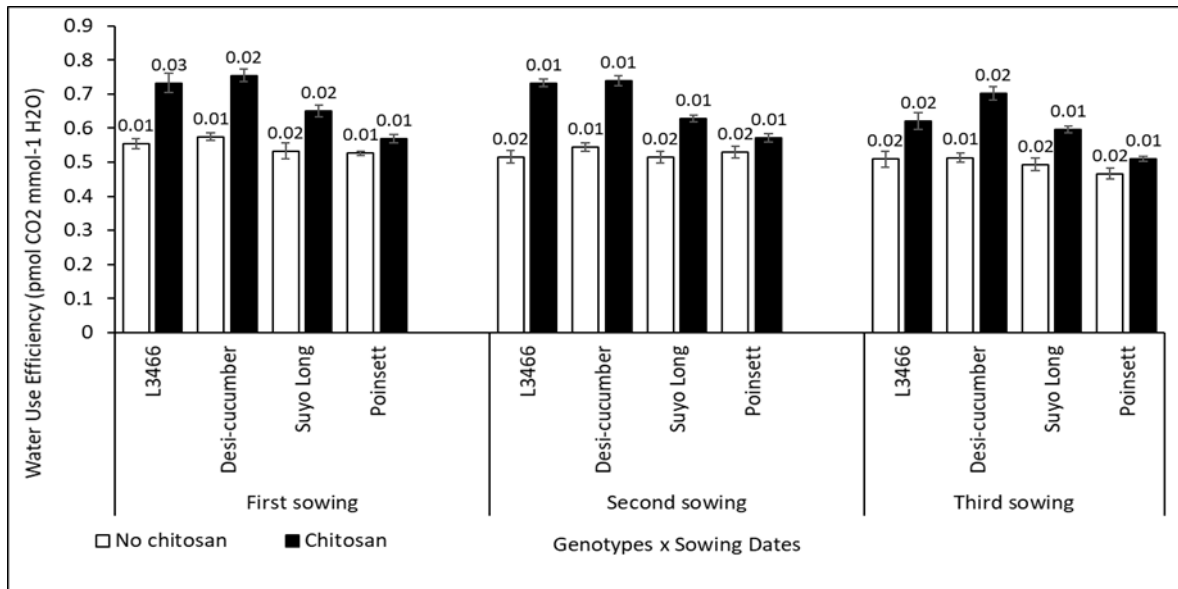


**Fig. 6** Influence of chitosan on transpiration rate of selected cucumber genotypes grown under different sowing dates

**Water use efficiency**

Cucumber genotype L3466 (0.73 pmol CO<sub>2</sub> mmol<sup>-1</sup> H<sub>2</sub>O) and Desi-cucumber (0.75 pmol CO<sub>2</sub> mmol<sup>-1</sup> H<sub>2</sub>O) revealed the highest water use efficiency (WUE) in the first sowing date when CHT was applied. Suoyo Long (0.49 pmol CO<sub>2</sub> mmol<sup>-1</sup> H<sub>2</sub>O) and Poinsett (0.47 pmol CO<sub>2</sub> mmol<sup>-1</sup> H<sub>2</sub>O) revealed the least efficiency in the third sowing date when no CHT foliar spray was done. L3466 (0.62 pmol CO<sub>2</sub> mmol<sup>-1</sup> H<sub>2</sub>O) and Desi-cucumber (0.70 pmol CO<sub>2</sub> mmol<sup>-1</sup> H<sub>2</sub>O) had substantially more WUE than Suoyo long (0.60 pmol CO<sub>2</sub> mmol<sup>-1</sup> H<sub>2</sub>O) and Poinsett (0.51 pmol CO<sub>2</sub>

mmol<sup>-1</sup> H<sub>2</sub>O) in the third sowing date without CHT application. Similarly, Suoyo Long and Poinsett showed an increased WUE with the CHT application. Susceptible genotypes were more noticeable under harsh environments of heat in the third sowing date, however, without chitosan treatment Suoyo Long and Poinsett could have died when temperature exceeded 47 °C. However, the genotypes did not differ in WUE for the first and the second sowing date, but it was substantially decreased at the third sowing date (Fig. 7).

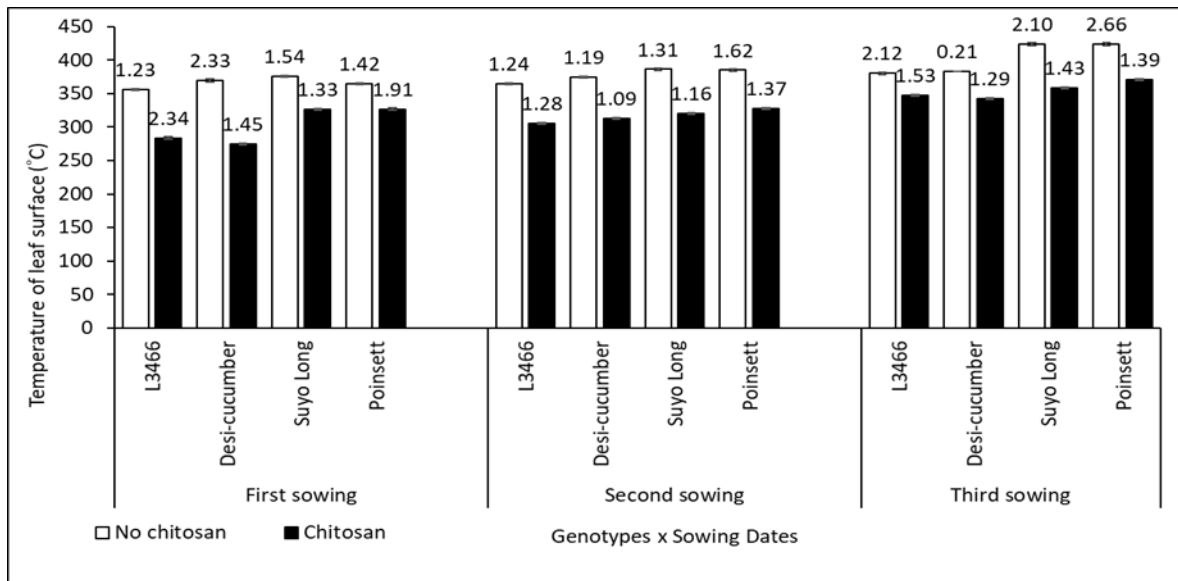


**Fig. 7** Influence of chitosan on water use efficiency of selected cucumber genotypes grown under different sowing dates

**Temperature of leaf surface**

During the first sowing date, Desi-cucumber (33.4 °C) had the lowest temperature of the leaf surface at par with L3466 (33.4 °C) during CHT application. Both heat-tolerant genotypes were not significantly different from each other.

Suyo Long (34.7 °C) and Poinsett (34.6 °C) also exhibited decreased temperature of leaf surface with CHT treatment in the first sowing time. While Suyo Long (34.7 °C) at par with Poinsett (34.7 °C) without CHT application in the third sowing date (Fig. 8).

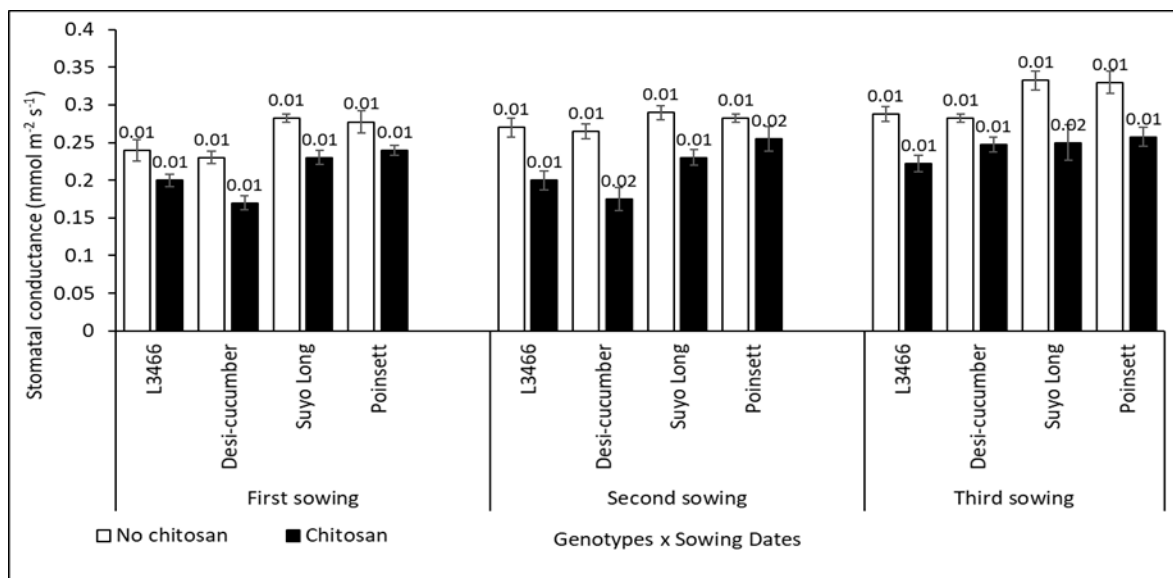


**Fig. 8** Influence of chitosan on temperature of leaf of selected cucumber genotypes grown under different sowing dates

**Stomatal conductance**

Desi-cucumber (0.17 mmol m<sup>-2</sup> s<sup>-1</sup>) had the minimum level of stomatal conductance followed by L3466 (0.20 mmol m<sup>-2</sup> s<sup>-1</sup>), in the first sowing date with CHT application. Suyo Long (0.30 mmol m<sup>-2</sup> s<sup>-1</sup>) at par with Poinsett (0.29 mmol

m<sup>-2</sup> s<sup>-1</sup>) without CHT application in third sowing date. All genotypes enhanced stomatal conductance in the third sowing date, while minimum stomatal conductance was seen in the first sowing date. The combined data analysis of genotypes and CHT did not show any significant interaction between CHT and genotypes (Fig. 9).

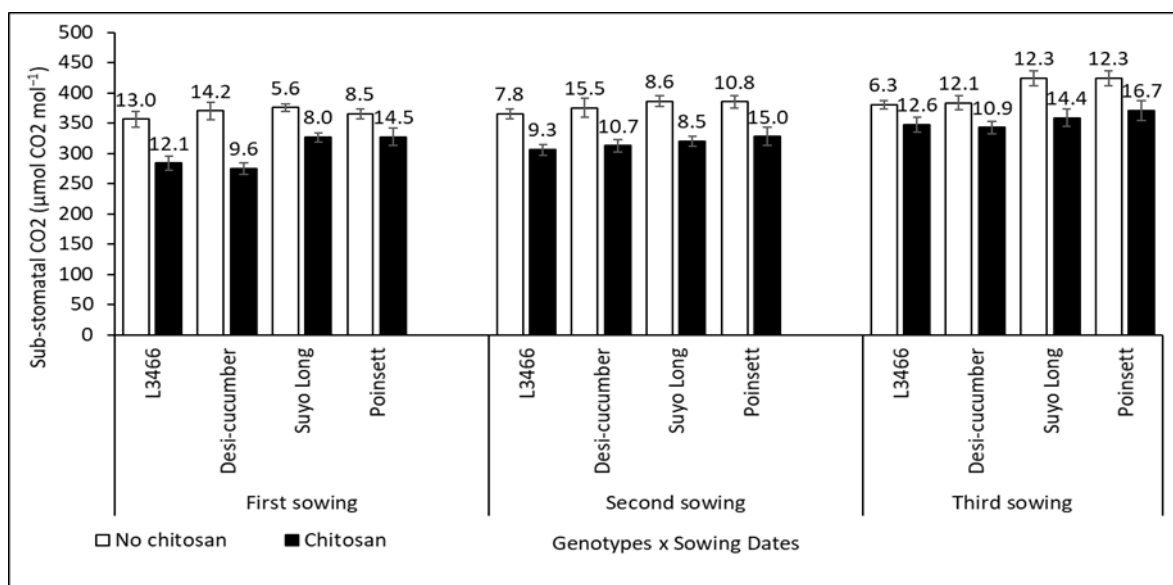


**Fig. 9** Influence of chitosan on stomatal conductance of selected cucumber genotypes grown under different sowing dates

**Sub-stomatal CO<sub>2</sub>**

It was revealed during the first sowing date that Desi-cucumber (275 μmol CO<sub>2</sub> mol<sup>-1</sup>) had the lowest sub-stomatal CO<sub>2</sub> (SSC) followed by L3466 (284 μmol CO<sub>2</sub> mol<sup>-1</sup>) with CHT application. Highest SSC was observed

in Suyo Long (424 μmol CO<sub>2</sub> mol<sup>-1</sup>) and Poinsett (424 μmol CO<sub>2</sub> mol<sup>-1</sup>) in the third sowing when no CHT was applied. The third showing date (15<sup>th</sup> April) revealed the highest sub-stomatal CO<sub>2</sub> as compared with the first (15<sup>th</sup> March) and the second sowing date (1<sup>st</sup> April) (Fig. 10).



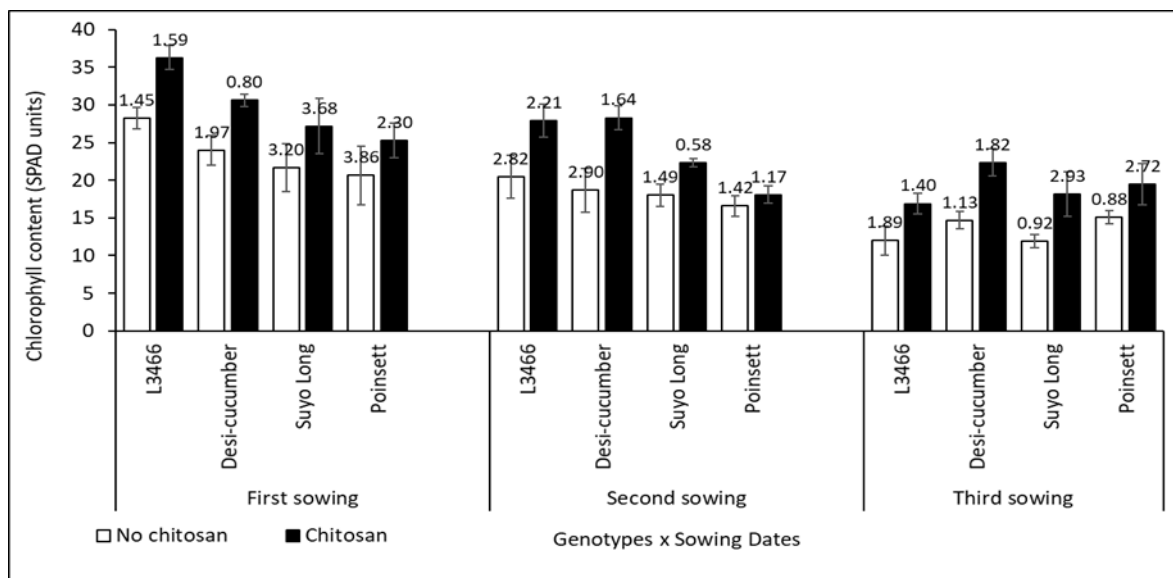
**Fig. 10** Influence of chitosan on sub-stomatal CO<sub>2</sub> of selected cucumber genotypes grown under different sowing dates

**Chlorophyll contents**

L3466 (36.3 SPAD units) had the highest chlorophyll contents followed by Desi-cucumber (30.6 SPAD units), during the first sowing date with CHT application. Suyo Long (11.9 SPAD units) and Poinsett (15.1 SPAD units) had lowed chlorophyll contents but when CHT was applied

Suyo Long (18.2 SPAD units) and Poinsett (19.5 SPAD units) also exhibited slightly higher chlorophyll content during 1<sup>st</sup> sowing time. However, L3466 (16.9 SPAD units) showed lower chlorophyll contents than Desi-cucumber (22.4 SPAD units) with CHT-treated plants in the third sowing time. Chlorophyll content decreased as sowing time increased (Fig. 11).



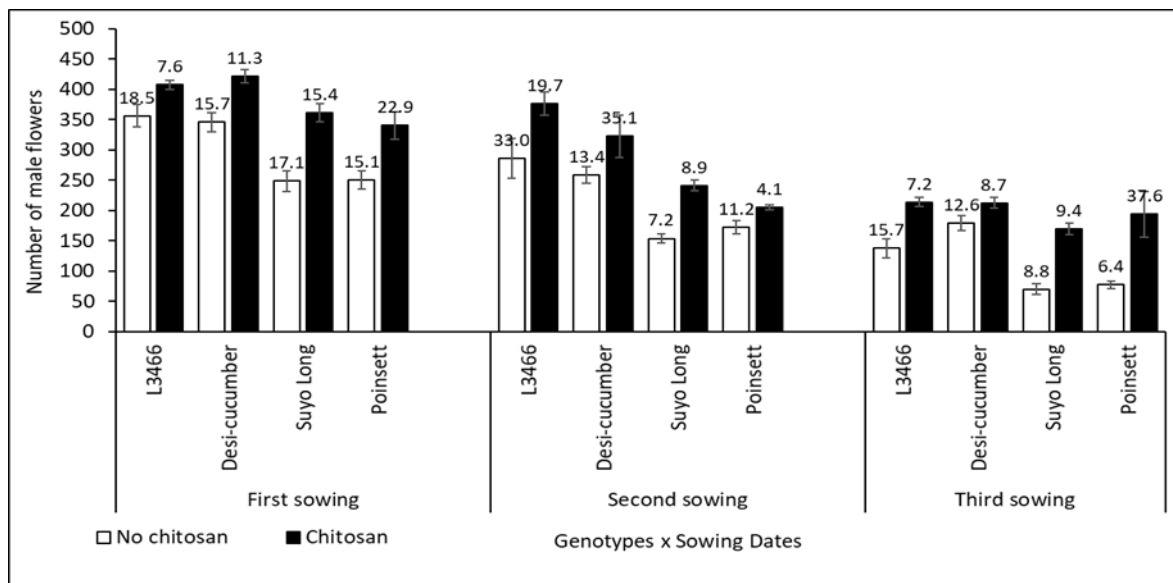


**Fig. 11** Influence of chitosan on chlorophyll contents of selected cucumber genotypes grown under different sowing dates

**Number of male flowers**

With the application of CHT, Desi-cucumber (422) had the most number of male flowers followed by L3466 (408). The least number of male flowers were exhibited by Suyo Long (156), without CHT application. L3466 (214) and, Desi-cucumber (213) were not significantly different in the

third sowing time when CHT was sprayed of leaves. But CHT treated plants of Desi-cucumber (213) had a remarkable difference with no CHT treatment (138). It was observed that later sowing dates reduced the number of male flowers (Fig. 12).

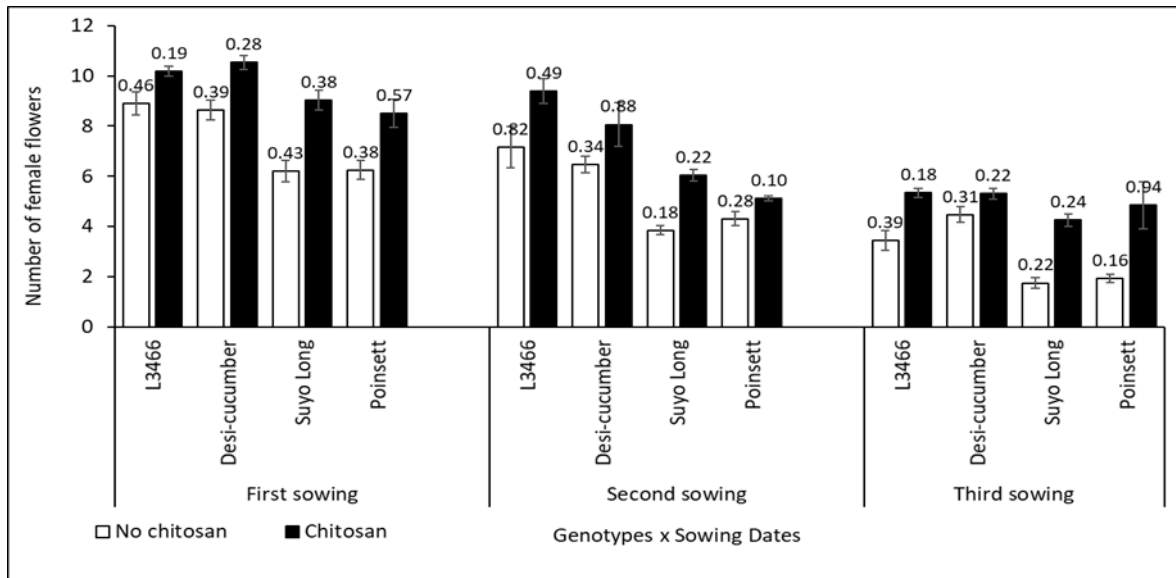


**Fig. 12** Influence of chitosan on number of male flowers of selected cucumber genotypes grown under different sowing dates

**Number of female flowers**

Desi-cucumber (10.54) had the most female flowers, at par with L3466 (10.19) with CHT application during the first sowing date. However, without CHT application Desi-

cucumber (8.64) and L3466 (8.91) had a relatively low number of female flowers. It was observed that Suyo Long (1.75), followed by Poinsett (1.93) provided a minimum number during the last sowing time (Fig. 13).

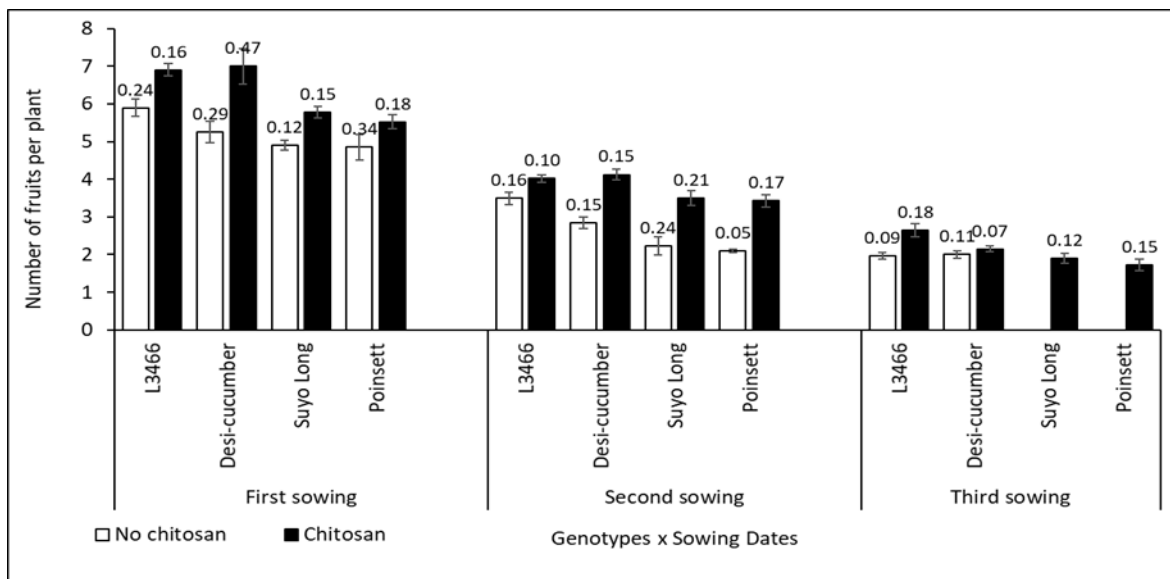


**Fig. 13** Influence of chitosan on number of female flowers of selected cucumber genotypes grown under different sowing dates

**Number of fruits per plant**

During 1<sup>st</sup> sowing time, it was depicted that Desi-cucumber (6.63) exhibited the highest number of fruits by L3466 (6.40) with CHT treated plants, while Suyo Long (5.78) and Poinsett (5.53) showed significantly low in the number regarding CHT treated plants. However, in the 3<sup>rd</sup> sowing time, Desi-cucumber (3.15) exhibited the highest number

of fruits by L3466 (3.23), while Suyo Long (2.60) and Poinsett (2.50) also revealed minimum value with CHT application in the third sowing date. Heat sensitive genotypes, Suyo Long and Poinsett could not survive at the reproductive stage and were unable to produce fruits under extreme conditions of heat (temperature above 47°C) in the last sowing time (15<sup>th</sup> March) (Fig. 14).



**Fig. 14** Influence of chitosan on number of fruit per plant of selected cucumber genotypes grown under different sowing dates

**Discussion**

During the present research, photosynthetic rates differed significantly among the genotypes under the hot environment. The photosynthetic rate was normally maintained until 40 °C, while a significant reduction was seen in all genotypes when environment ambient

temperature was increased. This enormous variation revealed that the photosynthetic process is affected by heat stress (Goraya et al., 2017). Photosynthesis can be entirely suppressed during heat stress due to three key sites involving photosystem II owing to the production of activation of reactive oxygen species (Nishiyama et al., 2005; Nishiyama et al., 2006). It was observed that hot

environmental conditions inhibit photosynthetic CO<sub>2</sub> assimilation rates due to alterations in the electron transport chain and decrease of the RuBisCO activation state which might be due to a reduction in stomatal conductance (Salvucci & Crafts-Brandner, 2004). It was observed that heat-tolerant varieties have a higher CO<sub>2</sub> assimilation rate as compared to heat-susceptible varieties. Partial closure of stomata enhanced resistance to environmental CO<sub>2</sub> diffusion to the chloroplasts (Camejo et al., 2005).

At 40 °C, tolerant and susceptible genotypes were observed to be significantly different in the concentration of sub-stomatal CO<sub>2</sub>. Such variation in sub-stomatal CO<sub>2</sub> was observed by Shaheen et al. (2016) which showed slight inhibition of the photosynthetic apparatus depicted the vast transformations in different heat tolerant and sensitive tomato genotypes. Equivalent results were observed in the present study when a significant difference was observed among cucumber genotypes, it clarifies that extra CO<sub>2</sub> is accumulated inside leaves during high-temperature stress, and thereby enhancing sub-stomatal CO<sub>2</sub>. This can produce reduced rates in respiration owing to a decrease in photosynthesis at a CO<sub>2</sub> compensation point (when CO<sub>2</sub> generated by respiration is totally utilized by photosynthesis).

The temperature of the leaf surface plays a vital role in assessing the heat tolerance ability of a genotype because each genotype maintained normal metabolic processes necessary for survival. It was revealed that heat-sensitive genotypes had higher leaf surface temperatures than heat-tolerant genotypes at the same temperature. Previous studies indicated the fact that heat susceptible genotypes had hotter leaf surfaces than heat-tolerant genotypes. These variations among genotypes are directly related to transpiration rate. It was seen that cell membrane thermostability, leaf temperature, and pollen grain viability, production, germination, size, moisture contents, and germ tube length were found to be major indicators for heat stress (Naveed et al., 2014).

Heat susceptible genotypes revealed a high transpiration rate when exposed to 40°C compared with heat-tolerant genotypes. High temperature produces high transpiration rates because water molecules gain energy to escape from the leaf surface. Raising temperature will increase water loss and lead to organ dehydration (Mazorra et al., 2002) which becomes a cause for osmotic adjustment of tissues. Osmotic adjustment due to stress might lead to a decline in hydraulic conductance in roots similarly decreasing ionic translocation, water, organic solutes, inhibiting photosynthesis, enhancing evapotranspiration, and reducing leaf osmotic potential. Plants respond to heat stress by adjustment of stomatal opening and closing to minimize water loss (Wahid et al., 2007). Extended periods of elevated temperature cause water loss continuously resulting in physiological drought; it is a point where transpiration surpasses the rate of water uptake from roots (Bittelli et al., 2001). In our study, it was demonstrated that high transpiration rates result in lower water use efficiency in a specific genotype. A substantial alteration was observed among various cucumber genotypes. Heat tolerant genotypes had higher water use

efficiency than heat susceptible genotypes. Evaluation of water use efficiency revealed heat tolerance was directly related to biomass production in plants. Further, CHT foliar application improved the heat tolerance capacity which is supported by the findings of Wahid et al. (2012). Chlorophyll biosynthesis is highly influenced by heat stress. Porphyrin biosynthesis is required for proper chlorophyll function during the seedling stage. In one experiment, 60% chlorophyll biosynthesis of cucumber cv. Poinsett seedlings were inhibited whereas about 70% of protochlorophyllide synthesis was inhibited during heat stress (42 °C). Roughly 46% more protochlorophyllide oxidoreductase was produced in heat-stressed seedlings compared to seedlings grown under normal temperature conditions (Tewari & Tripathy, 1998). CHT improved physiological traits which were also identified by Sharif et al. (2018) and despite its ability to produce resistance in plants against abiotic stress, it has proven environment friendly (Malerba & Cerana, 2020).

Current findings revealed a decrease in photosynthetic activity in sensitive genotypes might be due to deficiency of available water, which can be attributed to efficient photosynthetic apparatus function in heat-tolerant genotypes, similar to previous researches of Camejo et al. (2005).

Chitosan plays a vital role as an elicitor for mitigation of abiotic stress through the activation of signaling pathways i.e. NO, H<sub>2</sub>O<sub>2</sub>, and transduction pathways (Hidangmayum et al., 2019). The induction of the jasmonic acid pathway enhanced water use efficiency similarly to abscisic acid (ABA) (De-Ollas et al., 2015), due to the fact that stomatal closure increased levels of ABA, reducing loss of water by lowering the transpiration rate. CHT treatment was previously reported to stimulate physiological safekeeping of photosynthetic apparatus along with regulation of stomata via ABA synthesis. Production of secondary metabolites i.e. organic acids, sugars, amino acids was proven to maintain turgor under stress conditions (Hidangmayum et al., 2019). Comparable results were obtained for the activation of metabolites under salt stress by the application of CHT (Geng et al., 2010).

Current findings of 20-25 percent yield enhancement by the application of CHT regardless of the genotypes and sowing times. Janmohammad et al., (2014) demonstrated the ability of CHT to improve the growth and yield in lentils plants grown under drought conditions. These results were strengthened by Shehata et al. (2012), who stated that foliar application of yeast along with CHT potentially enhanced stem, foliage attributes alongside yield, and quality of cucumber.

## Conclusion

Results showed that CHT application (200 ppm) increased heat tolerance in cucumber by maintaining osmotic balance and cell turgor pressure. Physiologically heat-tolerant genotypes (L3466 and Desi-cucumber) were able to maintain water balance better when treated with CHT than susceptible genotypes (Suyo Long and Poinsett) which had a high loss of water. It was further noted that

CHT application induced heat resistance to a certain level of stress and was comparatively less effective under extremely high temperatures.

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