

## Sodium silicate mediated response of antioxidative defense system in *Lycopersicon esculentum* mill. under water stress

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### Abstract

The present study was designed to study the effect of water stress on *Lycopersicon esculentum* Mill. and role of sodium silicate in the protection of tomato plants under water deficit condition.

Different biochemical parameters such as photosynthetic pigments, protein, sugar, MDA content, proline, nitrate reductase activity and activities of antioxidant enzymes (SOD, CAT, APX and POX) were examined in tomato leaves at 40 and 60 DAS by the standard methods. The lycopene and  $\beta$ -carotene contents in tomato fruits were also analyzed at 60, 65 and 70 DAS.

Water stress significantly decreased relative water content (RWC), pigment content, sugar and protein contents in tomato leaves at 60 DAS but the accumulation of proline was stimulated in tomato leaves under water deficit condition. The activities of antioxidant enzymes such as SOD, CAT, APX and POX were significantly increased under (3d and 6d) water stress condition at 60 DAS.

This study offers first hand information on the water stress-induced oxidative stress in *Lycopersicon esculentum* and development of antioxidative defense system against drought. The results obtained clearly indicated the positive impact of sodium silicate in protection of tomato plants under water deficit condition.

**Keywords:** Anti oxidative defense system, *Lycopersicon esculentum*, Reactive oxygen species, Water stress.

### Introduction

Water stress is an important threat to plant growth and sustainable agriculture worldwide [1]. It has been estimated that drought severely reduces the yield and productivity of food crops worldwide up to 70% [2]. Water stress negatively influences crop growth and development through changes in various physiological and biochemical processes that ultimately decreases crop yield [3]. Water stress leads to oxidative stress in the plants due to stomatal closure which causes significant reduction in photosynthetic electron transport chain [4] and may produce reactive oxygen species (ROS). Excessive formation of reactive oxygen species (ROS) can damage plants by oxidizing their biomolecules [5]. Reactive oxygen species (ROS) such as superoxide anion ( $O_2^-$ ), hydrogen peroxide ( $H_2O_2$ ) and hydroxyl radical ( $OH^-$ ) are highly reactive which can directly attack on pigments, membrane lipids, proteins, nucleic acids and increases membrane leakage which may lead to cell death [6]. The degree of damage by ROS depends on the balance between the production of ROS and its removal by the antioxidant scavenging mechanism [7]. The higher plants

possess very efficient enzymatic and non-enzymatic anti oxidative defense mechanism that allow the scavenging of ROS and protection of cellular components from oxidative damage [8]. The antioxidant system plays a critical role in neutralizing the free radicals which may affect the cellular stability. The enzymatic antioxidants (such as superoxide dismutase, SOD; catalase, CAT; ascorbate peroxidase, APX) and non-enzymatic antioxidants (proline, lycopene and beta-carotene) are the main components of antioxidant defense system [9]. The enzymes such as superoxide dismutase (SOD, EC 1.15.1.1), is responsible for dismutation of  $O_2^-$  into  $H_2O_2$  and  $O_2$ , and CAT scavenge  $H_2O_2$  into  $H_2O$  and  $O_2$  [10]. According to Mittler [6], the capability of plant tissues to cope with water stress might be related to their strength to scavenge ROS by raising the activities of the antioxidant enzymes during water loss. *Lycopersicon esculentum* Mill. (tomato; family: Solanaceae) commonly known as The Poor Man's Apple, is one of the chief vegetable crops in India. Tomatoes are consumed in a number of ways including sauce, soup and fresh as salad [11]. Tomato are excellent source of antioxidants, fiber, carbohydrates, amino acids, minerals and vitamins [12]. The antioxidants present in tomato fruits are mainly lycopene and  $\beta$ -carotene which has been found defensive against cancer, pancreatic tumor and cardiovascular

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diseases [13]. Lycopene, the main carotenoid in tomato, is known as an important natural antioxidant with anti-carcinogenic properties [14]. Various researches showed that lycopene protects the skin from ultra-violet (UV) rays and offers defense against skin cancer [15].  $\beta$ -carotene acts as immunity booster and antioxidant and it plays significant role in various vital activities such as cell communication promotion, cancer prevention and eyesight improvement.

Silicon is recognized as quasi-essential element for plants because its deficiency results in various dysfunctions with respect to plant growth and proliferation [16]. Silicon acts as plant protectant, plays a pivotal role in enhancing the plants growth and productivity especially in stress condition [17]. The ameliorative role of silicon to adverse effects of drought has been reported in different crops such as rice [18], sugarcane [19], wheat [20], sorghum [21] and soybean [22]. A critical perusal of the literature revealed the intimate relationship between enhanced antioxidant enzyme activities and increase in resistance to environmental stresses as observed in several plant species such as rice [23], sugar beet [24] and wheat [25]. The CAT and SOD activities were increased in *Helianthus annuus* [26] and *Brassica napus* [27] under water deficit condition. However, no effort seems to have been made to study the protective role of sodium silicate in the development of antioxidative defense system in *Lycopersicon esculentum* Mill. which help in scavenging the lethal ROS under water stress condition. This study may explore the possible mechanism for sodium silicate mediated water stress tolerance and it may help in development of future strategies for the development of crop plants in drought prone areas.

## Materials and methods

The present experiments were conducted in the Plant Physiology Laboratory, Amity Institute of Biotechnology, Amity University, Noida.

### Geographical position of the study site

Noida is an administrative headquarters of Gautam Budh Nagar district. The study site is located at latitude 28° 32' N and longitude 77° 28' E, 200 m above the sea level.

### Collection of the tomato seeds

The certified, healthy and uniform seeds of tomato (*Lycopersicon esculentum* Mill. variety Pusa 120) were procured from Indian Agricultural Research Institute (IARI), New Delhi. The seeds were stored in sterilized polythene bags to avoid contamination.

### Preparation of different concentrations of sodium silicate

Sodium silicate [ $\text{Na}_2\text{O}_3\text{Si}_2\text{H}_2\text{O}$ ] (molecular weight: 284.20 g/mol) was purchased from LOBA Chemieprivate limited, Mumbai. Different concentrations of sodium silicate were prepared with distilled water and mixed thoroughly with the growth medium [2g/10kg (T<sub>1</sub>), 3g/10 kg (T<sub>2</sub>), 5g/10 kg (T<sub>3</sub>), 7g/10kg (T<sub>4</sub>) and 9g/10kg (T<sub>5</sub>)] in different pots for the treatment in comparison to control.

### Growth of tomato plants

The seeds of tomato (*Lycopersicon esculentum* Mill. Pusa 120) were sown in the earthen pots (30 cm deep and 30 cm in diameter), containing equal weights 10 kg of growth medium which was comprised of garden soil: cow manure (3:1). For the treatment, effective concentrations of sodium silicate such as 5g and 7g were added in 10 kg of growth medium (soil : cow manure). The plants were thinned to one plant per pot at 10 DAS and uniform watering (400 ml/pot) was continued for 55 days till flowering.

### Water stress treatment

The water stress treatment was given after the flowering stage (55 DAS) in the tomato plants:

#### Control

Normal watering in which tomato plants receive adequate water to maintain the soil moisture level at field capacity throughout their growth period.

#### Sodium silicate treatment

In sodium silicate treated pots, normal watering in which plants receive adequate water to maintain the soil moisture level at field capacity throughout their growth period.

### Water stress treatment in control and sodium silicate treated pots

Water stress treatment was given to the control and sodium silicate treated tomato plants at flowering stage by withholding the water supply for 3 and 6 days respectively.

### Relative water content (RWC)

For the measurement of relative water content, tomato leaves of control and treatment at 40 DAS and 60 DAS were cut into discs of uniform size, weighed for a fresh weight (FW) and were immediately floated on distilled water at 25°C in the darkness. After 12 h, turgid weight (TW) was measured and then discs were dried



in an oven at 80°C for 48 h for the dry weight (DW). The RWC was calculated by the modified method of Bars and Weatherly [28].  

$$\text{RWC (\%)} = (\text{FW}-\text{DW}) / (\text{TW}-\text{DW}) \times 100$$

### Estimation of photosynthetic pigment

The amount of chlorophyll can be determined in the tomato leaves by the method of Lichtenthaler [29]. The leaves (10 mg) of control and treatment were grounded with 10 ml of 80% acetone and centrifuged at 3000 rpm for 10 minutes. The optical density of the supernatant was measured at 645 and 663 nm and the amount of total carotenoids was determined at 470nm. The determination of chlorophyll a, chlorophyll b and total chlorophyll can be done by applying the following formula:

$$\text{Total Chlorophyll (mg/g)} = \frac{20.2 \times \text{OD}_{645} + 8.02 \times \text{OD}_{663} \times V}{1000 \times W}$$

$$\text{Chl a (mg/g)} = \frac{12.7 \times \text{OD}_{663} - 2.69 \times \text{OD}_{645} \times V}{1000 \times W}$$

$$\text{Chl b (mg/g)} = \frac{22.9 \times \text{OD}_{645} - 4.68 \times \text{OD}_{663} \times V}{1000 \times W}$$

Where, V = volume of the supernatant in ml, W = fresh weight of the leaves in g and OD = optical density.

### Chlorophyll stability index (CSI)

Chlorophyll stability index (CSI) was determined according to the method of Sairam et al. [30] and calculated by the formula: CSI = Total chlorophyll under treatment/ Total chlorophyll under control x 100

### Determination of electrolyte leakage

For electrolyte leakage, 0.2 g of tomato leaves were cut into discs 1 cm in diameter and placed into plastic tubes containing 50 ml of distilled water. After 24 hours, the EC of water containing the leaf sample was measured (C1) by using an electrical conductivity meter. The plastic tubes were then autoclaved at 120°C in an autoclave for 20 min and their EC was measured (C2). Electrolyte leakage was determined as: Electrolyte leakage (%) = C1/C2 x 100

### Lipid per oxidation

Lipid peroxidation was measured by estimating the malondialdehyde content (MDA) following the method of Heath and Packer [31]. Tomato leaves (200 mg) were homogenized with 5 ml of 0.01% w/v trichloroacetic acid (TCA) and centrifuged at 10,000 g for 10 min. One ml of supernatant was mixed with 4 ml of 0.5%

(w/v) thiobarbituric acid (TBA) prepared in 20% TCA. The mixture was heated in water bath at 95°C for 30 min followed by quick cooling and centrifuged at 10,000 g for 10 minutes. The absorbance of supernatant was recorded at 532 nm and corrected by subtracting the non-specific absorbance at 600 nm. MDA content was determined by using extinction coefficient of 155 mM<sup>-1</sup>cm<sup>-1</sup> and expressed as μmol g<sup>-1</sup>FW.

### Sugar content

The sugar content was estimated by the method of Hedge and Hofreiter [32]. About 0.25 g tomato leaves of control and treatment were homogenized in 2.5 ml of 95% ethanol. After centrifugation, the supernatant (1 ml) was mixed with 4 ml of anthrone reagent and heated on boiling water bath for 8 min. The absorbance was taken at 620 nm after rapid cooling and sugar was quantified with the standard curve prepared from glucose.

### Protein content

Quantitative estimation of protein content in tomato leaves was done following the method of Lowry et al. [33].

Stock solution of the following reagents were prepared :

- Alkaline sodium carbonate solution (0.2 % Na<sub>2</sub>CO<sub>3</sub> in 0.1 N Na OH).
- Copper sulphate - sodium potassium tartar ate solution (0.5% CuSO<sub>4</sub>. 5H<sub>2</sub>O in 1% sodium potassium tartarate).
- Alkaline copper reagent: Mixed 50 ml of reagent A and 1 ml of reagent B.
- Folin - Ciocalteu reagent, dilute the reagent with equal volume of water just before use.
- 1 N Na OH

Tomato leaves of control and treatment were homogenized with 1 ml of 1 N Na OH for 5 min at 100°C. Alkaline copper reagent (5 ml) was added to it and allowed the mixture to stand at room temperature for 10 min. 0.5 ml of Folin - Ciocalteu reagent was added immediately and mixed the contents in the tube. The absorbance of the solution was measured at 650 nm after 30 min. The amount of protein was calculated with reference to standard curve of lysozyme.

### Nitrate reductase activity

Nitrate reductase (NR) activity was measured by following the procedure of Jaworski [34]. Fresh leaves of tomato (0.25 g) were incubated in 4.5 ml medium which contained 100 mM phosphate buffer (pH 7.5), 3% (w/v) KNO<sub>3</sub>, 3N HCl and 0.02% (w/v) N-(1-Naphthyl) ethylene diamine dihydrochloride. The absorbance was recorded at 540 nm. NR activity was measured with standard curve prepared from NaNO<sub>2</sub> and expressed as mmol NO<sub>2</sub> mg protein<sup>-1</sup>h<sup>-1</sup>.



## Assay of non-enzymatic antioxidants

### Estimation of proline

Extraction and determination of proline was done according to the method of Bates et al. [35]. Tomato leaves of control and treatment (40 DAS and 60 DAS) were extracted with 3% sulphosalicylic acid and an aliquot was treated with acid-ninhydrin and acetic acid, boiled for 1 h at 100°C. The reaction mixture was extracted with 4 ml of toluene. The absorbance of chromophore containing toluene was determined at 520 nm. Proline content was expressed as  $\mu\text{mol g}^{-1}\text{FW}$  using a standard curve.

### Determination of Lycopene content

The control and treated fruits of tomato (3 gm) were grounded in liquid nitrogen and extracted with 10 ml of acetone: hexane (2:1) solution at 60, 65 and 70 DAS. The suspension was centrifuged at 5,000g for 10 min in 50 ml corex tubes. The upper hexane layer was removed and the absorbance of 1:10 dilution of this extract was determined by UV-Vis spectrophotometer at 453, 505 and 663 nm. The amount of lycopene was calculated from the standard curve prepared by the supplied lycopene from Sigma. Lycopene was calculated according to the following formula [36].

$$\text{Lycopene (mg/100ml)} = -0.0458 A_{663} + 0.372 A_{505} - 0.0806 A_{453}$$

### Estimation of $\beta$ -carotene content

The control and treated fruits of tomato (3 gm) were grounded in liquid nitrogen and extracted with 10 ml of acetone: hexane mixture (4:6) and filtered through filter paper. The absorbance of the filtrate was measured at 453, 505, 663 nm by UV-Vis spectrophotometer.  $\beta$ -carotene was calculated according to the following equation (Nagata and Yamashita with modification [37]).

$$\beta\text{-carotene (mg/100ml)} = 0.216 A_{663} - 0.304 A_{505} + 0.452 A_{453}$$

The  $\beta$ -carotene content was quantified with the standard curve prepared by the purified  $\beta$ -carotene procured from Sigma.

## Assay of enzymatic antioxidants

### Extraction and assay of antioxidant enzyme

The enzyme extract was prepared by homogenizing (0.25 g) tomato leaves with 0.1 M sodium phosphate buffer (pH 7.0) containing polyvinyl pyrrolidone. The homogenate was centrifuged at 4°C at 15,000 g for 30 min in cooling centrifuge. The supernatant was used for the assay of superoxide dismutase (SOD), catalase (CAT), peroxidase (POX) and ascorbate peroxidase (APX).

### Assay of SOD

Superoxide dismutase (EC 1.15.11) activity was determined by the nitroblue tetrazolium (NBT) photochemical assay method following Beyer and Fridovich [38]. 0.2 g fresh leaf tissue was homogenized in 1 % polyvinyl pyrrolidone (PVP) prepared in 50 mM potassium phosphate buffer (pH 7.0) and centrifuged at 15,000×g for 30 min at 4°C. The reaction mixture contained 0.5 ml clear supernatant, 2 ml 0.15 M Methylene diamine tetra acetic acid (EDTA), 20 mM methionine, 0.12 mM NBT, 0.5 ml 11.96  $\mu\text{M}$  riboflavin and 0.5 ml PVP. The optical density (OD) was determined colorimetrically against a blank at 560 nm. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the reduction of NBT.

Enzyme activity was calculated as:

$$\text{SOD units/ml} = [(V-v)-1] \times 200 \text{ units/g FW}$$

Where V= absorbance of respective reference and v = absorbance of respective test.

### Assay of CAT

Catalase activity (EC1.11.1.6) was assayed following by the method of Cakmak and Marschner [39]. The assay mixture (2 ml) contained 25 mM potassium phosphate buffer (pH 7.0), 10 mM  $\text{H}_2\text{O}_2$  and 0.5 ml enzyme extract. The rate of  $\text{H}_2\text{O}_2$  decomposition for 1 min was monitored at 240 nm and calculated using extinction coefficient of 39.4  $\text{mM}^{-1} \text{cm}^{-1}$  and expressed as enzyme unit  $\text{g}^{-1}\text{FW}$ . One unit of catalase was determined as the amount of enzyme required to oxidize 1  $\mu\text{M}$   $\text{H}_2\text{O}_2 \text{min}^{-1}$ .

Enzyme activity was calculated as:

$$\text{Activity FW/min} = 250 \times 10X/3$$

Where X is the observed OD.

### Assay of POX

POX activity (EC 1.11.1.7) was assayed by the method of McCune and Galston [40]. Fresh leaves (0.2 g) were homogenized in 0.1 M potassium phosphate buffer (pH 6.0) and centrifuged at 10,000×g for 20 min at 4°C. The reaction mixture contained 2.0 ml enzyme extract, 2 ml potassium phosphate buffer, 1.0 ml 0.1 N pyrogallol and 0.2 ml 0.02%  $\text{H}_2\text{O}_2$  and OD was determined at 430 nm. One unit of enzyme activity is defined as the amount which produced an increase of 0.1 OD per minute. Enzyme activity was calculated as:

$$\text{Total activity/g FW /min} = 10X \times 25$$

Where X is the observed OD.

### Assay of APX

Ascorbate peroxidase (EC 1.11.1.11) activity was measured according to Nakano and Asada [41] by estimating the rate of ascorbate oxidation. Reaction mixture (2ml) consisted of 25mM phosphate buffer (pH 7.0), 0.1mM EDTA, 0.25mM Sodium ascorbate, 1.0 mM  $\text{H}_2\text{O}_2$  and 0.2 ml of enzyme extract. The enzyme activity was determined using an extinction coefficient of



2.8mM<sup>-1</sup>cm<sup>-1</sup> by measuring the change in absorbance at 290nm for 1 min and expressed as enzyme unit g<sup>-1</sup>FW.

## Statistical analysis

All the treatments were arranged in a randomized block design with three replications. Data were statistically analyzed using analysis of variance (ANOVA) by using SPSS software (Ver. 10; SPSS Inc., Chicago, IL, USA). The treatment mean was analyzed by Duncan's multiple range test (DMRT) at p < 0.05.

## Results and Discussion

Drought is one of the most important environmental factors that influence plant growth and development and limit plant production. The decrease in water availability has an immediate impact on water status and affects different plant growth parameters via detrimental effects on water absorption, transport of water and solute to growing plant organs. The authors of the present investigation already reported significant reduction in various

morphological parameters of tomato plants under water stress condition and its alleviation by supplementation of sodium silicate [42]. Silicon improves plant growth under water stress condition by affecting a variety of metabolic processes such as improved water status of plant [43], regulation of plant defense system [44] and changes in leaf organelles ultra-structure [45]. The beneficial effects of silicon are also attributed to its deposition in cell wall of leaves and stems of plants that protect against multiple abiotic and biotic stresses [46].

### Relative water content

Relative water content is considered as a measure of plant water status, reflects the metabolic activity in tissues. In the present study, relative water content was significantly increased with increase in concentration of sodium silicate at 40 DAS with maximum increase in T<sub>2</sub> treatment but decreased significantly after 6 days water stress treatment (Table - 1 and 2). Our results are in agreement with Sonobe et al. [47] who reported that silicon application actively promoted water uptake that led to the development of high water potential.

**Table 1:** Effect of sodium silicate on the relative water content and electrolyte leakage in leaves of *Lycopersicon esculentum* Mill. at vegetative stage at 40 DAS.

Treatment	Relative water content (%)	Electrolyte leakage (%)
C	87.07±0.14	64.31±0.12
T <sub>1</sub>	92.84±0.46 (6.63)*	58.22±0.34 (9.47)
T <sub>2</sub>	93.61±0.92 (7.51)*	55.46±0.47 (13.76)

\*Stimulation percentage over control

Where; C=Control, T<sub>1</sub> = Pots containing sodium silicate (5g/10 kg soil)

T<sub>2</sub> = Pots containing sodium silicate (7g/10 kg soil)

Data are means of three replicates ± sem. Different letters in each group show significant differences at P < 0.05.

The studies have shown that growth of plants are directly proportional to the availability of water in the soil [48]. Water stress results in significant decline in relative water content [1] and causes loss of turgor which causes impaired mitosis, cell elongation and expansion resulted in reduced growth and yield of crop plants [49]. Water stress reduced the leaf growth and leaf area in many species of populus [50] and soybean [51]. The silicon deposited as colloidal gel in the conducting tissues i.e. xylem vessels and cell wall of leaves restricts the transpired water bypass flow and hence offers an obstacle to transpiration through cuticle [52]. Thus improves the water status of plants and keeping the leaves erect and increases light penetration hence improving photosynthetic efficiency of plants under water deficit situation. Pei et al. [53] reported that silicon sustains water potential of leaves in wheat plants under water stress at the similar level as that of the well watered plants. Therefore, it is obvious that a positive correlation exists between silicon uptake and water potential of plants under drought condition. The beneficial impact of silicon on water

potential of plants is also linked with decrease in cuticular transpiration that results in increase of leaf water potential under water deficit condition [53].

### Electrolyte leakage and lipid peroxidation

The leakage of membrane is caused by the uncontrolled enhancement of free radicals, which causes lipid peroxidation. Lipid peroxidation was measured in terms of MDA content which increased significantly with increase in severity of water stress. The damage to membrane permeability may be due to peroxidation of polyunsaturated fatty acids present in biomembranes resulting into formation of byproducts such as malondialdehyde. MDA is a product of membrane lipid peroxidation, is considered a reliable marker of oxidative stress thus, higher MDA contents is related to a higher degree of oxidative stress. However, plants relieved from severe water deficit due to the presence of sodium silicate in the



tomato leaves exhibited decline in lipid peroxidation in WS<sub>3</sub>+T<sub>1</sub> and WS<sub>6</sub>+T<sub>2</sub> but it was still higher than control. The MDA content in the tomato leaves at 60 DAS was: WS<sub>6</sub>> WS<sub>3</sub>> WS<sub>6</sub>+T<sub>2</sub>> WS<sub>3</sub>+T<sub>1</sub>> C (Figure - 1 and 2). Increased MDA accumulation has been correlated with reduction of relative water content and photosynthetic pigment content under prolonged drought condition [54]. Cell membranes are the first target of abiotic stresses and

maintenance of their integrity and stability under stress condition is major component of tolerance in plants [55]. The reduction in electrolyte leakage 9.47% and 13.76% was observed in T<sub>1</sub> and T<sub>2</sub> treatment over control. Maximum electrolyte leakage 39.42% and 35.07% was observed in WS<sub>6</sub> and WS<sub>3</sub> treatments (Table-2). Water deficit mediated increase in electrolyte leakage has been reported by several workers [56].

**Table 2:** Effect of sodium silicate on the relative water content and electrolyte leakage in leaves of *Lycopersicon esculentum* Mill. at flowering stage at 60 DAS.

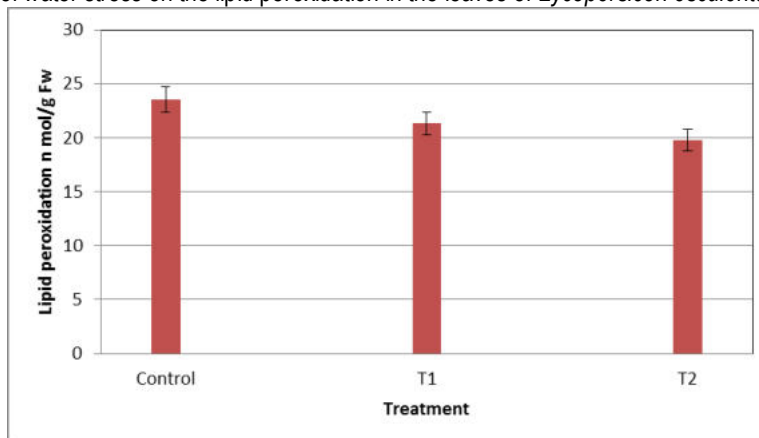
Treatment	Relative water content (%)	Electrolyte leakage (%)
C	89.20±0.74	65.42±0.26
WS <sub>3</sub>	71.42±0.82 (19.93)	88.36±0.38 (35.07)*
WS <sub>6</sub>	65.81±0.61 (26.22)	91.21±0.21 (39.42)*
T <sub>1</sub> + WS <sub>3</sub>	81.64±0.54 (8.48)	73.84±0.16 (12.87)*
T <sub>2</sub> + WS <sub>6</sub>	86.75±0.27 (2.75)	75.56±0.49 (15.49)*

\*Stimulation percentage over control

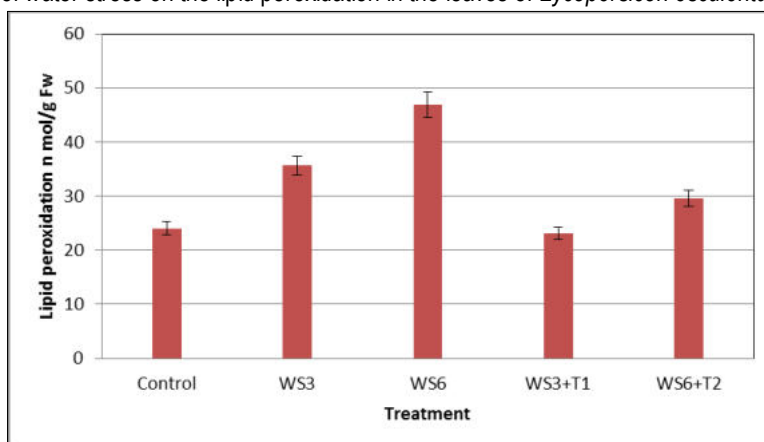
Where; C=Control , T<sub>1</sub> = Pots containing sodium silicate (5g/10 kg soil)

T<sub>2</sub> = Pots containing sodium silicate (7g/10 kg soil), WS<sub>3</sub> = Water stress treatment given for 3 days, WS<sub>6</sub> = Water stress treatment given for 6 days  
Data are means of three replicates ± sem. Different letters in each group show significant differences at P < 0.05.

**Figure 1:** Effect of water stress on the lipid peroxidation in the leaves of *Lycopersicon esculentum* Mill. at 40 DAS.



**Figure 2:** Effect of water stress on the lipid peroxidation in the leaves of *Lycopersicon esculentum* Mill. at 60 DAS.



### Pigment content and photosynthesis

Total chlorophyll content in tomato leaves were maximum in control but 27.84% increase in total chlorophyll content was recorded in T<sub>2</sub> treatment over control at 40 DAS (Table-3). The chlorophyll stability index (CSI) was also measured and it was highest in T<sub>2</sub> treatment

i.e. in the presence of high concentration of sodium silicate (Table-3). The increase in pigment content (chlorophyll a, b and total chlorophyll content) was in order: T<sub>2</sub>> T<sub>1</sub>>C at 40 DAS (Table - 3). At 60 DAS, total chlorophyll content showed the following order: C > T<sub>1</sub>+WS<sub>3</sub>>T<sub>2</sub>+WS<sub>6</sub>> WS<sub>3</sub>>WS<sub>6</sub> (Table - 4).

**Table 3:** Effect of sodium silicate on the pigment content in leaves of *Lycopersicon esculentum* Mill. at vegetative stage at 40 DAS.

Treatment	Chlorophyll a (mg/g FW)	Chlorophyll b (mg/g FW)	Total chlorophyll (mg/g FW)	Chlorophyll stability index(CSI)	Carotenoids (mg/g FW)
C	2.25±0.09	0.98±0.06	2.91±0.12	-	2.06±0.04
T <sub>1</sub>	2.32±0.01	1.08±0.08	3.53±0.11	82.39±0.32	2.15±0.10
T <sub>2</sub>	2.54±0.24	1.47±0.10	3.72±0.16	105.68±0.54	2.23±0.21

Where; C= Control , T<sub>1</sub> = Pots containing sodium silicate (5g/10 kg soil)

T<sub>2</sub> = Pots containing sodium silicate (7g/10 kg soil)

Data are means of three replicates ± sem. Different letters in each group show significant differences at P < 0.05.

**Table 4:** Effect of sodium silicate on the pigment content in leaves of *Lycopersicon esculentum* Mill. at flowering stage at 60 DAS.

Treatment	Chlorophyll a (mg/g FW)	Chlorophyll b (mg/g FW)	Total chlorophyll (mg/g FW)	Chlorophyll stability index(CSI)	Carotenoids (mg/g FW)
C	2.92±0.12	1.15±0.34	4.07±0.25	-	2.32±0.21
WS <sub>3</sub>	1.30±0.09	0.92±0.05	2.22±0.08	54.55±0.43	1.88±0.09
WS <sub>6</sub>	0.98±0.02	0.75±0.02	1.73±0.21	42.51±0.84	1.50±0.06
T <sub>1</sub> + WS <sub>3</sub>	1.86±0.06	0.98±0.10	2.84±0.39	69.78±0.92	1.92±0.13
T <sub>2</sub> + WS <sub>6</sub>	1.43±0.07	0.84±0.09	2.27±0.16	55.77±0.56	1.69±0.02

Where; C=Control , T<sub>1</sub> = Pots containing sodium silicate (5g/10 kg soil)

T<sub>2</sub> = Pots containing sodium silicate (7g/10 kg soil), WS<sub>3</sub> = Water stress treatment given for 3 days, WS<sub>6</sub> = Water stress treatment given for 6 days Data are means of three replicates ± sem. Different letters in each group show significant differences at P < 0.05.

The chlorophyll a and b are prone to soil dehydration [51]. Water stress leads to reduction in chlorophyll a, chlorophyll b and total chlorophyll thus causing irreversible water-deficit damage to photosynthetic apparatus [57]. The foliar application of potassium silicate stimulated chlorophyll content and photosynthetic capacity in bentgrass [58]. The chlorophyll stability index is an indicator of

the stress tolerance capacity of plants [59].The chlorophyll content decreased to a significant level at higher water deficit condition in sunflower plants [60] and *Vaccinium myrtillus* [61].

The activity of PSII helps to sustain photosynthesis process in leaves exposed to abiotic stresses and this pigment system is the primary target of damage caused by photoinhibition [62]. Water



deficit conditions considerably damage the oxygen evolving complex of PSII and the PSII reaction centers in most of the plants [63]. Such drought-induced damage to PSII reaction centers has been ascribed to the degradation of structural proteins [64]. It is evident that drought-induced decline in photosynthesis occurs primarily due to closure of stomata as it decreases intercellular CO<sub>2</sub> concentration in leaves, which in turn reduces the rate of CO<sub>2</sub> assimilation, hence causing an imbalance between the PSII photochemical activity and electron requirement for photosynthesis [65]. The application of silicon decreased the decomposition of photosynthetic pigments and significantly increased photosynthetic rate of rice plants under water deficit condition [66]. Tale-Ahmad and Haddad [67] reported that silicon increased photosynthesis in wheat plants under drought and this might be associated with the enhancement in activities of photosynthetic enzymes. The increase in the photosynthetic activities of drought stressed tomato plants by the treatment of sodium silicate might also be linked to the improved efficiency of photosynthetic enzymes such as ribulose-

bisphosphate carboxylase and NADP<sup>+</sup> dependent glyceraldehyde-3-phosphate dehydrogenase under drought condition [68].

### Carotenoid content

Carotenoids are integral constituents of the thylakoid membrane in chloroplast. Carotenoids have two major functions in photosynthesis, they protect chloroplast from photo-oxidative damage and also act as accessory light harvesting pigments because they absorb light energy and pass it to the chlorophyll molecules. They are also considered as non-enzymatic antioxidants which play an important role in the protection against oxidative stress [69]. The fruits of *Lycopersicon esculentum* Mill. contains lycopene and β-carotene and their contents were decreased under water stress condition but significantly increased with the sodium silicate treatment (Figure - 3 and 4).

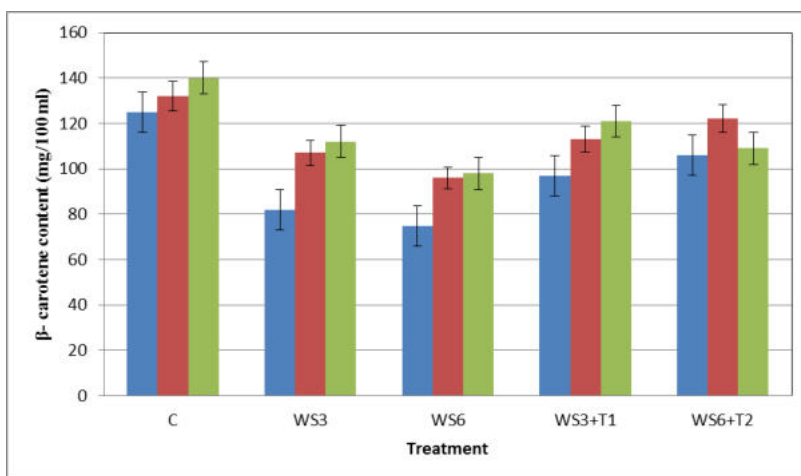


Figure 3: Effect of water stress on the β-carotene content in fruits of *Lycopersicon esculentum* Mill. at 60, 65 and 70 DAS.

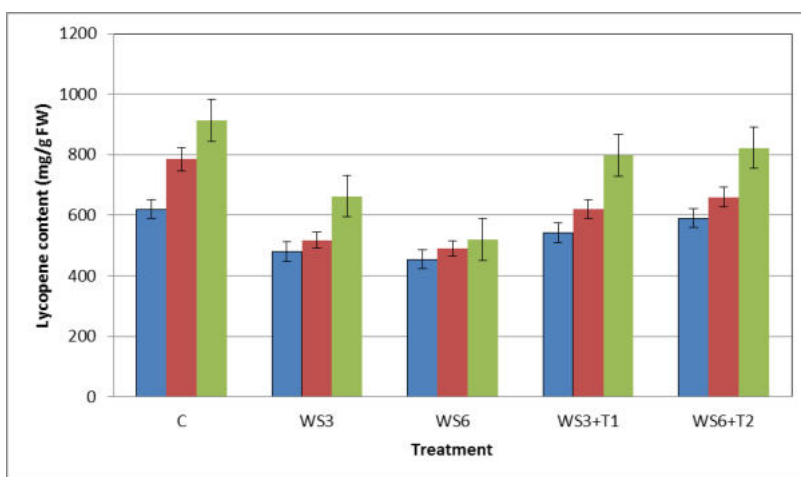


Figure 4: Effect of water stress on the lycopene content in fruits of *Lycopersicon esculentum* Mill. at 60, 65 and 70 DAS.





### Proline and Sugar contents

In the present study, proline content was significantly increased in the tomato leaves under water stress condition with maximum 58.42% increase in 6 d water stress treatment (Table - 6). At 60 DAS, proline content showed the following order: WS<sub>6</sub>> WS<sub>3</sub>> C > T<sub>2</sub>+WS<sub>6</sub>> T<sub>1</sub>+WS<sub>3</sub>. The proline and soluble sugars are the two most important compatible solute in plants [70]. Proline acts as an osmoprotectant as well as compatible solute and it acts as a redox-buffering agent possessing antioxidant property under stress condition [71]. Besides their role in osmotic adjustment, they may protect cellular membrane from damage and stabilize the structure and activities of proteins and enzymes. Proline also acts as low-molecular-weight chaperones and maintains the active conformation of macromolecules in stressed plants and participate in detoxification of ROS. Proline accumulation is positively related to drought tolerance [72] and it can activate the antioxidant defense mechanism in plants [4]. The proline accumulation in the tomato leaves under water stress condition may be associated with osmotic adjustment resulting inhibition of protein synthesis. There are reports that foliar applied proline ameliorated the adverse effect of water stress on growth and photosynthetic capacity of two maize cultivars [73].

The decrease in proline level in tomato leaves by sodium silicate treatment suggested the two possibilities that sodium silicate caused either relief from water stress or sodium silicate affected the activity of Δ<sup>1</sup>-pyrroline-5-carboxylate synthetase (P5CS) and proline dehydrogenase (PDH), two key enzymes which takes part in proline synthesis and degradation, respectively. Fariduddin et al. [74] reported that proline content in leaves exhibited an increase in response to drought stress in *Brassica juncea*. The reason of proline accumulation in water stressed leaves may be due to

protein breakdown [75], inhibition of protein synthesis [76] and inhibition of leaf development [77].

The sodium silicate treatment significantly increased sugar content in the leaves of *Lycopersicon esculentum* Mill. Total soluble sugar content decreased in water stressed tomato leaves it may be due to photosynthetic inhibition or stimulation of respiration [78]. Increase in sugar contents 2.85% and 4.67% was recorded in tomato leaves in T<sub>1</sub> and T<sub>2</sub> treatments respectively as compared with control (Table - 5 and 6). At 60 DAS, sugar content in tomato leaves showed the following order: C > T<sub>1</sub>+WS<sub>3</sub>> WS<sub>3</sub>> T<sub>2</sub>+WS<sub>6</sub>> WS<sub>6</sub>(Table-6). Elsheery and Cao [79] reported that mango cultivar which exhibited more active accumulation of soluble sugars revealed higher resistance to drought stress. The increase in the amount of soluble sugars also improved the drought tolerance capacity in sugarbeet [80] and black poplar [81].

### Protein content

The tomato plants treated with sodium silicate significantly increased total protein content in the leaves of *Lycopersicon esculentum* Mill. The total protein content 86.32 mg/g and 91.05 mg/g were recorded in tomato leaves in T<sub>1</sub> and T<sub>2</sub> treatments respectively as compared with 84.9 mg/g in control at vegetative stage (Table- 5 and 6). Water stress causes reduction in protein content (Rahman et al. 2004) and decline in protein content was dependent on the severity of water stress. At 60 DAS, protein content showed the following order: C > T<sub>1</sub>+WS<sub>3</sub>> WS<sub>3</sub>> T<sub>2</sub>+WS<sub>6</sub>> WS<sub>6</sub> (Table-6). The inhibition of photosynthesis and impaired metabolic activities resulted in decreased protein synthesis and it is evident from our experiments. Another probable reason for the decrease in protein content is its breakdown into amino acids which serve as osmolyte and defense enzymes [83].

**Table 5:** Effect of sodium silicate on the biochemical components of *Lycopersicon esculentum* Mill. at vegetative stage at 40 DAS.

Treatment	Proline (μmol/g FW)	Sugar (mg/g FW)	Protein (mg/g FW)	NR (μmol NO <sub>2</sub> /g FW h <sup>-1</sup> )
Control	9.57±0.08	92.52±0.96	84.90±0.27	9.24±0.12
T <sub>1</sub>	9.35±0.04 (2.29)	95.16±0.54 (2.85)*	86.32±0.52 (1.67)*	12.56±0.21 (35.93)*
T <sub>2</sub>	8.92±0.12 (6.79)	96.84±0.67 (4.67)*	91.05±0.64 (7.24)*	15.29±0.32 (65.48)*

\*Stimulation percentage over control

Where; C=Control, T<sub>1</sub> = Pots containing sodium silicate (5g/10 kg soil)

T<sub>2</sub> = Pots containing sodium silicate (7g/10 kg soil), WS<sub>3</sub> = Water stress treatment given for 3 days, WS<sub>6</sub> = Water stress treatment given for 6 days. Data are means of three replicates ± sem. Different letters in each group show significant differences at P < 0.05.

### Nitrate reductase activity

The nitrate reductase activity in the tomato leaves was adversely affected by water stress treatment as compared with control (Table

- 6). Significant reduction 30.65% and 38.07% in nitrate reductase activity was reported in 3d and 6d water stress treatment at 60 DAS but the enzyme activity was increased with supplementation of sodium silicate (Table - 6).



**Table 6:** Effect of sodium silicate on the biochemical components of *Lycopersicon esculentum* Mill. at flowering stage at 60 DAS.

Treatment	Proline ( $\mu\text{mol/g FW}$ )	Sugar ( $\text{mg/g FW}$ )	Protein ( $\text{mg/g FW}$ )	NR ( $\mu\text{mol NO}_2/\text{g FW h}^{-1}$ )
C	10.51 $\pm$ 0.07	94.63 $\pm$ 0.64	82.63 $\pm$ 0.44	15.92 $\pm$ 0.12
WS <sub>3</sub>	13.83 $\pm$ 0.09(31.59)*	83.12 $\pm$ 0.25(12.16)	76.28 $\pm$ 0.36(7.68)	11.04 $\pm$ 0.08(30.65)
WS <sub>6</sub>	16.65 $\pm$ 0.12(58.42)*	72.67 $\pm$ 0.16(23.21)	62.33 $\pm$ 0.21(24.57)	9.86 $\pm$ 0.09(38.07)
T <sub>1</sub> + WS <sub>3</sub>	9.21 $\pm$ 0.10(12.37)	89.25 $\pm$ 0.34(5.69)	78.10 $\pm$ 0.15(5.48)	14.23 $\pm$ 0.13(10.62)
T <sub>2</sub> + WS <sub>6</sub>	10.36 $\pm$ 0.02(1.43)	78.19 $\pm$ 0.29(17.37)	67.86 $\pm$ 0.10(17.87)	12.16 $\pm$ 0.28(23.62)

\*Stimulation percentage over control

Where; C=Control, T<sub>1</sub> = Pots containing sodium silicate (5g/10 kg soil)

T<sub>2</sub> = Pots containing sodium silicate (7g/10 kg soil), WS<sub>3</sub> = Water stress treatment given for 3 days, WS<sub>6</sub> = Water stress treatment given for 6 days. Data are means of three replicates  $\pm$  sem. Different letters in each group show significant differences at P < 0.05.

Nitrate reductase (NR) is an important cytosolic enzyme and its activity is sensitive to water stress [84]. The reduced nitrate reductase activity could be attributed to a decreased nitrate absorption by tomato plant roots due to dehydrated soil and transport of reduced nitrate from the roots to the leaves which consequently decreased the foliar nitrate concentration as reported in previous researches [85, 86]. The reduced photosynthetic rate or inhibited synthesis or low induction of enzymes may be responsible for reduction in nitrate reductase activity [87].

Superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and peroxidase (POX) are the main enzymes of the antioxidant defense system which help in scavenging of the lethal ROS. These defense enzymes act as stress markers. The increase in antioxidant enzymes during stress might be due to the signaling of ROS or oxidative homeostasis [88]. The activities of antioxidant enzymes (SOD, CAT, APX and POX) were increased significantly in tomato leaves in response to sodium silicate treatment. Although, significant increase was observed in all the treatments in comparison to control but maximum 84.12%, 123.2%, 64.02% and 84.21% stimulation was recorded for SOD, CAT, APX and POX activities in T<sub>2</sub>+ WS<sub>6</sub> treatment over the control at 60 DAS (Table -8).

### Antioxidant enzymes

**Table 7:** Effect of sodium silicate on the antioxidant enzyme activity of *Lycopersicon esculentum* Mill. at vegetative stage at 40 DAS.

Treatment	SOD ( $\text{EU g}^{-1}\text{ FW}$ )	CAT ( $\text{EU g}^{-1}\text{ FW}$ )	APX ( $\text{EU g}^{-1}\text{ FW}$ )	POX ( $\text{EU g}^{-1}\text{ FW}$ )
Control	23.36 $\pm$ 0.22	7.28 $\pm$ 0.14	8.69 $\pm$ 0.32	20.15 $\pm$ 0.27
T <sub>1</sub>	24.21 $\pm$ 0.34(3.64)*	8.65 $\pm$ 0.19(18.82)*	9.32 $\pm$ 0.49(7.25)*	20.88 $\pm$ 0.29(3.62)*
T <sub>2</sub>	26.69 $\pm$ 0.42(14.26)*	9.21 $\pm$ 0.23(26.51)*	10.81 $\pm$ 0.22(24.39)*	21.57 $\pm$ 0.41(7.05)*

\*Stimulation percentage over control

Where; C=Control, T<sub>1</sub> = Pots containing sodium silicate (5g/10 kg soil)

T<sub>2</sub> = Pots containing sodium silicate (7g/10 kg soil), WS<sub>3</sub> = Water stress treatment given for 3 days, WS<sub>6</sub> = Water stress treatment given for 6 days. Data are means of three replicates  $\pm$  sem. Different letters in each group show significant differences at P < 0.05.

**Table 8:** Effect of sodium silicate on the antioxidant enzyme activity of *Lycopersicon esculentum* Mill. at flowering stage at 60 DAS.

Treatment	SOD ( $\text{EU g}^{-1}\text{ FW}$ )	CAT ( $\text{EU g}^{-1}\text{ FW}$ )	APX ( $\text{EU g}^{-1}\text{ FW}$ )	POX ( $\text{EU g}^{-1}\text{ FW}$ )
C	24.18 $\pm$ 0.13	8.24 $\pm$ 0.02	9.45 $\pm$ 0.12	19.25 $\pm$ 0.23
WS <sub>3</sub>	32.96 $\pm$ 0.18(36.31)*	10.38 $\pm$ 0.14(25.97)*	10.12 $\pm$ 0.23(7.09)*	23.78 $\pm$ 0.21(23.53)*
WS <sub>6</sub>	42.18 $\pm$ 0.21(74.44)*	15.16 $\pm$ 0.23(83.98)*	12.34 $\pm$ 0.54(30.58)*	32.64 $\pm$ 0.45(69.56)*
T <sub>1</sub> + WS <sub>3</sub>	36.13 $\pm$ 0.45(49.42)*	12.65 $\pm$ 0.09(53.52)*	13.63 $\pm$ 0.62(44.23)*	28.59 $\pm$ 0.22(48.52)*
T <sub>2</sub> + WS <sub>6</sub>	44.52 $\pm$ 0.72(84.12)*	18.39 $\pm$ 0.04(123.18)*	15.50 $\pm$ 0.98(64.02)*	35.46 $\pm$ 0.16(84.21)*

\*Stimulation percentage over control

Where; C=Control, T<sub>1</sub> = Pots containing sodium silicate (5g/10 kg soil)

T<sub>2</sub> = Pots containing sodium silicate (7g/10 kg soil), WS<sub>3</sub> = Water stress treatment given for 3 days, WS<sub>6</sub> = Water stress treatment given for 6 days. Data are means of three replicates  $\pm$  sem. Different letters in each group show significant differences at P < 0.05.



The present study reveals that superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and peroxidase (POX) work synergistically in scavenging ROS species in tomato plant under water stress. Several enzymes of the defense system increased tremendously during water stress in order to avoid the damage caused by reactive oxygen species [89, 90]. Ahmad and Haddad [67] reported that application of silicon under drought stress significantly increased the activities of SOD, CAT and APX enzymes in wheat. SOD is considered to be the first line of defense against reactive oxygen species as it controls the first threshold of the water-water cycle of antioxidant system [91, 92]. It acts first on free radicals and converts them to hydrogen peroxide, CAT in peroxisomes and APX in the cell as whole have potential to convert H<sub>2</sub>O<sub>2</sub> into water and oxygen [93]. Shao et al. [94] reported that CAT is the principal enzyme that scavenges H<sub>2</sub>O<sub>2</sub> in cells. The high activity of CAT in the present study is similar with the results of drought tolerance in *Catharanthus roseus* [95], alfalfa [96], peanut [97] and canola cultivars [98]. The combined action of CAT and SOD also converts the toxic O<sub>2</sub><sup>-</sup> into H<sub>2</sub>O<sub>2</sub> then into water and molecular oxygen, averting the cellular damage under water stress condition [72]. The increased activities of SOD and CAT were observed in various plants like cucumber [99] and mustard [100] under abiotic stress condition. The increase in the antioxidant level was reported with increase in abiotic stress intensity in maize and soybean [101]. The higher activities of antioxidant enzymes improved drought tolerance capacity in mulberry [72], tea [102] and olive [4]. The above mentioned results are in agreement with i.e. increase in antioxidant enzymes in tomato leaves under 3d and 6d water stress treatment.

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## Conclusion

In the present study, the ability of tomato plants to overcome water stress relies on the upregulation of antioxidant enzymes. The positive relationship between the contents of osmotic solute (proline and soluble sugar) and antioxidant enzyme activities (SOD, CAT, APX and POX) were also observed in our study. It is clear from the present investigation that different biomolecules coordinate together in order to protect the tomato plants against water stress. The positive effects of sodium silicate in alleviation of water stress in tomato plants may suggest its active involvement in biochemical processes of plants. The results also suggest a potential use of sodium silicate treatment may cause over expression of the antioxidant genes and can be a suitable candidate for crop production in drought prone areas.

## Conflict of interests

Declared none.

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