Research Article

Quality indices of tomato plant as affected by water stress conditions and chitosan application

Abdul Basit¹, Hassnain^{1*}, Mehboob Alam¹, Izhar Ullah¹, Syed Tanveer Shah¹, Syed Ali Zuhair² and Inayat Ullah³

1. Department of Horticulture, Crop Production Sciences, The University of Agriculture, Peshawar-Pakistan

2. Department of Plant Breeding & Genetics, The University of Agriculture, Peshawar-Pakistan

3. Department of Agricultural Mechanization, The University of Agriculture, Peshawar-Pakistan

*Corresponding author's email: saemstar@gmail.com

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Abstract

Plants show physiological, morphological and chemical responses to microbial, physical or chemical factors which are known as elicitors. Water deficit stress is one of the most important abiotic stresses that affects plant physiological and morphological traits. Chitosan is a natural, less toxic and economical compound that is biodegradable and eco-friendly with various applications in agriculture. The present experiment was carried out to evaluate the effect of pre-harvest foliar application of chitosan on quality indices of tomato plant under different water stress conditions. Tomato (Cv. Rio Grande) plants were subjected chitosan concentrations (0, 50, 100, 150 mg L⁻¹) and water stress intervals (3, 6, 9 and 12 days) after 15 days of transplantation. Tomato plants treated with 6 days water stress interval recorded maximum fruit firmness, fruit juice content, total soluble solids, titratable acidity and ascorbic acid content and minimum of fruit juice pH. Similarly, chitosan application @ 100 mg L⁻¹ attained highest fruit firmness, percent juice content, total soluble solid, titratable acidity, ascorbic acid content and minimum fruit juice pH. It is concluded that 100 mg L⁻¹ chitosan application along with 6 days water interval had positive impact on quality indices of tomato.

Keywords: Chitin, Chitosan; Drought Stress; Quality; Stress tolerance; Tomato

Introduction

Tomato (*Lycopersicon esculentum* Mill.) is an important crop of Night shade family (2n=24 chromosomes). It predominately contains 93-96% water but rich in carotenoids (mainly lycopene), ascorbic acid, potassium and iron. The °Brix of tomato fruit ranges from 4% to 9% [1]. It was originated in the western coastal plain of South America [2]. The European imported tomato to

subcontinent of the Indo-Pak in the second half of 19th century. Tomato is grown all over the world where as China, USA, Turkey, Italy, Egypt, India, Spain, Brazil, Iran and Mexico are the major producers [3]. It is cultivated in the warm season and is categorized as annual plant. The average optimum temperature for its cultivation ranges from 25 to 29°C [4]. It is utilized in a variety of traditions such as sun dried

tomatoes, tomato juice, soup and ketchup as well as fresh salad [5]. Pakistan produces two crops of tomato annually, one crop is cultivated in spring and the second crop is cultivated in autumn. During 2013-14 the total cultivated area under tomato crop was 63.2 thousand hectares and the total production was 599.7 thousand tones. It is grown in all the four provinces of Pakistan. During 2013-14 tomato was cultivated in Khyber Pakhtoonkhwa, Punjab, Baluchistan and Sindh on area of 14.0, 7.8, 27.0 and 14.4 thousand hectares, respectively, and there production was 135.7, 100.1, 200.6 and 163.3 thousand tons, respectively [6].

Chitosan, a given name to a deacetylate form of chitin, is a natural biodegradable compound derived from crustaceous shells such as crabs and shrimps, whose main attributes corresponds to its poly-cationic nature [7]. Also as a high molecular polymer, nontoxic and bioactive agent, it has become a useful compound due to its fungicidal effects and elicitation of defense mechanisms in plant tissues [8]. Chitosan is a low acetyl form of chitin mainly composed of glucosamine, 2-amino-2-deoxy- β -D-glucose [9]. Recently, some researchers reported that chitosan enhanced plant growth and development [10]. They reported that application of chitosan increased vital enzyme activities of nitrogen metabolism (nitrate reductase, glutamine synthetase and protease) and improved the transportation of nitrogen (N) in the functional leaves which enhanced plant growth and development. Chitosan has been widely used for stimulation of plant defense [7]. Chitosan oligomers enter most regions of the cell, and subsequently induced changes in: cell membranes, chromatin, deoxyribonucleic acid (DNA), calcium, MAP kinase, oxidative burst, reactive oxygen species (ROS), pathogenesis related (PR) genes/proteins and phytoalexins [11]. Pre-harvest chitosan applications have been noted to be effective

in controlling postharvest fungal infection in strawberries [12]. Moreover, plants treated with chitosan may be less prone to stress evoked by un-favorable conditions, such as drought, salinity and low or high temperature [13].

Chitosan seems to act as a stress tolerance inductor it enhanced a hyper sensible reaction and lignification, inducing lipid peroxidation, and production of defense against pathogens when directly applied to plant tissue [14]. Seeds treated with chitosan reduced the mean germination time; increased germination index leads to improving seedling growth under low temperature stress and also reported that the application of chitosan reduced the vanadium toxicity when applied to wheat and barley in irradiated form [15]. During drought stress foliar application of chitosan helps to reduce the loss of water from the leaves by including stomatal type closing compounds, which are able to decrease water loss from the leaf by improving plant biomass or yield of crop. Foliar application of chitosan helps to reduce the water stress effect on yield which may be due to increase in stomatal conductance under water stress and its role in reducing transpiration rate [16]. Similarly, [12] reported that chitosan spray significantly maintained the keeping quality of strawberry fruit as compared with control.

Keeping in view the importance of chitosan, an experimental trial was scheduled to study the pre-harvest application of chitosan on quality indices of tomato under water stress conditions.

Materials and methods

Experimental site and procedure

An experiment on “Quality indices of tomato plant as affected by pre-harvest application of chitosan and water stress condition” was carried out at Ornamental Nursery, Department of Horticulture, The University of Agriculture Peshawar, located at 34°N latitude, 71°E longitude with an altitude of

350m above sea level and has a sub-tropical climate [17], during 2018. Complete Randomized Design (CRD) with two factors was used. Tomato (Cv. Roma) plants were sprayed with various concentrations of chitosan after two weeks of transplantation. Plants were subjected to four water-stress treatments: S₁= 3 days (100% field capacity (FC), S₂= 6 Days water stress intervals (70% water capacity; WC), S₃= 9 Days water stress intervals (50% WC) and S₄= 12 Days water stress intervals (30% WC). The seeds were sown in nursery trays in the last week of January; a slight but frequent irrigation was applied to seedlings with the help of hand sprayer. The seedlings trays were placed in a greenhouse. The seedlings were transplanted when they reach to 5-6 leaves stage and transplanted early in morning, and the seedlings were transplanted into plastic tubes and the tubes were transferred to a lathe house. The Plastic tubes were prepared one week before transplantation of the crop. All the stone, stub, root or any other material which may result in barrier to the crop were removed. Chitosan shows less solubility in water. Chitosan in required amount were dissolved in 100ml of double distill water with the addition of some drop of acid such as acetic acid to obtained four different concentrations of chitosan (0, 50, 100 and

150 (mg L⁻¹) and after properly dissolving, its volume was raised to 1000 ml. Different concentrations of chitosan were applied manually at different stages after water stress interval. The foliar spray was carried out with manual pump spray.

Physicochemical analysis

Fruit firmness (kg cm⁻²)

Firmness of the fruit was measured by using penetrometer (Wagner fruit firmness analyzer model FT-327) having the limit of 28. The instrument was set with 8 mm tip, which is sensible for fragile type tissue of fruit. Five fruit from each plant in each treatment were picked for determining firmness of fruit. After that the plunger was removed and the result on the dial was noted. A similar procedure was reviewed for the stripped area on the side of the similar fruit. Interpretation was noted as kilogram per centimeter square (kg cm⁻²).

Fruit juice content (%)

For finding the juice content the weight of the fruit was measured through electronic balance. After that fruit were crushed with the help of blender to obtain juice from it. The extracted juice was weighed and the readings were recorded. The following equation was used for determining percent juice content (%).

$$\text{Fruit juice content (\%)} = \frac{\text{Average juice weight (g)}}{\text{Average fruit weight (g)}} \times 100$$

Total soluble solids (⁰Brix)

Total soluble solids (TSS) for all treatments in each replications were calculated by the help of using handheld refractometer was measured by extracting juice of the fruit that was passed through a mesh in order to remove the seeds. A drop of extracted juice (from separate fruit) was spotted on spotless and dry prism of refractometer and led was closed properly. The readings were recorded as (⁰Brix).

Fruit juice pH

Five fruit are randomly taken from every treatment for determining pH of fruit. For this purpose an Electronic pH meter was used. The meter was the meter was placed in buffer solution before investigation then put in fruit juice for determining fruit juice pH. Prior to use the pH meter, its pH was adjusted to 7 by washing the tip of pH meter with tap water [18].

Titrateable acidity (%)

For determining titrateable acidity the following procedure given in [19] was used for finding titrateable acidity. The fruit juice

was utilized for purpose of titrateable acidity and the readings were measured as percent (%). The following formula was used for finding titrateable acidity.

$$\text{Titrateable acidity (\%)} = \frac{N \times T \times F}{D \times S} \times 100$$

Ascorbic acid content (mg 100g⁻¹)

For determining ascorbic acid content of the fruit the following procedure suggested by [20] was used.

Procedure

For determination of ascorbic acid contents of tomato fruit, 10ml juice sample was taken from extracted juice in graduated cylinder and for making 10% solution; oxalic acid was

added to make the volume up to 100ml. After that from this 10% solution (2,6-dichlorophenol indophenol) (NaHCO₃) was taken. As the pink color was appeared, burette readings were noted and the following formula was used for calculating vitamin C content.

The following formula were used for calculation:

$$\text{Ascorbic acid (mg 100g}^{-1}\text{)} = \frac{F \times T \times 100}{D \times S} \times 100$$

Where,

T = stand for Dye solution (ml) which is taken from burette

F = Dye Factor

S = Fruit juice (g) taken which are taken for dilution purpose

D = diluted sample (ml) used for titration

Statistical procedure

The collected data was subjected to Analysis of Variance (ANOVA) by using Complete Randomized design (CRD) for different variables suggested by [21]. For means comparison, data were analyzed at LSD ≤ 0.01 using statistical software package Statistix 8.1 [22].

Results and Discussion**Fruit firmness (kg cm⁻²)**

The statistical analysis of the data for fruit firmness that intervals of water stress and application of chitosan concentration significantly affected fruit firmness (kg cm⁻²) of tomato, while their interaction was found non-significant (Table 1). Maximum fruit firmness (3.77 kg cm⁻²) was recorded in plants treated with 6 days water stress interval, while minimum fruit firmness (2.58 kg cm⁻²) was recorded in plants treated in 12 days water stress interval (Fig. 1). Regarding chitosan maximum fruit firmness (3.94 kg

cm⁻²) was noted in fruit sprayed with 100 mg L⁻¹ chitosan foliar spray, while the minimum fruit firmness (2.72 kg cm⁻²) was recorded in control (Fig. 2). Firmness is one of the main qualitative characters seen by the consumer which is definitely major in overall produce. The decreasing trend in firmness regarding increasing in water stress is due to quick evaporation of water, which ultimately increases rate enzymatic activities and also enhances more respiration, which resulted in diseases fruit. According to our experiment optimum concentration of chitosan such as 100 and 150 mg L⁻¹ maintained higher firmness of tomato fruit. [23] reported that peach fruit treated with chitosan and calcium chloride decrease the swelling of fruit in early stages, and retained fruit freshness and firmness decreased the percent weight loss as well. The results of our experiment are also in line with by the findings of [24] who found that papaya fruit treated with higher concentration of chitosan resulted in maximum firmness as compared to untreated ones. Pre-harvest spray of chitosan showed a significant increase in fruit firmness with increasing the concentration of chitosan. The beneficial effect of the elevated chitosan concentration on firmness has been reported

for peach, Japanese pear, Kiwifruit [25]. Whereas, [12] also indicated that fruit from chitosan sprayed strawberry fruit were firmer and ripened at a slower rate as indicated by anthocyanin content and titratable acidity.

Fruit juice contents (%)

Various treatments of water stress intervals and foliar application of chitosan significantly affected juice content of tomato fruit while their interaction was found non-significant (Table 1). Maximum fruit juice content (62.04%) was observed in fruit plants treated with 6 days water stress interval statistically similar (60.86%) to plants treated with 3 days water stress interval. Whereas,

the lowest juice content (43.68%) was noted in the fruit plants treated with 12 days water stress interval (Fig. 1). Regarding chitosan concentrations, highest fruit juice content (57.56%) was noticed in fruit treated with 100mg L⁻¹ chitosan foliar spray, while lowest juice content (52.69%) was observed in control (Fig. 2). Drought stress has negative effect on juice content of tomato fruit because of low availability of water present in juice content. Our findings are correlated with the results of [26], who investigated that juice content of fruit decrease due to high water stress intervals.

Figure 1. Quality attributes of tomato as influenced by water stress interval

Figure 2. Quality attributes of tomato as influenced by chitosan levels

Chitosan reduce ripening process and inhibit fruit senescence which is directly related with various qualitative parameters such as improvement of aroma, taste and color decrease in titratable acidity, rise rate of sugar content as well as reduce fruit firmness which enhanced fruit juice content. The results of our experiment are correlated with the findings of [27] who reported that in tomato fruit juice content can be reduce by chitosan concentration it might be a layer of semi permeable our the surface of fruit by

marinating internal atmosphere due to low availability of oxygen for process of respiration and also it reduce the ethylene production.

Total Soluble Solid (°Brix)

The statistically analyzed data indicated that water stress and chitosan significantly affected total soluble solid (TSS) in tomato fruit, while interaction was found non-significant (Table 1). Highest TSS (4.76 °Brix) was noted in plants treated with 6 days water stress interval, while lowest TSS (3.92

⁰Brix) was recorded in plants treated with 12 days water stress interval (Fig. 1). Regarding chitosan application, maximum TSS (4.89 ⁰Brix) was observed in plants treated with 100mg L⁻¹, while minimum TSS (3.63 ⁰Brix) was noted in control (Fig. 2). Drought stress has a main role to increase total soluble solids in fruit like in citrus it enhances internal fruit quality because of increase in TSS [26, 28]. Total soluble solids is one of the major qualitative character which have direct linked with TSS content of a fruit is one of the main quality attributes which is related with dynamic feature for fruit quality. During storage stage starch contents reduces quickly

and covert to soluble sugars from different enzymatic action and according to our findings could be reported that increasing drought stress definitely increased TSS levels in tomato fruit. Our research findings are correlated with the findings of [29] who reported that in orange fruit total soluble solids can be enhanced by increasing water stress interval. TSS of tomato fruit significantly increased with optimum concentration of chitosan especially at 100 and 150 mg L⁻¹. These results are correlated with the findings of [30] who found that TSS showed positive trend in response to chitosan application.

Table 1. Mean Square value of quality attributes of tomato as influenced by water stress interval and chitosan levels

Sources		Mean Square (MS)					
		FF	JC	TSS	FJ pH	TA	AA
Water stress (W)	3	3.1695***	846.335***	1.886***	0.001 ^{ns}	0.0152***	9.474***
Chitosan level (C)	3	3.2487***	48.412***	3.761***	0.037***	0.0312***	61.39***
W x C	9	0.0404 ^{ns}	2.177 ^{ns}	0.188 ^{ns}	0.001*	0.0013 ^{ns}	3.497*
Error	32	0.033	1.359	0.097	0.004	0.0006	1.422
Total		47					

***:P≤0.001; *:P≤0.05; NS: Non-significant; FF: Fruit firmness; JC: Juice content; TSS: Total Soluble Solid; FJ pH: Fruit juice pH; TA: Titratable acidity; AA: Ascorbic acid

Fruit juice pH

The statistical analysis of data as regards fruit juice pH showed that different chitosan levels and interaction of water stress interval and chitosan concentration significantly affected pH of tomato fruit. Whereas, various water stress intervals had non-significant effect on fruit juice pH (Table 1). However, minimum fruit juice pH (5.37) was noted in plants treated with 6 days water stress interval, while maximum fruit juice pH (5.39) was noted in plants treated with 12 days water stress interval (Fig. 1). Regarding chitosan minimum fruit juice pH (5.33) was observed in tomato fruit treated with 100mg L⁻¹ while maximum fruit juice pH (5.46) was recorded in control (Fig. 2). In case of interaction, maximum fruit pH (5.47) was observed in chitosan untreated plant supplied with water

stress condition for 3 days. However, plants sprayed with chitosan @100mgL⁻¹ and water stress for 6 days showed lowest fruit juice pH (5.31) (Fig. 3). Water stress has no significant role in tomato fruit pH but it increases up to optimum level due to increasing in water stress intervals. Our findings are correlated with the results of [31] who observed that different water stress level showed non-significant effect on the pH of tomato fruit but it may increase slowly by increasing optimum water stress level. The pH of fruit were significantly affected by application of chitosan as it is related to acidic content of the fruit such as ascorbic acid as reported by [32]. After harvesting stages of fruit, organic acid increased and which results in higher pH and lower acidity [33]. According to the findings of [34] who reported that pH of fruit are

affected by increase of carbohydrates in tissue and organic acid of the fruit. Our outcomes demonstrate that chitosan decrease pH of fruit it might be because of rise in ascorbic acid contents of fruit. Our results show that ascorbic acid content in fruit juice was influenced by chitosan levels as decrease in fruit juice pH may be due to rise in ascorbic acid content of the fruit. The results of our experiment are matched with [35] who observed that chitosan make a semi permeable layer over the surface of fruit which by maintaining the availability of oxygen due to which respiration rate decreases. Eventually organic acid are safe to be consumed as a respiratory metabolites which results in low pH.

Titrateable Acidity (%)

Chitosan levels and water stress intervals significantly affected titrateable acidity of tomato fruit while their interaction was found non-significant (Table 1). Maximum titrateable acidity (0.496%) was recorded in fruit plant treated with 6 days water stress interval, whereas minimum titrateable acidity (0.415%) was observed in fruit plant treated with 3 days water stress interval (Fig. 1). Regarding chitosan application, higher titrateable acidity (0.511%) was observed in tomato fruit treated with 100mg L⁻¹ while minimum titrateable acidity (0.390%) was recorded in control (Fig. 2). Drought stress has a major role in increasing titrateable acidity of tomato fruit as it slightly increased due to optimum stress level but it may decreased by increasing water stress intervals. Water stress can be used as a culture practice to improve internal fruit quality by increasing acid content of fruit for reducing senescence of fruit. Our results are in similarity with the findings of [36] who reported that fruit have high titrateable acidity during water stress condition. With increased harvesting storage stages, the rate of reparation increases which ultimately promote ethylene production which cause

ripening and senescence of the fruit [37]. Total acidity and total sugars in fruit significantly increased in response to chitosan application compared to the control treatment it reduces respiration process and reduce organic acid catabolism which eventually results in slower reduction of titrateable acidity and fruit ripening. The results of our experiments are related with the findings of [38] who reported that when strawberry and raspberry fruit treated with chitosan it reduce ripening process and delay senescence.

Ascorbic Acid (mg 100g⁻¹)

Various treatments of chitosan concentrations, water stress intervals and their interaction significantly affected ascorbic acid content of tomato fruit (Table 1). Maximum ascorbic acid content (23.52 mg 100g⁻¹) was noted in fruit with 6 days water stress interval statistically followed by ascorbic acid content (22.69 mg 100g⁻¹) was given by fruit plants have 3 days water stress interval while minimum ascorbic acid content (21.43 mg 100g⁻¹) was observed in fruit plant treated with 3 days water stress interval (Fig. 1).

Regarding chitosan ascorbic acid content (25.35 mg 100g⁻¹) was recorded in fruit applied with 100 mg L⁻¹ chitosan. However minimum ascorbic acid content (19.85 mg 100g⁻¹) was founded in control (Fig. 2). Highest value of ascorbic acid (30.68mg100g⁻¹) was noted in chitosan untreated plant kept at water stress for 12 days. While minimum ascorbic acid value (16.70mg100g⁻¹) was recorded in chitosan untreated plant provided water stress for 3 days (Fig. 4). Ascorbic acid in fruit plants is known for its anti-oxidant activity for which key players are magnesium and phosphorus both catalyzes this activity [39]. A slight reduction in water supply maintain fruit quality without reducing the marketable fruit yield and vitamin C concentration was increased may be due to optimum water

stress interval. Our findings are also similar with the findings of [40] who proposed that higher level of ascorbic acid content was observed in tomato fruit under water stress condition. Reduction in oxidation of ascorbic acid due to limiting the gaseous exchange by the application of chitosan over the pores present on the fruit skin. Our results are also

correlated with findings of [41] who found that *Prunus avium* L. fruit having high content of ascorbic acid being treated with higher concentration of chitosan and it might delay the enzymatic activities because of low availability of oxygen which ultimately results in reducing ascorbic acid oxidation in fruit.

Figure 3. Fruit juice pH of tomato as affected by interaction of chitosan concentration and water stress interval

Figure 4. Ascorbic acid of tomato as affected by interaction of chitosan concentration and water stress interval

Conclusion

Among various water stress treatment 6 days

water stress interval show maximum fruit firmness (kg cm^{-2}), fruit juice content (%),

minimum fruit juice pH, maximum total soluble solids, titratable acidity (%) and ascorbic acid (mg 100g⁻¹) were observed. Tomato plants treated with chitosan foliar spray at the rate of 100 mg L⁻¹ resulted in maximum fruit firmness (kg cm⁻²), fruit juice content (%), minimum fruit juice pH, maximum total soluble solids, titratable acidity (%) and ascorbic acid (mg 100g⁻¹) were observed. Water stress treatment with 6 days interval combined with foliar spray of chitosan at the rate of 100 mg L⁻¹ are recommend for acquiring better quality of tomato under water stress condition.

Authors' contributions

Conceived and designed the experiments: Hassnain, Performed the experiment: Hassnain & A Basit, Analyzed the data: A Basit & Hassnain, Contributed materials/tools: Hassnain, I. Ullah & SA Zuhair, Wrote the article: A Basit & I Ullah, Supervised the experiment: M Alam.

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