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ORIGINAL ARTICLE

Improved growth, productivity and quality of tomato (*Solanum lycopersicum* L.) plants through application of shikimic acid

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Transpiration rate;
Yield

Abstract A field experiment was conducted to investigate the effect of seed presoaking of shikimic acid (30, 60 and 120 ppm) on growth parameters, fruit productivity and quality, transpiration rate, photosynthetic pigments and some mineral nutrition contents of tomato plants. Shikimic acid at all concentrations significantly increased fresh and dry weights, fruit number, average fresh and dry fruit yield, vitamin C, lycopene, carotenoid contents, total acidity and fruit total soluble sugars of tomato plants when compared to control plants. Seed pretreatment with shikimic acid at various doses induces a significant increase in total leaf conductivity, transpiration rate and photosynthetic pigments (Chl. a, chl. b and carotenoids) of tomato plants. Furthermore, shikimic acid at various doses applied significantly increased the concentration of nitrogen, phosphorus and potassium in tomato leaves as compared to control non-treated tomato plants. Among all doses of shikimic acid treatment, it was found that 60 ppm treatment caused a marked increase in growth, fruit productivity and quality and most studied parameters of tomato plants when compared to other treatments. On the other hand, no significant differences were observed in total photosynthetic pigments, concentrations of nitrogen and potassium in leaves of tomato plants treated with 30 ppm of shikimic acid and control plants. According to these results, it could be suggested that shikimic acid used for seed soaking could be used for increasing growth, fruit productivity and quality of tomato plants growing under field conditions.

1. Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most popular and widely consumed vegetable crops all over the world, and high-quality yield is an essential prerequisite for its economical success in the Saudi Arabia. Tomato has been recently gaining attention in relation to the prevention of some human diseases. This interest is due to the presence of carotenoids and particularly lycopene, which is an unsaturated alkylic compound,

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that appears to be an active compound in the prevention of cancer, cardiovascular risk and in slowing down cellular aging (Gerster, 1997; Di Cesare et al., 2012; Abdel-Monaim, 2012). Lycopene is found in fresh, red-ripe tomatoes as all-trans (79–91%) and cis- (9–21%) isomers (Shi et al., 1999; Boileau et al., 2002; Abdel-Fattah and Al-Amri, 2012).

Soils in the arid and semiarid regions like Saudi Arabia have little nutrient and mineral contents, which adversely affect plant growth and quality. One of the cost-effective strategies for counteracting deficiencies of soil minerals involves the application of chemical fertilizers. Increasing growth and quality of tomato plants through increasing the productivity per unit area as well as with expanding the cultivated area in newly reclaimed lands is the major important national target by application of cheap efficient strategies. Shikimic acid is the known precursor of aromatic amino acids, L-phenylalanine and L-tyrosine. These compounds are phenylpropane (C_6–C_3) derivatives as are the building units of lignin (Aldesuquy and Ibrahim, 2000). Phenylalanine is an excellent precursor in all plants but tyrosine in only really effective in the grasses (Stafford, 1974). The shikimic pathway, a collection of seven enzymatic reactions whose end product is chorismate, has been studied for many years in a variety of microorganisms and plants. In plants, chorismate is the precursor not only for the synthesis of aromatic amino acids (i.e. phenylalanine, tyrosine and tryptophan), but also for many secondary metabolites with diverse physiological roles (Weaver and Herrmann, 1997). Shikimic acid is used in several plants without side effects and also used in a large scale for growth enhancement and improving fruit quality of crop and vegetable plants for many years (Aldesuquy and Ibrahim, 2000; Elwan and El-Hamahmy, 2009).

In most plants, sucrose is the major product of photosynthesis and the major form of carbohydrate transported to non-photosynthetic organs (Favati et al., 2009). It can be involved in numerous metabolic pathways. Elwan and El-Hamahmy (2009) concluded that the quality of fruit pepper was positively correlated with the high amount of total soluble sugars. The shikimic acid pathway participates in the biosynthesis of plant phenolics (Logemann et al., 1995), where the most abundant classes of phenolic compounds in plants are derived from phenylalanine via elimination of an ammonia molecule to from cinnamic acid (Hahlbrock and Scheel, 1989). Phenolic compounds play an important role in the regulation of plant growth and metabolism and they are no longer considered to be a passive by-product. In some cases, phenolic treatment induces expression of the same genes and resistance against the same spectrum of pathogens as pathogen induced resistance (Lawton et al., 1996).

In the light of the above limited reviews, the present work aimed to evaluate the influence of seed presoaking with shikimic acid on growth parameters, fruit quality, transpiration rate, total leaf conductivity, photosynthetic pigments, mineral contents and productivity of tomato plants growing in field conditions.

2. Materials and methods

2.1. Plant and growth conditions

Seeds of tomato (*Solanum lycopersicum* Mill.) were surface sterilized in 7% sodium hypochlorite for 10 min, subsequently washed thoroughly with distilled water. The sterilized seeds were divided into four sets. Seeds of the 1st set were soaked in distilled water to serve as control, the other three sets (2nd, 3rd and 4th) were soaked in shikimic acid at 30, 60 and 120 ppm, respectively for about four hours, then washed with distilled water. All these treated seeds were left to germinate for 5 days on a moistened filter paper in dark at 25 °C. Uniform germinated seedlings were sown in a $8 \times 8 \times 9\ cm^3$ plastic plate containing moist-autoclaved vermiculite soil and left to grow in a greenhouse under controlled conditions. Three weeks later, plants were transplanted into plots ($25 \times 25\ cm^2$) in a randomized complete block design, three plots for each treatment and each plot had ten plants having inter row and inter plant spacing that were 70 and 40 cm, respectively, at the research experimental farm of the Facility of Science, King Saud University, Riyadh, Egypt in March 2012. The physical and chemical analyses of the soil used in this study are listed in Table 1. Soil characteristics were pH 7.58, electrical conductivity 1.51 ds cm^{-1}, total organic matter 0.74%, total nitrogen 70.0 mg kg^{-1}, total phosphorus 15.3 mg kg^{-1}, potassium 132 mg kg^{-1}, magnesium 114 mg kg^{-1} and calcium 560 mg kg^{-1}. All plants were watered as needed with tap water to maintain soil moisture near field capacity (75–80%) and fed once weekly with 35 g N m^{-2} as potassium nitrate and 35 g P m^{-2} as superphosphate as a nutritive solution. Harvesting (ten plants per each treatment) was carried out 12 weeks after transplant.

2.2. Measurements

2.2.1. Growth and fruit yield parameters

At harvest, shoot height and leaf number per plant were recorded. Fresh and dry (70 °C for 48 h) weights of shoots and roots were determined. Leaf area was measured using a leaf area meter (Li-Cor, Lincoln, NE, USA). Fruit number for each treatment was also recorded. Fruit thickness was measured by a caliper. Average weight of fruit's fresh and dry masses in each treatment was recorded.

2.2.2. Estimation of fruit quality

Total acidity in fruits for each treatment was determined in the supernatant obtained by extracting 10 g of fruit with distilled water according to the method of Wills and Ku (2002) using citric acid as a reference. Total soluble sugars in fruit extracts

Table 1 Physical and chemical analyses of soil used throughout this study.

Sand (%)	62
Silt (%)	15
Clay (%)	23
Soil texture	Sandy loamy
pH	7.58
E.C (ds m^{-1})	1.51
Organic matter (%)	0.74
N (mg kg^{-1})	70.0
P (mg kg^{-1})	15.3
K (mg kg^{-1})	132
Mg (mg kg^{-1})	114
Ca (mg kg^{-1})	560

were determined with antheron reagent spectrophotometrically at 620 nm according to Stewart (1974). Fruit carotenoids were extracted from the fruit pericarp by acetone (85%) and determined spectrophotometrically according to Lichtenthaler and Weliburn (1983). Vitamin C in the fruit extract was estimated according to Pearson (1970). Lycopene content in the fruit extracts was assessed by RP-HPLC (Allteck, Milano, Italy), the pigment was separated using a C_{18} luna column equipped with a luna C_{18} guard column, utilizing a solution of MeOH/THF as mobile phase. The flow rate was set at 2 ml/min and the elution of the compounds was obtained at isocratic conditions. Detection was performed at 450 nm and the peaks were tentatively identified by comparing their retention times to those of the lycopene standard (Sigma, USA).

2.2.3. *Estimation of photosynthetic pigments*

Photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) in leaves were assayed according to Hiscox and Israelstam (1979). The extraction was made from 100 mg of fresh sample in acetone (80%) in the dark at the room temperature and was measured with a UV/VIS spectrophotometer (Shimadzu UV-160, Kyoto, Japan).

2.2.4. *Nutrient analysis*

Oven-dried leaf plant tissues were grounded and sieved through a 0.5 mm sieve. A known weight of the grounded material was digested in a digestion flask containing a triple acid mixture (HNO_3:H_2SO_4:60% HCl_4, with a ratio of 10:1:4; respectively) for analysis of phosphorus and potassium. Phosphorus (P) was extracted by nitric–perchloric acid digestion and measured using the Vanadomolybdophosphoric acid colorimetric method (Jackson, 1973). Potassium (K) was assayed using a flame spectrophotometer (Corning 400, UK). Total nitrogen (N) was determined by the Kjeldahl method (Nelson and Sommers, 1973).

2.2.5. *Measurements of total leaf conductance and transpiration rate*

Total leaf conductance and transpiration rate of the tomato leaves were measured using Li-Cor, 6400XT, Lincoln, NE, USA.

2.3. *Statistical analysis*

A randomized block design with ten replicates was adopted. The data were statistically analyzed using one-way analysis of variance, and the means were separated by Duncan's multiple range test by the least significant difference (LSD, $P \leqslant 0.05$) method using Costat software (Cohort, Berkeley, CA).

3. Results and discussion

3.1. *Plant growth*

As compared to control plants, seeds presoaked with various concentrations of shikimic acid significantly increased shoot

Table 2 Effect of seed presoaking in shikimic acid on growth responses and leaf area of tomato plants.

Parameters	Presoaking treatments			
	Control (distilled water)	Shikimic acid (ppm)		
		30	60	120
Shoot fresh wt. (g/plant)	38.4[*,c]	50.5[b]	88.3[a]	75.4[a]
Root fresh wt. (g/plant)	10.2[d]	20.3[c]	32.4[a]	27.1[b]
Shoot dry wt. (g/plant)	4.03[c]	6.55[b]	10.08[a]	9.55[a]
Shoot dry wt. (g/plant)	1.21[c]	2.53[b]	3.44[a]	3.00[a]
Shoot height (cm/plant)	31.5[c]	35.1[b]	42.0[a]	39.5[ab]
No. of leaves/plant	107[c]	188[b]	276[a]	220[b]
Leaf area (cm^2/plant)	30.3[c]	39.8[b]	47.5[a]	42.3[b]

[*] Values in each row followed by the same letter(s) are not significantly different at $P \leqslant 0.05$ (Duncan's multiple range test).

Table 3 Effect of seed presoaking in shikimic acid on fruit yield and quality of tomato plants.

Parameters	Presoaking treatments			
	Control (distilled water)	Shikimic acid (ppm)		
		30	60	120
Number of fruits/plant	8.5[*,c]	14.0[b]	18.6[a]	15.5[b]
Fruit fresh wt. (g/plant)	102.6[c]	130.4[b]	150.4[a]	138.2[b]
Fruit dry wt. (g/plant)	7.12[c]	10.3[b]	14.9[a]	11.5[b]
Fruit thickness (mm)	7.1[bc]	7.6[b]	8.4[a]	8.1[a]
Total soluble sugars (mg/g dwt)	59.9[d]	67.2[c]	77.5[a]	70.8[b]
Total acidity (mg/L)	2333[d]	2733[c]	3883[a]	2931[b]
Vitamin C (mg/100 cm^3 juice)	244[bc]	254[b]	280[a]	250[b]
Carotenoids in fruit (mg/g dwt)	0.25[c]	0.37[b]	0.55[a]	0.40[b]
Lycopene (mg/100 g)	13.94[c]	16.58[b]	24.32[a]	18.02[b]

[*] Values in each row followed by the same letter(s) are not significantly different at $P \leqslant 0.05$ (Duncan's multiple range test).

height, number of leaves, leaf number, fresh and dry weight of tomato plants (Table 2). The magnitude of increase appears to depend mainly on the concentration used, whereas with the concentration increase there is a simultaneous increase up to the limit in the above growth parameters. 60 ppm of shikimic acid was most efficient in its ability to increase plant growth in comparison with other treatments. Shikimic acid being a precursor of phenolic compounds has been shown to be of great importance in the regulation of growth and they are no longer considered to be passive by-products (Jain and Srivastava, 1981). The improvement in growth parameters of tomato plants in response to shikimic acid application might be mediated through the increased longevity of leaves by retaining chlorophylls and increasing mineral contents which perhaps contributed to increased plant growth (Neera and Garg, 1989; Gupta, 1990). In addition, the results obtained here are in agreement with the results of Aldesuquy and Ibrahim (2000) who stated that seed priming with shikimic acid increased growth and yield of cowpea plants grown under greenhouse conditions through increasing the total soluble sugars protein content and photosynthetic activity.

The increase in the leaf area of tomato plants in response to shikimic acid application could have resulted from the rapid rate of movement of nutrients and hormones transported through transpiration stream from the root, which can accelerate the rate of leaf expansion in the developing leaves (Aldesuquy and Ibrahim, 2000). In connection with these results, it was reported that a low concentration of salicylic acid increases the growth of maize seedlings, with the higher concentration inhibiting it (Jain and Srivastava, 1981). Furthermore, Abo-hamed et al. (1987) reported that the soil drenched with salicylate led to an increase in the fresh and dry weight of shoot and at lower concentration appeared to enhance plant height and leaf area of wheat plant.

3.2. Fruit yield and quality

It is clear from Table 3 that the application of shikimic acid at various concentrations greatly affected the flower yield and quality of tomato plants. Thus seed presoaking with shikimic acid at all doses increased significantly fruit number, average fruit weight and fruit thickness of tomato plants compared with control plants. Our findings are supported by the results of Aldesuquy and Ibrahim (2000) who stated that shikimic acid induced an increase in growth and yield of cowpea plants. The beneficial effects of shikimic acid on fruit yield may have been due to the translocation of more photoassimilation to fruits, thereby increasing fruit weight (Gunes et al., 2007; Elwan and El-Hamahmy, 2009; Favati et al., 2009). In the same direction, the significant highest value of total acidity in fruit juice was increased by 66.4% with the treatment of 60 ppm of shikimic acid over the control treatment.

The data in Table 3 showed that high vitamin C and carotenoids were significantly increased in fruits of tomato plants treated with shikimic acid, particularly 60 ppm, compared with non-treated control plants. Since ascorbic acid (vitamin C) plays an important role as an antioxidant and protects the plant during oxidative damage by scavenging free radicals and ROS that are generated by various stresses (Schulthesis et al., 2002; Elwan and El-Hamahmy, 2009). Higher content of ascorbic acid might maintains relatively lower levels of ROS in pepper and tomato fruit resulting in less damage caused by ROS after stress. In addition, regarding the protection role of ascorbic acid against oxidative damage, several epidemiological and experimental studies have shown that the consumption of foods rich in vitamin C is associated with a decreased risk of several chronic diseases, including cardiovascular disease and cancer (Jacob and Sotoudeh, 2002).

In general, seed priming of tomato plants with shikimic acid significantly increased total soluble sugar content in fruits compared with control treatment (Table 3). These results are in agreement with the results of Ethness and Roitsch (1997) who demonstrated that carbohydrate metabolism in tomato plants is regulated by different plant hormones. Furthermore, shikimic acid is a precursor of many phenolic compounds and these substances are known to provide protection to auxins against oxidation (Schneider and Whitman, 1974). The increased levels of auxins may result in an increase in invertase enzymes as it has been demonstrated by Glaszou et al. (1966). This fact could explain the observed increase in total soluble sugar content in developing seeds.

Lycopene, which is responsible for the red color of tomatoes, is greatly affected by the application of shikimic acid. It has been shown that shikimic acid at various concentrations induced drastic increases in lycopene in tomato plants particularly at 60 ppm. These results are in agreement with the results of Garcia and Barret (2006) and Favati et al. (2009) who stated that the content of lycopene in fresh tomato fruit was strongly dependent on agricultural techniques and processing methods.

3.3. Photosynthetic pigments

It is clear from Table 4 that shikimic acid at all concentrations significantly increased the contents of the photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) in leaves of tomato plants when compared to non-treated control plants. Such stimulations in these contents were highly remarked at 60 ppm. The stimulative effect exerted by shikimic acid on pigment biosynthesis might presumably be due to the fact that shikimic acid increases the rate of transpiration and this will possibly increase the rate of transpiration of minerals and cytokinin from the root to the developing shoot (Aldesuquy and Ibrahim, 2000). Moreover, Uheda and Kuraishi (1978) found that kinetin increased both transpiration and chlorophyll synthesis. In this study, higher concentrations of lycopene corresponded to higher chlorophyll "a" values, confirming the correlation between lycopene and chlorophyll "a" (Arias et al., 2000).

3.4. Nutrient contents

The results in Fig. 1A–C showed that the application of shikimic acid significantly increased the concentrations of N, P and K in leaves of tomato plants comparing to control plants. The magnitude of increase in these nutrients was markedly observed at 60 ppm of shikimic acid. On the other hand, no significant differences were observed in N and K concentrations in leaves of tomato plants between plants treated with 30 ppm of shikimic acid and control plants. In connection to these results, Xu and Tian (2008) concluded that the application of nitrogen and salicylic acid at low concentration positively increased the foliage fresh and dry weight, fruit yield

Table 4 Effect of seed presoaking in shikimic acid on the content of photosynthetic pigments in leaves of tomato plants.

Presoaking treatments	Photosynthetic pigments (μg/g fresh wt.)			
	Chlorophyll "a"	Chlorophyll "b"	Carotenoids	Total
Control (distilled water)	1018[*,c]	790[c]	169[c]	1977[c]
Shikimic acid (ppm)				
30	1175[c]	850[c]	210[c]	2235[c]
60	1910[a]	1340[a]	320[a]	3570[a]
120	1765[b]	1110[b]	195[b]	3070[b]

[*] Values in each column followed by the same letter(s) are not significantly different at $P \leqslant 0.05$ (Duncan's multiple range test).

and mineral contents of pepper plants. Furthermore, the results in this study were in good agreement with those obtained by Aldesuquy and Ibrahim (2000) who demonstrated that seeds' retreatment with shikimic acid significantly increased mineral contents like P, N. Mg and K in leaves of cowpea plants under natural conditions.

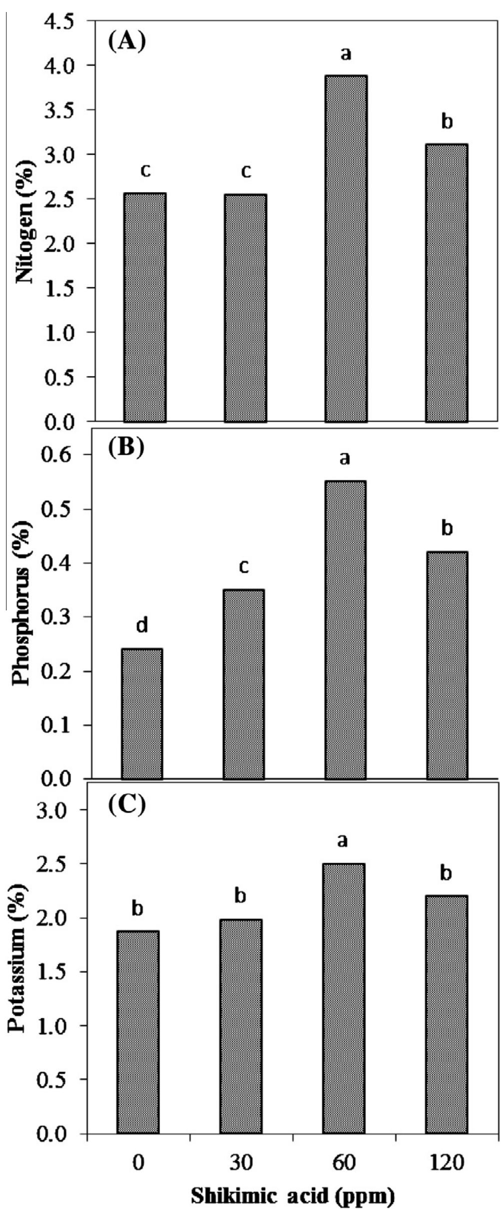

Figure 1 Effect of seed presoaking in shikimic acid on concentration of nitrogen (A), phosphorus (B) and potassium (C) of tomato plants. Data labeled with different letters are significantly different at $P \leqslant 0.05$.

3.5. Total leaf conductivity and transpiration rate

In general, presoaking of tomato seeds with shikimic acid at 30, 60 and 120 ppm induced a drastic increase in total leaf conductance and transpiration rate of tomato leaves when compared to control plants (Fig. 2A and B). The magnitude of response varied among the concentration of shikimic acid applied. 60 ppm gave the highest response in the transpiration rate of tomato plants among all treatments. The increase in

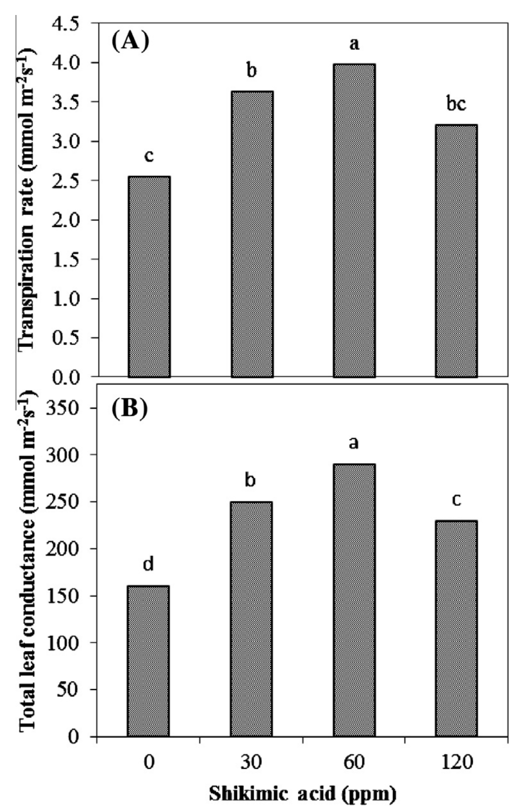

Figure 2 Effect of seed presoaking in shikimic acid on transpiration rate (A) and total leaf conductance (B) of tomato plants. Data labeled with different letters are significantly different at $P \leqslant 0.05$.

transpiration rate of tomato plants in response to shikimic acid application may result from the fact that shikimic acid increases the biosynthesis of phenolic compounds particularly coumarin (Saito et al., 1997) which increases the number of both stomata and epidermal cells and therefore increase the rate of water vapor loss through stomata and finally resulted in an obvious increase in total leaf conductance in plants (Gupta, 1992). These results of this study are in agreement with those obtained by Aldesuquy and Ibrahim (2000) in cowpea plants. The analysis of the results in this study (data not shown) revealed that the qualitative parameters of fruit yield and quality were positively related with the amount of transpiration rate and total conductivity in leaves of tomato plants.

4. Conclusions

This study has clearly concluded that seed priming with shikimic acid improves plant growth, and fruit quality of tomato plants grown under field conditions by increasing photosynthetic pigments, transpiration rate, enhancing vitamin C, lycopene and carotenoid contents, accumulation of sugars in fruits and nutrient contents of tomato plants. It was observed that 60 ppm of shikimic acid is needed for high yield and better fruit quality of tomato plants when compared to other treatments. In future, this study will be extended to include further investigations on the effect of shikimic acid on some metabolic pathways, different enzymes and endogenous hormonal levels.

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