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GROWTH AND PHYSIOLOGICAL RESPONSES OF TOMATO (*LYCOPERSICON ESCULENTUM* MILL.) TO SIMULATED ACID RAIN

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Abstract

This investigation was undertaken to ascertain the effect of simulated acid rain (SAR) on growth, yield and physiological parameters of tomato. SAR exposure (pH 3.0 and 4.0) caused white-to-tan spots on the abaxial and adaxial surface of tomato leaves. SAR exposure at pH 3.0 and 4.0 significantly suppressed pigment synthesis, shoot and root dry weights and yield of tomato. The effects were more pronounced at lower pH 3.0. Reducing and nonreducing sugars were significantly diminished to varying degree by SAR solutions of pH 3.0 and 4.0 and the effect being more accentuated at pH 3.0. Nonreducing sugars declined to a greater extent than did the reducing sugars and this effect was more pronounced in SAR-treatment of pH 3.0. SAR-exposure of pH 3.0 and 4.0 resulted in accumulation of soluble phenols as an induced mechanism against SAR stress. The results are discussed in the light of physiological responses of plants to abiotic stresses.

Introduction

Acid rain is a major polluting agent possibly harmful to terrestrial and aquatic ecosystems (Wellburn, 1989; Heij & Erisman, 1997; Driscoll *et al*., 2001; Brimblecombe *et al*., 2007). With regard to the effect on plants, it is known that acid rain alters the leaf physiology (Evans, 1982; Haines *et al.*, 1985; Agarwal & Agarwal, 1999) which in turn might influence the response of plants to other stresses such as the attack of pathogens (Bolla & Fitzsimmons, 1988). The results of simulated acid rain (SAR) treatment on different plant species (or even different varieties of the same species) obtained by various workers are often contradictory. Heagle *et al.*, (1983) investigated the effect of SAR in the field on growth and yield of soybeans and the chemical properties of soils. Although, SAR at pH 2.8 caused slight foliar injury, plant growth, pod yield, and seed protein content remained unaffected. Acid rain exposure of plants results in characteristic foliar injury symptoms, modified leaf anatomy, structural changes in the photosynthetic pigment apparatus and a decrease in chlorophyll a and b contents (Soares *et al.*, 1995; Siffel *et al.*, 1996; Silva *et al*., 2005; Sant´Anna-Santos *et al*., 2006). SAR exposure alters the ability of plant to take in CO_2 for photosynthesis that consequently inhibits the production of reducing sugars such as glucose (Velikova *et al*., 1999). Forsline *et al.*, (1983), Tong & Liang (2005) and Zhang *et al*., (2005) found a rapid decrease in soluble sugars following application of simulated acid rain at low pH (less than 3.5). By contrast, Shumejko *et al*., (1996) found no effect of SAR on the concentration of sugars in the needles of Scots pine.

Plants have evolved various mechanisms to defend themselves with various types of stresses, including biotic and abiotic stresses. One such mechanism involves accumulation of free phenols in the roots of plants in response to both biotic and abiotic stresses (Nicholson & Hammerschmidt, 1992; Ellard-Ivery & Douglas, 1996; Dixon &

Paiva, 1995; Abreu & Mazzafera, 2005; Olenchenko & Zagoskina, 2005). This investigation attempts to examine the effect of simulated acid rain (SAR) on growth, yield, pigment synthesis, levels of reducing and nonreducing sugars and soluble phenol accumulation in tomato plant.

Materials and Methods

Plant culture and treatments: Two-week-old tomato (*Lycopersicon esculentum* Mill.) cv Sun seedlings raised in steam sterilized soil were planted in each of the 18 cm diameter plastic pots containing 1.5 kg sandy loam (72% sand, 18.6% silt and 9.4% clay; pH 7.7) In each pot 200 g compost was mixed with the soil. Simulated acid rain (SAR) treatment was applied in the form of a solution prepared in accordance with Capron & Hutchinson (1986). SAR solution contained 20 µmol dm^{-3} KOH, 27 µmol dm^{-3} $CaSO_4$, 10 µmol dm^{-3} NaOH, 27 µmol dm^{-3} $FeCl_3$, 0.1 µmol dm^{-3} $PbCl_2$, 1.5 µmol dm^{-3} $ZnCl_2$, 0.18 µmol dm^{-3} $MnCl_2$ and 0.15 µmol dm^{-3} $CuCl_2$.

The desired pH was adjusted by using a mixture of 50µmol dm^{-3} H_2SO_4 and HNO_3. SAR solutions were adjusted to pH 3.0, 4.0 and 5.0 while deionized distilled water (pH 7.0) was used for controls. Seedlings were allowed to establish in pots for four weeks before any treatment was given. SAR exposure was in the form of twice a week shower of 200 ml acid rain solution that was simulated by sprinkling appropriate solutions from a hand-held atomizer. To expose the plants to SAR, pots were transferred to a separate chamber and returned to the greenhouse benches immediately following exposure.

Treatments and controls were replicated ten times and randomized on a greenhouse bench. Day/night temperature regime in the green house was 33/26ºC with 14 h photoperiod. Relative humidity during the experiment varied between 50-65%. Plants were irrigated on alternate days with 250 ml of tap water. Each pot was provided with 100 mg urea and 100 mg Potassium phosphate, one month after treatment as the soil was low in NPK. The plants were exposed to the designated SAR-solution twice a week.

Growth and yield: Plants were harvested at 68 days after first SAR-exposure and growth parameters, including root and shoot dry weights (70ºC for 24h) and tomato yield (fresh weight of fruits) were recorded. Five replicates were used for dry matter and yield measurements.

Chlorophyll and visual symptoms: Visual symptoms of toxicity if any were noted. Chlorophylls a and b contents of leaves were determined by extracting 1g of fresh leaves from each plant separately in 100 ml of 80% acetone at 20 and 40 days after treatment. The extract was filtered and optical densities were recorded at 663 and 645nm for the estimation of chlorophylls a and b respectively (Arnon, 1949).

Sugar content**:** Sugars were determined at 10, 20, 30, 40, 50 and 60 days following treatments. Total soluble sugars other than starch were extracted from fresh leaf material according to the procedure of Cerning & Guilhot (1973). Total soluble sugars were determined spectrophotometrically using 0.2 % anthrone in concentrated sulphuric acid as reagent following the method of Yemm & Willis (1954). Reducing sugars were estimated using alkaline-copper and arsenomolybdate as reagent in accordance with Nelson-Somogyi's modified method (Marais *et al*., 1966). The amount of nonreducing sugars was calculated as the difference between total soluble sugars and reducing sugars.

Soluble phenols: Soluble phenol contents were ascertained at 10, 20, 30, 40, 50 and 60 days after treatments. Levels of soluble phenols in roots were determined in accordance with Dihazi *et al.*, (2003). Root tissues (500 mg) were taken from each plant and homogenized in an ice bath with 2ml 80% methanol v/v. The homogenate was centrifuged three times at 6000 g for 3 min. One hundred µl of the supernatant was added to Folin-Ciocalteau reagent (0.5 ml) and 1 ml saturated Sodium carbonate. The mixture was incubated at 40^0C for 30 min. and the absorbance of the developed blue colour was read at 725 nm. Catechol was used as standard. The amount of soluble phenols was expressed as µg mg^{-1} fresh weight. All biochemical analyses were performed using samples from five replicates.

Statistical analysis: Data were subjected to statistical analysis following Zar (1999). One-way analysis of variance (ANOVA) or factorial analysis of variance (FANOVA) were performed appropriate to the experimental design used. Duncan's multiple range test was employed as a post-hoc procedure.

Results

Dry matter production and yield: Simulated acid rain (SAR) at pH 3.0 and 4.0 significantly (P at the most 0.05) suppressed shoot and root growth (dry weight) of tomato plants compared to controls (Table 1). Shoot growth was reduced by 24.5 and 20.4%, while root growth was decreased by 32.1 and 25.0% by SAR at pH 3.0 and 4.0 respectively compared to controls. Greater reduction in shoot and root dry weights ($p<0.01$) was found in SAR-treatment at pH 3.0 relative to SAR of pH 4.0 and root growth was more affected than the shoot growth. Fruit weight (yield) was significantly abated (P at the most 0.05) by SAR at pH 3.0 and 4.0 and the reduction in yield was 30.2 and 24.1 % at pH 3.0 and 4.0 respectively over the controls.

Plant symptoms and Leaf chlorophyll: SAR-treatments of pH 3.0 and 4.0 caused white-to-tan irregular lesions on both the abaxial and adaxial surface of tomato leaves. The foliar symptoms were more pronounced in SAR-treatment at the lowest pH 3.0, while such lesions were less pronounced in SAR at pH 4.0. The foliar injury symptoms began to appear earlier (*i.e.,* two weeks after the commencement of treatment) in SAR-solution at pH 3.0 and later (between 3 and 4 weeks) in SAR solutions of pH 4.0, and only a few less prominent lesions were seen at pH 5.0. Chlorophyll a and b were both significantly reduced by SAR-treatment (P at the most 0.05) relative to controls (Table 2) at pH 3.0 and 4.0. Greater reduction in the pigment synthesis was caused by SAR-solution of pH 3.0 compared to that of pH 4.0 while no significant difference in chlorophyll a and b content was recorded at pH 5.0. Chlorophyll a was more affected than chlorophyll b.

Sugar concentration: The level of reducing sugars decreased significantly (P at the most 0.05) by SAR-treatment relative to controls from 10^{th} day onwards following exposure to SAR of pH 3.0 and from 20^{th} day at pH 4.0 (Table 3). In SAR-treatment at pH 5.0, reducing sugars were significantly lower than the controls only at 30 and 40 days following treatment. Nonreducing sugars also declined significantly (P at the most 0.01) by SAR-exposures of various pH compared to controls at most of the sampling periods (Table 4). The most spectacular depletion of nonreducing sugars occurred in SAR exposure of pH 3.0.

Table 1. Effects of simulated acid rain (SAR) on dry matter production and yield of tomato at 65 days after treatment (mean ± standard deviation). Means in a column sharing the same letter are not significantly different at p≤0.05.

Treatments	Dry weight of shoot (g)	Dry weight of root (g)	Fruit weight per plant (g) fresh weight
Control	9.8[a] ±1.2	2.8[a] ±0.3	267.4[a] ±14.5
SAR pH 3.0	7.4[b] ±1.7	1.9[b] ±0.5	188.6[b] ±12.7
SAR pH 4.0	7.8.[b] ±1.3	2.1[a] ±0.4	203..0[b] ±15.4
SAR pH 5.0	9.5[a] ±1.4	2.4[a] ±0.6	258.7[a] ±18.2

Table 2. Effect of simulated acid rain (SAR) exposure at different pH on chlorophylls a and b content (mg/g FW) of tomato leaves at 20 and 40 days following the first treatment (mean ± standard deviation). Means in a column sharing the same letter are not significantly different at p≤0.05.

.Treatment	Day	Chlorophyll a	Chlorophyll b
Controls	20	0.765a ± 0.15	0.417a ± 0.13
	40	0.758a ± 0.12	0.423a ± 0.09
SAR pH 3.0	20	0.649b ± 0.14	0.378b ± 0.13
	40	0.653b ± 0.17	0.365 b± 0.15
SAR pH 4.0	20	0.733b± 0.10	0.384b ± 0.12
	40	0.724b ± 0.16	0.387b ± 0.08
SAR pH 5.0	20	0.748a ± 0.14	0.410a ± 0.14
	40	0.755a ± 0.15	0.404a± 0.12

Table 3. Effect of simulated acid rain (SAR) treatment at different pH on reducing sugar content of tomato leaves (mg/g FW) at various time periods after first treatment (mean ± standard deviation). Means in the columns sharing the same letter are not significantly different at p≤0.05.

Treatment	Days					
	10	20	30	40	50	60
Controls	17.4a ± 1.2	17.6a ± 0.9	18.2a ± 1.4.	17.3a ± 1.8	18.8a ± 1.4	18.1a ± 1.2
SAR pH 3.0	15.6b ± 1.4	14.5b ± 1.2	15.4b ± 0.9	14.7b ± 0.7	14.5b ± 1.3	13.9b ±1.0
SAR pH 4.0	16.3c ± 0.9	16.0c ± 1.5	17.5c ± 1.0	16.4c ± 0.9	15.0b ±1.5	16.6c ±1.4
SAR pH 5.0	16.8ac ± 1.3	17.2a ± 1.0	17.7c ± 1.7	16.8ac ±1.1	18.3a ± 1.6	18.5a ±0.9

Table 4. Effect of simulated acid rain (SAR) exposure at different pH on nonreducing sugar content of tomato leaves (mg/g FW) at various time periods after treatments (mean ± standard deviation). Means in the columns sharing the same letter are not significantly different at p≤0.05.

Treatment	Days					
	10	20	30	40	50	60
Control	11.7a ± 1.4	11.2a ± 1.1	10.2a ± 1.2	12..3a ± 0.9	11.5a ± 1.2	10.6a ±1.5
SAR pH 3.0	8.3b ± 0.9	7.2b ± 1.1	8.1b ± 0.8	8.4b ± 1.2	7.6b ± 0.9	7.4b ±1.0
SAR pH 4.0	8.7b ± 0.5	9.0b ± 0.7	9.4b ± 0.4	11.0b ± 0.5	10.7b ± 0.3	10.3a ±0.8
SAR pH 5.0	12..2a ± 0.9	11.4a ± 0.8	9.7ab ± 1.5	11.6ab ± 1.1	12.4a ± 1.3	10.9a ±1.6

Table 5. Effect of simulated acid rain (SAR) at different pH on soluble phenol content (µg/g FW) of tomato roots at various time periods after treatment (mean ± standard deviation). Means in the columns sharing the same letter are not significantly different at p≤0.05.

Treatment	Days					
	10	20	30	40	50	60
Control	354a ± 16	347a ± 14	351a ± 22	346a ± 16	350a ± 19	362a ±17
SAR pH 3.0	362a ± 15	496b ± 21	513b ± 26	461b ± 20	447b ± 19	456b ±23
SAR pH 4.0	364a ± 18	391c ± 17	408c ± 22	388c ± 19	379c ± 18	380ac ±21
SAR pH 5.0	349a ± 20	356a ± 18	349a ± 20	362a ± 16	338a ± 17	361a ±22

Soluble phenol concentration: Treatment of tomato plants with SAR (pH 3.0 and 4.0) resulted in high phenol accumulation in tomato roots over the controls (P at the most 0.05) at 20 days onwards following treatment (Table 5). Phenol accumulation was greatest in SAR-treatment at pH 3.0 compared with SAR of pH 4.0 while SAR at pH 5.0 did not exhibit significant accumulation in soluble phenols. Maximal amounts of soluble phenols were recorded on 30th and 40th day following the first SAR-treatment at pH 3.0 and 4.0. The level of soluble phenols was roughly one and a half times higher in SAR-treatment at pH 3.0 compared to controls at respective time periods.

Discussion

Simulated acid rain (SAR) at pH 3.0 and 4.0 caused characteristic white-to-tan irregular lesions on both the surfaces of tomato leaves which was associated with decreased chlorophyll content. This accords well with the earlier results of Sheridan & Rosenstreter (1973), Evans (1982), Percy (1986) and Khan & Khan (1994). Greater foliar injury occurred at SAR-treatment of lowest pH (3.0). Leaf chlorosis and lesions would compromise the photosynthetic capability of plants. Levels of photosynthetic assimilates,

including sugars, free amino acids and organic acids depend greatly on the level of photosynthetic pigments and their efficiency (Velikova *et al*., 19997; Hopkins, 2003). Shan (1998) demonstrated that acid rain reduces the efficiency of the use of chlorophyll in photosynthesis which may be linked to the increase in the rate of degradation of chlorophyll to pheophytin 'a' that results in reduction of net photosynthetic rate. Siffel *et al.,* (1996), Velikova *et al.,* (1999) and Yu *et al.,* (2002) demonstrated that SAR-exposure of leaves results in an increase in the intensity of chlorophyll fluorescence emission (*i.e*., changes in photosystem II activity) of the light-harvesting complex (LSH)) that indicates structural changes of photosynthetic pigment apparatus (thylakoid membrane system) resulting from direct foliage-mediated action of acid rain. Increased chlorophyll 'a' fluorescence results not only from acid rain exposure but also in response to different atmospheric pollutants such as SO_2, O_3 and NO_X and other stresses (Calatayud, 2007). Reduced photosynthetic rate would result in lower levels of photosynthetic assimilates and consequently reduction in plant growth (root and shoot dry matter) as well as the yield.

Both reducing and nonreducing sugars markedly declined in SAR exposure at pH 3.0 and 4.0 which corresponds with the findings of Ferenbough (1976), Forsline (1983) and Zhang *et al.,* (2005) who found reduced carbohydrate (sugar) production following SAR-treatment. Nonreducing sugars were depleted to a greater extent than did the reducing sugars. This corroborates the earlier results of Bolla & Fitzsimons, 1988) who recorded a remarkable decrease in nonreducing sugars in SAR treated pine seedlings at lower pH.

Simulated acid rain (SAR) treatment resulted in the accumulation of soluble phenols in tomato roots. It has been established that phenol metabolism is activated in plants reacting to various biotic and abiotic stresses (Nicholson & Hammerschmidt, 1992; Metraux & Raskin, 1993; Ellard-Ivery & Douglas, 1996; Dihazi *et al.,* 2003; Dixon & Paiva, 1995; Abreu & Mazzafera, 2005; Olenchenko & Zagoskina, 2005; Ganeva & Zozikova, 2007). Shumejko (1996) reported increased level of high molecular weight phenols in the needles of Scots pine while Suomela *et al.,* (1998) recorded increased low molecular weight phenolics in birch leaves. Our results showing enhanced production of free phenols in response to SAR exposure confirms the role of these compounds in activating plant defense system under stress condition. The production of free phenols was much accentuated at SAR of pH 3.0. Increased synthesis of phenols would require precursors of simple carbohydrates derived from glycolysis and pentose phosphate shunt for the synthesis of various phenolic acids *via* the shikimic acid pathway (Vermerris & Nicholson, 2006). This need for precursors for the biosynthesis of phenolics suggests diversion of *de novo* synthesized and stored carbohydrates (nonreducing sugars) away from pathway of energy production to pathways for synthesis of a chemical response. Use of energy resources to respond to stress could alter the ability of tomato plant to maintain its ability to respond to other forms of stresses.

Acid rain is known to alter leaf physiology, reduces the ability of plants to resist pathogens (Haines *et al.,* 1985, Bolla & Fitzsimons, 1988) and might, therefore, influence the response of tomato to other types of stresses including susceptibility to pathogens (Nicholson & Hammerschmidt, 1992; Khan & Khan, 1994). SAR treatment resulted in a significant depletion of sugars, particularly nonreducing sugars and increased phenol level. An inverse correlation was observed between sugar content and the levels of soluble phenols, which is presumably the consequence of divergence of precursors of sugar metabolism to the synthesis of secondary metabolities such as phenols. However, the observed relationship between sugar concentration in the leaves and phenol

accumulation in the roots can not necessarily be extrapolated to other species or even to different varieties as varieties of a crop often respond differentially to SAR (Johnston & Shriner, 1986; Forsline, 1983).

SAR-treatment at pH 3.0 and 4.0 markedly suppressed growth and yield of tomato presumably due to reduction of photosynthesis as a result of chlorosis, degradation of chlorophyll to pheophytin or reduced photosystem II activity and as a consequence lesser availability of assimilates (as evidenced by low levels of soluble sugars) and also because of the diversion of assimilates to pathways of secondary metabolism as explained earlier.

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