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A preliminary study on the effects of ozone exposure on growth of the tomato seedlings

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Abstract:

A study was conducted to evaluate the consequence of application of ozone for a diminutive period repeatedly on tomato seedlings to enhance their biomass**.** To study the kinetics of these stress-induced growths in more detail we used short pulses (2 min) of ozone (O_3) exposure at high concentrations as elicitor. The seedlings treated with different concentration of ozone (0.1, 0.2, 0.3, 0.4 ppm) are referred to as T_1, T_2, T_3 and T_4 seedlings respectively. The seedlings treated with ambient air are referred to as control seedlings. Among the treatments, T_2 seedlings have a positive effect of an increased leaf area, enlarged shoot size and root length on biomass production than T_1, T_3, and T_4 seedlings relative to control seedlings. Although T_3 seedlings show enlarged shoot length; the root length and leaf area was reduced than T_2 seedlings. The T_4 seedlings show greatly reduced root length, stunted shoot and shrunken leaf size than T_1, T_2 and T_3 seedlings. Hence, only the T_2 concentration of ozone affords 33% enhanced dry weight and strict biomass allocation to leaves**,** shoot and root**.** Further studies are needed to identify the mechanism of these preliminary conclusions.

Keywords: biomass**,** *Lycopersicon esculentum* cv. PKM1, ozone, reactive oxygen species.

Abbreviations*:* O_3- ozone; ROS-reactive oxygen species**;** ppb-parts per billion; ppm-parts per million.

Introduction

Tropospheric ozone (O_3) is a phytotoxic air pollutant that causes more damage to vegetation worldwide than all other air pollutants combined (Ashmore and Bell, 1991). The biological effects of O_3 on plants have been studied for more than 50 years. Chemical characteristics that are at the basis of its behavior are: high oxidizing power, diffusion coefficient similar to the one of CO_2 (and consequently a certain facility to penetrate the plant tissues), solubility in water 10 times higher than CO_2 and tendency to react with water in sub-basic environment (Izuta, 2006). O_3 can cause foliar injury, reduces the plant growth, productivity and changes in crop quality was reported (Chappelka and Samuelson, 1998). O_3 revealed itself as an important instrument for the study of plant responses to biotic and abiotic stress, and a valid alternative to more expensive and complicated treatments for the induction of resistance to several pathogens and abiotic stress, without presenting moreover particular problems in terms of environmental impact (Sudhakar et al., 2007a, b; Nagendra-Prasad et al., 2007). The environmental problem represented by tropospheric O_3 has lead during the last decades the efforts of researchers to

focus on the study of its effects on plants, and in some cases the investigation has brought to light unpredictable effects that can be utilized for convenient practical application (Eckey-Kaltenbach et al., 1994; Sudhakar et al., 2007a, b). O_3 generates hydroxyl free radicals, which are very reactive oxidizing agents used for the control of weeds and as an antimicrobial agent (pryor, 1999). The term 'hormesis' is derived from the Greek and has variously been cited as meaning 'to urge on, to impel and to excite'. Luckey (1980) provided a more functional definition for hormesis as signifying 'the stimulation by low doses of any potentially harmful agent'. The agents capable of bringing about these stimulatory effects may be either chemical or physical ones. In this article the term "hormesis" will be taken specifically as meaning beneficial effects arising from the application of low doses of O_3.

When the concentration of O_3 exceeds 0.1 ppm, foliar injury to sensitive vegetation was observed by Chappelka and Samuelson (1998). But, while we do our experimentation, application of O_3 repeatedly for a diminutive period efficiently increased the leaf surface area, shoot length and root length of the seedlings on biomass production. In the present paper we standardized the minimum concentration of ozone at which the biomass of the tomato seedlings proficiently increase. Leaf area expansion and enhanced biomass production is a desirable trait to get large amounts of plant material, which are often a prerequisite for scientific investigations. Although, increase in leaf length and leaf width have been shown before in cereal crop species by UV (Alqudat et al., 1998; Bos and Neuteboom, 1998; Nedunchezhian and Kulandaivelu, 1997), in this study the traits associated with inherent variation in shoot, root elongation and leaf area expansion associated with enhanced biomass production has been investigated in tomato plants for the first time by using O_3.

Materials and methods

Plant Material

Lycopersicon esculentum cv. PKM1 seeds were obtained from the TNAU - coimbatore, India. Healthy seeds of uniform size after surface sterilization with 0.1% mercuric chloride solution for 5 min were germinated and potted. Subsequently, well-established seedlings were shifted to closed top chamber. O_3 treatments were performed in the closed top chamber (122 cms in height x 122 cms in diam.) after the emergence of 3rd and 4th leaf.

Ozone generation

O_3 gas was generated by passing dry oxygen gas through a corona discharge type O_3 generator (V can Network model M221, India). The stability of the O_3 concentration in the closed top chamber during the O_3 exposure period was monitored carefully and estimated by UV photometric O_3 analyzer (Thermo Environmental Instruments, Franklin, MA, USA).

Ozone treatments

To study the kinetics of the O_3 stress-induced growths in more detail we used short pulses (2 min) of O_3 exposure at high concentrations as elicitor. Tomato seedlings were examined for their response to O_3 concentration of 0.1, 0.2, 0.3 and 0.4 ppm respectively for a time period of 2 min a day continuously for 10 days (as the O_3 is phytotoxic air pollutant, it is repeatedly passed for a diminutive period to analyze the plants response and increase in treatment time duration caused adverse effects whereas decrease in time duration did not show any reasonable effect on the plants. i.e. O_3 treatment duration was standardized as 2min repeatedly for 10 days) and the treated seedlings are referred to as T_1, T_2, T_3 and T_4 seedlings (i.e. $T_{1=}$ 0.1, $T_{2\,=}$ 0.2, $T_{3\,=}$ 0.3 and $T_{4\,=}$ 0.4 ppm of O_3 passed). The seedlings treated with ambient air are referred to as control seedlings. A minimum of 30 explants were cultured for each treatment and each experiment was repeated thrice.

Growth measurements

Seedlings were harvested on the 3rd week after O_3 exposure and the growth variables such as shoot elongation, root length, leaf area and biomass (dry weight) were analyzed after harvest. For dry-weight measurements, samples were dried at 80°C for 48 h.

Data analysis

The variance analysis in the results and the averages of the treatments were compared following Tukey test at the 5% significance level, using SAS (SAS Institute, 1996), and based on statistical theories by Gomes (2000).

Table 1. Data represents biomass allocation such as shoot length, root length, leaf area and fresh weight as a function of plant size of T_1, T_2 T_3, and T_4 tomato plants after treatment with various concentration of ozone relative to control plants

Seedlings	Shoot length (cm)	Root length (cm)	Leaf area (cm^2)	Dry weight (g)
Control	7.30 [cd]	2.94 [d]	56.34 [d]	3.96 [d]
T_1	8.74 [ab]	4.86 [c]	67.62 [b]	4.78 [b]
T_2	9.06 [a]	6.23 [a]	74.37 [a]	5.23 [a]
T_3	9.24 [a]	6.07 [ab]	71.43 [ab]	5.12 [a]
T_4	7.57 [c]	5.74 [b]	62.78 [c]	4.27 [c]

A minimum of 30 explants were cultured for each treatment and each experiment was repeated thrice. Where, T_1 = 0.1 ppm, T_2 = 0.2 ppm, T_3 = 0.3 ppm, T_4 = 0.4 ppm of ozone passed for a time period of 2 min a day continuously for 10 days (i.e. O_3 treatment duration was standardized as 2min repeatedly for 10 days) and for the control (C) treatment, ambient air was passed. Statistically the means of the three experiments were not significantly different ($P<0.05$). Values in the same column with different letters are significantly different at $P<0.05$ in accordance with Fisher's least significant difference test.

Results and discussion

In vegetable crop production, maximum yield is achieved when there is a better emergence and stand establishment of seedlings. When the plant is attacked by O_3, it puts in action a series of metabolic responses that can result in either induction of damage or resistance (Sudhakar et al., 2007a, b; Nagendra-Prasad et al., 2007). O_3 enters the plant through the stomata, diffuses in the apoplast and once there is rapidly decomposed, giving hydroxyl radical, superoxide, hydrogen peroxide and other ROS (Heath and Taylor, 1997). The formation of hydroxylic radical, in particular, is stimulated by the presence of Fe_{2+}, amines, thiolic groups, caffeic acid (Byovet et al., 1995), and of hydrogen peroxide by reaction of O_3 with unsaturated fatty acids (Pryor and Church, 1991). During the closed top chamber trials, seedlings exposed to acute O_3 exposure for diminutive period (2 min) promotes growth and increases the biomass. Among the four treatments, T_3 seedlings shows maximum increase in the shoot length of 27% followed by T_2 = 24%, T_1 = 18% and T_4 = 4% relative to control seedlings. Following the experimental period, root length got increased to a maximum of 93% in T_2 followed by 88 % in T_3, 78 % in T_4 and 50 % in T_1 relative to control seedlings (Table 1). Although T_3 seedlings posses enlarged shoot, the root length remains smaller than T_2 seedlings because it is likely that the high demand for carbon in the division and expansion zones of the growing leaves resulted in more carbon being used in the shoot instead of going to the roots as reported by Hu et al. (2000). Leaf area of the seedlings was increased in T_1 for about 20%, T_2 = 32.7%, T_3 = 27% and T_4 leaves = 12% relative to control seedlings (Table 1). Possibly, the maximum increase in length and width of T_2 leaves in the present study resulted from an increase in leaf meristem size of successive leaves and increases in apical dome size as described by Bos and Neuteboom (1998). Achieving a higher total leaf area is also important in determining a high ground cover, final biomass, and yield (López-Castañeda et al., 1996). Dry weight of the seedlings got increased to a maximum of 33 % in T_2 followed by T_3 = 30%, T_1 = 21% and T_4 = 8% relative to control seedlings (Table 1). Miller et al. (1998) observed that, elevated CO_2 usually increase partitioning of biomass to branches, decrease partitioning to pods, increase specific leaf weight, and decrease leaf area ratio, whereas O_3 suppressed leaf and root weight ratios, specific leaf weight and increased pod weight ratios, but both O_3 and CO_2 accelerated reproductive development. In addition, we have evidenced that UV significantly increased the shoot, root, leaf biomass in radish and the similar changes were reported for 16 rice cultivars from seven geographical regions (Teramura et al.,

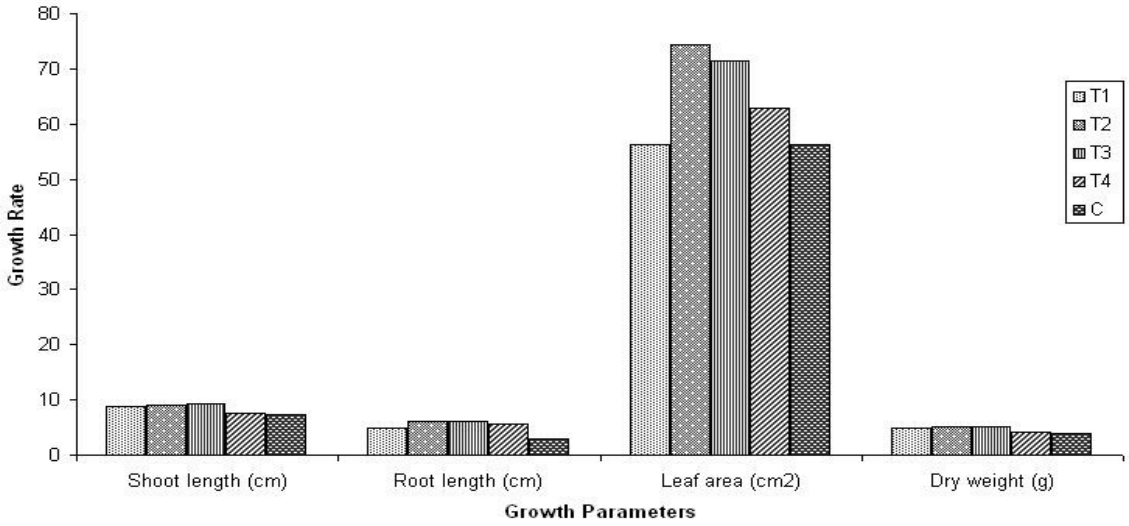

Fig 1. Graph showing comparison of the biomass (shoot length, root length, leaf area, dry weight) of T_1, T_2 T_3, and T_4 tomato plants after treatment with various concentration of ozone relative to control plants. A minimum of 30 explants were cultured for each treatment and each experiment was repeated thrice. Where, T_1 = 0.1 ppm, T_2 = 0.2 ppm, T_3 = 0.3 ppm, T_4 = 0.4 ppm of ozone passed for a time period of 2 min a day continuously for 10 days (i.e. O_3 treatment duration was standardized as 2min repeatedly for 10 days) and for the control (C) treatment, ambient air was passed.

1991) and for the seedlings of six temperate deciduous tree species (DeLucia et al., 1994).

O_3 enters the plant through the stomata, diffuses in the apoplast and once there is rapidly decomposed, giving hydroxyl radical, superoxide, hydrogen peroxide and other ROS (Heath and Taylor, 1997). Reactive oxygen species (ROS) are emerging as important regulators of plant development. There is now abundant evidence that ROS play roles in cell growth (Gapper and Dolan, 2006). There are two major contributors to growth: First, there is cell division, which increases the number of cells in an organ; second, there is expansion of those cells. Recent discoveries suggest that ROS may control development through their role in regulating cell growth. In addition, we have evidenced that in O_3 treated plants H_2O_2 level will be drastically increase, and the peroxidase will get activate to bring back the normal level of H_2O_2 (Sudhakar et al., 2007a). Peroxidase is an oxido-reductive enzyme that involve in wall binding Process. Pangopoulas et al.,(1990) also found that the hormone related changes in plant growth might also be due to an increase in the oxidative enzyme peroxidase.

Precise role of H_2O_2 in the wall differentiation process is unknown, but it may act by stimulating the activity of cellulose synthases that are active during the growth of the plant (Gapper and Dolan, 2006). Whereas ROS are clearly required for growth, spatial regulation of their production is important in determining organ shape and form (morphogenesis). Local accumulation of ROS at the tips of growing root hairs suggests that spatial regulation of ROS production is an important determinant of cell shape (Carol et al., 2005). Genetic evidence suggests that GTPases of the Rho class (called ROPs in plants) are involved in spatial regulation of ROS production and, by extension, spatial control of growth (Carol et al., 2005).

It has been suggested that ROS control cell expansion in maize (*Zea mays*) roots (Liszkay et al., 2004). Whereas there is clearly a role for ROS in root elongation, there is also evidence that NADPH oxidases derived ROS are required during the growth

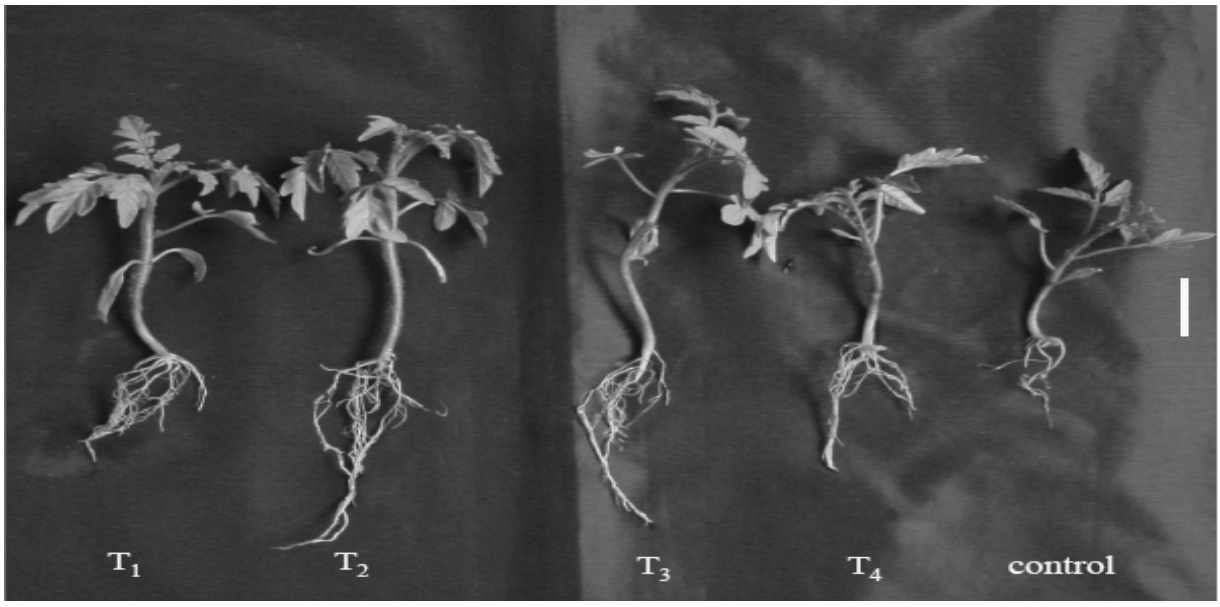

Fig 2. Photograph showing variation in growth due to different concentration of ozone treatments i.e. T_1 , T_2, T_3 and T_4 compared to control plants, without ozone treatment. [Bar 2 cm] A minimum of 30 explants were cultured for each treatment and each experiment was repeated thrice. Where, T_1 = 0.1 ppm, T_2 = 0.2 ppm, T_3 = 0.3 ppm, T_4 = 0.4 ppm of ozone passed for a time period of 2 min a day continuously for 10days (i.e. O_3 treatment duration was standardized as 2min repeatedly for 10 days) and for the control (C) treatment, ambient air was passed.

of other organs. During leaf expansion, a wave of ROS-dependent cell growth sweeps through the leaf (Rodriguez et al., 2002) This suggests that the rate of cell growth may be proportional to the amount of ROS produced in growing organs, but in contrast higher O_3 concentration affects the plant severely. The mechanism by which ROS action these different facets of development remains mysterious, but unraveling them will provide important insights into the mechanism by which ROS controls development. The cell wall plays an important role in cell expansion; loosening the wall allows cells to expand, whereas wall cross-linking can inhibit expansion. There is evidence that ROS are involved in both processes. They have been implicated in the loosening of cell walls in growing tissues (Liszkay et al., 2004) and in making cell walls stiff as growth ceases and cells differentiate (Ros- Barcelo et al., 2002).

Moreover, our findings indicate that T_2 concentration of O_3 stress caused morphological changes effectively by means of promotion of shoot length, root length and leaf area on increased biomass production (Fig 1, 2). O_3 concentration up to 0.3 ppm for 2 min repeatedly for 10 days didn't cause any foliar injury to the tomato seedlings. However, the concentration of O_3 above 0.3 ppm affects the plant physiology. The reason behind the biomass increase by T_2 treatments remains unknown, although it is assumed that some above said mechanisms may help in enhanced plant biomass promotion. We argue this because, in addition we have evidenced that similarly Nedunchezhian and Kulandaivelu, (1997) and Al-Oudat et al. (1998) reported that enhanced UV-B radiation increased leaf area of cowpea, broad bean and wheat plants than the untreated control plants.

This study indicate that, applying O_3 stress at a particular concentration can be used to get large amount of plant materials, which are often a prerequisite for isolating sufficient amounts of plant resources and organelles. As O_3 is a cheap source and easy to handle it can be applied to large number of plants at a same time by fumigation into huge chamber such as glass house, green house, closed top

chamber and open top field chamber using O_3 generator. The present work concerns itself with the issues that would have to be overcome if the concept were to be applied on a commercial basis and the protocol should be standardized for the other plants. Interestingly, there was no previous report on O_3 increasing the plant biomass. Further studies are needed to identify the mechanism of these preliminary conclusions and metabolic changes that occurred due to O_3 treatment.

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