



The role of elevated ozone on growth, yield and seed quality amongst six cultivars of mung bean



Nivedita Chaudhary, S.B. Agrawal*

Laboratory of Air Pollution and Global Climate Change, Department of Botany, Banaras Hindu University, Varanasi-221005, India

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ABSTRACT

Tropospheric ozone (O_3) can be deleterious to plants by decreasing crop yield and quality. Present study was conducted on six cultivars of mung bean (HUM-1, HUM-2, HUM-6, HUM-23, HUM-24 and HUM-26) grown under ambient O_3 (NFC) and elevated O_3 levels (ambient + 10 ppb; NFC+) in open top chambers (OTCs) for two consecutive years. Ozone monitoring data showed high mean ambient concentration of O_3 at the experimental site, which was above the threshold value of 40 ppb. Ozone exposure induced symptoms of foliar injury and also depicted accumulation of reactive oxygen species (ROS) which led to increased membrane damage vis-a-vis solute leakage. Root/shoot allometric coefficient (k), yield and seed quality showed negative response to O_3 . Differential response of mung bean cultivars against elevated O_3 was assessed by comparing the levels of antioxidants, metabolites, growth, total biomass and yield. Cultivar HUM-1 showed maximum sensitivity towards O_3 as compared to other cultivars. Findings of present study emphasized the possibility of selection of suitable O_3 resistant cultivars for the areas experiencing high concentrations of O_3 .

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1. Introduction

Tropospheric ozone (O_3) is recognized as one of the most phytotoxic secondary air pollutant. In the past few decades, an increase in the concentration of O_3 precursors are noted mainly because of combustion of fossil fuels, rapid industrialization and urbanization which substantially accelerate rise in O_3 concentration in lower atmosphere (Ashmore, 2005).

Tropospheric O_3 is projected to increase through 20–25% by the year 2050 and about 40–60% by 2100 (Meehl et al., 2007). Ozone induces number of plant responses including visible leaf injury, premature leaf senescence, reduced plant growth, physiological and biochemical alterations (Chaudhary and Agrawal, 2013) as well as reductions in yield and quality of seeds (Mishra et al., 2013a). Ozone effects on plants is associated with its entry via stomata and rapid reaction with the cellular components to produce reactive oxygen species (ROS) such as hydroxyl radical

($\bullet OH$), superoxide anion ($\bullet O_2^-$) and hydrogen peroxide (H_2O_2) having high oxidation potential to cause detrimental effects on plants (Schraudner et al., 1998). Production of ROS is a normal phenomenon in plants; however, more ROS generation is observed in plants exposed to elevated O_3 . In coming decades, the continued increase in O_3 concentration may cause potential threat to agricultural yield, particularly in China and India (Van Dingenen et al., 2009). It has been suggested that pulses are one of the most sensitive crops against O_3 (Mills et al., 2007).

In view of the above, the present study was focused to assess the impact of ambient and elevated levels of O_3 on the growth, biochemical and yield characteristics by selecting six cultivars of mung bean.

2. Material and methods

2.1. Study area

Experiment was conducted from April to June for two consecutive years (2010 and 2011) at the Botanical Garden of the Banaras Hindu University, Varanasi (25°18'N latitude, 82°01'E longitude and 76.2 m above sea level), situated in the Eastern Gangetic Plains of India. Soil of the experimental plot was alluvial, pale brown and sandy loam in texture (sand 45%, silt 28% and clay 27%) with a slightly alkaline pH (7.3).

Abbreviations: A, age; C, cultivars; DAG: days after germination; HI, Harvest index; LA, Leaf area; NFC, Non-filtered chambers receiving ambient air; NFC+, non-filtered chamber supplied with elevated O_3 (ambient + 10ppb O_3); NOL, Number of leaves plant⁻¹; OTCs, Open top chambers; PH, Plant height; ROS, reactive oxygen species; SP, Soluble protein; YRS, Yield response to stress

* Corresponding author. Tel.: +91 9415309682; fax: +91 542 2368174.

E-mail addresses: nivedita.bhu@gmail.com (N. Chaudhary),

sbagrawal56@gmail.com (S.B. Agrawal).

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Meteorological data were recorded periodically from the Indian Meteorological Division, BHU station, Varanasi, and variations were observed for both the experimental years.

Maximum temperature was recorded highest in the months of April, 2010 (42.8 °C), and May, 2011 (40.7 °C), while lowest was noticed during June (40.1 °C in 2010 and 37.0 °C in 2011). Minimum temperature also varied and ranged from 18.3 to 32.5 °C during the first year (2010) while, 14.2–29.7 °C in the second year (2011). During the first year of experiment, maximum relative humidity varied from 20 to 80% and minimum varied from 13 to 66%. In second year of experiment, maximum relative humidity ranged from 25 to 100%, while minimum varied from 18 to 92%. Total rainfall (April, May and June) was recorded 23.8 mm in 2010 while it was 257 mm in 2011. Sunshine hours ranged from 7.8 to 9.0 h during 2010 and 5.6–9.6 h during 2011.

2.2. Experimental set-up

The experiment was performed in specially designed open top chambers (OTCs) installed at experimental site by following the design of Bell and Ashmore (1986). Each OTC was of dimensions 1.5 × 1.8 m consisting of an aluminium framework covered by 0.25 mm thick polyethylene cover. Each OTC was connected to a heavy duty air blower via conducting duct, and flow rate of the blower was adjusted so as to provide three air changes per minute. Non-filtered chambers receiving ambient air [NFC] and non-filtered chamber supplied with elevated O₃ [ambient + 10 ppb O₃ i.e. NFC+] were used for growing different cultivars and there were three triplicate chamber for each treatment.

Climate models predict that mean surface O₃ concentrations may rise 20–25% globally by 2050, with concentrations in India and south Asia reaching comparable values by 2020 (Van Dingenen et al., 2009); therefore, in the present experiment, 10 ppb O₃ over ambient (20% of the ambient O₃) was selected to expose the plants.

Plants were exposed to elevated O₃ in the respective OTCs with the help of O₃ generators (Model Systrocom, India) attached to the respective blowers for proper mixing of O₃ with the air entering inside the chamber with daily O₃ fumigation duration of 6 h per day (09:00–15:00 h).

2.3. Raising of plants

Six cultivars of mung bean, *Vigna radiata* L. (HUM-1, HUM-2, HUM-6, HUM-23, HUM-24 and HUM-26) were obtained from the Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi. Inside the OTCs, sowing was done in three rows. After one week of germination, plants were thinned to maintain a distance of 15 cm. Seeds were sown in all the experimental set-ups using normal agronomical practices and recommended doses of fertilizers (20, 40 and 20 kg per hectare of N as urea, P as super phosphate and K as murate of potash, respectively) were added during field preparation. Manual weeding was done at regular intervals throughout the experiment.

2.4. Ozone monitoring

Ozone monitoring was done on an 8 h per day basis (9:00–17:00 h) at the experimental site. Air samples at the canopy height were drawn through a 15 m long inert Teflon tube (0.35 cm in diameter). Ozone concentrations were monitored by using UV absorption photometric O₃ analyzer (Model APOA 370, HORIBA Ltd, Japan).

An exposure index for O₃, that is AOT40 (accumulated O₃ over a threshold concentration of 40 ppb during daylight hours) was calculated according to the formula provided by Mills et al. (2007).

2.5. Plant sampling and analysis

Random samples of plants were taken in triplicate from each replicate chamber of treatments, that is NFC and NFC+ for all the cultivars (i.e. $n=9$ for each treatment) at 20, 40 and 60 days after germination (DAG).

2.5.1. Reactive oxygen species and membrane damage

Lipid peroxidation (LPO) was measured as malondialdehyde content according to the method of Heath and Packer (1968), solute leakage was measured according to Dijak and Ormrod (1982) by using the conductivity meter (Model-306, Systronics, India) and superoxide radical (O_2^-) production rate was measured according to the method of Elstner and Heupel (1976).

2.5.2. Metabolite contents and antioxidative enzymes

Total phenolics and protein contents in foliar samples were estimated by using the protocols given by Bray and Thorpe (1954) and Lowry et al. (1951), respectively. Ascorbic acid content was estimated by method of Keller and Schwager (1977).

Activities of ascorbate peroxidase (APX) assessed by the method of Nakano and Asada (1981), catalase (CAT) by the method of Aebi (1984), while glutathione reductase (GR) and superoxide dismutase (SOD) were determined by the method given by Schaedle and Bassham (1977) and Beauchamp and Fridovich (1971), respectively.

2.5.3. Growth characteristics

Plant height, leaf area and numbers of leaves were quantified to assess the growth characteristics of plants. Leaf area was measured using a portable leaf area meter (Model Li-3100, Li-COR, Inc., USA). Plant parts were separated and oven-dried at 80 °C till constant weight and then biomass of different parts was added to get the total biomass (g per plant) of each plant. The allocation pattern was studied by calculating the root/shoot allocation coefficient (k) (Grantz et al., 2006):

$$k = \frac{\text{RGR}_{\text{root}}}{\text{RGR}_{\text{shoot}}}$$

where, RGR denotes relative growth rate of root and shoot.

2.5.4. Yield attributes and seed quality

Harvesting was performed at 80 DAG for each cultivar and ten plants per treatment were sampled. Different yield parameters, such as number of seeds per plant, weight of seeds (g m^{-2}), number of pods per plant and weight of pods (g per plant) were recorded. Harvest index (HI) was calculated as the ratio of economic yield to above ground biomass of the plant.

For the nutrient analyses in seeds, dry powdered seeds samples (0.1 g) were digested in a mixture of HClO₄, HNO₃ and H₂SO₄ (triple acid digestion 5:1:1) by following the method of Allen et al. (1986). The digested samples were filtered through Whatman No. 42 filter paper and volume was maintained to 25 ml with double distilled water. Concentrations of Ca, Mg and K in the filtrate were determined with the help of Atomic Absorption Spectrophotometer (Model 2380, Perkin Elmer, USA). Total nitrogen was quantified by micro-Kjeldahl technique through automatic N analyser (Grehardt, Model KB8S, Frankfurt, Germany). Estimation of total soluble sugars and starch in seeds was conducted by following phenol/H₂SO₄ colorimetric assay (DuBois et al., 1956). Soluble protein (SP) content in seeds was estimated by following the method of Lowry et al. (1951).

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