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Protection of palak (*Beta vulgaris* L. var Allgreen) plants from ozone injury by ethylenediurea (EDU): Roles of biochemical and physiological variations in alleviating the adverse impacts

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ABSTRACT

Ameliorative effects of ethylenediurea (*N*-[2-(2-oxo-1-imidazolinidyl) ethyl]-*N*′ phenylurea, abbreviated as EDU) against ozone stress were studied on selected growth, biochemical, physiological and yield characteristics of palak (*Beta vulgaris* L. var Allgreen) plants grown in field at a suburban site of Varanasi, India. Mean eight hourly ozone concentration varied from 52 to 73 ppb which was found to produce adverse impacts on plant functioning and growth characteristics. The palak plants were treated with 300 ppm EDU at 10 days after germination at 10 days interval up to the plant maturity. Lipid peroxidation in EDU treated plants declined significantly as compared to non-EDU treated ones. Significant increment in F_v/F_m ratio in EDU treated plants as compared to non-EDU treated ones was recorded. EDU treated plants showed significant increment in ascorbic acid contents and reduction in peroxidase activity as compared to non-EDU treated ones. As a result of the protection provided by EDU against ozone induced stress on biochemical and physiological characteristics of palak, the morphological parameters also responded positively. Significant increments were recorded in shoot length, number of leaves plant^{-1}, leaf area and root and shoot biomass of EDU treated plants as compared to non-EDU treated ones. Contents of Na, K, Ca, Mg and Fe were higher in EDU treated plants as compared to non-EDU treated ones. The present investigation proves the usefulness of EDU in partially ameliorating ozone injury in ambient conditions.

1. Introduction

There are enough evidences that background tropospheric ozone concentrations are increasing on large geographical scale (Prather et al., 2003; Simmonds et al., 2004; Carslaw, 2005; Tiwari et al., 2008). Current levels of ozone are high enough to depress crop yield (Emberson et al., 2001; Ashmore, 2005). Increasing traffic load has increased the demand of fossil fuel resulting in increasing emissions of nitrogen oxides (NOx) and volatile organic compounds (VOCs), precursors of O_3. In view of increasing use of fossil fuel in transport sector in the future, concentration of O_3 is likely to increase in both developed and developing countries (Wang and Mauzerall, 2004).

In India, ambient O_3 concentrations are found to cause significant yield reductions (Agrawal et al., 2003b; Tiwari et al., 2005, 2006). Agrawal et al. (2003b) reported 34% reduction in yield of mungbean at a site experiencing 6 h average O_3 concentration of 55 ppb. Singh et al. (2009) reported yield reduction of 16.4% in mustard at 12 h average O_3 concentration ranging from 41.65 to 54.2 ppb during the growth period. Yield reductions of 15.6% and 11.4%, respectively were recorded in rice cultivars NDR 97 and Saurabh 950 at O_3 concentrations ranging from 30.5 to 45.4 ppb (Rai and Agrawal, 2008).

The biological effects of O_3 on plant growth and development have been extensively studied for the last 50 years. A majority of these studies were done using open top chambers (OTCs) which control the flow of pollutants inside the chambers. However, OTCs have effects of their own, often exaggerating the pollutant effects, which make it difficult to compare the results to truly ambient chamberless conditions (Manning et al., 2004).

Ethylenediurea (*N*-[-2-(2-oxo-1-imidizolidinyl) ethyl]-*N*′-phenylurea), abbreviated as EDU was first reported by Carnahan et al. (1978) to protect snap bean plants against O_3 injury in glasshouse. Thereafter, this chemical has been widely used in greenhouse, OTCs and in ambient conditions as an O_3 protectant chemical to assess the plant response to O_3 (Manning, 2000; Bortier et al., 2001; Agrawal et al., 2003a, 2005; Tiwari et al., 2005). EDU has been successfully used to assess the impact of O_3 on horticultural crops such as bean (Kostka-Rick and Manning, 1993a; Brunschon-Harti et al., 1995a; Elagoz and Manning, 2005), clover (Tonneijck and Van Dijk, 2002), mung bean (Agrawal et al., 2005), potato (Clarke et al., 1990; Eckardt and Pell, 1996; Hassan, 2006), radish (Kostka-Rick and Manning, 1993b; Hassan

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et al., 1995), tobacco (Manes et al., 1990), tomato (Varshney and Rout, 1998) turnip (Hassan et al., 1995), wheat (Tiwari et al., 2005; Wang et al., 2007; Singh and Agrawal, 2009), rice (Wang et al., 2007) and ash trees (Paoletti et al., 2008).

The mode of action of EDU is not completely understood. Bennett et al. (1978) found that EDU does not affect stomatal behavior of plants suggesting that antiozonant activity of EDU is biochemical rather than biophysical in nature. Agrawal and Agrawal (1999), however, found that stomatal resistance increased in snap bean plants having EDU + O_3 treatment as compared with plants treated with O_3. A recent study suggested that both biochemical and biophysical processes may be modified under EDU treatment as increased ascorbate peroxidase activity along with decreased stomatal conductance were recorded in ash trees treated with 450 ppm EDU (Paoletti et al., 2008). Reduction in stomatal conductance of EDU treated plants signifies lesser O_3 flux in the plants. Higher ascorbate peroxidase activity indicates a better ROS scavenging capacity of the EDU treated plants (Paoletti et al., 2008). Regner-Joosten et al. (1994) demonstrated that EDU does not enter the cell, but remains in the apoplast where it may act as a scavenger of O_3 or O_3 derived radicals. Lee et al. (1981) showed that EDU delays the senescence in leaves of red clover (*Trifolium pretense* L.) and this effect may arise through the activation of enzymes responsible for scavenging free radicals in the plant cells.

The protective nature of EDU stays for a relatively short period after application, either applied as a soil drench or foliar spray (Carnahan et al., 1978). No mobilization of EDU from old to new leaves has been detected (Weidensaul, 1980). Therefore, repeated application of EDU is suggested for protection against O_3 injury. The advantage of this technique is that no chamber or artificial enclosures are necessary to evaluate O_3 effects. EDU may also serve as a potential research tool in remote areas where electricity is not available and therefore is especially recommended for developing regions (Bytnerowicz et al., 1993; Tiwari et al., 2005). EDU, however, may be toxic at higher concentrations (Eckardt and Pell, 1996).

The present investigation was undertaken with the objective of assessing ambient O_3 injury in palak (*Beta vulgaris* L. var Allgreen) using ethylenediurea with particular reference to biochemical and biophysical variations in EDU treated and untreated plants. Palak is a leafy vegetable grown in suburban fringes for local consumption due to short shelf life. It is suggested to be sensitive to O_3 injury (Agrawal et al., 2003b). Suburban areas have been shown to frequently experience high O_3 concentrations (Agrawal et al., 2003b; Tiwari et al., 2005, 2008).

2. Materials and methods

2.1. Study area

The study was conducted at Agricultural Farm, BHU, a suburban area of Varanasi, located in the eastern Gangetic plains of the Indian subcontinent at 25°14′ N, 82°03′ E and 76.19 m above mean sea level. The experiment was carried out during summer season of 2005 in the months of May and June. This period of the year is characterized by mean monthly maximum temperature ranging from 37.93 to 40.85 °C and mean monthly minimum temperature between 25.5 and 27.5 °C. Total rainfall was 138.9 mm, which mostly occurred in the month of June. Maximum relative humidity varied from 68.5% to 80.5% and minimum relative humidity from 32.74% to 33.2%.

2.2. Ozone monitoring

O_3 concentration was monitored for 8 h daily (8.00–16.00 h) and readings were recorded at hourly interval using an automatic O_3 monitor (Model 400A, API, INC, USA). For each day, maximum and minimum O_3 concentrations were also recorded.

2.3. Plant material

The plant material used in the study was palak (*B. vulgaris* L. var Allgreen). This heavy yielding variety was developed by Indian Agricultural Research Institute (IARI), New Delhi. The yield of this variety is about 12.5 tonnes green hectare^{-1} in 6–7 cuttings made 15–20 day intervals.

2.4. Raising of plants

Field plots were prepared using standard agronomic practices. Recommended dose of fertilizers (120, 60 and 40 kg ha^{-1} of N, P and K as urea, superpotash and muriate of potash, respectively) were added during the preparation of field plots. Seeds were hand planted in rows in 10 plots, each of 1.5 × 1.5 m in size. The experiment had EDU (+EDU) and non-EDU (−EDU) treatments. The experimental design was randomized block design, each treatment replicating 5 times. Thus, the experiment used overall 10 plots, 5 for EDU treatment and 5 for non-EDU treatment. Single plot was taken as statistical units from which different plant replicates were taken for estimation of different parameters.

After germination, plants were thinned to one plant per 15 cm. EDU treatment of 300 ppm as soil drench was given 10 days after germination (DAG) from 9.00 to 10.00 h local time at 10 days interval up to the plant maturity. The concentration of 300 ppm EDU was chosen on the basis of preliminary experiments in pot conditions at 150, 300 and 450 ppm treatments at known O_3 concentration. In another experiment with wheat also, sensitive cultivar showed best protection against O_3 at 300 ppm EDU treatment (Tiwari et al., 2005). Palak is reported to be sensitive to O_3 during a transect study by Agrawal et al. (2003b). EDU solution was freshly prepared in deionized water. Initially up to 30 DAG, 100 mL of EDU was given to each plant, thereafter 200 mL EDU was applied. Plants were kept under identical water regimes.

2.5. Plant sampling and analysis

2.5.1. Biochemical characteristics

For biochemical analysis, 3 plants per plot were taken and parameters such as contents soluble protein (Lowry et al., 1951), ascorbic acid (Keller and Schwager, 1977) and phenol (Bray and Thorpe, 1954), lipid peroxidation measured in terms of MDA (malonaldihyde) contents (Heath and Packer, 1968) and peroxidase activity (Britton and Mehley, 1955) were analyzed at 30 and 60 DAG.

For estimating the nutritional quality, the plants were oven dried at 80 °C till a constant weight was obtained. Oven dried plant samples (3 replicates per treatment) were grinded in a stainless steel grinder and passed through a 0.5 mm sieve. These powered samples were used for determining total nitrogen, Na, K, Ca, Mg and Fe contents. Total nitrogen content was determined by Gerhaldt Automatic N Analyzer (Germany). For determination of Na, K, Ca, Mg and Fe contents, digestion of powered shoot samples was done by following the method given by Allen et al. (1974) and the respective nutrient content in the digested material was determined with the help of Atomic Absorption Spectrophotometer (Model 2380, Perkin Elmer, USA).

2.5.2. Physiological characteristics

Chlorophyll fluorescence was determined in 5 plants per plot between 10.00 and 11.00 h using portable Plant Efficiency Analyzer (PEA, MK2, 9414, Hansatech Instruments Ltd., England). Leaf clips for dark adaptation were placed on the adaxial side of the leaves