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Differences in responses of two mustard cultivars to ethylenediurea (EDU) at high ambient ozone concentrations in India

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ABSTRACT

Ethylenediurea (EDU) at two different concentrations (200 ppm and 400 ppm) was used as a tool to study the response of two local Indian cultivars (Kranti and Peela sona) of mustard (*Brassica rapa* syn. *B. campestris*) to ambient ozone conditions. During the experiment, the critical AOT 40 levels were exceeded in the area of study in the Indo-Gangetic plains. Seed weight, biomass, oil content of the seeds and chlorophyll content increased at both EDU treatment levels in both cultivars. Gas exchange parameters (stomatal conductance and photosynthesis) were not affected by the EDU treatment, while the responses of antioxidative enzymes varied between the cultivars. The results indicate that EDU-induced ozone protection in these mustard cultivars is mediated by an antioxidative defense system, and that the cultivars adopted different strategies against ozone stress. The cultivar Kranti, characterized by higher biomass accumulation and number of pods, showed stronger antioxidative defense through several enzymes throughout the experiment, whereas the cultivar Peela sona, characterized by earlier senescence and a greater resource allocation to seed weight, invested in enzymatic detoxification only during the vegetative phase. The seed oil content increased by 4–5% at higher EDU treatment in both cultivars, which causes concern for mustard oil production at current ozone levels in India. Obviously, wider screening, using controlled ozone treatments, of *B. campestris* cultivars is urgently needed in order to maintain and improve the production rates in this highly populated and polluted area of India.

1. Introduction

Tropospheric ozone (O_3) is an important phytotoxic pollutant, causing adverse effects on plants and the whole ecosystem (Ainsworth et al., 2012). It is also considered to be a greenhouse gas, affecting global climate change (Moore, 2009). Ozone concentration has increased from 10 ppb before the industrial revolution (Volz and Kley, 1988) to the present global mean of approximately 50 ppb (8 h summer seasonal average) (The Royal Society, 2008). It is expected to increase further at an annual rate of 0.3 ppb per year (Wilkinson et al., 2012). Various modelling studies suggest a trend of rapid increase in ozone in South and East Asia (Dentener et al., 2005; Derwent et al., 2006; The Royal Society, 2008). In India, the Indo-Gangetic plains (IGP) are one of the most threatened regions by the ozone-induced risk as a result of intense agriculture, land-use changes, industrialization, urbanization and population growth (reviewed in Oksanen et al., 2013). The regional chemistry-transport model (REMO-CTM) predicts higher ozone precursor (CO, NO_x) and ozone concentrations over the IGP, as compared to other parts of India (Roy et al., 2008). With the current ozone trends, relative crop production losses in Indian crops, wheat, rice, soybean, beans, barley, maize and potato, will increase in the near future. The decrease in production is estimated to vary from 10 to 48% for wheat, 5 to 28% for rice, 8 to 32% for soybeans and 27 to 52% for beans (reviewed in Oksanen et al., 2013).

Mustard is an important tropical oilseed crop and is used for various purposes, for example as cooking oil, as a spice, as a preservative, as seed meal for cattle and as a traditional medicine. India is the world's second largest producer of rapeseed-mustard after China (Hegde, 2005), but India is still importing around 40% of its annual edible oil needs (Boomiraj et al., 2010). To meet the estimated growing consumption of rapeseed-mustard, its production has to be increased from 7 million tonnes to 16.4–20.5 million tonnes by the year 2030 (Directorate of Rapeseed-Mustard

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Research, 2011). Simulation models on climatic change (warming and precipitation changes) suggest a 10% to even over 50% yield loss for mustard (Boomiraj et al., 2010).

The response of plants to ozone is complex, with adjustments in stomatal conductance and a wide range of detoxification and defense processes initiated to scavenge the reactive oxygen species (ROS) generated by ozone (e.g., Overmyer et al., 2008). Often, the long-term cumulative effects of ozone exposure differ from the short-term responses. Additionally, the developmental phase of the plant also affects the responses (Black et al., 2007). There are several studies on the impacts of ozone on *Brassica* sp., demonstrating this complexity. Ozone may adversely affect both the vegetative and the flowering phase of *Brassica* sp., and yield reduction largely depends on the induced compensatory processes that reduce or prevent the deleterious effects of ozone exposure (Bosac et al., 1994; Stewart et al., 1996; Black et al., 2007). Short-term exposure of the terminal raceme of *B. campestris* to 100 ppb ozone during the early flowering stage showed no effect on the flower and pod number, although the mature seed number pod^{-1}, seed yield pod^{-1} and seed yield plant^{-1} decreased under ozone exposure (Black et al., 2012). Singh et al. (2009) also suggested that an increased fertilizer dosage (1.5 times the recommended) can provide protection against the yield loss resulting from ambient ozone in *B. campestris*, indicating the importance of soil nutrients for defense.

Various studies conducted on the Indian crops suggest their high vulnerability to ozone-induced damage, but unfortunately genetic variation among the cultivars and varieties in response to ozone has scarcely been addressed (Oksanen et al., 2013). Genetic differences among cultivars and varieties in ozone responses have mostly been studied for wheat and rice (Singh and Agrawal, 2009; Picchi et al., 2010; Shi et al., 2009; Sawada et al., 2012). Recently, Tripathi and Agrawal (2012) reported that two cultivars of *B. campestris* responded differently to ozone (ambient + 10 ppb) in terms of yield, photosynthesis and oil content. Most studies on *Brassica* species have been conducted with elevated ozone concentrations in growth chambers (Bosac et al., 1994, 1998; Stewart et al., 1996; Black et al., 2012) or in open top chambers (Singh et al., 2009; De Bock et al., 2012; Tripathi and Agrawal, 2012). However, there are no long-term studies on *Brassica* cultivars in natural field conditions under prevailing ambient ozone levels throughout the growing season.

EDU (ethylenediurea; [*N*-(2-2-oxo-1-imidazolidinyl) ethyl]-N′-phenyl urea) has been used as a chemical protectant against ozone, i.e., as a research tool for evaluating the impacts of ozone on plants (Paoletti et al., 2009; Manning et al., 2011) and to reveal the different responses among the cultivars to ozone toxicity, as indicated for wheat (Singh and Agrawal, 2009), white clover (Singh et al., 2010b), black gram (Singh et al., 2010a), and radish (Pleijel et al., 1999) (see also the review by Oksanen et al., 2013). EDU do not confound effects of its own on plant growth in non-ozone conditions (Foster et al., 1983; Szantoi et al., 2007). EDU is an alternative to open-top chambers for assessing ozone sensitivity of crops in ambient conditions, particularly in remote areas with electricity limitations.

The present study was conducted with the aim of exploring the differences in ozone responses between two Indian *Brassica campestris* cultivars in terms of physiological, biochemical, growth and yield parameters. EDU treatments were used to ameliorate ozone effects in ambient field conditions in a highly polluted area of India. We hypothesized that (1) ozone concentrations in the IGP area are high enough to cause growth reductions and yield losses in *B. campestris*, (2) two *B. campestris* cultivars will have different responses to EDU treatment. (3) Antioxidative defense is one of the main mechanisms for combating ozone stress. (4) Stomatal conductance and photosynthesis will be ameliorated by EDU.

2. Materials and methods

2.1. Experimental site and plant material

The study was conducted at the National Botanical Research Institute, Lucknow, India, situated along the southern bank of the River Gomti (26°55′N latitude, 80°59′E longitude), and at an altitude of 113 m, during the months of November 2012–April 2013. Two cultivars of mustard, *Brassica rapa* Syn. *B. campestris* (L.) Czern and Coss (hereafter referred to as *B. campestris*), Kranti and Peela sona were chosen for the present study. Both the selected cultivars are widely grown by farmers in the IGP region and have a life span of approximately 125 days.

2.2. Experimental design

The field size was 400 m^2, divided into five plots, i.e., five repetitions, each having six subplots of size 3×1.5 m^2. Each treatment had its own subplots for both cultivars. The field was ploughed for sufficient aeration. Seeds were sown in the subplots in rows 30 cm apart; the distance between the subplots was 0.75 m. To obtain the recommended dosages of NPK (80:40:40 kg ha^{-1}) for mustard, the field was fertilized twice during the experiment: at the beginning with urea, single superphosphate and muriate of potash, and then at 30 days after germination (DAG) with urea. The field was irrigated regularly to maintain uniform soil moisture. The treatments had different levels of EDU: the control, in which water was used in place of EDU, 200 ppm EDU and 400 ppm EDU. EDU was obtained from Prof. W.J. Manning, University of Massachusetts, USA. EDU was applied as a foliar spray starting at 20 DAG, and this was repeated weekly until the final harvest. The entire foliage of each plant was sprayed until it was visibly saturated.

2.3. Ozone Monitoring and AOT 40 calculations

The monitoring of the ambient ozone was carried out with a 2B Tech Ozone Monitor (106-L) on 8 h d^{-1} (from 9.00 to 17.00) at the experimental plot throughout the growing season (from 27 November, 2012 to 1 April, 2013). AOT 40 (accumulated exposure over a threshold of 40 ppb) was used as the exposure index for the ozone concentration, as described by De Leeuw and Van Zantvoort (1997).

2.4. Biomass sampling

Plant sampling for biomass estimation was performed at three different phases; the vegetative phase at 33 DAG and the flowering phase at 63 DAG for both cultivars, while the harvest phase sampling was done at 88 DAG for Kranti and at 93 DAG for Peela sona. Five different plants were selected randomly from the subplots. In order to obtain an intact root system, a monolith was carefully dug out and first kept in water, then washed with running tap water to remove the adhered soil. The roots and the shoots were separated and dried in an oven at 65 °C until the weight reached a constant value.

2.5. Physiological parameters and chlorophyll measurements

Net photosynthesis (A), stomatal conductance (g_s), water use efficiency (WUE), maximal photochemical efficiency of PSII (F_v/F_m) and non-photochemical quenching (NPQ) were measured on the youngest fully mature leaves, at 49 DAG and 67 DAG, from three randomly selected plants of both cultivars in the each treatment. All measurements were performed using a Li-COR 6400 gas exchange portable photosynthesis system (Li-COR, Lincoln,