Ecotoxicology and Environmental Safety 115 (2015) 101–111

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Cultivar specific variations in antioxidative defense system, genome and proteome of two tropical rice cultivars against ambient and elevated ozone ☆

Abhijit Sarkar [a], Aditya Abha Singh [a], Shashi Bhushan Agrawal [a,*], Altaf Ahmad [b], Shashi Pandey Rai [c]

[a] Laboratory of Air Pollution and Global Climate Change, Department of Botany, Banaras Hindu University, Varanasi 221005, India
[b] Department of Botany, Aligarh Muslim University, Aligarh 202001, India
[c] Laboratory of Morphogenesis, Department of Botany, Banaras Hindu University, Varanasi 221005, India

ARTICLE INFO

Article history:
Received 18 October 2014
Received in revised form
3 February 2015
Accepted 4 February 2015
Available online 13 February 2015

Keywords:
Tropospheric ozone
Antioxidative enzymes
Native PAGE
DNA
Yield
Grain quality

ABSTRACT

For the past few decades continuous increase in the levels of tropospheric ozone (O_3) concentrations is posing to be a threat for agricultural productivity. Two high yielding tropical rice cultivars (Malviya dhan 36 and Shivani) were evaluated against different concentrations of O_3 under field conditions. Experimental design included filtered chambers, non-filtered chambers having ambient O_3 and 10 and 20 ppb elevated O_3 above the ambient. Study was conducted to assess differential response if any in induction of antioxidative defense system, genome stability, leaf proteome, yield and quality of the product in both the test cultivars. Superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX), and glutathione reductase (GR) were induced under ambient and elevated levels of O_3. Native polyacrylamide gel electrophoresis (PAGE) of SOD, CAT and POD also displayed increased enzymatic activity along with associated alterations in specific isoforms. Ascorbic acid, thiols and phenolics were also stimulated at ambient and elevated O_3. Structural alterations in DNA of rice plants due to O_3 affecting its genome template stability (GTS) was examined using RAPD technique. 2-D PAGE revealed 25 differential spots in Malviya dhan 36 and 36 spots in Shivani after O_3 treatment with reductions in RuBisCO subunits. Reductions in yield and change in the quality of grains were also noticed.

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1. Introduction

Tropospheric ozone (O_3) has long been documented as a foremost threat to agriculture globally (Booker et al., 2009; Cho et al., 2011; Singh et al., 2014a). Rapid urbanization, industrialization, increased vehicle use coupled with uncontrolled fossil fuel burning and unwise management of natural resources have led to an increase in the concentration of ozone precursors and thus to tropospheric O_3. Presently, background O_3 concentrations have doubled since the last century (Meehl et al., 2007) and there are evidences of increase in its annual mean values ranging from 0.1 to 1 ppb per year (Coyle et al., 2003). According to the IPCC (2007) report, mean daily O_3 concentration is estimated to have increased from around 10 ppb, prior to the industrial revolution, to a current level of approximately 60 ppb during hot summer months. There are numerous model based reports which predicted an increase in future O_3 concentrations associated with O_3-induced damage to agricultural crops at global level including India (Ainsworth, 2008; Emberson et al., 2009).

Ozone predominantly penetrates in internal environment of leaf tissues through stomatal openings where it generates a cascade of reactive oxygen species (ROS) in surrounding aqueous medium that causes membrane damage, alteration of gene expression, impairment of photosynthetic proteins, degradation of chlorophyll and alterations in metabolic activities (Booker et al., 2009; Fuhrer, 2009; Singh et al., 2014b). In response to this, plants adopt various defense mechanisms which includes an array of antioxidants which may be enzymatic/non-enzymatic biomolecules or low molecular weight compounds (Chen and Gallie, 2005). Ozone, being a potent oxidant, affects the genome structure of a living organism. It is documented that increased ROS

☆Present work forms a part of Council of Scientific and Industrial Research (CSIR), New Delhi funded research Project no. 24/292/06/EMR-II.
* Corresponding author. Fax: +91 542 2368174.
E-mail addresses: abhijitbhu@gmail.com (A. Sarkar), abha2512.singh@gmail.com (A.A. Singh), sbagrawal56@gmail.com (S.B. Agrawal), ahmadaltaf@yahoo.com (A. Ahmad), shashi.bhubotany@gmail.com (S.P. Rai).

http://dx.doi.org/10.1016/j.ecoenv.2015.02.010
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production is the primary cause of mutaginicity of DNA which eventually affects DNA stability (Bray and West, 2005; Labuschagne, 2007). Apart from DNA damage, elevated ROS production leads to impairment of photosynthesis as a consequence of progressive loss in the amount and activity of enzymes, e.g. ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) (Agrawal et al., 2002; Cho et al., 2008; Singh et al., 2014b). These damaging effects of O_3 are conclusively translated into yield reduction. Several researchers have documented reduction in rice yield due to O_3 exposure (Ariyaphanphitak et al., 2005; Shi et al., 2009; Sawada and Kohno, 2009). Further, nutritional quality of product is also recognized as a major functional trait for the well being of society. Few reports have registered that this parameter was also adversely affected in rice due to O_3 stress (Rai et al., 2010; Wang et al., 2012; Zheng et al., 2013).

Rice is cultivated in around 95 countries globally as the main staple crop, and provides major source of nutrition for more than half the world's population (IRRI, 2002). It is the most important food source throughout the world with Asia, Africa and Latin America as the major rice consuming countries (Maclean et al., 2002; Ainsworth, 2008). Rice is the staple food crop of South East Asian countries, providing 21% of the calorific needs of world's population (Fitzgerald et al., 2009). Therefore, considering the importance of rice as a major food source, the present study was designed to evaluate the response of two high yielding cultivars of rice (*Oryza sativa* L. Cv Malviya dhan 36 and Shivani) against O_3 stress by using open top chambers (OTCs) under near natural conditions. Study was done to evaluate cultivar specific response under ambient and elevated levels by using integrated approaches. We focused our investigation on assessment of changes in antioxidant defense system, stability of genome, proteomic responses, yield losses and alterations in the nutritional quality of the product. Till date, limited studies have addressed the response of rice plants against futuristic concentrations of O_3 under field conditions which is also supported by documentation of Rai et al. (2010) that field experiments conducted with rice are still limited as compared to other crops. So far, effect of O_3 on plant genome has not been studied in detail, thus findings of the present work could provide some new information related to genotoxic effect of O_3. Results of the present study may be of helpful to researchers in understanding the mechanism of O_3 action and related responses in rice plants vis-a-vis in screening and raising of resistant cultivars against O_3.

2. Material and methods

2.1. Plant material

Rice (*O. sativa* L.) was selected as the plant material. The rice cultivars tested in the present experiment are widely cultivated in Indo-Gangetic plain of India. Malviya dhan 36 is a semi-tall, mutant of Mahsuri with yield potential of 40–45 q ha^{-1} while Shivani is a dwarf, high yielding hybrid cultivar with yield potential of 55–60 q ha^{-1}.

2.2. Experimental design

The experiment was performed at the Agricultural Research Farm of Banaras Hindu University sited at the eastern Gangetic plains of India. It is located 76.19 m above mean sea level at 82°03′ E longitude and 25°14′N latitude. The soil of the experimental site is fertile with a sandy loam texture (sand 45%, silt 28% and clay 27%) and soil pH ranging between 7.2 ± 0.2. Total N content varied from 0.02% to 0.04%, organic carbon from 0.15% to 0.34% and available P between 0.05 and 0.09 mg g^{-1}.

The experiment was carried out in field conditions using Open top chambers (OTCs) constructed according to the design of Bell and Ashmore (1986). OTCs were ventilated with ambient air that passed through activated charcoal filters (filtered chambers: FCs), ambient non-filtered air (non-filtered chambers: NFCs), elevated levels of O_3 exposure (non-filtered chambers with 10 ppb (19,600 ng m^{-3}) O_3 elevation: NFCLOs and non-filtered chambers with 20 ppb (39,200 ng m^{-3}) elevated O_3: NFCHOs). Exposure of elevated O_3 was done with the help of O_3 generators (Model Systrocom, India) daily at the peak hours of O_3 concentration (10:00 h to 15:00 h) at the experimental site. The ozone generators were fed with pure oxygen (up to 95% purity) to generate O_3 through oxygen cylinders to provide oxygen rich environment around the air inlet to minimize the formation of NOx or other by products. There were three replicate chambers for each treatment for both the cultivars. At the age of 21 days, rice seedlings were transplanted in rows maintaining a distance of 15 cm. In each chamber, there were 36 plants. Recommended dose of fertilizers (120, 80 and 60 kg ha^{-1} N, P and K as urea, single super phosphate and muriate of potash, respectively) were used in present study. Harvesting of crop was done in the second week of October.

2.3. Meteorological parameters

Data regarding the meteorological parameters viz., maximum and minimum temperature, relative humidity, total rainfall and sunshine hours at experimental site were obtained from the Indian Meteorological Division (IMD), Banaras Hindu University.

2.4. Ozone monitoring

During experiment, O_3 monitoring was carried out for 12 h d^{-1} (06:00–18:00 h) at the experimental site in different OTCs by an O_3 analyzer (Model APOA 370, HORIBA Ltd., Kyoto, Japan) at regular intervals. Air samples were collected with the help of teflon tube (0.35 cm diameter) placed above canopy of the plants. AOT40 (accumulated ozone over a threshold concentration of 40 ppb) value was calculated according to the formula of Mauzerall and Wang (2001).

2.5. Foliar injury

Macroscopic symptoms due to O_3 treatment was recorded in terms of foliar injury which was observed on the leaf surface as (non-parasitic) interveinal yellowing or chlorotic stippling.

2.6. Sampling of plants, antioxidants, lipid peroxidation (LPO), total protein and native PAGE

Enzymatic and non-enzymatic antioxidants and lipid peroxidation were measured at three stages of development spectrophotometrically. First sampling was done at 25 days after transplantation (DAT); second at 50 DAT and third at 75 DAT. Three replicates of both cultivars were selected randomly from each chamber at specific sampling periods, hence making a set of nine replication for each treatment ($n=9$). Third fully expanded leaves from top of the plant canopy were selected for analyses. Protein for native PAGE assay and spectrophotometric analyses was isolated according to the procedure suggested by Singh et al. (2014b). Total protein was quantified by the method given by Lowry et al. (1951). For estimation of lipid peroxidation, ascorbic acid, phenolics, thiols and spectrophotometric detection of antioxidative enzymes, superoxide dismutase (SOD), peroxidase (POD), ascorbate peroxidase (APX), catalase (CAT) and glutathione reductase (GR) activity was assayed by the method provided by Singh et al. (2014b). Native PAGE analyses for SOD, CAT and POD at 50 DAT was done for

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