Toward Sustainable Agriculture through Integrated 'OMICS' Technologies: A Quest for Future Global Food Security

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'Food security' has always been a prime issue for the development and progress of human civilization. However, in spite of all technological, scientific, and agricultural achievements the secure and affordable supply of safe and nutritious food to the population is still in an alarming state. In the year 2007 alone, the number of hungry people increased by 75 million and expected to reach to 1.2 billion worldwide by 2017. Among the various reasons responsible for rising food crisis, 'global environmental change' can be considered as one of the most critical factors today. Among the environmental factors, increasing tropospheric or surface level ozone (O_3) levels has been recognized as a major cause for declining plant growth and crop yield. According to the IPCC (2007) report, concentration of tropospheric O_3 is estimated to have increased from approximately 10 ppb prior in the industrial revolution to a current level of about 60 ppb during summer months, and is predicted to increase 20−40% more by 2050 in the industrialized countries of the Northern Hemisphere. Like other tropical countries, India is also under the severe threat of O_3 pollution.

The present review mainly focuses on the responses of rice (*Oryza sativa* L. cultivars - Malviya dhan 36 and Shivani) and wheat (*Triticum aestivum* L. cultivars - Sonalika and HUW 510) plants to elevated levels of O_3-stress at Indian context through a combination of physiology and high-throughput proteomics analyses using open top chambers (OTCs). Experimental sets were prepared as: filtered chambers (FCs) with almost negligible O_3, non-filtered chambers (NFCs) with ambient O_3, non-filtered chambers with 10 ppb O_3 fumigation (NFCLOs), and non-filtered chambers with 20 ppb O_3 fumigation (NFCHOs). Notably, O_3 causes significant induction in major cellular antioxidants, and negatively affects photosynthetic machinery in both rice and wheat plants. Proteomics analysis revealed that O_3 strongly inhibits the expression of major photosynthetic and important energy metabolism proteins, and induced the defense or stress related proteins. Proteomics, writing simply, refers to the study of all the proteins in a cell, tissue or organism, and is part of three young, high-throughput 'omics' technologies of genomics (transcriptomics), proteomics, and metabolomics. Random Amplified Polymorphic DNA (RAPD) analysis also revealed significant damage in genome template stability of both the crops under O_3 stress, hence indicating toward the mutagenic ability of O_3.

We believe that present results are a small but necessary step forward in developing O_3-tolerant rice and wheat genotypes, which can be utilized in the future high O_3 world for their optimum growth and yield.

Key words: Global food security, Climate change, Tropospheric ozone, Agricultural crops, 'omics' approach

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Environmental Changes and Challenges: Major Constrains for 'Global Food Security'

'Food security' is defined as a situation in which all people at all the time have physical and economic access to sufficient, safe and nutritious food to meet their dietary needs and live an active healthy life (ODI, 1997). In short, 'food security' is a three step process and depends on ― the availability of food, overall access to the available food, and proper use of the accessed food. However, achieving complete food security continues to be a major challenge not only for the under-developed and developing nations, but also for the developed world. Though there are numerous causes behind the food security issues, the challenges related to 'global environmental change' are found to be most important and serious in impact. During the preparation of this article, a conference was organized (17th October, 2011) in the UK at the British Medical Association's (BMA's) headquarters (UK) titled, "The health and security perspectives of climate change ― how to secure our future wellbeing", indicating the growing concern of people on the grave threats posed by climate change (Black, 2011). A multitude of factors affect the climate change globally, but, man-made factors like air, water and soil pollutants are the most critical. Among the air pollutants, different studies have reported tropospheric ozone (O_3) as a wide spread and toxic air pollutant with severe impact on both plants (see also Cho *et al*., 2011) and animal health globally. It must be noted that unsustainable and over-stretched agriculture systems are also in part responsible for climate change, as agricultural activities also result in the release of greenhouse gasses thereby affecting climate, globally.

Trends in Tropospheric Ozone Concentrations: Past, Present and Future

Ozone Levels of Yesterday and Today: A Global View

Being a secondary gaseous pollutant, O_3 is produced due to photolysis of nitrogen dioxide (NO_2). In the free troposphere, O_3 formation depends on reaction of methane (CH_4), carbon monoxide (CO), and non-methane organic compounds with nitrogen oxides (NOx) (Fig. 1). These reactions are principally controlled by sunlight and temperature. Nitrogen dioxide diminishes when O_3 reaches its peak (Tiwari *et al*.,
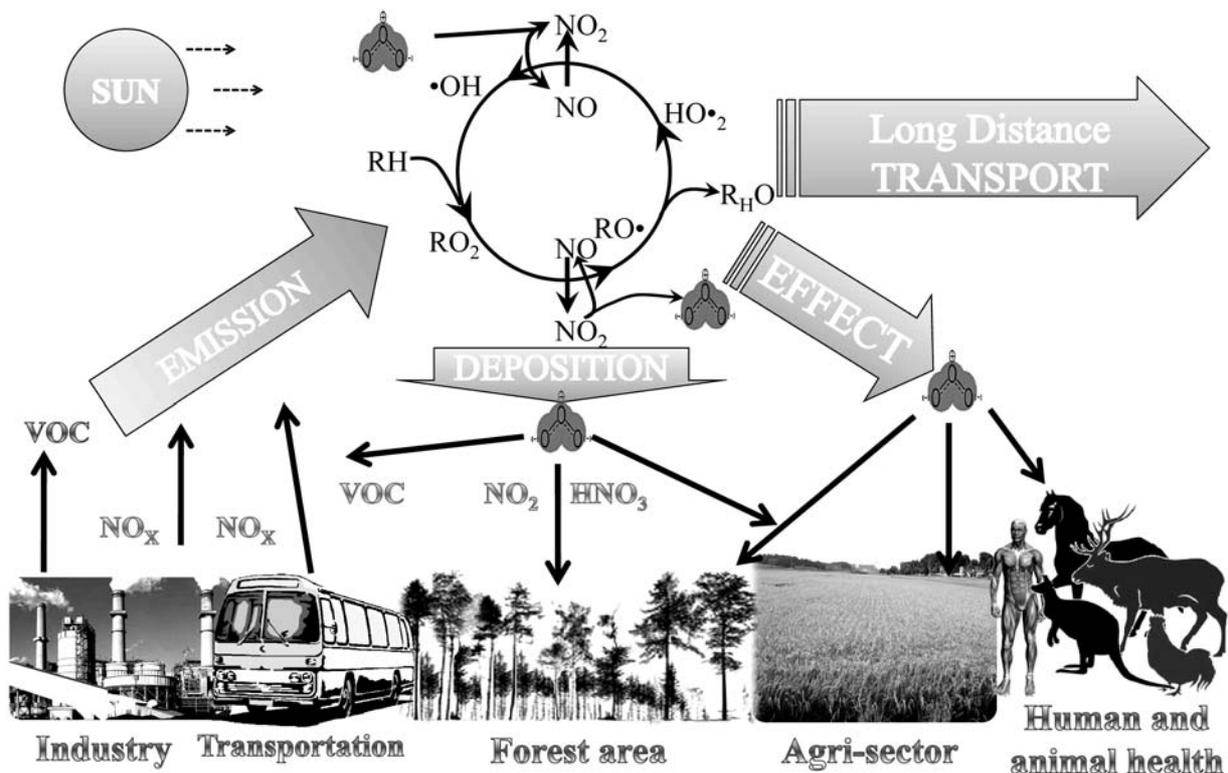
Fig. 1. Atmospheric cycle of tropospheric O_3 formation.

2008). In the ambient air, O_3 precursors play an important role during long range transport downwind from the sources (Fig. 1). Polluted air masses from urban and industrial areas can affect suburban and rural areas, even reaching to remote rural areas for considerable distances. High O_3 levels from one particular urban area can extend as far as 48 to 80 km (Krupa and Manning, 1988).

The background O_3 concentration has more than doubled in the last century (Meehl *et al.*, 2007), and there is an increase in annual mean values of O_3 ranging from 0.1 to 1 ppb per yr^{-1} (Coyle *et al.*, 2003). However, O_3 levels vary strongly with episodic peak concentrations during the warmest months in summer in the most polluted regions and maximum concentrations during spring prevailing at background sites (Vingarzan, 2004). In regions such as South East Asia exposed to summer monsoon that transports oceanic air with less O_3, the seasonal patterns show a peak during pre- and post-monsoon periods (He *et al.*, 2008).

In rural areas of Europe, mean O_3 concentrations reach 40 to 50 ppb from spring to summer (EMEP, 2004). In rural agricultural areas of the USA, mean O_3 concentrations reach between 50 and 60 ppb (90th percentile) (USEPA, 2006). Ozone concentrations over the mid- and high-latitude of the Eurasian and North American continents were 15−25 ppb in 1860, but increased to between 40 and 50 ppb even in remote areas and from 10−15 ppb to 20−30 ppb over the mid- and high-latitude Pacific Ocean (Lelieveld and Dentener, 2000).

In India, in spite of the favorable climatic conditions for O_3 formation, very limited data from systematic monitoring of O_3 are available. However, from the available reports, it is clear that O_3 concentration has been continuously increasing from 1992−2008 with higher peaks in rural areas. In a field transect study at urban sites of Varanasi, O_3 concentrations varied from 6−10.2 ppb during 1989−1991 (Pandey and Agrawal, 1992). During the same period, daytime O_3 concentrations (9 h mean) were reported to vary from 9.4 to 128.3 ppb at an urban site in Delhi (Varshney and Aggrawal, 1992). Sarkar and Agrawal (2010b), at a rural site of Varanasi during 2007−2008 and 2008−2009, recorded mean O_3 concentrations of 45.3 and 47.3 ppb, respectively. Emberson *et al.* (2009) reported that large parts of South Asia experience up to 50−90 ppb mean 7 hour (M 7) O_3 concentrations.

Projected Trends of Ozone Concentrations

Future trends of O_3 concentrations will depend on the anthropogenic emission path of precursors and on trends in temperature, humidity and solar radiation, but the effects of both factors will vary spatially (Zeng *et al.*, 2008). By 2030, average O_3 in surface air over much of the northern hemisphere may increase by 2 to 7 ppb across the range of IPCC SRES emission scenarios described in Nakicenovic and Swart (2000). By 2100 the two more extreme scenarios projected baseline O_3 increases of >20 ppb, while the other four scenarios yielded changes of -4 to $+10$ ppb (Prather *et al.*, 2003). Using 18 atmospheric models, Ellingsen *et al.* (2008), for the CLE scenario (current legislation in place), obtained an increase in AOT40 (accumulated exposure over a threshold of 40 ppb) by 21−38% by 2030 over the northern hemisphere, relative to 2000 and by 50% on the Indian subcontinent, but a decrease with current regional legislation in Europe. In UK, the annual average O_3 concentration is predicted to reach 30−40 ppb in rural areas leading to doubling of AOT 40 values by 2030 (Giorgi and Meleux, 2007). Global photochemical models project that under current legislation emission scenarios, parts of Asia will experience further significant increases in O_3 concentration up to 2030 (Dentener *et al.*, 2005). Meehl *et al.* (2007) projected an increase of 20−25% in O_3 concentrations between 2015 and 2050 and 40−60% by 2100 in Asia.

Impact of Tropospheric Ozone on Agricultural Production Worldwide

To quantify the impacts of O_3 on crop yield at national and regional scales, NCLAN (National Crop Loss Assessment Network) program in USA and EUCLAN (European Crop Loss Assessment Network) in Europe were conducted using open top chambers (OTCs). In the NCLAN program, most experimental studies have used chambered field approach as OTC modified for field use with crops by Heagle *et al.* (1979). Data from NCLAN exposure- crop response regression analyses indicated that at least 50% of the species/cultivars tested were predicted to exhibit 10% yield loss at 7 h seasonal O_3 concentrations of <50 ppb. Adams *et al.* (1998) estimated that yield losses due to O_3 exposures accounted for 2−4% of the total US crop production. During the EUCLAN program conducted in nine countries of Europe on a variety of crops including wheat, barley, beans, etc. and where the crops were grown in OTCs, the experimental re-

sults showed that yield reductions were highly correlated with cumulative exposure to O_3 above a threshold of 30−40 ppb during daylight hours.

Emberson *et al*. (2001) reported higher magnitude of yield loss in parts of Asia, Latin America and Africa with trends of increased emissions of O_3 precursors. In Pakistan, 29−47% yield reductions were reported for 6 varieties of wheat (Maggs *et al*., 1995; Wahid *et al*., 1995a), 28−42% for five varieties of rice (Wahid *et al*., 1995b) and 37−46% for 2 varieties of soybean (Wahid *et al*., 2001) due to different air pollutants in the ambient air. Exposure of O_3 at 80 ppb concentration for 1.5 h daily for 30 days showed yield reductions of 29.5% in *Vicia faba*, 20.6% in *Oryza sativa*, 13% in *Panicum miliaceum*, and 9.7% in *Cicer arietinum*.

It was observed that reductions in crop yield varied from 2−60%. Differential responses were recorded among different crops and their cultivars. Maximum reductions were found in soybean (40−60%) followed by wheat (20−40%), rice (10−20%), and minimum in barley (Feng and Kobayashi, 2009). Studies by Emberson *et al*. (2009), Feng and Kobayashi (2009), and Mills *et al*. (2007) found same trend of sensitivity, reporting legumes to be most sensitive and barley to be most resistant under O_3 exposure.

Impact of Ozone on Major Agricultural Crops: An Indian Perspective

India has already been identified a future O_3 -hot spot with a projected increase of 20−40% in next 40 years. In the following study, we have examined the effects of elevated levels of O_3 on two major Indian crops rice and wheat.

Experimental Procedure
Development of O_3 Gradient Exposure at Near-Natural Conditions using Open Top Chambers

The high-yielding cultivars of rice (Malviya dhan 36 and Shivani) and wheat (Sonalika and HUW 510) were selected as test plants. All four cultivars are highly popular in the North Indian region due to their medium life span (135 days for rice and 120 days wheat), high yield, shorter maturity, and pathogen resistant nature. Experiments were conducted at Agriculture Research Farm, Banaras Hindu University, India (24°14′ N, 82° 03′ E). The experimental field is located at 76.1 m above mean sea level with its sandy soil loam (pH 7.2). For both the crops seeds were grown and transplanted as required following the normal cultivation practices

with recommended doses of fertilizers. Manual weeding was done time to time, when required.

Then rice plants (seven-days-old after transplantation and transplanted at 21 days) and wheat plants (seven-days-old after germination) were fumigated with O_3 using OTCs (Fig. 2) at respective growth periods (during June to October, 2007 and 2008 for rice; and December to April, 2007−08 and 2008−09 for wheat). The OTCs were constructed according to the design reported by Bell and Ashmore (1986). These chambers have simple basic design and can be constructed easily and rapidly. The OTCs were 1.5 m in diameter and 1.8 m in height, covered with 0.25 mm thick transparent polypropylene sheets. Continuous air was supplied at three changes per minute via a high speed blower. For O_3 fumigation, the O_3 generators (Model Systrocom Ltd., Varanasi, Uttar Pradesh, India) were placed inside the blowers for respective OTCs. Two different levels of O_3 treatments were provided in OTCs ($n=6$) for each cultivar. Experimental OTCs were divided as: charcoal filtered air (FCs), non-filtered air (NFCs), non-filtered air with +10 ppb O_3 (NFCLOs) and non-filtered air +20 ppb O_3 (NFCHOs). With these four experimental designs, plants were exposed to a series of four different O_3 concentrations: (i) nearly no O_3, (ii) ambient O_3, (iii) ambient +10 ppb O_3, and (iv) ambient +20 ppb O_3 (Figure 2). Open plots (OPs, $n=6$) were also used to monitor the effects of chamber (OTCs) enclosures. The experimental setups were distributed in a completely randomized manner. Ozone fumigation in respective OTCs was performed with O_3 generators for 5 h day^{-1} at the peak O_3 period (10:00 h to 15:00 h) of local time.

During the experiment, continuous daytime O_3 monitoring was carried out for 12 h day^{-1} (between local time 06:00 to 18:00 h) in the experimental site at different experimental setups by using UV absorption photometric ambient O_3 analyzer (Model APOA 370, HORIBA Ltd., Kyoto, Japan). The O_3 analyzer was calibrated with a known O_3 source of 84 ppb (as span set) and charcoal filtered air (as zero set). Data were recorded and subjected for further calculations.

Plant Response Analysis: from Phenotypic to 'Omics' Level

Responses of both the test plants were analyzed at different levels, like phenotypic, growth, physiological, biochemical, genome, proteome, and yield (Fig.
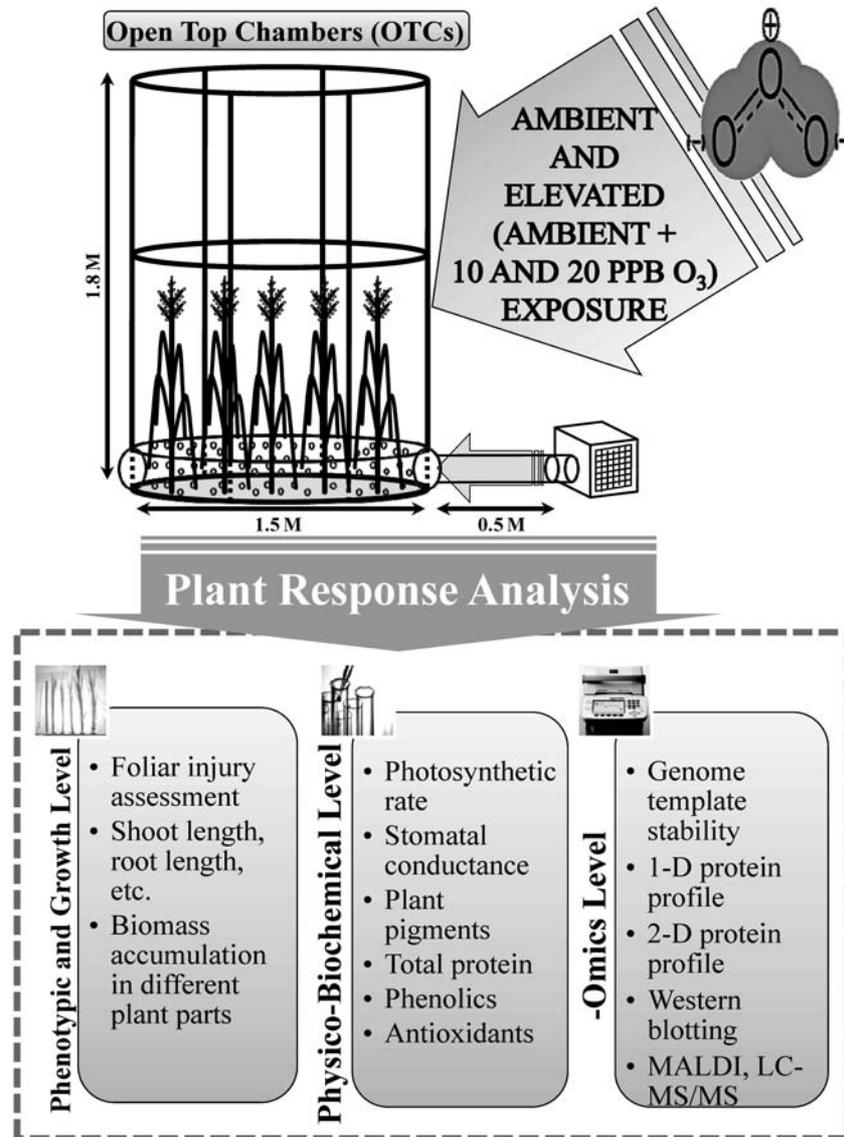
Fig. 2. Experimental strategy and work flow.

2). All the methodologies regarding the responses analyses were followed as described in Sarkar and Agrawal (2010ab), Sarkar *et al.* (2010), and Sarkar and Agrawal (2012).

Experimental Outcome

Most of the growth responses were severely affected by O_3 exposure in both the test crops. Foliar injury (non-pathogenic), in the form of interveinal chlorosis, was noticed in both the rice and wheat plants under ambient and elevated levels of O_3 exposure. Interestingly the magnitude of injury manifestation typically depends on the concentration and period of O_3 exposure in both the plants (Rai and Agrawal, 2008;

Sarkar and Agrawal, 2010a; Sarkar *et al.*, 2010; Sarkar and Agrawal, 2012). Growth parameters like shoot and root lengths, total number of leaves, total leaf area, etc. were significantly decreased at the later stages of plant's development in both the test plants under O_3 exposure. However, variety Shivani among rice cultivars and variety HUW 510 among wheat cultivars showed higher reduction in growth parameters under elevated levels of O_3 exposure as compared to the other test cultivars (Sarkar and Agrawal, 2010b; Sarkar and Agrawal, 2012). Physiological parameters like photosynthetic rate (ps), stomatal conductance (gs), chlorophyll a florescence kinetics (F_v/F_m) was
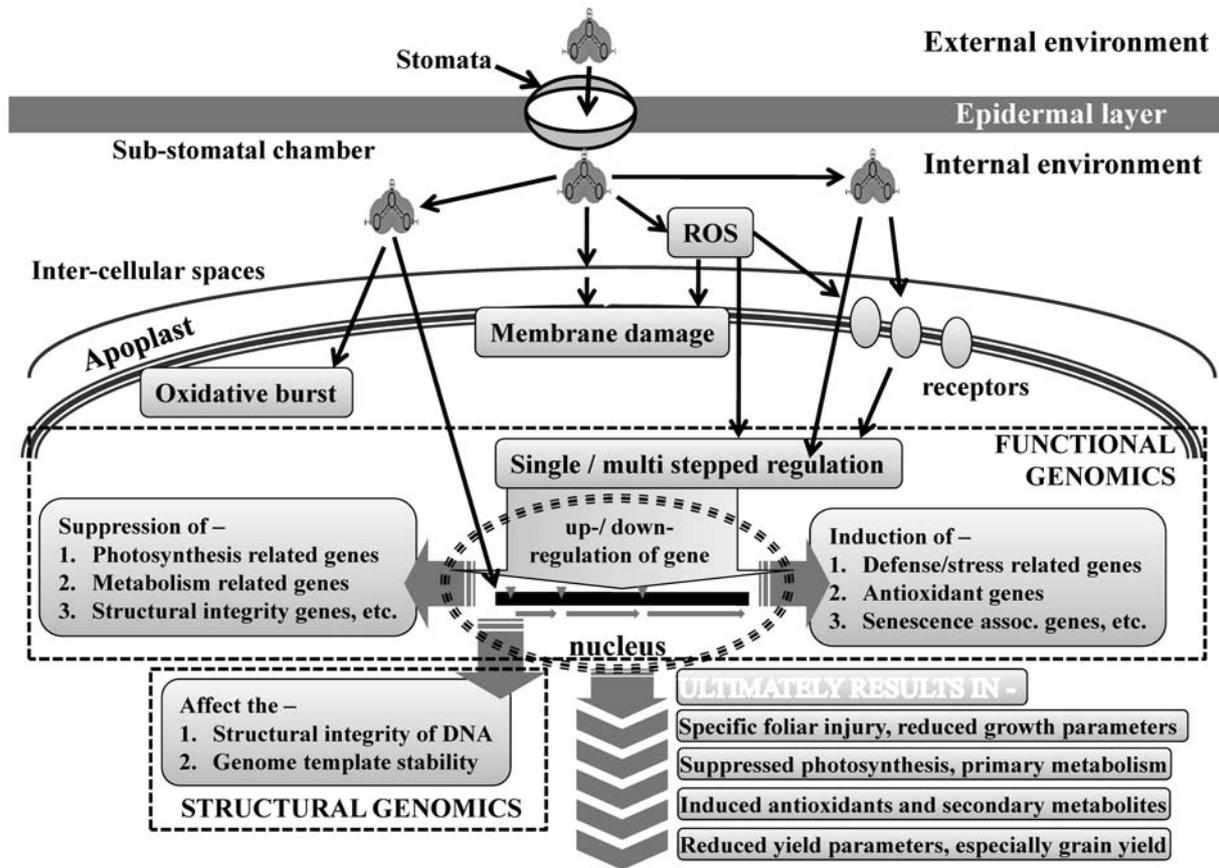
Fig. 3. Schematic illustration of the molecular response of test plants (rice and wheat) in relation to O_3 stress leading to the phenotypic changes and ultimately yield loss.

highly affected under O_3 exposure in both the test crops. Biochemical analysis revealed that O_3 induces anti-oxidative defense system, both enzymatic and non-enzymatic, in rice and wheat plants from early stage of plant growth after O_3 exposure (Sarkar *et al*., 2010; Sarkar and Agrawal, 2012). Results also showed that O_3 causes severe damage in reproductive parameters of the test crops, and this might be an important cause behind the yield reduction. RAPD analysis of the genomic DNA of both the test crops under varied exposure of O_3 pointed towards O_3 as a potent muta-gen, which can alter the genomic stability. Proteome analysis in both the crops showed suppression of some major photosynthetic and primary metabolism-related proteins, and induction of some anti-oxidative enzymes and defense/stress-related proteins under O_3 exposure (Sarkar *et al*, 2010; Sarkar and Agrawal, 2012). Yield and grain quality were also severely damaged in both rice and wheat (Rai *et al*., 2007; Sarkar and Agrawal, 2010b; Sarkar and Agrawal, 2012). Compiling all the

obtained results, it was quite clear that O_3, both the ambient (present) and elevated (future) levels, can cause severe damage to the crops (Fig. 3), and wheat appeared to be more sensitive than rice. However, the differential cultivar response in both the test crops under O_3 exposure might help the researchers to screen out a cultivar better suitable for the areas having higher levels of tropospheric O_3.

Concluding remarks

The overall results of our investigations clearly show-ed that O_3 acts as a major air pollutant in the Indian subcontinent and drastically affects the productivity, internal metabolism and growth of both the test crops, i.e. rice and wheat; however, the effect would be more severe in the coming future is our concern. Our cur-rent data also supported the findings of previous re-search conducted around the globe. It is quite obvious that plants will have to focus more on this toxic pol-lutant for their survival and retaining yield (the primary

interest of human society) in the future high O_3 world. Examples were discussed here on rice and wheat as a model system to investigate the effect of elevated O_3 using integrated approaches, phenotypical, physiological, and biochemical, under near-natural conditions provided in the OTCs. Elevated O_3 had a major influence on multiple cellular processes, which resulted in visible foliar injury on the leaves of wheat as phenotypical symptoms (Fig. 3). The OTCs have been extensively and authentically used for air pollution studies, and minor changes in microclimatic conditions used during the present experiment have proved its efficacy in unraveling the molecular events underlying the phenotypical and physiological changes due to O_3. Our initial studies pave the way for future "in-depth and field-based proteomics study", in relation to develop sustainable agricultural methods for global food security against environmental changes and challenges. Finally, and to emphasize, there is no unique approach/technology capable of addressing the problem of O_3 effects on the plants/crops, and therefore proteomics-generated information/resources will have to be integrated and correlated with other 'omics'-based approaches (transcriptomics and metabolomics), information, and conventional programs.

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