1. Introduction

The human race has evolved through centuries and civilized through many ways on their home planet Earth. Although travelling through the ‘ages’ – ‘man’ had learned the use of fire, utilized the nature and natural resources, gathered knowledge, practiced agriculture, developed industries, and gradually moved towards a superior modernized life. While climbing the steps of this ‘modernized civilization’, ‘man’ introduced a new term – ‘pollution’ to the world’s vocabulary. By definition, pollution is the “undesirable state of the natural environment being contaminated with harmful substances as a consequence of human activities” (source - http://wordnet.princeton.edu). But, at present, this ‘undesirable state of natural environment’ has turned into a major concern for the survival of life on Earth. Air, water, and soil – the three major natural resources, and fundamental backbone of Earth’s environment, have been found to be heavily ‘contaminated with harmful substances’ throughout the world. This does not imply all is contaminated and lost, but just to highlight how precarious the situation is for us humans.

Though, there is no historical account on the pollution on Earth, it was found while reviewing the available literatures that the incident of ‘air pollution’ is not a new event to our society. In an article, published in ‘Science’, John D. Spengler and Ken Saxton commented that – “....soot found on ceilings of prehistoric caves provides ample evidence of the high levels of pollution that was associated with inadequate ventilation of open fires....” (Spengler and Sexton, 1983). During the past couple of decades, rapid urbanization,
unplanned industrialization, fast growth in vehicle use, uncontrolled fossil fuel burning, and injudicious management of natural resources have transformed this indoor problem of ‘prehistoric’ origin into a serious environmental hazard of the present century. The ‘clean air’ of today (and tomorrow) is no more as like was a few decades ago. Looking back, this may have been unavoidable as the human race grew and progressed, paying a price for modernization. In a report on the health effects of air pollution, the World Health Organization (WHO) stated that about two million people die every year because of air pollution throughout the world, while many more suffer from breathing ailments, heart diseases, lung infections, and even cancer (WHO, 2008).

In general, air pollutants can be largely divided in two major categories depending on their formation. The first category is of the primary pollutants, which are emitted directly into the atmosphere, and are mostly present at higher concentrations in urban areas and close to large point emission sources, like carbon dioxide (CO₂). The second category is of the secondary pollutants, which are created by the reactions of primary pollutants under favorable environmental conditions, like tropospheric ozone (O₃) formed due to a series of photochemical reactions involving nitrogen oxides (NOx) and volatile organic compounds (VOCs) under bright sunlight. However, over past decades, it has been quite clearly understood that both these above mentioned air pollutants are also two major components of much discussed ‘global warming’, and hence ‘global climate change’ phenomenon. Tropospheric O₃, along with methane and black carbon, are key contributors to global warming, augmenting the radiative forcing of CO₂ by 65% (Penner et al., 2010). Global agri-production, hence food security, is under severe crisis due to the direct and indirect effects of both the pollutants. Studies have shown that O₃ and CO₂ generally enter plants through stomata, and subsequently affect the inter- and intra-cellular system by modifying the gene expression behavior (Fiscus et al., 2005; Bokhari et al., 2007; Cho et al., 2008; Sarkar et al., 2010). High throughput ‘-omics’ is a combination of potential and central techniques, which can answer many key biological questions in both plants as well as animals. In the present chapter, we mainly focused our ideas towards describing the in depth and up-to-date ‘transcriptomics’ analyses of major agricultural crops under the influence of O₃ and CO₂ rise. We hope that the overall coordinated picture of O₃ and CO₂ – responsive crop transcriptome might help to construct a future roadmap towards the development of next generation crops with optimized yield and other functions for future high-O₃ and CO₂-world.

1.1 Defining ‘global climate change’: Is it really changing?

According to the IPCC report, climate change refers to a statistically significant variation in either the mean/stable state of the climate and in its components/variability, persisting for an extended period (typically decades or longer). It might happen due to the natural internal processes of atmospheric components and/or external forcing and/or to persistent anthropogenic changes in the composition of the atmosphere and/or in the use and management of natural resources (source: http://www.ipcc.ch/ipccreports/tar/wg1/518.htm). In 2007, IPCC for the first time reported to the United Nations (UN) that the earth’s climate system has undoubtedly and significantly got warmer in the past years and will continue to be. According to the action groups of IPCC, the average annual temperature in the Pacific Northwest rose by 1.5° F in the 20th century and is expected to rise 0.5° F per decade in the first half of the 21st century. Now, this climate warming, hence climate change
at global level, has become an important issue and a hot debatable topic throughout the world, in both the developed and developing countries. Initially, it was only an ‘issue’ to be considered, then as a major ‘scientific issue’ to be studied, and after that as an principle ‘environmental policy issue’ to be endlessly debated. But in the past few years, it has been metamorphosed into a significant risk factor to be addressed by the global community, especially for those in the energy sector (Sioshansi and Oren, 2007). Although there are thousands of events that can be directly or indirectly correlated to the global warming and climate change, the prime incidents are as follows -

- Retreating mountain glaciers on all continents
- Thinning ice caps in the Arctic and Antarctic
- Rising sea level – about 6-7 inches in the 20th century
- More frequent heavy precipitation events (rainstorms, floods or snowstorms) in many areas, which affects the survival of both animal and plant life
- More intense and longer droughts over wider areas, especially in the tropics and subtropics
- Reduction in the yield production of major agricultural crops around the world, hence affecting food security and safety

### 1.2 Past and present trends in the concentrations of ambient O$_3$ and CO$_2$: Where we stop?

Interestingly, both the above mentioned gases are present in the earth’s atmosphere from ancient periods. However, 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 the summer months. Projections show that the level will rise 20 to 40% more by the year 2050 in the industrializing countries of the Northern hemisphere. As per the reports and projections, it is quite apparent that this secondary air pollutant will be a far more critical crisis in the coming future than the present time. On the other hand, CO$_2$ also increased in the atmosphere primarily since the industrial revolution, through the burning of fossil fuels in energy industries, transportation, households, and others. Both these O$_3$ and CO$_2$ are the principle greenhouse gases (GHGs) too. In the following sections, we will mainly discuss on the global trends in temporal and spatial distributions of both these pollutants.

#### 1.2.1 Tropospheric O$_3$ trend

In the ambient air, O$_3$ precursors play an important role during long range transport downwind from the sources. Polluted air masses from urban and industrial areas can affect suburban and rural areas, even reaching to remote rural areas traveling 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). Ozone formation also depends largely upon prevailing meteorological conditions of the area. Tiwari et al. (2008) reported positive correlation between mean maximum temperature/sunlight with O$_3$ concentration.

Background O$_3$ concentrations have more than doubled in the last century (Meehl et al., 2007). There is an increase in annual mean values of O$_3$ ranging from 0.1 to 1 ppb per year$^{-1}$ (Coyle et al., 2003). In the Northern hemisphere, O$_3$ is also influenced by the influx from the
However, O₃ varies 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 East Asia exposed to the summer monsoon which transports oceanic air with less O₃, the seasonal patterns show a peak during pre- and post-monsoon periods (He et al., 2008). During the day, O₃ concentration pattern depends on elevation and shows strong diurnal variations at lowland sites where its destruction dominates during the night and vertical mixing together with photochemical activity causes highest levels in the afternoon.

In rural agricultural areas of the USA, mean O₃ concentrations reach between 50 and 60 ppb (90th percentile) (USEPA, 2006). Concentrations over the mid and high-latitude of the Eurasian and North American continents were 15 - 25 ppb in 1860, but increased to 40 - 50 ppb even in remote areas and from 10 - 15 ppb to 20 - 30 ppb over the mid- and high-latitude Pacific Ocean, respectively (Lelieveld and Dentener, 2000). Measures taken to reduce O₃ precursor emissions, led to changes in O₃ levels in many rural and urban areas of Europe, North America, and Japan; the frequency of the highest values shows a declining trend, while lowest values are increasing (Jenkin, 2008). The US EPA has reported that emission reduction in O₃ precursors has been substantial over the past 29 years (US EPA, 2009). The percent change in emissions of NOx and VOCs were 40 and 47%, respectively, for the period 1980 - 2008.

In India, despite of the favorable climatic conditions for O₃ formation, very limited data from systematic monitoring of O₃ are available. O₃ concentrations are continuously increasing from 1992 to 2008 with higher peaks in rural areas. In a field transect study at urban sites of Varanasi, O₃ concentrations varied from 6 to 10.2 ppb during 1989-1991 (Pandey and Agrawal, 1992). During the same period, daytime O₃ concentrations (9 hr mean) were reported to vary from 9.4 to 128.3 ppb at an urban site in Delhi (Varshney and Agrawal, 1992). It was observed that 10 h ground level mean O₃ concentrations in Delhi varied between 34 to 126 ppb during the winter of 1993 (Singh et al., 1997). At Pune, an annual average daytime O₃ concentration of 27 ppb and hourly concentration between 2 and 69 ppb were reported during August 1991 to July 1992 (Khemani et al., 1995). Lal et al. (2000) studied the pattern of O₃ concentrations from 1991 to 1995 at an urban site of Ahmedabad (India), and reported that daytime mean O₃ concentrations exceeding 80 ppb were rarely observed. The monthly average O₃ concentrations ranged between 62 and 95 ppb in summer (April - June) and 50 and 82 ppb in autumn (October - November) at New Delhi (Jain et al., 2005). Emberson et al. (2009) reported that large parts of South Asia experience up to 50 - 90 ppb mean 7 h (M 7) O₃ concentration. Mittal et al. (2007) using the HANK model reported O₃ concentration varying from 25 to 100 ppb over the entire Indian region.

1.2.2 Tropospheric CO₂ trend

Atmospheric CO₂ is accelerating upward from decade to decade. For the past ten years, the average annual rate of increase is 2.04 ppm. This rate of increase is more than double the increase in the 1960s. However, other than being a potent GHG and the main basis of global warming, CO₂ is also a key substrate for plant growth. Interestingly, scientists observed nearly similar trends in the overall concentrations of ambient CO₂ throughout the globe, which means that everybody is under similar crisis. Uncontrolled
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Anthropogenic activities throughout the world have caused the atmospheric CO₂ to increase continuously from about 280 ppm at the beginning of the 19th century to 369 ppm at the beginning of the 21st century (Figure 1). Projections also range between about 450 and 600 ppm by the year 2050, but strongly depend on future scenarios of anthropogenic emissions (Woodward, 2002).

Fig. 1. Increasing trends in atmospheric CO₂ concentrations at global level. [data adopted from http://www.esrl.noaa.gov/gmd/ccgg/trends/]

2. Evaluation of O₃- and CO₂-effect on agricultural crops through modern OMICS approaches: A face-to-face mêlée

The effect of both O₃ and CO₂ on the various levels of crop’s responses till the yield has been very well studied so far (for detail, see review Cho et al., 2010). However, their specific effects on the genome, hence - transcriptome and proteome, has not been evaluated to a large extent. In the next section, we have made an attempt to portray the impact of both these pollutants on the agricultural crops through modern OMICS approaches depending on the available reports.

2.1 Multi-parallel OMICS approaches in modern biology

We are running through the golden era of genomics (study of whole ‘genome’ is loosely called ‘genomics’). The genomics era is also in position to use multiple parallel approaches for the functional analysis of genomes in a high-throughput manner. These parallel approaches surely results in an exceptionally swift and effective system for the analyses and deductions of gene(s) function in a wide range of plants, at the level of transcript (transcriptomics), protein (proteomics), and metabolite (metabolomics) (Figure 2). All together, these four approaches are commonly referred as the multi-parallel OMICS approaches in modern biology.
2.2 Case studies

2.2.1 Effect of ozone (O₃) on crops

2.2.1.1 Rice (Oryza sativa L.)

Among all the major crops, rice (Oryza sativa L.) has been studied most for its response to O₃-stress (Agrawal et al., 2002; Cho et al., 2008; Feng et al., 2008; Frei et al., 2010). Agrawal et al. (2002) first reported a detailed combined transcriptomics and proteomics response of rice plants under elevated O₃-exposure. Two-weeks-old rice (cv. Nipponbare) seedlings were exposed to 200 ppb of O₃ for three days in a controlled fumigation chamber. A drastic visible necrotic damage in O₃-exposed leaves and consequent increase in ascorbate peroxidase (APX) protein(s) accompanied by rapid changes in the immunoblotting analysis and two-dimensional gel electrophoresis (2-DGE) protein profiles were observed. They also reported nearly 52 differentially expressed proteins. Among which were the O₃-caused drastic reductions in the major leaf photosynthetic proteins, including the abundantly present ribulose-1, 5-bisphosphate carboxylase/oxygenase (RuBisCO) and induction of various defense/stress-related proteins. Most prominent change in the rice leaves, within 24 h post-treatment with O₃, was the induced accumulation of a pathogenesis related (PR) class 5 protein, three PR 10 class proteins, APX(s), superoxide dismutase (SOD), calcium-binding protein, calreticulin, a novel ATP-dependent CLP protease, and an unknown protein. Feng et al. (2008) also followed the established two-week-old rice seedlings experimental model of Agrawal and co-workers (2002) and exposed plants to 0, 40, 80, and 120 ppb O₃ for nine days. A drastic damage in the photosynthetic proteins (mainly large and small subunits of RuBisCO) and primary metabolism related proteins was found, whereas an induced expression of some major antioxidant (i.e., glutathione-S-transferase and MnSOD) and
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defense/stress-related proteins (i.e., PR5 and two PR10 proteins OsPR10/PBZ1 and RSOsPR10) were reported. In another independent study, Cho et al. (2008) also checked the expression profiles of genes in leaves of two-week-old rice seedlings exposed to 200 ppb O$_3$ for 1, 12, and 24 h using a 22K rice DNA microarray chip. A total of 1,535 genes were differentially expressed more than five-fold over the control. Their functional categories revealed genes involved in transcription, pentose phosphate pathway, and signal transduction at 1 h. Genes related to antioxidant enzymes, ribosomal protein, post-translational modification (PTM), signal transduction, jasmonate, ethylene, and secondary metabolism at 12 and 24 h play a crucial role in O$_3$-response (Cho et al., 2008). Recently, Frei et al. (2010) have tried to identify the possible mechanism of O$_3$-response in rice seedlings by characterizing two important quantitative trait loci (QTL), in two different chromosome segment substitution lines (SL15 and SL41). Their findings suggest that the activity of some major antioxidant genes might contribute significantly in the response strategy of rice plant under higher O$_3$-stress.

In contrast with the above laboratory-based experimental models, Sarkar and Agrawal (2010) had applied ‘field-based integrated OMICS’ approach to understand the background of O$_3$ response in two high-yielding cultivars (Malviya dhan 36 and Shivani) of mature rice plants under natural conditions. They found dependable phenotypical response, in the form of foliar injury, followed by definite changes in leaf proteome. Major damage was in the photosynthetic proteins (large and small subunits of RuBisCO) and primary metabolism-related proteins. Moreover, an induced expression of some antioxidant and defense/stress-related proteins were reported in the rice leaf proteome.

2.2.1.2 Wheat (Triticum aestivum L.)

Wheat (Triticum aestivum L.) is the third most important crop around the globe, and nearly two thirds of the world population depends on this crop for their primary nutrition supplement. Sarkar et al. (2010) employed ‘field-based integrated OMICS’ approach to understand the background of O$_3$ response in two wheat cultivars (cvs Sonalika and HUW 510) against elevated O$_3$ concentrations (ambient + 10 and 20 ppb) under near natural conditions using open top chambers (OTCs). Results of their study showed drastic reductions in the abundantly present RuBisCO large and small subunits. Western blot analysis confirmed induced accumulation of antioxidative enzymes like SOD and APX protein(s) and common defense/stress-related thaumatin-like protein(s). 2-DGE analysis revealed a total of 38 differentially expressed protein spots, common in both the wheat cultivars. Among those, some major leaf photosynthetic proteins (including Rubisco and Rubisco activase) and important energy metabolism proteins (including ATP synthase, aldolase, and phosphoglycerate kinase) were drastically reduced, whereas some stress/defense-related proteins (such as harpin-binding protein and germin-like protein) were induced.

2.2.1.3 Maize (Zea mays L.)

Maize (Zea mays L.) is another important crop at global context. Being a C$_4$ crop, its response to climate change has been always been a bit different from the other plants/crops. Torres et al. (2007) have performed a detailed investigation of O$_3$ response in maize (cv. Guarare 8128) plants through gel-based proteomics approach. In that experiment, 16-day-old maize plants (grown in controlled environment at green house) were exposed to 200 ppb O$_3$ for 72 h, and
then the response was compared with a controlled plant (grown under filtered pollutant-free air). Results showed that nearly 12 protein spots were differentially expressed under O₃ exposure, and can be exploited as marker proteins. Expression levels of catalase (increased), SOD (decreased), and APX (increased) were drastically changed by O₃ depending on the leaf stage, whereas cross-reacting heat-shock proteins (HSPs; 24 and 30 kDa) and naringenin-7-O-methyltransferase (NOMT; 41 kDa) proteins were strongly increased in O₃-stressed younger leaves. The study also enumerated leaf injury as biomarker under O₃ stress in maize leaves.

2.2.1.4 Bean (*Phaseolus vulgaris* L.)

Torres et al. (2007) also conducted a study on response of cultivated bean (*Phaseolus vulgaris* L. cv. IDIAP R-3) against O₃ stress using the same experimental protocol as for maize (see above) and the effects were evaluated using gel-based proteomics followed by MS and immunoblotting. Results showed that in bean leaves two SOD proteins (19 and 20 kDa) were dramatically decreased, while APX (25 kDa), small HSP (33 kDa), and a NOMT (41 kDa) were increased after O₃ fumigation.

2.2.1.5 Pepper (*Capsicum annuum* L.)

Lee and Yun (2006) applied cDNA microarrays to monitor the transcriptome of O₃ stress-regulated genes in two pepper cultivars [*Capsicum annuum* cv. Dabotop (O₃-sensitive) and cv. Buchon (O₃-tolerant)]. Ozone stress up-/down-regulated 180 genes more than three-fold with respect to their controls. Transcripts of 84 genes increased, transcripts of 88 others diminished, and those of eight either accumulated or diminished at different time points in the two cultivars or changed in only one of the cultivars. A total of 67% (120 genes) were regulated differently in O₃-sensitive and O₃-tolerant pepper cultivars, most being specifically up-regulated in the O₃-sensitive cultivar.

2.2.1.6 Linseed (*Linum usitassimum* L.)

Tripathi et al. (2011) analyzed the response of linseed plants under elevated O₃-stress through combined genomics and proteomics approaches. The results showed that 10 ppb elevation over ambient O₃ concentration can cause 50% damage in the genome stability of linseed plants. In line to the genome response, leaf proteome was also severely affected under O₃ stress, and the damages were mainly observed on the photosynthetic and primary metabolism-related proteins.

2.2.2 Effect of carbon dioxide (CO₂) on crops

2.2.2.1 Rice

Bokhari et al. (2007) had exposed 10-day-old rice (*O. sativa* L. spp *Indica* cv. 93-11) seedlings to 760, 1140, and 1520 ppm of CO₂ for 24 h, and assessed the response of test plants through 2-D gel-based proteomics followed by protein identification. Comparative analysis of leaf proteome revealed 57 differentially expressed proteins under elevated CO₂ in the rice leaf proteome. Majority of these differentially expressed proteins belonged to photosynthesis (34%), carbon metabolism (17%), protein processing (13%), energy pathway (11%), and antioxidants (4%). Several molecular chaperones and APX were found to be up-regulated under higher CO₂, whereas major photosynthetic proteins like RuBisCO and RuBisCO activase, and different proteins of Calvin cycle were down-regulated.
2.2.2.2 Wheat

Hogy et al. (2009) have studied the effect of elevated CO$_2$ on the grain proteome of wheat (T. aestivum L. cv. Triso) in a completely free-air CO$_2$ enrichment (FACE) setup. Results of this experiment revealed a total of 32 proteins were affected. Out of them, 16 proteins were up-regulated and 16 proteins were down-regulated. Among the up-regulated proteins, triticin precursor, putative avenin-like beta precursor, serpin, peroxidase 1, alpha-chain family 11 xylanase, starch synthase I, and cytosolic glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were the major proteins, whereas among down-regulated proteins, globulin (Glb 1) storage protein, low-molecular weight glutenin, ATP synthase $\beta$ subunit, and alpha-tubulin were the major changed proteins.

3. Concluding remarks

The projected levels of O$_3$ and CO$_2$ are critically alarming, and have become a major issue of concern for food security worldwide. Scientific evidences indicate that crop plants are in general sensitive to both these air pollutants, but in different ways.

Plant resistance to O$_3$ involves a wide array of response ranging from the molecular and cellular level to the whole plant level. Significant effects of O$_3$ are early leaf senescence, decreased photosynthetic assimilation, altered stomatal behavior, decreased growth and productivity, and reduced carbon allocation to roots and changes in metabolic pathways. Genotype differences in response to O$_3$ are related to stomatal behavior, anti-oxidative potential hormonal regulation, and carbon allocation during reproduction affecting the yield responses. Detailed understanding of genotypic response is crucial in predicting the long-term impacts of O$_3$ on agriculture in global context, including the breeding of resistant cultivars. Several potential O$_3$ biomarkers have been identified, which could be exploited to screen and develop O$_3$-tolerant varieties in future (Figure 3). However, in case of CO$_2$, it is an integrated compound for plant’s survival. So, at the initial stages, any increment in the ambient CO$_2$ levels showed a positive response towards plants yield, but also raised many questions. The behavior of RuBisCO, key enzyme of photosynthesis, is still under debate at higher CO$_2$ levels (Figure 3).

While reviewing the available reports on O$_3$ and CO$_2$ air pollutants, we found that both the stresses leave some kind of specific ‘signature’ on the crops response and that the ‘signature’ is not crop dependent. However, it must be emphasized that there are only limited OMICS studies available on crop responses to O$_3$ and CO$_2$. Future work in our laboratories and those around the world will help provide new and much needed insight into the nature of the plant response to air pollutants and ways and means to help circumvent their deleterious effects. It is quite clear that we will need proper engineering of crops to combat the emerging problem, and research, analysis, and reviews on initial crop-pollutants interaction have pointed toward some important functional traits required while considering the next-generation crops:

i. Crops should have efficient and effective stomatal behavior to properly maintain the balance of external gas influx. As per the present research outcomes, we can see that crops prefer avoidance more than developing resistance towards any stress.

ii. Crops should possess efficient photosynthetic system with higher catalytic ability to generate energy for combating the prevailing unfavorable atmosphere.

iii. Crops should have improved detoxification system and superior stress tolerant molecular network within the cell.
Finally, as both O₃ and CO₂ stress leave a specific signature on the crop plant transcriptome, inputs from genome-wide analysis could be effectively exploited for further crop improvement in vivo, and the objective of our on-going studies.

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This book provides us a thorough overview of Crop Plant with current advance in research. Divided into two sections based on the chapters contents. Chapter 1 provides information about markers and next generation sequencing technology and its use. Chapter 2 is about how we can use Silicon for Drought tolerance. Chapter 3 is to deal with the major problem of rising CO2 and O3 causing environmental pollution. Chapter 4 covers the phenomena of RNAi and its use, application in crop science. Chapter 5 is a review for boron deficiency in soils and how to deal with it for better crops. Chapter 6-10 provide some information regarding recent works going on in crop science.

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