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Preface

Genotoxicity refers to the property of chemical agents to induce changes in the genetic information within a cell causing changes which may result in mutations. Genotoxicity is similar to mutagenicity except that genotoxic effects are not necessarily always associated with mutations. Oxidative stress is a complex chemical and physiological phenomenon that accompanies virtually all biotic and abiotic stresses in higher plants and develops as a result of overproduction and accumulation of reactive oxygen species (ROS).

The book "*Induced Genotoxicity and Oxidative Stress in Plants*" is intended to deliver information on genotoxicity induced by a wide spectrum of genotoxic agents and relative oxidative damage in plants with special reference to the metabolism of reactive oxygen species (ROS). Genotoxicity is the ability of different agents to cause damage to genetic material. However, the damage induced in the genetic material includes not only DNA, but also the cellular components related to the functionality and behavior of chromosomes within the cell. For example, proteins involved in the repair, condensation, and decondensation of DNA in the chromosomes, or other structures, such as the mitotic spindle, responsible for distribution of the chromosomes during cell division. Oxidative stress is a complex chemical and physiological phenomenon that accompanies virtually all biotic and abiotic stresses in higher plants and develops as a result of overproduction and accumulation of reactive oxygen species. Stresses induced by various agents may lead to over production of ROS resulting in progressive oxidative damage and ultimately cell death. Despite their destructive activity, they are well-described as second messengers in a variety of cellular processes, due to tolerance to various environmental stresses. Whether ROS would serve as a signaling molecule or could cause oxidative damage to the tissues depends on the delicate equilibrium between ROS production, and their scavenging. Different genotoxic agents, that enter the ecosystem either naturally or through anthropogenic events, tend to impose serious threats on its biotic components specially the sedentary flora. Plants are exposed to many stress factors including chemical compounds and radiation affecting their seed germination, seedling growth, and floral and fruit development. These stress factors can adversely affect the quality and quantity of the product leading to morphological, anatomical, physiological, biochemical, and molecular damage to plants.

In this book, we have chapters that emphasize on the role of different genotoxins and stress induced by them. The chapters are contributed by experienced, highly dignified, and internationally acclaimed scientists, researchers, and academicians around the world. The chapters are designed in such a way that it clarifies the concepts of the specified themes. The book is aimed at several audiences, from breeders to agronomists, from students to researchers, from teachers to academicians working in the fields of agriculture, plant science, environmental biology, and biotechnology. Each group of reader will have different technical backgrounds and expertise. Therefore, the chapters are written on three levels, namely introduction, main text supported by relevant figures and tables, and bibliography.

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About the Editors

Dr Zeba Khan obtained her master's and Ph.D. degree from Aligarh Muslim University. Her professional journey started with joining as a Senior Project Officer in the National Bureau of Plant Genetic Resources, New Delhi, India. She also worked as a Postdoctoral Research Associate under DST-PURSE funded Project at Aligarh Muslim University. During her postdoctoral tenure, she extensively studied the role of nanoparticles on plants and their genotoxical impacts on plant ecology. Her specific research aims in understanding the role of various physical and chemical agents that are responsible for induced genotoxicity and stress in plants as a response to change in the environment and in a way utilization of Genetics and molecular techniques to improve yield and quality of agronomically important crops. During her post-master's tenure, she worked on mutation breeding in Chicory which is an important medicinal herb. She has published a number of research papers, review articles, chapters in reputed journals and books, and presented papers in various conferences, seminars, and symposiums. She has been honored for her outstanding performance in research and had received the young scientist (2018) and environmentalist of the year award (2019) by IFCEE.

Dr. Mohd. Yunus Khalil Ansari worked as a professor in the Department of Botany, A.M.U, Aligarh and retired as a chairperson on 20.08.2018. He has been associated with the teaching in cytogenetics and molecular biology. His research areas are cytogenetics, mutation breeding and biotechnology. He produced several important mutants in more than thirty plants. He has been actively involved in research and published around seventy scientific research articles in reputed national and international journals and was the coauthor of three books. He attended many national and international conferences/symposia and workshop. Professor Ansari has produced 15 Ph.D.'s and 7 M.Phil. students. He was a co-principal investigator in the Department of Biotechnology sponsored project "in vitro propagation of *Pterocarpus* sp. and *Saraca* sp." and worked as a co-investigator in a project sponsored by the Ministry of Environment and Forest on the preserving 30 endangered plants.

Durre Shahwar received her Ph.D. in Botany from Aligarh Muslim University, Aligarh, India. She is the recipient of the Maulana Azad National Fellowship and National Fellowship from the University Grants Commission, New Delhi. Dr Shahwar has been awarded the Junior Scientist of the year award (2018) by International Foundation for Environment and Ecology, Kolkata in 5th International Conference on Environment and Ecology, University Gold Medal for securing first rank in M.Sc. (Botany) in 2014. She has published several research articles and book chapters in peer reviewed national and international journal. She has also participated in various national and international conferences and received life membership of scientific bodies in India. Her research interests are cytogenetic, plant breeding and molecular biology (proteomics and genomic). She is actively engaged in mutation breeding for genetic improvement of legume crop.

Induced Genotoxicity and Oxidative Stress in Plants: An Overview

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Afshana, Mudasir A. Dar, and Zafar A. Reshi

Abstract

Being sedentary, plants always face a vast array of environment-related factors in the form of ultraviolet rays, higher salt concentrations, water scarcity and dehydration, high water potentials, extremely low and high temperature among other air and soil-borne chemicals. Besides this, an increase in the production of industrial wastes, encompassing toxic heavy metals and metalloids constantly put heavy stress loads on plants. Majority of these agents have, very recently, been implicated to harmfully alter the chemical and physical aspects of DNA. This is deemed to happen as a consequence of oxidative stress and reactive oxygen species (ROS) outburst. Consequent to the DNA alterations and genome instability, plants face numerous cytotoxic complicacies which negatively impact their health and hence, yield. Most importantly, the toxic agents induce ROS production, damage other cellular macromolecules, including the vital photosynthetic apparatus. Surging industrialization and widespread use of chemical fertilizers, despite inlaid with some positives, have recently been perceived as serious challenges for plants to cope up with around the globe. To get on well and adapt with the genotoxic agents and the follow-up stress, wide range of efficient counteracting mechanisms spanning over morpho-anatomical, hormonal and biochemical features got evolved in plants. Interestingly, at the molecular level, heavy metal generated genotoxicity and allied disruptions are more than efficiently overcome by changing the activity profile of stress-responsive genes. Another potent way of overcoming genotoxic stress and genomic instability in plants is via epigenetic modifications. Recent advancements in our understanding of environmental stress-induced toxicity and the follow-up compensatory responses (both transcriptional and epigenetic) are anticipated to recognize the

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crucial avenues in the target pathways for elevating the resistance and endurance of crop plants to different environmental stresses.

Keywords

Genotoxicity · Heavy metals · Drought · Salinity · Reactive oxygen species (ROS) · UV radiations

Abbreviations

ABA	Abscisic acid
ADP	Adenosine di-phosphate
AFLP	Amplified fragment length polymorphism
AP-PCR	Arbitrarily primed polymerase chain reaction
APX	Ascorbate peroxidase
ASc	Ascorbate
ATP	Adenosine tri-phosphate
BER	Base excision repair
CAT	Catalase
DNA	Deoxyribonucleic acid
DR	Direct repair
DSBR	Repair of double-strand DNA breaks
FQs	Fluoroquinolones
GR	Glutathione reductase
GSH	Glutathione synthetase
GSSG	Glutathione disulphide
HR	Homologous recombination
LHC	Light harvesting complex
MMR	Mismatch repair
mRNA	Messenger ribonucleic acid
NER	Nucleotide excision repair
NHEJ	Non-homologous end joining
PC	Phytochelatins
PCD	Programmed cell death
POX	Peroxidase
PS	Photosystem
RAPD	Random amplified polymorphic DNA
ROI	Reactive oxygen intermediates
ROS	Reactive oxygen species
RuBP	Ribulose 1,5-bisphosphate
SA	Salicylic acid
SOD	Superoxide dismutase
SSR	Simple sequence repeats
UV	Ultraviolet

1.1 Introduction

Being unable to move plants always are bound to cope with a great variety of environmental constraints, limiting their growth and hence, yield (Dutta et al. 2018). Amongst these constraints, harmful UV radiations, salinity, industrial wastes containing toxic heavy metals are most serious with prominent negative impacts on crop plants. A disproportionate fraction of these stress-inducing environmental factors are known to disrupt the physical and chemical parameters of genetic material (DNA). Thus, by altering the genetic material (genotoxic), these are expected to disrupt the morpho-physiology and biochemistry of the subject plants a great deal. Interestingly though all the genotoxic materials change the structure and chemical aspects of DNA, but only some are able to cause mutations. This may better be paraphrased thus, 'All mutagens are genotoxic, but it's not the other way round'. To cope up with the stress causing genotoxic stuff in the environment, plants have evolved enormous counteracting mechanisms which efficiently reduce the level of oxidative stress and greatly help scavenge the harmful reactive oxygen species (ROS). In this chapter, we are interested to understand the influence of various genotoxic agents (physical and chemical) on the performance of crop plants, particularly their yield and how do plants get over with the serious and harmful consequences of genetic material altering agents. Progressive industrialization concomitant with global climate change and other anthropogenic activities has added to the hostilities of atmosphere, hydrosphere and lithosphere, which severely affect the crop plants (Wright and Nyberg 2015). In view of this, environmental stresses and the associated issues like delayed growth and drastic crop yield reduction have emerged as one of the major concerns for the world. Increasing population and the negative impacts of heavy metal-induced stress on plant health impose tremendous roadblocks in meeting the world's ever rising food demands (Wani et al. 2018). Harmful implications of industrial development can be more than compensated by breeding stress-tolerant crop plants in future.

1.2 Different Genotoxic and Oxidative Stress-Causing Agents

1.2.1 Heavy Metals

Heavy metals in the soil compete with essential mineral nutrients for binding sites and are thus absorbed on the root surface (Ramkumar et al. 2020). Straight away after they enter the cells of plants, multifaceted effects of toxic heavy metals in the form of structural and functional disruptions of genetic material and proteins occur. This is materialized directly through attacks on thiol substituents of protein molecules drastically altering their conformational and functional aspects (Bertin and Averbeck 2006). It is well known that heavy metals induce oxidative damages in plasma membranes and other macromolecules including photosynthetic apparatus via increased production of reactive oxygen species (ROS). Decreased membrane

endurance, significant reduction in photosynthetic yield, besides other physiological and biochemical disruptions, is believed to be an immediate outcome of reactive oxygen species formation due to heavy metals. Other important implications linked with ROS production include curtailment in the production of different pigments, imbalanced hormone synthesis, disturbed nutritional status, halted genetic material copying and delayed cell cycle (Sharma et al. 2012). Subject to type and concentration of heavy metal and developmental stage of the plant being exposed, a wide range of stress responses are seen in plant cells. In effect sophisticated heavy metal modulating and ROS scavenging pathways operate in plants to withstand their chemical toxicity (Chan et al. 2016).

Heavy metals can affect developmental progression, pace and timing of senescence and production of energy-rich molecules because they are highly active. Due to indiscriminate utilization of heavy metals in industries and agro-technology, their high bioaccumulation and toxic features are among the key abiotic stress agents for life forms (Shah et al. 2010). Many abnormalities in the genetic information have been reported to occur due to either high metal concentration or their unbalanced and inappropriate proportion in different cellular compartments. Toxic metals and other important mineral elements reach cells by common mechanisms of absorption and uptake processes. The amount of heavy metals consumed by plants varies greatly depending upon their concentration and speciation in the soil water. These move from the soil solution to root surfaces, enter the root cells and ultimately reach the shoots through the transpiration stream (Imtiyaz et al. 2016). Excessive metal concentrations induce toxic implications via (1) altered cell membrane permeability; (2) sulphydryl (-SH) cation reactions; (3) reaction affinity with phosphate moieties of ADP or ATP molecules; and (4) critical ion substitution (Kumar et al. 2017).

The effect of their devastating impact on plants mainly includes a powerful and rapid disruption of the developmental progression in both upper and lower plant parts (Alaoui-Sosse et al. 2004). Most importantly they can also cause a drastic reduction in the efficiency of assimilatory apparatus and in some instances evoking premature ageing (Alaoui-Sosse et al. 2004). Heavy metal exposed plants also possess small and thick belowground parts which appear to be or loosely organized (Casella et al. 1988). Amongst all these effects, reduced growth and prior onset of ageing and senescence are taken as the most severe consequences of chronic heavy metal exposure in plants. Meanwhile, the important knowledge of heavy metal induced eco-physiological alterations may have great implications for future research in improving crop yields of plants.

Data from in vivo and, in particular, in vitro research have shown that heavy metals are capable of releasing protein, lipid and thylakoid membrane element components necessary for photosynthetic operation. Previous studies have shown that a surplus of heavy metals get strongly linked with plasma membrane and other cellular structures via oxygen and amino acids (histidine, tryptophan and tyrosine) especially after illumination (Maksymiec 2007). Consequently, the PS II quinone acceptor sites, and/or TyrZ to P680 + electron donation, and electron flow through PSII reaction centre cyt b559 are disturbed. Certain studies have shown the Mg of chlorophyll in many plant species is being substituted with some highly toxic heavy

metals. The decrease in chlorophyll synthesis, following exposure to heavy metals, can ensue because of suppressed synthesis in chlorophyll forming enzymes (Maksymiec 2007). Hg primarily acts on Cu-substituting plastocyanin in its molecule, thus trying to block the electron's passage to PSI (Radmer and Kok 1974). Some in vitro conformational modifications in light harvesting complex II (LHCII) arise due to a complex of cadmium, mercury, lead and some associated proteins (Ahmed and Tajmir-Riahi 1993). Thus, as per Krupa and Baszynski (1995) changes in the various sections of the photosynthetic apparatus could be partly due to the direct intervention of huge amounts of heavy metals.

1.2.1.1 Toxic Implications of some Heavy Metals in Plants

Metal contaminants can be present in soil, air or water and by far soil is the most heavy metal polluted part of the biosphere due to the fact that these metals remain there for longer durations (Lasat 2002). Because of their possible adverse ecological consequences, contamination of croplands by these heavy metal elements and ensuing crop yield reductions has emerged as a grave among the environmentalists. In view of their prevalence in soils and huge toxicity in crop plants, heavy metals are aptly named as soil contaminants.

The warning of heavy metal contamination began with the effects of mercury ingestion which caused Minamata disease. Liu et al. (1994) reported that in many plant species, high concentrations of heavy metals have been found to be chromotoxic and mutagenic. In plants such as *Allium cepa* (Liu et al. 1994) and *Zea mays* L., heavy metal like iron (Pb) usually affects the root growth and cell division (Sagbara et al. 2020).

With the onset of the industrial age, the issue of metal genotoxicity has gained new dimensions. To cope up with the emerging uses and demands for novel materials, huge quantities of new mineral elements, which are not used before, are being mined world over. Such metals are released by air, water and soil into the biosphere and eventually impact the physiological processes of plants, animals and humans. Notwithstanding the fact that radioactive and organic wastes generated toxicity exceeds the heavy metal pollutants mobilized from all combined sources, the potential toxic implications on crop plants and the bioaccumulation of heavy metals along food chains cannot be underestimated (Pacyna et al. 2016).

Several experiments have been done recently in different microbes and animals to test and assess the levels of metal inflicted genotoxicity. Though previously only a few reports highlighted the apparent genotoxic consequences of heavy metal contamination in plant systems, it is now well understood that arsenic, lead and mercury cause a number of breakages (clastogenic) in chromosomes and in some instances alter the genetic material (mutagenic). Besides causing a number of chromosomal and DNA defects, heavy metals are well known for decreasing the rate of division in plant cells (Liu et al. 1995). The degree and extent of genetic material alterations and chromosomal deformities, besides depending on the heavy metal concentration also relies on its oxidation status and exposure time. It has been reasonably concluded that the effect of heavy metals is more apparent and easily recognizable when plants are subjected to high metal concentration treatments for a longer time (Patra et al.

2004). Another twist in the story of heavy metal effects on plants is that the intensity of toxicity is conditioned to diploid chromosome number, lengthwise expansion of chromosomes and the occurrence of metacentric chromosomes (Ma and Uren 1995).

Among the heavy metals Cd, Hg and Pb are known to have immensely harmful and long-lasting genotoxic impacts in plants (Chaoui et al. 1997). For instance, higher oxidation state mercury (Mercuric form), which has a potential capability of getting associated with the genetic material through covalent linkages, causes exchange of sister chromatids in chromosomes (Beauford et al. 2006). Additionally, in a concentration-dependent manner, it causes a significant drop in mitotic index and increases the incidences of aberrations in chromosomes (Patra et al. 2004). Considering the impact of heavy metals on the yield of crop plants, quite recently scores of studies focussed on evaluating the genotoxicity of plants after being exposed to highly toxic heavy metals like Mg, Pb, Cu, Mn and Cd have been carried out. These studies hugely rely on cytological (chromosome abnormalities and formation of micronuclei), molecular (comet assay) and cutting-edge molecular genetic advancements (RAPD, AP-PCR, AFLP, SSR, etc.) (Enan 2006). Heavy metals such as cadmium, lead, chromium and zinc are found to cause drastic negative impacts on seed germination and radical length in *Cicer arietinum* (Gupta et al. 2006). Despite obvious morpho-anatomical anomalies in this species, other cytological defects like bridge formation, laggards, stickiness and fragmentation of chromosomes were also reported (Siddiqui 2015). Likewise, increase in Cd concentration, besides causing membrane lipid peroxidation via ROS, has been implicated in causing genome instability through significant double-stranded DNA breaks in *Vicia faba* (Lin et al. 2007).

1.2.1.2 Response of Plants to Heavy Metal Induced Oxidative Stress

Species survival and persistence of the global biodiversity fundamentally counts on genomic stability due to several protective and repair mechanisms. Due to unprecedented human population explosion and the consequent change in the global environmental and climatic scenarios, enormously huge loads of stress are being directed on plants. Despite lacking the means of locomotion and other avoidance mechanisms plants, however, employ unique defensive and scavenging mechanisms to negate the harshness and hostility of the environment. A rapid outburst of reactive oxygen species intermediates (oxidative outburst) encompassing H_2O_2, $\dot{O}_2$ and $\dot{O}H$ is by and large the most frequent response of plants to environmental stresses like drought, temperature, salinity, radiation, metal, among others (Bolwell et al. 1995). There is a hypothesis named 'general adaptation' syndrome which advocates that different stress types evoke a similar response in plants. This hypothesis holds that the adaptive response in plants depends on the production of reactive oxygen intermediates (ROI) (Leshem and Kuiper 1996). Though disastrous to a number of cellular constituents especially DNA leading to genotoxicity through mutations and apoptosis (Bray and West 2005), ROIs are also known to impart defence (Alvarez et al. 1998), enhance growth and development (Van der Zalm and Schopfer 2004), cause programmed cell death (PCD) (Breusegem and Dat 2006) and initiate responsive signal transduction cascades (Pitzschke and Hirt 2006). One of the principal

counteractive strategies plants opt to respond many adverse environmental stresses is their inherent adaptive response. Most notably the plants which were long thought to be non-responsive have been found to possess diverse adaptive stress response (Panda and Panda 2002). Not surprisingly, therefore, plant cells when subjected to non-cytotoxic low doses of genotoxic substances, they get resistance against heavy doses of either the same or different genotoxin. This behaviour of plants towards genotoxins is specifically termed as genotoxic adaptation. Very recently, however, the above phenomenon has been named as 'conditioning hormesis' in plants (Calabrese et al. 2007). In a range of both prokaryotic and eukaryotic systems, low non-toxic doses of metals, high energy ionizing radiation, oxidative agents, besides other alkylating substances and neutrons trigger comprehensive genotoxic adaptations (Dimova et al. 2008). This has been primarily assessed and tested in the anomalies of spindle association, chromosomal abnormalities, generation of micronuclei, and assays regarding comet and homologous recombination phenomenon (Cortes et al. 1994). Though in vague, breakthroughs in molecular genetic studies hold that the function of genome protection and stability is due to a network of DNA repair pathways, some special proteins, unique polypeptides and epigenetic modifications (Dimova et al. 2008).

Heavy metals are one of the major agents causing lipid peroxidation and bio-membrane damages. The chief decomposition by-product of lipid (polyunsaturated fatty acids) peroxidation, malondialdehyde (MDA) in plants, is considered to be invoked largely due to the heavy metal generated stress (Hassan et al. 2017). For combating heavy metal toxicity, plants, therefore, produce varied types of high affinity low molecular weight thiols which strongly bind damage-causing heavy metals (Ghori et al. 2019). Amongst all these thiols, the most important and common thiols produced in plants include glutathione (GSH) and cysteine. GSH, whose synthesis occurs by the enzymes γ-glutamyl cysteine synthetase (GSH1) and glutathione synthetase (GSH2), both supported by ATP, is a sulphur containing tripeptide represented as γ-glutamate-cysteine-glycine. Besides being a precursor of phytochelatin, GSH significantly also detoxifies cadmium and nickel (Çelik et al. 2020). Phytochelatin polypeptides (γ-Glu-Cys)nGly(n = 2–11), which contain a large proportion of cysteine amino acids, possess strong metal affinities. These phytochelatins, which occur in a wide range of organisms including plants, fungi and many others (Grill et al. 1985; Gekeler et al. 1989) are formed due to the activity of unique enzyme named as phytochelatin synthases. Phytochelatins, in plants, are known to form strong complexes with some deleterious heavy metals in the cell cytoplasm and then subsequently move them into the vacuole (Kumar et al. 2017), offering immense protection.

The detoxification mechanisms evolved in plants in response to heavy metals involves binding (chelation) and in some cases sub-cellular localization. Multiple heavy metal detoxification mechanisms, acting in coordination and intricately networked, help plant to survive in heavy metal contaminated environments via repair of damages to their genome (Moura et al. 2012). Surprisingly, both short- and long-term processes underlying these repair mechanisms are operative in plants at various levels. Amongst the immediate or short-term processes include the rapid

changes in the transcriptional status of stress-regulated genes, ultimately affecting plants metabolism and physiology (Wada et al. 2004). In contrast, the long-term heavy metal initiated plant cell responses comprise various types of genetic modifications among which epigenetic modulations are significantly implicative (Schroeder et al. 2013). Need-based expression changes in stress-induced genes, which is long debated to be an intimate consort of stress response in plants, involves both universal and gene-specific regulatory mechanisms. Quite rationally it's therefore impressed upon that coordinated and profusely networked domains of stress perception and signalling pathways, involving cross talks at various steps, are actually behind the scenes of counteractive plant responses to different heavy metals (Wada et al. 2004).

1.2.1.3 Glutathione-Induced Stress Tolerance in Plants against Heavy Metals

In almost every part of the cell including cytoplasm, chloroplast, endoplasmic reticulum, vacuole and mitochondria, glutathione (GSH) has been reported to occur (Vogelsang and Dietz 2020). It is the most common non-proteinaceous thiol group present in plant cells and its wide range of biochemical functions have largely been assigned due to the thiol group. The nucleophilic nature of thiol group grants GSH the ability to form links, named as mercaptide linkages, with both metals and some select electron loving molecules (electrophiles). Unique chemical behaviour, relatively high stability and considerably large solubility in water allows the plants to use this compound in overcoming the negative impacts of oxidative stress of heavy metals, alongside some organic chemicals of endogenous or exogenous nature (Sarwar et al. 2017). Many studies suggest that overexposure to harmful metals directly or indirectly through their influence on metabolism leads to the formation of ROS. In plant systems, GSH acts by controlling the levels of one potentially severe oxygen species H_2O_2 (Gechev et al. 2006). By doing this a significant fraction of reduced form (GSH) gets converted to its oxidized state (GSSG), which is mandatory for the operation of some redox signalling pathways in plant systems (Millar et al. 2003). This change in the relative amounts and hence the ratios of reduced to oxidized forms (GSH/GSSG) of glutathione, indicating the cellular redox balance is thought to be associated with ROS perception in plants. Reduced glutathione (GSH) with strong antioxidant properties directly reduces most of the ROS generated during stress episodes (Millar et al. 2003).

In addition to scavenging most of the ROS, GSH also functions as an immediate precursor for the formation of phytochelatin. Phytochelatins (PCs) which are small peptides possessing unique metal linking properties were at the outset found in the higher plant cell suspensions, exposed to Cd (Su et al. 2020). Following this many other eukaryotes including higher plants were shown to contain PCs (Gekeler et al. 1989). In addition to Cd, heavy metals like Hg, Cu, Zn, Pb and Ni were also reported to induce PC formation. Formation of PCs from GSH in plant cells when treated with heavy metals involves phytochelatin synthase (PCS) enzyme. Straight away multitudes of physiological studies have implicated the physiological importance

of PCs in metal detoxification pathways alongside the maintenance of ionic balance (Hirata et al. 2005).

1.2.2 Ultraviolet Radiations

Genome stability, an important predictor of plant developmental progression and health, is closely linked with crop productivity. However, a wide range of well-known genotoxic agents (both chemicals and radiations) cause chemical and physical alterations in DNA structure and hence decrease its stability (Prasad et al. 2008). The genotoxic agents change genome integrity via oxidations in the individual bases, severely affecting the vital DNA copying processes and information transfer to mRNA(transcription) which causes the cell to die (Cadet and Davies 2017). Amongst the radiations, UV-B from sunlight with strong penetration power affects the plants and animals. These radiations are known to inhibit growth and development in plants due to reduced genome stability via oxidation and formation of cross-links between DNA bases (Bornman et al. 2019). Consequent upon these integrity and stability issues of genome, a spectrum of other physiological changes like recession in normal protein formation patterns, destruction of plasma membrane constituents and photo-assimilatory complexes occur that negatively influence the developmental pace of the whole organism. On the whole, the radiation-induced DNA damages can have a wide range of genotoxic and cytotoxic implications on the overall performance of plant cells. Left unrepaired, DNA structure and stability anomalies are expected to induce a series of functional and metabolic disruptions in plant cells (Burdak-Rothkamm and Rothkamm 2018).

1.2.2.1 Repair of DNA Damage Caused by Oxidative Stress and Induced Genotoxicity

To get along and adapt to the harmful effects of radiation caused DNA damages, the plant cells possess an in-built array of DNA repair systems, credibly increasing the chances of unaltered genetic transmission across generations (Vishwanatha et al. 2016). On recognizing the DNA damage, the eukaryotic cells delay their division and instead enter a checkpoint to repair the damages through the activation of a signal transduction cascade. The checkpoint proteins, including a conglomerate of sensor kinases, adaptors and many down-regulated effector protein kinases, help the cells to respond to DNA damages before entering the division phase (Petsalaki and Zachos 2020).

Several DNA repair pathways, working at different levels, are operative in an organism. They can be categorized as: (A) Direct repair (DR) which is essentially an enzyme (photolyase)-mediated, light-dependent photo-reactivation process (Jiang et al. 1997); (B) Mismatch repair (MMR), comprising base excision repair (BER) and nucleotide excision repair (NER) systems; in this repair system, damaged DNA bases and nucleotides are removed and replaced with correct ones (Shuck et al. 2008) and (C) Repair of double-strand DNA breaks (DSBR), which depends on the process of non-homologous end joining (NHEJ) and homologous recombination

(HR) (Puchta and Hohn 1996). All these pathways, though specific and uniquely efficient, are crucial to ensure the continued existence and stability of genomes. However, some kind of links in the execution of different DNA repair pathways has been reported in a number of studies. Molinier et al. (2008), using a genetic approach found a crosstalk of (DR), a prospected nexus between NER and HR mechanisms, with RAD1–RAD10 endonuclease intervention has also been stressed upon (Dubest et al. 2002). In spite of some initiatives taken, detailed understanding of plant-specific DNA repair mechanisms had to go a long way.

1.2.3 Temperature

1.2.3.1 High Temperature Stress

Higher temperature stress and its adverse impacts on physiology (photosynthesis, respiration), metabolism of proteins and other important membrane constituents severely limit the growth and distribution of plants in natural environments (Georgieva 1999). During high temperature, oxidative stress occurs due to overproduction of reactive oxygen species (ROS) which modifies the synthesis of macromolecules and nucleic acids (Khan and Shahwar 2020). Raised temperatures cause injury to plant cells by enough formation of active oxygen species like superoxides, peroxides and hydroxyl radicals, impairing the structure as well as function of vital cellular constituents (Van Breusegem et al. 2001; Liu and Huang 2005). Upon exposure to extremes of temperature, an outburst of highly active oxygen species production occurs in plants cells which subsequently result in cell damage and undesirable physiological alterations. Long-term exposures to temperature extremes and the consequent increase in ROS formation can drastically cause enzyme inactivation, lipid peroxidation, protein and DNA damages. For compensating the negativity of higher temperatures in plant species, a number of detoxification mechanisms (enzyme or non-enzyme dependent) have evolved which convert a considerable fraction of harmful oxygen entities to relatively benign molecules (Sairam and Tyagi 2004). Enzymatic antioxidants like superoxide dismutase, catalase, peroxidase, ascorbate peroxidase and glutathione reductase actively detoxify the highly reactive superoxide and H_2O_2 (Mittler 2002). Treatment of plants with salicylic acid (SA), abscisic acid (ABA) and calcium chloride additions shows some promise of enhancing the thermal resistance in a number of crop plants (Larkindale and Knight 2002; Chakraborty and Tongden 2005). Increase in thermal tolerance is particularly vital and indispensable for plants as they can't move to favourable environments in response to the daily temperature fluctuations.

Photochemical reactions and associated carbon metabolism reactions are more likely to get affected if temperatures go beyond 30 °C (Wang et al. 2009). Additionally, the water status of leaf cells and intracellular carbon dioxide are markedly affected due to high temperature generated heat stress-induced stomatal closure (Greer and Weedon 2012). All these effects in consortia lead to an apparent reduction in photosynthetic rate and hence delays developmental progression by

stalling growth. While the underpinning procedure involved in photosynthetic inhibition due to heat stress in plants is largely unclear, reduction in the rate of carbon fixation during photosynthesis due to inhibition of RUBP is believed to be mostly the most plausible reason (Kurek et al. 2007). One more likely explanation suggests that the heat stress significantly halts the process of electron transfer in light reaction of photosynthesis and decreases the operation of rubisco enzyme (Makino and Sage 2007). Amongst all the photosynthetic components PSII (crucial for photosynthetic electron transport in photosynthesis) is the worst affected by elevated temperature stress (Havaux 1996). In chloroplasts, the most severely affected enzymes due to heat stress are PSII, Rubisco and ATP synthase (Asthir 2015).

1.2.3.2 Low Temperature Stress

Cell damage, decreased production and limited distribution of plants in natural environments are also thought to be an immediate outcome of low temperature (0–15 °C) stress (Theocharis et al. 2012). Cold stress initiated damages in the cellular structures of non-adapted plants are observed very early (few hours after subjecting to cold). Moreover, it is a well-known fact that cold temperature treatment for a small duration induces only some transitory alterations while long-term exposures cause necrosis or death. Cold acclimation in plants has been recently related to the attainment of resistance to low temperatures (Theocharis et al. 2012). Reorganization of molecular and physiological features is believed to be the key behind cold tolerance and cold counteractive measures in some plants.

In addition to direct damages to cellular constituents, cold also severely impacts PSII restoration and damage repair. A number of reports confirmed that low-temperature stress inhibits the repair of PSII rather than causing photo damage to it. Protein labelling studies in Synechocystis cells showed a considerable suppression in de novo synthesis of D1 protein at lower temperatures (Allakhverdiev and Murata 2004). Another well-known fact is that extreme low temperature blocks the formation of D1 protein of PSII that is intensely associated with the assembly of photo system II constituents and repair (Kanervo et al. 1997).

1.2.3.3 Temperature Stress-Related Antioxidant Responses in Plants

By and large, the major outcome of oxidation related stresses in plants includes surged ROS production which consequently disturbs the structural and metabolic balances (Munné-Bosch and Alegre 2002). However, to a considerable extent these negative effects of temperature in a large number of plants are compensated (Janská et al. 2010). Plants are known to alter their metabolism for protecting vital proteins and other indispensable cellular structures, maintaining their turgor and osmotic balances (osmotic adjustments) and in some cases cause the modification of antioxidant system to properly stabilize the redox balance and maintenance of cellular equilibrium (Janská et al. 2010; Hasanuzzaman et al. 2013). Quite surprisingly temperature initiated stress effects in a large number of plant species have been observed to be alleviated by changes in the activity profile of a set of temperature stress-responsive genes (Semenov and Halford 2009).

Plants are believed to increase their thermostability and antioxidant potential just to reduce the incidence of temperature-related structural and physiological perturbations (Xu et al. 2013). A wide range of essential antioxidant enzymes in plant cells are drastically affected within the temperature range of 0–50 °C. The activity of CAT, SOD and APX increases upto a temperature of 50 °C and thereafter shows a considerable decline. On the contrary, the activity of POX and GR diminishes with rising temperature and have been shown to perform better in the temperature range of 20–50 °C (Chakraborty and Pradhan 2011).

Besides depending on the exposure time, magnitude of temperature also influences the response of antioxidant formation in many plant species. For instance, the Pepper plants, treated with 8 °C for 3 consecutive days show the oxidation and peroxidation associated symptoms during the first day (Airaki et al. 2012). During the first 24 h, formation of CAT and APX gets invoked, raising the concentration of Asc and GSH. The oxidative stress-related effects in pepper plants got receded in the second and third day of low temperature treatment owing largely to early adjustment of their antioxidant metabolism during the early hours due to adjustment of their antioxidant metabolism (Airaki et al. 2012).

1.2.4 Pesticides

In the face of development and expansion of our economy, we have unknowingly put our life supporting natural resources like water and soil at risk. Among the plethora of industries polluting the precious water and soil resources, pesticide formulation plants are highly perilous. Worldwide as well as in India pesticides like organo-chlorines and phosphates are well-represented contaminants of aquatic and terrestrial ecosystems (Jayaraj et al. 2016). Pesticides present in soils and water in the form of suspended or dissolved particles get accumulated in the edible parts of crop plants, causing a serious threat to the well-being of humans. Recent spike in agriculture production through mechanisation and indiscriminate use of hazardous pesticides and chemical fertilizers have tremendously contributed to water pollution in developing countries. Many pesticide residues which are known to have harmful DNA alteration potencies cause serious mutations (Rahman and Debnath 2015).

Pesticides include a broad range of chemicals used to protect crop plants from fungi, insects, herbs, etc. Amongst these fungicides, herbicides and insecticides constitute the mostly widely used chemicals effective against disease caused by fungi, herbs and insects, respectively (Dhanamanjuri et al. 2013). Unfortunately, the excess use of these chemical pesticides has led to their accumulation in the soil (Ahemad 2011), thereby reducing the fertility of soil. Furthermore, the indiscriminate use of these chemicals is known to have induced significant resistance in the insect pests and other fungi, reducing their effectiveness which is reflected in their tremendous usage. Also it has been ascertained that most of these agrochemicals, besides removing harmful agents, also decline the population of some beneficial insects (Kim et al. 2017). Out of the total 4.6 million tonnes of pesticides used annually worldwide, almost 85% are alone used in agricultural fields (Zhang et al.

2011). Moreover, amongst all kinds of pesticides, herbicides and fungicides are disproportionately used globally (De et al. 2014). Large-scale use of these agrochemicals is supposed to have some serious consequences in plants with apparent disruptions of important physiological and biochemical processes. This occurs due to disruption of membrane structure, reduced photosynthetic yield, and compromised pigment production, disruption of hormone and nutrient status, and halting of DNA synthesis, gene expression and cell proliferation (Shakir et al. 2016). Exposure to herbicide 2,4-D in chicory has been found to induce chromosomal variations in chicory (Khan et al. 2009) A serious concern related to herbicide use is that many of these act non-specifically (Xia et al. 2006), causing considerable economic losses in multiple crop farming. Agrochemicals have been reported to affect plant health by casing genotoxic damage of fundamentally important bio-molecules including DNA by spiking up the pace of reactive oxygen species production (ROS) (Sies 2015). ROS-induced cellular damages especially of membrane proteins and nucleic acids eventually cause a wide spectrum of oxidative and genotoxic responses in plant cells. In response to pesticide-mediated oxidative stress and cellular damages, plant cells exhibit some antioxidant defences (Banerjee et al. 2001). These defences which are both enzymatic (superoxide dismutase, catalase, ascorbate peroxidase and glutathione reductase) and non-enzymatic (phenylpropanoids, carotenoids, glutathione and proline) effectively inactivate and detoxify the harmful free radicals which are later on scavenged (Yusuf et al. 2011). Besides, agrochemicals have also been implicated to have some cytotoxic effects in a number of plant species (Pandey 2008). Excessive exposure to pesticides in *Allium cepa* and *Vicia faba* has been known to cause serious chromosome structural aberrations (Mesi and Kopliku 2013). These structural alterations in chromosomes are reflected in the form of mutations (Fatma et al. 2018). Owing to the above fact, agrochemicals are widely assessed for their mutagenic potencies in crop plants (Larramendy et al. 2015). Therefore, in addition to reducing crop pests, many of the agrochemicals are strongly associated with some chronic crop damages and are hence absolutely concerning. These severe drawbacks of chemical pesticides call for the creation of alternatives which are target specific, environment friendly, cost effective and above all without any genotoxic side effects (Rahman and Debnath 2015). Despite a handful of studies, precise comprehension of the underlying pesticide-induced crop damage mechanisms is yet to be understood. In an attempt to investigate the various kinds of cytotoxic and genotoxic effects of pesticides on the genome of crop plants, *Trigonella foenum graecum L.* (fenugreek), native to tropical regions, was being exposed to fungicides like tricyclazole and thiabendazole and insecticides including plethora and slash-360. It was found that the exposure fungicides and insecticides in this plant species causes a number of abnormalities among which chromosomal breakdown, membrane disruption and generation of ROS are highly consequential (Mahapatra et al. 2019).

1.2.5 Salinity

Salt stress is regarded as one of the major global issues having detrimental effects on crop plants. According to an estimate almost 50% of the global agricultural land will be harmed due to rising salt quantities (Wang et al. 2003; Bartels and Sunkar 2005). Escalated salt concentration in soils is strongly associated with a number of crop injuries among which oxidative stress, formation of reactive oxygen species and membrane protein disruptions are concerning (Munns 2006; Muchate et al. 2016). Building up of excess salts in the root systems of plants, through stoppage of water and mineral uptake, disturbs the osmotic equilibrium (Paranychianakis and Chartzoulakis 2005). It has further been reported that excess salts leads to enormous harmful effects on the integrity and functioning of DNA, RNA, represses synthesis of proteins, impedes the continuity of cell cycle, retards germination of seeds and decreases the productivity (Rodríguez-Eugenio et al. 2018; Anuradha and Rao 2001). To ensure their survival, plants constantly adapt by activating a series of genes including protein kinases. These protein kinase genes have recently been shown to function in various signal transduction cascades which govern cell proliferation and initiation of stress response (Zhu 2016). Currently newly identified variants of nutrients and fertilizers are being given exogenously to plants by researchers to improve their salt tolerance and hence productivity (Zhu 2016). There is concrete evidence in favour of l-carnitine exogenous treatment scaling up the pace of cell cycle by increasing mitosis under saline circumstances (Surai 2015). During episodes of salt stress in mammalian cell lines, it has been observed that l-carnitine activates a number of antioxidant enzymes which are actively associated in the manufacture of numerous protective molecules (Surai 2015). By controlling cell cycle through some unknown transitions, antioxidant compounds enhance the salt tolerance in plants and thus reduce the incidence of salinity associated oxidative damages (Benjamin et al. 2019). Similar studies by Charrier et al. (2012) suggest that in *Arabidopsis thaliana*, carnitine treatment of seedlings greatly supports development, besides giving protection against excess salts and the associated oxidative damages. In view of the stimulatory effect of carnitine on seed germination and cell proliferation in *Arabidopsis thaliana*, its 1 mM concentration is appropriately suggested to be the best stress reducing remedy in other plant cells.

It has been observed that when cells located at the root tips of barley were treated with high salt concentrations, they undergo chromosome breakdown. A handful of studies revealed that abnormally high salt levels are mutagenic due to induction of structural aberrations or even changing the number of chromosomes (Tabur and Demir 2010). Quite interestingly, it has been well reported that increased concentration of salts raises the percentage of chromosome abnormality (Marakli et al. 2014). Amongst all sorts of abnormalities, disorderly prophase was the most prominent type of chromosomal alteration in salt stressed seeds of barley. Furthermore, salt stress has been acknowledged to generate a significant number of ring-shaped chromosomes in this species. Surprisingly the prior treatment of salt stressed root meristem tips of barley with l-carnitine significantly reduced the frequency of

oxidative stress initiated chromosomal anomalies and other genotoxic effects (genotoxic index).

1.2.6 Antibiotics

There is a growing concern among the scientific community regarding an increase in the traces of pharmaceutical products in the environment (Pico and Andreu 2007). So far a number of drugs have been reported to occur in soil sediments, wastewaters of domestic and industrial origin, natural water bodies and interestingly in the living organisms of aquatic ecosystems (White and Rasmussen 1998). Many antibiotics are known to occur in huge amounts in organic fertilizers (Hamscher et al. 2002), domestic sewage and sludge treated soils (Golet et al. 2003). It is well known that a significant fraction of drugs including antibiotics find their way into the wastewaters through the excreta. Drugs like fluoroquinolones (FQs) have been detected in appreciable amounts in the raw sludge and water samples of natural reservoirs in Switzerland (Golet et al. 2002, 2003). Furthermore, addition of this drug laden sewage sludge to the agricultural soils pollutes the soil and underground water resources (Hamscher et al. 2005).

The ever-increasing ecological concern related to the presence of pharmaceutical traces in the wastewaters of hospitals is that several antibiotics and cytostatic drugs exhibit DNA damaging properties in both prokaryotic and eukaryotic cells (Giuliani et al. 1996). It has been found that the wastewaters of health care institutes contain considerable quantities of ciprofloxacin which was later found to be the principal genotoxic agent in these effluents (Hartmann et al. 1999). Drugs like fluoroquinolones were shown to cause untimely replication of genetic material, induce DNA cuts, inflict chromosome damages and form micronuclei (Bredberg et al. 1991). Considering the huge genotoxic potential of quinolones and fluoroquinolones, evaluation of their impacts on plant roots through direct exposure was impressed upon. Subsequently a test based on micronuclei formation in *Vicia faba* was devised by Marcato-Romain et al. (2009) to assess the genotoxic implications of drugs like quinolones and fluoroquinolones. This test is enough sensitive for the assessment of both clastogenic and aneugenic effects of drugs on plant genomes (El Hajjouji et al. 2007). Micronuclei basically arise because of chromosomal cuts and abnormal mitosis.

An important group of antibiotics having structural resemblances to nalidixic acid (NA) effectively interact with the DNA gyrase enzyme and inhibit its activity (Curry et al. 1996). Another group of highly active compounds affecting a broad range of bacterial species include the fluorinated quinolones and naphthyridines where the seventh carbon position is linked to a cyclic amino group as its enrofloxacin (ENR) (Radl 1990) and its principal metabolite ciprofloxacin (CIP) (Gorla et al. 1999). The mammalian topoisomerase II which is similar to other gyrase enzymes and many other enzymes assisting replication are known to strongly cross-react with quinolones (Bredberg et al. 1991). It is supposed that this compound invariably leads to stabilization of Gyrase-DNA complexes which subsequently causes