3 Biochemical and Molecular Responses of Plants Exposed to Radioactive Pollutants

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Abstract

Radioactive substances are naturally existing rare elements that emit radiation of high energy (ionizing and non-ionizing) capable of transforming the physiological and biochemical attributes of living organisms. However, the extent of natural release is slow and also not sufficient enough to affect the biosystems. The exploitation of these radionuclides for electricity, medicine, agriculture, nuclear weapons, and geological and scientific research for human well-being has led to enhanced release and accumulation of radiation in natural environment, ultimately affecting the metabolic functioning. The most prominent effect is caused by ionizing radiation (high energy) when compared with non-ionizing radiation (low energy). Ionizing radiation causes water radiolysis and produces hydroxyl radicals (reactive oxygen species [ROS]), which in turn cause oxidative stress in living cells. The interaction of radiation-induced ROS with biological organic compounds causes chromosomal aberrations (inversions/deletions), DNA damage, reduction in growth, and developmental abnormalities. Responding to ionizing radiation, plants trigger the antioxidant defense system and produce antioxidative molecules such as glutathione and ascorbate as well as antioxidative enzymes such as catalase (CAT), glutathione reductase (GR), superoxide dismutase (SOD), and ascorbate peroxidase (APOD). Antioxidative biomolecules support plants in scavenging the free radicals generated in their cells and protect them from the harmful effects of radiation. Radionuclides, particularly neutrons-alpha-beta particles and gamma rays, have been used in artificial mutation breeding. Artificial mutation using physical mutagens is a powerful tool in developing new and unique plant varieties. In this chapter, radioactive substances, their accumulation in the environment, interaction with plants, and their sensible aspects are discussed.

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3.1 Introduction

Radioactive substances and their associated radiation are present in the earth's atmosphere since its origin. It is assumed that life originated in a radioactive environment that had ionizing radiation (Zakariya and Kahn 2014). Radioactive substances are unstable natural substances that decay and emit ionic radiation continuously into their surroundings. Naturally occurring radioactive materials (NORMs) are present in the earth's crust, walls of buildings, food we eat, water we drink, and the air we breathe (Zakariya and Kahn 2014). It is noteworthy to mention that houses made up of bricks and stones have high radiation levels compared to wooden homes. The impact of radiation on living systems depends on the dose and duration of exposure to radiation. Doses and sources of ionizing radiation differ from time to time and place to place. However, naturally existing radionuclides and radiation released from them into their surroundings are not enough to affect the biological system.

Nowadays, radioactive substances are widely being used in several areas such as medicine, electricity, agriculture, industry, and research (Zakariya and Kahn 2014), thus contributing significantly to the society. Extensive and unplanned exploitation of these radioactive substances is resulting in the accumulation of radiation in surroundings at a higher rate. Natural environment is receiving radiation particularly from nuclear testing, radiation used to diagnose diseases, and cancer therapy. Small quantities of radiation are also released from coal and nuclear power plants. Accidental release, nuclear testing, uncontrolled use of radionuclides for medicinal purpose and research, and lack of proper strategy for the disposal of radionuclide waste are resulting in the deposition of the wastes in air, soil, and water; currently, the proper harvesting and safe disposal of radionuclide waste is worldwide concern. Continuous efforts to use these radioactive substances and their radiations for various purposes of human development have witnessed an associated health risk not only to humans but also to plants. Plants respond variously to ionizing and non-ionizing radiations depending on the dose and duration of radiation exposure. Animals, particularly humans, can escape the radiation exposure by leaving the place or by protecting themselves; however, plants cannot escape the radiation exposure because they are static and cannot change their position.

Plants uptake these radionuclide wastes from the soil along with water and leaves also absorb them from air. Radionuclides with high energy interact with metabolic pathways and alter the molecular nature of plants, ultimately altering the biochemical products. Plants counteract the reactive oxygen species (ROS and oxidative stress) generated by radiation exposure by producing antioxidative biomolecules. Radiations have varied impacts on the physiological and biochemical attributes of plants, and are positively correlated to the type, dose, and duration of radiation exposure. It has been reported that low doses of radiation have stimulatory effects, intermediate doses have harmful effects, whereas high doses can bring about a significant decrease in the growth, development, and productivity of plants (Kovalchuk et al. 2000). Furthermore, Holst and Nagel (1997) proposed that different plants respond variously to radiation depending on their age, morphology, species, physiology, and genomic organization. Radiation is always not harmful; rather, it is sometimes very fruitful. Radiation is widely used in plant breeding programs and has been proved to be very fruitful in producing new hybrid vigor varieties. In this chapter, the types and sources of radiation, the interaction with plants, and the biochemical responses of plants to radioactive pollutants will be discussed.

3.2 Radioactivity and Radioactive Substances

Radioactivity is defined in terms of the disintegration of atoms. In other words, radioactivity is the property of an element to emit particles or/and radiations spontaneously into its surroundings that cannot be altered using heat, electricity, temperature, pressure, or any other external force (Hazra 2018). Elements exhibiting radioactivity are called radioactive substances. The atom consists of a centrally placed positively charged proton and a neutral neutron (nuclei), and negatively charged electron in its outer orbit. The nuclei of elements having protons disintegrate and release energy in the form of radiation. The unit of radioactive decay is Becquerel, and one Becquerel is equal to one disintegration per second. The decay of radioactive substances continues till a stable element is formed. The time taken to decay half of the radionuclides is termed as 'half-life' of that element and it differs for different radionuclides. Half-life varies from seconds to billions of years. For example, the half-life of ^{131}I is eight days, ^{238}U is 4.5 billion years, and ^{40}K is 1.25 billion years.

Radioactive substances emit three kinds of radiations: alpha (α), beta (β), and gamma (γ) particles or radiation (Fig. 3.1). Ionizing radiations are the electromagnetic waves that have the capability to pass through matter, thereby inducing the matter electrically charged or ionized. Alpha particles (alpha radiation or alpha decay) are high-energy positively charged particles (+2) consisting of two protons

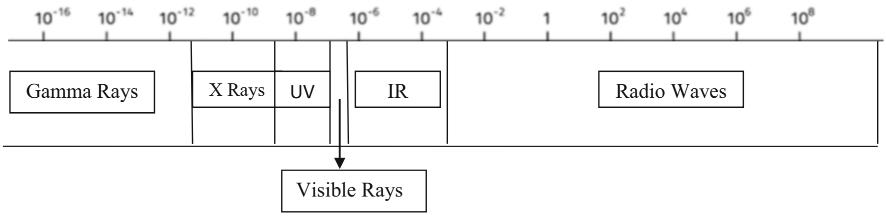

Fig. 3.1 Wavelength (in m) of different rays

and two neutrons with a molecular mass of four units (He atom). Examples of alpha particles releasing radioactive substances are Uranium (U) and Radium (Ra). Beta particles (beta radiation or beta decay) are high-energy, high-speed negatively charged electrons, i.e., negatrons (β^-) or positrons (β^+) with a molecular mass of that of H. Beta particles have more penetrating power in comparison to alpha particles. Gamma rays are neutral and have very strong power of penetration and can penetrate the human body.

3.3 Types of Radiation

Radiation is a charged or neutral energy wave or particle that transports in the form of either electromagnetic waves or energetic particles (United Nations Scientific Committee on the Effects of Atomic Radiation [UNSCEAR] 2010; Smičiklas and Šljivić-Ivanović 2016). There are basically two types of radiations, ionizing radiation and non-ionizing radiation (Table 3.1).

3.3.1 Ionizing Radiation

Ionizing radiations are electromagnetic radiations with high energy and shorter wavelength. They are capable of ionizing the atoms or molecules of the medium or substances through which they pass. Charged molecules in the medium generated by the ionizing radiation break the chemical bonds of proteins, DNA, and other

Table 3.1 Comparison of alpha, beta, and gamma rays

Properties	Alpha ray (α)	Beta ray (β)	Gamma ray (γ)
Nature	High-speed helium nucleus	High-speed electrons	High-speed electromagnetic radiation
Mass	6.65×10^{-27}kg	9.20×10^{-31}kg	Negligible
Charge	+2	−1	No charge
Velocity	Less than the velocity of light (ranges between 1.4×10^7 ms^{-1} to 2.1×10^7 ms^{-1})	Nearly equal to the velocity of light (about 1.8×10^8 ms^{-1})	Equal to the velocity of light in free space (equal to 3×10^8)
Penetration power	Low	Moderate	High
Ionizing power	Greater than beta and gamma rays	Very low	Very low
Effect of electric and magnetic fields	Deflects toward negative plate	Deflects toward positive plate	No deflection
Luminescence	Produces fluorescence and phosphorescence	Produces phosphorescence	Produces phosphorescence

biological organic molecules thereby causing alteration in the metabolism of living systems.

3.3.2 Non-ionizing Radiation

Non-ionizing radiations are comparatively higher wavelength particles with low energy. They are not capable of ionizing or converting the atoms or molecules of the medium. However, the energy present in the non-ionizing radiations is capable of exciting the atoms or molecules of the medium through which they pass, causing the molecules to vibrate faster.

3.4 Sources of Radioactive Radiation

There are basically two sources of radiation, natural and man-made.

3.4.1 Natural Radiation

There are three main sources of natural radiation (Table 3.2):

1. Cosmic radiation—The sun and stars continuously release charged particles (+ & -), which interact with the earth's atmosphere and magnetic field. This interaction results in the production of radiation to which living organisms including plants are exposed. Common examples of radionuclides produced after the interaction of cosmic rays with atmosphere are ^{3}H, $^{7,10}Be$, ^{14}C, ^{26}Al, and ^{39}Ar (Agency for Toxic Substances and Disease Registry [ATSDR] 1999; Smičiklas and Šljivić-Ivanović 2016). Such natural radiation varies on earth's surface due to the variation in the elevation in different parts of the world. Altitude and to a lesser extent latitude are the major factors on which radiation exposure due to cosmic rays depend. Cosmic radiation generally includes beta and gamma radiations.

Table 3.2 Sources of radiation and their contribution (%) in natural environment (United State Nuclear Regulatory commission [USNRC])

Type of radiation	Source of radiation	Contribution (%)
Natural radiation	Radon	55
	Cosmic	08
	Terrestrial	08
	Internal	11
Man-made radiation	Medical diagnostics	11
	Nuclear medicine	04
	Consumer products	03

2. Terrestrial radiation—Terrestrial radiation is present on the earth's surface, that is, soil, water, and vegetation as rocks, minerals, and soil contain NORMs. Radioactive materials such as ^{238}U, ^{232}Th, and ^{40}K are present in the earth's crust, which release radiations continuously into their surroundings, exposing the living organisms. Terrestrial radiation also varies from place to place on the earth's surface due to the variation in the availability of radioactive substances around the world.
3. Internal radiation—Internal sources of radiation are present inside the living body. They result from the consumption of food, water, and air carrying radioactive substances. These radiations do not vary significantly among species or person to person. Such radiations generally include ^{40}K, ^{14}C, and ^{210}Pb.

3.4.2 Man-Made Radiation

Man-made radiations are those radiations which are produced by radioactive substances used at a large scale for human benefits. The important sources of such radiations are medical diagnostic sources (X-rays, nuclear medicine, and radiation therapy) and consumer products such as tobacco (Thorium), building materials, sources of fuel, smoke detectors, luminous watches and dials, electron tubes, and fluorescent starters. Nuclear fuel cycling and residual wastes from the testing of nuclear weapons (Chernobyl) are also the major sources of radiation on earth's surface. During the above-described processes, radioactive substances release radiation of high energy to which living organisms including plants are exposed. The rate of release of radiation and its accumulation in the atmosphere is increasing rapidly due to over unplanned exploitation of radioactive substances for human development.

3.5 Radioactive Pollution in Soil

Radioactive pollution or contamination is defined as the undesired accumulation of radioactive substances on surfaces of materials or within solids, liquids, gases, or biota (International Atomic Energy Agency [IAEA] 2007). Soil is the major receiving pool of emitted radionuclides. Soil receives radionuclides as radioactive wastes released during exploitation of radioactive substances for nuclear energy, nuclear weapon testing, medicine, agriculture, research, etc. It has been reported that soils contaminated with radionuclides lose their natural property of soil fertility for good agricultural produce (Aleksakhin 2009). Soil is the major factor that influences the growth and development in plants, and may be degraded rapidly due to the disposal of radionuclides, mostly at or around the site of the institute or industry using radioactive substances for human developmental processes. The quality of soil in terms of fertility is characterized by its physical, chemical, and biological properties

(Brady and Weil 2002; Osman 2013). The interaction between soil and radioactive pollutants is determined by the physical, chemical and biological properties of soil and nature of pollutants. The binding and retention of pollutants in soil is governed by the five basic components including water, minerals, gases, organic matter, and microorganisms. Soil captures the radioactive pollutants by physical (reversible) sorption carried out by the charges on soil surface and chemical (irreversible) sorption carried out by high-affinity, specific interactions and covalent bond formation (Sparks 2003; Sposito 2008). In this way, plants are exposed to radioactive pollutants and their products or radiations.

3.6 Absorption and Interaction of Radionuclides with Plants

The accumulation in the atmosphere depends on the availability of radiation sources. The higher the industrial and institutional practices, greater is the accumulation of radiation in that site (man-made radiation). When the availability of terrestrial sources of radiation is maximum, the availability and accumulation of radiation and exposure to living organisms is also maximum (terrestrial radiation). In this way, plants are exposed to radiations differently around the world.

The biosynthetic activity of plants is governed by at least 15–20 basic parameters of the plant physiology and environment. Minimum biosynthetic activity is essential for the absorption and accumulation of the radionuclides inside plants, which is possible only when the basic parameters are in the appropriate range. Plants interact with radionuclides either at their soil-root zones or the aerial-shoot zone (Fig. 3.2). Radioactive pollutants suspended in the air as particles or aerosols (and gases) are absorbed by the shoots of the plants (foliar absorption) and those present in the rhizosphere are absorbed by the roots (Koranda and Robison 1978). They showed that the uptake of ^{99}Sr and ^{137}Cs by the soil-root system is governed by the presence of organic matter, inorganic colloids (clay), and other competing elements of the soil. The activity of the plants for radionuclides depends on their retention in the atmosphere and the soil. It is evident that at the time of nuclear testing, radionuclides are released into the atmosphere and they remain suspended in the atmosphere for a certain period. During this period, plants accumulate the radionuclides in their body through foliar absorption (Koranda and Robison 1978). Radionuclides enter the plant body from the air either in the form of a solution or as gases. The solution reaches the leaf and finally the leaf tissue, whereas gases carrying radionuclides enter through the stomatal opening. After certain period of stay in the atmosphere, the radionuclides reach the soil from where they are absorbed by the plants through their root systems.

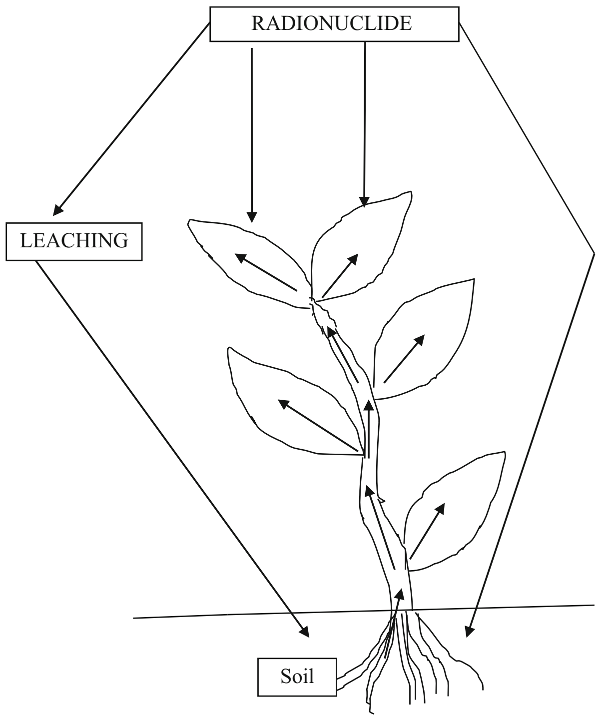

Fig. 3.2 Diagrammatic representation of radionuclide uptake by plants

3.7 Impact of Radiation on the Physiological, Biochemical, and Molecular Attributes of Plants

Ionizing radiations have significant impact on the physiological, biochemical and molecular nature of plants (Table 3.3). The impact of ionizing radiations is controlled by the dose and duration. It has been discussed earlier that lower doses of an ionizing radiation might not negatively affect plant growth and development whereas higher doses have a negative or lethal effect on plants. The information on the effects of ionizing radiations is of wide interest because of their application in agriculture, horticulture, environmental protection, and space science (Caplin and Willey 2018).

3.7.1 Effects of Ionizing Radiation at the Molecular Level

It has been reported that radiation induces mutations, and, hence has been widely used in the development of hybrid plants since the concept of mutation was proposed by Hugo de Vries. The concept of the theory of mutation of Hugo de Vries was

Table 3.3 Effects of radiations on the different aspects of plants

Effect	
Denaturation of	Morphological, physiological, and biochemical response
• DNA	• Generation of defense against oxidative stress (DAOS)
• Protein	• Synthesis of antioxidant enzymes and molecules
• Organic molecules	• Stunted growth
• Chloroplasts	• Mutational breeding
• Cell membrane	• Chromosomal/DNA rearrangements
• Cell wall	• Activation of specific genes
• Biosynthetic pathway	

published by Hubrecht (1904). Hugo de Vries proposed that mutations can be induced in plants using X-rays. More than 2500 cultivars currently used as food have been developed by mutagenesis induced by high doses of ionizing radiation (IR 10 s of Gy or more) (Cheng et al. 2014). Ionizing radiation is still playing a significant role in the development of improved varieties of crops such as rice and wheat (Caplin and Willey 2018; Cheng et al. 2014). Cheng et al. (2014) showed that 9.19% genome sequences of Red-1 varieties of rice (rich in beneficial ingredients), developed by gamma irradiation, was altered. They further showed that point mutation was the main factor responsible for alteration.

Experimental studies have suggested that irradiation of plants with gamma rays inhibits their growth and is associated with the synthesis of auxin and DNA. Further, it was postulated from experimental studies that (a) DNA is a prerequisite for auxin biogenesis, that is, DNA is required for auxin synthesis; (b) auxin is required for DNA formation; and (c) radiation affects other cellular entities essential for both DNA and auxin synthesis (Jan et al. 2012; Lage and Esquibel 1995; Momiyama et al. 1999). Ionization radiation brings about mutation in plants and is of wide interest for plant breeders (mutation breeding). Mutation breeding is one of the significant tools for the development of high yielding and qualitative plant varieties. Mutation breeding involves three types of mutagenesis generated either by treatment of ionizing radiation or chemical mutagen. The three kinds of mutagenesis are: (i) induced mutagenesis; (ii) site-directed mutagenesis (mutation at a specific site in the DNA molecule); and (iii) insertion mutagenesis (DNA insertion) (Forster and Shu 2012; Kharkwal and Shu 2009; Oladosu et al. 2016). Ionizing radiation has a high incidence of double-stranded breaks (DSBs) in DNA compared to other radiation or mutagens (Caplin and Willey 2018). Plants were exposed to a high degree of ionizing radiation during their early period of colonization of land surface as compared to today's level of ionizing radiation (Caplin and Willey 2018). Ionization radiation brings about DSBs that may result in the deletion of DNA segments (Kovalchuk et al. 2000, 2004; Sato et al. 2006). However, single-stranded breaks are also frequent due to the exposure to ionizing radiation (Cheng et al. 2014). Sato et al. (2006) conducted an experiment in which they treated rice with gamma rays and ethylmethanesulfonate (EMS) to obtain mutants. They showed that the point mutation rate was lower when treated with gamma radiation but higher when treated with EMS. Conversely, knockout mutation was higher when treated with gamma radiation compared that with EMS. It has been reported that acute doses of IR exposure (10–100 s Gy) produce a 'net' rate of mutation from 10^{-9} base pair mutation per Gy to 6.13×10^{-6} bp mutation per 500 Gy (Sato et al. 2006). It has also been demonstrated that low doses of chronic ionizing exposure to plants have high rates of mutation compared to acute high doses. Kovalchuk et al. (2000) experimentally demonstrated that wheat, planted in Chernobyl NPP-affected soil and exposed to ionizing radiation of 0.3 Gy for a growing season of 100 days showed six-fold increase in its mutation rate.

Further studies to understand the effect of ionizing radiation (IR) on plants showed that IR induces changes in the gene expression of plants. It has been reported that acute high doses of IR exposure may change 100–1000 s of genes (Caplin and Willey 2018). The most notable information on changes in gene expression due to IR exposure involves the induction of DNA repair gene and antioxidant defense machinery of the plant system. Kim et al. (2014) reported that genes with changed expression have significant contribution in catalytic activity, endomembrane system, and are active in metabolism. They proposed that gamma irradiation brings about significant changes in gene transcripts and expression. They demonstrated that, in *Arabidopsis thaliana*, out of the 20,993 genes used as microarray probes, a total of 496 genes were up-regulated whereas 1042 were down-regulated by gamma irradiation. It has been reported that the exposure of the plant to 200 Gy of gamma irradiation showed alteration in gene expression responsible for sugar and starch metabolism (Hwang et al. 2014). Hwang et al. (2014) performed the experiment to study the effect of gamma rays, cosmic rays, and ion beams on rice. They proposed that the overall expression patterns were similar for gamma rays and ion beams but was different for cosmic rays. They further reported that changes in gene expression were related to sucrose–starch metabolism, finally resulting in an increased content of sugar and starch in all the three types of irradiation used in the experiment.

Further studies showed that exposure of plants to acute IR results in the up-regulation of genes responsible for DNA repair, oxidative stress response, and signal transduction pathways, whereas chronic exposure has no effect on the changes in gene expression (Caplin and Willey 2018). A similar observation of variation in physiological and gene expression of *Arabidopsis* plants was observed for acute and chronic exposure of plants with γ-irradiation (Goh et al. 2014). They demonstrated that exposure of *Arabidopsis* seedlings to 200 Gy γ-irradiation in an acute manner for 1 h or 24 h, or in a chronic manner for 1, 2 or 3 weeks resulted in a decrease in the plant height, silique number, and silique length. The up-regulation of gene expression in response to acute and chronic exposure to γ-irradiation involved gene encoding for zinc finger proteins, heat shock factors, NADPH oxidase, WRKyY DNA-binding proteins, and calcium-binding proteins (Goh et al. 2014). They further reported that out of the four antioxidant enzymes, catalase (CAT), peroxidase (POD), ascorbate peroxidase (APOD), and superoxide dismutase (SOD) studied for γ-irradiation, CAT and POD exhibited a decreased cellular activity for both acute and chronic exposure. Studies conducted by Kimura et al. (2008) on rice seedling leaves suggested that low-dose exposure to IR in the affected area in Chernobyl showed an up-regulation of gene expression related to defense mechanisms, cell wall synthesis, and secondary metabolite synthesis.

3.7.2 Effect of Ionizing Radiation on the Physiology and Biochemistry of Plants

Ionizing radiation plays a significant role in the radiolysis of water compared to the photolysis of water during photosynthesis. High doses of IR results in an increased rate of lysis of water, resulting in the generation of a high amount of free radicals, that is, ROS (Kovács and Keresztes 2002) such as superoxide radicals (O^{2-}), hydroxyl radicals (OH^{-}), and peroxide (H_2O_2) (Apel and Hirt 2004). Kovács and Keresztes (2002) demonstrated that gamma rays bring about softening of fruits and finally breaking of middle lamella of the cell. These radicals react simultaneously with the structural and functional organic molecules such as proteins, lipids, and nucleic acids and bring about an alteration in the cellular biosynthetic pathway (Salter and Hewitt 1992). Apel and Hirt (2004) describe that plants have developed mechanisms to synthesize antioxidant enzymes and molecules to combat stress and also generate ROS purposefully as a signal molecule to control pathogenic defense mechanism, programmed cell death, and stomatal behavior. Gamma irradiation has been reported to reduce the chlorophyll content in *Nicotiana tabacum* by 55.9% (Wada et al. 1998). Plants have developed mechanisms to encounter the oxidative stress created in the cellular compartments by producing a high amount of antioxidants (Willey 2016; Jan et al. 2012). These oxidative stresses are capable of degrading the protein and the metabolic activity in plants.

The impacts of IR-induced oxidative stress in plants include alteration in morphology, anatomy, biochemistry, and physiology of plants (Ashraf et al. 2003). Ashraf et al. (2003) demonstrated that basmati rice treated with gamma radiation showed a decline in seedling shoot and root lengths, panicle fertility, and grain yield. These morphological variations were negatively correlated to irradiation and were dose-dependent. Further observation suggested that changes in the cellular redox potential created due to oxidative stress brings about changes in the dilation of thylakoid membranes, alteration in photosynthesis, activation of antioxidant producing biosynthetic pathway, and accumulation of phenolic compounds (Kovacs and Keresztes 2002; Ashraf 2009; Wi et al. 2007). It has been reported that the induction of seeds with high doses of gamma irradiance resulted in decreased protein and carbohydrate contents due to the increased metabolic and hydrolyzing enzyme activities in the germinating seeds (Barros et al. 2002; Maity et al. 2004; Jan et al. 2012). The treatment of *Dacus carrota* L. with gamma irradiation resulted in an increased uptake of glucose, pyruvate, and a decreased uptake of acetate and succinate (Jan et al. 2012). Bourke et al. (1967) reported that gamma irradiation resulted in a decrease in all amino acids except serine and valine.

Plants have developed biosynthetic mechanisms to encounter the oxidative stress created in their cellular compartments by producing a high amount of antioxidants

(Willey 2016). They synthesize enzymes containing sulfur such as amino acids (cystine, cysteine) and SOD to disarm the free radicals and ultimately protect the plants from oxidative stress (Qin et al. 2000; Jan et al. 2012). Qin et al. (2000) reported that the change in the activity of SOD and POD in ^{60}Coγ-ray and EMS-treated seeds of *Lathyrus sativus* was directly linked to the concentration of radiation. Zhang et al. (2016) showed that the treatment of *Arabidopsis* seeds resulted in a reduction in the root and shoot lengths due to the production of superoxide radicals and hydrogen peroxide. Simultaneously, the production of antioxidant enzymes was also up-regulated in *Arabidopsis* in response to the low-energy N(+) beam. They reported no effect of radiation or EMS on the CAT activity. Different types of antioxidant enzymes and molecules are synthesized and expressed in plants in response to ionizing radiation. Some of them have been discussed below:

3.8 Antioxidant Enzymes and Molecules

3.8.1 Superoxide Dismutase (SOD)

SOD plays a vital role in combating the oxidative stress generated by ionizing radiation in plants. It has been demonstrated that higher the content of SOD, CAT, and POD in the cellular pool, lower is the vulnerability of plants to the secondary effect of radiation. SOD probably acts as an electron donor in transition metal radiation-affected cells/tissues and protects the irradiated cells by sensitizing them against the effects of H_2O_2 (Jan et al. 2012). It has been observed that the treatment of *Vigna radiata* (L.) R. Wilczek with 20–200 Gy gamma irradiation showed sharp changes in both SOD and POD (Roy et al. 2006). It was seen that gamma irradiation of *V. radiata* resulted in a reduced height of seedling and germination frequency. Roy et al. (2006) also demonstrated that the RAPD analysis of gamma-irradiated plants (200 Gy) exhibited new bands, indicating DNA damage. Pramanik (1997) demonstrated the correlation between the morphological damage, such as the decrease in seedling height in *Plantago ovata,* and gamma irradiation. Also, dose-dependent gamma irradiation was related to changes in SOD activity. Changes in SOD isozyme pattern in response to oxidative stress is an indication of the development of radioprotection mechanism inside plants. The correlation between radiation doses and antioxidant enzyme activities has been demonstrated in vivo and in vitro by several workers (Singh 1974). Gupta et al. (1993) demonstrated the correlation between the expression of Cu/Zn SOD in tobacco leaves and stress. They further suggested that plants can withstand severe stress and can maintain their normal photosynthetic activity by producing the SOD isozyme. They showed that transgenic plants can retain their rate of photosynthesis 20% more than untransformed plants. They concluded that the SOD generated in the chloroplast plays a vital role in providing support to plants in tolerating stress.

Further study on irradiation suggest that the production of antioxidant molecules or enzymes are linked with the alteration in gene expression in response to stress created by radiation. Overexpression of SOD in irradiated cells is due to the induction of genes or alleles responsible for SOD enzyme synthesis (Inzé and Van Montagu 2002). Pramanik (1997) reported that the PAGE gel analysis of the SOD activity of irradiated calli showed an appearance of extra bands (R_f value – 0.59). In some cases, disappearance of certain bands immediately after exposure to γ-irradiation has also been observed, which may be associated with the degradation of certain biomolecules (Sen Raychaudhuri and Deng 2000) or switching off of the metabolic pathway (Jan et al. 2012). Zaka et al. (2002) showed that the overexpression of antioxidant enzymes, particularly POD, CAT, GR, SOD, and G6PDH, or molecules is directly linked to gamma irradiation. They further showed that SOD and G6PDH in particular play a significant role in the protection of *Stipa capillata* from oxidative stress created by ROS. In this way, plants disarm the oxidative stress by producing SOD.

3.8.2 Peroxidase (APX; EC 111.1.11) and Catalase (CAT; EC 1.11.1.6)

The two other important antioxidant enzymes are POD and CAT, produced in response to ionizing radiation. The interaction of plants with the irradiated rays creates a stress in their cellular activity due to the production of oxidative radicals. Several studies suggest that ionizing radiation results in the production of hydrogen peroxide at a higher rate (Wi et al. 2006). Wi et al. (2006) reported an increased content of H_2O_2 in pumpkin (*Cucurbita ficifolia bouche*) with high doses of gamma irradiation. Hydrogen peroxide continued to be present in xylem vessels, plasma membrane, middle lamella, and also in parenchyma cells (Jan et al. 2012). The biochemical activity demonstration of irradiated cells showed an increased level of POD enzyme in the irradiated cells. A similar observation of the expression of POD enzymes associated with gamma irradiation has been reported by different workers in different plants such in garlic bulbs (Croci et al. 1994) and the root disks of sweet potato (Ogawa and Uritani 1970). Croci et al. (1994) reported that gamma irradiation of garlic cloves resulted in a decrease in the total DNA content of inner sprouts immediately and after 100 days of irradiation, whereas the total RNA, protein, and carbohydrate contents of the inner sprouts were not changed. They proposed that DNA is the most sensitive component of the cell to radiation exposure.

POD enzyme protects plants by disarming the effect of H_2O_2 by eliminating them (particularly lipid hydrogen peroxide) from the cellular pool. The overall equation of peroxyl radical removal by POD enzyme is as follows:

$$H_2O_2 + DH_2 \rightarrow 2H_2O + \mathrm{D}$$

There are several reports in the literature on the overproduction of POD enzyme in the irradiated cells of plants and their role in scavenging the oxidative radicals (Khanna and Maherchandani 1981). Khanna and Maherchandani (1981) proposed that lower doses of gamma radiation stimulated the POD activity in chickpea whereas a decrease in the POD activity was observed for higher doses of gamma irradiation. CAT is another enzyme which plays a significant role in the elimination or scavenging of free peroxyl radicals in plants. Aly and El-Beltagi (2010) showed that antioxidants can prevent plants from oxidative radical damage generated due to IR exposure. They observed the stimulation of POD, APOX, CAT, SOD, and GST under the influence of gamma irradiation and it was positively correlated dose-dependent. They also reported an increase in the malondialdehyde (MDA) content associated with gamma irradiation. The up-regulation of genes for CAT, POD, Cu/Zn SOD, GST, and the down-regulation of cytosolic and stromal APX have been reported in *Nicotiana tabacum* L. (Cho et al. 2000). Cho et al. (2000) reported that the gamma irradiation of tobacco showed varied responses. According to them, certain group of genes (glutathione-*S*-transferase, POD, SOD, and CAT) showed stimulating response, whereas other groups (cytosolic APOD, stromal APOD, and TMK-1 receptor like-kinase) showed reduction. There were also certain groups of genes that exhibited either no response or irregular response. These included pathogenesis-related proteins, tobacco Ca^{2+}-dependent protein kinase, the β-subunit of translational initiation factor 2B, and a chitinase-related receptor-like kinase (Cho et al. 2000). The overall reaction mechanism involving CAT and radicals is mentioned below:

$$ROOH \overset{catalase}{\rightarrow} H_2O + ROH + A(Jan\, et\, al.\, 2012)$$

$$2H_2O_2 \overset{catalse}{\rightarrow} 2H_20 + O_2$$

The investigation of the effect of irradiation on biochemical properties showed enhanced rate of production of POD and CAT with a consequential decline in growth (except at 5 krad which showed growth) of wheat irradiated with high doses of ionizing radiation (Chaomei and Yanlin 1993). They showed that irradiation of wheat plants above 20 krad resulted in an increased activity of both POD and acid phosphatase activity. The CAT activity was higher at 5 krad and 20 krad. Several reports are now available on the production of antioxidant enzymes such as POD, SOD, CAT, and APX, associated with the exposure of plants with ionizing radiation (Singh et al. 1993; Foyer et al. 1997; Zaka et al. 2002). Singh et al. (1993) demonstrated that phenolic content, polyphenol oxidase, and POD were positively correlated with different doses of gamma irradiation in sugarcanes. Foyer et al. (1997) suggested that thiol/disulphide exchange reactions involving glutathione pool and H_2O_2 play a crucial role in modulating metabolism and changes in gene expression corresponding to environmental and biotic stresses. It has been reported that chronic exposure of gamma irradiation to *Arabidopsis thaliana* has no effect on the concentration of non-enzymatic antioxidants, ascorbate, and glutathione (Vandenhove et al. 2009). Štajner et al. (2009) reported that gamma irradiation resulted in a decrease in the total antioxidant activity (15.7%) and an increase in MDA and OH^- by 21.6 and 79.33%, respectively, in soybean compared to non-irradiated soybean.

3.8.3 Glutathione Reductase Activity (GR; EC 1.6.4.2)

A similar response of increased content of GR activity in plants was observed with gamma irradiation. The increased content of GR activity was reported in roots and shoots of three *Trigonella* L. genus irradiated with gamma radiation (Jan et al. 2012). Foyer et al. (1991) reported that the GR activity of transgenic *Nicotiana tabacum* var. Samsun was two- to ten-folds higher than the non-transgenic control tobacco plant. Synthesis and production of GR in irradiated plants is governed by gene regulation. Studies suggest the correlation between enhanced content of GR activity with an increase in the transcription rate of encoding genes (Foyer et al. 1995). They proposed that overexpression of GR activity in chloroplast is responsible for the increased antioxidant activity, ultimately supporting the Poplar plant in disarming the oxidative stress.

3.8.4 Ascorbate and Glutathione

Plants synthesize ascorbate to achieve their optimal growth and metabolic activity. It has been demonstrated that irradiation of plants has variable response associated with ascorbate synthesis (Vitamin C). In some plants, exposure to gamma radiation showed either no response or decrease in ascorbic acid (ascorbate; AA) such as in potato and strawberries, papaya, mango, strawberry, and litchi (Graham and Stevenson 1997; Beyers et al. 1979). Graham and Stevenson (1997) reported increase in dehydroascorbic acid (DHAA) content immediately after irradiation in strawberry plant. It has been reported that exposure of plants to ionizing radiation results into conversion of ascorbic acid into dehydroascorbic acid (DHAA) (Diehl 1990; Kilcast 1994; Jan et al. 2012).

Glutathione is another antioxidant molecule which supports plants in disarming the effect of oxidative stress. Halliwell and Gutteridge (1989) reported that glutathione protects plants from oxidative stress by directly interfering with free radicals. The overall reaction of glutathione with DHAA has been shown below:

Correlation between glutathione levels and ionizing radiation has been variously studied.

$DHAA + 2GS \rightarrow Ascorbate + GSSG$ (Jan et al., 2012)

$$GSSG + NADPH \rightarrow 2GSH + NADP$$

3.9 Conclusions

From the above discussion, it may be concluded that the research on the effect of ionizing radiation on metabolic adaptation of plants will certainly prove to be fruitful in maintaining the environmental condition sustainable. It will also make a platform for the development of new varieties of essential plants. The exploitation of radionuclides for the generation of energy, medical diagnosis and treatment, nuclear weapons, etc., is the need for the present generation and it will certainly increase the level of ionizing radiation in our surrounding, making it difficult for survival. Hence, it is the urgent need of the present research to focus on the findings and develop radiation-resistant, radiation-tolerant plants, which can minimize the level of radiation, making the environment sustainable.

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