The Oxidative and Antioxidative Response in *Glycine max* (L.) Merr. Exposed to Various Abiotic Stress

Apurva Panwar[1], Priti Chauhan[2], Jyoti Srivastava[3]

[1, 2]Ph.D Scholar, Department of Bioscience and Biotechnology Banasthali University, P.O. Banasthali Vidyapith, Rajasthan, 304022, India

[3]Associate Professor, Department of Bioscience and Biotechnology Banasthali University, P.O. Banasthali Vidyapith, Rajasthan, 304022, India

Abstract: ***Soyabean [Glycine max (L.) Merr.] is considered essential due to its high oil production and nutritive value. However, because of the different abiotic and biotic stress, the plant experiences from accumulation of reactive oxygen species (ROS) that results in severe oxidative damage. The effect of abiotic stress such as heavy metals, salinity, temperature and drought were detected on soyabean during crop production. Abiotic stress produces several morphological changes in root, stem and leaf of the plants. Plants possess homeostatic cellular mechanism to regulate the level and concentration of various abiotic factors by using several enzymatic and non enzymatic responses. The cell wall and plasma membrane are the main barriers against the metal pollutants and some other compound present in plants like phytochelatin based sequestration etc. Exposure of heavy metal to the plants could lead multiple toxic effects by inducing reactive oxygen species (ROS), which inhibit most cellular processes at various levels of metabolism. The present study summarizes the response of Glycine max to different biotic, abiotic factors as well as heavy metal pollutants.***

Keywords: Abiotic stress, biotic stress, antioxidant, Soyabean

1. Introduction

Soyabean is a species of legume belongs to *Fabaceae* family that is indigenous to East Asia, China and Manchuria. It is one of the most important sources of oil and protein in the 19th century and hence it is widely grown for its nutritious value worldwide. Soyabean originated from China around 1100 to 1700 B.C. but was introduced into early Europe only in the 17th Century [1]. In the summer 1910; Alfred Jones shipped soyabeans for culture trials to West Africa. The main producers of *G. max* are the Brazil (27%), United States (35%), China (6%), Argentina (19%) and India (4%) [2]. Soybean was first grown in Ghana in 1909. It has gained worldwide importance as a primary source of vegetable oil and protein. Soyabean production constitutes around 55% of the total world production of oilseeds and figures around 170 to 185 million tons. It contains about 40 % high quality proteins (as against 7.0 % in rice, 12 % in wheat, 10 % in maize and 20-25 % in other pulses) and 20 % oil [2].

Soybean seed is one of the most nutritious legumes and has higher energy value, because it contains good amount of mineral and about 37% of high-quality proteins, which is almost twice of the meat proteins, 4 times of the egg proteins and 12 times of the milk proteins. It also has 18% of unsaturated fat, vitamins A, E, F and B and is one of the good sources of lecithin, essential substances for the cell because it dissolves the bad cholesterol and helps in the absorption of vitamins. Soybean is a major protein source for humans and other animals. About 90% of soluble proteins in soybean seeds are globulins and more than 70% of globulins are 'glycinin' (11S globulin) and 'β-conglycinin' (7S globulin). Glycinin is relatively rich in sulfur containing amino acids such as methionine and cysteine (3% to 4.5%) and is stored primarily in cotyledons of seeds where it is deposited in protein bodies. Soybean, with over 40% protein and 20% oil, has now been recognized all over the world as a potential supplementary source of edible oil and nutritious food [3]. Foy in 1992 [4] reported that aluminium toxicity hampers crop production in tropical and subtropical areas and it is a primary factor in limiting plant growth in acid soils.

2. Abiotic Stress

Stress is an altered physiological condition caused by factors that tend to obliterate the equilibrium. Many chemical and physical changes produced by different stress conditions [5]. Abiotic stress is previously known as a major limiting factor in plant growth and will soon become more severe as desertification cover more and more of the worlds terrestrial area [6]. Abiotic stresses such as high salinity, drought and particularly heavy metal can impose limitations on crop productivity and limit land availability for agriculture [7]. Particularly, these are more responsible for most of the reduction that differentiates yield potential from harvestable yield [8]. Among abiotic stressors, heavy metal contamination represents a global environmental problem endangering humans, animals and plants. Abiotic factors may alter the quality and magnitude of plant defences or modify the plant's physiology and signalling pathways. Tolerance to abiotic stresses is very complex, due to the complexity of interactions between stress factors and various molecular, biochemical and physiological phenomena affecting plant growth and development [9].

In order to alleviate the stress and re-establish cellular homeostasis and antioxidant capacity, plants have developed highly effective mechanisms to regulate the uptake, accumulation, distribution, and detoxification of heavy metal ions such as the accumulation of secondary metabolites, the production of volatile compounds and changes in protein expression [10]. This response is usually not only accompanied by an alteration in the gene expression pattern, but also by inevitable qualitative and quantitative changes in proteins [11].

Salt stress

Salt stress is one of the most important abiotic stresses that adversely affect natural productivity and causes significant crop loss worldwide. It has been considered as a serious constraint on agricultural productivity due to it involves the morphological and developmental changes. Salinity stress affects plant growth and also development processes like seed germination, seedling growth and vegetative growth, flowering and fruit set and finally leads to tissue death and ultimately plant dies [12] [13]. Salinity reduces the uptake and transport of nitrate [14][15] that is necessary for protein synthesis [16]. Serrano and his co-workers [17] experimentally proved that salt stress affects the integrity of cellular membranes, activities of enzymes and the functioning of the plant photosynthetic apparatus. It also causes osmotic stress and ion ionic imbalance [18].

Although, there is a great diversity of responses among cultivars to salinity [19] stress can provoke several metabolic alterations in plants such as lipid peroxidation, reduction in chlorophyll content, increase in ROS and anti-oxidative enzyme activity all such alterations are accompanied by reduced plant growth [20]. Salinity also limits the photosynthesis which can increase oxygen induced cellular damage due to increase ROS generation [21]. To counteracts the ROS overproduction under stress, defence systems that scavenge cellular ROS have been developed in plants to cope with oxidative stress via the non-enzymatic and enzymatic systems [22]. Higher plants have multiple protective mechanisms against salt stress including ion homeostasis, osmolyte biosynthesis, ROS scavenging, water transport and transducers of long-distance response Coordination.

Drought Stress

Drought has been known as one among the most limiting environmental stresses on plant growth and productivity. Drought stress causes biochemical changes such as osmolite and specific protein accumulation caused by changes in the cellular and molecular level [23]. Plant responses to drought leads to some adaptive changes such as: growth rate, stomatal conductance, osmotic potential network, and antioxidant defences and also changes in metabolism and the expression of several genes that are thought to play an important role on adaptive response of plants to water stress [24].

Some of the genes responsive to drought stress, high salinity and cold temperatures at the level of transcription (m RNA) have been widely reported [25]. A change in protein expression, accumulation and synthesis of protein has been observed in several plant species under conditions of drought stress during the growth [26]. Late embryogenesis abundant (LEA) protein especially with a molecular weight of 10-30 kDa involved in the protection of higher plants from damage caused by environmental stresses, especially drought [27].

Heavy metal stress

Exposure to heavy metals has been documented to induce changes in the expression of plant proteins [28]. Accumulation of heavy metals not only decreased nodulation [29] and growth of leguminous plants [30] but also has multiple direct and indirect effects on plant growth and alters many physiological functions and biochemical reactions. Excess HMs causes the enhanced production of reactive oxygen species (ROS) in the plant tissue [31].

Heavy metal ions act as elicitors in plant defence reactions For example: glyceollins, isoflavonoid, phytoalexins which are involved in the interaction between soybean (*Glycine max*) and the phytopathogenic oomycete *Phytophthora sojae* were induced by mercury ($HgCl_2$) treatment. There is evidence that plant's ability to mitigate the negative impacts of redox reactive heavy metal stress, by increased antioxidative protection appears to be limited [32]. Authors suggested within the framework of the Graduate Research School was to evaluate plant responses to heavy metal stress, in particular considering the elicitation of volatile production and upregulation of phytohormone levels by heavy metal salts [33]. In this context, oxylipins may not only play an important role during plant herbivore interactions, but also mediate responses after encountering abiotic stress.

3. Plant Responses against Abiotic Stress

Phytoharmone in response of plant stress

Plant hormones are involved in many physiological and developmental processes play a crucial role in the adaptation to abiotic stress as shown by the regulation of hormone synthesis in the presence of heavy metals [34]. For example, plants exposed to toxic levels of Cd, Cu, Fe, and Zn produce higher levels of ethylene, but Co does not have the same effect [35]. Cd and Cu stimulate ethylene synthesis by upregulating ACC synthase expression and activity [36]. Abscisic acid (ABA) is an important phytohormone in plants, which regulates root and seed development, seed germination, and biotic and abiotic stress responses. ABA interacts with other phytohormones to mediate plant performance under biotic and abiotic stress condition [37]. Cu and Cd also induce the rapid accumulation of jasmonic acid (JA) in *Phaseolus coccineus* [35]. Salicylic acid (SA) also involved in heavy metal stress responses; it protects roots from lipid peroxidation caused by Cd toxicity [38].

Plant defence response against heavy metal stress

As a first line of defence against heavy metals, plant roots secrete exudates into the soil matrix to chelate metals and to prevent their uptake inside the cells [39]. For example, Ni-chelating histidine and citrate are present in root exudates and these reduce the uptake of Ni from soil binding sites on the root cell wall. It allows the metal exchange that influences the availability of ions for uptake and diffusion into the apoplast [40] [41]. The cell wall pectic sites, hystidyl groups, extracellular carbohydrates (callose) and mucilage prevent heavy metals uptake into the cytosol. According to Ernst *et al*., 1992 [42] the cell wall has only a minor impact on metal tolerance.

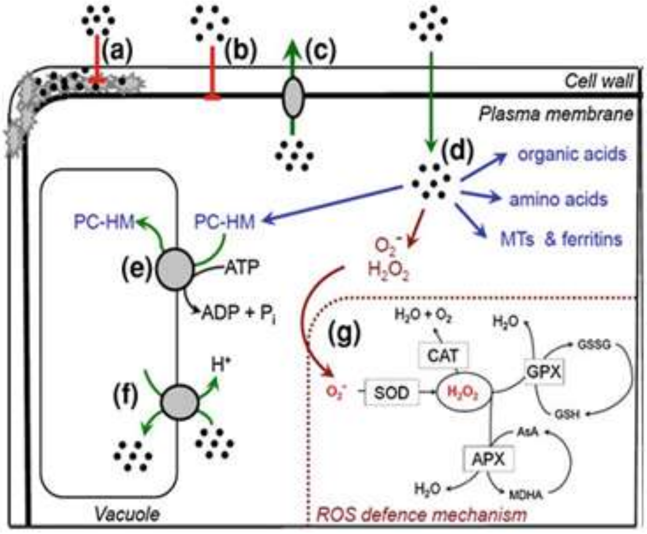

Figure 2.1 The response to heavy metal toxicity in higher plants [16].

Glycine betaine (GB) is synthesized abundantly in the chloroplast, where it plays a significant role in the defence and regulation of the thylakoid membrane by maintaining photosynthetic attributes [43]. In many crop plants the natural GB accumulates at levels that can counterbalance the adverse effects of various environmental stresses. In plants, NO and CO have already been identified as signalling molecules which involve in antioxidative defence [44]. Phytochelatins (PC) also could reduce cytoplasmic toxicity by complexing intracellular metals. Biliverdin IXα (BV) and bilirubin both play an important role against oxidative damage. Sugar alcohols like pinitol also play an important role in intracellular osmotic adjustment as well as in scavenging free radicals.

4. Defence Against Antioxidative Stress and Oxidative Stress

ROS are produced continuously as by products of various metabolic pathways that are localized in different cellular compartments such as chloroplast, mitochondria and peroxisomes. According to Gratao and his co-workers (2008) [45] whether ROS will act as damaging, protective or signalling factors depends on the delicate equilibrium between ROS production and scavenging at the proper site and time. In particular, H_2O_2 acts as a signalling molecule in response to heavy metals and other stresses [46]. Accumulation of ROS as a result of various environmental stresses is a major cause of loss of crop productivity worldwide [47].

ROS affect many cellular functions by damaging nucleic acids, oxidizing proteins, and causing lipid peroxidation [48]. Plants have developed robust mechanisms including enzymatic or nonenzymatic scavenging pathways to counter the deleterious effects of ROS production [49]. The presence of ROS scavenging enzymes of the catalase and ascorbate peroxidase families is needed to maintain redox homeostasis. The antioxidant properties of plants exposed to various stress factors have been studied [50] but studies related to the effect of heavy metal induced stress on vitamin levels in plants are limited. To combat the oxidative damage, plants have the antioxidant defence system that comprising of numerous enzymes such as catalase (EC 1.11.1.6), peroxidases (EC 1.11.1.7), superoxide dismutases (EC 1.15.1.1) and the nonenzymic constituents tocopherol, ascorbate and reduced glutathione which remove, neutralize and scavenge the ROS and compounds of low molecular weight [51].

5. Enzymatic Oxidant

Superoxide dismutase (SOD)

SOD is a main antioxidant enzyme that causes dismutation of superoxide radicals at almost diffusion limited rates to produce H_2O_2 and is the first line of defence against oxidative stress in plants [52]. Therefore, it has a crucial role in the defence mechanism against free radical toxicity [53]. Superoxide dismutase is divided into three main groups on the basis of the metal cofactor.

1) **Copper or Zinc**: It present in plants, mainly in the chloroplasts and cytosol. In these enzymes, basically copper and zinc works as cofactor.
2) **Manganese superoxide dismutase:** This enzyme is localized in peroxisomes and mitochondria. Here manganese as its cofactor.
3) **Iron superoxide dismutase:** They are localised in chloroplast in plants and are absent in animals [54]. They play important in shielding mangroves from excess irradiance. After NaCl treatment SOD activity increases and then H_2O_2 is produced [55].

Catalase (CAT)

Plant cells are equipped with defence machinery such as catalases and ascorbate peroxidises [56]. Catalases (EC1.11.1.6), a heme containing tetrameric enzymes that are generally localized in glyoxysomes or peroxisomes in the germinating seeds of the plants and it act as scavenger of ROS during beta oxidation of fatty acids [57], salt stress and other abiotic stress conditions. Therefore, plants have various catalase isozymes that are encoded by some genes: two isozymes in castor oil plant [58], Arabidopsis [59] and three in tobacco [60]. Two different classes of catalases are present.

a) **Class I catalases-** It is found in both monocot and dicot plants that play important role in glyoxysomal and photorespiratory functions [57].
b) **Class II catalases-** These are mostly found in dicot plant that play a crucial role in different stress responses such as pathogen infection, wounding and salt stress [61].

6. Alternative H_2O_2 dismutating pathways

Ascorbate peroxidase (APX)

APX are antioxidants that perform the same functions as catalases. However, unlike catalases, APX catalyses the removal of ascorbate in higher plants by different mechanism [62]. It uses two ascorbate molecules to reduce hydrogen peroxide to water molecules, with the consequent generation of two monodehydroascorbate (MDHA) molecules [51]. APXs are localized in four different organelles such as ‘thylakoid membrane bound APX in chloroplasts’ (t-APX), ‘stroma APX’ (s-APX), ‘glyoxysomes’, ‘peroxisomes’, ‘membrane bound APX’ (m-APX), ‘mitochondrial membrane bound form’ (mit-APX) and lastly 'cytosolic APX' (c-APX) [63].APX also plays a critical role during drought and salt stress [64].

Guaicol Peroxidase (GPOX)

GPOX can be differentiating enzymes on the basis of sequences and physiological functions. It putrefies 'indole-3-acetic acid' and has a vital role in the biosynthesis of lignin or defence response against abiotic and biotic stresses by destroying hydrogen peroxide. The activity of this enzyme varies that depends upon the stress condition and plant species. It prefers aromatic electron donors 'guaiacol' and 'pyragallol' usually oxidizing ascorbate at the rate of around one percent that of guaiacol [65].

Peroxidase (POX)

POX acts as H_2O_2 scavenger and present in high amount in all plants. They generate phenoxy compounds from cinnamic acid in the cell wall of plants. It is also involved in other defence mechanisms in plants, including responses to insects [52] and in a coordinated response known as the oxidative burst [66]. In 2003, [67] it is reported that the enhancement of peroxide activity in salinized cells of *S. nudiflora* specified that these cells had a higher disintegration of H_2O_2 under salt stress

Monodehydroascorbate reductase (MDHAR)

It is a 'flavin adenine dinucleotide' enzyme that is localised in different cell organelles such as cytosol, chloroplast, peroxisomes and mitochondria where, it acts as scavenger of H_2O_2.This enzyme function in the presence of NAD(P)H [65]. In 2004, Schutzendubeland his co-workers [68] have been reported that MDHAR activity increased or decreased when exposure of cadmimum to *Pinus sylvestris* and *Populus Canescens* respectively.

Glutathione peroxidase (GPX)

GPXs are associated with large family of diverse isozymes that use GSH to reduce H_2O_2 and organic and lipid hydroperoxides, and hence protect the plant cells from different oxidative stress [69]. It is reported a family of seven related proteins in endoplasmic reticulum (ER), cytosol, mitochondria and chloroplast such as AtGPX-1 to AtGPX-7 in *Arabidopsis*. According to some studies, under salt and heavy metal stress inserted the radish phospholipid hydroperoxide GPX gene (Rs-PHGPx) into ayeast PHGPx deletion mutant and found that PHGPx mRNA levels suddenly enhance in plant tissues.

Dehydroascorbate reductase (DHAR)

This enzyme regenerates ASH from the oxidized state and it responsible for the maintenance of cellular ASH (redox state) that is crucial for tolerance to different biotic or abiotic stresses which leads to the production of reactive oxygen species. Yin and his co-workers (2010) [70] suggested that over expression of DHAR enzyme also increase plant tolerance to Aluminium by regulating high level of ASH.

Glutathione S transferase (GST)

It plays important role in different functions of cells such as apoptosis regulation, detoxification of hydroxyl ions and peroxides with the help of GSH that produces scavenger of genotoxic and cytotoxic compounds which can damage the different proteins and nucleic acid [22].

Non-Enzymatic Antioxidant

Other than anti-oxidative enzymes, non-enzymatic antioxidants also have a crucial role in scavenging free radicals that are generating in the plant tissues during different abiotic stress conditions. The non-enzymatic component comprises molecules such as α-tocopherol, ascorbic acid, glutathione and carotenoids that can scavenge Reactive oxygen species (ROS). Ascorbic acid and tocopherols have a poor ability to donate electrons and thereby transfer of single hydrogen atoms making them efficient antioxidants [71]. Reduced glutathione is a powerful reluctant and hence a very efficient scavenger of ROS. In 1997, Cheeseman and his co-workers are reported that superoxide radicals generated in plants are scavenged non-enzymatically by reduced ascorbate and glutathione [72]. Carotenoids scavenge free radicals that are produced owing to higher excitation energy from chlorophyll during photosynthesis reactions [71].

Ascorbic acid

It is widely distributed, water soluble antioxidant that minimizing the damage caused by reactive oxygen species in plants. It is present in all plant tissues, usually being higher in photosynthetic cells and meristematic tissues and some fruits. It has been reported that its reduced state ASH mostly remains in the leaves and chloroplast under normal physiological conditions [73]. It is also known as a most powerful reactive oxygen species (ROS) scavenger. The ASH redox system consists of L-ascorbic acid, DHA and MDHA. On the other hand, in high amount of cadmium decrease in the ASH in the nodules and roots of *G. max* has also been observed [73].

Glutathione (GSH)

It occurs abundantly in reduced form GSH and the steadiness between the GSH and GSSG is a vital component in maintaining cellular redox state in plant tissues [74]. It is present in different cell organelles like mitochondria, cytosol, vacuole, endoplasmic reticulum, peroxisomes, apoplast and chloroplasts where it helps to shield the photosynthetic apparatus from oxidative damage (Jimenez *et al*., 1998; Mittler *et al*., 1992). It is particularly important in plant because GSH plays a key role in the antioxidative defence system by regenerating another potential water soluble antioxidant like ASH, via the ASH-GSH cycle [75]. It is mandatory to maintain the normal reduced state of cells so as to neutralize the inhibitory effects of reactive oxygen species and induced oxidative stress. It is a potential scavenger of O_2, hydrogen peroxides and most dangerous reactive oxygen species like hydroxyl ions [51].

Proline

Proline also acts as a potent antioxidant and potential inhibitor of PCD. It is a good osmoprotectant and helps in membrane stability by stabilizing the protein. An interrelation was also shown between increased proline content and developed tolerance to the abiotic stress factors. Therefore, proline can now be considered as nonenzymatic antioxidants of those plants, microbes and animals require alleviating the adverse effects of reactive oxygen species [76]. In plants, proline also mitigate the effect of singlet oxygen and free radical induced damages and performs an crucial role in protection of proteins against denaturation

[77]. Free proline has been proposed as a metal chelator, an inhibitor of lipid peroxides or hydroxyl redical and O_2 scavenger [6].

Proline is also an effective quencher of reactive oxygen species that is formed under salt, dehydration and metal stress conditions in all plants including algae [77]. Hong and his co-workers (2005) [78] reported that a significant lower level of malondialdehyde that is a marker for free radical production was observed in proline overproducing plants.

α- Tocopherol (Vitamin E)

Tocopherol recognized as potential scavengers of reactive oxygen species and lipid radicals that is confined to the biomembrane where they play both antioxidant and nonantioxidant functions [79]. Four different isomers (α, β, γ, δ) are found in plants, out of them, α-tocopherol has the highest anti-oxidative activity due to the presence of three methyl groups in its structure [80]. Oxidative stress activates the expression of genes responsible for the synthesis of tocopherols in higher plants.

Carotenoids

Carotenoids are pigments that are located in plants and microorganisms. It is a lipid soluble antioxidant plays a majority of functions in plant metabolism as well as oxidative stress tolerance. An increase in Carotenoid contents was also reported afterward Cd stress [75].

Flavonoids

Flavonoid occurs widely in the plant kingdom. Flavonoids usually accumulate in vacuole as glycosides, but they also found as exudates on the surface of leaves and other aerial plant parts. Flavonoids are among the most important bioactive secondary metabolite of the plant. Most flavonoids outperform prominent antioxidant, such as ASH and α-tocopherol [81]. Flavinoids serve as reactive oxygen species scavengers by overcome the effect of free radicals before they damage the cell therefore, it have crucial role in the plants under adverse environmental condition There is substantial increase in flavonoid levels under biotic and abiotic stress such as metal toxicity, drought, nutrient deprivation and wounding [82].

Ferritin

Ferritin, a prevalent multimeric, iron storage protein. They have considerable role during oxidative stress in plants and halophytes by shielding the chloroplast [61]. Different isoforms of ferritin gene have been reported in soybean and cowpea [83] three in *Lupinus luteus* (Strozycki *et al.* 2003) two in maize and tobacco [84]. Ferritin exclude increased amount of free iron and prevents formation of OH radicals [84]. Ferritin accumulation was found in the sites where high amount of H_2O_2 present [85].

Cadmium (Cd)

Cadmium (Cd) is a poisonous pollutant that can easily be taken up by plant roots and transported to the leaves therefore, effecting the plant growth and development, alternation in photosynthesis rate, water use efficiency and the uptake of micronutrients and macronutrients leading to reduction in crop production due to plant affected by several diseases such as chlorosis etc [86]. The main difficulty with the Cadmium is that it can be transferred to the food chain and hazardous to the human health [87].

In recent years, according to some evidences it plays a key role in heavy metal toxicity [88] [89]. Generally, Cd is accumulated in roots, different vegetal parts [90] and small portion is transported to the aerial parts and other cellular compartments [48]. The tolerance of plants to Cd involves metal detoxification processes, such as complexation with phytochelatins [48] [86].

Lead (Pb)

Lead (Pb^{2+}) is potent environmental pollutants that pose a serious threat to the environment or human and animal health [91] has attracted considerable attention due to its widespread distribution. Lead contamination in soil and potential risk to the environment leads to the considerable attention of scientists due to it not only stimulated the changes of soil microorganism's activities but also affected the change of physiological indices therefore, resulted in yield decline [92].

Pb^{2+} accumulated in different plant tissues particularly in the root tissues [93]. It have multiple direct and indirect effects on the plant's biochemical and physiological activities like growth and metabolism, reduction in photosynthesis and membrane disorganization along with visible symptoms including stunted growth and small leaves [94].

Zinc (Zn)

Out of seventeen essential elements, zinc is now being recorded most important micronutrient for the development and plant growth in crop production. It have significant role in the synthesis of nucleic acid, protein, membrane integrity, enzyme activation and helps in the utilization of nitrogen and phosphorus in plant. Plant availability of soil Zn is affected by soil type, soil pH, soil moisture, organic matter, mineralogy and Zn diffusion and plant uptake [70]. Darwish *et al.*, (2002) [18] reported that treatment of Zn gave the highest seed, oil yield/fed in soybean. Yasari (2012) [95] studied the effect of Zn on the soyabean crop such as variation in amount of seed oil, protein content and percentage depends upon the addition of Zn that is directly adding them in to the soil or sprinkling them on the crop.

Activity of PSII is inhibited when Mn is replacing by Zn in the thylacoid membrane. Generally, in the membrane sixteen molecules of Mn and Zn per four hundred chlorophyll molecules are present. However, during stress condition in high concentrations of zinc, the ratio of manganese and zinc changes to twelve molecules of Mn and thirty molecules Zn atoms [96].

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