RESPONSES TO ZINC (Zn^{2+}) STRESS BY SELECTED RICE (*Oryza sativa* L.) VARIETIES OF NORTH EAST INDIA

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

SAGOLSHEM PRIYOKUMAR SINGH

MZU REGISTRATION NO: 1600797

Ph.D. REG NO: MZU/Ph.D./1031 of 26.05.2017

DEPARTMENT OF BOTANY

SCHOOL OF LIFE SCIENCES

DECEMBER, 2021

RESPONSES TO ZINC (Zn^{2+}) STRESS BY SELECTED RICE (*Oryza sativa* L.) VARIETIES OF NORTH EAST INDIA

BY

SAGOLSHEM PRIYOKUMAR SINGH

DEPARTMENT OF BOTANY

SUPERVISOR

Dr. Y. TUNGINBA SINGH

DEPARTMENT OF BOTANY

MIZORAM UNIVERSITY

SUBMITTED

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS OF THE DEGREE OF DOCTOR PHILOSOPHY IN BOTANY OF MIZORAM UNIVERSITY MIZORAM

MIZORAM UNIVERSITY

(A Central University Established by an Act of Parliament of India)

Y. Tunginba Singh Ph.D.
Associate Professor

Department of Botany
School of Life Sciences
Tanhril, Aizawl-796004,
Mizoram, India
Phone: +91 9862604012
Email: tunginba@mzu.edu.in

CERTIFICATE

This is to certify that the thesis work entitled, "**Responses to zinc (Zn^{2+}) stress by selected rice (*Oryza sativa* l.) varieties of North East India**," submitted by Sagolshem Priyokumar Singh (MZU/Ph.D./1031 of 26.05.2017) in partial fulfillment of the requirement for the degree of Doctor of Philosophy in Botany is a record of bonafide work carried out by him under my supervision and guidance.

(Dr. Y. TUNGINBA SINGH)

Supervisor

DECLARATION BY THE CANDIDATE

I, **Sagolshem Priyokumar Singh**, hereby declare that the subject matter of this thesis is the record of work done by me, that the contents of this thesis did not form basis of the award of any previous degree to me or to the best of my knowledge to anybody else, and that the thesis has not been submitted by me for any research degree in any other Universities/Institute.

This is being submitted to the Mizoram University for the degree of Doctor of Philosophy in Botany

SAGOLSHEM PRIYOKUMAR SINGH

Candidate

(Dr R. LALFAKZUALA)

Head of Department

(Dr. Y. TUNGINBA SINGH)

Supervisor

AKNOWLEDGEMENT

I would like to express my heartfelt appreciation to my supervisor, Dr. Y. Tunginba Singh, Associate Professor, Department of Botany, Mizoram University for his unwavering support of my Ph.D. studies and research, as well as his patience, encouragement, passion, and vast knowledge. His advice was invaluable throughout the course of my research and thesis writing.

I am thankful to Professor S.K. Mehta and Dr. R. Lalfakzuala, Dept of Botany for making their laboratory available for my works. My earnest gratitude to all the faculty and staff of the Department of Botany, Mizoram University for their constant help and support.

I am very thankful to my labmates – Vanlalsanga, Laldinfeli Ralte, Hmingremhlua Sailo, Vanlaldina Khiangte, Malsawmthanga and Lalremliani for their support, constant help during my research work. I would like to express my thanks to all research scholars of the Department of Botany.

My special thank goes to my mother for loving me and for giving me this wonderful life. I love you and am forever grateful to you for being my mother.

SAGOLSHEM PRIYOKUMAR SINGH

Preface

Rice is an important staple food crop for half of the world population and many abiotic stresses significantly reduce crop production and quality of many agricultural food crops. Among the abiotic stresses heavy metal toxicity is counted as one of the important detrimental factors with respect to its effect on the environment as well as food safety. Some metals are important for normal growth and development of plants at low concentrations such as Zn, Mn, Ni, and Cr but when these metals are over accumulated in the environment it becomes a serious issue for the plant and animals.

Metals are naturally found in the surrounding environment through natural process such as weathering of rocks. Rapid industrialization and expansion of agricultural activities also significantly enhance heavy metal pollution in soil, water and air. Excessive amount of heavy metal is accumulated in the soil due to a wide variety of anthropogenic activities like disposal of high metal wastes in improperly protected landfills, leaded gasoline and lead based paints, application of large amount of fertilizer, animal manures, sewage sludge, compost, pesticides, coal combustion residues, petrochemicals, and atmospheric deposition.

Hence, soil and water toxicity due to heavy metal contamination has become an important constraint for crop production and quality. The present study was taken up to assess heavy metal stress response by two local rice varieties of north east India. The main objectives of my study are:

1. To estimate germination percentage and growth rate of rice varieties under Zn stress.

2. To determine photosynthetic efficiency and antioxidant activities of rice under Zn stress.

3. To analyse DNA damage in rice using molecular makers under Zn stress.

Analysis of Zn stress was performed using two pigmented local rice varieties Chakhao and Kawnglawang from Manipur and Mizoram respectively. The results obtained from the study revealed that Zn stress had slight impact on chlorophyll content, total soluble protein, and antioxidant enzyme activity. However, genotoxicity analysis using RAPD-PCR revealed that Zn stress did not have a significant impact on DNA damage. The present study suggests that the physiological and biochemical parameters like seed germination percentage, chlorophyll content, total soluble protein, antioxidant enzyme along with the genotoxicity analysis using RAPD primer can be used as good biomarkers in heavy metal toxicity studies. At the same time, selection of metal tolerant cultivars can be done for future rice breeding programs.

CONTENTS

Chapter 1

Introduction

1.1 Rice

Rice is a cereal food crop belonging to the family Poaceae. *Oryza sativa* and *Oryza glaberrima* are the two main rice species that are domesticated and they are native to tropical and subtropical southern Asia and southern Africa (Crawford and Shen, 1998). *O. sativa* is the widely cultivated species while a small amount of *O. glaberrima* species is being grown in West Africa for the last 3500 years (FAO, 1993). The actual origin of rice domestication is not yet definitely known, though it is believed that rice domestication occurred independently in China, India, and Indonesia and then from there it has given rise to three races: *japonica*, *indica*, and *javanica* (also known as bulu in Indonesia). There are reports that rice was cultivated in India between 1500 and 2000 B.C. and in Indonesia around 1648 B.C (FAO, 1993). It is shown by archaeological studies that tropical or indica rice was cultivated in Ho-mu-tu Chekiang Province, China around 7000 years ago (Chang, 1983). It is believed that originally rice was cultivated without submersion, but due to mutations, it has become a semi-aquatic plant. Rice can grow in diverse environmental conditions but it grows faster and more abundantly in wet and warm conditions (Gnanamanickam, 2009). Rice ecosystems can be classified into four types: irrigated, rainfed lowland, deep water, and rainfed upland. Rice grown under irrigated land is most common and contributes 55% of the overall production (Bernier et al., 2008).

Rice is the major staple food of Asia and part of the Pacific. The Asia-Pacific region produces and consumes over 90% of the world's rice. Rice consumption per capita has begun to decline in middle and high-income Asian countries such as the Republic of Korea and Japan as prosperity and urbanization have increased

(Papadematrious 2000). However, about a quarter of Asia's population remains impoverished, with significant unmet rice demand and rice consumption is increasing more rapidly in this region. At the moment, Asia's population is expanding at a rate of 1.8 percent per year, and it is unlikely to stabilize until the middle of the next century. A population prediction for the year 2025 shows a 51 percent rise on average, and up to 87 percent in some circumstances, over the base year 1995 (Bos et al., 1994). Over a 45-year period (1950 to 1995), the annual growth rate for rice consumption in the Asia-Pacific Region has maintained pace with demand, owing to increased yields rather than increased land. During this time, improved varieties have had a considerable impact in an ever-increasing order (Khush, 1995). From 261 million tonnes in 1950 (with Asian production of 240 million tonnes) to 573 million tonnes in 1997 (with Asian production of 524 million tonnes), the global rice supply has more than doubled (FAO 1997).

1.2 Heavy metal

Heavy metals are inorganic chemicals with atomic mass greater than 20amu and a density higher than 5 gcm^{-3}, and they impart cytotoxic, genotoxic, and mutagenic effects on humans, animals, and plants (Flora et al., 2008). Due to the increase of heavy metal accumulation in the soil, there is an increase in the risks of food safety and health, which is an important concern to the public as well as government agencies (McLaughin and Singh, 1999; Mandal and Suzuki, 2002). Rice is an important staple food consumed daily by more than half of the world population especially in Asian countries and heavy metal contamination is a severe abiotic stress that affects the production of rice (Nguyen and Ferrero 2006; Acquaah 2007, Huang et al. 2013,). Two main factors are responsible for heavy metal accumulation, natural sources (e.g., wind-blown dust, decaying vegetation, forest fires, sea spray) and human activities (e.g., mining, metal production; wood production, and phosphate fertilizer). Naturally, the sources of heavy metals in the soil are the parent rocks. Weathering of rock is regarded as an important source of heavy metals in the environment. The environmental condition and composition of rocks directly

influence the process of weathering (Abdu et al., 2011). Some of the rock minerals change their crystalline structure after weathering and make them available to be absorbed by the soil or transported towards surface water or groundwater (Parth et al. 2011). Heavy metals found in the soil can be categorized into two types: essential elements and non-essential elements. Essential elements are those that are required for normal plant growth, such as Fe, Mn, Zn, Cu, Mg, Mo, and Ni and non-essential elements such as Cd, Sb, Cr, Pb, As, Co, Ag, Se and Hg, are those that do not have known biological and physiological functions (Schutzendubel and Polle, 2002; Rascio and Navari-Izzo, 2011; Tangahu et al., 2011; Zhou et al., 2014). Essential elements play important role in the growth, metabolism, development, and structure of enzymes and proteins at low concentrations (Zengin and Munzurohlu, 2005). However, excess amounts of these metals can hamper normal functioning of cells and disturb the metabolic processes such as displacement of building blocks of protein structure, hindering functional groups of important cellular molecules, disturbance in the functionality of important biomolecules such as pigments or enzymes (Emamverdian et al., 2015).

1.3 Heavy metal contamination

Heavy metals are found unpretentiously in the soil from pedogenetic processes of weathering of parent materials at levels that are regarded as trace (<1000mg kg^{-1}) and rarely toxic (Pierzynski 2000; Kabata-Pendias 2001). Soil environment is generally contaminated due to the rapidly increasing rate of generation of heavy metals by anthropogenic activities as compared with the natural ones and the concentration and chemical form of heavy metals found in the soil environment become more bioavailable to the plants (D'Amore et al 2005; Wuana, et al. 2011). An excessive amount of heavy metal is accumulated in the soil due to a wide variety of anthropogenic activities like disposal of high metal wastes in improperly protected landfills, leaded gasoline and lead-based paints, application of large amounts of fertilizer, animal manures, sewage sludge, compost, pesticides, coal combustion residues, petrochemicals, and atmospheric deposition (Basta et al., 2005;

Khan et al 2008; Zhang et al 2010). A large quantity of fertilizers is used in order to improve soil nutrient condition or to change soil pH to make nutrient more bioavailable (Bradl 2005) and the compounds used to make this fertilizer contain a trace amount of heavy metals as impurities, so continuous use of these fertilizers enhance more accumulation of heavy metals in the soil (Jones et al., 1981). Similarly, various kinds of pesticides are extensively used to control insects and pests in high production agriculture where they are either treated with seeds or applied in soil and their application in soil has led to an increase deposition of heavy metal in that soil (Bradl 2005).

1.4 Bioavailability of Heavy metals

The bioavailability of metals in the soil depends on the physical, chemical, and biological processes and interactions between them and also depends on their distribution between solid and solution phase (Rieuwerts et al 1998; Fijalkowski et al 2012). The process of binding heavy metals, and their bioavailability depends on various soil parameters such as

A. Granulometric composition
B. Organic matter content
C. pH value
D. Sorption capacity
E. Content of macro and micronutrients
F. Oxidation-reduction potential
G. Activity of microorganisms
H. Bioavailability for plants and animals
I. Resistance of the soil

pH is regarded as one of the important factors influencing concentrations of soluble and metal availability by plants (Brallier et al 1996). At low pH, metal tends to form soluble organometal or as free ionic species and increase its bioavailability and at

higher pH values metal tends to decrease its solubility (Chuan et al., 1996; Thornton 1996; Olaniran et al., 2013). Redox potential is the measurement of the tendency of an environment to oxidize or reduce substrates. Redox reactions in soils are controlled by the aqueous free electron activity, which can also be expressed in terms of the redox potential (E_h). High redox potentials are associated with dry, well-aerated soil, while low redox potentials are associated with soil rich in organic matter and waterlogging (Evans, 1989). Soil texture also plays an important role in influencing metal solubility and their role can be expressed in terms of types of soils like clay, silt, and sand fractions (Qian et al., 1996). Clay fraction and granulometric composition of soil have a high potential to bind with heavy metals (Fijalkowski et al 2012). Soil organic matter is incorporated with humic substances or humus, and non-humic substances and they are accumulated in the soil as a result of the decomposition of plant materials (Foth, 1978). Soil organic matter content has small impact on metal binding but it is potential in retention of atmospheric metal inputs in the surface of the soil (Zimdahl and Skogerboe 1997; Rieuwerts et al, 1998). Thus, different factors may influence the solubility of metals in soil but initially, available metal solubility largely depends on metal physico-chemical properties, particle size, and their sources (Jones and Jarvis, 1981).

Uptake of metals by the plants is also influenced by many factors such as temperature, soil pH, soil aeration, competition between the plant species, the type of plant and its size, the root system, the availability of elements in the soil or foliar deposits, the type of leaves, soil moisture and plant energy supply to the roots and leaves (Yamamoto and Kozlowski 1987). Metals such as Mn, Cu, Zn, Mo, and Ni in appropriate concentrations are important for the normal growth and development of plants since they act as cofactors and catalytic components of proteins and enzymes (Moustakas and Ouzounidou 1994). However, their accumulation can alter many vital plant processes, such as mineral nutrition (Ouzounidou et al., 1992) transpiration (Poschenrieder et. al., 1989), photosynthesis (Lidon and Henriques, 1991), enzyme activities-related to metabolism (Nussbaum et. al., 1988),

biosynthesis of chlorophyll (Lidon and Henriques,1991), nucleic acids metabolism (Doncheva et. al., 1996) and seed germination (Ouzounidou et. al., 1992).

1.5 Effects of Heavy metals

Excessive amount of heavy metals in plants severely affects the accumulation and transportation of important elements in plants which often leads to the change in the morphological characters of plants such as changes in the leaf area, shoot length, and root length etc. (Pant et al 2014, Seneviratne et al 2019). Further, increase in accumulation of heavy metals changes the environment for normal growth of plants (Doni et al 2014). Heavy metal accumulation in plants not only disturb the morphological and physiological characters but also alters the various anatomical characteristics like dissolution and reduction in parenchymatous cells, mesophyll cells, and decrease in the number of xylem vessels as well as in the diameter of root, stem and leaf (Batool et al 2015).

Seed germination, one of the most significant stages in a plant's life, is sensitive to the chemical and physical conditions of the rhizosphere (Bewley 1997). Although the seed coat can act as a primary barrier defending harmful effects of heavy metals, most seeds and seedlings show a decline in germination in response to heavy metal stress causing a major concern for agricultural and forestry practices (Adrees et al. 2015). Earlier studies reported that through inhibition of storage food mobilization, reduction in radical formation, disruption of cellular osmoregulation, and the degradation of proteolytic activities, heavy metals cause inhibition of seed germination and seedling development (Adrees et al. 2015; Karmous et al. 2015; Arif et al 2019).

Previous studies also reported that there had been significant alteration in the metabolic process of photosynthesis, respiration, gaseous exchange, and nutrient translocation, which ultimately decreased plant growth under heavy metal stress (Sharma et al. 2005; Gomes et al. 2011). Excessive accumulation of heavy metal also

inhibits the normal functioning of photosynthetic apparatus such a leaf tissue, cytosolic enzymes, chloroplast membranes, photosynthetic pigments and causes interruption in the photosynthetic carbon reduction cycle (Rai et al., 2016). Chlorosis and retardation of plant growth are frequently seen when a plant is grown under a heavy metal polluted environment, which indicates the disturbance in the biosynthesis of important photosynthetic pigments (Rai et al., 2016). An earlier report also showed the decline in chlorophyll content in plants due to heavy metals such Hg, Cu, Cr, Cd, and Zn, which in turn caused a decrease in photosynthetic rate (Aggarwal et al., 2012). Thus, heavy metal exposure disturbs the normal biosynthesis of important photosynthetic pigments such as chlorophylls and carotenoids.

Proteins are large and complex biomolecules that comprise of one or more long chains of amino acids that play many important functions within an organism. They have a vast array of functions such as catalyzing metabolic reactions, DNA replication, transporting molecules, cellular signaling, membrane fusion, structural support, and protection etc. (Saraswathy and Ramalingam, 2011). The structure of a protein generally determines the function it is going to perform. The physical and chemical conditions of the protein environment have a great impact on the conformation of a protein. Exposure to extreme temperature, reactive molecules, heavy metal ions, and other stresses triggered disturbance in the protein folding process of newly synthesized protein and also influenced the misfolding of protein (Golbberg 2003; Saraswathy and Ramalingam, 2011; Guo and Zhou 2016).

Genotoxicity is a term used in genetics to describe the damaging effect on a cell's genetic material (DNA, RNA) that compromises the cell's integrity. A genotoxin is a substance that has the ability to cause genotoxicity such as radiation, chemicals etc. (Shah 2012). Genotoxic metal binding to the cell nucleus causes promutagenic damage including DNA base modifications, inter-and intra-molecular cross-linkage of DNA and proteins, DNA strand breaks, rearrangements, and depurination. Chemical reactions driving this damage, and the resulting mutations,

are characteristics of an oxidative DNA attack (Kasprzak 1995). The genotoxicity of heavy metals has been reported in many plants. Currently, a wide variety of DNA markers/techniques have been applied for understanding heavy metal toxicity at the DNA level. Many PCR- based techniques have been developed for the analysis of DNA in the field of genotoxicity (Liu et al., 2007).

The random amplified polymorphic DNA (RAPD) method is a PCR- based technique that is simple, fast, reliable and capable of detecting not only point mutations but also temporary alteration of DNA (Ahmad et al., 2012). This method involves amplification of random segments of genomic DNA using short arbitrary primers without the need for prior knowledge of genomic DNA (Welsh and McClelland 1990; Williams et al., 1990). It has been successfully used in species and strain identification (Bardakci and Skibinski 1994; Cocconcelli et al., 1995), genetic diversity analysis (Koh et al., 1999), genetic marker-assisted breeding (Liu et al., 1999), genetic variation detection (Keshava et al., 1999), and evaluation of genotoxicity level induced by environmental pollutants (Rocco et al., 2010, 2011, 2012). The assessment of genomic template stability (GTS) is a measure of DNA damage and mutation such as deletion or addition of DNA sequences, structural modification, point mutation, and mutation by polyploid variability. The polymorphic DNA bands obtained from RAPD analysis can considerably aid in the creation of molecular markers for the detection of damaged or mutant DNA in plants cells. GTS is a qualitative indicator of RAPD profile differences (Younis et al., 2020).

1.6 Plant response against heavy metals

Plants employ various defense strategies for tolerance or detoxification whenever there is a stress condition including heavy metal stress. As a first step towards dealing with metal intoxication, plants adopt an avoidance strategy to overcome stress via restricting metal uptake from soil or excluding it, preventing metal entry into plant roots (Viehweger 2014). The primary response to heavy metal stress in plants is the production of reactive oxygen species (ROS). ROS are the

byproducts of aerobic metabolic processes. When aerobic organisms use molecular oxygen, a number of oxygen-containing reactive species are formed, which are collectively referred to as reactive oxygen species (ROS). Both the physiological and pathology of aerobic life are influenced by ROS (Li et al., 2016). ROS are always produced as a result of electron leakage onto O_2 from electron transport processes in the chloroplast, mitochondria, and plasma membranes, or a byproduct of numerous metabolic pathways located in different cellular compartments. Several environmental stresses such as salinity, drought, metal toxicity, chilling, and UV-B radiations lead to overproduction of ROS in plants due to disturbances in cellular homeostasis (Sharma and Dubey 2007; Heyno et al., 2011). ROS comprises free radicals such as superoxide anion (O_2^-), hydroxyl radical (•OH), as well as non radical molecules like hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), etc. (Sharma et al., 2012). ROS are generated in several cellular compartments such as chloroplast, mitochondria, peroxisomes, apoplast, and plasma membrane (Huang et al., 2019; Singh et al., 2019). Different kinds of environmental factors influence the overproduction of ROS such as high light, high or low temperature, salinity, drought, metal toxicity, nutrient deficiency, and pathogen attacks (Tripathy and Oelmuller, 2012).

ROS are highly reactive chemical molecules and they are produced as a byproduct of aerobic metabolism and they play important role in cell signaling and homeostasis. The pathway for the generation of ROS in plants can be either directly through Haber-Weiss/Fenton reactions, or by the activation of NADPH oxidase, or by inhibiting enzymes through the displacement of essential cation (Shahid et al., 2014). Increased generation of ROS amid environmental conditions can endanger cells by triggering lipid peroxidation, protein oxidation, nucleic acid damage, enzyme inhibition, activation of programmed cell death (PCD) pathway, and ultimately cell death (Verma and Dubey 2003; Meriga et al., 2004; Sharma and Dubey 2005; Maheshwari and Dubey 2009; Mishra et al., 2011; Srivastava and Dubey 2011). When the ROS production in the cell exceeds the cellular detoxification capacity, the cell undergoes a state of oxidative stress that stimulates

the oxidation of important biomolecules, such as DNA, proteins, lipids, and carbohydrate (Sharma et al., 2012). ROS have been implicated as second messengers in intracellular signalling cascades that mediate a variety of plant responses, including stomatal closure (Neill et al., 2002; Kwak et al., 2003; Yan et al., 2007), programmed cell death (Bethke and Jones 2001; Mittler 2002), gravitropism (Jung et al., 2001), and the acquisition of tolerance to both biotic and abiotic stressors, at low/moderate concentrations (Torres et al., 2002; Miller et al., 2008). Plants use redox-sensitive proteins, calcium mobilization, protein phosphorylation, and gene expression to sense, transduce, and transform ROS signals into suitable physiological responses. Through the oxidation of conserved cysteine residues, ROS can be recognized directly by critical signaling proteins like tyrosine phosphatase (Xiong et al., 2002). Many signaling components such as protein phosphatase, protein kinases, and transcription factors can be regulated by ROS, and it can interface with other signal molecules and pathways that are part of the signaling network that controls response downstream of ROS (Neill et al., 2002). The balance between oxidant generation and antioxidant removal determines the strength, lifespan, and size of the ROS signaling pool (Sharma et al., 2012).

Plants have a sophisticated antioxidative defense system to scavenge ROS that includes both non enzymatic and enzymatic components. Diverse organelles, such as chloroplasts, mitochondria, and peroxisomes, produce and scavenge ROS, and ROS-scavenging routes from different cellular compartments are coordinated in plant cells (Pang and Wang 2008). Potentially harmful oxygen metabolites are produced at low levels under normal conditions, and there is an optimal balance between ROS production and quenching. A number of negative environmental variables can disrupt the balance between ROS production and quenching, resulting in a rapid increase in intracellular ROS levels (Noctor et al., 2008; Sharma et al., 2019). However, plants produce antioxidants to overcome the oxidative injury induced by heavy metals (Zabalza et al., 2008). Some of the antioxidants which are generated during heavy metal stress include several ROS-removing enzymes such as SOD, CAT, GPX, APX, GR, and low molecular mass antioxidants scavengers such

as ascorbate (ASC) and GSH (Baker, 1987). Antioxidative enzyme activity appears to be a key component of plants' antioxidant defense mechanism against metal-induced oxidative injury in metal stressed plants (Shah et al., 2001). Plant response to metal exposure varies based on plant species, tissues, development stages, metal type, and concentration. The activation of a set of defense mechanisms involving enzymatic and nonenzymatic components is one of the primary responses of plants (Cakmak and Horst 1991; Bhaduri and Fulekar 2012).

1.7 Heavy metal impact on health

Heavy metal contamination of soil may increase risks and hazards to humans and the ecosystem through direct ingestion or contact with contaminated soil, the food, drinking of contaminated groundwater, reduction in food quality via phytotoxicity, reduction in land usability for agricultural production causing food insecurity (Valke et al., 2005; Wuana et al 2011). Rice being an important staple food crop, it is important to study the nutritional composition and quality of rice grain. Heavy metal pollution in paddy fields has become an important issue as an important environmental pollutant and its characteristic bioaccumulation and non-biodegradable properties (Hoisa et al., 2015). Due to the high potential risk to the ecosystem and human health, heavy metal contamination is counted as a potential pollutant in rice and it is regarded as one of the major toxic substances (Jozefczak et al., 2012; Hoisa et al., 2015). Using wastewater as a mode of irrigation in the agricultural soil of paddy fields is a widespread practice in developing countries, which rapidly increases the uptake of metals in crops. This rapid elevation of heavy metals in crops decreases the food quality and becomes a menacing issue to health for the consumers (Murtaza et al., 2015). Industrialization, urbanization, and rapid economic development have resulted in increased industrial and agricultural activity around the world. Toxic heavy metals may be released into the water, air, and soil as a result of such actions. Growing human foods in heavy metal-contaminated media causes bioaccumulation of these elements in human food chains, which then reach the human body (Ali et al., 2019). Contamination of heavy metals in the human food

chain poses a threat to human health, as evidenced by cases from the twentieth century. In Japan, intake of Hg-contaminated fish and Cd-contaminated rice caused Minamata disease (MD) and itai-itai sickness, respectively (Nishijo et al., 2017).

To safeguard human health from the detrimental impacts of toxic heavy metals, the bioaccumulation and biomagnification of heavy metals in human food chains should be regularly monitored (Ali et al., 2019). Although heavy metal biomagnification is a contentious topic in metal ecotoxicology, multiple studies have found heavy metal biomagnification in particular food chains. An organism at higher trophic levels in food chains is more vulnerable to biomagnification of certain metals in food chains. Biomagnification can result in the larger concentration of trace metals in organisms of higher trophic levels, posing a health concern to these organisms or their human consumers (Barwick and Maher 2003).

Rice (*Oryza sativa*), being a model organism, is chosen to investigate the physiological and biochemical changes against heavy metal stress. Northeast India harbors many diverged landraces of rice. However, to the best of my knowledge, these rice varieties are not studied in terms of heavy metal stress. The present study, for the first time, aims to investigate the physiological and biochemical responses of selected rice varieties of North East India under different concentrations of Zn. This study will provide information about the sensitivity and tolerance capability of rice varieties of North East India against Zn stress.

1.8 Objectives:

1. To estimate germination percentage and growth rate of rice varieties under Zn stress.

2. To determine photosynthetic efficiency and antioxidant activities of rice under Zn stress.

3. To analyse DNA damage in rice using molecular makers under Zn stress.

Chapter 2

Review of Literature

Heavy metal contamination of soil becomes toxic when they are migrated to groundwater or taken up by flora and fauna and this process adversely affects the whole ecosystem. Heavy metals are naturally generated from parent rocks but anthropogenic activities such as use of municipal wastewater for irrigation, low quality and excess input of fertilizers and smelting, and fossil fuel combustion etc. result in the increased heavy metals in agricultural soil. All the ingredients of soil if present in higher concentrations than the normal amount may cause contamination or serious effect on the ecosystem, which might result in significant damage and cause unpreventable contamination in the systems. And heavy metals are the most important group among these agents, which can be damaging even at trace amounts (Oner and Celik, 2011). The negative effect on the development and quality of plants is higher if the heavy metal contaminated agricultural areas are used for crop plantation (Qishlaqi, 2008). Accumulation of heavy metal in the plant is more when the plant is exposed to the heavy metal contaminated areas and it also causes serious health risk to consumers via the food chain (Okoronkwo et al., 2005).

Zn is one of the important essential micronutrients and plays an important role in plant cell growth and development (Lin et al., 2015). And it is the second most abundantly found transition metal in a living organism (Marschner et al., 2011). Zn is found naturally in the soil through pedogenetic processes of mother rocks leaching (Wuana and Okieimen 2011). Naturally, the background concentration of Zn in soils and rocks is in the range between 10 and 300 ppm while its concentration in rivers is very less (< 0.01-0.2 ppm) (Noulas et al., 2018). Zn is released in the surrounding environment through the natural erosion process while the volcanic eruption has an important contribution to the dispersal of Zn in the environment (Prasad 2008).

Furthermore, industrial activities increase the Zn contamination in addition to the natural sources. Major anthropogenic activities such as mining, smelting, sewage sludge, and persistent use of Zn fertilizers are some of the important causes of Zn pollution in the environment (Balafrej et al., 2020).

Zn can be found in different forms, usually as a free ion (Zn^{2+} and $ZnOH^{+}$) or complexed with organic matters in soil. Many factors such as soil pH, organic matters, total soil Zn content, soil temperature and moisture, root distribution, and rhizosphere can affect the Zn availability to plants (Baruah 2018). Soil pH can be an important factor regarding the bioavailability of Zn since it can correlate with many biological and other chemical properties of soil (Behera and Shukla 2015). Soil organic matter enhances Zn solubility and reduces fixation which results in increase availability of Zn to the plant roots (Cakmak 2009). However, high organic matter content in soils like in peat and musk soils tends to decrease the Zn availability because of Zn binding property on the solid-state of humid substances (Balafrej et al., 2020). Soil water content can be another important determining factor for the availability of Zn to plants (Patnaik et al., 2008). Flooding of soil leads to the reduction of Zn availability due to the dissolution of P and the formation of insoluble compounds with Mn, Fe, CO_3, and S under anaerobic conditions (Alloway 2004). Flooding of soil notably reduces Zn concentration and also affects Zn availability by changing pH (Baruah 2018). According to Yoo and Jame (2003), flooding significantly reduces Zn concentration in leaves of rice (*Oryza sativa*), wheat (*Triticum aestivum*), and barley (*Hordeum vulgare*).

Root's interaction with the rhizosphere can be an important factor for the availability of Zn. Root activity induces lot of changes in the soil rhizospheric properties, like the pH, chemical equilibrium, mobility, microbial activity, and bioavailability (Compant et al., 2010; Seshadri et al., 2015). An earlier study reported that there were wide range of differences in metal uptake and translocation depending on crop plants' abilities to absorb and accumulate heavy metals in their

body parts and even between cultivars of the same plant species (Satpathy et al., 2014).

Excess and deficiency of Zn are differentiated with a minute concentration range and it has frequently mistaken the toxicity and deficiency of Zn stress in plants due to similar visual symptoms (dos Santos et al., 2019). So, it is a common mistake to add more Zn in the soil thinking that there is a deficiency of Zn when there is already an excessive amount of this element in toxic level (dos Santos et al., 2019). Zn is a vital component of special proteins known as Zn fingers that bind to DNA and RNA and help in their regulation and stabilization. In plants, Zn plays an important role as a functional, structural, or regulatory cofactor of various enzymes like carbonic anhydrase, carboxypeptidase, Zn-superoxide dismutase, oxidoreductases, transferases, and hydrolases that are responsible for many metabolic processes in plants (Brown et al., 1993; Mousavi et al., 2013; Balafrej et al., 2020).

Zn is an important micronutrient and plays an important role in plant development, reproduction, signaling, and approximately 15-20 mg kg^{-1} dry weight of Zn is required for the proper growth of most crops (Cakmak et al., 1996). An excess amount of Zn beyond the required concentration can be toxic to flora and fauna, and humans (Cambier et al., 2009). Reactive oxygen species (ROS) are produced in plants as a normal part of the metabolic process of chloroplasts, mitochondria, and peroxisomes (Berni et al., 2019). Under stress conditions, ROS production is increased which ultimately results in oxidative stress that causes damage to lipids, protein, and DNA and ultimately leads to cell death (Kanazawa et al., 2000). Oxidative stress refers to the condition caused due to imbalance in production and accumulation of ROS in cells and the inability of the biological system to detoxify the overproduction of ROS (Schieber and Chandel 2014; Pizzino et al., 2017).

The seed germination stage is the most sensitive process where seeds first exchange interface with the surrounding environment and at this stage they are relatively sensitive to the changing environmental conditions (Solanki and Dhankhar 2011). Many environmental factors including biotic and abiotic factors affect the seed germination of many crop plants which is why it is the most important physiological process (Moosavi et al., 2012) and seed coat is softened at the time of imbibition and germination and this makes the seed more accessible to various stresses (Kranner and Colville 2011). The severity of metals on seed germination depends on the metal's ability to penetrate the seed coats and reach embryonic tissues and also the physical and chemical properties of metal ions (Márquez-García et al., 2013). Effect of heavy metals on seed germination also depends on the anatomy and structure of the seed coats of different plant species (Munzuroglu and Geckil 2002). Metals like Cu and Cd had been reported to inhibit the water uptake and as a result, the germination process was hampered (Kranner and Colville 2011).

Heavy metals enter the plant system along with the nutrient absorbed by the root from the soil (Dal Corso et al., 2013). The main physiological change in response to heavy metal exposure is the reduction in plant growth (Hu et al., 2013). Reduction in photosynthesis and respiration due to heavy metal exposure in plants is often accompanied by change in leaf structure and physiology. In addition, heavy metal exposure affects the metabolic processes and reduction in energy production and also affects transpiration and transportation of materials between the various organs (Ying et al. 2010). Heavy metal stress can also disturb the nutrient and water uptake by the roots that ultimately affects various developmental processes in plants such as flowering, embryogenesis, and seed formation by affecting the normal functioning of root and leaf (Shahid et al., 2015). Some metals such as Fe, Cu, and Zn are important nutrient of plants since they play important roles as cofactor for various enzymes that are required for the normal functioning of photosynthesis (Kovacik et al., 2010; Shanmugam et al. 2011). Metals are hazardous to plants primarily by destroying chloroplasts and interfering with photosynthesis. Metal ions interfere with photosynthetic enzymes and chloroplast membranes, causing

photosynthesis to be inhibited (Aggarwal et al., 2012). Heavy metal deposition in leaves, affects the function of the stomata and thus impacts photosynthesis and transpiration rates in higher plants thereby reducing photosynthesis indirectly. Heavy metal reduction of photosynthetic pigments has an indirect effect on photosynthesis, which is why the use of non-destructive technologies and ease of measurement allow photosynthetic pigments to be frequently used to determine stress for regulatory purposes (Aggarwal et al., 2012).

Plants and other photoautotrophs utilize chlorophyll as a major photosynthetic pigment. The word chlorophyll comes from the Greek words *chloros*, which means green, and *phyllon*, which means leaf. Chlorophyll gets its distinctive green colour from its capacity to absorb light in the blue and red regions, while attenuating light in the green region to a considerably lesser level. The primary job of Chlorophyll is to collect light in order to fuel photosynthesis and transform the absorbed light energy into chemical energy, which is then stored as sugars. Chlorophyll comes in a variety of forms in photosynthetic organisms. Chlorophyll a and b are found in higher plants in a 3:1-4:1 ratio (Solovchenko et al., 2019). Chlorophyll a is the most important constituent in oxygen production by photosynthetic plants, while the main function of chlorophyll b is the absorption of blue light energy (Yang et al., 2020). Chlorophyll has a cyclic tetrapyrrole ring as its backbone, which is a huge symmetrical planar structure in which four pyrrole rings are connected together by methine (C=) bridges and four nitrogen atoms are coordinated with a central metal atom, magnesium (Mg) (Solovchenko et al., 2019). The specialised photosystems, photosystem I (PSI) and photosystem II (PSII), are found in the thylakoid membrane systems of cyanobacteria and plant and algal chloroplasts, where the reaction centres photochemically convert light energy into usable chemical energy in the form of ATP and NADPH through photosynthetic electron transport (PET). The primary light-harvesting complexes (LHCs) associated with PSI and PSII in plants and green algae are supramolecular, integrated thylakoid membrane pigment–protein complexes that absorb light and then transmit it to reaction centres (Nelson and Ben-Shem 2004; Merchant and Sawaya 2005). Photochemistry traps this energy within

PSII reaction centres by forming a charge-separated state in which P680 is photo-oxidized (P680 + photon → $P680^+ + e^-$). As a result, $P680^+$, nature's most powerful oxidising agent, oxidises water and produces O_2, which is required by all other aerobic species (Huner and Grodzinski 2011). Carotenoids are also plant pigments that give them their yellow, bright red, and orange colours and play an important role in the modulation of plant growth and development, as well as plant environment interaction (Xie et al., 2019).

Heavy metals have long been known to have an impact on the photosynthetic machinery and several studies reported that Cd, Cu, Zn, and Pb affect photosynthesis (Zlobin et al., 2015; Shahid et al., 2015; Zhang et al., 2020). According to Aggarwal et al. (2012), heavy metals such as Hg, Cu, Cr, Cd, and Zn have the potential to decrease the chlorophyll content. Reduction in photosynthetic pigment may be due to the high redox potential of many heavy metals that inhibit the biosynthesis pathways of photosynthetic pigments (Chandra and Kang 2016). Several studies also reported the reduction of chlorophyll content due to heavy metal stress in cyanobacteria, unicellular chlorophytes (*Chlorella*), gymnosperms such *Picea abies* and angiosperms, such as *Zea mays, Quercus palustrus* and *Acer rubrum*, sunflower, and almond (Chandra and Kang 2016; Zengin and Munzuroglu 2006; Elloumi et al., 2007). Changes in photosynthetic pigment contents are linked to visual signs of plant sickness and photosynthetic productivity; hence chlorophyll concentration is frequently evaluated in plants to determine the influence of environmental stress (Parekh et al., 1990).

Excessive heavy metal accumulation causes oxidative stress and genotoxicity, which results in cytotoxicity and damages many biological components such as lipids, proteins, and nucleic acids. Over-production of ROS can also change the intrinsic properties of membranes such as fluidity, ion transport, loss of enzyme activity, protein cross-linking, inhibition of protein synthesis, DNA damage, and ultimately result in cell death (Sharma et al., 2012). In order to attenuate the harmful

effects of heavy metal and oxidative stress, plants must control the production and removal of ROS (Sharma et al., 2012). Excess amount of ROS production directly or indirectly affects proteins by modifying them in various ways. Direct modification of protein activity can be acquired through nitrosylation, carbonylation, disulphide bond formation, and glutathionylation and indirect modification of protein can be achieved through conjugation with breakdown products of fatty acid peroxidation (Yamauchi et al., 2008). In addition, excessive ROS production causes site-specific amino acid alteration, peptide chain fragmentation, aggregation of cross-linked reaction products, changed electric charge, and enhanced protein proteolysis susceptibility (Sharma et al., 2012). ROS are one of the most common causes of DNA damage, as they exacerbate oxidative damage to nuclear, mitochondrial, and chloroplast DNA. Oxidative attack on DNA causes deoxyribose oxidation, strand breakage, nucleotide loss, a range of changes in the organic bases of the nucleotide, and DNA-protein crosslinks (Auten and Davis 2009). Furthermore, oxidative DNA damage can cause alteration in the nucleotides of one strand, resulting in DNA stand mismatches. Plants, on the other hand, generate antioxidants to counteract the oxidative damage caused by heavy metals. Several ROS-removing enzymes such as SOD, CAT, GPX, APX, GR, and low molecular mass antioxidant scavengers, such as ascorbate (ASC) and glutathione (GSH), are produced under heavy metal stress (Michalak 2006; Rastgoo et al., 2011; Sharma et al., 2012).

Rice is an important staple food that is consumed throughout the world and heavy metal contamination in paddy fields hamper the yield as well as the quality of rice grain (Satpathy et al., 2014). Accumulation of heavy metal can induce several cellular stress responses and also can damage different cellular components such as membranes, proteins, and DNA (Gjorgieva et al., 2013). Recent advances in molecular biology have led to the development of a number of selective and sensitive assays for DNA analysis in ecogenotoxicology. Random Amplified Polymorphic DNA (RAPD) is a DNA-based technique that can evaluate variation at the DNA level, and differences can clearly be shown when comparing DNA fingerprints from individuals exposed or nonexposed to genotoxic agents (Savva 1998; Gjorgieva et

al., 2012). Heavy metal toxicity has a variety of effects on the growth and development of plant. There are reports available on plant height, chlorophyll content, protein content, lipid peroxidation, and antioxidative enzyme activity under heavy metal stress, which can provide information at the population level, but only a few have been reported on DNA marker approaches (Gupta and Sarin 2009; Cenkci et al., 2010). The measure of parameters at the population level helps to interpret the data at molecular level. The majority of research on DNA damage caused by heavy metals has concentrated on chromosome aberration, comet assays, and micronucleus assays, although these only look at total nuclear DNA (Lin et al., 2007; Souguir et al., 2011). PCR-based molecular markers such as RAPD and AFLP, which provide evidence of DNA mutation, are more sensitive. RAPD profiles are one of the most extensively used marker approaches for determining the level of genotoxicity in organisms, and they can be useful for a preliminary assessment of toxic effects on populations (Wolf et al., 2004; Liu et al., 2007; Gupta and Sarin 2009; Cenkci et al., 2010; Korpe and Aras 2011). Although, concerns have been raised about the RAPD technique's reliability and reproducibility, but after proper optimization it is a reliable, sensitive, and reproducible assay for detecting DNA damage or mutations in a short period of time at a low cost that can be used in genotoxicity and carcinogenesis studies after proper optimization (Atienzar and Jha 2006). Some earlier research also reported the analysis of genotoxicity in rice using RAPD markers under arsenic (Ahmad et al., 2012) and cadmium (Liu et al., 2007) stress. They concluded that RAPD analysis in conjunction with other biochemical parameters could be used as a powerful eco-toxicological tool in biomonitoring heavy metal pollution.

Studies had shown that the nutritional quality of certain traditional rice landraces had higher values than those produced by conventional and modern techniques that could be attributed to their high bioactive compound contents (Mbanjo et al., 2020). Similarly, previous studies also reported the presence of high anti-oxidant activity in pigmented rice grains, which can be used as an efficient scavenging compound against reactive oxygen species (Chakuton et al., 2012; Zhang

et al 2015; Petroni et al., 2017; Ghasemzadh et al., 2018). Because of this, some of the traditional pigmented rice varieties have effective anti-diabetic property and hence can be useful in the management of diabetes mellitus (Hemamalini et al., 2021). Bioactive compounds present in pigmented rice grain have the capacity to reduce cancer cells viability and the inclusion of pigmented rice in diet can prevent breast cancer (Liang et al., 2014).

The Northeast region of India comprises of states of Assam, Arunachal Pradesh, Meghalaya, Manipur, Mizoram, Tripura, Nagaland, and Sikkim. The region has a rich rice diversity of more than 10,000 rice cultivars both aromatic and non-aromatic varieties and it is one of the major biodiversity hotspots in the world (Mao et al., 2009). Some rice cultivars of Northeast India possess high genetic diversity and contain high amount of Zn and Fe and they can serve as good candidates for future rice breeding programs (Vanlalsanga et al., 2019). Chakhao is an black aromatic, and glutinous rice cultivar found in Manipur, and it has high anthocyanin, polyphenols, and Zn content (Roy et al., 2014; Asem et al., 2015; Chanu et al., 2016; Asem et al., 2017). Kawnglawng is a popular pigmented local rice variety of Mizoram and is widely consumed in every parts of Mizoram (Lalmuanpuii et al., 2021).

Chapter 3

Materials and Methods

Seeds of two local rice varieties (Chakhao and Kawnlawng) were collected from the local rice farmers. Chakhao was collected from a local farmer of Kakching District, Manipur and Kawnglawng was collected from Lengpui, Mamit District, Mizoram. Seeds were surface sterilized in 10% Sodium Hypochlorite solution for 10 min and washed thoroughly for 3-4 times with distilled water. The seeds were then soaked for 24 h in distilled water.

3.1 Germination percentage:

Surface sterilized seeds were spread over Petri dishes lined with filter paper containing different concentrations of Zn. For the treatments, sulphate salts of Zn ($ZnSO_4$) were used in three different concentrations (5mM, 10mM, and 15mM). Germination percentage was analyzed by taking 30 seeds per treatment in petri plates and thirty seeds without Zn treatment served as control. After 14 days, seeds were counted for germination percentage.

Surface sterilized rice seeds were grown for fourteen days in Petri plates and rice seedlings were exposed to different concentrations of Zn (5mM, 10mM, and 15mM) for 2 days and rice seedlings without Zn treatment served as control. Further analysis such as chlorophyll content, protein content, antioxidant activity, enzyme activity, and DNA isolation for genotoxicity study were performed from these rice seedlings.

3.2 Photosynthetic pigments:

The amount of photosynthetic pigments in terms of chlorophyll a and chlorophyll b was determined according to the method of Hartmut *et al.* (1983). Fresh leaf tissues (0.2g) were homogenized in 3ml ethanol (95%, v/v). The homogenate was centrifuged at 500g for 10 min and the supernatant was extracted. Then, 9ml ethanol (95%, v/v) was added to 1ml aliquot of supernatant. And, the mixture was determined by monitoring the absorbance at the wavelengths 665 and 649 nm in a spectrophotometer. The amount of photosynthetic pigment content was calculated using the following equations:

Chlorophyll a (C_a) = 13.95 A_{665} – 6.88 A_{649}

Chlorophyll b (C_b) = 24.96 A_{649} – 7.32 A_{665}

3.3 Protein Estimation:

Total protein of the extract was estimated following Lowry's method (Lowry et al., 1951).

3.4 Antioxidant enzyme assay:

Leaf samples (0.5g) were taken per treatment and homogenized in 8ml of 50mM Potassium phosphate buffer (pH 7.8) under ice-cold conditions. Homogenate was centrifuged at 10,000 g for 20 min at 4^0C and the supernatant was used for the determination of enzyme activity.

3.4.1 Catalase (CAT) activity:

CAT activity was measured by a reduction in absorbance at 240nm due to the decline of extinction H_2O_2. The 3ml reaction mixture containing 2.8 mL Phosphate

buffer (25mM, pH 7.0), 0.1 mL H_2O_2 (0.4%) and 0.1 mL enzyme extract was used. The reaction was started with the addition of H_2O_2 (Aebi 1983).

3.4.2 Ascorbate peroxidase (APX) activity:

APX activity was measured according to Nakano and Asada (1981). The assay depended on the decrease in absorbance at 290 nm as Ascorbate was oxidized. The 3 mL reaction mixture consisted of 2.7 mL Phosphate buffer (25mM, pH 7.0), 0.1 mL ascorbate (7.5 mM), 0.1 mL H_2O_2 (0.4%) and 0.1 mL of enzyme extract. The reaction started with the addition of H_2O_2.

3.4.3 Superoxide dismutase (SOD) activity:

SOD activity was assayed by measuring its ability to inhibit the photochemical reduction of Nitroblue tetrazolium (NBT) (Rao and Sresty 2000). NBT reaction solution contained 50 mmol $L^{-`}$ Phosphate buffer (pH 7.8), 13 mmol $L^{-`}$ Methionine, 75 µmol $L^{-`}$ NBT, 2 µmol $L^{-1}$ Riboflavin, 0.1 mmol $L^{-`}$ EDTA. The reaction mixture was 3.1 mL, which contained 3mL NBT reaction mixture and 0.1 mL enzyme extract. Reaction was started by adding 2 µmol L^{-1} Riboflavin and placing the reaction tubes under 15W fluorescent lamps for 15 min. A complete reaction mixture without the enzyme extract served as a control. The photoreduction of NBT was measured at 560 nm.

3.5 DNA Isolation:

DNA was isolated from plant leaves following Edwards et al., (1991). Briefly, 100mg of the leaf was macerated in a 1.5 ml centrifuge tube for 10-30 sec and 400µl of Extraction buffer (200 mM Tris HCl pH 7.5, 250 mM NaCl, 25 mM EDTA, 0.5% SDS) was added. The sample was vortex for 1 min and centrifuged at 13,000 rpm for 5 min. The supernatant was collected and mixed with an equal amount of cold

isopropanol and kept at room temperature for 2 mins. Then, the mixture was centrifuged at 13,000 rpm for 5 mins and the dried pellet was dissolved in 100 µl TE buffer.

3.6 RAPD-PCR:

PCR amplification was performed in a Veriti-96- well thermal cycler (Thermo Fisher Scientific). The amplification conditions were set as, initial denaturation at 94 ^{0}C for 5 min, 35 cycles of denaturation at 94 ^{0}C for 30 sec, annealing temperature 34 ^{0}C for 30 sec, extension at 72 ^{0}C for 1 min followed by final extension at 72^{0}C for 15 min. The amplified product was run on a 1.5% agarose gel and viewed in a Alphalmager Mini (Protein Simple, USA). Sizes of amplified bands were ascertained by comparing with molecular weight marker (100 bp) using Alpha View software (Protein Simple, USA).

3.7 Estimation of genomic template stability (GTS):

Changes in the RAPD profiles were expressed as GTS, a qualitative measure showing the obvious changes in the number of RAPD profiles. The GTS was calculated by the formula:

$$GTS = (1 - \frac{a}{n}) \times 100$$

where a is the average number of changes in DNA profiles and n is the number of bands selected in control DNA profiles.

3.8 Statistical analysis

The statistical analysis was performed using Microsoft excel. The results were subjected to one-way analysis of variance (ANOVA) and Bonferroni test was used for comparison between pairs of treatments.

Chapter 4

RESULT

4.1 Germination percentage:

Zn treatment did not have a significant impact on germination in the studied rice varieties. Germination percentage was found to be 100%, however, Zn treatment did reduce the growth of shoot and root lengths.

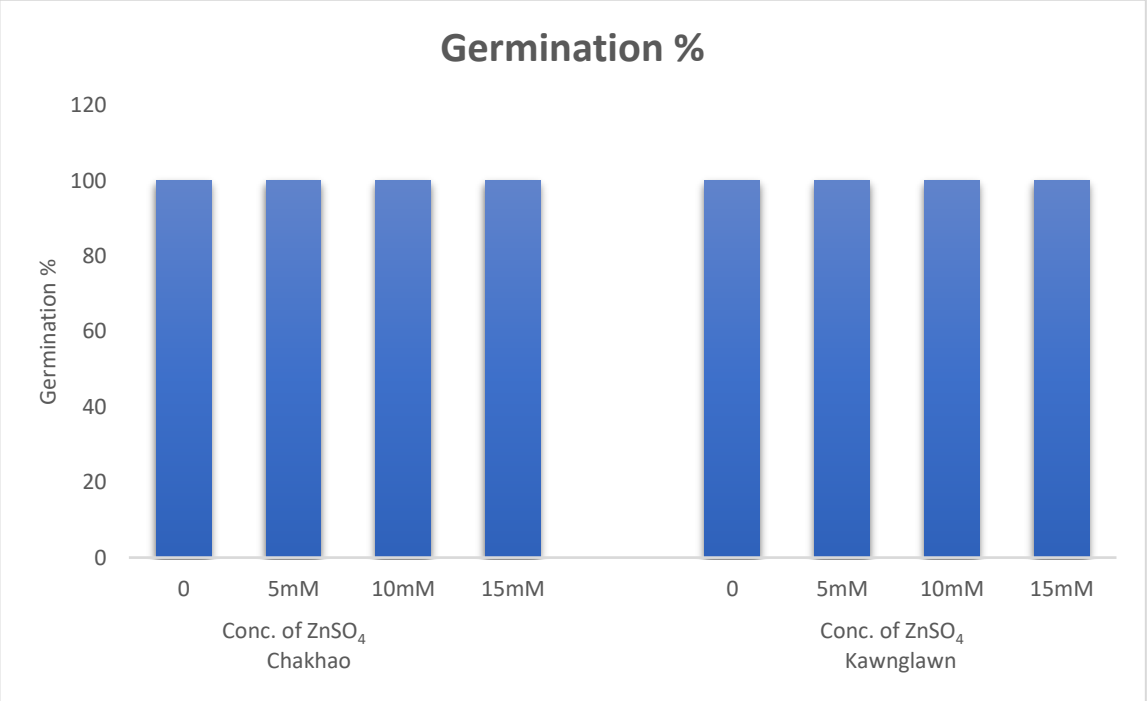

Figure 1: Germination percentage after 14 days of Zn treatment

Figure2: Chakhao: 1-Control, 2-5mM, 3-10mM, and 4-15mM $ZnSO_4$ (Germinated rice seedling after 14 days of Zn treatment)

Figure3: Kawnglawng: 1-Control, 2-5mM, 3-10mM, and 4-15mM $ZnSO_4$ (Germinated rice seedling after 14 days of Zn treatment)

4.2 Effects of $ZnSO_4$on Photosynthetic pigments and protein content:

The present studies showed that Zn treatment had significant impact on the photosynthetic pigments (Chlorophyll 'a' and Chlorophyll 'b'). When 14 days old rice seedlings were exposed to different concentrations of $ZnSO_4$ (5mM, 10mM, and 15mM) for 48 h, the chlorophyll 'a' and chlorophyll 'b' contents in both the studied rice cultivars (Kawnglawng and Chakhao) were reduced significantly with the increasing concentration of $ZnSO_4$ ($p \leq 0.05$) (Fig. 4 and 5). Control plant showed 1.64 ± 0.03 mg/L and 1.67 ± 0.03 mg/L of chlorophyll 'a' in Chakhao and Kawnglawng respectively. Reduction in chlorophyll 'a' content was found the highest in the rice expose to 15mM concentration of $ZnSO_4$ (Chakhao – 1.4 ± 0.03 and Kawnglawng – 1.21 ± 0.03) as compared with the control. Then, Chlorophyll 'b' content was found to be 0.62 ± 0.05 mg/L and 0.58 ± 0.08 mg/L in the control plants of Chakhao and Kawnglawng respectively. The maximum decline in chlorophyll 'b' content was found in rice plants treated 15mM $ZnSO_4$ (Chakhao – 0.39 ± 0.06 mg/L and Kawnglawng – 0.4 ± 0.08 mg/L). Protein content was found to be 1.14 ± 0.01 mg/FW and 1.06 ± 0.006 mg/FW in the control rice plants of Chakhao and Kawnglawng respectively. The maximum reduction in protein content was found in rice exposed to 15mM $ZnSO_4$ (Chakhao – 0.96 ± 0.008 mg/FW and Kawnglawng – 0.88 ± 0.008 mg/FW) (Fig. 6). The present studies revealed that the protein content was reduced in the rice treated with different concentrations of Zn as compared with the control and the result was found to be significant ($p \leq 0.05$) (Fig. 6).

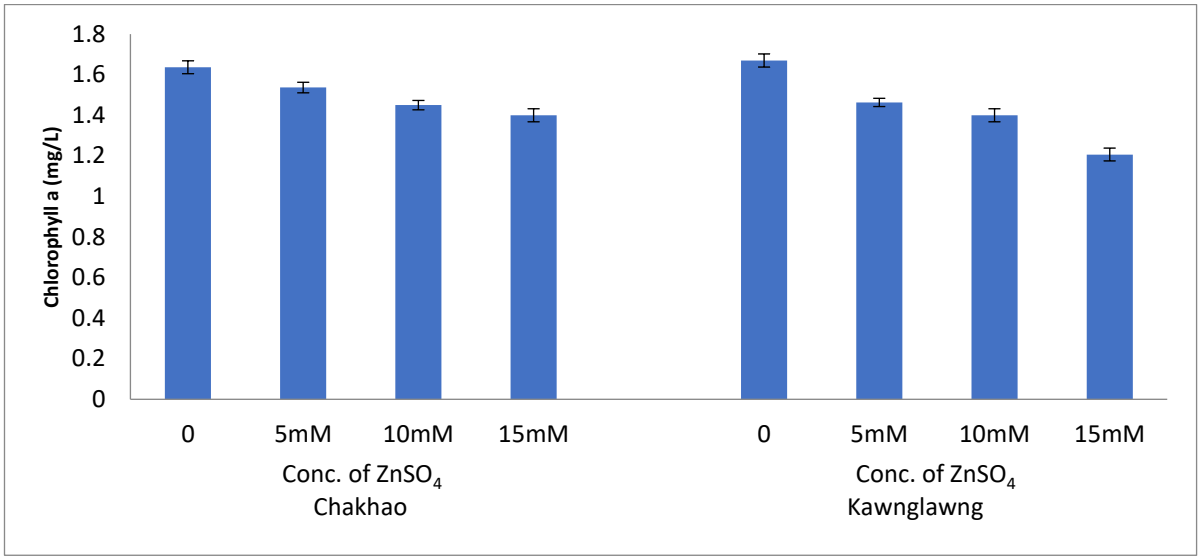

Figure4: Chlorophyll 'a' content in rice under different concentration of Zn stress. Values are mean ± SE based on three independent experimental data ($p \leq 0.05$).

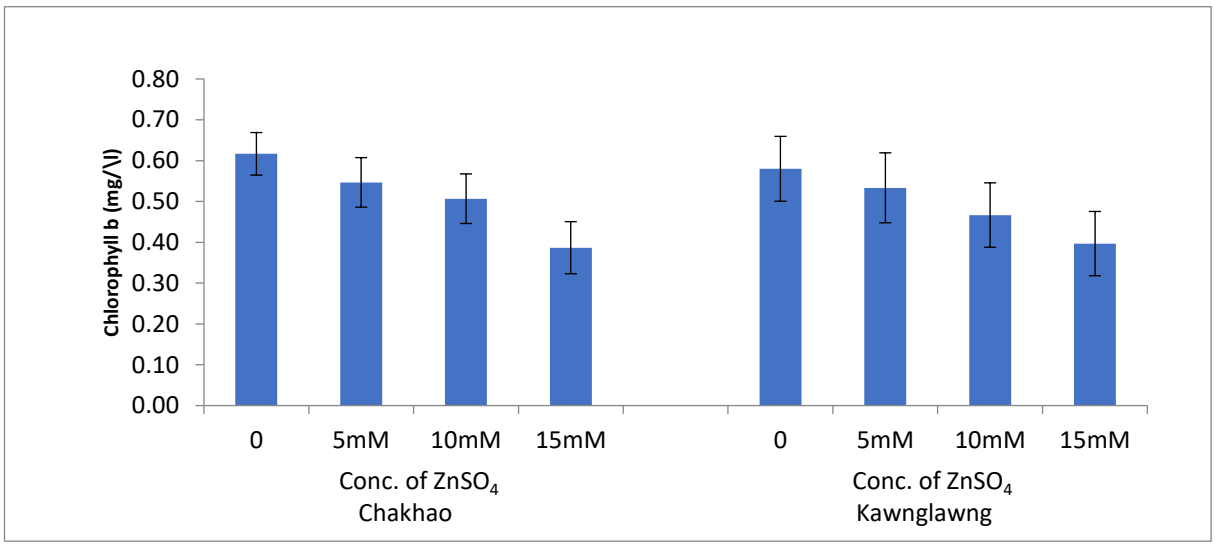

Figure 5: Chlorophyll 'b' content in rice under different concentration of Zn stress. Values are mean ± SE based on three independent experimental data ($p \leq 0.05$).

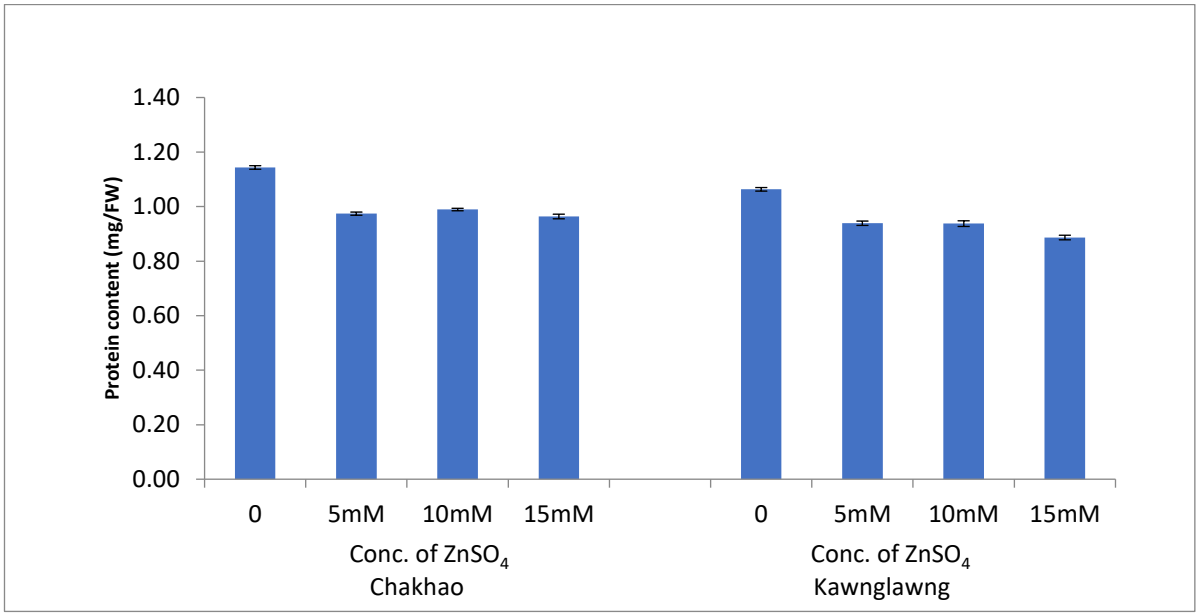

Figure6: Total protein content in rice under different concentration of Zn stress. Values are mean ± SE based on three independent experimental data ($p \leq 0.05$).

4.3 Effects of $ZnSO_2$ on Antioxidant activity:

4.3.1 Catalase (CAT)

Catalase plays an important role in preventing the accumulation of H_2O_2 in a plant cell. The present study showed that rice verities (Chakhao and Kawnglawng) treated with different concentration of Zn (5mM, 10mM, and 15mM) has a significant impact on the CAT activity. It was found that under Zn stress, CAT activity significantly increased in both varieties of rice- Chakhao (1.36 ± 0.18 unit/min/mg protein to 2.25 ± 0.13 unit/min/mg protein) and Kawnglawng (1.55 ± 0.15 unit/min/mg protein to 2.71 ± 0.18 unit/min/mg protein) with increasing concentrations of Zn when compared with the control (Fig. 7). CAT activity was found to be the highest in rice seedlings treated with 15mM $ZnSO_4$ in both the rice varieties and the result was found to be significant ($p \leq 0.05$).

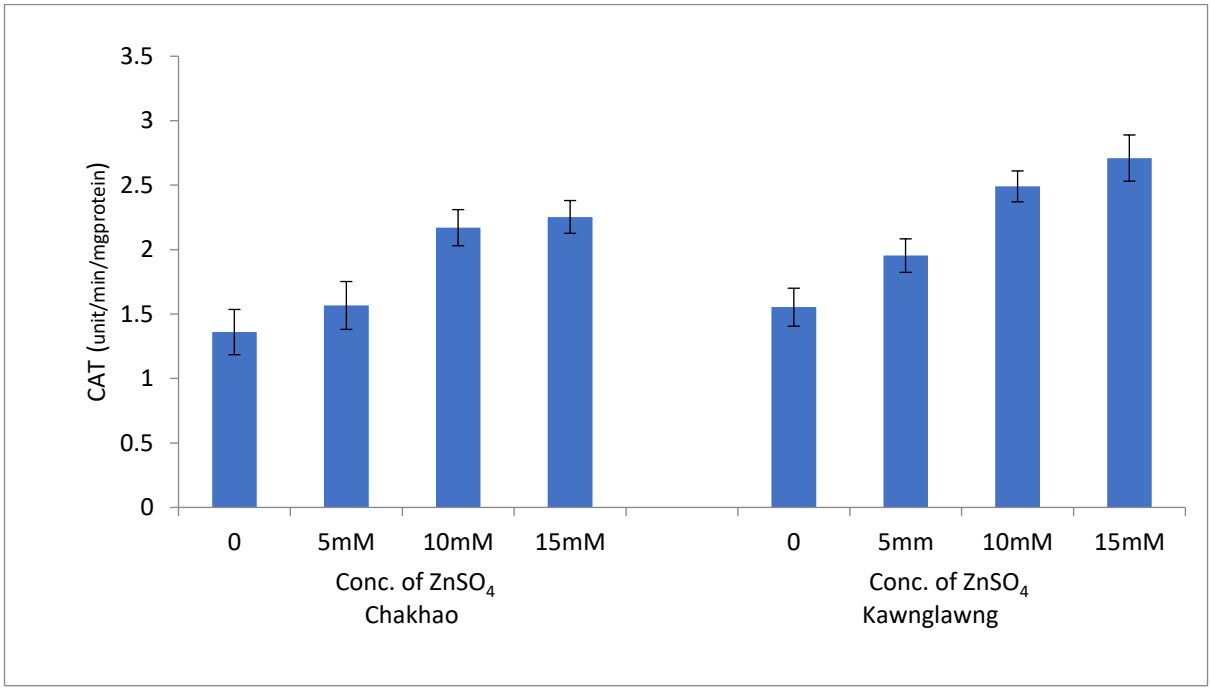

Figure7: Catalase (CAT) activity under different concentration of Zn stress. Values are mean ± SE based on three independent experimental data ($p \leq 0.05$).

4.3.2 Ascorbate peroxidase (APX)

It was observed in my study that rice seedling exposed to different concentrations of $ZnSO_4$ (5mM, 10mM, and 15mM) significantly increased the APX activity with increasing concentration in both the rice varieties- Chakhao (0.32 ± 0.02 unit/min/mg protein to 0.52 ± 0.02 unit/min/mg protein) and Kawnglawng (0.39 ± 0.02 to 0.55 ± 0.02) as compared with the control (Fig. 8). APX activity was found to be the highest in rice seedling treated with 15mM in both the rice varieties and the result was found to be significant ($p \leq 0.05$).

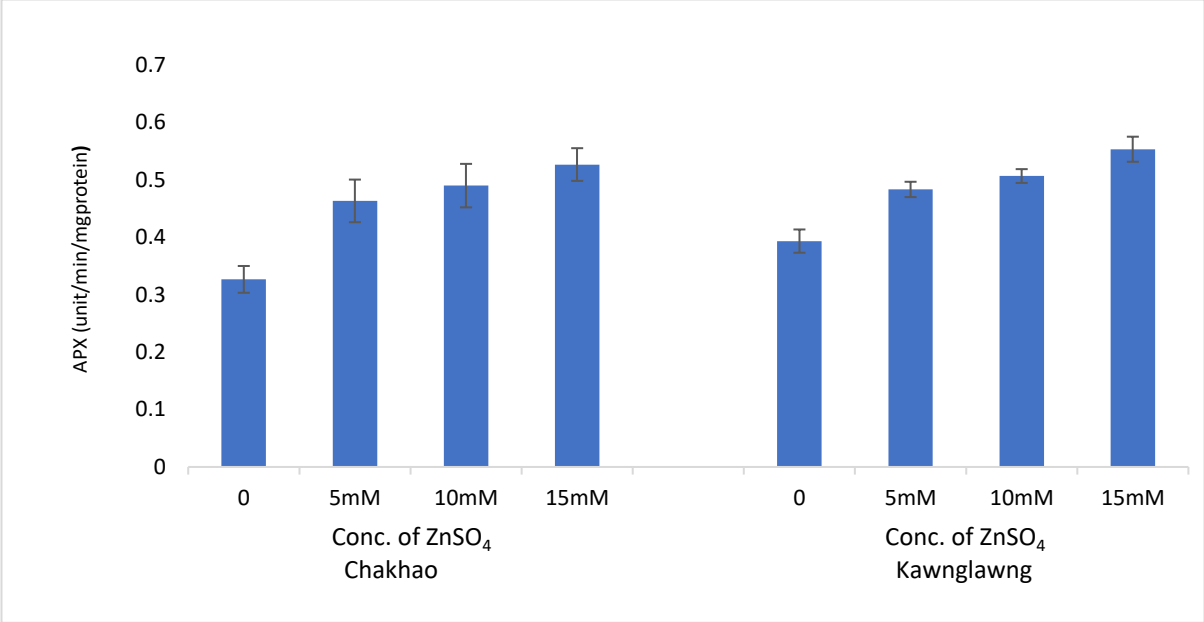

Figure8: Ascorbate peroxidase (APX) activity under different concentration of Zn stress. Values are mean ± SE based on three independent experimental data ($p \leq 0.05$).

4.3.3 Superoxide dismutase (SOD) activity:

The present study showed that rice verities (Kawnglawng and Chakhao) treated with different concentration of $ZnSO_4$ (5mM, 10mM, and 15mM) had an impact on the SOD activity. It was found that under Zn stress, SOD activity increased in both the varieties- Chakhao (0.97 ± 0.02 unit/mg FW to 1.31 ± 0.02 unit/mg FW) and Kawnglawng (0.79 ± 0.02 unit/mg FW to 0.55 ± 1.36 unit/mg FW) with increasing concentrations of Zn when compared with the control (Fig. 9) and the result was found to be significant ($p \leq 0.05$). SOD activity was found to be the highest in rice seedling treated with 15mM $ZnSO_4$ in both rice varieties.

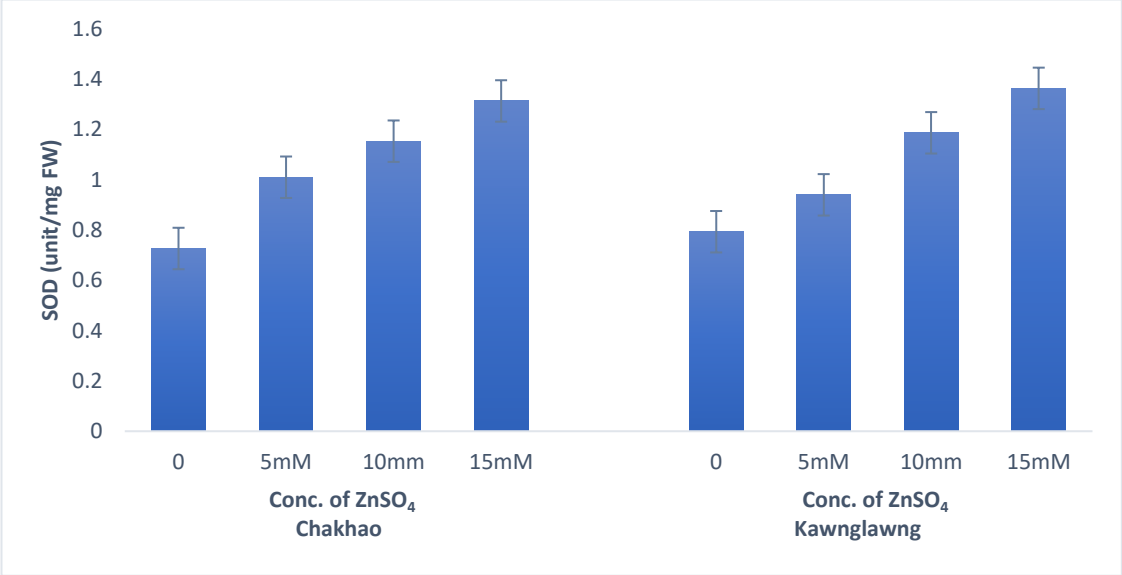

Figure9: Superoxide dismutase (SOD) activity under different concentration of Zn stress. Values are mean ± SE based on three independent experimental data ($p \leq 0.05$).

4.4 Genotoxicity of Zn stress:

In order to analyze the genetic effects of Zn stress on the rice varieties- Kawnglawng and Chakhao, an RAPD-PCR analysis was performed. Fourteen days old rice seedlings were expose to different concentrations of $ZnSO_4$(5mM, 10mM and 15mM) for 48 h and DNA was extracted from leaves. Five RAPD primers were employed to screen the rice genomes for genotoxicity. In Chakhao and Kawnglawng control plants, the RAPD profile revealed 29 bands and 28 bands, respectively. The changes in RAPD profiles generated due to Zn stress in both varieties such as appearance/disappearance of new bands and variation in band intensity were found when compared with control experiments.

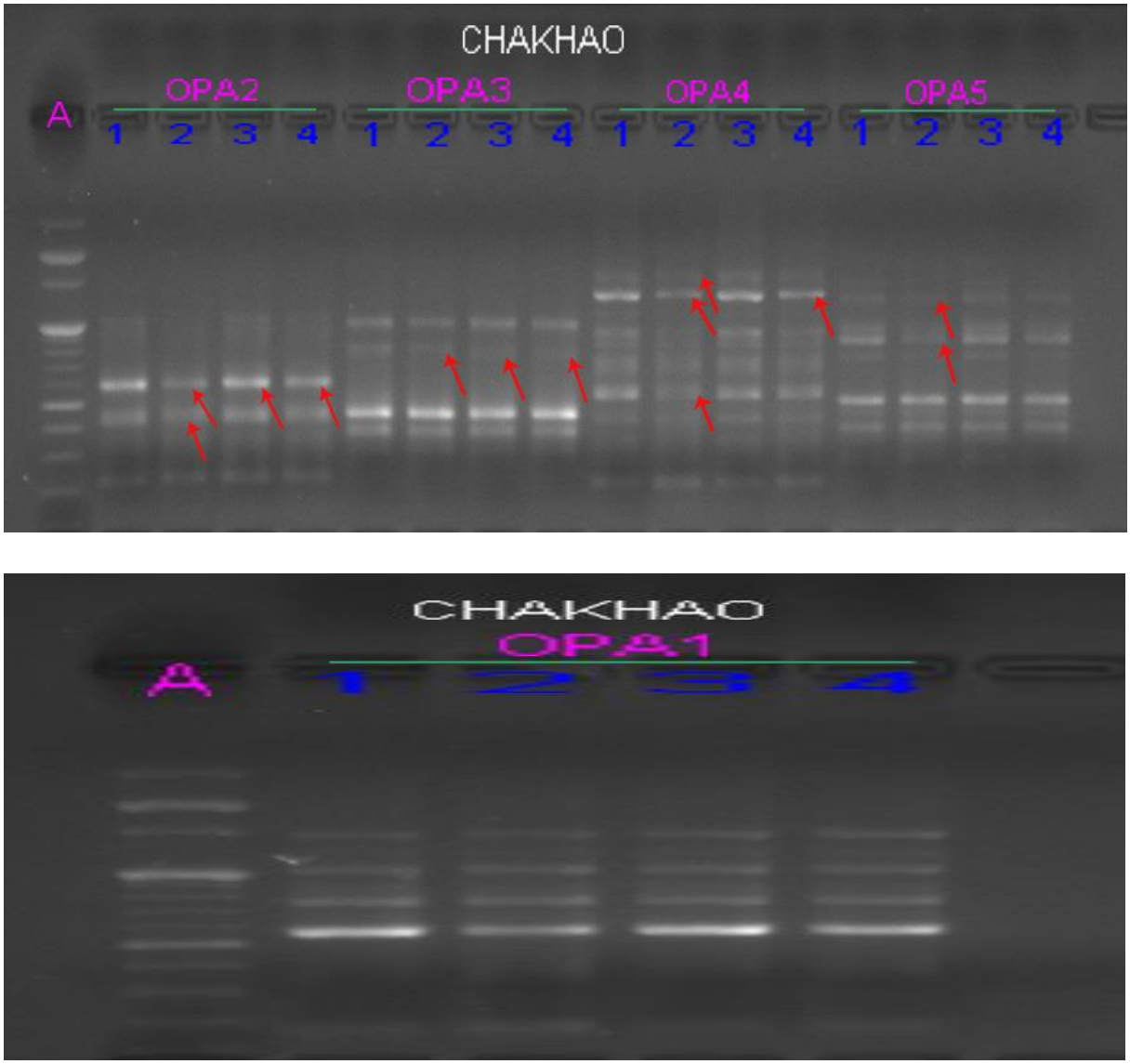

Figure10: RAPD profile of Chakhao under different concentrations of Zn stress. (Lane A indicates 100bp DNA marker, Lanes: 1-Control, 2-5mM, 3-10mM, and 4-15mM $ZnSO_4$)

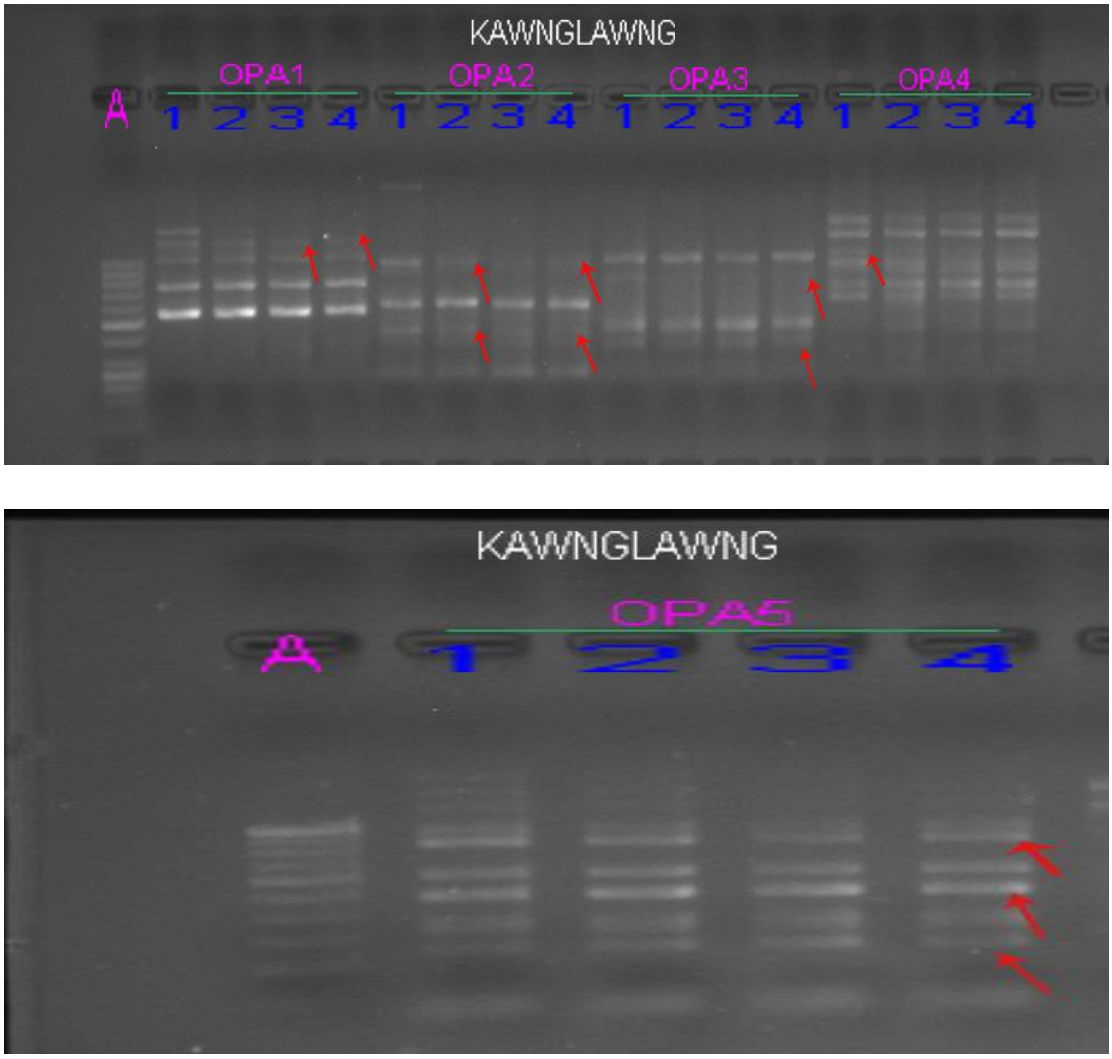

Figure11: RADP profile of Kawnglawng under different concentrations of Zn stress. (Lane A indicates 100bp DNA marker, Lanes: 1-Control, 2-5mM, 3-10mM, and 4-15mM $ZnSO_4$)

4.5 Estimation of genomic template stability:

The genomic template stability (GTS) value, a qualitative measure that showed changes in RAPD profile was calculated for every five primers in both the rice cultivars (Table 3). From the analysis, it was found that the rice cultivar Chakhao shows 100% GTS, which means that under Zn stress (at 15mM) there was no effect on the genome stability other than an increase and decrease in band intensity. Then, Kawnglawng rice cultivar treated with 15mM $ZnSO_4$ showed 94.2% GTS at three primers and 100% at two primers with an overall GTS of 96.2%. The change in GTS indicated that Zn stress had a slight negative effect to the Kawnglawng genome.

Table 1: Changes of total bands in control and polymorphic bands (Chakhao)

SL.	Primer	Control	5mM				10mM				15mM			
			a	*b*	*c*	*d*	*a*	*B*	*c*	*d*	*a*	*b*	*C*	*d*
1	OPA1	5	0	0	0	0	0	0	0	0	0	0	0	0
2	OPA2	4	0	0	2	0	0	0	0	1	0	0	1	0
3	OPA3	4	0	0	1	0	0	0	1	0	0	0	1	0
4	OPA4	8	0	0	3	1	0	0	0	0	0	0	1	0
5	OPA5	8	0	0	2	0	0	0	0	0	0	0	1	0
Total bands		29	0	0	7	1	0	0	1	1	0	0	4	0
a+b		0					0				0			

a indicates appearance of new bands, *b* disappearance of normal bands, *c* decrease in band intensities, *d* increase in band intensities, *a+b* polymorphic bands,

Table 2: Changes of total bands in control and polymorphic bands (Kawnglawng)

SL.	Primer	Control	5mM				10mM				15mM			
			a	b	c	d	a	b	c	d	a	B	c	d
1	OPA1	5	0	0	0	0	0	0	1	0	0	1	0	0
2	OPA2	5	0	0	2	0	0	0	0	0	0	1	1	0
3	OPA3	4	0	0	0	0	0	0	0	0	0	0	0	2
4	OPA4	8	0	0	0	0	0	0	0	0	1	0	0	0
5	OPA5	6	0	0	0	0	0	0	1	0	0	0	1	2
Total bands		28	0	0	2	0	0	0	2	0	1	2	2	4
a+b		0					0				3			

a indicates appearance of new bands, *b* disappearance of normal bands, *c* decrease in band intensities, *d* increase in band intensities, *a+b* polymorphic band

Table 3: Changes of GTS for all primers

No. of primers	CHAKHAO				KAWNGLAWNG			
	0	5mM	10mM	15mM	0	5mM	10mM	15mM
OPA1	100	100	100	100	100	100	100	94.2
OPA2	100	100	100	100	100	100	100	94.2
OPA3	100	100	100	100	100	100	100	100
OPA4	100	100	100	100	100	100	100	94.2
OPA5	100	100	100	100	100	100	100	100
Mean	100	100	100	**100**	100	100	100	**96.52**

Chapter 5

Discussion

The present study was taken up to investigate the Zn toxicity in two local rice varieties (Kawnglawng and Chakhao) of Northeast India. Assessment of toxicity level was done based on the germination percentage, photosynthetic pigment, total protein content, antioxidant activities, and DNA damage evaluated using a PCR base RAPD profile. Excess accumulation of heavy metals in agricultural land has become a growing concern for the general public because of its severe impact on soil ecosystems and harmful effect on human health (Payus and Talip 2014). Naturally, Zn is found in the soil at low concentrations, ranging from 10 to 300 mg kg^{-1}, with an average of approximately 50 mg kg^{-1} (Mortvedt 2000). Below this concentration, Zn is an important micronutrient for plant metabolism and growth. Zn serves as a structural and catalytic component in a variety of processes, including cell division, cell growth, and protein synthesis (Jain et al., 2013). It is involved in chromatin structure, gene expression and regulation, nucleic acid, carbohydrate, lipid, and protein metabolism, as well as photosynthetic carbon metabolism (Gai et al., 2017; Noulas et al., 2018). Zn is also necessary for the production of tryptophan, an auxin precursor amino acid (Tsonev and Lidon 2012).

Seed germination is the most delicate step of plant growth, as it is the stage that seeds first interact with the environment, and they are particularly susceptible to changing environmental conditions (Solanki and Dhankhar 2011). In the present investigation, rice exposure to Zn stress does not have a significant impact on the germination percentage, and germination percentage was found to be 100% in both the rice variety. However, at higher concentration Zn stress significantly inhibited root formation and shoot growth. Inhibition in root formation and shoot growth could be due to the heavy metal interfering with the stressed plants' overall growth

performance (Kikui et al., 2005; Panda et al., 2009; Buendía-González et al., 2010; Gangwar et al., 2010, 2011; Gangwar and Singh, 2011; Eleftheriou et al., 2012; Hayat et al., 2012; Hasanuzzaman et al., 2012; Anjum et al., 2014). Cell division and elongation are both involved in root growth. In this context, after exposure to heavy metals, a decrease in mitotic activity has been reported in various plant species, resulting in slowed root growth (Fontes and Cox 1998; Doncheva et al., 2005; Sundaramoorthy et al., 2010; Hossain et al., 2012; Thounaojam et al., 2012). My findings are in agreement with previous studies that had shown the inhibitory and harmful effects of several heavy metals (Pb, Cd, and Zn) on seed germination and growth in rice and other crop plants (Kopyra and Gwozdz 2003; Mahmood et al., 2007; He et al., 2014)

In my study, Zn stress considerably affected the chlorophyll content in rice seedlings. Chlorophyll 'a' and 'b' contents were significantly decreased with the increasing concentration of Zn levels in both the rice verities as compared to control. A maximum decline of 14% (in Chakhao) and 27% (in Kawnglawng) chlorophyll 'a' content was found in rice exposed to 15mM $ZnSO_4$ as compared with the control plants. Similarly, a maximum reduction in chlorophyll 'b' content was found at 37% in Chakhao and 31% in Kawnglawng rice plants treated with 15mM $ZnSO_4$ as compared with the control plants. Reduction of photosynthetic pigments under Zn stress may be due to inhibition or disturbance in the biosynthesis or enhanced degeneration of thylakoids (Vassilev et al., 2007). Furthermore, a decrease in chlorophyll content could be attributed to lower biomass or oxidative stress-induced increased lipid peroxidation of chloroplast membranes (Ma et al., 2013). An earlier study also reported similar results of a reduction in photosynthetic pigments under Zn stress in rice (Salah et al., 2015). Similar studies on other plants such as wheat, red cabbage, and tea also showed that chlorophyll a, b and carotenoid contents reduced under Zn stress (Vajpayee et al., 2000; Hajiboland and Amirazad 2010; Mukhopadhyay et al., 2013). Likewise, other heavy metals such as Cd, Cr, Pb, Ni, As, etc. also reported to have a significant influence on the reduction of photosynthetic pigments (Rao and Sresty 2000; Miteva and Merakchiyska 2002;

Panda et al., 2003; Panda and Choudhary 2005; Aslam et al., 2014; Jawad Hassan et al., 2020; Shah et al., 2020; Yang et al., 2021). The inhibition of chlorophyll biosynthesis enzymes such as δ-aminolaevulinic acid dehydrates and protochlorophyllide reductase is thought to be the cause of reduction in chlorophyll content in plants exposed to heavy metal stress (Mukhopadhyay et al., 2013).

The present study shows that rice exposure to Zn affects the protein content. Minor reduction in the protein content was found as compared with the control when the rice was treated with different concentrations of Zn and the result was found significant ($p \leq 0.05$). A decrease in protein synthesis or an increase in the rate of protein breakdown could be the cause of the decrease in protein content (Balestrasse et al., 2003). Earlier studies also reported that the heavy metal (Cadmium, Copper, and Nickel) stress in plants such as tomatoes and pea can significantly affect the total protein content (Yucel and Cuneyt 2019; Bavi et al., 2011). Heavy metals are considered to hinder a protein's biological functions by bonding to it and altering its native structure (Hossain and Komatsu 2013). Cadmium (Cd), for example, can inhibit thiol transferase function, causing oxidative damage, potentially by binding to cysteine residues in its active sites (Hasan et al., 2017).

Environmental factors such as soil salinity, drought, temperature extremes, and heavy metals are known to induce oxidative damage to plants, either directly or indirectly, by causing an increased level of reactive oxygen species (ROS) (Malecka et al., 2001; Shah et al., 2001). Heavy metals cause oxidative stress by producing reactive oxygen species such as superoxide radicals (O_2-), hydrogen peroxide (H_2O_2), hydroxyl radicals (HO^*), and singlet oxygen ($^1O_2$) (Luna et al., 1994; Prasad et al., 1999). Over-production of ROS can alter various biomolecules such as nucleic acid, protein, lipids, and amino acids. In response to the increasing ROS production, the level of antioxidant enzymes such as SOD, CAT, APX, and POD also increase, which is an important protective mechanism to reduce oxidative damage (Panda et al., 2011; Song et al., 2013; Manikandan et al., 2015). Oxidative stress is a regulated

process in which the balance between the oxidative and antioxidant capacity determines the plant's fate. The antioxidant defense system provides effective protection against active oxygen and free radicals under normal conditions but under stressful conditions, the antioxidant defense system capacity increases (Srivastava 1999). Results from my study on the antioxidant enzyme activities showed that CAT increased in the rice plants exposed to Zn stress. CAT was found to increase with the increasing concentrations of Zn (5mM, 10mM, and 10mM) as compared to control and the maximum increase of 65% (Chakhao) and 74% (Kawnglawng) of CAT was found in rice treated with 15mM $ZnSO_4$. The activity of CAT in both the cultivars was significantly ($p \leq 0.05$) higher in Zn treated plants as compared to the control shown in (Fig. 7). Increase in CAT activity under stress conditions such as salinity (Jaleel et al., 2007; Kibria et al., 2017; Hamzeh-Kahnoji et al., 2021), drought (Sarker and Oba 2018; Zhu et al., 2020; JamshidiGoharrizi et al., 2020) and heavy metal (Malar et al., 2016; AbdElgawad et al., 2020; Tang et al., 2020) has been reported in other plants. CAT is primarily responsible for scavenging hydrogen peroxide (H_2O_2) produced during several routes under normal and stressful conditions (Mittler 2002; Foyer and Noctor 2005). Hydrogen peroxide (H_2O_2) is eliminated by CAT enzymes by breaking it down directly into water and oxygen.

The current study found that when rice plants were exposed to Zn stress, their APX activity also increased. Increasing the concentration of Zn treatment enhanced the level of APX activity in both the studied rice varieties. A high level of APX activity was found in rice seedlings exposed to the highest concentration of $ZnSO_4$ (15mM) in both the rice varieties suggesting that this enzyme might be used as an inherent defensive mechanism to protect them from Zn induced oxidative damage. The APX activity under 15mM of Zn was about 62% in Chakhao and 41% in Kawnglawng. The activity of APX in both the cultivars was significantly ($p \leq 0.05$) higher in Zn treated plants as compared to the control shown in (Fig. 8). Earlier studies also showed the increase in APX activity under a variety of stressful conditions such as drought (Sharma and Dubey 2005; Raja et al., 2020; Khazaei and Estaji 2020), salinity (Hefny and Abdel Kader 2009; Jabeen et al., 2021), UV

irradiation (Han et al., 2009) and heavy metal stress (Liu et al., 2007; Malar et al., 2016; Alam et al., 2021). APX is one of the most widely distributed antioxidant enzymes in plant cells, and isoforms of APX have a higher affinity for H_2O_2 than CAT, making APXs effective scavengers of H_2O_2 under stress.

The function of enzymatic and non-enzymatic antioxidants, the stability of membranes, and the protection of protein sulfhydryl groups, all require an optimal quantity of Zn. However, both Zn deficiency and excess affect redox equilibrium in plants (Cheah et al., 2020). Zn plays an important role in promoting growth at ideal concentrations but inhibits growth at greater or lower levels by interfering with the plant's common metabolic functions. SOD is a metalloenzyme that catalyzes superoxide anion dismutation to oxygen and hydrogen peroxide. Aerobic organisms require such enzymes as a defense system to survive (Beyer et al., 1991). Its activity regulates the relative levels of O_2^- and H_2O_2, the two Haber–Weiss reaction substrates, lowering the likelihood of OH radical production, which is highly reactive and can harm membranes, proteins, and DNA (Bowler et al., 1992). SOD is usually classified into three isoenzymes based on the metal cofactors. Cu/Zn-SOD is found in the cytoplasm of eukaryotic cells, Mn-SOD is found in the mitochondria of prokaryotic and eukaryotic cells, and Fe-SOD is found in prokaryotes (Tian and Zhong 2005). The majority of SOD is found in the cytoplasm, followed by mitochondria. As a result, antioxidant capacity exists in many sections of plant cells (Lin et al., 2015). In my present study, a significant ($p \leq 0.05$) increase in the SOD activity was found in rice growing under toxic levels of Zn stress. Maximum increase of 81% in Chakhao and 72% in Kawnglawng was found in the rice treated 15 mM Zn (Fig. 9). It is well known that antioxidant systems protect cells from damage caused by harmful oxygen species such as superoxide radicals, H_2O_2, and hydroxyl radicals (OH), which are all produced under environmental and xenobiotic stressors (Asada 1994; Foyer et al., 1994; Lozano et al., 1996). A significant increase in SOD activity might be due to the de-novo synthesis of enzymatic protein (Lozano et al., 1996). A similar result of increased SOD activity in response to salinity (Comba et

al., 1998) and heavy metal toxicity (Shah et al., 2001) was also reported earlier. SOD activity improves and maintains its ability to eliminate free radicals under adverse situations, allowing cells to protect themselves from and withstand the harmful effects of adversity from the outside environment (Lin et al., 2015).

In order to improve the effective evaluation and proper environmental monitoring of potential genotoxic pollutants, sensitive and selective technologies are required to detect toxicant-induced mutations in the genome's wide range of biota. The effectiveness of such approaches can be enhanced even further by linking molecular/cellular response to higher-order alterations like reductions in population parameters (Atienzar et al., 1999; Theodorakis et al., 2006). At both the biochemical and molecular levels, alterations in DNA produced by genotoxic substances can be detected using several molecular markers (Savva 1998). RAPD detects changes in genomic DNA and clearly illustrates the detection of pollution-induced DNA damage. RAPD is a reliable, sensitive, and repeatable method that can detect a wide range of DNA damage (e.g., DNA adducts, DNA breakage) as well as point mutations, making it useful in genotoxicity and carcinogenesis research (Atienzar and Jha 2006). Genotoxic effects of Zn stress in rice was evaluated using a PCR base RAPD profile. A total of five RAPD primers (OPA 1, OPA 2, OPA 3, OPA 4, and OPA 5) were employed to analyze the change in genomic DNA. The present study showed a change in RAPD patterns comprised of a loss and/or gain of bands under Zn stress in both the studied rice varieties (Table 1 and Table 2). RAPD pattern generated from Chakhao under Zn stress did not show any loss or gain of bands as compared with the control. But in Kawnglawng, there was a change in the RAPD pattern due to the loss of bands. The loss of normal RAPD bands could be linked to genotoxic induced DNA damage, point mutations, or complicated chromosomal rearrangements (Atienzar et al., 1999; Liu et al., 2007; Atienzar et al., 2000). Metals have been demonstrated to cause a variety of DNA damage in previous studies under heavy metal stress, including single and double-strand breaks, changed nucleotide, point, and deletion mutations, and DNA protein cross-linkage. In this study, change in the RAPD pattern in the form of increase and decrease of band intensity was also

noticed. Previous studies also reported similar outcomes (Ahmad et al., 2012; Manikandan et al., 2015). According to Liu et al. (2005), change in band intensity and missing bands in RAPD patterns are likely to be caused by variations in oligonucleotide priming sites owing to chromosomal rearrangements rather than point mutations or DNA damage in primer binding sites and interaction of DNA polymerase with damaged DNA. Alteration in DNA due to mutation can also affect the kinetics of PCR events. The occurrence of new amplified PCR products may be due to some oligonucleotide priming sites becoming available to oligonucleotide primers after structural alteration or because of structural modification in genomic DNA sequence (Liu et al., 2007). According to Atienzar et al. (2002), a loss of an amplified PCR band can only occur if the same structural change occurs in 75-90% of the cells. DNA lesions such as bulky adducts are likely to have a negative impact on RAPD profiles. They can cause DNA structural changes as well as hinder DNA polymerization and/or stop the Tag polymerase in the PCR reaction, resulting in a reduction in RAPD band intensity or, in case of substantial DNA damage, the loss of amplified products (Atienzar et al., 2002).

RAPD analysis can detect transient DNA alterations that may or may not eventually appear as mutations. Classic genotoxic techniques, such as the comet and micronucleus assay, are less sensitive than molecular marker studies such as RAPD or amplified fragment length polymorphism (AFLP). The use of several biomarkers can significantly improve the identification of genotoxic effects. Change in RAPD or AFLP patterns caused by genotoxins are due to the modifications in genomic template stability that may be directly related to the change in some measures including total soluble protein level, root growth, and chlorophyll content. Genomic template stability (GTS) analysis can be an important genotoxicity study tool in order to understand the level of DNA damage, the efficiency of DNA repair and replication. GTS analysis from my study showed 100% GTS value for all the primer in the rice variety Chakhao (Table 3). The average %GTS for all the primers in the rice variety Kawngalwng was found at 100% (5mM), 100% (10mM), and 96.52% (15mM) (Table 3). The changes observed in RAPD profiles could be regarded as

modifications in GTS and it is related to the level of DNA damage, the efficiency of DNA repair, and replication (Rocco et al., 2011; Ahmed et al., 2012). Therefore, it can be concluded from my result that the genotoxicity effect of Zn stress is very less in the studied rice varieties. Few earlier studies reported a reduction in GTS values in rice exposed to different concentrations of Arsenic (Ahmed et al., 2012) and Cadmium (Liu et al., 2007). The high change in GTS in the tolerant variety could be owing to the molecular machinery's ability to adjust to As(III) stress being more efficient, resulting in greater alterations in the DNA profile, however, this has to be looked into more carefully and also associated with grain yield (Ahmed et al., 2012). A high level of DNA damage does not always imply a lower level of genomic template stability (in comparison to a low level of DNA changes), because DNA repair and replication may be hampered by the pollutant-induced adducts' excessive, fatal effects. A toxic effect can totally suppress a biological response if a population's existence is threatened; nevertheless, genomic template stability cannot be completely compromised since DNA damage induction may not rise linearly (plateau effect). Furthermore, because genomic template stability may be linked to several types of DNA damage, such as DNA adducts, mutations, rearrangements, and so on, predicting a dose–response connection would be problematic (Rocco et al., 2014).

Rice cultivation necessitates healthy soil and ample water, but the overuse of chemical fertilizers and pesticides in paddy fields has already induced a burden on soil and water quality, resulting in significant toxic metal accumulations. Therefore, it is important to understand the toxic effect of heavy metals in plants. The goal of this study was to understand more about how local rice varieties/cultivars react to varied Zn stress. Effect of Zn stress on biochemical markers like chlorophyll content, protein content, and antioxidant enzymes like CAT, APX, and SOD activity were studied. A RAPD-PCR analysis was also carried out in order to better understand the impact of Zn stress on DNA. It was found that Zn stress had a considerable impact on rice chlorophyll concentration, protein content, and antioxidant enzymes. A genome toxicity investigation of Zn stress on the rice varieties Chakhao and Kawnglwang revealed no significant damage to the rice genome. Heavy metal stress

had been associated with considerable changes in the RAPD profile in previous investigations (Liu et al., 2007; Ahmed et al., 2012; Aslam., et al., 2014). The current investigation shows that Chakhao and Kawnglawng rice varieties have a strong tolerance to heavy metal stress (high antioxidant enzyme activities coupled with high GTS percentages), and hence suggests that these two local rice varieties from northeast India could be used as good candidates for future rice breeding programs against Zn stress.

Chapter 6

Conclusion

Rice is a major dietary source for half of the world population and plays a significant role in nutritional contribution to the consumer. As a result it is important to understand the quality and nutritional composition of rice. Heavy metals are nonbiodegradable, persistent inorganic chemical constituents that have cytotoxic, genotoxic, and mutagenic effects on humans, animals, and plants by influencing and contaminating food chains, soil, irrigation, or potable water, aquifers, and the surrounding environment. Heavy metals can be classified into two groups: (1) essential micronutrients such as Fe, Mn, Zn, Cu, Mg, Mo, and Ni which is required for normal growth of the plant, (2) nonessential elements such as Cd, Sb, Cr, Pb, As, Co, Ag, Se, and Hg which do not have known biological and physiological functions.

Heavy metal (HMs) pollution is regarded as one of the major concerns for soil and water, causing varieties of toxic and stress effects on plants and ecosystems. Rice grain produced through paddy soils contaminated with heavy metals such as As, Al, Cu, Cd, Pb, Hg, Mn, Se, and Zn is a major source of heavy metal consumption by humans in most countries. As a result, the gradual aggregation of heavy metals in rice grains and subsequent transmission to the food chain poses a significant threat to agriculture and human health. Many studies in recent years have explored the influence of heavy metals toxicity on rice at multiple levels, including molecular, biochemical, physiological, cellular, and tissue, and found a link between heavy metals toxicity and declining rice productivity.

Heavy metal has the ability to react with many essential cellular components such as DNA, protein, and enzymes, resulting in a variety of stress responses in plants such as oxidative stress, which is the root cause of plant cell death. Oxidative stress causes severe morphological, metabolic, and physiological changes in plants, such as DNA strand breakage, protein defragmentation, and photosynthetic pigment degradation, all of which can lead to cell death. Plants, in turn, have a variety of methods to reduce heavy metal toxicity. Plants have antioxidant defense mechanisms that can be divided into two groups: enzymatic antioxidants (such as SOD, CAT, GPX, GR) and nonenzymatic antioxidants (such as AsA, GSH, carotenoids, alkaloids, tocopherols, proline, and phenolic compounds), which together act as free radical scavengers to mitigate the harmful effects of heavy metal agglomeration in the cells. Earlier studies also reported an increase in various antioxidant activities such as CAT, APX, SOD, and POD under heavy metal toxicity (Panda et al., 2011; Song et al., 2013). These antioxidant potentials can be measured using a variety of in vivo and in vitro approaches, to examine the ROS-damaging effects of antioxidant enzymes.

The present study was taken up to understand the responses of two pigmented local rice cultivars, Chakhao and Kawnglawng of Northeast India to heavy metal stress (Zn). Chakhao, which means delicious rice in Manipuri, is a black scented rice of Manipur, and it has recently attracted scientific attention due to its increased nutraceutical qualities such as antioxidant, anticarcinogenic, fibre, vitamin, and mineral contents. Black rice has high anthocyanin concentration in the pericarp, which possesses antioxidant properties. This rice has long played a vital role in the indigenous Meitei population of Manipur's socio-cultural customs in India. Recently, Chakhao was also given the Geographical Indication (GI) label. A Geographical Indication (GI) is a designation that distinguishes products that originate or are produced in a certain place and have a unique characteristic that can be traced back to its location. Kawnglawng is a local rice cultivar of Mizoram and it is widely popular among the Mizo community. The Mizo people's diet is entirely rice-based, with rice serving as the main meal and other items such as vegetables and meat

serving as side dishes. Kawnglawng is used specially in making *Chhangban*, a traditionally popular processed food and it is prepared by dehusking rice in wooden motar and then the clean rice is soaked in clean water for an hour to even a whole night, depending on one wish. The rice is then dried in the sun for a period of time. It is ground again to get a fine powdered grain. The powdered grain is sieved for a better result. The finer the grain, the more sticky it is. After that, the very fine powdered grain is treated. Water is added to the powdered grain, which is then formed into sticky balls and wrapped in the leaves of the hnahthial plant (*Phrynium capitatum*).

Heavy metal toxicity in these rice was investigated using different physiological and biochemical parameters such as seed germination, chlorophyll content, total soluble protein, antioxidant enzyme response, and DNA damage using a PCR based method. The present showed that under varied Zn concentrations, the germination percentage of rice was not affected and germination percentage was found to be 100%. The chlorophyll content was found significantly reduced in the rice treated with different concentrations of Zn in both the rice varieties. Protein content was also found slightly reduced in rice exposed to Zn stress. Results from my study showed that Zn stress had a significant impact on rice antioxidants enzymes such as CAT, APX, and SOD. All the antioxidant enzyme activity increased significantly with the increasing concentration of Zn and the maximum activity was found in rice treated with 15mM Zn. Zn stress's genotoxic effects in rice were assessed utilizing a PCR-based RAPD profile. The average GTS value was found to be 100% for all the primers in the rice cultivar Chakhao. And the GTS for all the primers in the rice variety Kawngalwng was found 100% in rice treated with 5mM and 10mM and 96.52% in 15mM with an average GTS of 98.84 %. Results suggest that molecular, physiological, and enzyme assays could be used together as a reliable and powerful biomarker to detect the genotoxic effect of heavy metals in different varieties of plants. The present study concludes that Chakhao and Kawnglawng have a strong tolerance to heavy metal stress (high antioxidant enzyme activities coupled with high GTS percentages), and suggests that these two local rice varieties from

northeast India could be used as good candidates for future rice breeding programs against Zn stress.

Appendix

Anova: Single Factor						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
control	3	0.98	0.326667	0.001633		
5mM	3	1.39	0.463333	0.004133		
10mM	3	1.47	0.49	0.0043		
15mM	3	1.58	0.526667	0.002433		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.068567	3	0.022856	7.313778	0.011117	4.066181
Within Groups	0.025	8	0.003125			
Total	0.093567	11				
	Chakhao -APX					

Anova: Single Factor						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
control	3	1.18	0.393333	0.001233		
5mM	3	1.45	0.483333	0.000533		
10mM	3	1.52	0.506667	0.000433		
15mM	3	1.66	0.553333	0.001433		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.040625	3	0.013542	14.90826	0.001223	4.066181
Within Groups	0.007267	8	0.000908			
Total	0.047892	11				
	Kawnglawng-APX					

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
control	3	4.08	1.36	0.0925
5mM	3	4.7	1.566667	0.103333
10mM	3	6.51	2.17	0.0589
15mM	3	6.76	2.253333	0.048533

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.754492	3	0.584831	7.713747	0.00955	4.066181
Within Groups	0.606533	8	0.075817			
Total	2.361025	11				

Chakhao-CAT

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
control	3	4.66	1.553333	0.065033
5mM	3	5.86	1.953333	0.050533
10mM	3	6.75	2.25	0.0432
15mM	3	7.2	2.4	0.0961

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.254158	3	0.418053	6.561121	0.015042	4.066181
Within Groups	0.509733	8	0.063717			
Total	1.763892	11				

Kawnglawng-CAT

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
control	3	4.91	1.636667	0.003033
5mM	3	4.61	1.536667	0.002033
10mM	3	4.35	1.45	0.0016
15mM	3	4.2	1.4	0.0031

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.097158	3	0.032386	13.26394	0.001798	4.066181
Within Groups	0.019533	8	0.002442			
Total	0.116692	11				

Chakhao- Chlorophyll a

Groups	*Count*	*Sum*	*Average*	*Variance*
1.68	2	3.33	1.665	0.00605
1.43	2	2.96	1.48	0.0008
1.41	2	2.79	1.395	0.00605
1.21	2	2.41	1.205	0.00605

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.218838	3	0.072946	15.39754	0.011587	6.591382
Within Groups	0.01895	8	0.004738			
Total	0.237788	7				

Kawnglawng-Chlorophyll a

Anova: Single Factor						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
control	3	1.85	0.616667	0.008133		
5mM	3	1.64	0.546667	0.011033		
10mM	3	1.52	0.506667	0.011033		
15mM	3	1.16	0.386667	0.012233		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.083625	3	0.027875	2.627651	0.122114	4.066181
Within Groups	0.084867	8	0.010608			
Total	0.168492	11				
	Chakhao- chlorophyll b					

SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
control	3	1.74	0.58	0.0189		
5mM	3	1.6	0.533333	0.022033		
10mM	3	1.4	0.466667	0.018633		
15mM	3	1.19	0.396667	0.018633		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.057492	3	0.019164	0.98025	0.448923	4.066181
Within Groups	0.1564	8	0.01955			
Total	0.213892	11				
	Kawnglawng-chlorophyll b					

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
control	3	3.430364	1.143455	0.000126
5mM	3	2.922421	0.97414	0.000103
10mM	3	2.967371	0.989124	6.23E-05
15mM	3	2.890955	0.963652	0.000217

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.0643485	3	0.021449	168.7241	1.43E-07	4.066181
Within Groups	0.001017	8	0.000127			
Total	0.0653655	11				

Chakhao-Protein

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
control	3	3.189878	1.063293	0.000128
5mM	3	2.816786	0.938929	0.000197
10mM	3	2.812291	0.93743	0.000325
15mM	3	2.659459	0.886486	0.000214

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.050937	3	0.016979	78.62508	2.81E-06	4.066181
Within Groups	0.001728	8	0.000216			
Total	0.052665	11				

Kawnglawng-Protein

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
control	3	2.18	0.726667	0.001233
5mM	3	3.03	1.01	0.0169
10mm	3	3.46	1.153333	0.003233
15mM	3	3.94	1.313333	0.002233

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.558492	3	0.186164	31.5532	0.00	4.066181
Within Groups	0.0472	8	0.0059			
Total	0.605692	11				

Chakhao-SOD

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
control	3	2.38	0.793333	0.002233
5mM	3	2.82	0.94	0.0097
10mm	3	3.56	1.186667	0.010133
15mM	3	4.09	1.363333	0.001633

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.579292	3	0.193097	32.59025	7.81E-05	4.066181
Within Groups	0.0474	8	0.005925			
Total	0.626692	11				

Kawnglawng-SOD

References

AbdElgawad, H., Zinta, G., Hamed, B.A., Selim, S., Beemster, G., Hozzein, W.N., Wadaan, M.A., Asard, H. and Abuelsoud, W (2020) Maize roots and shoots show distinct profiles of oxidative stress and antioxidant defense under heavy metal toxicity. *Environmental Pollution 258*:113705.

Abdu N, Agbenin JO, Buerkert A (2011) Geochemical assessment, distribution and dynamics of trace metals in urban agricultural soils under long-term wastewater irrigation in Kano, northern Nigeria. *Journal of Plant Nutrition and Soil Science* 173:447–458.

Acquaah G (2007) Principles of Plant Genetics and Breeding. Oxford, UK: Blackwell.

Adrees M, Ali S, Rizwan M, Zia-ur-Rehman M, Ibrahim M, Abbas F, Farid M, Qayyum M, Irshad MK (2015) Mechanisms of silicon-mediated alleviation of heavy metal toxicity in plants: a review. *Ecotoxicology and Environmental Safety* 119:186-197.

Aebi HE (1983) Catalase. *Methods of enzymatic analysis*.

Aggarwal A, Sharma I, Tripati BN, Munjal AK, Baunthiyal M, Sharma V (2012) Me tal toxicity and photosynthesis. In: Photosynthesis: overviews on recent progress & future perspectives. 1st ed. New Delhi: I K International Publishing House Pvt. Ltd; p. 229–236.

Ahmad MA, Gaur R, Gupta M (2012) Comparative biochemical and RAPD analysis in two varieties of rice (Oryza sativa) under arsenic stress by using various biomarkers. *Journal of hazardous materials* 217:141-148.

Alam, P, Balawi TH, Altalayan FH, Hatamleh AA, Ashraf M, Ahmad P (2021) Silicon attenuates the negative effects of chromium stress in tomato plants by modifying antioxidant enzyme activities, ascorbate–glutathione cycle and glyoxalase system. *Acta Physiologiae Plantarum* 43(7):1-17.

Ali H, Khan E, Ilahi I (2019) Environmental chemistry and ecotoxicology of hazardous heavy metals: environmental persistence, toxicity, and bioaccumulation. *Journal of chemistry*.

Alloway BJ (2012) Heavy metals in soils: trace metals and metalloids in soils and their bioavailability (Vol. 22). Springer Science & Business Media.

Anjum NA, Gill SS, Gill R, Hasanuzzaman M, Duarte AC, Pereira E, Ahmad I, Tuteja, R, Tuteja N (2014) Metal/metalloid stress tolerance in plants: role of ascorbate, its redox couple, and associated enzymes. *Protoplasma* 251(6):1265-1283.

Arif N, Sharma NC, Yadav V, Ramawat N, Dubey NK, Tripathi DK, Chauhan DK and Sahi, S (2019) Understanding heavy metal stress in a rice crop: toxicity, tolerance mechanisms, and amelioration strategies. *Journal of Plant Biology* 62(4):239-253.

Asada K (1994) Production and action of active oxygen species in photosynthetic tissues. In: Foyer CH, Mullineaux PM, eds. Causes of Photooxidative Stress and Amelioration of Defence Systems in Plants. Boca Raton, USA: CRC Press, 77-104.

Asem ID, Imotomba RK, Mazumder PB (2017) The deep purple color and the scent are two great qualities of the black scented rice (Chakhao) of Manipur. *Advances in International Rice Research, ed. JQ Li (London: Intech Open)*, 125-136.

Asem ID, Imotomba RK, Mazumder PB, Laishram JM (2015) Anthocyanin content in the black scented rice (Chakhao): its impact on human health and plant defense. *Symbiosis* 66(1):47-54.

Aslam R, Ansari MYK, Choudhary S, Bhat TM, Jahan N (2014) Genotoxic effects of heavy metal cadmium on growth, biochemical, cyto-physiological parameters and detection of DNA polymorphism by RAPD in Capsicum annuum L.–An important spice crop of India. *Saudi Journal of Biological Sciences* 21(5): 465-472.

Atienzar FA, Conradi M, Evenden AJ, Jha AN, Depledge MH (1999) Qualitative assessment of genotoxicity using random amplified polymorphic DNA: comparison of genomic template stability with key fitness parameters in Daphnia magna exposed to benzo [a] pyrene. *Environmental Toxicology and Chemistry: An International Journal* 18(10):2275-2282.

Atienzar FA, Cordi B, Donkin ME, Evenden AJ, Jha AN, Depledge MH (2000) Comparison of ultraviolet-induced genotoxicity detected by random amplified polymorphic DNA with chlorophyll fluorescence and growth in a marine macroalgae, *Palmaria palmata*. *Aquatic Toxicology* 50:1–12.

Atienzar FA, Jha AN (2006) The random amplified polymorphic DNA (RAPD) assay and related techniques applied to genotoxicity and carcinogenesis studies: a critical review. *Mutation Research* 613:76-102.

Atienzar FA, Jha AN (2006) The random amplified polymorphic DNA (RAPD) assay and related techniques applied to genotoxicity and carcinogenesis studies: a critical review. *Mutation Research* 613:76–102.

Atienzar FA, Venier P, Jha AN, Depledge MH (2002) Evaluation of the random amplified polymorphic DNA (RAPD) assay for the detection of DNA damage and mutations. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis* 521(1-2), 151-163.

Auten RL, Davis JM (2009) Oxygen toxicity and reactive oxygen species: the devil is in the details. *Pediatric research* 66(2):121-127.

Baker AJ (1987) Metal tolerance. *New phytologist* 106:93-111.

Balafrej H, Bogusz D, Triqui ZEA, Guedira A, Bendaou N, Smouni A, Fahr M (2020) Zinc hyperaccumulation in plants: A review. *Plants* 9(5):562.

Balestrasse KB, Benavides MP, Gallego SM, Tomaro ML (2003) Effect of cadmium stress on nitrogen metabolism in nodules and roots of soybean plants. *Functional plant biology* 30(1):57-64.

Bardakci F, Skibinski DOF (1994) Application of the RAPD technique in tilapia fish: species and subspecies identification. *Heredity* 73(2):117-123.

Baruah R (2018) Towards the bioavailability of zinc in agricultural soils. In Role of Rhizospheric Microbes in Soil (pp. 99-136). Springer, Singapore.

Barwick M, Maher W (2003) Biotransference and biomagnification of selenium copper, cadmium, zinc, arsenic and lead in a temperate seagrass ecosystem from Lake Macquarie Estuary, NSW, Australia. *Marine Environmental Research* 56(4):471-502.

Basta NT, Ryan JA, Chaney RL (2005) Trace element chemistry in residual-treated soil: key concepts and metal bioavailability. *Journal of Environmental Quality* 34(1):49–63.

Batool A, Ziaf K, Amjad M (2015) Effect of halo-priming on germination and vigor index of cabbage (Brassica oleracea var. capitata). *Journal of Environmental and Agricultural Sciences* 2(7):8.

Bavi K, Kholdebarin B, Moradshahi A (2011) Effect of cadmium on growth, protein content and peroxidase activity in pea plants. *Pakistan. Journal of Botany* 43(3):1467-1470.

Behera SK, Shukla AK (2015) Spatial distribution of surface soil acidity, electrical conductivity, soil organic carbon content and exchangeable potassium, calcium and magnesium in some cropped acid soils of India. *Land Degradation & Development 26*(1):71-79.

Berni R, Luyckx M, Xu X, Legay S, Sergeant K, Hausman JF, Guerriero G (2019) Reactive oxygen species and heavy metal stress in plants: Impact on the cell wall and secondary metabolism. *Environmental and Experimental Botany* 161:98-106.

Bernier J, Atlin GN, Serraj R, Kumar A, Spaner D (2008) Breeding upland rice for drought resistance. *Journal of the Science of Food and Agriculture* 88(6):927-939.

Bethke PC, Jones RL (2001) "Cell death of barley aleurone protoplasts is mediated by reactive oxygen species," *Plant Journal*25(1)19–29.

Bewley JD (1997) Seed germination and dormancy. *The plant cell* 9(7):1055.

Beyer W, Imlay J, Fridovich I (1991) Superoxide dismutases. *Progress in nucleic acid research and molecular biology* 40:221-253.

Bhaduri AM, Fulekar MH (2012) Antioxidant enzyme responses of plants to heavy metal stress. *Reviews in Environmental Science and Bio/Technology* 11(1):55-69.

Bos E, Vu MT, Massiah A, Bulatao RA (1994) World population projections 1994-: estimates and projections with related demographic statistics. Johns Hopkins University Press.

Bowler C, Montagu MV, Inze D (1992) Superoxide dismutase and stress tolerance. *Annual review of plant biology* 43(1):83-116.

Bradl HB (2005) Sources and origins of heavy metals. In *Interface science and technology* 6:1-27. Elsevier.

Brallier S, Harrison RB, Henry CL, Dongsen X (1996) Liming effects on availability of Cd, Cu, Ni and Zn in a soil amended with sewage sludge 16 years previously. *Water, Air, and Soil Pollution* 86(1):195-206.

Brown PH, Cakmak I, Zhang Q (1993) Form and function of zinc plants. In Zinc in soils and plants. *Springer, Dordrecht* 93-106.

Buendía-González L, Orozco-Villafuerte J, Cruz-Sosa F, Barrera-Díaz CE, Vernon-Carter EJ (2010) Prosopis laevigata a potential chromium (VI) and cadmium (II) hyperaccumulator desert plant. *Bioresource Technology* 101(15):5862-5867.

Cakmak I (2009) Enrichment of fertilizers with zinc: An excellent investment for humanity and crop production in India. *Journal of trace elements in medicine and biology 23*(4):281-289.

Cakmak I, Horst WJ (1991) "Effect of aluminium on lipid peroxidation, superoxide dismuatse, catalase, and peroxidase activities in root tips of soybean (*Glycine max*). *Physiologia Plantarum* 83(3):463–468.

Cakmak I, Öztürk L, Karanlik S, Marschner H, Ekiz H (1996) Zinc-efficient wild grasses enhance release of phytosiderophores under zinc deficiency. *Journal of Plant nutrition 19*(3-4):551-563.

Cambier P, Schvartz C, Oort FV (2009) Contamination of peri-industrial agrosystems and ecosystems by metals.

Cao X, Harris W (2010) Properties of dairy-manure-derived biochar pertinent to its potential use in remediation. *Bioresource technology* 101(14):5222-5228.

Cenkci S, Cigerci IH, Yildiz M, Ozay C, Bozdag A, Terzi H (2010) Lead contamination reduces chlorophyll biosynthesis and genomic template stability in *Brassica rapa* L. *Environmental and Experimental Botany* 67:467–473.

Chakuton K, Puangpronpitag D, Nakornriab M (2012) Phytochemical content and antioxidant activity of colored and non-colored Thai rice cultivars. *Asian Journal of Plant Sciences* 11(6):285-293.

Chandra R, Kang H (2016) Mixed heavy metal stress on photosynthesis, transpiration rate, and chlorophyll content in poplar hybrids. *Forest Science and Technology* 12(2):55-61.

Chang TT (1983) The origins and early cultures of the cereal grains and food legumes. *The origins of Chinese civilization*, 65-94.

Chanu CS, Yenagi NB, Math KK (2016) Nutritional and functional evaluation of black rice genotypes. *Journal of Farm Sciences*, *29*(1): 61-64.

Cheah BH, Chen YL, Lo JC, Tang IC, Yeh KC, LIN YF (2020) Divalent nutrient cations: friend and foe during zinc stress in rice. *Authorea Preprints*.

Chuan MC, Shu GY, Liu JC (1996) Solubility of heavy metals in a contaminated soil: effects of redox potential and pH. *Water, Air, and Soil Pollution* 90(3):543-556.

Cocconcelli PS, Porro D, Galandini S, Senini L (1995) Development of RAPD protocol for typing of strains of lactic acid bacteria and enterococci. *Letters in Applied Microbiology* 21(6):376-379.

Comba ME, Benavides MP, Tomaro ML (1998) Effect of salt stress on antioxidant defence system in soybean root nodules. *Functional Plant Biology* 25(6):665-671.

Compant S, Clément C, Sessitsch A (2010) Plant growth-promoting bacteria in the rhizo-and endosphere of plants: their role, colonization, mechanisms involved and prospects for utilization. *Soil Biology and Biochemistry* 42(5):669-678.

Crawford GW, Shen C (1998) The origins of rice agriculture: Recent progress in East Asia. *Antiquity* 72: 858–866.

D'Amore JJ, Al-Abed SR, Scheckel KG, Ryan JA (2005) "Methods for speciation of metals in soils: a review. *Journal of Environmental Quality* 34(5):1707–1745.

DalCorso G, Fasani E, Furini A (2013) Recent advances in the analysis of metal hyperaccumulation and hypertolerance in plants using proteomics. *Frontiers in plant science* 4:280.

Doncheva S, Amenos M, Poschenrieder C, Barcelo J (2005) Root cell patterning: a primary target for aluminium toxicity in maize. *Journal of experimental botany* 56(414):1213-1220.

Doni F, Isahak A, Zain CRCM, Yusoff WMW (2014) Physiological and growth response of rice plants (Oryza sativa L.) to Trichoderma spp. inoculants. *AMB Express* 4(1):1-7.

dos Santos LR, da Silva BRS, Pedron T, Batista BL, da Silva Lobato AK (2020) 24-Epibrassinolide improves root anatomy and antioxidant enzymes in soybean plants subjected to zinc stress. *Journal of Soil Science and Plant Nutrition* 20(1):105-124.

Edwards K, Johnstone C, Thompson C (1991) A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. *Nucleic acids research* 19(6):1349.

Eleftheriou EP, Adamakis ID S, Melissa P (2012) Effects of hexavalent chromium on microtubule organization, ER distribution and callose deposition in root tip cells of *Allium cepa* L. *Protoplasma* 249(2):401-416.

Elloumi N, Zouari M, Chaari L, Jomni C, Rouina BB, Abdallah FB (2014) Ecophysiological responses of almond (*Prunus dulcis*) seedlings to cadmium stress. *Biologia* 69(5):604-609.

Emamverdian A, Ding Y, Mokhberdoran F, Xie Y (2015) Heavy metal stress and some mechanisms of plant defense response. *The Scientific World Journal*.

Evans LJ (1989) Chemistry of metal retention by soils. *Environmental Science and Technology* 23:1046–1056.

FAO (1993) Forest resources assessment 1990: tropical countries. FAO.

FAO (1997) Selected indicators of food and agricultural development in the Asia-Pacific Region, 1986-96; FAO-RAP Publication No. 1997/23, 206 pp. Regional Office for the Asia and the Pacific, FAO Bangkok, Thailand.

Fijałkowski K, Kacprzak M, Grobelak A, Placek, A (2012) The influence of selected soil parameters on the mobility of heavy metals in soils. *Inżynieria I Ochronaśrodowiska* 15:81-92.

Flora SJS, Mittal M, Mehta A (2008) Heavy metal induced oxidative stress & its possible reversal by chelation therapy. *Indian Journal of Medical Research* 128(4):501.

Fontes RLF, Cox FR (1998) Zinc toxicity in soybean grown at high iron concentration in nutrient solution. *Journal of Plant Nutrition* 21(8):1723-1730.

Foth HD (1978) Fundamentals of Soil Science, 6th edn. John Wiley and Sons, New York.

Foyer CH, Lelandais M, Kunert KJ (1994) Photooxidative stress in plants. *Physiologia Plantarum* 92: 696-717.

Foyer CH, Noctor G (2005) Redox homeostasis and antioxidant signaling: a metabolic interface between stress perception and physiological responses. *The Plant Cell* 17(7):1866-1875.

Gai APC, dos Santos DS, Vieira EA (2017) Effects of zinc excess on antioxidant metabolism, mineral content and initial growth of *Handroanthus impetiginosus* (Mart. ex DC.) Mattos and Tabebuia roseoalba (Ridl.) Sandwith. *Environmental and Experimental Botany* 144:88-99.

Gangwar S, Singh VP (2011) Indole acetic acid differently changes growth and nitrogen metabolism in Pisum sativum L. seedlings under chromium (VI) phytotoxicity: implication of oxidative stress. *Scientia horticulturae* 129(2): 321-328.

Gangwar S, Singh VP, Sheo MP, Maurya JN (2010) "Modulation of manganese toxicity in Pisum sativum L. seedlings by kinetin." *Scientia horticulturae* 126(4)467-474.

Gangwar S, Singh VP, Srivastava PK, Maurya JN (2011) Modification of chromium (VI) phytotoxicity by exogenous gibberellic acid application in Pisum sativum (L.) seedlings. *Acta Physiologiae Plantarum* 33(4):1385-1397.

Ghasemzadeh A, Karbalaii MT, Jaafar HZ, Rahmat A (2018) Phytochemical constituents, antioxidant activity, and antiproliferative properties of black, red, and brown rice bran. *Chemistry Central Journal* 12(1):1-13.

Gjorgieva D, Kadifkova-Panovska T, Mitrev S, Kovacevik B, Kostadinovska E, Bačeva K, Stafilov T (2012) Assessment of the genotoxicity of heavy metals in *Phaseolus vulgaris* L. as a model plant system by Random Amplified Polymorphic DNA (RAPD) analysis. *Journal of Environmental Science and Health* 47(3):366-373.

Gjorgieva, D., Kadifkova Panovska, T., Ruskovska, T., Bačeva, K., & Stafilov, T. (2013). Influence of heavy metal stress on antioxidant status and DNA damage in Urtica dioica. *BioMed Research International, 2013*.

Gnanamanickam, SS (2009) Rice and its importance to human life. In *Biological control of rice diseases.Springer, Dordrecht* pp. 1-11.

Goldberg AL (2003) Protein degradation and protection against misfolded or damaged proteins. *Nature* 426(6968):895-899.

Gomes MP, Marques TCLLDS, Nogueira MDOG, Castro EMD, Soares ÂM (2011) Ecophysiological and anatomical changes due to uptake and accumulation of heavy metal in Brachiaria decumbens. *Scientia Agricola* 68:566-573.

Gupta M, Sarin NB (2009) Heavy metal induced DNA changes in aquatic macrophytes: Random amplified polymorphic DNA analysis and identification of sequence characterized amplified region marker. *Journal of Environmental Sciences* 21(5):686-690.

Hajiboland R, Amirazad F (2010) Growth, photosynthesis and antioxidant defense system in Zn-deficient red cabbage plants. *Plant, Soil and Environment* 56(5):209-217.

Hamzeh-Kahnoji Z, Ebrahimi A, Sharifi-Sirchi GR, Majidi-Hervan E (2021) Monitoring of morphological, biochemical and molecular responses of four contrasting barley genotypes under salinity stress. *Journal of the Saudi Society of Agricultural Sciences*.

Han C, Liu Q, Yang Y (2009) Short-term effects of experimental warming and enhanced ultraviolet-B radiation on photosynthesis and antioxidant defense of *Picea asperata* seedlings. *Plant Growth Regulation* 58(2):153-162.

Hartmut K, Lichtenthaler A and Welburn R (1983) Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochemical Society transactions* 11: 591-92

Hasan M, Cheng Y, Kanwar MK, Chu XY, Ahammed GJ, Qi ZY (2017) Responses of plant proteins to heavy metal stress—a review. *Frontiers in plant science* 8:1492.

Hasanuzzaman M, Hossain MA, da Silva JAT, Fujita M (2012) Plant response and tolerance to abiotic oxidative stress: antioxidant defense is a key factor. In *Crop stress and its management: perspectives and strategies* Springer, Dordrecht 261-315.

Hayat S, Khalique G, Irfan M, Wani AS, Tripathi BN, Ahmad A (2012) Physiological changes induced by chromium stress in plants: an overview. *Protoplasma 249*(3):599-611.

He J, Ren Y, Chen X, Chen H (2014) Protective roles of nitric oxide on seed germination and seedling growth of rice (Oryza sativa L.) under cadmium stress. *Ecotoxicology and environmental safety* 108:114-119.

Hefny M, Abdel-Kader DZ (2009) Antioxidant-enzyme system as selection criteria for salt tolerance in forage sorghum genotypes (*Sorghum bicolor* L. Moench). In *Salinity and water stress* (pp. 25-36). Springer, Dordrecht.

Hemamalini C, Sam S, Patro TSSK (2021) Awareness and consumption of small millets.

Heyno E, Mary V, Schopfer P, Krieger-Liszkay A (2011) Oxygen activation at the plasma membrane: relation between superoxide and hydroxyl radical production by isolated membranes. *Planta* 234(1):35-45.

Hojsa I, Braegge C, Bronsky J, Campoy C, Colomb V, Decsi T, Domellöf M, Fewtrell M, Mis NF, Mihatsch W, Molgaard C (2015) Arsenic in rice: a cause for concern. *Journal of pediatric gastroenterology and nutrition* 60(1):142-145.

Hossain MA, Piyatida P, da Silva JAT, Fujita M (2012) Molecular mechanism of heavy metal toxicity and tolerance in plants: central role of glutathione in detoxification of reactive oxygen species and methylglyoxal and in heavy metal chelation. *Journal of Botany*.

Hossain Z, Komatsu S (2013) Contribution of proteomic studies towards understanding plant heavy metal stress response. *Frontiers in Plant Science* 3:310.

Hu Y, Nan Z, Su J, Wang N (2013) Heavy metal accumulation by poplar in calcareous soil with various degrees of multi-metal contamination: implications for phytoextraction and phytostabilization. *Environmental Science and Pollution Research* 20(10):7194-7203.

Huang H, Ullah F, Zhou DX, Yi M, Zhao Y (2019) Mechanisms of ROS regulation of plant development and stress responses. *Frontiers in Plant Science* 10-800.

Huang Z, Pan XD, Wu PG, Han JL, Chen Q (2013) Health risk assessment of heavy metals in rice to the population in Zhejiang, China. *PloS one* 8(9):75007.

Hüner NPA, Grodzinski B (2011) "Photosynthesis and photoautotrophy." 315-322.

Jabeen Z, Fayyaz HA, Irshad F, Hussain N, Hassan MN, Li J, Rehman S, Haider W, Yasmin H, Mumtaz S, Bukhari SAH (2021) Sodium nitroprusside application improves morphological and physiological attributes of soybean (*Glycine max* L.) under salinity stress. *Plos one*, 16(4):0248207.

Jain A, Sinilal B, Dhandapani G, Meagher RB, Sahi SV (2013) Effects of deficiency and excess of zinc on morphophysiological traits and spatiotemporal regulation of zinc-responsive genes reveal incidence of cross talk between micro- and macronutrients. *Environmental science & technology* 47:5327–5335.

Jaleel CA, Gopi R, Manivannan P, Panneerselvam R (2007) Responses of antioxidant defense system of *Catharanthus roseus* (L.) G. Don. to paclobutrazol treatment under salinity. *Acta Physiologiae Plantarum* 29(3):205-209.

JamshidiGoharrizi K, Amirmahani F, Salehi F (2020) Assessment of changes in physiological and biochemical traits in four pistachio rootstocks under drought, salinity and drought+ salinity stresses. *Physiologia plantarum 168*(4):973-989.

Jawad Hassan M, Ali Raza M, Ur Rehman S, Ansar M, Gitari H, Khan I, Wajid M, Ahmed M, Abbas Shah G, Peng Y, Li Z (2020) Effect of cadmium toxicity on growth, oxidative damage, antioxidant defense system and cadmium accumulation in two sorghum cultivars. *Plants*, *9*(11), p.1575.

Jones LHP, Jarvis SC (1981) The fate of heavy metals. *The chemistry of soil processes* 593-620.

Jones LHP, Jarvis SC, Green DJ, Hayes MHB (1981) The fate of heavy metals in the chemistry of soil processes. New York.

Jozefczak M, Remans T, Vangronsveld J, Cuypers A (2012) Glutathione is a key player in metal-induced oxidative stress defenses. *International journal of molecular sciences* 13(3):3145-3175.

Jung Hee Joo, Yun Soo Bae, and June Seung Lee (2001) “Role of auxin-induced reactive oxygen species in root gravitropism,” *Plant Physiology* 126(3)1055–1060.

Kabata-Pendias A, Pendias H (2001) Trace Metals in Soils and Plants, CRC Press, Boca Raton, Fla, USA, 2nd edition.

Karmous I, Bellani LM, Chaoui A, El Ferjani E, Muccifora S (2015) Effects of copper on reserve mobilization in embryo of *Phaseolus vulgaris* L. *Environmental Science and Pollution Research* 22(13):10159-10165.

Kasprzak KS (1995) Possible role of oxidative damage in metal-induced carcinogenesis. *Cancer Investigation* 13:411–430.

Keshava C, Keshava N, Zhou G, Whong WZ, Ong T M (1999) Genomic instability in silica-and cadmium chloride-transformed BALB/c-3T3 and tumor cell lines by random amplified polymorphic DNA analysis. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis* 425(1):117-123.

Khan S, Cao Q, Zheng YM, Huang YZ, Zhu YG (2008) Health risks of heavy metals in contaminated soils and food crops irrigated with wastewater in Beijing, China. *Environmental pollution* 152(3):686-692.

Khazaei Z, Esmaielpour B, Estaji A (2020) Ameliorative effects of ascorbic acid on tolerance to drought stress on pepper (*Capsicum annuum* L) plants. *Physiology and Molecular Biology of Plants* 26(8):1649-1662.

Khush GS (1995) Modern varieties - their real contribution to food supply and equity. Geojournal 35(3):275-284.

Kibria MG, Hossain M, Murata Y, Hoque MA (2017) Antioxidant defence mechanisms of salinity tolerance in rice genotypes. *Rice Science* 24(3):155-162.

Kikui S, Sasaki T, Maekawa M, Miyao A, Hirochika H, Matsumoto H, Yamamoto, Y (2005) Physiological and genetic analyses of aluminium tolerance in rice, focusing on root growth during germination. *Journal of Inorganic Biochemistry* 99(9):1837-1844.

Koh TL, Khoo G, Fan LQ, Phang VPE (1999) Genetic diversity among wild forms and cultivated varieties of discus (*Symphysodon* spp.) as revealed by random

amplified polymorphic DNA (RAPD) fingerprinting. *Aquaculture* 173(1-4):485-497.

Kopyra M, Gwóźdź EA (2003) Nitric oxide stimulates seed germination and counteracts the inhibitory effect of heavy metals and salinity on root growth of Lupinus luteus. *Plant Physiology and Biochemistry* 41(11-12):1011-1017.

Korpe DA, Aras S (2011) Evaluation of copper-induced stress on eggplant (Solanum melongena L.) seedlings atthe molecular and population levels by use of various biomarkers. *Mutation Research* 719 29–34.

Kováčik J, Klejdus B, Hedbavny J, Bačkor M (2010) Effect of copper and salicylic acid on phenolic metabolites and free amino acids in Scenedesmus quadricauda (Chlorophyceae). *Plant Science* 178(3):307-311.

Kranner I, Colville L (2011) Metals and seeds: biochemical and molecular implications and their significance for seed germination. *Environmental and Experimental Botany 72*(1): 93-105.

Kwak JM, Mori IC, Z. M. Pei ZM (2003) "NADPH oxidase AtrbohD and AtrbohF genes function in ROS-dependent ABA signaling in *Arabidopsis*," *EMBO Journal*, vol. 22, no. 11, pp. 2623–2633, 2003.

Lalmuanpuii R, Zodinpuii B, Lalbiaknunga J (2021) Traditional food preparation of wild edible vegetables among the ethnic groups of Mizoram, North East India. *Journal of Ethnic Foods* 8(1):1-11.

Li R, Jia Z, Trush MA (2016) Defining ROS in biology and medicine. *Reactive oxygen species (Apex, NC)* 1(1): 9.

Liang Y, Gao Y, Lin Q, Luo F, Wu W, Lu Q, Liu Y (2014) A review of the research progress on the bioactive ingredients and physiological activities of rice bran oil. *European Food Research and Technology* 238(2):169-176.

Lidon FC, Henriques FS (1991) Limiting step on photosynthesis of rice plants treated with varying copper levels. *Journal of plant physiology* 138(1):115-118.

Lin AJ, Zhang XH, Chen MM, Cao Q (2007) Oxidative stress and DNA damages induced by cadmium accumulation. *Journal of Environmental Science* 19: 596–602.

Lin H, Ya-li P, Jun C, Liang L (2015) "Effect of heavy metal stress on antioxidase enzymes." In *Proceedings of international conference on manufacturing science and engineering (ICMSE 2015)* 871-876.

Liu W, Yang YS, Zhou Q, Xie L, Li PA, Sun T (2007) Impact assessment of cadmium contamination on rice (*Oryza sativa* L.) seedlings at molecular and population levels using multiple biomarkers. *Chemosphere* 67(6):1155-1163.

Liu Y, Wang X, Zeng G, Qu D, Gu J, Zhou M, Chai L (2007) Cadmium-induced oxidative stress and response of the ascorbate–glutathione cycle in Bechmeria nivea (L.) Gaud. *Chemosphere* 69(1):99-107.

Liu ZJ, Li P, Argue BJ, Dunham RA (1999) Random amplified polymorphic DNA markers: usefulness for gene mapping and analysis of genetic variation of catfish. *Aquaculture* 174(1-2):59-68.

Lowry OH, Rosbrough NJ, Farr AL, Randall RJ. *Journal of Biological and Chemical* 193:265-1951.

Lozano R, Azcon R, Palma JM (1996) SOD and drought stress in *Lactua sativa*. *New Phytologist* 136:329–331.

Luna CM, Gonzalez CA, Trippi VS (1994) “Oxidative damage caused by an excess of copper in oat leaves. *Plant and Cell Physiology* 35(1)11–15.

Luna CM, González CA, Trippi VS (1994) Oxidative damage caused by an excess of copper in oat leaves. *Plant and Cell Physiology* 35(1):11-15.

Ma C, Chhikara S, Xing B, Musante C, White JC, Dhankher OP (2013) Physiological and molecular response of *Arabidopsis thaliana* (L.) to nanoparticle cerium and indium oxide exposure. *ACS Sustainable Chemistry & Engineering* 1(7):768-778.

Maheshwari R, Dubey RS (2009) Nickel-induced oxidative stress and the role of antioxidant defense in rice seedlings. *Plant Growth Regulation* 59(1):37-49.

Mahmood T, Islam KR, Muhammad S (2007) Toxic effects of heavy metals on early growth and tolerance of cereal crops. *Pakistan Journal of Botany* 39(2):451.

Malar S, Vikram SS, Favas PJ, Perumal V (2016) Lead heavy metal toxicity induced changes on growth and antioxidative enzymes level in water hyacinths (*Eichhornia crassipes* Mart). *Botanical studies* 55(1):1-11.

Malecka A, Jarmuszkiewicz W, Tomaszewska B (2001) Antioxidative defense to lead stress in subcellular compartments of pea root cells. Acta BiochimPolon 48:687–698

Mandal BK, Suzuki KT (2002) Arsenic round the world: a review. *Talanta 58*(1):201-235.

Manikandan R, Sahi SV, Venkatachalam P (2015) Impact assessment of mercury accumulation and biochemical and molecular response of Mentha arvensis: a potential hyperaccumulator plant. *The Scientific World Journal*.

Márquez-García B, Márquez C, Sanjosé I, Nieva FJJ, Rodríguez-Rubio P, Muñoz-Rodríguez AF (2013) The effects of heavy metals on germination and seedling characteristics in two halophyte species in Mediterranean marshes. *Marine pollution bulletin* 70(1-2):119-124.

Marschner H (2011) *Marschner's mineral nutrition of higher plants*. Academic press.

Mbanjo EGN, Kretzschmar T, Jones H, Ereful N, Blanchard C, Boyd LA, Sreenivasulu N (2020) The genetic basis and nutritional benefits of pigmented rice grain. *Frontiers in genetics* 11:229.

McLaughlin MJ, Singh BR (1999) Cadmium in soils and plants. In *Cadmium in soils and plants* Springer, Dordrecht (1-9).

Merchant S, Sawaya MR (2005) The Light Reactions: A Guide to Recent Acquisitions for the Picture Gallery. *Plant Cell* 17:648–663.

Meriga B, Reddy BK, Rao KR, Reddy LA, Kishor PK (2004) Aluminium-induced production of oxygen radicals, lipid peroxidation and DNA damage in seedlings of rice (*Oryza sativa*). *Journal of plant physiology* 161(1):63-68.

Michalak A (2006) Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. *Polish Journal of Environmental Studies* 15(4).

Miller G, Shulaev V, Mittler R (2008) "Reactive oxygen signaling and abiotic stress," *Physiologia Plantarum* 133(3)481–489.

Mishra S, Jha AB, Dubey RS (2011) Arsenite treatment induces oxidative stress, upregulates antioxidant system, and causes phytochelatin synthesis in rice seedlings. *Protoplasma* 248(3):565-577.

Miteva, E, Merakchiyska, M (2002) Response of chloroplasts and photosynthetic mechanism of bean plants to excess arsenic in soil. *Bulgarian Journal of Agricultural Science*.

Mittler R (2002) "Oxidative stress, antioxidants and stress tolerance," *Trends in Plant Science* 7(9)405–410.

Mittler R (2002) Oxidative stress, antioxidants and stress tolerance. *Trends in plant science* 7(9):405-410.

Moosavi SA, Gharineh MH, Afshari RT, Ebrahimi A (2012) Effects of some heavy metals on seed germination characteristics of Canola (*Barassica napus*), Wheat (*Triticum aestivum*) and sunflower (*Carthamus tinctorious*) to evaluate phytoremediation potential of these crops. *Journal of Agricultural Science* 4(9):11.

Mortvedt JJ (2000) Bioavailability of micronutrients. In: Sumner ME (ed) Handbook of Soil Science. *CRC Press, Boca Raton* 71–88.

Moustakas M, Ouzounidou G (1994) stressed wheat plants is due to Al"-induced elemental loss. *Plant Physiology Biochemistry 32*(4):527-532.

Mukhopadhyay M, Das A, Subba P, Bantawa P, Sarkar B, Ghosh P, Mondal TK (2013) Structural, physiological, and biochemical profiling of tea plantlets under zinc stress. *Biologia Plantarum* 57(3):474-480.

Munzuroglu, O, Geckil HİKMET (2002) Effects of metals on seed germination, root elongation, and coleoptile and hypocotyl growth in *Triticum aestivum* and *Cucumis sativus*. *Archives of Environmental Contamination and Toxicology 43*(2):203-213.

Murtaza G, Javed W, Hussain A, Wahid A, Murtaza B, Owens G (2015) Metal uptake via phosphate fertilizer and city sewage in cereal and legume crops in Pakistan. *Environmental Science and Pollution Research* 22(12):9136-9147.

Nakano Y, Asada K (1981) Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant and cell physiology* 22(5):867-880.

Neill S, Desikan R, Hancock J (2002) Hydrogen peroxide signalling. *Current opinion in plant biology* 5(5):388-395.

Nelson N, Ben-Shem A (2004) The Complex Architecture of Oxygenic Photosynthesis. Nature Reviews Molecular Cell Biology 5:971–982.

Nguyen NV, Ferrero A (2006) Meeting the challenges of global rice production. pp 1-9.

Nishijo M, Nakagawa H, Suwazono Y, Nogawa K, Kido T (2017) Causes of death in patients with Itai-itai disease suffering from severe chronic cadmium poisoning: a nested case–control analysis of a follow-up study in Japan. *BMJ open* 7(7):015694.

Noctor G, Veljovic-Jovanovic SONJA, Driscoll S, Novitskaya L, Foyer CH (2002) Drought and oxidative load in the leaves of C3 plants: a predominant role for photorespiration? *Annals of Botany* 89(7):841-850.

Noulas C, Tziouvalekas M, Karyotis T (2018) Zinc in soils, water and food crops. *Journal of Trace Elements in Medicine and Biology* 49:252-260.

Nussbaum, S., Schmutz, D., & Brunold, C. (1988). Regulation of assimilatory sulfate reduction by cadmium in Zea mays L. *Plant Physiology*, *88*(4), 1407-1410.

Okoronkwo NE, Igwe JC, Onwuchekwa EC (2005) Risk and health implications of polluted soils for crop production. *African Journal of Biotechnology 4*(13).

Olaniran AO, Balgobind A, Pillay B (2013) Bioavailability of heavy metals in soil: impact on microbial biodegradation of organic compounds and possible improvement strategies. *International journal of molecular sciences* 14(5):10197-10228.

Oner O, Sukru K, Mehmet C (2011) Evaluation of groundwater quality and contamination around fluorite mineralization kaman region, central anatolia, turkey. *Geochemistry* (1):81-95.

Ouzounidou G, Eleftheriou EP, Karataglis S (1992) Ecophysical and ultrastructural effects of copper in Thlaspi ochroleucum (Cruciferae). *Canadian Journal of Botany* 70(5):947-957.

Panda P, Nath S, Chanu TT, Sharma GD, Panda SK (2011) Cadmium stress-induced oxidative stress and role of nitric oxide in rice (*Oryza sativa* L.). *Acta Physiologiae Plantarum* 33(5):1737-1747.

Panda SK, Baluška F, Matsumoto H (2009) Aluminum stress signaling in plants. *Plant signaling & behavior* 4(7):592-597.

Panda SK, Chaudhury I, Khan MH (2003) Heavy metals induce lipid peroxidation and affect antioxidants in wheat leaves. Biol Plant 46:289–294

Panda SK, Choudhury S. "Chromium stress in plants." *Brazilian journal of plant physiology* 17 (2005): 95-102.

Pang CH, Wang BS (2008) Oxidative stress and salt tolerance in plants. In *Progress in botany* (pp231-245). Springer, Berlin, Heidelberg.

Pant PP, Tripathi AK (2014) Impact of heavy metals on morphological and biochemical parameters of Shorearobusta plant. *Ekológia*, 33(2):116.

Papademetriou MK (2000) Rice production in the Asia-Pacific region: issues and perspectives. *Bridging the rice yield gap in the Asia-Pacific region* 220.

Parekh D, Puranik RM, Srivastava HS (1990) Inhibition of chlorophyll biosynthesis by cadmium in greening maize leaf segments. *Biochemie und Physiologie der Pflanzen* 186(4):239-242.

Parth V, Murthy NN, Saxena PR (2011) Assessment of heavy metal contamination in soil around hazardous waste disposal sites in Hyderabad city (India): natural and anthropogenic implications. *Journal of Environmental Research and Management* 2:27–34

Patnaik MC, Raju AS, Raj GB (2008) Effect of soil moisture regimes on zinc availability in a red sandy loam soil of Andhra Pradesh. *Journal of the Indian Society of Soil Science* 56(4):452-453.

Payus C, Talip AFA (2014) Assessment of heavy metals accumulation in paddy rice (Oryza sativa). *African Journal of Agricultural Research* 9(41):3082-3090.

Petroni K. Landoni M, Tomay F, Calvenzani V, Simonelli C, Cormegna M (2017) Proximate composition, polyphenol content and anti-inflammatory properties of white and pigmented Italian rice varieties. *Universal Journal of Agricultural Research* 5(5):312-321.

Pierzynski GM, Sims JT, Vance GF (2000) Soils and Environmental Quality, CRC Press, London, UK, 2nd edition.

Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V, Squadrito F, Altavilla D, Bitto A (2017) Oxidative stress: harms and benefits for human health. *Oxidative medicine and cellular longevity*.

Poschenrieder C, Gunse B, Barcelo J (1989) Influence of cadmium on water relations, stomatal resistance, and abscisic acid content in expanding bean leaves. *Plant Physiology* 90(4):1365-1371.

Prasad AS (2008) Zinc in human health: effect of zinc on immune cells. *Molecular medicine* 14(5):353-357.

Prasad KVSK, Saradhi PP, Sharmila P (1999) Concerted action of antioxidant enzymes and curtailed growth under zinc toxicity in *Brassica juncea*. *Environmental and experimental Botany* 42(1):1-10.

Qian J, Shan X-Q, Wang Z-J and Tu Q (1996) Distribution and plant availability of heavy metals in different particle size fractions of soil. *The Science of the Total Environment* 187:131–141.

Qishlaqi A, Moore F, Forghani G (2008) Impact of untreated wastewater irrigation on soils and crops in Shiraz suburban area, SW Iran. *Environmental monitoring and assessment* 141(1):257-273.

Rai R, Agrawal M, Agrawal SB (2016) Impact of heavy metals on physiological processes of plants: with special reference to photosynthetic system. In Plant Responses to Xenobiotics. *Springer, Singapore* 127−140.

Raja V, Qadir SU, Alyemeni MN, Ahmad P (2020) Impact of drought and heat stress individually and in combination on physio-biochemical parameters, antioxidant responses, and gene expression in *Solanum lycopersicum*. *Biotechnology* 10(5):1-18.

Rao KVM, Sresty TVS (2000) Antioxidant parameters in the seedlings of pigeon pea (Cajanus cajan, (L.) Millspaugh) in response to Zn and Ni stresses. *Plant Science* 157:113–128.

Rascio N, Navari-Izzo F (2011) Heavy metal hyperaccumulating plants: how and why do they do it? And what makes them so interesting? *Plant science* 180(2):169-181.

Rastgoo L, Alemzadeh A, Afsharifar A (2011) Isolation of two novel isoforms encoding zinc-and copper-transporting P1B-ATPase from Gouan (Aeluropuslittoralis). *Plant Omics Journal* 4(7):377-383.

Rieuwerts JS, Thornton I, Farago ME, Ashmore MR (1998) Factors influencing metal bioavailability in soils: preliminary investigations for the development of a critical loads approach for metals. *Chemical Speciation & Bioavailability* 10(2): 61-75.

Rocco L, Frenzilli G, Fusco D, Peluso C, Stingo V (2010) Evaluation of zebrafish DNA integrity after exposure to pharmacological agents present in aquatic environments. *Ecotoxicology and Environmental Safety* 73(7):1530-1536.

Rocco L, Frenzilli G, Zito G, Archimandritis A, Peluso C, Stingo V (2012) Genotoxic effects in fish induced by pharmacological agents present in the sewage of some Italian water-treatment plants. *Environmental toxicology 27*(1):18-25.

Rocco L, Izzo A, Zito G, Peluso C, Stingo V (2011) Genotoxicity in zebrafish (Danio rerio) exposed to two pharmacological products from an impacted Italian river. *Journal of Environmental and Analytical Toxicology* 1(103):2161-0525.

Rocco L, Valentino IV, Scapigliati G, Stingo V (2014) RAPD-PCR analysis for molecular characterization and genotoxic studies of a new marine fish cell line derived from Dicentrarchus labrax. *Cytotechnology* 66(3):383-393.

Roy S, Banerjee A, Pattanayak A, Roy SS, Rathi RS, Misra AK, Ngachan SV & Bansal K (2014) Chakhao (delicious) rice landraces (*Oryza sativa* L.) of North-east India: collection, conservation and characterization of genetic diversity. *Plant Genetic Resources* 12(3):264–272.

Salah SM, Yajing G, Dongdong C, Jie L, Aamir N, Qijuan H, Weimin H, Mingyu N, Jin H (2015) Seed priming with polyethylene glycol regulating the physiological and molecular mechanism in rice (*Oryza sativa* L.) under nano-ZnO stress. *Scientific Reports* 5(1):1-14.

Saraswathy N, Ramalinga P. (2011) *Concepts and techniques in genomics and proteomics*. Elsevier.

Sarker U, Oba S (2018) Catalase, superoxide dismutase and ascorbate-glutathione cycle enzymes confer drought tolerance of Amaranthus tricolor. *Scientific reports 8*(1):1-12.

Satpathy D, Reddy MV, Dhal SP (2014) Risk assessment of heavy metals contamination in paddy soil, plants, and grains (*Oryza sativa* L.) at the East Coast of India. *BioMed research international*.

Savva D (1998) Use of DNA fingerprinting to detect genotoxic effects. *Ecotoxicology and Environmental Safety 41*(1):103-106.

Schieber M, Chandel NS (2014) ROS function in redox signaling and oxidative stress. *Current biology 24*(10):R453-R462.

Schutzendubel A, Polle A (2002) Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization. *Journal of experimental botany* 53(372): 1351-1365.

Seneviratne M, Rajakaruna N, Rizwan M, Madawala HMSP, Ok YS, Vithanage M (2019) Heavy metal-induced oxidative stress on seed germination and seedling development: a critical review. *Environmental Geochemistry and Health* 41(4):1813-1831.

Seshadri B, Bolan NS, Naidu R (2015) Rhizosphere-induced heavy metal (loid) transformation in relation to bioavailability and remediation. *Journal of soil science and plant nutrition* 15(2):524-548.

Shah K, Kumar RG, Verma S, Dubey RS (2001) Effect of cadmium on lipid peroxidation, superoxide anion generation and activities of antioxidant enzymes in growing rice seedlings. *Plant Science* 161(6):1135-1144.

Shah K, Kumar RG, Verma S, Dubey RS (2001) Effect of cadmium on lipid peroxidation, superoxide anion generation and activities of antioxidant enzymes in growing rice seedlings. *Plant Science* 161(6):1135-1144.

Shah SU (2012) Importance of Genotoxicity & S2A guidelines for genotoxicity testing for pharmaceuticals. *IOSR Journal of Pharmacy and Biological Sciences* 1(2):43-54.

Shah T, Munsif F, D'amato R, Nie L (2020) Lead toxicity induced phytotoxic impacts on rapeseed and clover can be lowered by biofilm forming lead tolerant bacteria. *Chemosphere* 246:125766.

Shahid M, Khalid S, Abbas G, Shahid N, Nadeem M, Sabir M, Aslam M, Dumat C, (2015) Heavy metal stress and crop productivity. In *Crop production and global environmental issues* Springer, Cham. 1-25.

Shahid M, Pourrut B, Dumat C, Nadeem M, Aslam M, Pinelli E (2014) Heavy metal-induced reactive oxygen species: phytotoxicity and physicochemical changes in plants. *Reviews of Environmental Contamination and Toxicology* 232:1-44.

Shanmugam V, Lo JC, Wu CL, Wang SL, Lai CC, Connolly EL, Huang JL, Yeh KC (2011) Differential expression and regulation of iron-regulated metal transporters in Arabidopsis halleri and Arabidopsis thaliana–the role in zinc tolerance. *New Phytologist* 190(1):125-137.

Sharma P, Dubey RS (2005) Drought induces oxidative stress and enhances the activities of antioxidant enzymes in growing rice seedlings. *Plant growth regulation* 46(3):209-221.

Sharma P, Dubey RS (2007) Involvement of oxidative stress and role of antioxidative defense system in growing rice seedlings exposed to toxic concentrations of aluminum. *Plant cell reports* 26(11):2027-2038.

Sharma P, Jha AB, Dubey RS (2019) Oxidative stress and antioxidative defense system in plants growing under abiotic stresses. In Handbook of Plant and Crop Stress, Fourth Edition (pp. 93-136). CRC press.

Sharma P, Jha AB, Dubey RS, Pessarakli M (2012) Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *Journal of botany*.

Singh A, Kumar A, Yadav S, Singh IK (2019) Reactive oxygen species-mediated signaling during abiotic stress. Plant Gene 18:100173.

Solanki R, Dhankhar R (2011) Biochemical changes and adaptive strategies of plants under heavy metal stress. *Biologia* 66(2):195-204.

Solovchenko A, Yahia EM, Chen C (2019) Pigments. In *Postharvest Physiology and Biochemistry of Fruits and Vegetables. Woodhead Publishing* 225-252.

Song R, Zhao CY, Liu J, Zhang J, Du YX, Li JZ, Sun HZ, Zhao HB, Zhao QZ 2013. Effect of sulphate nutrition on arsenic translocation and photosynthesis of rice seedlings. *Acta physiologiae plantarum* 35(11):3237-3243.

Souguir D, Ferjani E, Ledoigt G, Goupil P (2011) Sequential effects of cadmium on genotoxicity and lipoperoxidation in Vicia faba roots. *Ecotoxicology* 20:329–336.

Srivastava HS (1999) Biochemical defence mechanisms of plants to increased levels of ozone and other atmospheric pollutants. *Current Science* 76:525–533.

Srivastava S, Dubey RS (2011) Manganese-excess induces oxidative stress, lowers the pool of antioxidants and elevates activities of key antioxidative enzymes in rice seedlings. *Plant Growth Regulation* 64(1):1-16.

Sundaramoorthy P, Chidambaram A, Ganesh KS, Unnikannan P, Baskaran L (2010) Chromium stress in paddy:(i) nutrient status of paddy under chromium stress;(ii) phytoremediation of chromium by aquatic and terrestrial weeds. *Comptes rendusbiologies* 333(8):597-607.

Tang J, Zhang L, Zhang J, Ren L, Zhou Y, Zheng Y, Chen A (2020) Physicochemical features, metal availability and enzyme activity in heavy metal-polluted soil remediated by biochar and compost. *Science of the Total Environment* 701:134751.

Tangahu BV, Sheikh Abdullah SR, Basri H, Idris M, Anuar N, Mukhlisin M (2011) A review on heavy metals (As, Pb, and Hg) uptake by plants through phytoremediation. *International Journal of Chemical Engineering*.

Theodorakis CW, Lee KL, Adams SM, Law CB (2006) Evidence of altered gene flow, mutation rate, and genetic diversity in redbreast sunfish from a pulp-mill-contaminated river. *Environmental science & technology* 40(1):377-386.

Thornton I (1996) Risk assessment related to metals: The role of the geochemist. Report of the International Workshop on Risk Assessment of Metals and their Inorganic Compounds, Angers, France, November 1996.

Thounaojam TC, Panda P, Mazumdar P, Kumar D, Sharma GD, Sahoo L, Sanjib P (2012) Excess copper induced oxidative stress and response of antioxidants in rice. *Plant physiology and biochemistry* 53:33-39.

Tian CM, Zhong QP (2005) Advance in current research of superoxide dismutase. *China Tropical Medicine* 8.

Torres MA, Dangl JL, Jones JDG (2002) "*Arabidopsis* gp91^{phox} homologues Atrbohd and Atrbohf are required for accumulation of reactive oxygen intermediates in the plant defense response," *Proceedings of the National Academy of Sciences of the United States of America* 99(1)517–522.

Tripathy BC, Oelmüller R (2012) Reactive oxygen species generation and signaling in plants. *Plant signaling & behavior* 7(12): 1621-1633.

Tsonev T, Lidon FJC (2012) Zinc in plants—an overview. *Emirates Journal of Food & Agriculture* 24(4):322–333.

Vajpayee P, Tripati RD, Rai UN, Ali MB, Singh SN (2000) Chromiun accumulation reduces chlorophyll biosynthesis, nitrate reductase activity and protein content of Nymphaea alba. Chemosphere 41:1075–1082

Valko MMHCM, Morris H, Cronin MTD (2005) Metals, toxicity and oxidative stress. *Current medicinal chemistry* 12(10):1161-1208.

Vanlalsanga, Singh SP, Singh YT (2019) Indigenous rice of Northeast India harbor rich genetic diversity as measured by SSR markers and Zn/Fe content. *BMC genetics* 20(1):1-13.

Vassilev A, Nikolova A, Koleva L, Lidon F (2011) Effects of excess Zn on growth and photosynthetic performance of young bean plants. *Journal of phytology*, *3*(6).

Verma S, Dubey RS (2003) Lead toxicity induces lipid peroxidation and alters the activities of antioxidant enzymes in growing rice plants. *Plant Science* 164(4):645-655.

Viehweger K (2014) How plants cope with heavy metals. *Botanical Studies*, 55(1):1-12.

Welsh J, McClelland M (1990) Fingerprinting genomes using PCR with arbitrary primers. *Nucleic acids research* 18(24):7213-7218.

Williams JG, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic acids research* 18(22):6531-6535.

Wolf HD, Blust R, Backeljiau T (2004) The use of RAPD in ecotoxicology. A Review. *Mutation Research* 566:249–262.

Wuana RA, Okieimen FE (2011) Heavy metals in contaminated soils: a review of sources, chemistry, risks and best available strategies for remediation. *International Scholarly Research Notices*.

Xie J, Yao S, Ming J (2019) Variations in chlorophyll and carotenoid contents and expression of genes involved in pigment metabolism response to oleocellosis in citrus fruits. *Food Chemistry* 272:49–57.

Xiong L, Schumaker KS, Zhu JK (2002) Cell signaling during cold, drought, and salt stress. *The plant cell* 14:S165-S183.

Yamamoto F, Kozlowski TT (1987) Effects of flooding, tilting of stems, and ethrel application on growth, stem anatomy and ethylene production of *Pinus densiflora* seedlings. *Journal of Experimental Botany* 38(2):293-310.

Yamauchi Y, Furutera A, Seki K, Toyoda Y, Tanaka K, Sugimoto Y (2008) Malondialdehyde generated from peroxidized linolenic acid causes protein modification in heat-stressed plants. *Plant physiology and biochemistry* 46(8-9):786-793.

Yan J, Tsuichihara N, Etoh T, Iwai S (2007) "Reactive oxygen species and nitric oxide are involved in ABA inhibition of stomatal opening," *Plant, Cell and Environment*. 30(10)1320–1325.

Yang S, Ulhassan Z, Shah AM, Khan AR, Azhar W, Hamid Y, Hussain S, Sheteiwy MS, Salam A, Zhou W (2021) Salicylic acid underpins silicon in ameliorating chromium toxicity in rice by modulating antioxidant defense, ion homeostasis and cellular ultrastructure. *Plant Physiology and Biochemistry* 166:1001-1013.

Yang Y, Zhang L, Huang X, Zhou Y, Quan Q, Li Y, Zhu X (2020) Response of photosynthesis to different concentrations of heavy metals in Davidia involucrata. *PloS one* 15(3):0228563.

Ying RR, Qiu RL, Tang YT, Hu PJ, Qiu H, Chen HR, Shi TH, Morel JL (2010) Cadmium tolerance of carbon assimilation enzymes and chloroplast in Zn/Cd hyperaccumulator *Picris divaricata*. *Journal of plant physiology* 167(2):81-87.

Yoo MS, James BR (2003) Zinc extractability and plant uptake in flooded, organic waste-amended soils. *Soil science* 168(10):686-698.

Younis A, Ramzan F, Ramzan Y, Zulfiqar F, Ahsan M, Lim KB (2020) Molecular markers improve abiotic stress tolerance in crops: a review. *Plants* 9(10):1374.

Yücel G, Cüneyt AK I (2019) Effects of some of heavy metals on total protein amount and peroxidase activity in *Solanum lycopersicum* Mill. *Journal of Scientific Perspectives* 3(4):371-378.

Zabalza A, Gálvez L, Marino D, Royuela M, Arrese-Igor C, González EM (2008) The application of ascorbate or its immediate precursor, galactono-1, 4-lactone, does not affect the response of nitrogen-fixing pea nodules to water stress. *Journal of plant physiology* 165(8):805-812.

Zengin FK, Munzuroglu, O (2005) Effects of some heavy metals on content of chlorophyll, proline and some antioxidant chemicals in bean (Phaseolus vulgaris L.) seedlings. *Acta BiologicaCracoviensia Series Botanica* 47(2):157-164.

Zengin, F. K., & Munzuroglu, O. (2006). Toxic effects of cadmium (Cd++) on metabolism of sunflower (Helianthus annuus L.) seedlings. *Acta Agriculturae Scandinavica Section B-Soil and Plant Science*, *56*(3), 224-229.

Zhang H, Shao Y, Bao J, Beta T (2015) Phenolic compounds and antioxidant properties of breeding lines between the white and black rice. *Food Chemistry* 172:630-639.

Zhang H, Zhang LL, Li J, Chen M, An RD (2020) Comparative study on the bioaccumulation of lead, cadmium and nickel and their toxic effects on the growth and enzyme defence strategies of a heavy metal accumulator, *Hydrilla verticillata* (Lf) Royle. *Environmental Science and Pollution Research* 1-13.

Zhang MK, Liu ZY, Wang H (2010) Use of single extraction methods to predict bioavailability of heavy metals in polluted soils to rice. *Communications in Soil Science and Plant Analysis* 41(7):820-831.

Zhang Z, Henderson C, Gurr SJ (2004) *Blumeria graminis* secretes an extracellular catalase during infection of barley: potential role in suppression of host defence. *Molecular plant pathology* 5(6):537-547.

Zhou B, Yao W, Wang S, Wang X, Jiang T (2014) The metallothionein gene, TaMT3, from Tamarixandrossowii confers Cd2+ tolerance in tobacco. *International journal of molecular sciences* 15(6):10398-10409.

Zhu Y, Luo X, Nawaz G, Yin J, Yang J (2020) Physiological and biochemical responses of four cassava cultivars to drought stress. *Scientific reports*, *10*(1), 1-12.

Zimdahl RL, Skogerboe RK (1977) Behavior of lead in soil. *Environmental Science & Technology* 11(13):1202-1207.

Zlobin IE, Kholodova VP, Rakhmankulova ZF, Kuznetsov VV (2015) *Brassica napus* responses to short-term excessive copper treatment with decrease of photosynthetic pigments, differential expression of heavy metal homeostasis genes including activation of gene NRAMP4 involved in photosystem II stabilization. *Photosynthesis research* 125(1):141-150.

BIO-DATA

1. Name : Sagolshem Priyokumar Singh
2. Father's Name : Sagolshem Shyamsundar Singh
3. Mother's Name : Nongmaithem Radhamani Devi
4. Date of Birth : 14-02-1989
5. Sex : Male
6. Marital Status : Single
7. Permanent Address : Mutum Phibou Mayai Leikai, Imphal West, Manipur, 795132
8. Phone / Mobile : +91-9615413198
9. E-mail : priyosagolshem@gmail.com
10. Education Qualification :

Sl. No.	Exam Passed	Year	Name of Board / University	Specialization or Subjects	% obtained
1	HSC / 10th	2004	Board of Secondary Education Manipur (BOSEM)	Mathematics, English, Sc, Social Sc, Manipuri, Computer Sc.	45.33
2	SSC/ 10+2	2006	Council of Higher Secondary Education Manipur	English, Manipuri, Computer Sc, Physics, Chemistry, Biology	57.40
3	GRADUATE	2010	Manipur University (MU)	B.Sc. Botany Honors	48.77
4	MASTERS	2013	University of Pune	M.Sc. Botany	64.20

Conference proceeding

1. Presented paper on "Impact Assessment of Zinc Stress on Pigmented Rice Cultivars of Northeast India" at 2nd Annual Convention of North East (India) Academy of Science and Technology (NEAST) & International Seminar on Recent Advances in Science and Technology (ISRAST) (16th -18th November 2020)
2. Presented paper on "Influence of Lead stress on Antioxidant activity and DNA damage in pigmented Rice of Northeast India" at International Conference on Biotechnology for Environment and Health (ICBEH) 25 to 27 November 2021.

Seminar and workshop attended

1. National Seminar on "Biodiversity, Conservation and Utilization of Natural Resources with References to Northeast India (BCUNRNEI)" during 30th - 31st March 2017 organized by Department of Botany, Mizoram University.
2. Training course on "Introduction to Molecular Entomology" during 7th-12th August, 2017 at Hyderabad organized by Agri Biotech Foundation.
3. Workshop on "Statistical and Computing Methods for Life -Science" during 5thy -10th march, 2018 organized by Department of Botany, Mizoram University and Indian Statistical Institute, Biological Anthropology Unit, Kolkata at Department of Botany, Mizoram University.

List of Paper Publications

1. **Sagolshem Priyokumar Singh** and Y. Tunginba Singh* (2021) Impact assessment of zinc stress on pigmented rice (Oryza sativa) cultivars in Northeast India. *Res. On Crops*. 22(1): 18-23.
2. Vanlalsanga, **Sagolshem Priyokumar Singh**, Y. Tunginba Singh (2019) Indigenous rice of Northeast India harbor rich genetic diversity as measured by SSR markers and Zn/Fe content. *BMC genetics* 20(1):1-13.

PARTICULARS OF THE CANDIDATE

NAME OF THE CANDIDATE : Sagolshem Priyokumar Singh

Degree : Ph.D.

TITLE OF THESIS : Responses to zinc (Zn^{2+}) stress by selected Rice (*Oryza Sativa* L.) varieties of North East India

Date of Admission : .08.2016

APPROVAL OF RESEARCH APPROVAL

1. DRC : 24.04.2017
2. BOS : 01.05.2017
3. SCHOOL BOARD : 26.05.2017

MZU REGISTRATION No. : 1600797

PH. D REGISTRATION No. & DATE : MZU/Ph.D./1031 of 26.05.2017

(Dr. R. LALFAKZUALA)

Head

Department of Botany

ABSTRACT

RESPONSES TO ZINC (Zn^{2+}) STRESS BY SELECTED RICE (*Oryza sativa* L.) VARIETIES OF NORTH EAST INDIA

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

SAGOLSHEM PRIYOKUMAR SINGH

MZU REGISTRATION NO: 1600797

Ph.D. REG NO: MZU/Ph.D./1031 of 26.05.2017

DEPARTMENT OF BOTANY

SCHOOL OF LIFE SCIENCES

DECEMBER, 2021

RESPONSES TO ZINC (Zn^{2+}) STRESS BY SELECTED RICE (*Oryza sativa* L.) VARIETIES OF NORTH EAST INDIA

BY

SAGOLSHEM PRIYOKUMAR SINGH

DEPARTMENT OF BOTANY

SUPERVISOR

Dr. Y. TUNGINBA SINGH

DEPARTMENT OF BOTANY

MIZORAM UNIVERSITY

SUBMITTED

IN PARTIAL FULFILLMENT OF THE REQUIREMENT OF THE DEGREE OF DOCTOR OF PHILOSOPHY IN BOTANY OF MIZORAM UNIVERSITY MIZORAM

ABSTRACT

Rice is an important staple food used daily by more than half of the world's population, particularly in Asia. And, heavy metal poisoning is a severe abiotic stress that impacts rice production. The important sources of heavy metal contamination are natural sources (wind-blown dust, decaying vegetation, forest fires, sea spray) and anthropogenic activities (mining, metal production; wood production, and phosphate fertilizer). Metals found in the soil can be categories into two types: essential elements and non-essential elements. Metals like Zn, Mn, Ni, and Cr are essential elements as they are needed in small amounts by both plants and animals, and they are also important for the physical growth and development of agricultural plants like rice. Non-essential elements are those that do not have known biological and physiological functions and they include Cd, Sb, Cr, Pb, As, Co, Ag, Se, and Hg. Although some metals such as Zn, Cr, Ni, and Mn are important minerals, their toxicity to humans and animals are of great concern when they are present in higher concentrations.

Rice grain produced from paddy soils contaminated with heavy metals (HMs) such as As, Al, Cu, Cr, Cd, Pb, Hg, Mn, Se, and Zn is a major source of HM consumption by humans in most nations. As a result, the gradual aggregation of HMs in rice grains and subsequent transmission to the food chain poses a significant threat to agriculture and human health. Several studies in recent years have looked at the influence of HMs toxicity on rice at multiple levels, including molecular, biochemical, physiological, cellular, and tissue, and found a link between HMs toxicity and declining rice productivity. As a result, it is critical to understand how HMs interact with rice crops at all levels, from the cell to the whole plant, and to create effective ways to mitigate these stress reactions.

In the present study, two popular aromatic local rice varieties of northeast India, Kawnglawng (Mizoram) and Chakhao (Manipur) were analyzed for their reposed

against Zn stress. Physiological and biochemical parameters like seed germination percentage, chlorophyll a and b content, total soluble protein, and antioxidant enzyme activity such as Catalase (CAT) activity, Ascorbate peroxidase (APX) activity, and Superoxide dismutase (SOD) activity were investigated under Zn stress by exposing the rice at different concentrations of $ZnSO_4$ (5mM, 10mM and 15mM). The genotoxicity of Zn stress was also evaluated using a PCR base RAPD technique and the Genomic template stability (GTS) was calculated from the RAPD profiles.

Results from my study revealed that Zn stress did not have a significant impact on seed germination percentage as both the rice varieties showed 100% germination. However, Zn stress inhibited shoot growth and root formation in both the studied rice varieties. Then, decrease in chlorophyll a and b content was found with increasing concentration of Zn and rice treated with 15mM Zn sulphate concentration was found to have the minimum levels of chlorophyll a and b. Total soluble protein content was also found to decrease with the increasing concentration of Zn. HMs are considered to hinder protein's biological functions by bonding to it and altering its native structure. The reduction in chlorophyll content in plants exposed to heavy metal stress is also hypothesized to be caused by inhibition of chlorophyll biosynthesis enzymes such as δ-aminolaevulinic acid dehydrates and protochloro-phyllide reductase.

Antioxidant activity in response to Zn stress showed high CAT, APX, and SOD activities and it was also found that antioxidant activities significantly increased with the increasing concentration of Zn. Heavy metals induced oxidative stress by overproduction of reactive oxygen species (ROS) and in order to scavenge these ROS, plants produced antioxidant enzymes such as CAT, APX, and SOD as a defense mechanism. Finally, genotoxicity analysis using RAPD-PCR and GTS showed that Zn stress did not have a significant impact on DNA damage. Hence, these rice varieties can be selected as potential parental lines for breeding against Zn stress.