THE EFFECT OF SODIUM SELENITE ON SALINITY STRESSED Triticum aestivum

BY

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CERTIFICATION

This is to certify that this project work was carried out by Ojo Mercy Boyede of the Department of Plant Science and Biotechnology, Federal University Oye-Ekiti, Ekiti state. The report has been read and approved as meet the requirement for the award of Bachelor of Science (B.Sc) Degree in plant science and Biotechnology, Federal University Oye Ekiti, Ekiti state.

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DEDICATION

This project is dedicated to God almighty the Alpha and Omega for the breath of life, wisdom knowledge and understanding. I am forever grateful to him.

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All thanks to God Almighty the maker of the whole universe who happens to be the author and the finisher of my faith who has also made this project a success by sustaining my life.

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ABSTRACT

The investigation was aimed to determine the responses of wheat to selenite under salinity stress. Wheat cultivar was collected from NACGRAB (National Centre for Genetic Resource and Biotechnology) Ibadan, Oyo. The seed were sterilized and treated with different concentration of selenite (50mg/l, 100mg/l and 150mg/l). By soaking after eight hours of soaking seed was properly rinsed with distil water. Top soil was collected, air dried and was filled into a perforated polythene bags (4kg) planting was done and after two weeks of germination salinity stress was introduced at different concentration (S0, S100mM and S200mM) After four weeks of salinity stress introduction growth parameters such as plant height, numbers of leaf and tillers per plant were determined till maturity. It was observed that selenite had a significant effect on numbers of leaf than on tillers both on plant under salinity stress and those not stressed. Biochemical analysis was done on wheat to determine how selenite has improved the antioxidant activities that determine the effect of salinity stress. It was deduced that selenite had a significant effect on Superoxide dismutase, carotenoid, catalase, Malondiaidehyde but had no significant effect on Ascorbate peroxide while there was a significant difference in the green pigment such as chlorophyll a and b in plant treated with selenite 100mg/l under salinity stress 200mM above all other treatment. Plant treated with selenium 100mg/l and under salinity 100mM show a significant difference on yield such as the fresh weight, dry weight, grain weight and numbers of grain.

CHAPTER ONE

1.0 INTRODUCTION

Background of the study

Wheat *(Triticum aestivum)* is the most extensively grown cereal crop in the world, wheat is an annual grass growing to between ½ to 1 ¼ meters in height, with a long stalk that terminate in a tightly formed cluster of plump kernels enclosed by a bear of bristly spike (Smith, 2010). It is grown all over the world for it highly nutritious and useful grain, as one of the top three most produces crops. It is use in the production of many important foods such as bread, biscuit, feeds, confectionary, amongst many, utilization. The crop which has been cultivated for over 10,000 years probably, originated in Fertile Crescent around 9600 BCE along with other staples. Wheat is commonly known as the king of cereals (Kotal *et al.,* 2010). *Triticum aestivum* is the major source of wheat that contributed to total of 95% wheat cultivation in the world (Shewry, 2009).It is highly preferred by consumer for its nutritious flour which is mainly used for the baking of different varieties of bread and other baked product (Bushuk, 1998).

Wheat is a major cereal crop in many parts of the world and it is commonly known as king of cereals. It belongs to Poaceae family and globally after maize, wheat is the second most produced food among the cereal crops, rice ranks third. High substrate salinity is a major limiting factor for plants in coastal habitats and germination being one of the most critical periods in life cycle of halophytes (Gilles *et al.,* 2001; Rubio-Casal *et al.,* 2003). The nutritional aspect of wheat is addressed through its macronutrient and micronutrient component. These groups consist of carbohydrates, protein and lipids, for macronutrient, grains consists of approximately 75% carbohydrate and therefore many believed that the important of carbohydrate and fibers within wheat takes precedence over their concentration of vitamins, minerals, and photochemical. The second major macro nutrient wheat is protein. the wheat flour contain starch(65-75%) protein (12-14%) most of the essential amino acid and fat(1.5-2% minerals(1.5-2%) vitamin B complex, vitamin E vitamin vitamin K and crude fibres (2-2%) (Izanloo,2008; shewry, 2009).

Selenium is important for human nutrition Selenium (Se) is an important element that was discovered in 1817, and since the 1960s, it has been regarded as an essential micronutrient for both animals and humans, playing among other functions a relevant role in the antioxidant system of mammals. Inadequate blood Se levels in the human body are a well-known concern in many parts of the world. This malnutrition problem is often due to Se-poor diet, probably as a result of the low Se availability in soils where crops are growing. Nowadays, it is known that not only the total content, but also the inorganic and organic forms of Se contained in foods

The essentiality of selenium to higher plants is still under debate (Terry *et al.,* 2000). Selenium can increase the tolerance of plants to UV-induced oxidative stress, delay senescence, and promote the growth of ageing seedlings (Xue et al., 2001; Pennanen et al., 2002). Recently it has been shown that selenium has the ability to regulate the water status of plants under conditions of drought (Kuznetsov et al., 2003). Hartikainen et al. (2000) reported about growth promoting effect of selenium in ryegrass. Senescence stress is partly counteracted with enhanced antioxidation which is associated with an increase glutathione peroxidase (GSH-Px) activity. Although some studies have evaluated the effect of hardness, temperature, pH and other parameters on selenium toxicity, sulphate has perhaps been most widely studied in relation to selenium uptake and toxicity in aquatic and terrestrial organisms (Sappington, 2002). Selenium and sulphur are nutrients with very similar chemical properties and their uptake and assimilation proceed through common pathways (Eapen in D'Souza, 2005).

1.2 STATEMENT OF PROBLEM AND JUSTIFICATION

Wheat which is one of the world most useful and economically important food staple. Despite it great economic valve, an increase in salinity stress poses threats to the availability of this crop plant. Hence sodium selenite which is an element with great important effect on the improvement of with capacity to withstand this salinity effect and not only to withstand but also do well in the aspect of yield.

Selenium (Se) is an essential micronutrient with a range of physiological and anti-oxidative properties. Priming of plant seeds is an easy, low-cost, low-risk, and effective approach to improve plant tolerance under stressful environments. Very little has been done to evaluate the overall role of Selenite seed priming on survival of salinity stressed wheat

1.3 Aims of the study

The overall aim is to determine the responses of *Triticum aestivum to* selenite under salinity stress

1.3.1. OBJECTIVES

- To determine the effect of selenite on growth and yield of wheat under salinity stress
- To investigate the activities of antioxidant enzymes and chlorophyll contents under salinity stress
- To determine the effect of selenite on nutritional composition and lipid of wheat under salinity stress

CHAPTER TWO

LITERATURE REVIEW

2.0. SELENIUM

The element selenium (Se) is placed in the subgroup VIA of the periodic table. It was discovered in 1817 by Swedish chemist JonsJakob Berzelius, who named the new element from the Greek word 'Selene', meaning Goddess of the Moon (Terry et al., 2000). Selenium is widely used in industry for the manufacture of several products, such as semiconductors, rectifiers, photovoltaic cells (because of its ability to convert light energy into electrical energy),corrosion-resistant alloys, pharmaceutical substances, and pigments (red and orange color) for paints, ceramics, and glass-making process (Mehdi et al., 2013). Selenium is of metabolic importance in cyanobacteria and in some plants, being involved in their antioxidative processes. Selenium is widely distributed on the Earth's surface and available for plants in at least small traces. Cultivation of plants enriched with selenium could be an effective way of producing selenium rich foodstuffs and thereby increase health benefits. The essentiality of selenium to higher plants is still under debate. Selenium can increase the tolerance of plants to UV-induced oxidative stress, delay senescence, and promote the growth of ageing seedlings.

Selenium (Se) is a trace element playing an essential role in human and animal health. It is necessary for the synthesis of the amino acid selenocysteine, which is involved in the formation of approximately 25-35 proteins (called selenoproteins) that are critical in mammal metabolism (Rayman, 2012; Oliver and Gregory, 2015V, selenium deficiency in the human organism has been linked with thyroid gland dysfunctions, irreversible brain damage, peripheral vascular diseases, chronic and degenerative osteoarthropathy(Combs,m2000; Pilon -Smits and Quinn, 2010; Davis *et al.,* 2012; Fairweather-Tait et al., 2011; Rayman, 2012; Riaz and Mehmood, 2012; Cardoso et al., 2015). This safe range of dietary intakes of Se is considered narrow (Tan et al., 2002). Although Se deficiency in the animal and human body leads to various diseases and disorders, an excessive Se intake is also capable of causing adverse effects to health (Yang et al., 1983; Goldhaber, 2003; Aldosary et al., 2012; Riaz and Mehmood, 2012). Besides the selenoproteins, non-protein organic forms of Se have been also shown to have important benefits to human health. Recently, particular attention has been given to the anti-carcinogenic effects of some methylated organic compounds of Se. Although

there are studies reporting on the chemo preventive properties of some non-methylated organic forms of Se (Ryan-Harshman and Aldoori, 2005; Rayman, 2012; Riaz and Mehmood,2012), several researches have been able to demonstrate a potent anticancer activity of monomethylated Se forms, especially *Se*-methylselenocysteine and γ-glutamyl-*Se*methylselenocysteine(Ip and Ganther, 1990; Ip et al., 2000; Dong et al., 2001; Medina et al., 2001; Lee et al.,2006; Wang et al., 2009).

One of the safest and most important way to increase Se intake is consuming Se-enriched foods, which is possible though crops with higher Se content in their edible parts. Therefore, aiming to increase the Se intake by population in Se-poor areas, the biofortification of staple crops with Se has a great relevance. Basically, biofortification is defined as a process to increase the nutritional values of crops in terms of essential nutrients for animal and human nutrition, such as vitamins, iron (Fe), zinc (Zn), iodine (I), and Se. The biofortification crops with Se may be performed of different ways .Genetic biofortification involves the traditional and molecular plant breeding approaches to choose cultivars of a given specie that are efficient to accumulate Se. In addition, agronomic biofortification refers to the use of Se-containing fertilizers (applied to soil and/or by folia spray) to increase contents of Se in staple plant foods .Genetic and agronomic biofortification of staple crops have been extensively studied and have showed to be good approaches to increase the natural intake of Se by animals and humans (Pilon-Smits and Quinn, 2010; Ramos et al., 2010; Fairweather-Tait et al., 2011; Ávilaet al., 2013).

A good example of using fertilizers containing Se to increase the Se intake in foods and feeds has been currently observed in Finland. The low available Se contents in the Finnish soils result in an insufficient amount of Se in take by animals and humans. Hence, this country started to add Se in fertilizer since 1984 to reduce the occurrence of Se deficiency in the population, through a policy established by the Ministry of Agriculture and Forestry. Several studies have pointed out that the increased Se content in the edible parts of staple crops is closely linked with the decrease of health problems associated with Se deficiency, following the inclusion of Se into the fertilizers in Finland. The positive effects of adding Se in fertilizers to bio-fortify crop plants and to improve the health of the Finnish population may be seen with details in a publication elsewhere (Alfthan et al., 2015).

2.1 CURRENT AND FUTURE NEEDS OF SELENIUM

The increase in Selenium intake by population with the use of Se-containing fertilizers in Finland has been well documented, being a satisfactory strategy to be performed in other countries where the natural Se content or availability in soils is low, such as in Brazil. In this context, it has to be mentioned that the Ministry of Agriculture, Livestock, and Supply included Se as a possible micronutrient to be added in Brazilian fertilizers, according the Normative Instruction number 46 recently published. However, such action of applying fertilizers with Se needs to be carried out with caution, taking into account that the dose range of dietary Se requirement for humans is narrow. Therefore, soil characteristics that affect Se availability, such as interactions with other elements and sorption-desorption reactions with minerals and organic matter, need to be well understood. Such knowledge will assist to establish forms and doses of Se to be added in fertilizers as an important way to improve the Se intake by population in Se-poor sites. Moreover, it will necessary to build up a monitoring plan to assess contents of Se in soils and in agricultural crops, as well as in the human body, to regulate further adjustments, avoiding excessive Se intake by population (i.e. Se intake rise more than the nutritional recommendations for humans). Influence of different soaking times with selenium on growth, metabolic activities of wheat seedlings under low temperature stress decomposition of H2O2 was followed by a decline in absorbance at 240 nm.

2.2 BENEFICIARY EFFECT OF SELENIUM TO HUMANS

Some regions, suffer from a relative deficiency of selenium (Pirc and Šajn, 1997). Cultivation of plants enriched with selenium could be an effective way of producing selenium rich foodstuffs and thereby increase health benefits (Ip and Lisk, 1994; Finley *et al.*, 2001; Lyons et al., 2005). Selenium has an important role in the prevention of atherosclerosis, specific cancers, arthritis, and altered immunological functions. The beneficial effects of selenium are dependent on the chemical form. Selenomethionine (SeMet) is known to be the most readily assimilated form of selenium (Patrick, 2004). Supplementation of human diet with selenium yeast, containing SeMet as the main chemical form, significantly reduced the occurrence of prostate cancer (Duffield-Lillico et al., 2003).

2.3. EFFECT OF SELENIUM ON PLANTS

The essentiality of selenium to higher plants is still under debate (Terry et al., 2000). Selenium can increase the tolerance of plants to UV-induced oxidative stress, delay senescence, and promote the growth of ageing seedlings (Xue et al., 2001; Pennanen et al., 2002). Recently it has been shown that selenium has the ability to regulate the water status of plants under conditions of drought (Kuznetsov et al., 2003). Hartikainen et al. (2000) reported about growth promoting effect of selenium in ryegrass. Senescence stress is partly counteracted with enhanced antioxidation which is associated with an increase glutathione peroxidase (GSH-Px) activity. Although some studies have evaluated the effect of hardness, temperature, pH and other parameters on selenium toxicity, sulphate has perhaps been most widely studied in relation to selenium uptake and toxicity in aquatic and terrestrial organisms (Sappington, 2002). Selenium and sulphur are nutrients with very similar chemical properties and their uptake and assimilation proceed through common pathways (Eapen in D'Souza, 2005).

2.3.1 ABILITY OF PLANTS TO ACCUMULATE SELENIUM

Selenium has not been classified as an essential element for plants, although its role has been considered to be beneficial in plants capable of accumulating large amounts of the element (Terry et al., 2000). Uptake and accumulation of selenium by plants is determined by the chemical form and concentration, soil factors such as pH, salinity and CaCO3 content, the identity and concentration of competing ions, and the ability of the plant to absorb and metabolize selenium (Kabata Pendias, 2001). Actively

2.4. SORPTION BEHAVIOR OF SELENIUM IN SOILS

Sorption behavior of Se depends on, among other factors, Se speciation. As mentioned early, selenate is less sorbed than selenite, thus, the last form is strongly adsorbed on solids, such as Fe/Al oxyhydroxides, having low mobility in soils. Several studies have showed that selenite is adsorbed much more strongly than selenate on different solid surfaces, such as manganese oxide (Saeki et al., 1995), hydroxyapatite (Monteil-Rivera et al.; 2000), and soils (Eich-Greatorex et al., 2010). The difference in adsorption behavior of selenate and selenite reflects different Se contents that are plant-available, being the selenate much more phyto-available. In terms of the adsorption mechanisms, selenate forms mainly outer-sphere complexes, which

2.5 PLANT GROWTH UNDER SELENIUM

Plant growth is one of the essential integral indicators of the plant physiological condition. Growth rates of wheat (Triticum aestivum L., cv. Triso) plants depended on the concentration of selenium added to soil. The height and fresh weight of plant shoots in treatment (Se0.4) were 116 and 125%, respectively, compared to those of control plants. However, the increase in soil content of selenium in treatment (Se0.8) caused the reduction of the above parameters by 32 and 62%, respectively, with regard to the control values. The negative influence of Pb was manifested in retardation of plant growth; the extent of growth retardation depended on the content of stressor in soil In treatment (Pb50), the plant height and the shoot fresh weight were 20 and 21% lower than in control plants. In treatment (Pb100), these parameters were lowered by 36 and 29%, respectively. The effect of combined application of selenium and lead on plant growth also depended on the content of elements added to soil. When applied at a low concentration, selenium alleviated the negative effect of lead. For example, in plants subjected to treatment (Pb50 + Se0.4), the plant height and its fresh weight retained their control values or were slightly above these levels. However, the introduction of selenium at a higher concentration in treatment (Pb50 + Se0.8) enhanced the negative impact of the stressor. Particularly strong negative synergism of lead and selenium was evident in plants withstanding the treatment (Pb100 + Se0.8); in this treatment, the height and biomass of aerial organs decreased to 44 and 38%, respectively, compared to control values (Table 1). The plant roots were affected by the presence in soil of $Pb2^{+}$ and $Se6^{+}$ at various concentrations in the same manner as the aerial organs, but the negative impact was expressed to a greater extent. It should be noted that the root length in plants exposed to treatment (Se0.4) was approximately 20% lower than in control plants, whereas the root fresh weight was 15% higher is bound by non-specific anion exchange, whereas selenite is bound by ligand exchange, creating inner-sphere complexes that are not reversible (McBride, 1994).

In the case of selenate, besides bound by outer-sphere complex, which is its predominant adsorption mechanism, researches have also shown contributions of inner-sphere complexes, generating a mixture of outer- and inner-sphere surface complexes, as reported on iron oxides and hydroxides (Peak and Sparks, 2002) (Jordan et al., 2013). Selenate and selenite may compete with organic acids as well as with other anions, such as phosphate and sulfate for soil adsorption sites. This fact is relevant for agro ecosystems, particularly, for tropical soils taking into account that these soils usually receive high amounts of phosphate and sulfate, which come up from agricultural products applied on soils, such as fertilizers and gypsum.

Because Selenium can be in several interactions in the environment, its sorption behavior differs among soils, where the soil management or production systems are different. Therefore, local researches evaluating variables that may alter the behavior of Se in a particular environment need to be better understood. Ogaard et al. (2006) reported that cattle manure affects selenium behavior in soils, decreasing the sorption of Se species, selenate and selenite. There are studies in literature showing the effect of phosphate under Se adsorption. Most of these studies showed that Se become more plant available following phosphate addition, i.e., Se sorption decreases as phosphate increases, which is well reported for Japanese soils (Nakamaru et al.; 2006; Nakamaru and Sekine, 2008). It has to be mentioned that this trend is more pronounced for selenite than selenate. This occurs due to chemical similarities between phosphate and the selenite anion (Eich-Greatorex et al., 2010), while the anion selenate tends to compete with sulfate. These information may assist explain the results found by Nakamaru and Sekine (2008), where it was verified that selenite sorption did not change after an increase in the sulfate concentration.

2.5.1 GRAIN YIELD OF SELENIUM TREATED-WHEAT

Crop productivity is the rate at which a crop accumulates organic matter which depends primarily on the rate of photosynthesis and conversion of light energy to chemical energy by green plants (Reddy,2004). According to analysis of variance (Table2) significant differences was observed for irrigation treatments ($P<0.01$), various Se spraying treatments ($P<0.05$) and interactions between irrigation and Se spraying ($P<0.05$) on grain yield. The highest grain yield was produced with normal irrigation and 36 g.ha-1Se spraying (S0Se2) with 6435 kg.ha-1, and the least was belonged to non-irrigation at 50% flowering stage and pure water spraying treatment (S2Se0) with 3596 kg.ha-1 grain yield. Se application at both stress treatment (S1Se1,S1Se2 and S2Se1 ,S2Se2) caused a significant decrease in grain yield compared with no Se treatments (S1Se0 and S2Se0) which indicates the importance of Se in compensation of drought damages during plant growth and its role in increasing grain yield. The higher grain yield in Se treated plots was because of more number of grain per spike as well as TKW and LAI. Several reports indicated that either soil or foliar application of micronutrients had positive correlation with wheat yield (Habib,2009; Wroble, 2009). The yield of wheat is composed of three components i.e. number of spikes, kernelsper spike and kernels weight. Though, kernel weight does exert an influence on grain yield but its effect is lower than spikes and kernels per spike. According to the results is showed by nejata et al. (2009), maize grain yield was 27%lower under stress than irrigation conditions and Selenium

spraying increased grain yield by 27.1and 5% under stress and normal conditions, respectively. Under stress, the grain yield of SC700 was 6.925 t.ha-1 without Selenium spraying and 8.524 t/ha with Selenium spraying. Under normal condition, the grain yield of SC700 was 15.828 t.ha-1 without Selenium spraying and 16.894 t.ha-1 with Selenium spraying which was the highest. Chandler and Singh (2008), observed that grain yield and biological yield particularly showed maximum sensitivity to moisture stress. It is reported that Leaf spraying with Selenium at flowering stage and shortly afterward significantly increases yield, so that under drought stress, yield and thousand-grain weight increased but grain number/head decreased (Visic,2006). The experiments shows that under stress condition, Selenium spraying increases the activities of the enzymes superoxide dismutase and catalase as well as lipid level of peroxidase. Other studies showed that plant treatment with Selenium could improve its drought-tolerance, so that it could be due to the increase in activity level of antioxidant enzymes (Timothy, 2001).

2.6 RELATIVE WATER CONTENT

Drought stress had a significant effect on RWC. The analysis of data showed that with the increase in the duration of water stress period there was a progressive decrease in the relative water content of flag leaves. RWC decreased by 6.1 and 13.4% for non-irrigation at 50% flowering and 50% stem elongation stages (table 2). The intensity of the response to water stress depends on the stress severity and its duration, as well as the plant developmental stage. Wheat crop needs water for the entire period of growth, but some stages are more vulnerable to water shortage and moisture stress during this period may result in significant yield losses, noteworthy in this regard are the phases of crown root initiation, booting and early grain fill period (Iqbal and Bano, 2009). Khakwani *et al.*, (2011) in a study about Drought tolerance screening of wheat varieties by inducing water stress conditions, reported that RWC of all varieties was significantly decreased when subjected to stress conditions as compared to control. They stated that leaf area of all varieties decreased significantly in both drought conditions. Leaf area of plants grown under 35% FC deceased 21-42% and decreased 44-64% when these varieties were grown under 25% FC. Deepak and Wattal (1995) indicated that as moisture stress intensified, leaf water potential and content as well as leaf area growth and development were significantly decreased. Kumar and Singh (1996) showed that moisture stress decreased leaf conductivity and this decrease was larger in lower leaves than in upper

ones. On the other hand, transpiration rate was similar between two cultivars and decreased as moisture stress intensified, under which a positive correlation was found between transpiration rate and leaf conductivity which eventually affected leaf relative water content. According to results of Bayoumi*et al.* (2008), RWC may be attributed to differences in the ability of the variation to absorb more water from the soil and/or the ability to control water loss through stomata. Se spraying at drought stress conditions had desirable effect on RWC and increased it. Dhillon (2002), reported that selenium foliar spraying increases antioxidant enzymes and improve plant drought resistance. It was shown that Se has the ability to regulate the water status of plants under conditions of drought (Kuznetsov *et al.*, 2003) and that the protective effect of Se under drought stress conditions was achieved by increasing the water uptake capacity of the root system (Kuznetsov *et al.*, 2003). Eskandari Zanjani et al. (2012) in a study about Effects of Zeolite and Selenium Application on Pumpkin under Drought Stress, reported that in drought stress treatments the highest amount of RWC and the lowest of WSD were obtained in the presence of zeolite and Se together.

2.7 SALINITY

Salinity is a serious problem for crop production in the globe since 20% of cultivar and 50% of irrigated lands are affect by salinity (Hasegawe et.al; 2000; Munns and Tester 2008). Research on salinity has been done extensively throughout the world as it is the main abiotic stress and great challenges for the crops growers and the world. Salinity is a dangerous threat to crops growth and yield which also is found in wheat although it is known to be somewhat to salinity stress. Salinity is the major environmental factor that limits plant growth and primary productivity in aquatic ecosystems (Moradi et al., 2013). In coastal water bodies, salinity can vary seasonally and can be influenced by changes in water levels, precipitation, evaporation (Schallenberg et al., 2003), hydrological alterations (Howard & Mendelssohn, 1999) and anthropogenic activities (Roache et al., 2006). Exposure to salinity may cause several morphological, physiological and biochemical changes in plants, due to excess ions and water deficit (Greenway & Munns, 1980; Maskri et al., 2010). The most common effects in plants are toxicity, diminished CO2 assimilation and enhanced generation of reactive oxygen species (Chawla et al., 2013). Changes in fundamental processes have also been observed, such as growth, photosynthesis, protein synthesis and lipid metabolism (Parida & Das, 2004).

Plant growth and development are adversely affected by salinity – a major environmental stress that limits agricultural production. This chapter provides an overview of the physiological mechanisms by which growth and development of crop plants are affected by salinity. The initial phase of growth reduction is due to an osmotic effect, is similar to the initial response to water stress and shows little genotypic differences. The second, slower effect is the result of salt toxicity in leaves. In the second phase a salt sensitive species or genotype differs from a more salt tolerant one by its inability to prevent salt accumulation in leaves to toxic levels. Most crop plants are salt tolerant at germination but salt sensitive during emergence and vegetative development. Root and shoot growth is inhibited by salinity; however, supplemental Ca partly alleviates the growth inhibition. The Ca effect appears related to the maintenance of plasma membrane selectivity for K over Na. Reproductive development is considered less sensitive to salt stress than vegetative growth, although in wheat salt stress can hasten reproductive growth, inhibit spike development and decrease the yield potential, whereas in the more salt sensitive rice, low yield is primarily associated with reduction in tillers, and by sterile spikelets in some cultivars.

The salinity problem has been aggravated by the requirement of irrigation for crop production in arid and semiarid environments. It is estimated that at least 20% of all irrigated lands are salt-affected (Pitman and Läuchli, 2002). About 17% of the cultivated land is under irrigation; yet, irrigated agriculture contributes more than 30% of the total agricultural production (Hillel, 2000). The total global area of salt-affected soils has recently been estimated to be approximately 830 million hectares (Martinez-Beltran and Manzur, 2005). The different types of soil salinity that impact agricultural productivity, i.e. irrigation-induced salinity and 'transient' dry-land salinity have been characterized in detail by Rengasamy (2006), with special emphasis on Australia. Clearly, soil salinity is one of the major environmental stresses that limit agricultural productivity worldwide

High salinity concentrations in plants also generate changes in plant productivity (Doganlar et al., 2010; Hasegawa et al., 2000), nutrient imbalances (Ashraf, 2009), accumulation of osmoprotective compounds, such as proline (Bohnert et al., 1995), and changes in nitric oxide (NO) content (Zhang & Blumwald, 2001). Na+ acts on the activation of a wide range of enzymes in plants, is involved in membrane osmosis, and can also replace K^+ in some osmotic and metabolic functions. Cl^- plays an important role in photosynthesis,

enzyme activation, osmotic regulation and cell division (Ashari-Esna & Gholami, 2010). Excessive Na^+ and Cl^- concentrations affect the absorption of many essential nutrients such as K^+, Ca^{2+}, Mg^{2+} and Na^+ (Abdallah et al., 2016; Iqbal et al., 2015). This occurs through competitive interactions affecting the ionic selectivity of cell membranes (Stoeva and Kaymakanova, 2008) and photosynthetic activity (Parida et al., 2002), reducing stomata opening and leading to decreases in intracellular CO2 (Munns & Tester, 2008).

Rashman et al. (2000) showed that yield component of wheat cultivars were significantly ($p<0$; 005) affected by different salinity levels. Agronomic characters i.e. plant height, tiller plant numbers of grain plant 100- grain weight, grain and straw yield and harvest index of two salt tolerant cultivar (mutant, and lu 265) were reduced less than were reduced less than salt sensitives cultivars (yecora and ws711.)

Padole et al. (1995) stated in field trials in the rabi (winter) season of 1982 to 1984 with wheat cultivar HMD-15553 was grown on soil of low medium or high salinity and it was irrigated with normal, moderately high saline water, grain yield was not adversely affected by saline water of 1.5 dm-1 where it decreased with saline or sodicity water of 4.0-4.2 dsm-1 and SAR1.7 or 8.6 growing wheat in low saline(7.6dsm-1) or 8.6 sodic (ESP 22-77) soil had little effect on yield where as yield was decreased in highly saline (15.7dsm-1) and highly sodic.

Haggai et al. (1984) studies the effect of salinity on yield of wheat applying salinity ranging from very low to very high levels increase in soil salinity and soil sodicity reduce the number of ear bearing and non ear bearing tillers, the numbers of spikelets per ear 100 grain weight and per ear 100 grain weight and straw and grain yield.

2.7.1 EXTENT AND DISTRBUTION OF SOIL SALINITY

According to Ghassemi et al. (1995), salt affected soils cover at least 20% of the world's cultivated land. Incidentally, most of the countries of south and south East Asia, Africa and South America lying in arid and semi-arid climates are affected by this threat. On global basis around one third of the total world's irrigated land (Epstein et al. 1980) and 7% of the world's total land area is considered salt-affected (Munns and Tester 2008). Out of 21.5 million hectares of salt affected land area in Asia, about 12 million hectares is saline and 9.5 million hectares sodic. According to another estimate, about30% world's rice growing land is affected by problem of soil salinity (Ahmad and Prasad, 2011). Salt-affected soils are found in almost

all continents (Almansouri et al. 2001) and under all climatic conditions, however, they are relatively more widespread in the arid and semi-arid climates compared to humid regions. The problem of salinity has also been reported in the tropical belts of Africa and Latin America, and even in the Polar Regions.

2.7.2 EFFECTS OF HIGH NA^+ IONS ON PLANT GROWTH UNDER SALT STRESS

The accumulation of Na+ in leaves and shoots results in a range of osmotic and metabolic problems for plants. Osmotic effects could occur due to high concentration of Na+ in the leaf apoplast as Na^+ enters leaves from the xylem stream and it accumulates to toxic levels, since leaving it behind, water transpires from the leaf surfaces. This mechanism of Na^+ toxicity was first proposed by Oertli (1968), and direct supporting evidence has been provided by x-ray microanalysis measurements of Na^+ in the apoplast of rice leaves by Flowers et al. (1991). These authors calculated that there was about 600 mol m-3 Na^+ in the apoplast of leaves of rice plants that were moderately salt stressed. With high Na^+ conc. in the leaf apoplast and/or vacuole, plant cells have difficulty in maintaining low cytosolic Na^+ and, perhaps low Na^+/K^+ ratios (Dubcovsky et al. 1996). Leaves are more vulnerable to Na^+ than roots, simply because Na^+ accumulates to higher conc. In shoots compare to roots. Na^+ is transported to the shoot in the rapidly moving transpiration stream in the xylem, but can only be returned back to roots via the phloem. There is limited evidence of extensive recirculation of shoot Na^+ ions to roots, suggesting that Na^+ transport is largely unidirectional and results in progressive accumulation of Na^+ in leaves with extended exposure to salt stress. Metabolic toxicity of Na^+ is largely result of its ability to compete with K^+ for binding sites forcellular function. More than 50 enzymes are activated by K^+, and Na^+ can not substitute this role (Bhattacharya 2005). Moreover, protein synthesis requires high conc. of K^+ due to K^+ requirement for the binding of tRNA to ribosome (Blumwald 2000) and probably other aspects of ribosome function (WynJones 1981). The disruption of protein synthesis by elevated conc. of Na^+ appears to be an important cause of damage by Na^+. Some effects of high soil Na^+ are also resulted in deficiency of other nutrients (Silberbush and Lipps 1991), or of interactions with other environmental factors, such as drought, which aggravate the problems of Na^+ toxicity. Specifically, other nutrient deficiencies can occur because elevated Na+ inhibits the uptake of other nutrients by (i) disrupting the uptake of other nutrients directly by interfering with transporters in the root plasma membrane, such as K^+ selective ion channels; and (ii) inhibiting root growth by the effects of Na^+ on soil properties (Bhardwaj et al. 2009). Under salt stress Na^+ enters into plant tissues and leads to injurious effects such as imbalance in

intracellular ionic concentrations (Ghosh et al. 2011), on set of oxidative stress (Ghosh et al. 2012), and inhibition of the photosynthesis process (Kumar et al.2000). In woody perennials such as citrus and grapevines, Na^+ is confined in the woody roots and stems, whereas, Cl- accumulates in the leaves and is most damaging to these plants often by inhibiting the process of photosynthesis. cell, as all active metabolic processes and the organelles doing these processes are placed in cytosol. If too much amounts of Na+ enter a plant, it will ultimately accumulate to toxic levels in leaves causing premature senescence and reduction in the photosynthetic capacity of the plant, which further inhibit plant growth (Achary et al. 2012).Another important feature of tolerant species is to check the flow of Na^+ ions into the leaves andmaintain higher K^+/Na^+ or $Ca2^+/Na^+$ ratios under salinity (Yamaguchi and Blumwald 2005). Ascytoplasmic Na+ is toxic above threshold levels, it is excluded by plasma-membrane Na^+/H^+ antiporters that are energized by the proton gradients generated by the plasma membrane ATPase (Blumwald 2000). Cytoplasmic Na^+ may also be compartmentalized by vacuolar Na^+/H^+ antiporters into the cell vacuoles. These transporters are energized by the proton gradients generated by the vacuolar H^+ - ATPase and H^+ - PPiase. In *Arabidopsis thaliana*, Na^+ efflux is mediated by a plasma membrane Na^+/H^+ antiporter (SOS1). The salt overly sensitive (SOS) pathway has been comprehensively studied and it has been found that prevalence of salt stress leads to a Ca2+oscillation that activate the SOS3-SOS2 kinase complex. This activated kinase complex then phosphorylates and activates SOS1 and tonoplast Na^+/H antiporter (AtNHX1). It has been reported by Wang et al. (2003) that long-distance Na^+ t+ansport between roots and shoots is controlled by SOS1 by loading and unloading Na^+ from the xylem sap, as well as Na^+ efflux in root. Transgenic Arabidopsis plants over-expressing SOS1 have reduced Na^+ accumulation in xylem stream and shoot, and increased salt tolerance of plant (Shinozaki and Shinozaki2007) Gene networks involved in drought stress response and tolerance.

2.8 SALT STRESS AND PLANT GROWTH

Salinity stress has a major impact on plant growth and development. Processes such as seed germination, seedling growth and vigour, vegetative growth, are adversely affected by high salt concentration, that ultimately cause poor plant growth (Sairam and Tyagi, 2004). Plant growth responds to salinity in two phases: a rapid, osmotic phase that inhibits growth of young leaves, and a slower, ionic phase that accelerates senescence of mature leaves. Salt stress is a condition where excessive salts in soil solution cause inhibition of plant growth or even their death.

Various environmental factors negatively affect plant growth and development and finally the biological yield of the crop, at harvest. These factors include salinity, drought, heavy metal toxicities and temperature extremes which limit the crop productivity worldwide. Salinity is one of the most important abiotic stress, limiting crop production in arid and semi-arid regions, where soil salt content is naturally high and precipitation may be insufficient for leaching (Zhao et al., 2007). Soil salinity can be defined as the concentration of dissolved mineral salts in soil solution as a unit of volume or weight basis (Ghassemi et al., 1995) whereas, the sodicity expresses the presence of sodium, attached (exchangeable) to clay particles (plates) of the soil matrix. Sodic soils, by definition, contain excessive concentrations of exchangeable sodium (Bernstein, 1975). Sodicity differs from salinity by being specific to only one salt (sodium) rather than a range of salts and it is a measure of ions on clay surfaces rather than in the solution. Because sodium chloride (NaCl) is the dominant salt in alkaline soils, therefore sodium exists in the soil solution as well as on clay surfaces. Consequently, salinity and sodicity usually occur together. Soil salinity stresses the plants in two ways: higher concentrations of salts in the soil make it harder for roots to extract water (osmotic stress), and secondly high salt level within the plant may be harmful (specific ion toxicity) (Munns and Tester, 2008; Hussain et al., 2008). According to the Food and Agriculture Organization (FAO) Land and Nutrition Management Service (2008), over 6% of the world's land is affected by either salinity or sodicity which accounts for more than 800 million ha of land. Low precipitation, high surface evaporation, weathering of native rocks, irrigation with saline water, and poor cultural practices are among the major contributors to the increasing salinity. Secondary salinization, in particular, exacerbates the problem where once productive agricultural lands are becoming unfit to cultivation due to poor quality irrigation water. Salt stress has various damaging effects on plant physiological processes such as increased respiration rate, ion toxicity (Sudhir and Murthy, 2004), changes in C and N metabolism (Kim et al., 2004a), mineral distribution, membrane instability (Marschner, 1986), membrane permeability (Gupta et al., 2002) and decreased efficiency of photosynthesis (Hayat et al., 2010c), reduced leaf area, dry mass and stomatal conductance which ultimately lead to decrease in the plant productivity.

2.8.1 SALT SENSITIVITY OF WHEAT AT VARIOUS GROWTH STAGE

It has long been known that salinity reduces the growth rate of the entire wheat plant and its specific organs, but it also affects plant development. The architecture

of expanding wheat leaves from recently emerged seedlings subjected to 200 mM NaCl was greatly affected (Hu et al., 2005b). By close examination of the transverse section of leaf 4, investigators found that salinity reduced the cross sectional area, width and radii of epidermal and mesophyll cells along the leaf axis, indicating that adverse effects from salinity were occurring during leaf initiation. The duration of plant development is also affected by salinity. The salt sensitivity of wheat at various growth stages was evaluated by Maas and Poss (1989a) by imposing salt stress [-0.05 to -1.25 MPa (1.4–28 dS/m)], using a combination of NaCl and CaCl salts, either 10, 56, or 101 days after planting (referred to as vegetative and spikelet differentiation, reproductive, and maturation stages, respectively). At each developmental stage, the stress was imposed for a 45-day duration and then removed. Salt stress retarded leaf development and tillering but hastened plant maturity. When grain yield data were compared among treatments, 'Aldura' and the more tolerant variety 'Probred', became less sensitive to salinity the later plants were stressed, even though the duration of stress was held constant. Salt stress, imposed while the shoot apex is in vegetative stage, can adversely affect spike development and decrease yields of wheat (Maas and Grieve, 1990). When wheat was salt-stressed during spike or panicle differentiation, reproductive development was stimulated but the number of spikelets was reduced. They found that salt stress accelerated the development of the shoot apex on the mainstem and decreased the number of spikelet primordia. The terminal spikelet stage occurred about two weeks earlier in salt-stressed wheat as compared to non-stressed controls. Anthesis also occurred earlier in salt-stressed plants but tillering was delayed several days. The investigators found that salt stress increased the phyllochron (the interval between appearance of successive leaves on the main stem based on thermal time) and reduced the number of leaves initiated on the main stem. Salt stress decreased the yield potential mostly by reducing the number of spike-bearing tillers. This conclusion was also reached by El-Hendawy et al. (2005) in a comprehensive evaluation of numerous wheat cultivars using cluster analysis. Therefore Maas and Grieve (1990) concluded that salinity stress needs to be avoided prior to and during spikelet development on all tiller spikes if full yield potential is to be achieved. Grieve et al. (2001) conducted another salt stress release study on spring wheat where salinity was imposed and withdrawn, before or after, three growth stages;

Late leaf primordial initiation, 2) double ridge stage, and 3) terminal spike formation. They found that grain yields were maximized when salt stress was delayed until after the terminal spike formation or by withdrawing stress at the late leaf primordial stage or double ridge stage. They found that short periods of salt stress during organogenesis have irreversible consequences on wheat growth and development. In a more in-depth examination of semi dwarf wheat varieties, Grieve et al (1993) used a three-piece linear-spline model and found that salinity decreased the rate of leaf primordium initiation but did not affect the duration of this phase. On the other hand, they found salinity reduced the duration of the spikelet primordium initiation phase, even though it had no effect on the rate of spikelet primordium initiation. This combination of effects resulted in less leaves and caused a reduction in the number of grain-bearing spikelets, severely affecting the yield potential of these wheat types.

Additional studies on wheat were conducted to examine salinity's effect on reproductive physiology. Khan and Abdullah (2003) found that pollen viability in two wheat cultivars differing in salinity tolerance was reduced 24–37%; depending upon cultivar. They also suggested that 80–90% of the carbon that fills wheat grains comes from current photosynthesis and not from stored vegetative carbon sources. While most of the carbon that is filling grains comes from active photosynthetic sources, the carbon is not distributed uniformly among tillers. Grieve et al (1992) analyzed the main spike yield components of salt-stressed wheat and found that grain yield from the main spike of two semi dwarf Mexican wheat varieties increased up to 15% more in salt-stressed plants (-0.65 MPa OP) than non stressed plants. They found that decreases in kernel numbers per spike were offset by increases in kernel weight. Therefore moderately salt-stressed wheat plants distributed their carbohydrates preferentially towards the main stem tillers. Other studies were directed towards ion relations in salt-stressed grain crops. Maas and Poss (1989a) found that K uptake was severely inhibited by salt stress imposed to wheat during the vegetative growth stage but not at later stages, even though the more tolerant variety 'Probred' accumulated less Na than the more sensitive "Aldura'. The effect of NaCl salinity on salt accumulation and reproductive

development in the meristem of wheat and barley was studied by Munns and Rawson (1999). They selected two varieties of each species differing in salt tolerance to observe changes in the development of the apex as it changed from vegetative

to reproductive growth. Apices were analyzed for ion contents when most of the spikelet primordia had been produced and the process of differentiation into floral organs had started. Potassium concentrations were unaffected by salinity (up to 175mM NaCl). In addition, they concluded that Na and Cl concentrations were too low to affect metabolism. Nevertheless, despite the small effect of salinity on apex ion relations, salinity still affected reproductive development; fewer spikelet primordia formed and the final spikelet numbers at ear emergence were reduced. In summary, a mature wheat plant is a consequence of sequential developmental processes that are characterized by changes in shoot apex morphology. The yield components such as tillers per plant, number of spikelets per spike and individual grain weights, are developed sequentially as the crop develops. If salt stress is applied before and during the shoot apex transition from vegetative to reproductive stage, it can significantly affect vegetative and reproductive development. Salt stress can hasten reproductive development but also can adversely affect spike development and decrease the yield potential of wheat.

2.8.2 Vegetative growth stage

Most of the literature indicates that plants are particularly susceptible to salinity during the seedling and early vegetative growth stage as compared to germination. Examples are found in barley (Ayers *et al.*, 1952), corn (Maas *et al.*, 1983), cotton (Abul-Naas and Omran, 1974), cowpea (Maas and Poss, 1989), melon (Botia *et al.*, 2005), New Zealand spinach (Wilson *et al.*, 2000), red orach (Wilson et al., 2000), rice (Pearson and Ayers, 1966), sorghum (Maas *et al.*, 1986), tomato (del Amor *et al.*, 2001), and wheat (Maas and Poss, 1989). In greenhouse experiments with corn and wheat, the total shoot biomass of salt-stressed plants relative to non-stressed plants was much lower than salinity's overall effect on relative grain yield (Maas *et al.*, 1983; Maas and Poss, 1989). Although it may not be true for most crops, some investigators found that salt tolerance among melon cultivars during early seedling growth correlated well with salt tolerance based on fruit yield at the end of the season (NersonandParis, 1984).

2.9 SALT STRESS AND PHOTOSYNTHESIS

Growth of the plants is dependent on the level of photosynthates and, therefore, environmental stresses affecting photosynthesis also affect growth (Taiz and Zeiger, 1998; Manikandan and Desingh, 2009). A positive relationship between photosynthetic capacity and growth has already been reported for various plants, grown under saline conditions e.g., Abelmoschus esculentus (Saleem et al., 2011), Triticum aestivum (James et al., 2002), Zea mays (Crosbie and Pearce, 1982), Asparagus officinalis (Faville *et al.,* 1999), Gossypium hirsutum (Pettigrew and Meredith, 1994), Phaseolus vulgaris (Seemann and Critchley, 1985) and Cynodon dactylon (Akram et al., 2007). Iyengar and Reddy (1996) attributed the decrease in photosynthetic rate to salinity stress induced following factors:

1. Dehydration of cell membranes which reduce their permeability to CO2 due to osmotic stress caused by high salt concentration in soil and water inactivating the photosynthetic electron transport via shrinkage of intercellular spaces.

2. Specific ion toxicity caused particularly by Na^+ and Cl- ions. Cl- inhibits photosynthetic rate through its inhibition of NO3-N uptake by the roots (Banuls et al., 1991).

3. The reduction in CO_2 supply because of the closure of stomata results in restricting the availability of CO_2 for carboxylation reactions (Brugnoli and Bjorkman, 1992) and also minimizes the loss of water through transpiration and this affects light-harvesting and energy-conversion systems thus leading to decrease in chloroplast activity.

4. Enhanced leaf senescence, induced by salinity 5. Changes in enzyme activity, induced by the alterations in cytoplasmic structure 6. Negative feedback by reduced sink activity.

2.9.1 SALT STRESS AND THE LEVEL OF IONS AND NUTRIENT CONTENT OF PLANT

The salt ions like Na+, Cl-, SO4-2 present in the soil compete with the uptake of other nutrient ions like K+, Ca2+ etc. which results in the nutritional disorder and eventually leads to reduce the quality and yield of plants (Grattan and Grieve, 1999). Higher NaCl concentration has been reported to increase the level of Na+ and Cl- ions and decrease those of Ca2+, K+ and Mg2+ in various plants (Bayuelo-Jimenez et al., 2003). In the plant cells under the normal conditions (non-saline), there is 100 to 200 mM K+ and 1 to 10 mM Na+, an environment in which the enzymes function optimally. However, the higher ratio of Na+ to

K+ and accumulation of total salts at an elevated level inactivate these enzymes and inhibit protein synthesis. Moreover, Na+ displaces Ca2+ from the cotton root hair plasma membrane, resulting in the change in membrane permeability that can be noticed by the leakage of K+ from the cells (Cramer et al., 1985). Decrease in the content of Ca2+ and Mg2+ in the leaves of Brugueira parviflora has also been reported under salt accumulation (Parida et al., 2004). Plant acquisition and utilization of necessary nutrients particularly that of K+ and Ca2+ may also impair under saline conditions (e.g. ion deficiency) causing changes in the ratios of K+/Na+ and Ca2+/Na+, thus further lowering the growth and productivity of plants (Zhu et al., 2001). Some ions also serve as buffer to counter the effect of salinity on the accumulation of other ions. For example, when excess Ca2+ or NH4 is added to the growth medium containing high salinity, growth and nutrient accumulation can be stimulated, compared to the control (Cramer, 2002).

2.9.2 SALT STRESS AND PLANT WATER RELATIONS

As the salinity increases in the soil, its water potential decreases (Meloni *et al.*, 2001; Gulzar et al., 2003) which reduces water uptake leading to slower growth (Munns *et al.*, 2006). The plants ultimately get stunted (Wang and Nil, 2000), where plant height, fresh and dry mass of leaves, stem, and roots and yield are reduced (Ali Dinar et al., 1999; Chartzoulakis and Klapaki, 2000). However, at low or moderate salt concentration, plants adjust osmotically by accumulating solutes, thereby lowering the water potential and maintain a potential gradient for the influx of water. Leaf water potential decreases in response to salt stress in *Chenopodium quinoa* Willd, (Eisa et al., 2012), *Shepherdia argentea* (Qin et al., 2010) and *Iris lacteal* (Wen-Yuan et al., 2012). It has been reported that significant decrease in relative water content (RWC) occurred in response to salt stress in Beta vulgaris (Ghoulam et al., 2002) and Brassica species (Singh et al., 2010). Water use efficiency (WUE) also decreased with increasing levels of NaCl in Thymus vulgaris L. (Najafian et al., 2009) and Brassica juncea (Hayat et al., 2011).

2.9.3 SALT STRESS AND ANTIOXIDANT SYSTEM IN PLANT

Salinity and drought stress are well known to induce oxidative stress through the production of superoxide radicals by the process of Mehler reaction. These free radicals initiate the chain of reactions that produce more harmful oxygen radicals (Hsu and Kao, 2003). These reactive oxygen species (ROS) are continuously generated during normal metabolic processes in mitochondria, peroxisomes and cytoplasm which disturb normal

metabolism through oxidative damage of lipids, proteins, and nucleic acids when produced in excess (Hernandez et al., 2001; Ahmad et al., 2010). Plants throughout their life are prone to oxidative damage caused by environmental factors due to their sessile nature (Hippeli and Elstner, 1996). There is a constant need for efficient mechanisms to compensate the possible oxidative damage to cellular components. Plants have evolved efficient systems for ROS removal, which include specific ROS-scavenging antioxidative enzymes and small non-enzymatic molecules that act as ROS scavengers such as ascorbate, glutathione, □-tocopherol, flavonoids, anthocyanines, polyphenolic compounds and carotenoids. To overcome salt-mediated oxidative stress, plants detoxify ROS by upregulating antioxidative enzymes like superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), glutathione reductase (GR) and glutathione peroxidase (GPX). In plants, superoxide dismutase scavenges superoxide anions and converts them to hydrogen peroxide (Alscher et al., 2002). Catalase, the second line of defence, converts lethal hydrogen peroxide to water and molecular oxygen. Another versatile antioxidant enzyme is ascorbate peroxidase which utilizes ascorbate (AsA) as electron donor and scavenges H2O2 in water-water and ascorbate glutathione cycles. Hydrogen peroxide is reduced to water by APX and thus plays a vital role in cell defense mechanism (Kangasjarvi et al., 2008; Ashraf, 2009). Glutathione reductase catalyses the NADPH-dependent reduction of oxidized glutathione (GSSG) to its reduced (GSH) form (Meister, 1988). Glutathione reductase activity is thought to increase the ratio of NADP+/NADPH. The NADP+ accepts electrons from the photosynthetic electron transport chain (Bishop, 1971). Thus, the flow of electrons to O2 and the formation of O_2 can be minimized. The harmonized activities of the multiple forms of these enzymes in different subcellular compartments achieve a balance between the rate of formation and removal of ROS, and sustain H2O2 at the required levels for cell signalling. It is now widely accepted that the degree of oxidative cellular damage in plants, exposed to abiotic stress is controlled by the operative capacity of the antioxidative systems (Turkan et al., 2005). A correlation between antioxidant capacity and salinity tolerance has been reported in several plant species such as *Oryza sativa* (Demiral and Turkan, 2005), Beta vulgaris (Bor et al., 2003), Lycopersicon esculentum (Hayat et al., 2010), Sesamum indicum (Koca et al., 2007), Portulaca oleracea (Yazici et al., 2007), Plantago maritima (Sekmen et al., 2007), Brassica napus (Ashraf and Ali, 2008), Populus cathayana (Yang et al., 2009a), Helianthus annuus (Noreen et al., 2009), Panicum miliaceum (Sabir et al., 2011), Triticum aestivum (Ashraf et al., 2010a), and Carthamus tinctorius (Siddiqi, 2010). Furthermore, transgenic plants overexpressing ROS-scavenging enzymes, such as SOD (Alscher et al., 2002), APX (Wang et

al., 1999), GR (Foyer et al., 1995) and GPX (Roxas et al., 1997, 2000) showed enhanced tolerance to osmotic, temperature, photo-inhibition and oxidative stresses. Proline accumulation is believed to improve adaptation of plants to salt and drought stresses by scavenging free radicals and stabilizing membranes to maintain the conformation of proteins under stress conditions (Chen and Dickman, 2005). Proline is also reported to play a significant role in reducing the photo-damages of thylakoid membranes by scavenging the superoxide radicals (Ashraf and Foolad, 2007; Banu et al., 2009). Proline accumulation under dehydrated conditions is mainly due to increased biosynthesis and decreased degradation. Enhanced synthesis of proline under drought or salinity conditions has been involved in the alleviation of the stress in various plants such as Cynodon dactylon (Hameed and Ashraf, 2008), Pisum sativum (Noreen and Ashraf, 2009), Brassica juncea (Hayat et al., 2011a), Saccharum officinarum (Chaum and Kirdmanee, 2009), Panicum miliaceum (Sabir et al., 2011), Lycopersicon esculentum (Hayat et al., 2010c).

2.9.4 SALT STRESS AND YIELD

Abiotic stresses are the major factors for reducing the crop yield (Munns and Tester, 2008; Reynolds and Tuberosa, 2008). Specifically, salt stress has been reported to cause substantial yield losses in the agriculture world-wide (Ashraf et al., 2008). Bray and his co-workers (2000) reported that salinity and drought reduce the yield potential of annual crops by 51-82%. Similarly, Ashraf et al. (2008) reported that high salt level in soils cause a significant reduction in the yield of a wide variety of crops world over. Different yield components (pod number per plant, seeds per pod and seed weight) of Vigna radiata were significantly affected by salinity stress (Nahar and Hasanuzzaman, 2009). These components were negatively correlated with salinity levels. In *Oryza sativa*, grain yield was lost significantly by different salinity levels (Hasanuzzaman et al., 2009; Mahmood et al., 2009). Furthermore, Rad et al. (2012) reported that the salinity levels significantly decreased the filled panicle length, number of filled grains per filled panicle, number of spikelets per filled panicle and total number of spikelets per panicles in Oryza sativa. The salt stress reduced umbel number per plant, 1000 seed weight and see yield in Foeniculum vulgar Mill. (Rahimi et al., 2012), grain yield in Phaseolus vulgaris (Ghassemi-Golezani et al., 2012) and Triticum aestivum (Asgari et al., 2012). This reduction may be attributed to low production, expansion, senescence and physiologically less active green foliage (Wahid et al., 1997), and reduced photosynthetic rate (Seemann and Critchley, 1985). Moreover, reduced viability of pollen under stress condition could result in the failure of seed set (Abdullah et al., 2001).These reports suggest that salt

stress is one of the key challenges to crop production, and thus some specific means should be devised to improve crop productivity on saline soils and it should be given a major research priority. Although a multitude of means have already been suggested to improve crop salt tolerance, an integrated approach comprising conventional breeding and molecular marker-assisted breeding techniques seems to be more effective in improving crop salt tolerance (Munns and Tester, 2008).

2.9.5 IMPACT OF SALT STRESS ON GERMINATION

Salt stress affects germination percentage, germination rate and seedling growth in different ways depending on plant species (Ungar, 2005; Gul et al., 1999). It was reported that maximum germination of the seeds of halophytic plant so occurred in distilled water or under reduced salinity (Gul et al.,1999; Khan et al.,2003) and it has been found that germination percentage was reduced with a high NCl concentrations (Tobe et al,2001;Pujol et al, 2000 ; Rubio-Casal et.al., 2003). Excess salinitya with the plant root zone has a deleterious effect on plant growth and 8% germination at 1027mol/l level Mooring *et al*,1971). High level of salinity significantly reduced pigment content in leaves (Al-Sobhi et al., 2006). According to a report that seed germination of Suaeda salsa seeds decreased significantly with increased salinity.

Germination percentage of wheat cultivars was significantly affected by the salt stress ($p<0.05$). Germination percentage was reduced from 125mM NaCl salt concentration onwards for almost all the varieties. Seed germination was found to be highest in distilled water or RAJ-4123 variety. Rate of germination of wheat cultivars were significantly affected due to salt stress from 75mM Nacl salt concentration onwards.

There is considerable reduction in the rate of germination for almost all the varieties except the results were reciprocal for HD- 2045 wheat cultivar in case of 125mM and 150 mM concentration. The results of salt stress was almost prominent from 100mM salt concentration onwards for all the five wheat varieties resulting into mean daily germination (MDG). From the results of this present investigation it can be concluded that seeds of five different seeds of different wheat cultivars were susceptible to higher concentrations of salt solutions in germination stage which was supported by the works of (Ungar et al., 1996; Gul et al., 1999). The results regarding germination percentage, germination rate and mean daily germination the results were significant ($p<0.05$) for all the varieties. The reduced level of seed germination may be due to (i) loss of viability at higher salinity level (ii) delaying

germination of seeds at salinities that cause some stress to but not percent germination (Gulzar et al., 2001). A similar report of reduced level of germination of Suaeda salsa seeds under increased salinity level(Duan, 2007). Salinity caused a significant ($p<0.05$) reduction on root length and shoot length at the higher NaCl concentration .Increase in the salinity from 0 to 25mM NaCl had no effect on plant root and shoot length , while further increase from 50mM onwards significantly reduced the root length and shoot length . The effect of salt stress was completely inhibitory at 125mM and 150 mM NaCl concentrations for almost all the varieties except HD-6859 wheat cultivar. Growth processes are specially sensitive to the effects of salt, so that growth rates and biomass production provide reliable criteria for assessing the degree of salt stress and the ability of a plant to withstand it as reported by (Amor et al, 2005). In the current investigation the higher level of salinity has a more pronounced effect on root length with respect to shoot length as roots are directly exposed to salt solution. The reduction in root and shoot development may be due to toxic effects of the higher level of NaCl concentration as well as unbalanced nutrient uptake by the seedlings. High level of salinity may have also inhibit the root and shoot elongation due to slowing down the water uptake for overall osmotic adjustments of the plant body under high salt stress condition. Regarding fresh weight and dry weight the effect of salt stress was pronounced from 50mM Nacl concentration onwards. It was completely inhibitory from 75mM onwards for RAJ –4101. The proportion of fresh weight and dry weight allocated to root, shoot and leaf (whole plant body) decreased with increased NaCl levels.

2.9.5.1 GERMINATION AND SEEDLING EMERGENCE

Although most plants are tolerant during germination, salinity stress delays this process even though there may be no difference in the percentage of germinated seeds from one treatment to another (Maas and Poss, 1989a). It is this observation that categorizes this developmental stage for most crops as 'salt tolerant'. For example, salinity up to 10 dS/m actually stimulated the germination of Limonium perezii seeds, a commercially grown ornamental flower, yet salinities above 6 dS/m reduced stem length, adversely affecting quality and marketability (Carter et. al., 2005). Even though salinity delays germination, higher salt concentrations will eventually reduce the percentage of germinated seeds (Mauromicale and Licandro, 2002). While most crops show enhanced tolerance to salinity during germination, this is not true for sugar beet, a crop categorized as salt tolerant which is somewhat sensitive to salinity at germination (Läuchli and Epstein, 1990). There are even differences in tolerance among cultivars (Ahmad et al., 2005; Bayuelo-Jimenez et al., 2002)

and these differences do not necessarily correspond to seasonal tolerance, as shown for melon (Nerson and Paris, 1984), bean (Bayuelo-Jimenez et al., 2002) and rice (Heenan et al., 1988). On the other hand, salt tolerant barley varieties germinated faster and showed a much higher germination percentage than the more sensitive ones (Tajbakhsh et al., 2006). Regardless, salt tolerance screening at germination pro studies have been conducted in the laboratory using Petri-dish like containers with germination paper saturated with solutions that vary in salinity. While easy to observe germination, such artificial environments are uncharacteristic of field conditions (Esechie et. al., 2002). In soil decreased due to the uptakable selenium content. The lowestselenium dose significantly (by 10%) increased the catalase activity (Nowaket al. 2004).

2.9.5.1 SALT SENSITIVITY IN RELATION TO DEVELOPMENTAL GROWTH STAGE

It has long been recognized that a crop's sensitivity to salinity varies from one developmental growth stage to the next (Bernstein and Hayward, 1958). Although there are exceptions, the majority of the research indicates that most annual crops are tolerant at germination but are sensitive during emergence and early vegetative development (Läuchli and Epstein, 1990; Maas and Grattan, 1999). As plants mature, they become progressively more tolerant to salinity, particularly at later stages of development. While these statements are generally true (with the exception of perhaps a few crops), it is important to emphasize that the definition of salt tolerance is not the same for each growth stage. During germination and emergence, tolerance is based on percent survival, while during the later developmental stages, tolerance is usually based on relative growth reductions. Salinityaffectsbothvegetativeandreproductivedevelopmentwhichhasprofound implications depending on whether the harvested organ is a stem, leaf, root, shoot, fruit, fiber or grain. Salinity often reduces shoot growth more than root growth (Läuchli and Epstein, 1990) and can reduce the number of florets per ear, increase sterility and affect the time of flowering and maturity in both wheat (Maas and Poss, 1989) and rice (Khatun et al. 1995). Since salt-tolerance from an agronomic or horticulturist perspective is based on the yield of the harvestable organ, relative to that in non-stressed environments, understanding how salinity affects vegetative and reproductive development is important for developing management strategies that can minimize stress at critical times.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 COLLECTION OF PLANT MATERIAL

Wheat (Triticum aestivum) cultivar namely NGBO1120 was obtain from National Center form Genetics Resource and Biotechnology (NACGRAB) Ibadan Oyo state. The seeds were sterilized with ethanol (20%) for 3 minute as described by Hernandez (1995) then it was rinsed thoroughly with distilled water. The experiment was carried out in the department of plant science and biotechnology. Federal University Oye- Ekiti, Ekiti State, Nigeria.

GERMINATION TEST

Germination test was conducted on seed with different concentration of sodium selenite (0mg/l, 50mg/l, 100mg/l and 150mg/l). seed was soaked in sodium selenite of different concentration for eight hours and was air dried then cotton was placed in petrish dish and seed was planted in each petrish dish according to concentration of selenite. Radicle and plumule was measured using ruler. And numbers of plant was counted according to germination percentage.

3.2 PROPAGATION OF WHEAT PLANT

3.2.1 TREATMENT OF SEEDS WITH SODIUM SELENITE

The accessions (seed) was then treated with sodium selenite at different concentration (50mg/l,100mg/land 150mg/l) by soaking for 8 hours after which was thoroughly then it was rinsed thoroughly with distilled water and air dry.

3.2.2 PREPARATION OF SOIL FOR PLANTING

Air dried-soil was measured 4kg and placed in a perforated polytene bags for different concentration of sodium selenite treatment. The already air dried soil was water with 1400ml

of water which was the water holding capacity of the air dried soil. Holes were bored with a nail, and soil was use to cover the seeds. The seeds were water adequately.

3.2.3 INTRODUCTION OF SALT STRESS (NaCl)

The NaCl was applied by dissolving as follows

200mM (millimolar).....11.7g/l

100mM (milli-molar).....5.8g/l

The 11.7g/l was dissolved in 1000ml of water and the 5.8g/l was dissolved in 1000ml of water Each pot was water with 100ml of salt. The stress was introduced into the soil as irrigation for four times. After one month of growth of wheat plant within 2days interval, the growth morphological features were also measure between the interval of two weeks. The features that were measured include the plant height and numbers of tillers. After two month of maturity of the plant analysis was carried on the leave to check for level of chlorophyll, catalase, Superoxide dismutase (SOD), malondialhyde MDA, and APX when seeds were fully matured another analysis was carried out on the following content protein and ascorbic acid, in order to know the impact of sodium selenite on wheat under salinity stress.

3.3 Catalase (CAT)

Catalase activity can be determined in the plasma using Aebi's method (Aebi, 1984). Fifty microliter of the sample is added to a cuvette containing 450 µL of phosphate buffer (0.1M, pH 7.4) and 500 µL of 20 mM H_2O_2. Catalase activity is measured at 240 nm for 1 min using spectrophotometer. The molar extinction coefficient of H_2O_2, 43.6 M cm^{-1} was used to determine the catalase activity. One unit of activity is equal to 1 mmol of H_2O_2 degraded per minute and is expressed as units per milligram of protein.

Calculation:

$$\text{Units/ml} = \frac{\Delta A/\text{min} \times d \times 1}{V \times 0.0436}$$

d = dilution of original sample for Catalase Reaction

V = Sample volume in Catalase Reaction (ml)

$0.0436 = \varepsilon^{mM}$ for hydrogen peroxide

1 = Total reaction volume

3.4 Superoxide dismutase (SOD)

This method was described by Mccord and Fridovich (1969) and can be applied for determination of antioxidant activity of a sample. To 200 µL of the lysate, 2.5 ml of 75 mM of Tris–HCl buffer (pH 8.2), 30 mM EDTA and 300 µL of 2 mM of pyrogallol are added. An increase in absorbance is recorded at 420 nm for 3 min by spectrophotometer. One unit of enzyme activity is 50% inhibition of the rate of autooxidation of pyrogallol as determined by change in absorbance/min at 420 nm. The activity of SOD is expressed as units/mg protein.

Calculation

Increase in absorbance per minutes = $A_3 - A_0/2.5$

Where A_0 = absorbance after 30 seconds

A_3 = absorbance after 150 seconds

% inhibition = 100 – 100 x (increase in absorbance for substrate/increase in absorbance for blank)

1 unit of SOD activity was given as the amount of SOD necessary to cause 50% inhibition of the oxidation of adrenaline.

3.5 ASCORBATE PEROXIDASE

APX (EC 1.11.1.11) activity was measured according to the methods of Nakano and Asada, 1981. The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, 0.1 mM hydrogen peroxide, and 0.1 mL of enzyme extract in a total volume of 1 mL. The concentration of oxidized ascorbate was calculated by the decrease in absorbance at 290 nm. The absorption coefficient was 2.8 mM−1 cm−1. One unit of APX was defined as 1 mmol mL−1 ascorbate oxidized min−1 (Hassan et al 2006)

3.6 Protein Determination

Protein determination was carried out according to the method of Lowry *et al.*, (1951) as described by Holme and peck, (1998).

Procedure: 0.2ml of the test sample was added to 2.1ml of alkaline copper reagent which was freshly prepared by mixing 2% Na_2CO_3 in 0.1M NaOH, 1% $CuSO_4.5H_2O$ and 1% Na,K tartrate.$4H_2O$, (98:1:1, by volume). The mixture was vortexed and allowed to stand for 10 minutes after which 0.2 ml of Folin-Ciocalteau colour reagent was added. The resulting reaction mixture was vortexed and allowed to stand at room temperature in a dark locker for about an hour after which the absorbance of the mixture was read at 550 nm against a reagent blank. The blank was made up of 0.2 ml of distilled and appropriate volume of the diluents and colour reagent. The protein concentration of the test samples were estimated from a standard curve obtained using bovine serum albumin (BSA). To 0.2 ml of five different concentration (50, 100. 150, 200 and 250 μg/ml) of BSA was added to appropriate volumes of diluents and colour reagent as given above. The values obtained were then used to plot a curve of Absorbance against BSA concentration using linear regression.

Mg protein/ml = Asample x Dilution factor/E

Where E is the slope of the best-fit linear regression line obtained from the graph of the standard curve for BSA.

3.7 Determination of MDA

Total amount of lipid peroxidation products present in the brain samples was estimated by the thiobarbituric acid (TBA) method which measures the malondialdehyde (MDA) reactive products according to the method of Ohkawa et al., (1979).

Procedure

To 0.5 ml of samples was added 0.5 ml of phosphate buffer (0.1 M, pH 8.0) and 0.5 ml of 24% TCA. The resulting mixture was incubated at room temperature for 10 min, follow by

centrifugation at 2000 rpm for 20 min. To 1 ml of supernatant was added 0.25 ml of 0.33% TBA in 20% acetic acid and the resulting mixture was boil at 95°C for 1 hr. The resulting pink colour gproduct was cool and absorbance was read at 532nm.(Extinction coefficient of MDA, $\varepsilon 532 = 1.53 \times 10^5\ M^{-1}\ cm^{-1}$)

3.8 Determination of Vitamin C Content

Ascorbic acid Standard Solution: Prepare 1000µg/ml solution of ascorbic acid by dissolving 0.25g of ascorbic acid in 250ml of distilled water. A fresh stock solution stock solution was prepared each time and diluted to get 10µg/ml solution before use.

Iron (III) - phenanthroline reagent: Accurately weighed 0.198g (1mM) of AR grade 1, 10-phenathroline monohydrate was mixed with 1ml of 1N hydrochloric acid in a 100 ml flask. Then 0.16g of iron (III) ammonium sulphate was added and the contents were dissolved in a few ml of water. The solution was then diluted to the mark with water.

PROCEDURE

Pipette 0.2, 0.4, 0.6, 0.8 and 1.0 ml aliquots of 10µg/ml solution of ascorbic acid into test tube 2-6 and make up to 1 ml with distilled water. To test 7-10, pipette 1ml of extracted lycopene. Add 2.0 ml of Iron(III)- phenanthroline reagent to all the test tube and make up the volume to 5 ml with distilled water. Test tube 1(blank) will contain 2.0 ml of Iron(III)- phenanthroline reagent and make up to 5 ml with distilled water

Absorbance was read at 515nm against the blank after 10 minutes, and the concentration of vitamin C in the sample was determined from the calibration curve.

Estimation of Chlorophyll Content by Arnon Method

A known weight (100mg) of sample was homogenized in 10 ml of Acetone and centrifuged at 5000 RPM. The supernatant was collected and the absorbance was read at 663, 645 and 470 nm respectively in spectrophotometer. The amount of chlorophyll was calculated as follows. Chlorophyll and carotenoid content determination. The extraction of leaf pigments was

performed with 80% acetone, and the absorbance at 664 and 648 nm was measured with an Amersham spectrophotometer (Amersham Biosciences, Piscataway, NJ, USA). The chlorophyll a, chlorophyll b, and total chlorophyll quantities were calculated according to the method of Arnon (21). Total carotenoid content was measured at 470 nm. The pigment concentrations were expressed as μg g−1 fresh weight (FW).

CHAPTER FOUR

4.0 RESULT

4.1 **Germination Test**

High significant difference was observed in the number of plumule (3.3) and radicle (4.3) of the plants treated with 50mg/l sodium selenite. However, high germination percentage (90%) was found in plants without selenite and with 100mg/l selenite (table 1).

Table 1: Effect of selenite on germination of wheat seed

Treatment	Number of radicle	Number of plumule	Germination Percentage (%)
CNT	2.86	2.8	90
Se50	4.29	3.29	80
Se100	3.71	2.79	90
Se150	3.29	3.07	60

4.2 **Growth Performance of selenite primed wheat under salinity stress**

There was a significant difference in number of tillers and leaves under control and sodium selenite level at weeks 4 and 6 (fig 1 and 2). Plants treated with selenite concentration 50mg/l with and without salt stress was significantly high (<20) in number of leaves than other treatments at weeks 4 and 150mgl with and without salt stress was significantly high (<18). There were no significant differences in number of tillers with or without selenite and salinity stress. However at week 8 and 10, plants treated with 150mg/l selenite and 100mM salinity stress had a high (<30) significant difference in number of leaves when compared with other treatments (fig 3 and 4).

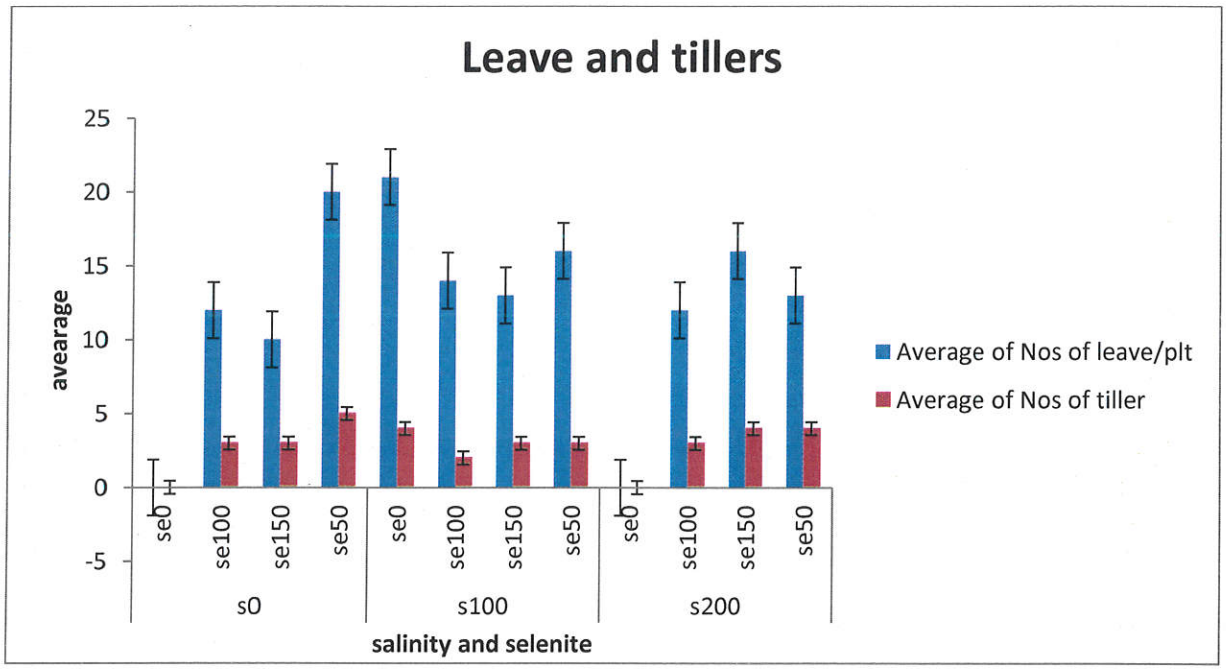

Figure 1: Numbers of leafs numbers of tillers of plant with selenite treatment under salinity stress on four weeks of germination

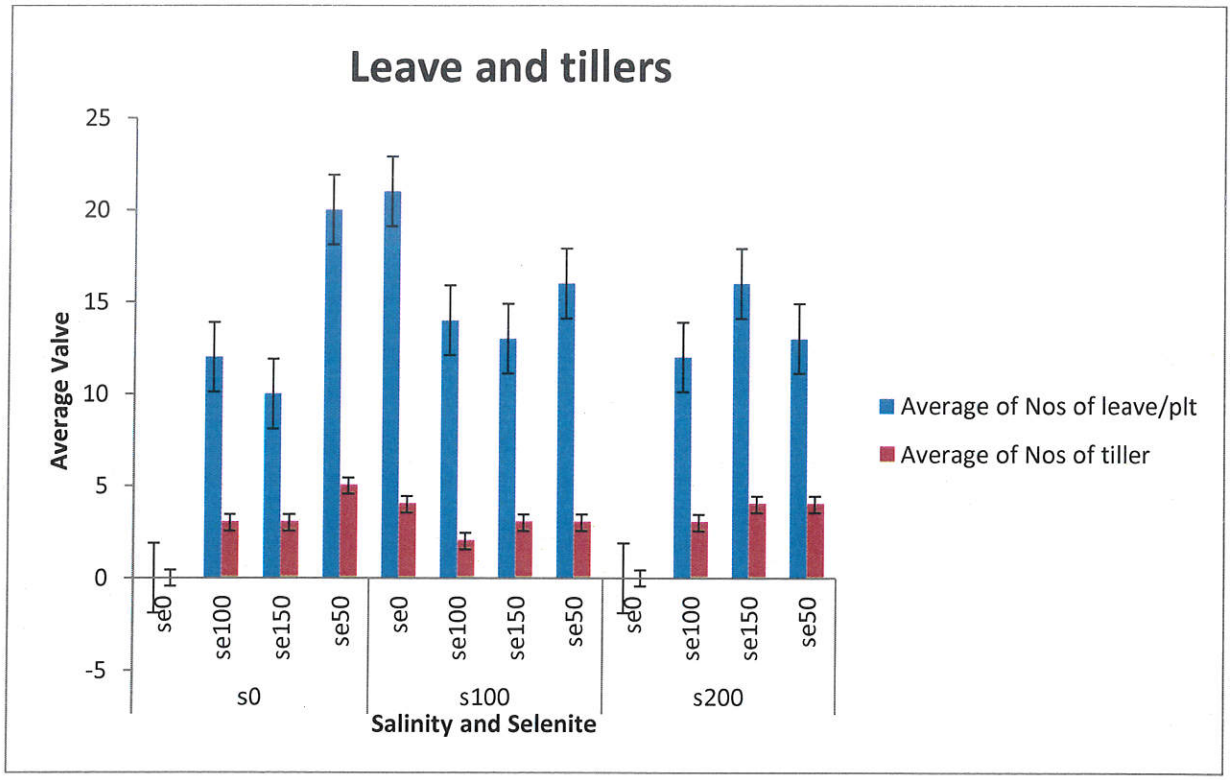

Figure 2: effect of selenite on numbers of leaves and tillers of salinity stressed wheat at six weeks after planting

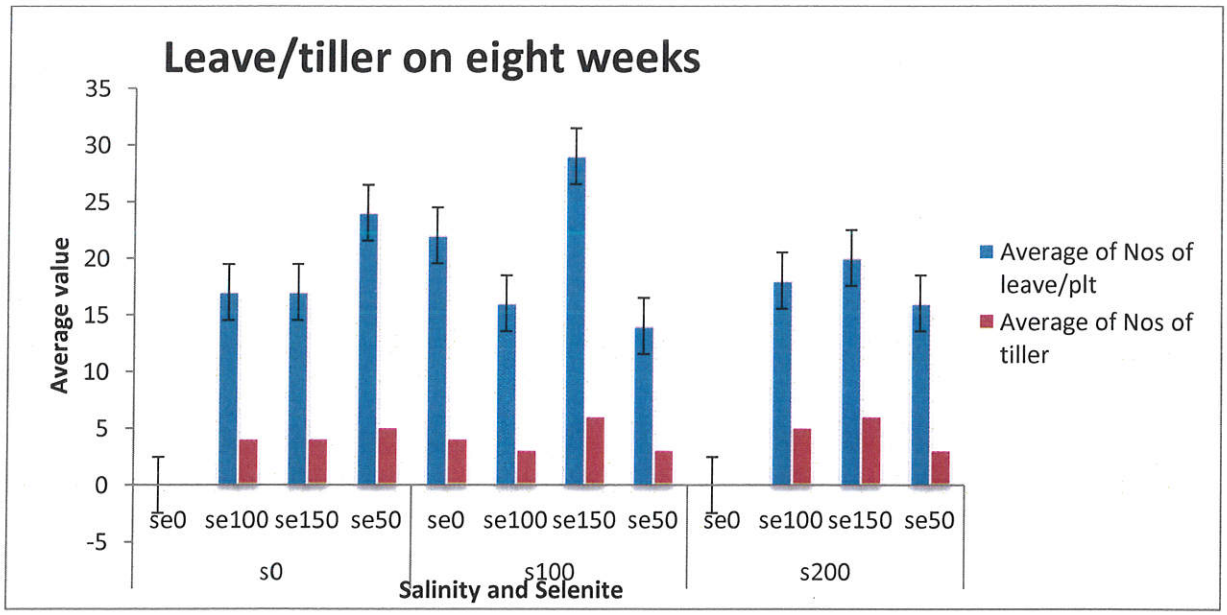

Figure 3: Numbers of leaf and tillers with selenite treatment under salinity stress on eight weeks of germination

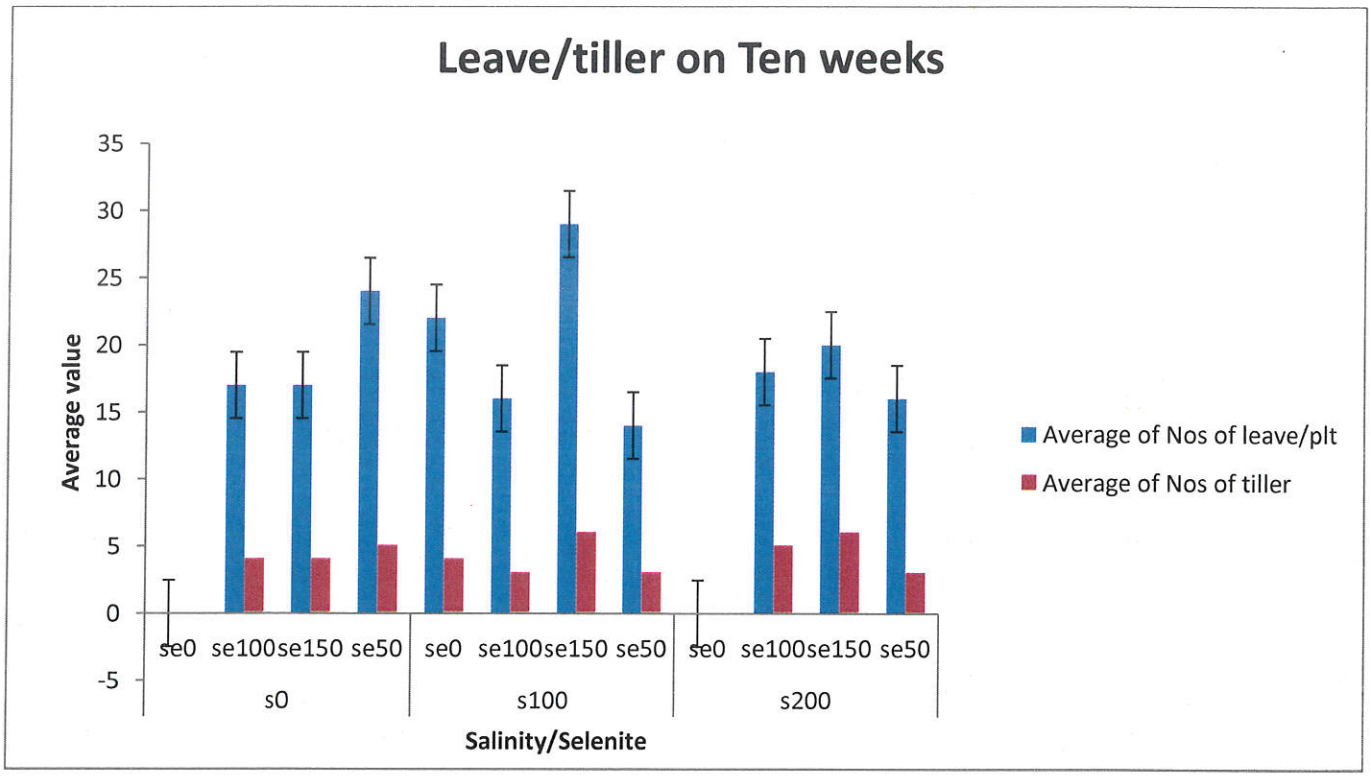

Figure 4: Numbers of leaf and tillers with selenite treatment under salinity stress on ten weeks of germination.

4.3 Yield of selenite primed wheat as affected by salinity stress

Table 2 shows the effect of selenite on salinity stressed wheat plant. Yield of wheat with selenite treatment 100mg/l with no salinity stress is significantly high in its fresh weight (16.5) dry weight (3.19) compared to all other treatment. Wheat treated with 100mg/l selenite under salinity stress of 100mm is significantly high in shoot weight (11.77g), grain weight (3.29g) and numbers of grain (134) than control and other treatments. Plants treated with 50mg/l selenite and salinity stress 100mM is significantly low in it fresh weight (0.68) dry weight (0.08) above all other treatments. Plants treated with 100mg/l selenite under salinity stress of 200mM are significantly low in shoot weight (0.70g) and numbers of grain (23) than other treatments.

Table 2: Effect of selenite on yield of wheat

Salt	Selenium	Dry root weight per plant (g)	Dry Shoot weight per plant (g)	Grain weight per plant (g)	Number of grain per plant
S	S0	0.43e	7.30b	1.05d	60.00e
S100	S0	0.19f	5.00c	0.54ef	52.00ef
S200	S0	0.09g	3.90cd	0.32f	35.00g
S	Se 50	0.69cd	8.25b	2.58b	65.00e
S100	Se 50	0.08	4.95c	2.00bc	69.00e
S200	Se 50	0.31ef	4.61c	1.78c	48.00f
S	Se100	3.19a	6.79bc	1.75c	49.00f
S100	Se100	1.78b	11.77a	3.29a	134.00a
S200	Se100	0.49e	6.26bc	0.70e	23.00h
S	Se150	0.34ef	6.52bc	2.74b	84.00d
S100	Se150	0.59d	11.45a	2.60b	106.00b
S200	Se150	0.87c	11.50a	3.09a	97.00c

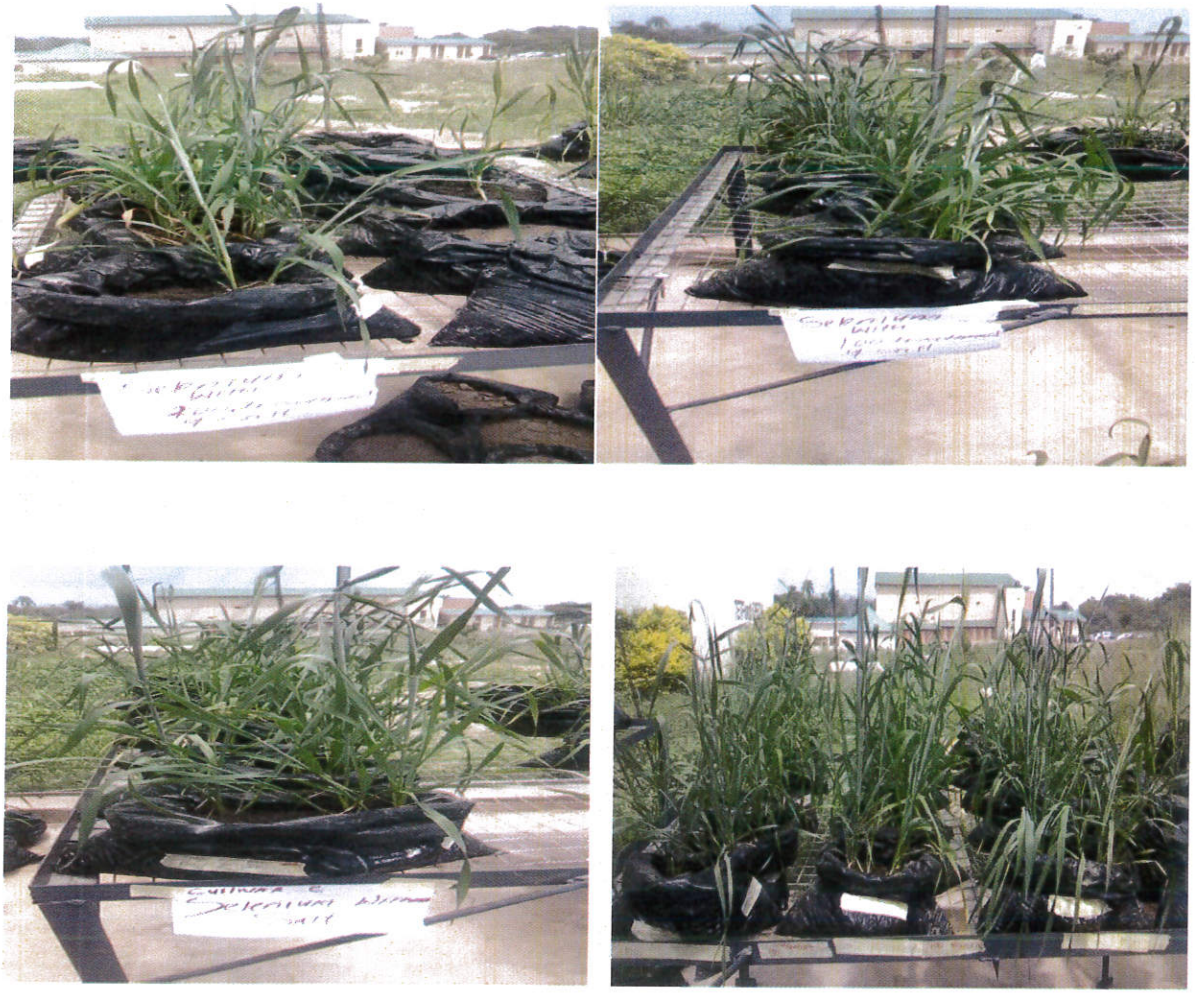

Plate 1: Photographs of *Triticum aestivum* which has been treated with selenium at different concentration (0mg/l, 50mg/l, 100mg/l and 150mg/l) also under the influence of salinity stress of different concentration(omM, 100mM and 200mM) in it vegetative stage.

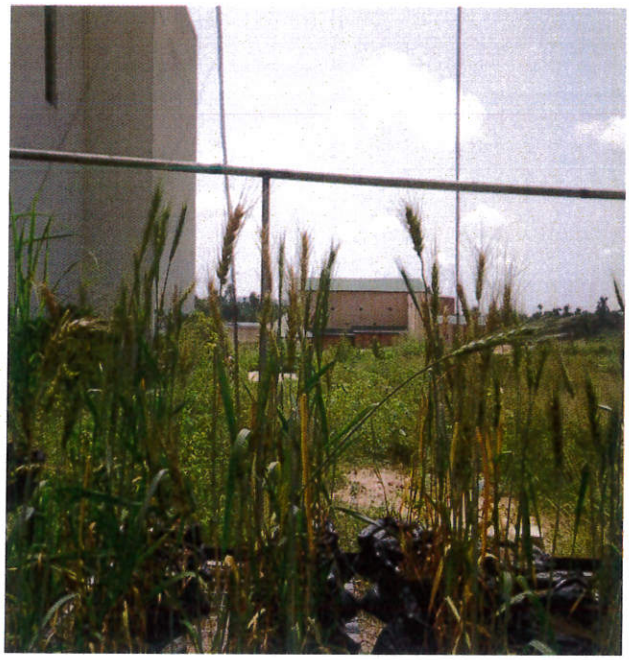

Plate 2: photographs *of Triticum aestivum* in it reproductive stage

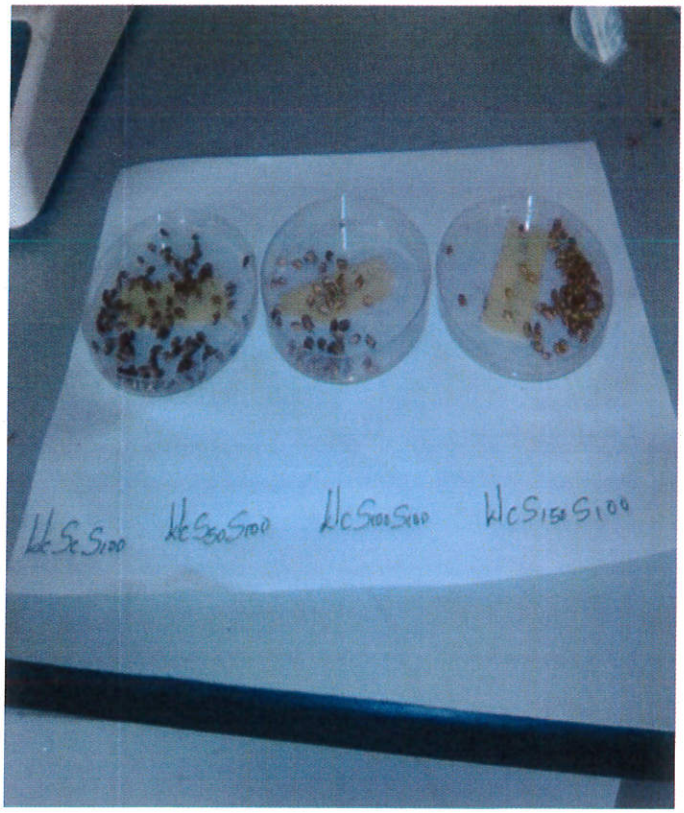

Plate 3: photographs of seeds of *wheat*

with different CONC of selenite at 100mM of salinity

Plate 3: photographs of seeds of *wheat*

with different CONC of selenite at 100mM of salinity

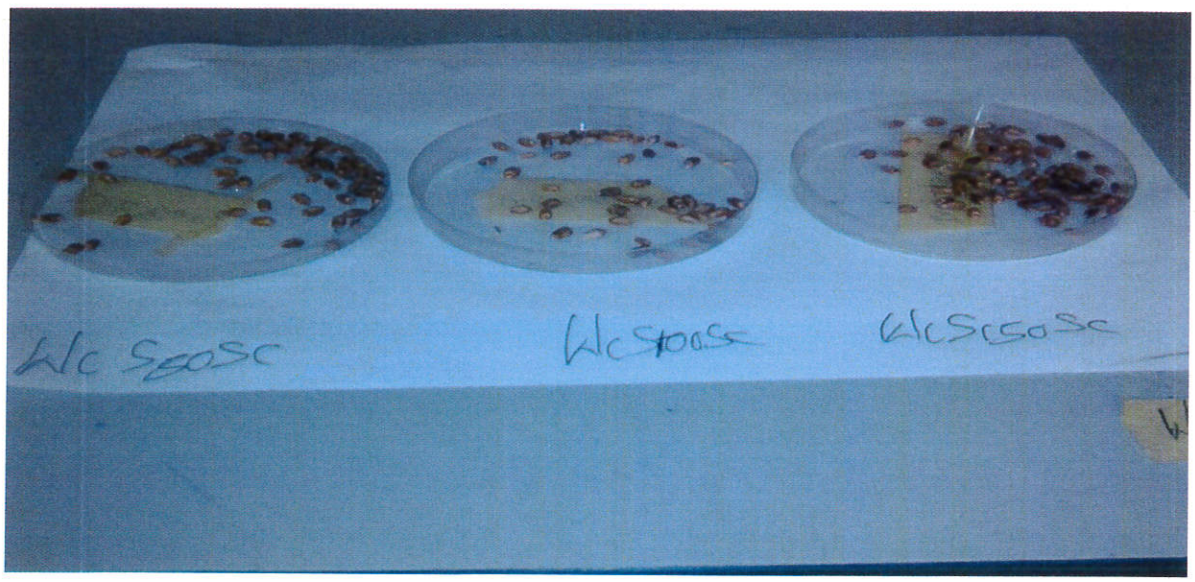

Plate 5: Photographs of seed of wheat with different concentration of sodium selenite with no salinity stress

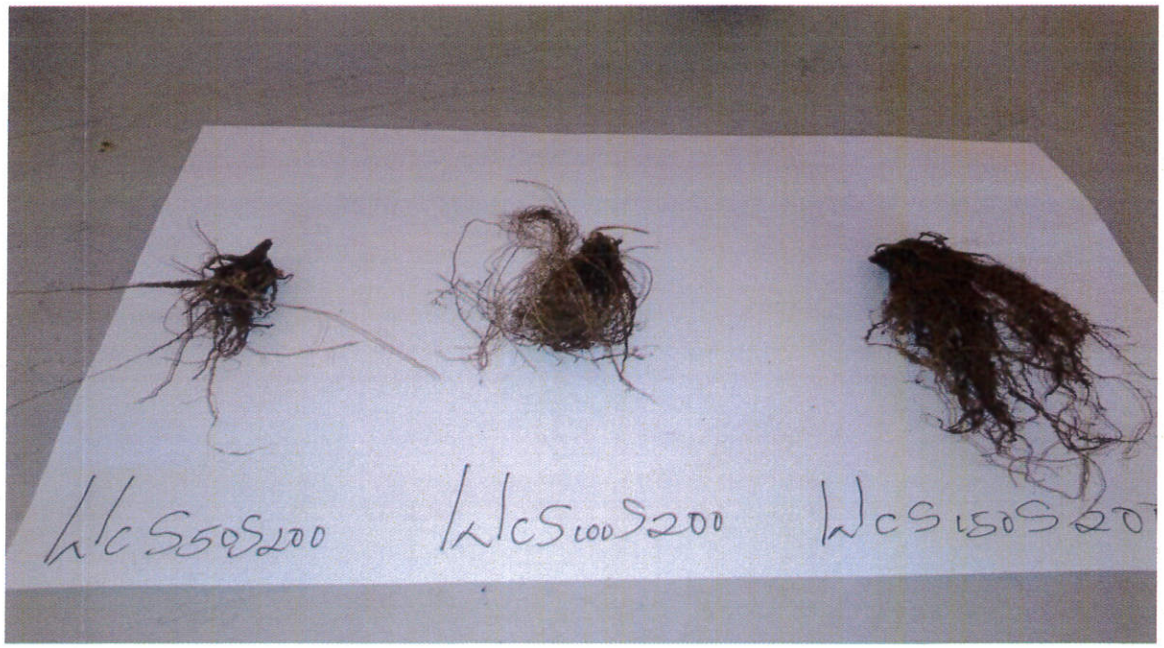

Plate 6: photographs of freshly collected root of wheat of different concentration of sodium selenite with 200mm of salt irrigation

Plate 7: photographs of freshly collected root of Wheat of different concentration of sodium selenite which was irrigated with 100mm of sodium chloride.

4.3 Chlorophyll Content of Wheat

When there was no salinity stress, chlorophyll a and b in plants treated with 50mg/l selenite were significantly higher than other treatments. When salinity stress was introduced, chlorophyll a and b contents of plant treated with 150mg/l and 100 mM salt and 100mg/l selenite and 200 mM salt were significantly high than treatment (fig 5) .

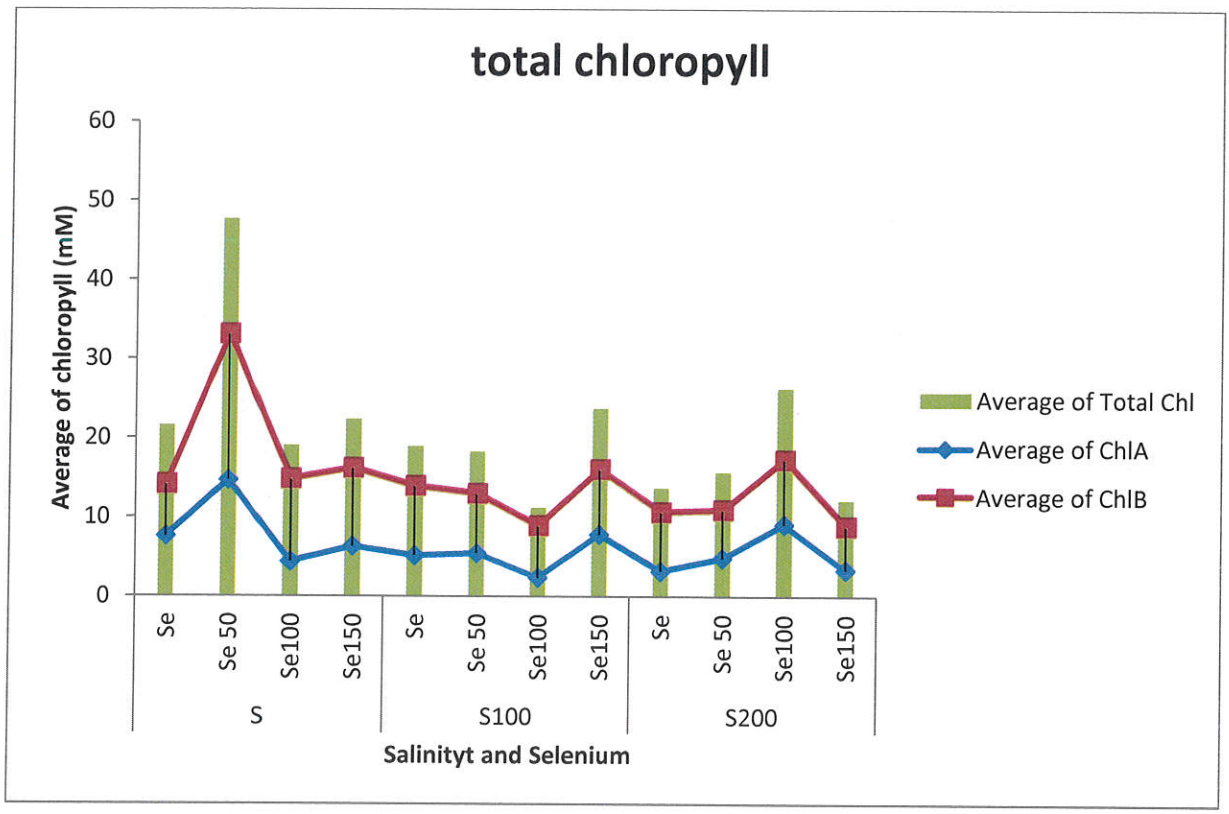

Figure 5: Effect of salinity on chlorophyll content of the leaf of wheat with Selenite treatment

4.4 Carotenoid Contents

When salinity was induced, total carotene was significantly high in plants treated with 50mg/l and 100mg/l selenite. Though, plants treated with 50 mg/l of selenite had the highest total carotene when there was no salinity stress nevertheless, selenite had a significant difference on total carotene of wheat plants with or without salinity stress (fig 6).

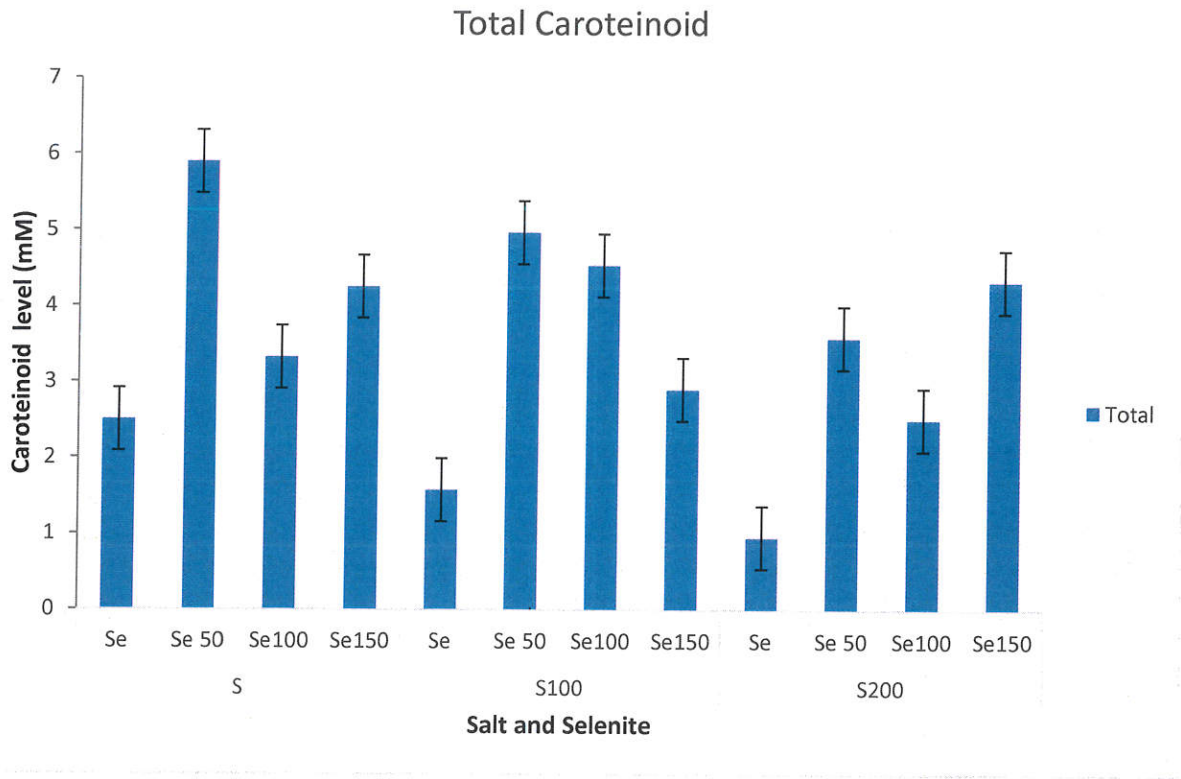

Figure 6: Carotenoid content of leave of wheat under the influence of Salinity stress with Selenite treatment

4.5 Malondialdehyde (MDA) Content of Selenite Treated Wheat Plants

Low MDA content was observed in plants treated with 50mg/l and without selenite when salinity stress was severe i.e. 200mM salt. When there was no salinity stress, selenite 50 mg/l had a significant high MDA compared with other treatments. During salinity stress, plants with 100mg/l selenite were significantly greater than other treatments (fig 7).

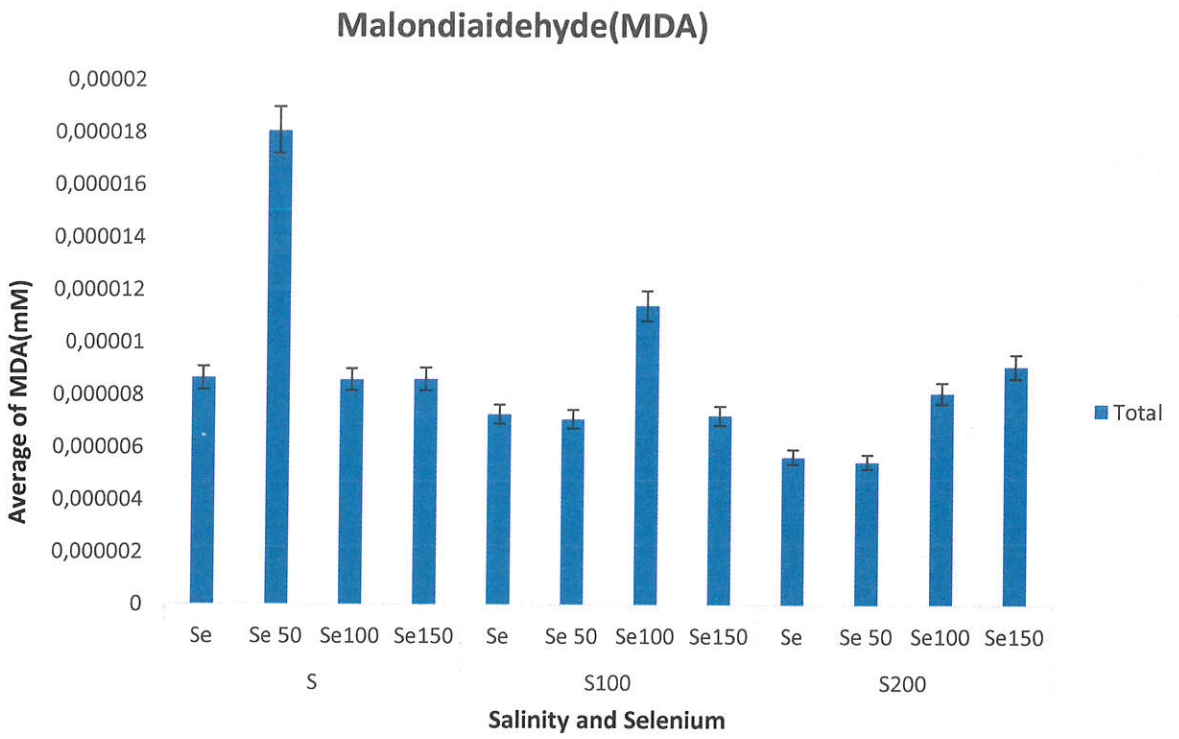

Figure 7: Malondialdehyde (MDA) Content of leave of wheat under the influence of Salinity stress with Selenite treatment

4.6 Antioxidant Enzymes of Wheat under the treatments of Salinity and Selenite

Ascorbate Peroxidase (APX)

Ascorbate peroxidase was significantly high in plant without salinity than other treatment. Selenite significantly lowered the activity of APX when salinity was introduced (fig 8).

Superoxide Dismutase (SOD)

Superoxide dismutase was significantly high in plants treated with 150mg/l with or without salinity. When salinity stress was severe i.e. 200mg/l, SOD activity was increased as the selenite concentration increase progressively (fig 9).

Catalase (CAT)

When salinity stress was induced, plants treated with 100mg/l selenite had the highest level of catalase activity whereas plants treated with 50mg/l selenite were significantly high when there was no salinity stress (fig 10).

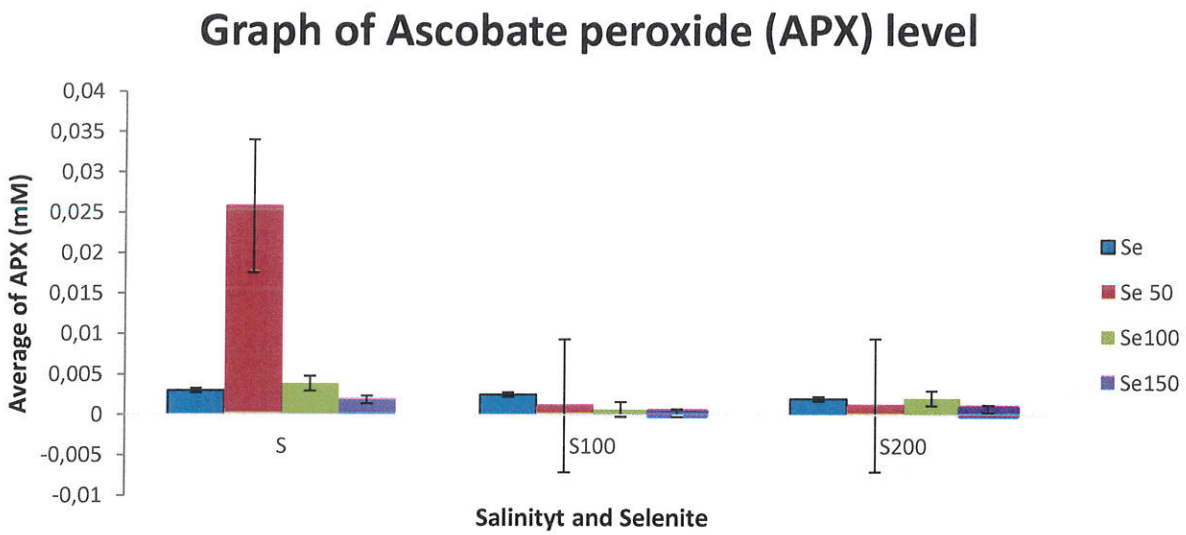

Figure 8: Ascorbate Peroxidase (APX) content of leave of wheat under the influence of Salinity stress with Selenite treatment

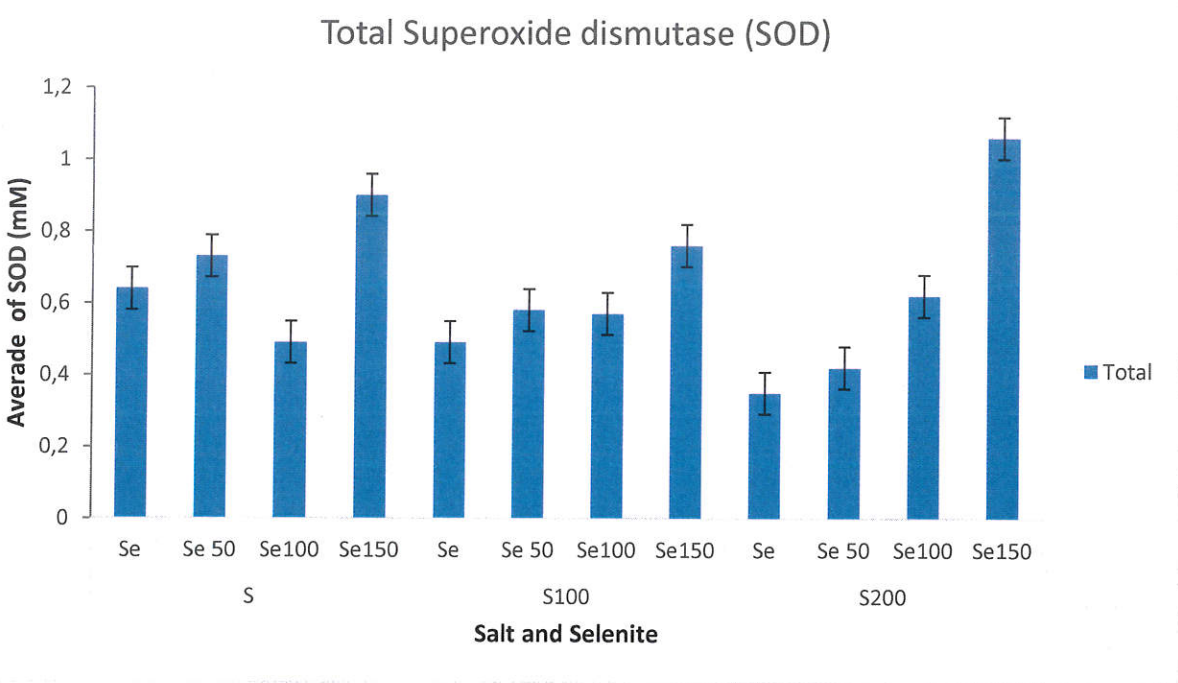

Figure 9: Superoxide dismutase (SOD) content of leave of wheat under the influence of Salinity stress with Selenite treatment

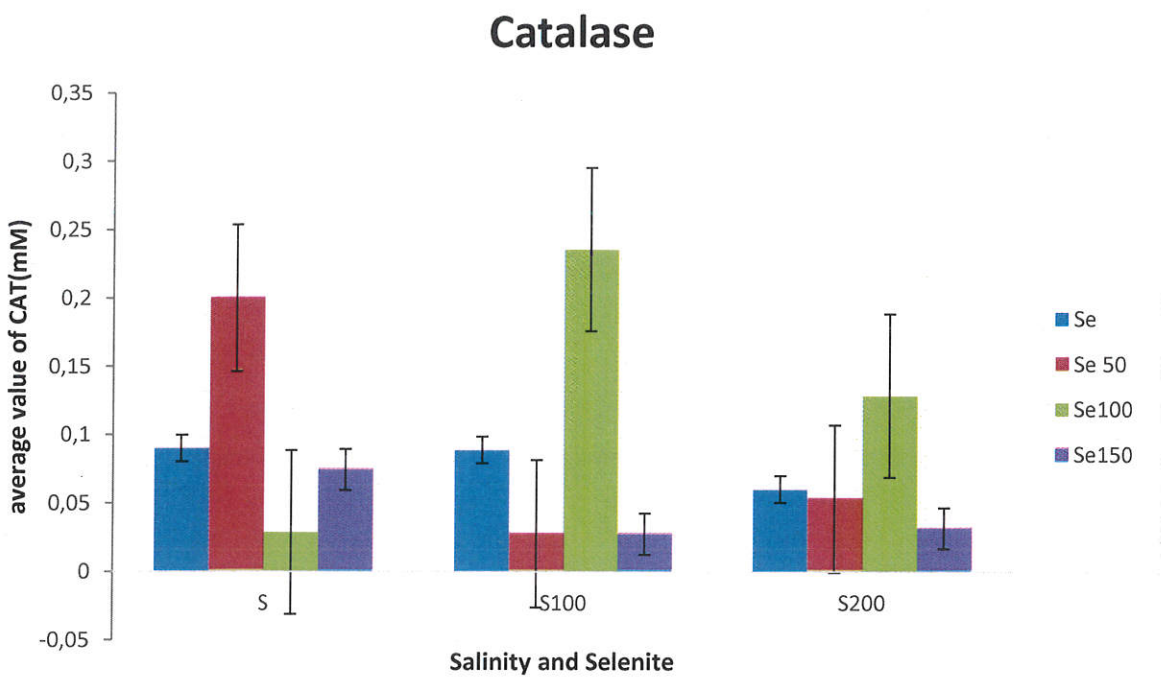

Figure 10: Catalase content of leave of wheat under the influence of Salinity stress with Selenite treatment.

4.7 DISSCUSSION AND CONCLUSIONS

Selenium (Se) is an essential micronutrient with a range of physiological and anti-oxidative properties. Priming of plant seeds is an easy, low-cost, low-risk, and effective approach to improve plant tolerance under stressful environments. Very little has been done to evaluate the overall role of Selenite seed priming on survival of salinity stressed wheat. Selenium has not been classified as an essential element for plants, although its role has been considered to be beneficial in plants capable of accumulating large amounts of the element (Terry *et al.*, 2000). Uptake and accumulation of selenium by plants is determined by the chemical form and concentration, soil factors such as pH, salinity and $CaCO_3$ content, the identity and concentration of competing ions, and the ability of the plant to absorb and metabolize selenium (Kabata Pendias, 2001).

High significant difference was observed in the number of plumule and radicle of the plants treated with 50mg/l sodium selenite. However, high germination percentage (90%) was found in plants under control and with 100mg/l of selenite treatment. This shows that sodium selenite improved the growth of the wheat seedling. Selenite had a significant difference on the number of leaves both on the cultivars with or without salinity stress at different level of developmental stages.

There was a significant difference(49mM) on the total chlorophyll and in chlorophyll a (10mM) and b (37mM) in plant treated with selenite 50mg/l when salinity was introduce selenite improve the plant treated with 100mg/l under salinity stress 200nM at this point selenite had a significant difference (22mM) on the plant above others under salinity stress. Selenite had a significant difference on plant under salinity or no salinity stress in it carotene status (Timothy,2001). As selenite was able to boost the antioxidant activities of carotene both in a stress and non-stress state (Timothy,2001).

There was no significant difference on the APX level of wheat plant under salinity stress. Selenite had a significant difference on catalase both on plant under salinity stress and no salinity stress. Selenite had a significant effect on the plant treated with selenite150mg/l under salinity stress of 200mM in SOD activities, selenite improve this plant at a low SOD content thereby preventing damage of SOD

CONCLUSION

Selenite had a significant difference on the antioxidant activities SOD (Superoxide dismutase), CAT, MDA, chlorophyll content, carotenoid of wheat plant either on salinity stress plant and no salinity stress plant as the selenite was able to improve the capacity of this antioxidant to with stand against salinity stress and also from preventing them from damage. However selenite had no significant difference on APX in wheat plant under salinity stress. Selenite improved the number of leave and grain yield of wheat plants.

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