PHYSIOLOGICAL, BIOCHEMICAL AND MOLECULAR PARAMETERS OF SALT TOLERANCE IN DIFFERENT GENOTYPES OF OATS (*Avena sativa* L.)

Thesis

Submitted to the Punjab Agricultural University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE
in
BOTANY
(Minor Subject: Biochemistry)

By

Kamaljeet Kaur
(L-2017-BS-254-M)

Department of Botany
College of Basic Sciences and Humanities

LUDHIANA-141004

2019

CERTIFICATE - I

This is to certify that the thesis entitled **"Physiological, Biochemical and Molecular Parameters of salt tolerance in different genotypes of oats (*Avena sativa* L.)"** submitted for the degree of Master of Science in the subject of **Botany** (Minor subject: **Biochemistry**) of the Punjab Agricultural University, Ludhiana, is a bonafide research work carried out by **Ms. Kamaljeet Kaur (L-2017-BS-254-M)** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

_____

(Dr. (Mrs.) Namarta Gupta)
Major Advisor
Associate Professor
Department of Botany
College of Basic Science and Humanities
Punjab Agricultural University
Ludhiana-141004, India

CERTIFICATE - II

This is to certify that the thesis entitled, **"Physiological, Biochemical and Molecular parameters of salt tolerance in different genotypes of oats (*Avena sativa* L.)"** submitted by **Ms. Kamaljeet Kaur** (Admission No. **L-2017-BS-254-M**) to the Punjab Agricultural University, Ludhiana, in partial fulfillment of the requirements for the degree of **Master of Science** in the subject of **Botany** (Minor subject: **Biochemistry**) has been approved by the Student's Advisory Committee along with External Examiner after an oral examination on the same.

_____
(Dr. (Mrs.) Namarta Gupta)
Major Advisor

_____
(Dr. (Mrs.) Neera Garg])
External Examiner
Professor
Department of Botany
Panjab University
Chandigarh

_____
(Dr. (Mrs.) Seema Bedi)
Head of the Department

_____
(Dr. (Mrs.) Gurinder Kaur Sangha)
Dean Postgraduate Studies

ACKNOWLEDGEMENT

Firstly, I owe my debt to God for showering ultimate blessings on me to see the lining in very dark cloud and helping me to become able to present this humble contribution to the knowledge of science.

I express my insufficiency to trace out appropriate words to express my deep sense of gratitude to my esteemed Major Advisor, ***Dr. (Mrs.) Namarta Gupta****, Associate Professor who nested me by showering valuable guidance, constant encouragement and benevolent help rendered throughout the study without which it would have been impossible to accomplish the present work. I have real appreciation and regards for her warm, friendly behaviour, motherly affection and sense of forgiveness throughout my association with her. It was my proud privilege and unforgettable experience to work under the supervision of such a dedicated guide.*

I am extremely thankful to Head of Department ***Dr. (Mrs.) Seema Bedi****, Professor-cum-Head, Department of Botany for her able guidance and suggestions during the entire degree.*

I express my deep sense of gratitude to the respected members of my advisory committee. This thesis would not have been possible without the help, immaculate guidance, constructive reviews and continuous support of ***Dr. (Mrs.) Meenakshi Goyal****, Assistant Biochemist,* ***Dr. (Mrs.) Nirmaljit Kaur****, (Dean PG's Nominee) and* ***Dr. (Mrs.) Navjyot Kaur****, Assistant Plant Physiologist,* ***Dr.(Mrs.) Yogesh Vikal****, Senior Geneticist.*

I feel elated in expressing thanks to ***Dr. Rahul Kapoor****, Assistant Forage Breeder, Department of Plant Breeding and Genetics for helping during tenure of my study and Statistical Analysis.*

I find no words to acknowledge in so formal manner the unending patience, encouraging moral support, affection and inspiration rendered by my respected parents, ***S. Ajaib Singh*** *and* ***Sd. Manjeet Kaur*** *and my brother* ***Harpreet Singh****. Today what I am, I owe to them and my family as their selfless sacrifices will remain indelible in my life.*

Loving thanks to my dear friends, ***Simarpreet****,* ***Priyanka****, ,* ***Rancy****,* ***Rohit, Ravneet*** *and* ***Manjeet Kaur*** *as we mutually engaged in making sense of the various challenges we faced and in providing encouragement to each other at those times when it seemed impossible to continue. I am very thankful to* ***Gurwinder Kaur*** *for her help in research work and continuous support during my degree.*

The cooperative attitude of non-teaching staff and technical assistance provided by ***Karam Sir*** *and* ***Ashok Sir*** *is worth appreciating and greatly acknowledged.*

I am also indebted to the respondents for their cooperation and generous response during data collection. In the end, I am thankful to all those who may not have been mentioned but are not forgotten.

Needless to say, errors and omissions if any are all mine.

(Kamaljeet Kaur)

Title of the Thesis	:	Physiological biochemical and molecular parameters of salt tolerance in different genotypes of oats *(Avena sativa* L.)
Name of the Student and Admission No.	:	Kamaljeet Kaur L-2017-BS-254-M
Major Subject	:	Botany
Minor Subject	:	Biochemistry
Name and Designation of Major Advisor	:	Dr. (Mrs.) Namarta Gupta Associate Professor
Degree to be Awarded	:	M.Sc.
Year of award of Degree	:	2019
Total Pages in Thesis	:	66 + Vita
Name of University	:	Punjab Agricultural University, Ludhiana-141004, Punjab, India

ABSTRACT

The effect of salt stress was studied on physiological, biochemical and molecular parameters in different genotypes viz. Kent, OL-10, OL-1862, OL-1869, OL-125, OL-1966, OL-1876, OL-1895 of oats (*Avena sativa* L.). When seeds of oats were subjected to different salt concentrations (at 25mM, 50mM, 75mM, 100mM, 125mM, 150mM, 175mM, 200mM) of NaCl, various physiological parameters such as shoot length(lamina and sheath), root length, dry weight, fresh weight, seed vigour I, seed vigour II declined with the increase in salt stress level in all the genotypes whereas the biochemical parameters such as total soluble sugars, total protein content and activities of antioxidant enzymes increased with the increase in salt stress level in all genotypes.

Keywords: *Avena sativa*, salt stress, salt treatments, physiological response, antioxidant enzymes, genotypes.

_____ _____

Signature of Major Advisor **Signature of the Student**

ਖੋਜ ਗ੍ਰੰਥ ਦਾ ਸਿਰਲੇਖ	:	ਜਵੀ ਦੇ ਵੱਖ-ਵੱਖ ਜੀਨੋਟਾਇਪਾਂ ਵਿੱਚ ਲੂਣ ਸ਼ਹਿਣਸ਼ੀਲਤਾ ਦੇ ਫਿਜਾਲਜੀਕਲ, ਬਾਇਓਕੈਮੀਕਲ ਅਤੇ ਮੌਲੀਕਿਊਲਰ ਮਾਪਦੰਡ
ਵਿਦਿਆਰਥੀ ਦਾ ਨਾਂ ਅਤੇ ਦਾਖ਼ਲਾ ਨੰਬਰ	:	ਕਮਲਜੀਤ ਕੌਰ (ਐਲ-2017-ਬੀ.ਐਸ-254-ਐਮ)
ਪ੍ਰਮੁੱਖ ਵਿਸ਼ਾ	:	ਬੌਟਨੀ
ਸਹਿਯੋਗੀ ਵਿਸ਼ਾ	:	ਜੀਵ ਰਸਾਇਣ
ਮੁੱਖ ਸਲਾਹਕਾਰ ਦਾ ਨਾਂ ਅਤੇ ਅਹੁੱਦਾ	:	ਡਾ. (ਮਿਸਿਜ਼) ਨਮਰਤਾ ਗੁਪਤਾ ਸਹਿਯੋਗੀ ਪ੍ਰੋਫੈਸਰ
ਡਿਗਰੀ	:	ਐੱਮ.ਐੱਸ.ਸੀ.
ਡਿਗਰੀ ਨਾਲ ਸਨਮਾਨਿਤ ਕਰਨ ਦਾ ਸਾਲ	:	2019
ਖੋਜ ਪੱਤਰ ਵਿੱਚ ਕੁੱਲ ਪੰਨੇ	:	66 + ਵੀਟਾ
ਯੂਨੀਵਰਸਿਟੀ ਦਾ ਨਾਮ	:	ਪੰਜਾਬ ਖੇਤੀਬਾੜੀ ਯੂਨੀਵਰਸਿਟੀ, ਲੁਧਿਆਣਾ-141004, ਪੰਜਾਬ, ਭਾਰਤ

ਸਾਰ ਅੰਸ਼

ਜਵੀ ਦੇ ਵੱਖ-ਵੱਖ ਜੀਨੋਟਾਇਪ, ਕੈਂਟ, OL-10, OL-1862, OL-1869, OL-125, OL-1966, OL-1876, OL-1895 ਵਿੱਚ ਲੂਣ ਤਨਾਅ ਦਾ ਫਿਜਾਲਜੀਕਲ, ਬਾਇਓਕੈਮੀਕਲ ਅਤੇ ਮੌਲੀਕਿਊਲਰ ਮਾਪਦੰਡਾਂ ਉੱਪਰ ਪ੍ਰਭਾਵ ਦਾ ਅਧਿਐਨ ਕੀਤਾ ਗਿਆ। ਜਵੀ ਦੇ ਬੀਜਾਂ ਨੂੰ ਵੱਖ-ਵੱਖ NaCl ਲੂਣ ਮਾਤਰਾ (25 mM, 50 mM, 75 mM, 100 mM, 125 mM, 150 mM, 175 mM, 200 mM) ਨਾਲ ਕਈ ਫਿਜਾਲਜੀਕਲ 'ਮਾਪਦੰਡ ਜਿਵੇਂ ਸ਼ੂਟ ਲੰਬਾਈ (ਲੇਮੀਨਾ ਅਤੇ ਸ਼ੀਥ), ਜੜ੍ਹਾਂ ਦੀ ਲੰਬਾਈ, ਸੁੱਕਾ ਭਾਰ, ਤਾਜ਼ਾ ਭਾਰ, ਸੀਡ ਵਿਗਰ-I, ਸੀਡ ਵਿਗਰ II ਸਾਰੇ ਜੀਨੋਟਾਇਪ ਵਿੱਚ ਲੂਣ ਮਾਤਰਾ ਵਧਣ ਦੇ ਨਾਲ ਘਟਦੇ ਗਏ ਜਦਕਿ ਬਾਇਓਕੈਮੀਕਲ ਮਾਪਦੰਡ ਜਿਵੇਂ ਕੁੱਲ ਘੁਲਣਸ਼ੀਲ ਸ਼ੂਗਰ, ਕੁੱਲ ਪ੍ਰੋਟੀਨ ਮਾਤਰਾ ਅਤੇ ਐਂਟੀਆਕਸੀਡੈਂਟ ਇੰਜਾਇਮ ਕਿਰਿਆ ਸਾਰੇ ਜੀਨੋਟਾਇਪਾਂ ਵਿੱਚ ਲੂਣ ਤਨਾਅ ਵਧਣ ਦੇ ਨਾਲ ਵਧੇ ।

ਮੁੱਖ ਸ਼ਬਦ: ਜਵੀ, ਲੂਣ ਤਨਾਅ, ਲੂਣ ਉਪਚਾਰ, ਫਿਜਾਲਜੀਕਲ ਪ੍ਰਤੀਕਿਰਿਆ, ਐਂਟੀਆਕਸੀਡੈਂਟ ਇੰਜ਼ਾਇਮ, ਜੀਨੋਟਾਇਪ ।

ਮੁੱਖ ਸਲਾਹਕਾਰ ਦੇ ਹਸਤਾਖਰ **ਵਿੱਦਿਆਰਥੀ ਦੇ ਹਸਤਾਖਰ**

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

CHAPTER-I

INTRODUCTION

Oat (*Avena sativa* L.) is a cereal crop belonging to family Poaceae. It is a minor and cool season crop that has been used as a foodstuff for both humans and livestock. Oat is among the valuable functional crops with numerous nutritional, industrial and health benefits. It is an excellent source of dietary fiber β into the diet. Oats are getting increasingly popular at the breakfast tables of Indian homes. Nutritionally oats are an excellent source of soluble fiber β in the range of 2-6 % (Bruce 2008). Oat β–glucan have high therapeutic properties like anti activity against diabetes, hyperlipidaemia, hypertension and inflammatory state. Oats along with soluble fiber β–glucan are rich in lipids, protein, B vitamins, minerals, plants, and specific micronutrients and also it acts as a unique source of polyphenols. It is also an excellent source of energy, unsaturated fatty acids and lower in carbohydrates than other whole grains.

Among different environmental stresses which are drought, flooding, salinity, heat, cold, Salinity is major constraint to the farming efficiency (Shukla *et al* 2012). Salt stress is a main abiotic stress which affects the crop production. It affects more than 6% of the world`s total land area (Gao *et al* 2016). In Punjab, about 80% of total salt affected area is found in Bathinda and Ferozepur districts. Using groundwater for irrigation is one of the reasons that causes accumulation of salts on land and makes it unfit for cultivation purposes (Singh 2004). Salinity of soil may be attributed to the seepage from Rajasthan feeder canals and appears to be the cause for the development of water logging and salinity in these areas. An appreciable portion of Punjab still suffers from high salinity level.

Salinity affects crop development by affecting many physiological, biochemical and metabolic processes (Willenborg *et al* 2005). Salt stress by overload of salts in soil or irrigation water affects the different development processes which includes photosynthesis, ion regulation, water relations, etc (Ashraf 2004). Because of high concentration of salts it is difficult for roots to take up water (Zhang *et al* 2018). Salinity, with the components of osmotic stress and ion toxicity, also causes oxidative stress. Accumulation of salts leads to osmotic stress, cell dehydration, nutrient imbalance, unstability of cellular membranes and reduce leaf photosynthetic activity (Shahid *et al* 2012). The damaging effect of salt stress on number of leaves, also increases with the increase in concentration of salts (Ha *et al* 2008). By changes in salt ion concentration, kind of salt present, or kind of plant species, the fresh and dry weight of the shoot system are affected either negatively or positively (Memon *et al* 2010). Protein amount can also be affected largely because of salinity (Kapoor and Srivastava

2010). Salinity leads to the raise of soluble sugars and proline content in the leaves of oat (Wang and Song 2006). Salt stress results in decreased leaf area, dry weight of shoot and leaf chlorophyll content (Zhao *et al* 2017*).*

Salinity can effect numerous processes in plants, which include the photosynthesis regulation, balance of ions, activity of antioxidant enzymes, osmotic agents (which are soluble sugars, proline and organic acids) and hormonal mechanisms (Prisco *et al* 2006). The contact of salt with mineral nutrients can cause nutrient inequity and deficiency in plant species. This can cause disorganization of membrane, photosynthesis inhibition, disruption in nucleic acid synthesis, accumulation of toxic metabolites and reactive oxygen species (Erdal 2012). The reduction in development of the plants under salt stress is because of nutrient disturbances, affecting the accessibility, transfer and partitioning of nutrients. In response to salinity, the productivity of ROS, such as singlet oxygen, superoxide, hydroxyl radical, and hydrogen peroxide, is improved (Ahmad and Prasad 2012). Salinity-induced ROS formation results in oxidative damages in a range of cellular components such as proteins, lipids, and DNA, affects essential cellular functions of plants.

Plants form the protective mechanism against oxidative stress. Enzymatic antioxidant system, is one of these protective mechanisms having the action of a amount of enzymes which are superoxide dismutase (SOD) and peroxidase (POD). Oat is sensitive to salinity in the growing seasons, especially during the germination and seedling stages (Han *et al* 2014). In salt stress conditions, protein gets accumulated maintaining osmoregulation in plants (Jamil and Rha 2013). Like in the other cereal species, the protein content in oat grains is much influenced by the environmental state as well as by the variety. Catalase activity, proline and free amino acids increase whereas protein content decreases with the raise in the duration of salinity in pearl millet. These environmental conditions are the reason for the buildup of various reactive oxygen species (ROS) such as superoxide radical (O_2), hydroxyl radical (OH), perhydroxy radical (HO_2), aloxy radical (RO), hydrogen peroxide (H_2O_2), singlet oxygen (O_2), thus making a condition of oxidative stress (Sneha *et al* 2014).

It is found that salt stress slow down germination rate (Kabar and Baltepe 1987), decreases root and stem elongation (Dash and Panda 2001) and reduce fresh weight and water content (El-Mashad and Kamel 2001).

Salt stress has 3 possible effects on plants which are as (i) reduction in water potential (ii) toxicity of any Na^+ and Cl^- absorbed (iii) intrusion with the absorbance of necessary nutrients. Maintenance of healthier nutrition with K^+ and Ca^{2+}, while limiting Na^+ uptake, is more significant attribute contributing to high salt stress tolerance in

plants.

Salt stress affects photosynthesis activity by decreasing carbon dioxide availability and decrease in amount of photosynthetic pigments (Ashraf and Harris 2013). Salinity, in general, has inhibitory effects on germination of seeds (Zhang 2010, Kaveh 2011 and El-Sabagh 2016) due to hyper osmotic stress and hyper-ionic toxicity (Hasegawa *et al* 2000). A large reduction in percentage germination, germination rate, root-shoot length and fresh weight of root and shoot is caused by salinity stress (El-Shaieny 2015). Germination proportion slowly decreased in all oat cultivated plants as the amount of salinity increased from 25 to 100 mM (Chauhan *et al* 2016). The physiological and molecular reaction in oat plant to long term salt stress have been studied by *Zhang et al* (2017). Transcription factor's are required to have a large role in salt tolerance in Oat (Hu *et al* 2017). Carding *et al* (1992) reported that (RT-PCR) is a very sensitive and exact method which is helpful for the finding of rare transcripts or for the samples analysis available in small quantity. Marone *et al* (2001) described a semi quantitative RT-PCR protocol to extract RNA from as small as 10,000 cells and to measure the expression levels of various targets of mRNAs from each sample. Plant genome contain large number of transcription factors which were regulated by multiple signaling pathways in response to salinity stress. These transcription factors induced set of stress-responsive genes and impart stress endurance to plants. Stress responsive genes are essential tool to understand the molecular mechanisms under salinity stress. These are also used for improving the salt tolerance of crops and producing transgenic plants by genetic engineering which are tolerant to different environmental stresses.

Thus investigation was planned to study the effect of salt stress on some physiological, biochemical and molecular parameters in different genotypes of oat (*Avena sativa* L.).

CHAPTER-II

REVIEW OF LITERATURE

Abiotic stress has become one of the important factors declining crop yield worldwide. The physiological, biochemical and molecular all processes are effected by abiotic stress in plant life from the early phase germination of seed to maturity and at last cause huge loss in the economic yield of plants. It was observed that about 70% yield of crops is badly affected by abiotic stress (Mantri *et al* 2012). Rapid population growth and subsequent food shortage especially in Asia and Africa and advancing salinity in arable land due to climate change have increased the importance of finding salt tolerant genotypes (Blumwald *et al* 2004).

Salt stress is one of the main abiotic stress affecting cultivation, with more than 80 million hectares of irrigated land affected worldwide. Salt tolerance is taken as percent survival of plants during germination or emergence stage, whereas at later developmental phases, fall in relative development rate is associated with salt tolerance of plants (Lauchli and Grattan 2012). Presence of high salinity in soils inhibits crop growth and yield and is a frequent constraint to agriculture in arid and semi-arid areas. Increased Na^+ and Cl^- content material in plant tissue can increase oxidative stress which causes deterioration in chloroplast ultrastructure as well as loss in chlorophyll (Khosravinejad *et al* 2009). Salinity leads to disturbances in plant metabolism, which consequently led to reduction of plant growth and productivity (Shafi *et al* 2009, Jaleel *et al* 2008). Genotypes growing under saline conditions might be stressed due to nutrient imbalance by depression in uptake and shoot transport (Marschner 1995). Salt stress tolerance in many crop species is correlated with a low Na+ concentration in the shoots, particularly in leaf tissues. Salts are present in upper strata of soil. Increase of sodium and chloride ions in soil along with other ions such as calcium, magnesium and sulphate ions causes salinity. Na^+ dominates amongst cations and Cl^- in anions in majority of saline soils to the volume that NaCl incorporates of approximately 50-80% of the overall soluble salts (Mekawy *et al* 2015).

Plant development is severely affected by salt stress at the seedling and early vegetative growth phases as compared to germination period in a numeral plants for example melon (Botia *et al* 2005), tomato (Del Amor *et al* 2001), red orach and spinach (Wilson *et al* 2000), cowpea (Maas and Poss 1989), sorghum (Maas *et al* 1986), corn (Maas *et al* 1983), rice (Pearson and Ayers 1966), and cotton (Abul-Naas and Omran 1974). Oat is sensitive to salt stress in the growing seasons, particularly during the germination and seedling stages (Verma *et al* 2017 and Han *et al* 2017). Increased salt

stress caused a major decrease in germination percentage, germination rate, root-shoot length and fresh weight of root and shoot (Jamil *et al* 2007)

2.1 Effect of salt stress on physiological parameters

2.1.1 Seed Germination and Vigour Parameters

Germination is considered as a first developmental step in the life cycle of a plant and is symbol for production of a new generation (Bewley 1997). Salt stress affects many morphological, physiological and biochemical processes including germination of seed, growth and yield of crop plants. The most sensitive stage to salinity is germination and seedling stage and with the increase of age, tolerance to salinity is increased (Ghorbani *et al* 2018). Crop plants in general are tolerant to salt stress at germination stage. Tajbakhsh *et al* (2006) reported that selection of crop plants for salt stress at germination period is necessary for determining salt tolerance potential of crops, as this period mainly determines crop establishment. Salt stress causes interruption in seed germination and consequently extends the mean germination time (MGT) (Tilaki *et al* 2011).

Germination and seedling establishment are vital stages and these stages influences both quality and quantity of crop yields (Subedi and Ma 2005). Some reports explain the decrease in germination of seed and subsequent development in different oat cultivars due to salt stress (Verma *et al* 1986). In salt stress studies, germination and plantlet development phases are focused and taken into the considerations when the salinity tolerance of a plant is determined (Ghoulam and Fares 2001). The germination stoppage on salty environments from the fact that water intake into the seed is hindered. Salt stress, in general, has inhibitory effects on germination of seeds (El-Sabagh *et al* 2018) due to hyperosmotic stress and hyper-ionic toxicity. Higher levels of salt usually cause reduction in percentage of germination (Mauromicale and Licandro 2002). It has been observed in various studies that germination of seed significantly decreases with increase in salt stress in the growth medium (Jamil 2012). The highest germination percentage was observed in the control among all of the treatment combinations, as reported by Naher and Alam (2010). . In sorghum, seed germination and seedling establishment is more sensitive during salt stress (Krishnamurthy *et al* 2003). Thus, germination is a critical stage of the plant life and resistance against salinity.

Earlier growth stages are more sensitive to salinity stress as compared to subsequent ones. As the plant attains maturity, they develop more resistance to counter the problem due to salinity. The resemblance of potassium with sodium was the vital factor disrupting normal metabolic functions as observed in salt sensitive cultivar. The tolerant one maintained certain potassium levels relative to sodium uptake. Salt stress adversely affects seed germination which may be due to internal osmotic or restricted

imbibtion rather than from ion toxicity effects (Alkraki 2001). Plants of two heritably varied spring wheat cultivars (cv. S-24 and MH-97) exhibited variable reaction to salt stress and were more sensitive at early developmental growth phases as compared to later growth phases (Ashraf *et al* 2012).

Seedling Length

Seedling is the most susceptible stage in the life cycle of plant life and germination determines when and where seedling development begins (Baskin and Baskin 2014, Gioria 2016). Inhibitory effects of salt stress are more evident on shoot growth as compared to root growth. The first organs to be affected by salt stress are plant roots. In oat, salt stress also cause changes in protein profiles in roots (Zhang *et al* 2016). Germinated seed radicles are primarily affected by excess salts due to water deficiency and specific ionic toxicity (Rengel 1992). Reducing the root zone by salt stress during the growing season is beneficial for ensuring the development and yield of crops. Length of root and shoot decreases with the increase in salinity level that may be due to slowing down the water absorption by the plants (Demir and Arif 2003).

During salt stress, less affected roots provide sufficient water and nutrients. High fibrous rooting in alfalfa is a trait with potential of usefulness as a salinity stress avoidance mechanism. Root growth has been used as a standard for screening of alfalfa salinity tolerance (Vaughan *et al* 2002). Salt stress decreases plant development through osmotic and toxic effects, and high sodium uptake ratio values cause sodicity, that increases soil resistance, decreases growth of root, and decreases water movement through the root with decrease in hydraulic conductivity. The reduction in shoot and root lengths is due to decreased physiological activities resulting from water and nutrients stress occurring under high salinity stress. The increase in root to shoot ratio or decline in shoot to root ratio is a general response to salinity stress, related to factors associated with water stress (osmotic effect) rather than a salt-specific effect (Hsiao *et al* 2000). During salinity stress, less affected roots provide sufficient water and nutrients. Better growth of roots is essential adaptive response in crops (Sheldon *et al* 2016). The increased NaCl concentration markedly reduced the leaf thickness and diameter of vessels, thus reducing the lamina length as seen in sugar beet genotypes (Skorupa *et al* 2019).

Seedling Biomass

A major decrease in leaf area and dry mass was occured in wheat plants when exposed to salt stress at the reproductive phase (Zheng *et al* 2013). Effects of salt stress on plant biomass have been suggested to be an important criteria for evaluating salt tolerance in crop plants (Bao *et al* 2009). A common reduction in fresh weight and dry weight has been seen in all plants subjected to salinity stress, but it is particularly

visible in the aerial part of plant. Various harmful effects of salinity stress contain the decrease in germination rate and development of seedling and the increase in the leaf area which ultimately decrease the photosynthetic area and production of biomass (Nasibi *et al* 2018). Further experiments in tomato plants irrigated with high salt concentrations explain that early events throughout the osmotic phase of salinity stress promote leaf senescence prior to the massive accumulation of toxic ions. Decline in seedling fresh weight with increasing salinity has been seen in maize (Majeed *et al* 2014, Hoque *et al* 2015). High salt concentrations decreased the dry weights of the affected crops like chickpea (Al-Mutawa 2003).

Seed Vigour

Seed vigour is a physiological property which governs the capability of a seed to produce a seedling quickly in soil and the extent to which that seed tolerate a variety of ecological factors (Perry 1980). Perry (1972) concluded that seeds which have high vigour are more tolerant to environmental pressure. The harmful effects of salt stress were more established by analyzing vigour index.

Germination test determine the germination ability of seed masses. Relation of different seedling growth parameters to seed yield and yield component under salty environment are significant for growth of salt tolerant varieties for production under salty stress environment (Dadar *et al* 2014). Turgor potential is completely linked with plant development, and thus, decline in turgor is the main reason of suppressed plant development under salty environment (Yadav *et al* 2011).

2.1.2 Effect of salt stress on biochemical parameters

It is recognized that salt stress induces oxidative stress in plant life at sub cellular level (Hernandez *et al* 2003). Salinity is typically accompanied by oxidative stress due to generation of reactive oxygen species (ROS) (Tsugane *et al* 1999 and Isayenkov 2012).Various studies have reported that salinity stress induces an accumulation of superoxide radicals (O_2) and hydrogen peroxide (H_2O_2) in different cell compartments, includes chloroplasts, mitochondria and apoplastic space, that correlates with increases in some oxidative stress parameters, like as lipid peroxidation and protein oxidation (Flowers and Colmer 2015). Understanding the physiological and biochemical responses under salinity helps to improve the salt tolerance of the crop species under such conditions (Chinnusamy *et al* 2005). Quick biochemical conversions in seeds are a prime feature which protects the seed from damage (Saeedipour and Moradi 2011).

Total soluble sugars

Total soluble sugar play a dual function with towards ROS, whichever promoting ROS production or participating ultimately in ROS scavenging mechanisms

by NADPH generating pathways, for example the oxidative pentose-phosphate pathway (Couee *et al* 2006). Total soluble sugar play role as signals, helpful in sensing and controlling for the plant not only photosynthetic action but also cellular redox balance (Sulmon *et al* 2015). Oat plants conducted osmotic adjustment by accumulating soluble sugars and proteins under various salt stresses.

The accumulation of soluble sugars helped reduction in the osmotic potential, maintain plant capability of absorbing water and maintain cell development. The salt tolerant species have a capability to oppose salinity stress during the accumulation and biosynthesis of compatible solutes. These substances raise the overall osmotic pressure inside the cells and allow the cells to maintain both turgor as well as the driving gradient for water uptake. Therefore compatible solutes such as proteins, amino acids, carbohydrates and quaternary ammonium compounds serve up significant roles as osmotic balancing agents and plant cell stabilizers (Rhodes and Hanson 1993, Holmstrom *et al* 2000). Accumulation of sugars, a characteristic of mature seeds appears to be central to the development of desiccation tolerance (Hoekstra *et al* 2001).

Accumulation of sugars in different parts of plants is enhanced in response to a variety of environmental stresses (Gorham *et al* 1981, Prado *et al* 2000 and Wang *et al* 1996). It was observed that increase in total soluble sugars and other osmolytes provide optimism to increase plant tolerance to abiotic stresses such as salinity, cold and drought (Rathinasabapathi 2000).

Total soluble proteins

Proteins which serve as a reservoir of energy may also help in adjustment of osmotic potential in plants subjected to salinity. They may be synthesized de novo in response to salt stress or may be present constitutively at low concentration (Ahmad and Satyawati 2008). It has been found that soluble protein content of leaves decreases in response to salinity (Wang and Nii 2000, Parida *et al* 2002). Hence, osmotic potential in plant cells gets disturbed due to decrease in soluble proteins. Mild salinity stress increased total soluble proteins but decreased these clearly under severe salt stress in mulberry (Agastian *et al* 2000). Similarly Doganlar *et al* (2010) studied salt induced decrease in total soluble proteins in three tomato species. When rice plants were exposed to salt stress, a major decrease in soluble proteins was seen (Amirjani 2010).

In contrast, a numeral reports existing in the literature which depict the increase in soluble proteins under salt stress. For example, wheat plants exhibited increase in soluble proteins due to varying levels of sodium chloride salinity (Afzal *et al* 2013). Salt-induced increase in soluble proteins occured in cowpea below various concentrations of salt in the growth medium (Lobato *et al* 2009). Similarly, there is a higher endogenous levels of total soluble proteins in response to mild salt

concentrations in the growth medium in radish plants (Turan *et al* 2010). Increase in total soluble proteins at various growth phases was also studied in wheat plants under sodium chloride salt. Sairam *et al* (2002) noted that in salt tolerant cultivars of barley, sunflower finger millet, and rice there were a high content of soluble proteins.

Various changes in activities of various antioxidant enzymes under salinity stress have been reported (Dolatabadian and Saleh 2009). For example, a salt stress increases a catalase (CAT) activity, whereas a superoxide dismutase (SOD) activity is not changed in cashew leaves (Ferreira-Silva *et al* 2012). In plant cell, antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) have been considered as a defensive team, whose combined purpose is to protect cells from oxidative damage (Mittler 2002). Increased SOD, POD and CAT activities are closely related to salt tolerance of many plants as reported in various researches (Rahnama and Ebrahimzadeh 2005, Azevedo Neto *et al* 2006, Koca *et al* 2007). Athar *et al* (2008) reported production of a wide range of free-radicals in wheat under salt stress, and suggested that higher antioxidant potential contribute in protecting the plant from stress. The raise in enzyme activity could effectively decrease the harm to cell membrane. The higher the activity, the stronger adapting capability of a plant to adversity (Liu 2007). Under salinity, POD could remain have high activity for a large time but less than 35 days in order to decrease the damage of salt stress to the plant.

Plants acquired inherent mechanisms to counteract stress-induced ROS-generation, which includes antioxidant enzymes such as catalase (CAT), peroxidase (POD) which neutralize stress-induced excess H_2O_2 converting it into H_2O (Mostofa *et al* 2015 and Sofo *et al* 2015). Reactive oxygen species production under salinity and their scavenging by the activation of antioxidants have been well documented for crops like wheat (Sairam *et al* 2005 and Wahid *et al* 2007), rice (Vaidyanathan *et al* 2003) and beans (Palma *et al* 2009). Although removal of ROS by activation of antioxidants is genetically controlled yet it can be improved by adopting physiological approaches.

Antioxidant enzymes

Antioxidant enzymes including SOD, CAT and POD activity increases in order to detoxify ROS and to provide salt tolerance (Sekmen *et al* 2012 and Balal *et al* 2012). In French bean, the enhanced activities of CAT and POX under salt stress suggest an effective scavenging of Hydrogen peroxide and tolerance against salt stress (Agarwal and Pandey 2004). SOD is considered as the most essential enzymatic constituent of the antioxidant system as it governs the alteration of lethal superoxide to H_2O_2 and O_2. Plants grown under salty environment tend to have better activity of superoxide dismutase (Ashraf *et al* 2009). Superoxide dismutase is the family of metalo enzymes

which catalyses the disproportion of superoxide O_2 to molecular oxygen and hydrogen peroxide (Scandalios 1993). Increase in SOD and the ASC-GSH cycle components occurs in the chloroplasts, mitochondria and peroxisomes from salt-treated (100 mM NaCl) *Lycopersicon pennellii* plants, while in the cultivated tomato (*Solanum lycopersicom),* which is susceptible to 100 mM sodium chloride, a fall occurred in the majority of these antioxidant defences (Mittova and Tal 2003). Peroxidases are present in cytosol and most organelles of plant cells, and mainly involved in oxidative induced H_2O_2 dissociation.

It has been found that SOD activity to be increased under stresses in different tissues of various plants (Gill and Tuteja 2010). Swapna (2003) and Weisany *et al* (2012) reported increased peroxidase activity in response to increasing levels of NaCl concentrations.

Catalase is tetrameric heme that catalyzes the breakdown of hydrogen peroxide to water (H_2O) and oxygen. This is an important enzyme which protects the cell from oxidative damage by ROS. It has significant role in removal of H_2O_2 generated in peroxisomes by oxidase involved in β-oxidation of fatty acids and photorespiration (Kumari *et al* 2009). Rice genotypes with higher concentrations of antioxidants have been reported to have greater tolerance of salt stress (Moradi and Ismail 2007).

2.1.3 Salt stress effect on gene expression

To cope with the detrimental effects of various abiotic stresses, crops have evolved many mechanisms to increase their tolerance including physical adaptions, interactive molecular and cellular changes. There has been a considerable effort to improve the tolerance to salinity in major food crops. The foundation of this is an understanding of the mechanisms of salinity tolerance and the development of appropriate selection criteria. Salinity reduces plant growth and yield by two mechanisms, osmotic stress and ion cytotoxicity (Tavakkoli *et al* 2010). RNA is isolated followed by the synthesis of first strand of cDNA, which then is subjected to PCR. It has been significantly used to determine expression of genes of interest in different plants subjected to salt or other abiotic stresses. Antioxidant genes are upregulated upon salt accumulation.

PCR is the simplest approach to study differential expression of genes under varying levels of salt stress, various time intervals and different developmental stages in various plant tissues.

Stress perception and signalling is the initial step in the plant response to salinity stress at molecular level. Recent studies have shown that stress sensing and signalling components can play significant roles in regulating the expression of salt tolerance genes and transcription factors, and the over expression of these genes in

several plants resulted in better stress tolerance (Deinlein *et al* 2014). A easy method is reported here for the semi-quantitative assay of mRNAs in the presence of an exogenous mRNA (pBR322 transcript) as a standard. This method uses the co-reverse transcription and co-amplification (co RT-PCR) of the target and standard mRNAs. This procedure enables transcripts to be compared when the differentiation process affects the transcription pattern of the β-actin housekeeping gene, which is usually used internal standard. β-actin is one of the most important HKGs used for normalizing gene expression levels (Bustin 2000)

Transcription factors are regulators for gene expression so they offer better target for transgenic exploitation. Na^+ exclusion is unlikely is the main mechanism for salt resistance in a crop because it has been observed that genetically modified wheat did not show higher yields under salinity (Genc *et al* 2015). Transcriptome analysis had a vital role in elucidating the complexity of gene expression regulation. Among numerous transcriptome analysis methods, microarray technologies and rna-seq have become the default popular methods of choices for genome wide transcriptome studies. While RNA-seq has recently become a preferred technique of choice in whole transcriptome analysis, microarrays represent a well-established technology and extensively used in the last decades and have provided a great deal of candidate genes for genetic engineering (Gao *et al* 2013).

Quantitative analysis of RNA is essential to the understanding of the mechanisms that regulate gene expression. The-polymerase chain reaction (PCR) after a first step of reverse transcription (RT) was adapted to overcome the low sensitivity of Northern analysis. Polymerase chain reaction is based on the exponential amplification of a target sequence. Therefore, many quantitative and semi-quantitative procedures were developed.

The RT-PCR is basically a qualitative method to differentiate between occurrence and nonexistence of a transcript under different conditions. This method is usually used to check expression pattern of specific genes in different tissues using gene-specific primers. The expression of antioxidant enzyme genes under salinity stress has been studied in earlier studies. Tolerant and sensitive genotypes of prairie june grass had been exposed to moderate stress by withholding watering for 7 days. RNA is isolated followed by the synthesis of first strand of cDNA, which is subjected to PCR. It is extensively used to determine expression of genes of interest in different plant tissues under salt or other abiotic stresses (Jamil *et al* 2005). Ueda *et al* (2004) compared previous consequences on expression profiling under salt stress after which the osmotic stress-specific gene expression was identified using the barley custom designed cDNA microarray.

There is a relationship between antioxidant enzyme activity and salinity tolerance. Superoxide dismutase (SOD) enzymes are metalloenzymes by nature and they catalyze the conversion of the superoxide anions to H_2O_2 and O_2. SOD transcription factor is the first line of defense against ROS scavenging by removing O^2 from the compartments (Takahashi and Asada 1983). Ahmed *et al* (2013) studied the SOD activity in Tibetan wild *Hordeum vulgare* genotypes under drought and salinity stress and concluded that the expression of SOD was significantly increased under salinity and drought stress alone and combined treatments during anthesis period. SODs expression in wild-type plants subjected to salt stress was upregulated.

CHAPTER-III

MATERIAL AND METHODS

3.1 LOCATION

The present investigation entitled "Effect of salt stress on physiological, biochemical and molecular parameters of oats in different genotypes" was conducted in PG laboratory, Department of Botany and Molecular Biology Laboratory, School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana.

3.2 PROCUREMENT OF SEEDS

Seed of OL-10, Kent, OL-1862, OL-1895, OL-1966, OL-125, OL-1869, OL-1876 of oat (*Avena sativa* L.) were collected from fodder section of department of plant breeding and genetics, Punjab Agricultural University, Ludhiana which were used for study to ensure germination of seed, growth of seedling and biochemical changes when different salt solutions were added to petri dishes in which seeds were placed. This experiment was done to study the consequence of salt stress with various concentrations on germination of seed, vigour parameters and biochemical parameters in oat seedlings.

3.3 Treatments and Experimental Design

Eight salt concentrations were:

T1 : Control

T2 : 25mM

T3 : 50mM

T4 : 75mM

T5 : 100mM

T6 : 125mM

T7 : 150mM

T8 :175mM

T9 : 200mM

Oat seeds were surface sterilized by 0.1 % solution of mercuric chloride, followed by thorough washing by distilled water to avoid any fungal infection. To stimulate salinity stress, the germination paper in petri dishes was moistened with solutions of various salt levels (Control, 25mM, 50mM, 75mM, 100mM, 125mM, 150mM, 175mM and 200mM NaCl). The petri dishes were incubate at 25°C and 60±15 % relative humidity for 10 days to record the effect of salinity on germination parameters. Two salinity levels were selected based on their effect on physiological parameters of the seeds.

3.4 OBSERVATIONS

After 10 days on different physiological attributes of germination, data was

taken. For biochemical analysis, 10 days old seedlings were used. From seedlings taken at random, parameters given below were recorded:

3.5 PHYSIOLOGICAL PARAMETERS

3.5.1 Percentage Germination

In every petri plate, ten seeds with two replications of each treatment were used randomly and placed in petri dishes containing germination paper under the hood. The dishes were placed in germinator with 25°C average temperature under 65% relative humidity. When the emergent root reached 2 mm length, seeds were considered germinated. First count after 4 days and final count after 6 days of normal seedlings was recorded and expressed in germination percentage and result was evaluated.

$$G\% = \frac{\text{Sum of germinated seeds}}{\text{Total number of seeds tested}} \times 100$$

3.5.2 Length of seedling (cm)

From each treatment five seedling were taken randomly and length of root, shoot were measured.

3.5.2.1 Root length (cm)

Five normal seedling from every treatment were selected for measuring length of root on 10th day of germination and average of these was computed in cms.

3.5.2.2 Shoot length (cm)

Shoot length was measured from five seedlings which were used for root length measurement and average of these was computed in cms.

3.5.3 Fresh Weight of Seedling (mg)

Seedling fresh weight was taken in milligrams of five randomly selected seedlings by balance. Average of weight was computed in milligrams.

3.5.4 Dry Weight of Seedling (mg)

Seedling dry weight of five randomly selected seedling was taken in milligrams and dried using oven for 17 hours at 110°C and by using weighing balance their dry weight was taken and average of these was computed in milligrams.

3.5.5 Seed vigour index

Vigour index of seeds were calculated as suggested by (Abdul Baki's Anderson 1973).

Vigour Index I = Germination (%) x Seedling length (cm)

Vigour Index II = Germination (%) x Seedling dry weight (g)

3.5.6 Statistical Analysis

Data was collected and analyzed statistically by using CPCS-1 computer package at 5 % CD.

3.5.7 Biochemical Attributes

3.5.7.1 Total soluble sugar content (Dubios *et al* 1956)

Reagents

Solution A: 80 percent ethanol

Solution B: 5 percent phenol

Solution C: Concentrated H_2SO_4

Extraction

Tissue of recognized weight were taken and crushed in Solution A and centrifugation at 5000rpm, followed by another extraction in solution A for whole extraction and supernatants were collected which were used for the total soluble sugars estimation

Estimation

Extract (0.1ml) was taken, addition of 1 ml of 5 percent phenol was done and after 10 min there was a addition of 5 ml of concentrated H_2SO_4 slowly with constant stirring. Then at room temperature, tubes were cooled down under running water. Then at 490 nm, absorbance was measured against blank. The amount of total soluble sugars was calculated using pure glucose (10-100µg) and expressed in mg g^{-1} FW.

3.5.7.2 Total soluble protein content (Lowry *et al* 1951)

Reagents

A: 2 percent sodium carbonate in 0.1 N NaOH.

B: 0.5 percent copper sulphate in 1 percent sodium potassium tartarate.

C: Prepared the mixture of 50 ml of reagent A and 1 ml of reagent B.

D: Folin-ciocalteau reagent (1N) diluted to 1:1 ratio with water.

Extraction: The tissue was homogenized in 5 ml of 0.1 N sodium hydroxide then centrifuged at 5000 rpm for 10 min. Extraction process was repetitive two times and make entire volume 10 ml. The collected protein extract was treated with 1 ml of 1 percent TCA and kept undisturbed for 24 hours at 4˚C and centrifugation at 5000 rpm for 20 minute and precipitated protein were dissolved in 0.1 N sodium hydroxide.

Estimation: 5 ml of reagent C was added to 0.2 ml of protein extract and mix was continuously shaken and placed at room temp. for about 10 min. After this addition of 0.5 ml of Folin Ciocalteau phenol reagent was done and set aside for 30 min. Blue colour was occurred and measured at 570 nm beside blank. Standard curve prepared by using bovine serum albumin (BSA) and expressed in mg protein g^{-1} fresh weight.

3.5.7.3 Extraction of catalase

i) **Extraction buffer (0.1M Sodium phosphate buffer (pH 7.5) containing 1 percent (w/v) insoluble PVP and 1Mm EDTA):** 25 ml of 0.2M sodium phosphate buffer (61 ml of 0.2 m sodium phosphate monobasic and 39 ml of 0.2M sodium

phosphate dibasic were mixed and diluted to 200ml) was diluted to 100 ml. To this added I gm of PVP and 37mg of EDTA.

b) Extraction

0.5g fresh tissue was crushed in 5ml cold (4°C) extraction buffer in pre chilled pestle and mortar then centrifugation at 20,000 rpm for 10 min and supernatant was used as enzyme extract.

Assay for catalase enzyme (Chance and Maehley 1955)

Reagents

- **0.1M Sodium phosphate buffer (pH 7.0)**
- **H_2O_2**: In 100ml of distilled water, 825µl of Hydrogen peroxide was dissolved

Assay

In 1.9ml chilled 0.1M sodium phosphate buffer (pH 7.0) 0.1ml enzyme extract was added. The reaction was initiated by addition of 1ml of Hydrogen peroxide. The absorbance decrease at 240 nm was calculated at 10 sec intervals for 1 minute. Catalase activity was expressed as µmoles of hydrogen peroxide decomposed /min/g FW of tissue.

3.5.7.4 Estimation of peroxidase (Shannon *et al* 1966)

Extraction

a) Reagents

i) **Extraction buffer (0.1M Tris-HCl buffer containing 1mM EDTA, 1 percent (w/v) PVP and 10µM β-mercaptoethanol):** To 0.1M Tris-Hcl buffer (3.6gm in 300ml of distilled water) there was a addition of 3.7 mg EDTA, 139.2µl β-mercaptoethanol and 1gm PVP.

b) Extraction

0.2g tissue was crushed in 2ml of cold (4°C) extraction buffer in pre chilled pestle and mortar then centrifugation at 20,000 rpm for 10 min and supernatant was used as enzyme extract.

Analyze

a) Reagents

i) **Guaiacol:** In 200ml 0.1M sodium phosphate buffer pH 6.5, 111µl guaiacol was dissolved (68.5ml of sodium phosphate monobasic and 31.5ml of sodium phosphate dibasic were mixed and diluted to 100ml)

ii) **H_2O_2:** 825µl Hydrogen peroxide was dissolved in 10ml distilled water

b) Procedure

In spectro photometric cuvette add 3ml of chilled guaiacol and 0.1ml enzyme extract. The reaction was initiated by addition of 0.1ml of Hydrogen peroxide increase

in absorbance was measured at 470 nm at 30 sec intervals for 3 min.

3.5.7.5 Extraction and assay of SOD (Marklunds and Marklunds 1974)

a. Reagent

Extraction buffer (0.1M Potassium phosphate buffer (pH 7.8) containing 1 percent (w/v) insoluble PVP and 1Mm EDTA): 0.1M potassium phosphate buffer of 100 ml (16 ml of 0.1 M potassium phosphate monobasic and 84 ml of 0.1M potassium phosphate dibasic were mixed) was diluted to 200 ml. In this there was a addition of 1 gm of PVP and 37mg of EDTA.

b. Extraction

Extraction was done from samples with 0.1M potassium phosphate buffer (pH 7.5) containing 1 percent PVP, 1mM EDTA and 10mM β- mercaptoethanol and centrifuged for 10 min at 10,000 rpm.

Analyze for SOD

Reagents

I. 6mM Pyrogallol

II. 6mm EDTA

III. 0.1M Tris- HCl buffer

b. Procedure

The reaction mixture contained 1.5ml of Tris-HCl buffer, 0.5 ml of 6 mM EDTA, 1ml of 6mM pyrogallol solution and 0.1 ml of enzyme extract. The reaction product was measured at 420 nm after at 30 sec interval upto 3 min.

3.5.8 RNA isolation preparation

Isolation of RNA was difficult from isolation of DNA because of contamination of RNase which degrades RNA. To conquer the contamination of RNase, treatment of 0.1 % DEPC water was given to all glassware and plastic ware and placed these for the night at 37°C. The solution was autoclaved at 20 psi for 24 minutes after mixing.

DEPC water treatment

Dip, micro centrifuge tubes, tips and mortar pestle in 0.1 percent DEPC water for the night. Then fill all the tips in tip boxes and micro tubes in jam jars and sited these in hot air oven for drying at 80 °C for the night and autoclaved at 121 °C for 24 min.

Collection of Sample

Because of RNase contamination, the most critical step for collection of sample is RNA isolation. Tissue for RNA isolation were used from one comparatively salt-sensitive and one comparatively salt-tolerant oat seedlings subjected under salt and control conditions. Isolation of RNA was done by using Trizol reagent from plants under salt stress and treated as control. The quality of isolated RNA was seen on 1.2

percent agarose gel and with the help of Thermo Scientific Nanodrop™ 1000 Spectrophotometer quantity was recorded.

Total RNA isolation from tissue

Isolation of RNA was done from seedlings of two genotypes (comparatively salt-sensitive and comparatively salt-tolerant) at control and 100mM NaCl conditions.

For extraction of RNA, Trizol was used.

Protocol

Required reagents:

- DEPC-treated water
- Trizol Reagent
- Liquid nitrogen
- 70% ethanol
- Isopropyl alcohol

RNA extraction

- Total RNA extraction were carried from whole plants with Trizol reagent. The tissues were compressed in liq. nitrogen by a pre-chilled mortar and pestle.
- Approximately 50-100 mg powdered form of tissue was transferred to DEPC treated 2 ml tubes.
- Tissue was crushed with 1 ml Trizol reagent immediately.
- Then for 5 minutes, crushed sample was incubated at room temp.to allow total dissociation of nucleoprotein compound.
- In 1 ml of Trizol reagent, 200 µl chloroform was added. Capped the tube strongly.
- Samples were dynamically mixed through shaking the tubes by hand for 15 seconds.
- Then placed them for incubation for 3 min. at room temp. Centrifugation at 12000xg for 15 minute at 4°C.
- By angling the tube at 45°, aqueous phase was removed and pipetting the solution out.
- In another tube, aqueous phase was transferred.
- 500 µl of 100 percent isopropanol was added in aqueous phase per ml of Trizol reagent then incubate at room temperature for 10 min.
- Centrifuged the samples at 12000x g for 10 min. at 4°C.
- Leaving the RNA pellet, removed the supernatant from tube.
- Pellet was cleaned with 1 ml of 75 per cent ethanol per ml of Trizol reagent which was used in the initial homogenization.

- Then vortex briefly, followed by centrifugation at 7500xg for 5 minute at 4°C. Remove the supernatant.
- Air dried the RNA pellet for 5 to 10 minute. Do not dry the pellet by vacuum centrifuge

Quantitative analysis

Two different methods of Quantification of RNA are:

a) Quantification of RNA using Thermo Scientific Nanodrop™ 1000 Spectrophotometer. It was done using standard protocol and absorbance was recorded at 260/280 nm.

b) Quantification of RNA using agarose gel 1.2 percent electrophoresis

- Quantity of RNA was checked on 1.2 percent agarose gel.
- Agarose powder was dissolved in MOPS buffer and 37 percent formaldehyde was added in this.
- Till the solution turn into clear solution heat it and cooled at 60 °C.
- Put in 1 µg/ml of EtBr to final concentration.
- Agarose solution was transferred to gel mould besides combs and leave it for solidification for 30-40 min.
- Then loading was done with autoclaved 50 percent glycerol in 1:1 ratio by micropipette.
- After the loading, the gel was subjected to electrophoresis at constant voltage (75 V) for 1 hour and visualized under UV transilluminator.

MOPS buffer preparation

10X MOPS buffer was prepared by addition of MOPS, sodium acetate and Ethylene diamine tetra acetic acid (EDTA) in 0.1% DEPC water and a 7.0 pH was adjusted with 1N NaOH solution.

cDNA synthesis from RNA

As RNA is a single stranded molecule it is very unstable, thus by forming its complementary strand and transformed into cDNA through reverse transcription using 1st strand cDNA synthesis kit (SRL) is essential to create it double-stranded. Process was followed as per manufacturer's protocol.

Protocol

Synthesis of First strand cDNA

1. Addition of subsequent reagents in the arranged order:
   - Template RNA = poly(A) mRNA=3µg
   - Prime = oligo (dT)15 primer=1µl
   - DEPC-treated water= 7.4µl

- Total amount = 12.4µl

2. Mix up, centrifuge and placed for incubation at 70°C for 5 minute. Cool on ice and spin down, put the vial again for chill.

3. Arrange subsequent cDNA Synthesis. Mix up and put the given below components in the indicated order:

- 5x first-strand buffer= 4 µl
- dNTPs(10 mM each)= 1 µl
- RNasin= 0.6 µl
- M-MLV= 1 µl

4. Mix and centrifuge.

5. Incubate for 60 minute at 42°C for oligo(dT)15. 60 minute incubate at 37°C for random hexamer primed synthesis.

6. At the end heating at 70°C for 5 minute.

cDNA Confirmation

Synthesized cDNA from RNA of PCR-positive plant tissue was confirmed using 26S (forward and reverse) rRNA. PCR reaction mix (12 µl) having cDNA template (4 µl), 25 µM $MgCl_2$ (0.3 µl), 5 µM FP and RP (1.0 µl each), Master mix (4.0 µl) and sterile water (1.7 µl) (Table.1).

Table 1: PCR

	Sequence	**Time (minutes)**	**Temperature (°C)**
•	first denaturation	5	94
•	Denaturation	1	94
•	Primer annealing	1	55
•	Extension	2	72
•	Repeat steps	35 cycle	II-IV
•	Extension	7	72
•	Storage	Infinite	4

Amplification was seen on 3% agarose gel and quantification was seen using Thermo Scientific Nanodrop™1000 Spectrophotometer.

Confirmation of cDNA using actin primer:

Actin is a housekeeping gene. These genes are abundantly and constantly expressed. cDNA presence was confirmed by using actin primer. The reaction was carried out in a thermo cycler with the following content shown in (Table. 2).

Table 2: Confirmation of cDNA using actin primer

cDNA	4 µl
Fast cycling master mix	4 µl
F primer	1.0 µl
R primer	1.0 µl
$MgCl_2$	0.3 µl
Nuclease free water	1.7 µl

The reaction condition followed by 35 cycles was applied:

- after 5 min of initial denaturation at 94°C,
- 1 min at 94°C for Denaturation
- 1 min at 60°C for Annealing
- 2 min at 72°C for Extension
- 7 min at 72°C for Final Extension

Agarose Gel Electrophoresis

1. The PCR product was analyzed by running on 3% agarose gel in 0.5X-TAE Buffer.
2. For 3 percent agarose gel, 3g of agarose is added in 100 ml of TAE-buffer. Boil the mixture for 2-3 minutes in hot oven.
3. Now, ethidium bromide was added in molten agar. Pour the agarose mixture in plate and adjust the comb. Wait till it solidify.
4. The sample (5 µl) was mixed and loaded. Along with these Gene Ruler 1kb plus DNA ladder was also run in one lane.
5. The run was performed at constant voltage (80V for 2 hrs). Agarose gels were visualized under UV-transilluminator and photographed under Gel documentation system.

CHAPTER-IV

RESULTS AND DISCUSSION

Laboratory studies

The present study was planned to see the effect of salt stress on physiological, biochemical and molecular parameters in different genotypes of oat (*Avena sativa* L.). Seeds of OL-10, Kent, OL-1862, OL-1895, OL-1966, OL-125, OL-1869, OL-1876 genotypes were subjected to different salt stress levels (25mM, 50mM, 75mM, 100mM, 125mM, 150mM, 175mM, 200mM) and observations were recorded on various physiological parameters such as percent germination, seedling length, seed vigour, seedling biomass and biochemical parameters such as total soluble sugars, proteins, antioxidant enzymes like catalase (CAT), peroxidase (POX), superoxide dismutase (SOD) from 10 days old seedlings. Also, molecular studies were carried on one sensitive and one tolerant genotype under selected salt stress levels.

4.1 EFFECT OF SALT STRESS ON PHYSIOLOGICAL, BIOCHEMICAL AND MOLECULAR PARAMETERS

4.1.1 Percent germination

The effect of salt stress on percent germination in different genotypes of oat is presented in (table. 3). Present results indicate that percentage germination decreased significantly with increase in salt concentration in all the genotypes of oat. The germination percentage was declined to almost negligible at 200mM in all the genotypes. As the NaCl concentration increased from 25mM to 100mM, germination percentage declined from 90 to 49.3% in OL-10, 100 to 80% in Kent, 95 to 61% in OL-1862, 83 to 74% in OL-1895, 100 to 60% in OL-1966, 91.6 to 61.6% in OL-125, 96.6 to 53.3% in OL-1869, 100 to 75% in OL-1876. In OL-1895 genotype maximum percentage germination was seen. In OL-10 genotype minimum percentage germination was seen. Differential response was seen at 75mM and 100mM. At 100mM genotypes OL-1895, Kent, OL-1876, OL-125, OL-1862 recorded a less reduction (10.8, 20, 25, 32.7, 35.7 percent respectively) in percent germination indicating these to be comparatively salt tolerant as compared to the genotypes OL-10, OL-1869, OL-1966 which recorded more reduction (45.2, 44.8, 40 %) indicating their comparative sensitivity to salt stress.

Similar decline in seed germination by salt stress has been reported in earlier works by (Chauhan 2019 and Berk and Ozkan 2016, Oyiga *et al* 2016, Long *et al* 2015). Salt stress can repress all growth-related factors (seedling length, seedling biomass) which in turn limit biomass and yield by limiting germination and development (Han *et al* 2013).

Table 3: Effect of salt stress on percent germination in oat (*Avena sativa* L.)

Genotypes / Salt Conc.	OL-10	Kent	OL-1862	OL-1895	OL-1966	OL-125	OL-1869	OL-1876
Control	90±5.0	100±0.0	95±0.0	83±7.6	100±0.0	91.6±7.6	96.6±5.8	100±0.0
NaCl(25mM)	71±6.6.	100±0.0	90±0.0	78.3±7.6	100±0.0	90±5.0	96.6±5.8	98.3±2.9
NaCl(50mM)	65±5.0	95±5.0	85±5.0	75±5.0	100±0.0	87.3±4.6	88±3.5	90±0.0
NaCl(75mM)	65±5.0	84.3±4.0	75±5.0	75±0.0	96±5.3	81.6±2.9	81.6±2.9	80±5.0
NaCl(100mM)	49.3±6.6	80±5.0	61±1.2	74±5.3	60±5.0	61.6±7.6	53.3±5.8	75±5.0
NaCl(125mM)	48.7±2.3	70±0.0	35±5.0	44±5.3	50±5.0	60±5.0	39.3±1.2	70.6±1.2
NaCl(150mM)	0±0.0	58.3±5.8	25±5.0	35±5.0	47.3±4.6	51.6±7.6	23.3±5.8	70±0.0
NaCl(175mM)	0±0.0	45.7±4.0	20.3±4.5	30±0.0	23.3±5.8	36.6±5.8	0±0.0	55±5.0
NaCl(200mM)	0±0.0	26.7±5.8	20±5.0	17.6±6.8	0±0.0	0±0.0	0±0.0	28.3±2.9
CD at 5%	X=2.22 Y=2.36 XY=6.67							

X=Genotype

Y=Salt Concentration

Germination of seed, seedling appearance and early survival are mainly sensitive to salt stress (Nasri *et al* 2015). Tejovathi *et al* (1988) reported that the ability of germination of seed and emergence under salt stress indicates its genetic potential for salt tolerance.

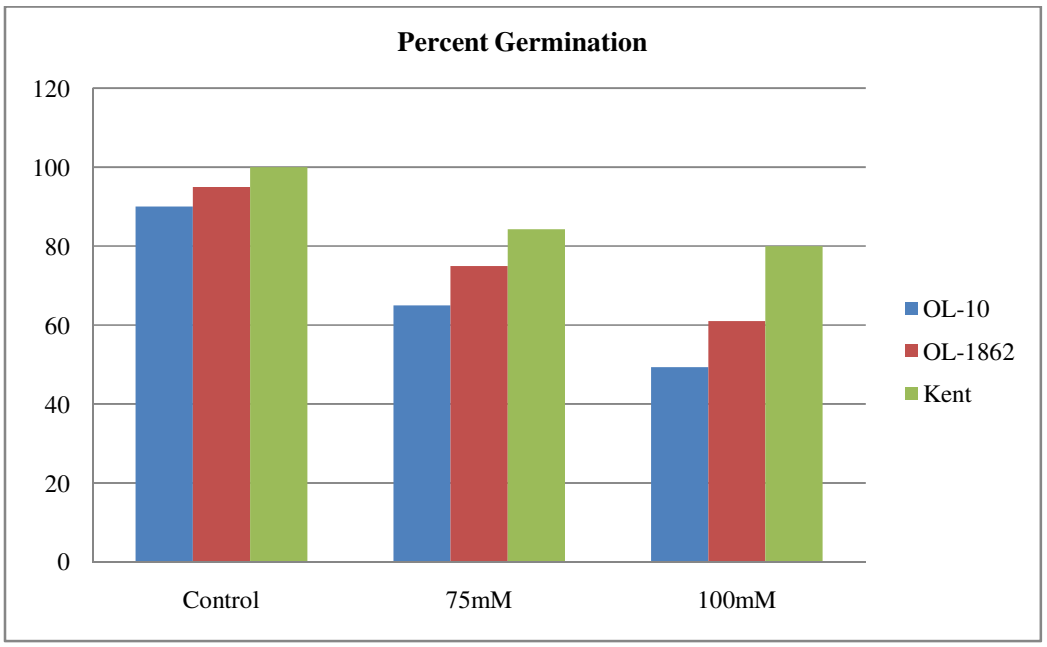

Fig. 1: Effect of salt stress on percent germination in oat (*Avena sativa* L.)

4.1.2 Seedling length (cm)

The seedling elongation rates are necessary for plants exploring for water and mineral nutrients. The relative root and shoot lengths were analyzed, to further investigate the effect of salt stress on *Avena sativa*.

4.1.2.1 Shoot and Root length (cm)

The effect of salt stress on shoot length and root length in different genotypes of oat is presented in (table 5 and 6). Present results indicate that shoot and root length decreased significantly with increase in salt concentration in all the genotypes of oat. The germination percentage was declined to almost negligible at 200mM in all the genotypes. As the NaCl concentration increased from 25mM to 100mM, shoot length in general declined from 16.3 to 6.8 cm in OL-10, 17.6 to 10.8 cm in Kent, 16.8 to 8.7 cm in OL-1862, 18.9 to 11.2 cm in OL-1895, 21.1 to 5.5 cm in OL-1966, 16.8 to 6.6 cm in OL-125, 16.1 to 5.4 cm in OL-1869, 16.0 to 9.8 cm in OL-1876. The maximum shoot length was shown by genotype Kent. Minimum shoot length was shown by genotype OL-1966. Differential response was seen at 75mM and 100mM . At 100mM genotypes Kent, OL-1876, OL-1895, OL-10, OL-1862 recorded a less reduction (38.6, 38.7, 40.7, 48.2, 58.2 percent respectively) in percent germination indicating these to be comparatively salt tolerant as compared to the genotypes OL-1966, OL-1869, OL-125

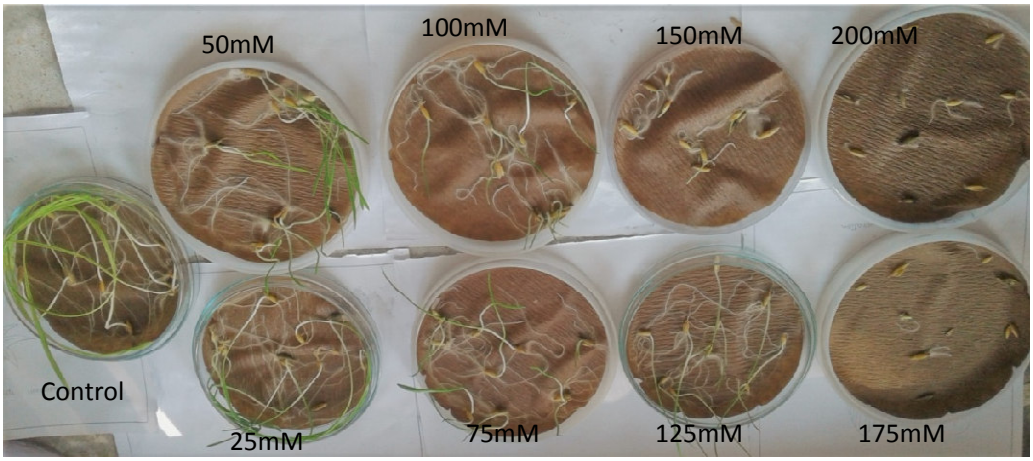

Plate 1: Germination of oat (*Avena sativa* L.) grown in petri dishes.

Plate 1a: Effect of salt (25, 50, 75, 100, 125, 150, 175 and 200 mM NaCl) stress on growth of root and shoot of *Avena sativa*. Photograph showing effect on seedling after 10 days of treatment.

Table 4: Effect of salt stress on seedling length (cm) in oat (*Avena sativa* L.)

Genotypes / Salt Conc.	OL-10	Kent	OL-1862	OL-1895	OL-1966	OL-125	OL-1869	OL-1876
Control	28.6±1.2	29.3±0.7	27.0±0.7	30.3±1.0	34.4±1.6	31.3±0.6	25.2±0.5	27.5±1.8
NaCl(25mM)	26.3±1.8	27.3±0.4	23.3±1.0	25.7±1.5	26.2±0.7	26.3±0.7	16.1±1.0	25.0±0.1
NaCl(50mM)	23.5±0.4	23.3±0.9	21.1±0.6	22.4±1.5	21.9±0.2	25.3±0.9	15.6±1.1	24.0±0.8
NaCl(75mM)	19.7±0.8	23.3±0.9	19.4±0.5	21.2±0.8	16.5±0.4	25±5.2	15.1±1.2	21.9±1.6
NaCl(100mM)	13.3±1.4	19.6±0.5	15.2±0.7	16.2±0.9	12.4±0.5	13±2.1	12.9±0.9	18.3±1.0
NaCl(125mM)	6.3±1.6	16.0±0.8	11.9±1.5	13.9±0.3	11.1±0.6	10±1.0	11.5±0.6	16±0.0
NaCl(150mM)	0±0.0	11.7±0.5	9.2±0.6	10.6±0.6	8.7±0.9	9.1±0.7	8.5±0.7	11.0±1.5
NaCl(175mM)	0±0.0	9.03±0.6	6.0±0.8	5.8±0.6	5.3±0.9	6.7±0.4	0±0.0	8.2±1.3
NaCl(200mM)	0±0.0	7.43±0.5	4.4±0.4	3.7±0.6	0±0.0	0±0.0	0±0.0	4.5±0.6
CD at 5%	X=0.58 Y=0.61 XY=1.75							

X=Genotype

Y=Salt Concentration

Table 5: Effect of salt stress on shoot length (cm) in oat (*Avena sativa* L.)

Genotypes / Salt Conc.	OL-10	Kent	OL-1862	OL-1895	OL-1966	OL-125	OL-1869	OL-1876
Control	16.3±0.3	17.6±0.4	16.8±1.1	18.9±0.1	21.1±0.5	16.8±0.1	16.1±0.6	16.0±1.0
NaCl(25mM)	15.1±0.9	16.1±0.1	13.9±0.8	16.9±0.6	16.0±0.1	14.7±0.4	8.1±0.4	13.7±0.7
NaCl(50mM)	14.1±0.4	14.3±0.4	12.1±0.6	14.7±0.7	12.3±0.6	13.7±0.6	7.7±0.1	13.0±0.1
NaCl(75mM)	11.0±1.0	14.3±0.4	12.3±0.1	14.0±0.2	9.1±0.6	11.8±0.8	7.4±0.4	11.9±0.8
NaCl(100mM)	6.8±1.0	10.8±0.4	8.7±0.4	11.2±0.2	5.5±0.4	6.6±1.3	5.4±0.1	9.8±0.6
NaCl(125mM)	2±1.0	8.5±0.5	5.6±1.0	9.5±0.0	5.2±0.5	3.7±0.3	5.0±0.5	7.5±0.5
NaCl(150mM)	0±0.0	5.7±0.4	4.5±0.3	6.9±0.1	3.6v±0.5	3.1±0.3	2.7±0.4	5.0±0.7
NaCl(175mM)	0±0.0	4.4±0.5	3.4±0.5	2.7±0.4	2.1±0.3	2.3±0.2	0.0±0.0	2.7±0.5
NaCl(200mM)	0±0.0	3.4±0.1	2.0±0.1	2.3±0.3	0.0±0.0	0.0±0.0	0.0±0.0	1.5±0.2
CD at 5%	X=0.57 Y=0.29 XY=0.82							

X=Genotype

Y=Salt Concentration

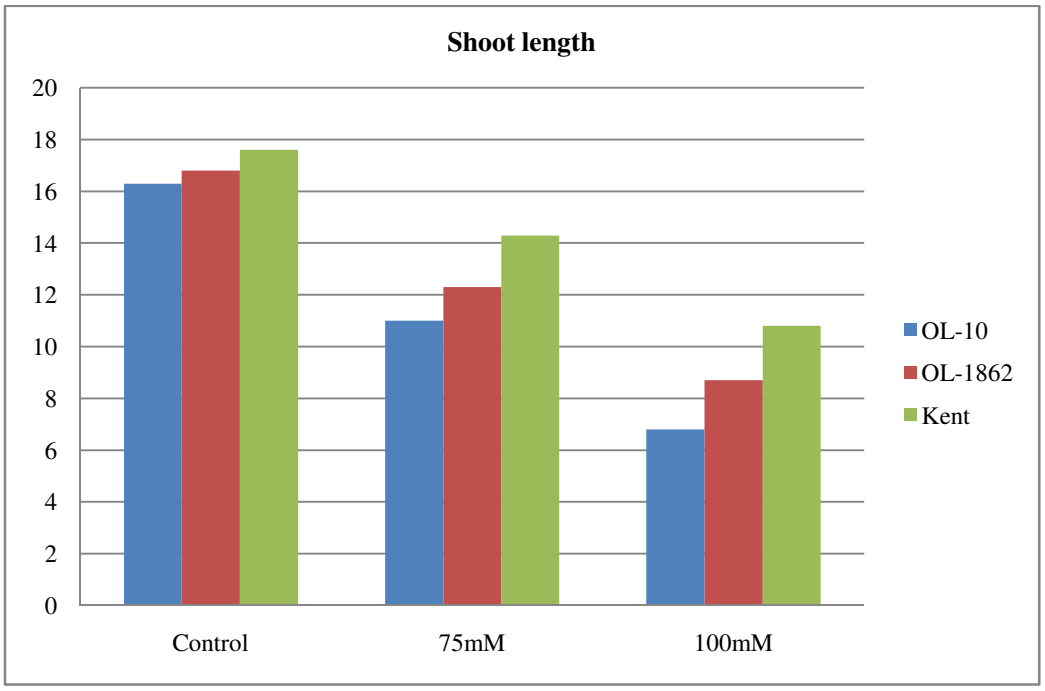

Fig. 2: Effect of salt stress on shoot length (cm) in oat (*Avena sativa* L.)

which recorded more reduction (74.4, 66.4, 60.7%) indicating their comparative sensivity to salinity stress.

4.1.2.1.1 Lamina length (cm)

Decrease in shoot length can be attributed to reduction in sheath length and lamina length. The effect of salt stress on lamina length in different genotypes of oat is presented in (table 5a). The effect of salt stress was much pronounced on lamina length as compared to sheath length. Present results indicate that lamina length decreased significantly with increase in salt concentration in all the genotypes of oat. The lamina length was declined to almost negligible at 200mM in all the genotypes. As the NaCl concentration increased from 25mM to 100mM, lamina length in general declined from 14.7 to 5.8 cm in OL-10, 17.4 to 12.3cm in Kent, 15.3 to 6.7cm in OL-1862, 10.4 to 3cm in OL-1895, 17.1 to 3cm in OL-1966, 13.0 to 4.0 cm in OL-125, 12 to 4.6 cm in OL-1869, 11.3 to 8 cm in OL-1876. The maximum lamina length was shown by OL-1876 at 25 to 100mM concentration. Minimum lamina length was shown by OL-1966 at 25 to 100mM concentration. Differential response was seen at 75mM and 100mM. At 100mM genotypes OL-1876, Kent, OL-1862, OL-10, OL-1869 recorded a less reduction (29.2, 29.3, 56.2, 60.5, 61.6 percent respectively) in lamina length indicating these to be comparatively salt tolerant as compared to the genotypes OL-1966, OL-1895, OL-125 which recorded more reduction(82.4, 71.1, 69.2%) indicating their comparative sensitivity to salt stress.

Table 5a: Effect of salt stress on lamina growth in oat (*Avena sativa* L.)

Genotypes / Salt Conc.	OL-10	Kent	OL-1862	OL-1895	OL-1966	OL-125	OL-1869	OL-1876
Control	14.7±1.1	17.4±1.1	15.3±0.8	10.4±0.4	17.1±1.4	13.0±0.5	12±1.0	11.3±0.9
NaCl(25mM)	12±1.0	15.5±1.1	11.2±0.5	9.2±0.5	12.2±0.7	11.6±0.8	4.3±0.8	7.7±0.3
NaCl(50mM)	10.2±0.4	12.8±1.0	12.7±1.1	7.8±0.3	12.8±0.2	8.9±0.1	4.1±0.8	9.6±0.4
NaCl(75mM)	7.7±0.4	12.6±0.2	10.4±1.1	4±0.7	4.5±0.6	8.6±0.6	1.7±0.3	6.8±0.1
NaCl(100mM)	5.8±1.1	12.3±0.6	6.7±0.2	3±0.3	3±1.0	4.0±0.7	4.6±0.5	8±1.0
NaCl(125mM)	4.8±1.7	9±1.0	4.5±0.3	3±0.3	2.7±0.2	1.8±0.2	0.5±0.3	5±1.0
NaCl(150mM)	0±0.0	3.2±0.6	2.6±0.2	1±0.2	1.3±0.2	1.4±0.4	2.8±0.2	1.6±0.6
NaCl(175mM)	0±0.0	3.1±0.7	1.6±0.5	1±0.3	0.8±0.3	0.4±0.4	0±0.0	0.9±0.1
NaCl(200mM)	0±0.0	1.9±0.2	1.6±0.4	0±0.0	0±0.0	0±0.0	0±0.0	0.8±0.1
CD at 5%	X=0.34			Y=0.36		XY=1.02		

X=Genotype

Y=Salt Concentration

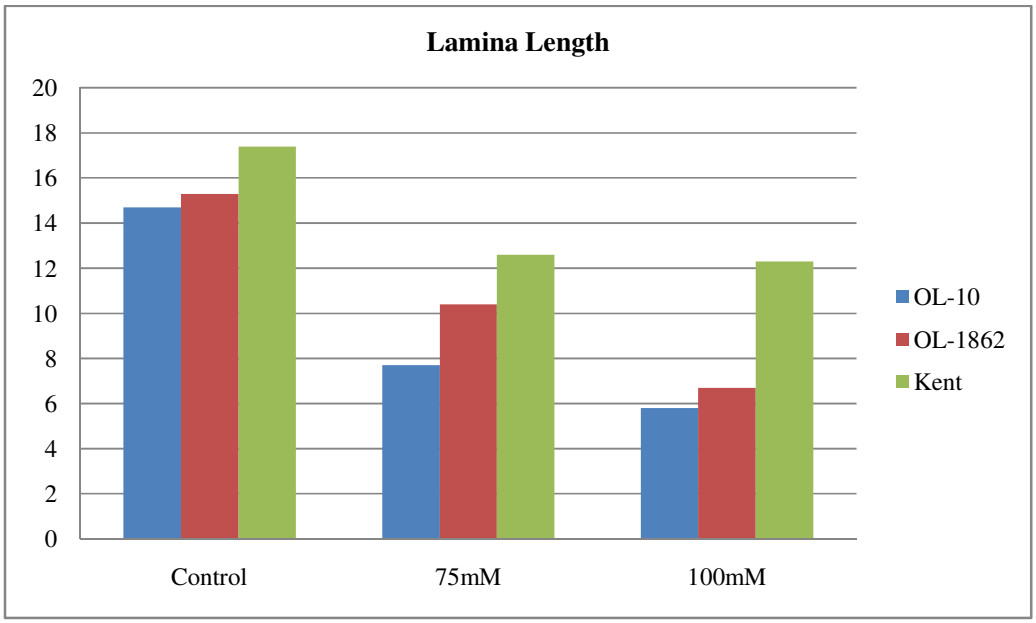

Fig 2a: Effect of salt stress on lamina length (cm) in oat (*Avena sativa* L.)

4.1.2.1.2 Sheath

The effect of salt stress on sheath length in different genotypes of oat is presented in (table 5b). Present results indicate that sheath length decreased significantly with increase in salt concentration in all the genotypes of oat. The sheath length was declined to almost negligible at 200mM in all the genotypes. There was not much difference in sheath length at 75mM and 100mM. As the NaCl concentration increased from 25mM to 100mM, sheath length in general declined from 5.6 to 3.5 cm in OL-10, 6.9 to 5 cm in Kent, 5.9 to 4.3cm in OL-1862, 5 to 4.4cm in OL-1895, 5.6 to 3.6 cm in OL-1966, 5.3 to 2.2 cm in OL-125, 5.1 to 2.8 cm in OL-1869, 4.8 to 3.5 cm in OL-1876. The maximum sheath length was shown by OL-1895 at 25 to 100mM concentration. Minimum sheath length was shown by OL-125 at 25 to 100mM concentration. At 100mM genotypes OL-1895, Kent, OL-1876, OL-1862, Kent, OL-1966 recorded a less reduction (12, 27.0, 27.1, 27.5, 35.7 percent respectively) in sheath length indicating these to be comparatively salt tolerant as compared to the genotypes OL-125, OL-1869, OL-10 which recorded more reduction (82.4, 71.1, 69.2%) indicating their comparative sensitivity to salt stress.

Similar results were reported in rice by (Abbasi *et al* 2004). The decrease in shoot- root lengths is because of decreased physiological activities resulting from water and nutrients stress occurring under high salt stress. These processes results in small shoot. Salt stress leads to trouble in metabolism of plant, which consequently lead to decrease in plant growth and production (Shafi *et al* 2009).

Table 5b: Effect of salt stress on sheath length in oat (*Avena sativa* L.)

Genotypes / Salt Conc.	OL-10	Kent	OL-1862	OL-1895	OL-1966	OL-125	OL-1869	OL-1876
Control	5.6±0.2	6.9±0.7	5.9±0.3	5±1.0	5.6±0.4	5.3±0.6	5.1±0.3	4.8±0.1
NaCl(25mM)	5.4±0.2	5.7±0.3	5.5±0.4	4.9±0.1	5.6±0.6	5.3±0.6	4.1±0.3	4.7±0.2
NaCl(50mM)	5.3±0.6	5.4±0.2	4.7±0.6	4.6±0.1	5.0±0.1	4.7±0.4	3.7±0.3	4.1±0.3
NaCl(75mM)	4.5±0.1	5.2±0.2	4.8±0.2	4.4±0.1	4.7±0.2	3.8±0.7	3.6±0.3	3.8±0.6
NaCl(100mM)	3.5±0.3	5±0.1	4.3±0.2	4.4±0.1	3.6±0.2	2.2±0.1	2.8±0.2	3.5±0.4
NaCl(125mM)	2.6±0.4	5.0±0.1	4.1±0.2	4.4±0.1	2.5±0.2	2±0.0	1.7±0.2	2.6±1.2
NaCl(150mM)	0±0.0	3.5±0.3	2.7±0.2	3.7±0.6	2.5±0.2	2±0.1	0.7±0.2	2±0.7
NaCl(175mM)	0±0.0	2.5±0.3	1.8±0.1	2.7±0.2	1.8±0.3	1.3±0.6	0±0.0	1.3±0.5
NaCl(200mM)	0±0.0	1.7±0.2	1.5±0.3	2±0.0	0±0.0	0±0.0	0±0.0	0.7±0.2
CD at 5%	X=0.19 Y=0.21 XY=0.59							

X= Genotype

Y= Salt concentration

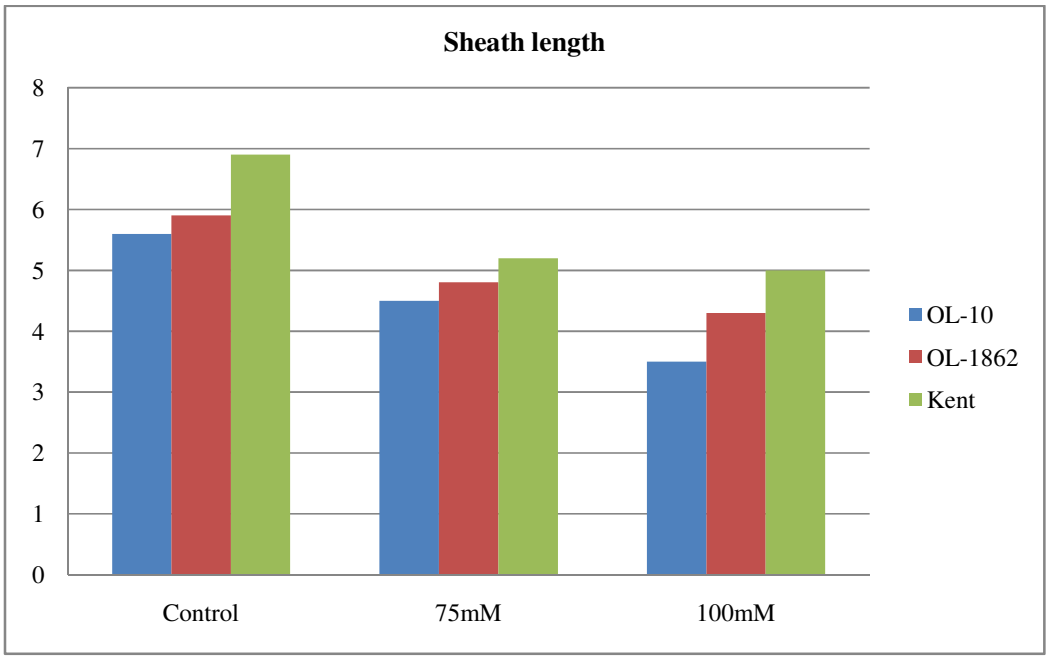

Fig 2b: Effect of salt stress on sheath length (cm) in oat (*Avena sativa* L.)

Similarly in root length as the NaCl concentration increased from 25mM to 100mM it declined from 10.2 to 6.5 cm in OL-10, 12.3 to 8.8 cm in Kent, 11.7 to 6.5 cm in OL-1862, 11.4 to 7.2 cm in OL-1895, 13.3 to 6.9 cm in OL-1966, 14.5 to 6.1 cm in OL-125, 9.1 to 6.1 cm in OL-1869, 11.5 to 8.5 cm in OL-1876 from 25mM to 100mM NaCl respectively. The maximum root length was shown by genotype OL-1869. Minimum root length was shown by genotype OL-125. Differential response was seen at 75mM and 100mM. At 100mM genotypes OL-1869, OL-1876, Kent, OL-10, OL-1862 recorded a less reduction (17.5, 26.0, 28.4, 36.2, 44.4 percent respectively) in root length indicating these to be comparatively salt tolerant as compared to the genotypes OL-125, OL-1865, OL-1966 which recorded more reduction (74.4, 66.4, 60.7%) indicating their comparative sensitivity to salt stress. Reduction in root length was less than the shoot length. Similar results were seen in barley seedlings by Agami (2014).

Shoot length of OL-10, Kent and root length of OL-1869, OL-1876 were non significantly affected at 25mM NaCl. However root and shoot length was significantly affected at highest levels of salinity (75 and 100 mM). The decrease with increasing salt stress may be due to limited supply of metabolites to young growing tissues because metabolic production is significantly perturbed at high salinity stress, probably due to the toxic effects of salt (Yousofinia *et al* 2012). The decrease in root length with increase in salt stress might be due to more inhibitory effect of salt to root and shoot growth (Anbumalarmathi and Mehta 2013) in rice. With increasing salinity levels, root and shoot length decreased. These findings are related to those discussed by Gupta and Srivastava (1989), who concluded that root were less affected than shoot in wheat. The

Table 6: Effect of salt stress on root length (cm) in oat (*Avena sativa* L.)

Genotypes / Salt Conc.	OL-10	Kent	OL-1862	OL-1895	OL-1966	OL-125	OL-1869	OL-1876
Control	10.2±1.5	12.3±0.4	11.7±0.4	11.4±1.0	13.3±1.1	14.5±0.6	9.1±0.2	11.5±0.8
NaCl(25mM)	9.4±0.9	11.2±0.5	9.4±0.4	8.8±1.0	10.2±0.7	11.6±0.4	8±1.0	11.3±0.7
NaCl(50mM)	9.4±0.4	9±1.0	9±0.0	7.7±1.1	9.6±0.5	11.6±0.4	7.9±1.0	11±0.8
NaCl(75mM)	7.4±0.4	9±1.0	8.5±0.5	7.2±0.6	7.4±0.4	13.1±5.1	7.7±0.8	10±1.0
NaCl(100mM)	6.5±0.4	8.8±0.1	6.5±0.5	5±1.0	6.9±0.1	6.1±0.8	7.5±0.9	8.5±0.5
NaCl(125mM)	4.3±0.9	7.5±0.3	6.3±0.5	4.4±0.3	5.9±0.1	6.1±0.8	6.5±0.5	8.5±0.5
NaCl(150mM)	0±0.0	6±0.2	4.7±0.3	3.7±0.5	5.2±0.4	6.0±0.8	5.8±1.0	6±1.0
NaCl(175mM)	0±0.0	4.6±1.0	2.6±0.5	3.1±0.3	3.2±0.6	4.4±0.4	0±0.0	5.5±1.0
NaCl(200mM)	0±0.0	4.0±0.5	2.4±0.4	1.4±0.3	0±0.0	0±0.0	0±0.0	3.3±0.6
CD at 5%			X=0.47		Y=0.50		XY=1.42	

X=Genotype

Y=Salt Concentration

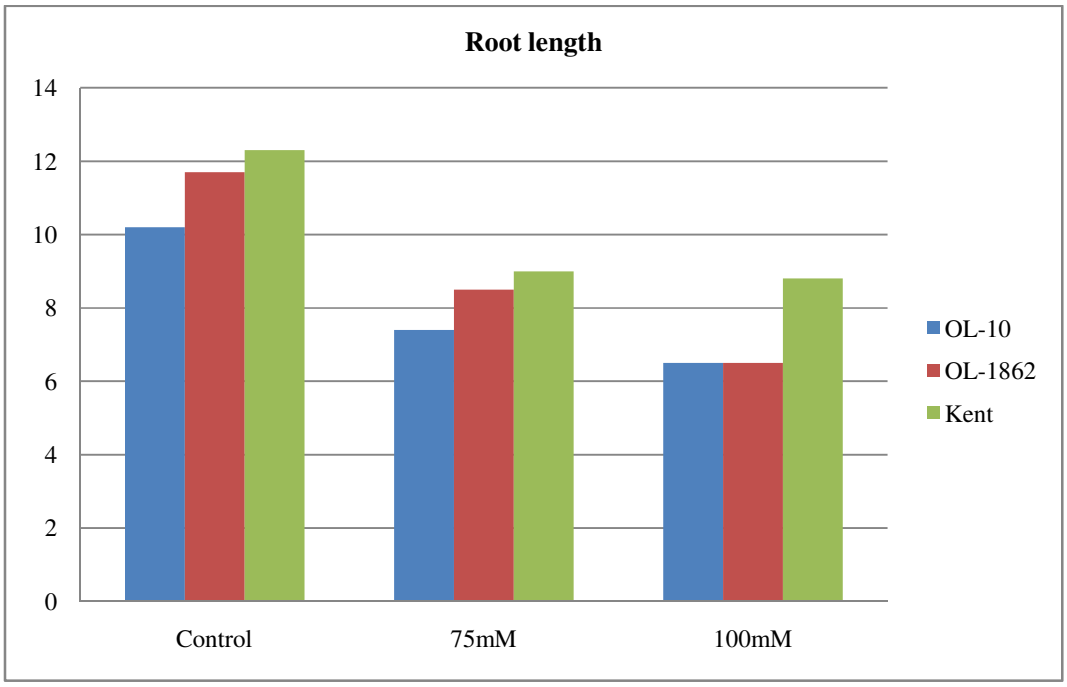

Fig. 3: Effect of salt stress on root length (cm) in oat (*Avena sativa* L.)

inhibitory effect of salinity on growth rate and development of seedlings was reported in castor bean (Rajput *et al* 2015). Reduction in growth is caused by salt stress which reduced the ability of plants to absorb water (Nawaz *et al* 2010). Similar results are reported in maize seedlings where root length and shoot length decrease with salt stress (Billah *et al* 2018). Seedling is the most vulnerable stage in the life cycle of plants and germination determines when and where seedling development begins (Lianes *et al* 2005). Reduction in root-shoot length may be due salt toxicity and disproportion in nutrient absorption by the seedling as suggested by Bybordi and Tabatabaei (2009).

Seedling biomass

4.1.3 Fresh and Dry weight of seedling (mg)

The effect of salt stress on fresh weight and dry weight in different genotypes of oat is presented in table 7 and 8. Present results indicate that fresh and dry weight decreased significantly with increase in salt concentration in all the genotypes of oat. The germination percentage was declined to almost negligible at 200mM in all the genotypes. As the NaCl concentration increased from 25mM to 100mM, fresh weight in general declined from 277.5 to 80.3 mg in OL-10, 301.5 to 135.4 mg in Kent, 280.2 to 107.7 mg in OL-1862, 332.3 to 155.5 mg in OL-1895, 415.3 to 203.2 mg in OL-1966, 285.3 to 176.4 mg in OL-125, 306.3 to 240.6 mg in OL-1869, 335.3 to 317.5 mg in OL-1876 from 25mM to 100mM NaCl respectively. The maximum fresh weight was shown by genotype OL-1876. Minimum fresh weight was shown by genotype OL-10. Differential response was seen at 75mM and 100mM. At 100mM genotypes OL-1876,

Table 7: Effect of salt stress on fresh weight (mg) in oat (*Avena sativa* L.)

Genotypes / Salt Conc.	OL-10	Kent	OL-1862	OL-1895	OL-1966	OL-125	OL-1869	OL-1876
Control	277.5±2.7	301.5±3.9	280.2±5.8	332.3±2.1	415.3±3.5	285.3±4.7	306.3±3.0	335.3±1.4
NaCl(25mM)	207.3±7.8	219.8±4.9	255.2±5.3	324.9±4.4	413.3±3.2	252.0±3.2	302.5±1.8	334.5±0.9
NaCl(50mM)	190.0±4.8	213.3±6.0	195.3±4.8	318.4±2.4	367.6±1.9	250.4±0.7	302.3±2.5	331.3±1.3
NaCl(75mM)	82.5±3.8	178.4±7.5	175.9±5.3	175.7±5.1	208.3±7.6	248.6±0.8	252.2±2.3	322.5±2.1
NaCl(100mM)	80.3±1.3	135.4±5.2	107.7±7.7	155.5±5.2	203.2±5.1	176.4±1.4	240.6±4.8	317.5±2.3
NaCl(125mM)	70.8±5.1	75.7±4.4	78.9±5.9	144.0±3.5	185.3±5.3	164.5±4.4	68.4±2.4	198.2±2.5
NaCl(150mM)	0±0.0	71.3±6.3	65.4±5.3	133.2±3.3	155.4±5.4	140.9±1.2	47.4±2.3	127.3±2.1
NaCl(175mM)	0±0.0	65.3±4.6	61.1±3.2	155.4±5.4	105.5±5.3	137.5±2.2	0±0.0	99.0±4.0
NaCl(200mM)	0±0.0	55.6±4.7	45.3±5.3	85.5±5.4	0±0.0	0±0.0	0±0.0	66.7±1.0
CD at 5%	X=2.19			Y=2.32		XY=6.57		

X=Genotype

Y=Salt concentration

Table 8: Effect of salt stress on dry weight (mg) in oat (*Avena sativa* L.)

Genotypes / Salt Conc.	OL-10	Kent	OL-1862	OL-1895	OL-1966	OL-125	OL-1869	OL-1876
Control	21.8±1.8	36±1.0	26±1.0	45.3±1.5	34.6±1.3	25.7±1.2	27.7±0.8	45.4±1.1
NaCl(25mM)	20±1.0	23.3±0.9	17±1.0	25.8±1.0	32±2.0	21.3±1.1	26±1.0	45±1.0
NaCl(50mM)	16±2.0	19.5±1.0	15.9±0.5	25±1.0	27.9±2.1	20±1.0	26±1.0	42±2.0
NaCl(75mM)	7.8±0.7	18.3±0.6	9.6±0.4	21.5±1.1	23.7±0.9	20±1.0	25.7±1.1	32.4±1.0
NaCl(100mM)	6.4±0.4	15±1.0	7.6±0.3	21±1.0	23±1.0	16.3±1.2	24.7±1.1	26.5±1.2
NaCl(125mM)	6±1.0	8.9±0.1	7±1.0	4.5±1.0	21±1.0	16.3±1.1	11.6±1.0	24.6±1.0
NaCl(150mM)	0±0.0	4.7±0.4	2±0.6	4±1.0	5.8±0.4	13±1.0	7.5±0.2	21.6±1.4
NaCl(175mM)	0±0.0	4.7±0.6	1.5±0.5	3.5±1.1	2±1.0	5.7±0.4	0±0.0	11.5±1.1
NaCl(200mM)	0±0.0	2±1.0	0±0.0	1.8±0.3	0±0.0	0±0.0	0±0.0	11.3±0.9
CD at 5%	X=0.53			Y=0.56		XY=1.60		

X=Genotype

Y=NaCl concentration

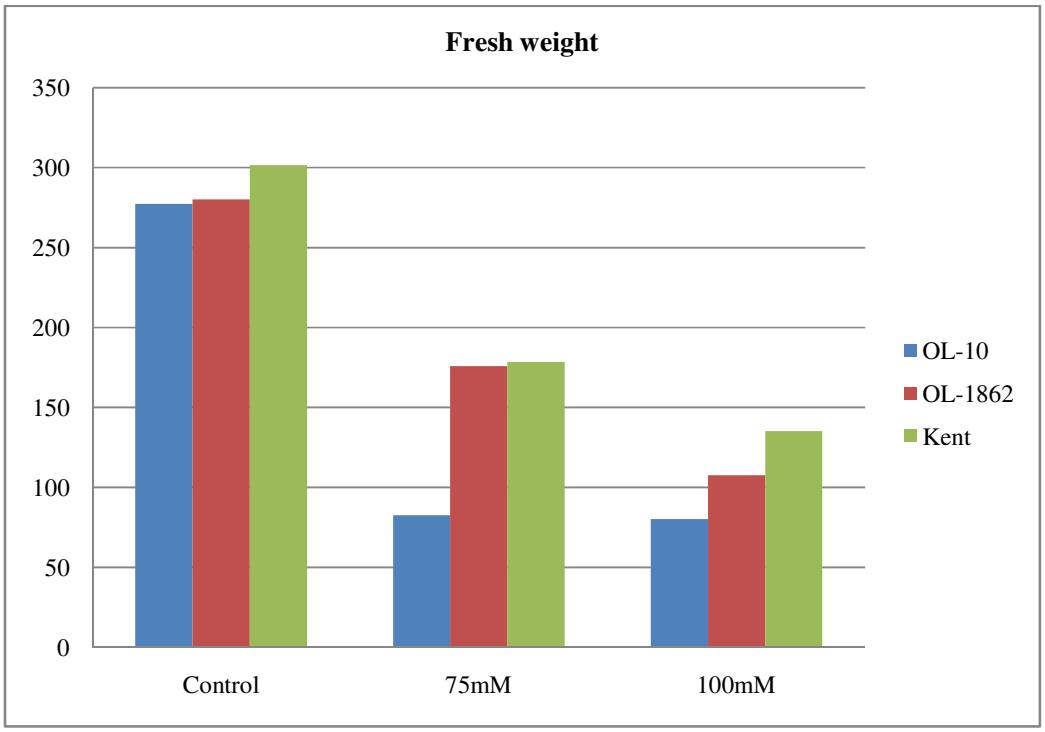

Fig. 4: Effect of salt stress on fresh weight (mg) in oat (*Avena sativa* L.)

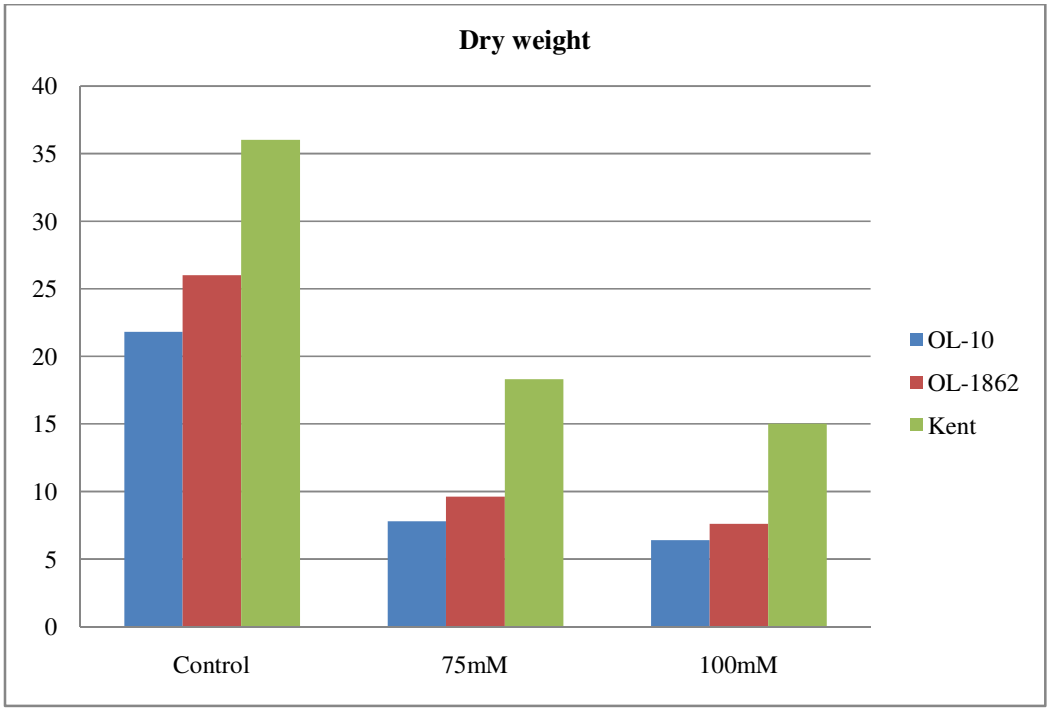

Fig. 5: Effect of salt stress on dry weight (mg) in oat (*Avena sativa* L.)

OL-1869, OL-125, OL-1966, OL-1895 recorded a less reduction (5.3, 21.4, 38.1, 51.0, 53.2 percent respectively) in fresh weight indicating these to be comparatively salt tolerant as compared to the genotypes OL-10, OL-1862, Kent which recorded more reduction (71.0, 61.5, 55.0%) indicating their comparative sensitivity to salt stress. Reduction in fresh biomass at higher concentration might be due to poor absorption of water from the growth medium due to physiological drought (Nedjimi *et al* 2006 and Seckin *et al* 2009). Fresh weights were decreased with an increase in the level of sodium chloride as a result of reduced photosynthesis and membrane stability (Mozafariyan *et al* 2013). In oat water deficit and salt treatments also caused reduction in fresh weight of seedling, which is similar to several reports (Ramezani *et al* 2011).

Similarly in dry weight as the NaCl concentration increased from 25mM to 100mM it declined from 21.8 to 6.4 mg in OL-10, 36 to 15 mg in Kent, 26 to 7.6 mg in OL-1862, 45.3 to 21 mg in OL-1895, 34.6 to 23 mg in OL-1966, 25.7 to 16.3 mg in OL-125, 27.7 to 24.7 mg in OL-1869, 45.4 to 26.5 mg in OL-1876 from 25mM to 100mM NaCl respectively. The maximum dry weight was shown by genotype OL-1869. Minimum dry weight was shown by genotype OL-1862. At 100mM genotypes OL-1869, OL-1966, OL-125, OL-1876, OL-1895 recorded a less reduction (10.8, 33.5, 36.5, 41.6, 53.6 percent respectively) in dry weight indicating these to be comparatively salt tolerant as compared to the genotypes OL-1862, OL-10, Kent which recorded more reduction (70.7, 70.6, 58.3%) indicating their comparative sensitivity to salt stress. NaCl-imposed stress reduced shoot and root dry weights in wheat genotypes (Zhang *et al* 2011) and (Xu *et al* 2008). Munns (2002) reported that a significant reduction in total dry weight under salinity was due to Na^+ and Cl^- accumulation and osmotic stress. Dadkhah and Grrifiths (2005) attributed such a decrease in dry weight to greater reduction in uptake and utilization of mineral nutrients by plants under salinity stress. The decrease in dry weight of plants under salty conditions which can be attributed to decrease rate of photosynthesis, as suggested by Jafari *et al* (2009).

4.1.5 Seed Vigour I

Seed vigour I is the product of percent germination and seedling length. The effect of salt stress on seed vigour I and seed vigour II in different genotypes of oat is presented in (table 9 and 10). Present results indicate that seed vigour I and seed vigour II decreased significantly with increase in salt concentration in all the genotypes of oat. The seed vigour I and II was declined to almost negligible at 200mM in all the genotypes. As the NaCl concentration increased from 25mM to 100mM, seed vigour I in general declined from 2433 to 750.2 in OL-10, 2930 to 1567.2 in Kent, 2714 to 811.0 in OL-1862, 2267.7 to 1193.6 in OL-1895, 3436.7 to 743.5 in OL-1966, 2864.3 to 788.5 in OL-125, 2437.3 to 685.7 in OL-1869, 2743.3 to 1461.3 in OL-1876 from 25mM to

Table 9: Effect of salt stress on seed vigour I in oat (*Avena sativa* L.)

Genotypes / Salt Conc.	OL-10	Kent	OL-1862	OL-1895	OL-1966	OL-125	OL-1869	OL-1876
Control	2433±63.9	2930±70.0	2714±99.9	2267.7±114.7	3436.7±158.2	2864.3±209.1	2437.3±180.3	2743.3±180.1
NaCl(25mM)	1873.9±257.6	2726.7±41.6	2097±94.0	1930±110.6	2620±65.6	2365.8±112.9	1552.7±15.5	2461.7±75.2
NaCl(50mM)	1529.8±124.4	2215.8±187.6	1789.4±127.0	1862.3±78.5	2190±17.3	2208.1±172.0	1374.8±139.4	2163±72.7
NaCl(75mM)	1283±119.8	1960.0±51.6	1458.2±114.9	1659.5±106.1	1584.9±116.5	2023.8±377.4	1232.8±100.3	1819.2±66.7
NaCl(100mM)	750.2±47.4	1567.2±86.2	811.0±92.2	1193.6±44.5	743.5±88.8	788.5±173.2	685.7±51.6	1461.3±118.3
NaCl(125mM)	309.5±87.7	1117.7±56.1	412.8±9.9	610.7±81.8	556.8±83.5	606.2±91.5	452.4±27.6	1181.3±80.1
NaCl(150mM)	0±0.0	683.8±91.6	229.8±41.4	372.8±72.0	415.6±76.0	472.5±95.5	195.3±34.0	796±53.8
NaCl(175mM)	0±0.0	414±62.0	124.7±42.9	174±18.7	123.7±35.5	245±45.5	0±0.0	492.2±146.8
NaCl(200mM)	0±0.0	198.7±48.0	86.8±20.6	59.9±24.1	0±0.0	0±0.0	0±0.0	127±24.2
CD at 5%	X= 56.9			Y=60.4		XY=170.9		

X=Genotype,

Y=NaCl concentration

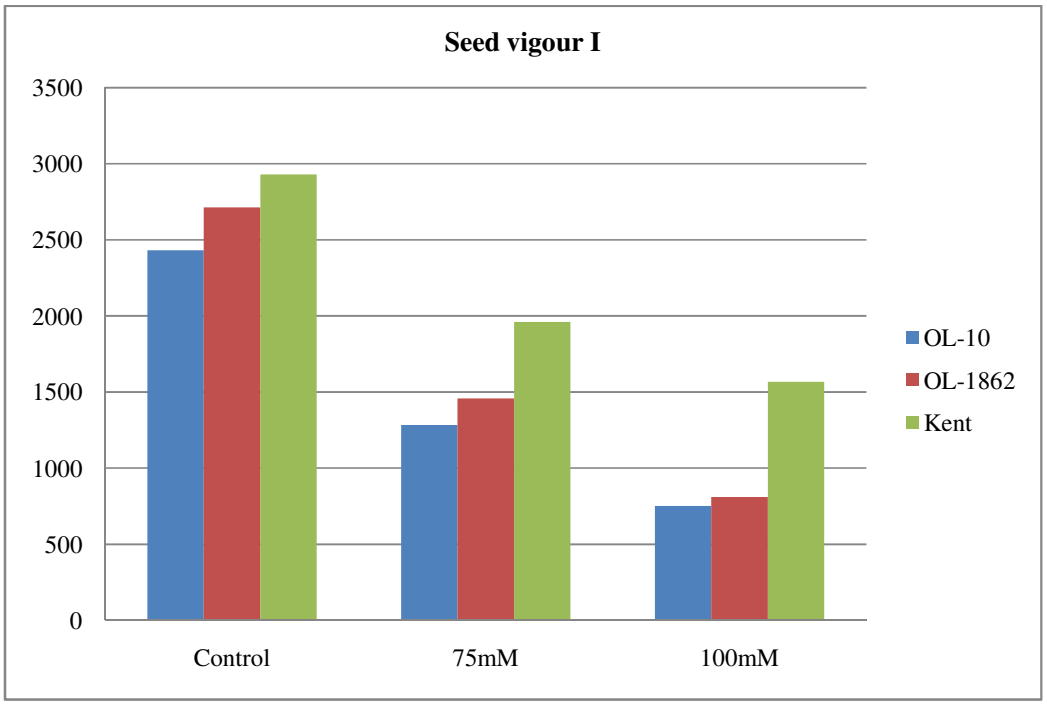

Fig. 6: Effect of salt stress on seed vigour I in oat (*Avena sativa* L.)

100mM NaCl respectively. The maximum seed vigour I was shown by genotype Kent. Minimum seed vigour I was shown by genotype OL-1966. Differential response was seen at 75mM and 100mM. At 100mM genotypes Kent, OL-1876, OL-1895, OL-10, OL-1862 recorded a less reduction (46.5, 46.7, 47.3, 69.1, 70.1 percent respectively) in seed vigour I indicating these to be comparatively salt tolerant as compared to the genotypes OL-1966, OL-125, OL-1869 which recorded more reduction (78.3, 72.4, 71.8%) indicating their comparative sensitivity to salt stress. Similar results of decreasing vigour index with salinity have been reported in spinach by (Keshavarzi *et al* 2011) and in maize by (Janmohammadi *et al* 2008).

4.1.6 Seed Vigour II

Seed vigour II is the product of percent germination and dry weight. Similarly seed vigour II in general declined from 2 to 0.3 in OL-10, 3.6 to 1.2 in Kent, 2.3 to 0.3 in OL-1862, 3.3 to 1.4 in OL-1895, 3.4 to 1.4 in OL-1966, 2.3 to 1 in OL-125, 2.6 to 1.3 in OL-1869, 4.5 to 1.9 in OL-1876 from 25mM to 100mM NaCl respectively. The maximum seed vigour II was shown by genotype OL-1869. Minimum seed vigour II was shown by genotype OL-1862. Genotypes OL-1869,OL-125, OL-1895, OL-1876, OL-1966 recorded a less reduction (50, 56.5, 57.5, 57.7, 58.8 percent respectively) in seed vigour II indicating these to be comparatively salt tolerant as compared to the genotypes OL-1862, OL-10, Kent which recorded more reduction (86.9, 85, 66.6%) indicating their comparative sensitivity to salt stress.

Table 10: Effect of salt stress on seed vigour II in oat (*Avena sativa* L.)

Genotypes / Salt Conc.	OL-10	Kent	OL-1862	OL-1895	OL-1966	OL-125	OL-1869	OL-1876
Control	2±0.1	3.6±0.1	2.3±0.1	3.3±0.2	3.4±0.1	2.3±0.1	2.6±0.2	4.5±0.1
NaCl(25mM)	1.4±0.1	2.3±0.1	1.5±0.1	1.9±0.1	3.2±0.2	1.9±0.1	2.5±0.3	4.4±0.3
NaCl(50mM)	1±0.2	1.8±0.2	1.3±0.1	2.0±0.3	2.7±0.3	1.7±0.2	2.2±0.1	3.7±0.2
NaCl(75mM)	0.5±0.1	1.4±0.2	0.7±0.1	1.7±0.2	2.3±0.1	1.6±0.1	2.0±0.1	2.5±0.3
NaCl(100mM)	0.3±0.1	1.2±0.1	0.3±0.0	1.4±0.2	1.4±0.1	1±0.2	1.3±0.2	1.9±0.2
NaCl(125mM)	0.3±0.1	0.6±0.0	0.2±0.0	0.1±0.1	1±0.1	1.0±0.2	0.4±0.0	1.7±0.1
NaCl(150mM)	0±0.0	0.2±0.1	0±0.0	0.1±0.1	0.2±0.0	0.4±0.3	0.1±0.1	1.5±0.1
NaCl(175mM)	0±0.0	0.1±0.1	0±0.0	0.1±0.1	0.0±0.0	0.2±0.1	0±0.0	0.7±0.2
NaCl(200mM)	0±0.0	0±0.0	0±0.0	0.0±0.0	0±0.0	0±0.0	0±0.0	0.3±0.2
CD at 5%	X=0.69			Y=0.73			XY=0.20	

X=Genotype

Y=Salt Concentration

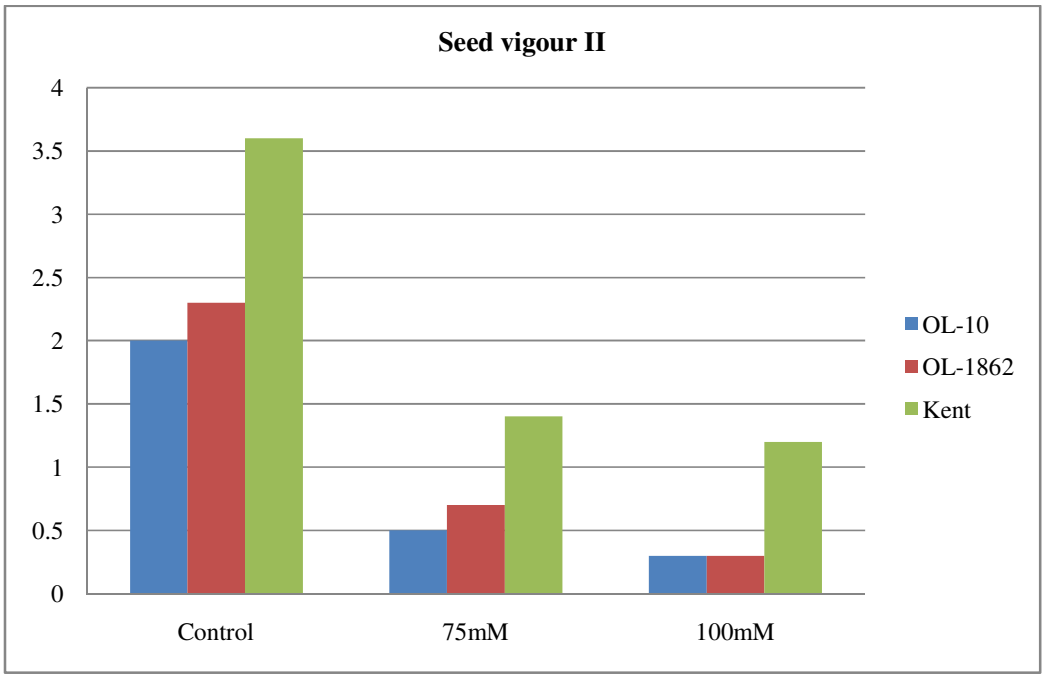

Fig. 7: Effect of salt stress on seed vigour II in oat (*Avena sativa* L.)

Biochemical Parameters

Differential response was observed at 75mM and 100mM NaCl with respect to the physiological parameters. So the biochemical parameters of the selected tolerant, moderate and sensitive genotypes were evaluated at these concentrations.

4.1.7 Total Soluble Sugars (mg g^{-1} FW)

As shown in table 11 total soluble sugars were recorded to increase in the stressed seedlings as compared to the control in all the genotypes. The increase in the soluble sugar content was more in the comparatively tolerant genotype (Kent) and moderate genotype (OL-1862) as compared to the sensitive genotype (OL-10). In

Table 11: Effect of salt stress on total soluble sugar content (mg g^{-1} FW) in oat (*Avena sativa* L.)

Genotypes / Salt Conc.	OL-10	Kent	OL-1862
Control	34.9±3.6	55.4±2.2	46.4±1.2
75mM	45.5±3.2	82.5±3.7	71.9±2.3
100mM	52±2.8	106.7±4.1	105.4±3.1
CD at 5%	X=3.0	Y=3.0	XY=5.2

X=Genotype

Y= Salt concentration

tolerant genotype Kent total soluble sugars increased from 55.4 in control to 82.5 in 75mM and 106.7 in 100mM NaCl. Similarly the total soluble sugars in moderately tolerant genotype OL-1862 increased from 46.4 to 71.9 and 105.4 at control, 75mM, 100mM and in the sensitive genotype OL-10, the soluble sugars increased from 34.9 in control to 45.5 in 75mM and 52 in 100mM NaCl.

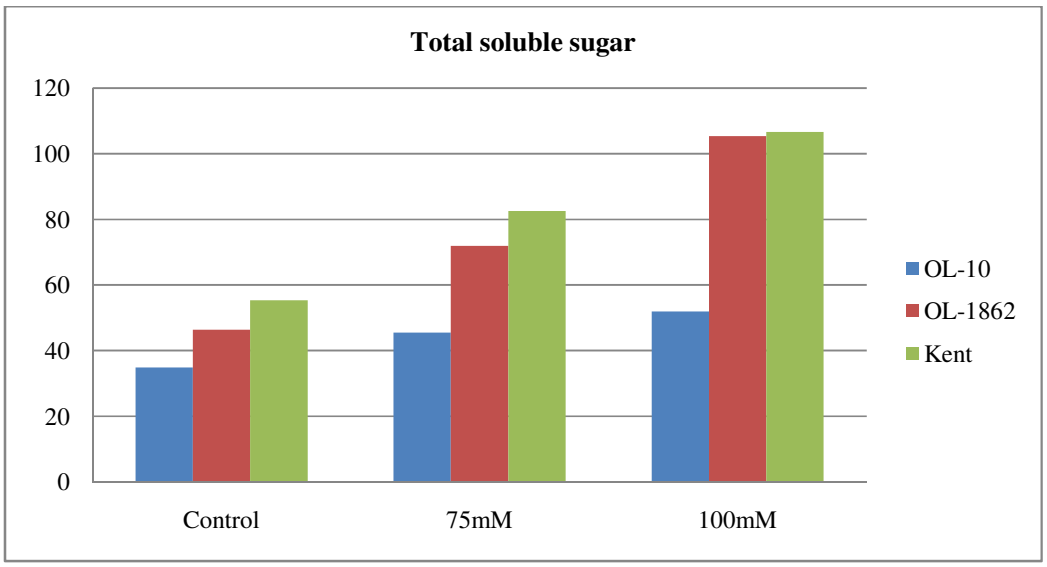

Fig. 8: Effect of salt stress on total soluble sugar content (mg g^{-1} FW) in oat (*Avena sativa* L.)

Ashraf and Fatima (1995) reported that salt resistant accessions accumulates more soluble sugars than the sensitive accessions in *Carthamus tinctorius*. Watanabe *et al* (2000) reported that in the genus *Populus*, most salt resistant species accumulate more soluble sugars in leaves than sensitive ones in the presence of salinity stress. Similar results were found in sunflower (Ashraf and Tufail 2008). Wahid (2004) reported that the most tolerant variety accumulates more soluble sugars in leaves indicate that soluble sugars play a key role in salt tolerance in sugarcane. Higher total soluble sugars have been reported in salt-tolerant genotypes than salt-sensitive ones (Nemati *et al* 2011, Khan *et al* 2015). It has been reported in wheat and barley that salinity stress caused an increase in the soluble sugar content of the seedlings (Li *et al* 2011). Sugars function as signals, helpful for plant in sensing and controlling the photosynthetic activity and cellular redox balance (Sulmon *et al* 2006). Soluble sugar acts as an osmotic agent to improve plant resistance to environmental stress (Fu *et al* 2010). This increase in the sugar levels of stressed plants help in effective osmoregulation under stress conditions (Gill *et al* 2003) and is involved in the osmotic adjustments. Sugar can act as an osmolyte to preserve membrane integrity and act as a signaling molecule to induce metabolic rearrangements and regulatory networks under stressful environmental stress (Krasensky and Jonak 2012).

4.1.8 Total Soluble Proteins (mg g^{-1} FW)

As shown in (table 12) total soluble proteins were recorded to increase in the stressed seedlings as compared to the control in all the genotypes. The increase in the soluble protein content was more in the comparatively tolerant genotype (Kent) and moderate genotype (OL-1862) as compared to the sensitive genotype (OL-10). In tolerant genotype Kent total soluble proteins increased from 19.7 in control to 22.6 in 75mM and 27.5 in 100mM NaCl. Similarly the total soluble proteins in moderately tolerant genotype OL-1862 increased from 19.4 in control to 22 in 75mM and 26.2 in 100mM and in the sensitive genotype OL-10, the soluble sugars increased from 19.4 in control to 21.5 in 75mM and 25.8 in 100mM NaCl.

Table 12: Effect of salt stress on total soluble protein content (mg g^{-1} FW) in oat (*Avena sativa* L.)

Genotypes / Salt Conc.	OL-10	Kent	1862
Control	19±1.2	19.7±2.3	19.4±3.9
75mM	21.5±2.4	22.6±3.5	22±3.0
100mm	25.8±2.2	27.5±2.1	26.2±2.1
CD at 5%	X=NS	Y=2.5	XY=NS

X= Genotype

Y=Salt concentration

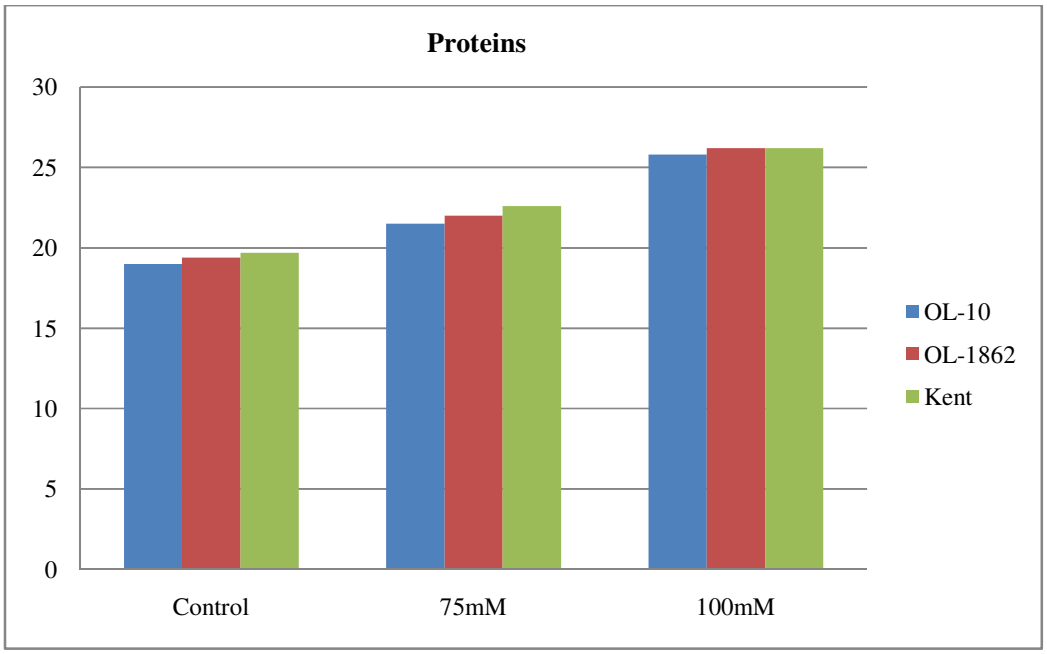

Fig. 9: Effect of salt stress on total soluble protein content (mg g^{-1} FW) in oat (*Avena sativa* L.)

Increased accumulation of total soluble proteins in response to saline stress was reported by (Liu *et al* 2016) in *Nitraria tangutorum*. Protein accumulated in plant may be involved in osmotic adjustment as well as in activation of antioxidant enzymes to decrease the amount of reactive oxygen species (ROS) produced by salt stress. Proteins accumulated in salt-stressed plants may provide storage form of nitrogen for the reutilization when stress is over (Singh *et al* 1987 and Qasim *et al* 2003). Increase in soluble protein content under salt stress may be the result of enhanced synthesis of specific stress-related proteins. Studies have revealed that protein synthesis could be affected by salt stress (Sousa *et al* 2003).

4.1.9 Catalase activity (ΔA min^{-1} g^{-1} FW)

CAT is important enzymatic component of the antioxidant system. It catalyzes the decomposition of hydrogen peroxide to water and oxygen (Cristina *et al* 2008). As shown in (table 13) catalase activity was recorded to increase in the stressed seedlings as compared to the control in all the genotypes. The increase in catalase activity was more in the comparatively tolerant genotype (Kent) and moderate genotype (OL-1862) as compared to the sensitive genotype (OL-10). In tolerant genotype Kent catalase activity increased from 22.1 in control to 86.3 in 75mM and 118 in 100mM NaCl. Similarly the catalase activity in moderately tolerant genotype OL-1862 increased from 17.3 to 24.4 and 41.9 at control, 75mM, 100mM and in the sensitive genotype OL-10, the catalase activity increased from 12.4 in control to 15.4 in 75mM and 29.9 in 100mM NaCl.

Table 13: Effect of salt stress on catalase activity (ΔA min^{-1} g^{-1} FW) in oat (*Avena sativa* L.)

Genotypes / Salt Conc.	OL-10	Kent	OL-1862
Control	12.4±1.9	22.1±0.9	17.3±0.9
75mM	15.4±2.0	86.3±2.2	24.4±2.2
100mM	29.9±3.0	118±1.9	41.9±2.0
CD at 5%	X=1.9	Y=1.9	XY=3.3

X=Genotype

Y=Concentration

Increasing activities of antioxidant enzymes and improving antioxidant metabolism in plants was one of the most important ways to enhance salt tolerance of plants (Mao *et al* 2004). CAT is involved in scavenging of H_2O_2 during salt stress and other abiotic stress conditions (Willekens *et al* 1997) and is considered as a major

enzyme detoxifying H_2O_2 in tomato fruits (Murshed *et al* 2014). CAT is often related to an enhanced tolerance to salt stress (Mittova *et al* 2004 and Gao *et al* 2008). In some salt-tolerant plants, increased in catalase activity have been recored after increasing NaCl, such as those described in myrtle, suggesting increased photo respiratory activity (Acosta-Motos *et al* 2017).

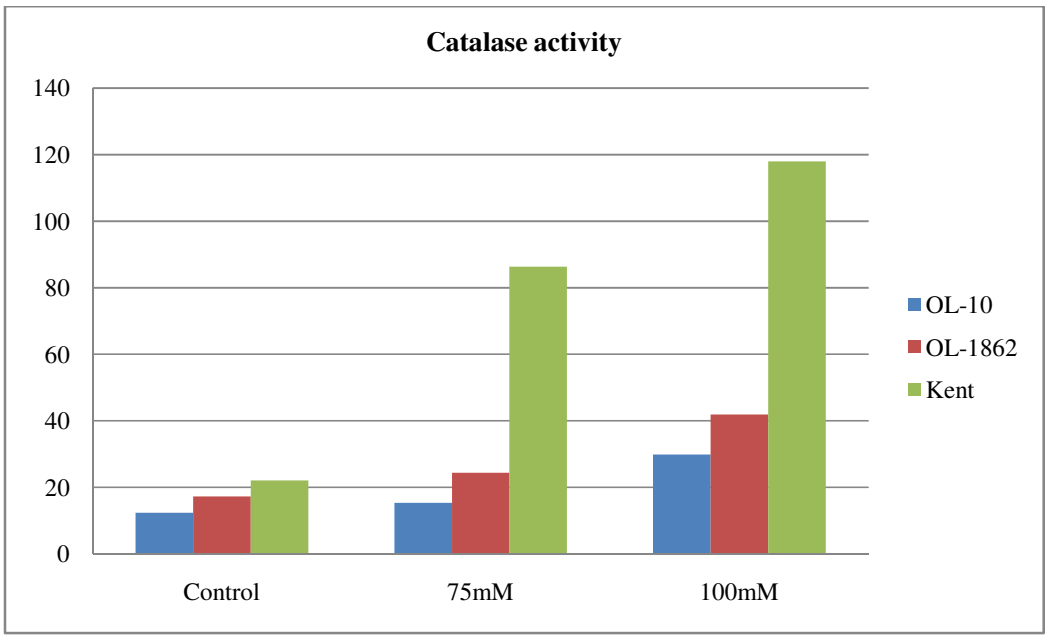

Fig. 10: Effect of salt stress on catalase activity (ΔA min^{-1} g^{-1} FW) in oat (*Avena sativa* L.)

4.1.10 Peroxidase activity (ΔA min^{-1} g^{-1} FW)

As shown in (table 14) peroxidase activity was recorded to increase in the stressed seedlings as compared to the control in all the genotypes. The increase in peroxidase activity was more in the comparatively tolerant genotype (Kent) and

Table 14: Effect of salt stress on peroxidase activity (ΔA min^{-1} g^{-1} FW) in oat (*Avena sativa* L.).

Genotypes / Salt Conc.	OL-10	Kent	OL-1862
Control	0.02±0	0.04±0	0.03±0
75mM	0.04±0	0.05±0	0.04±0
100mM	0.06±0	0.09±0	0.07±0
CD at 5%	X=0.9	Y=0.9	XY=NS

X=Genotype

Y= Salt concentration

moderate genotype (OL-1862) as compared to the sensitive genotype (OL-10). In genotype Kent peroxidase activity increased from 0.04 in control to 0.05 in 75mM and 0.09 in 100mM NaCl. Similarly the peroxidase activity in moderately tolerant genotype OL-1862 increased from 0.03 to 0.04 and 0.07 at control, 75mM, 100mM and in the sensitive genotype OL-10, the peroxidase activity increased from 0.02 in control to 0.04 in 75mM and 0.06 in 100mM NaCl.

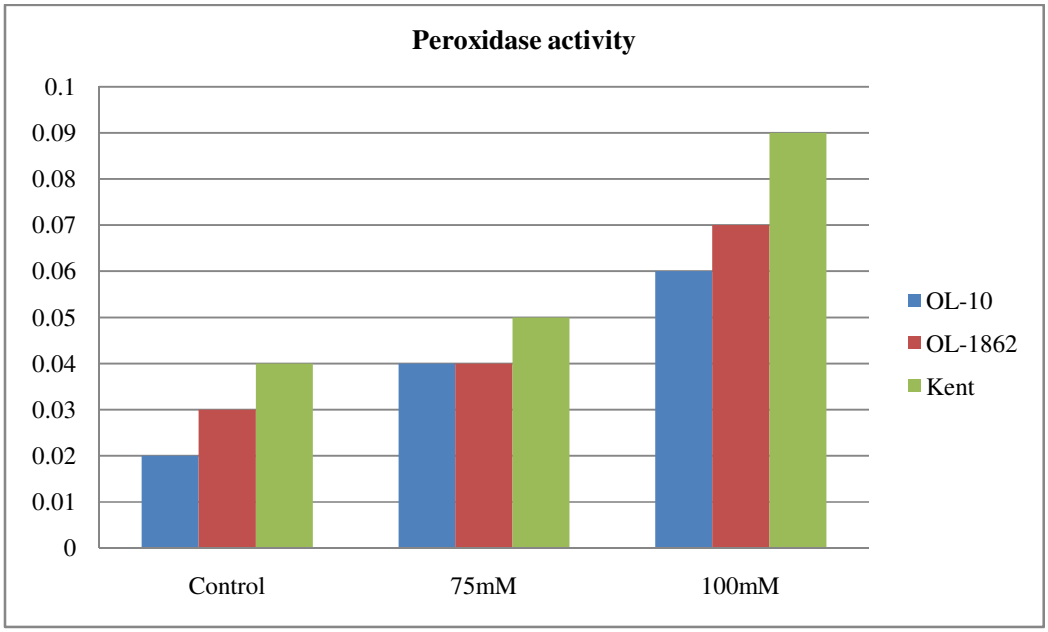

Fig.11: Effect of salt stress on peroxidase activity (ΔA min^{-1} g^{-1} FW) in oat (*Avena sativa* L.)

With increase in stress time, Peroxidase activity was higher in the early periods and lower in later periods, which showed that under salt stress, Peroxidase could remain high activity for a long time in order to lighten the damage of salt stress to the plant and soil salt in a content. The activity of Peroxidase changed at different growth stages. The observations of an increase of Peroxidase activity at the seedling stage, after treatment with NaCl, are similar to rice and poplar which were treated with NaCl (Zhou *et al* 2010). In potato seedlings, new POD isoenzymes appeared in response to salt stress (Rahnama and Ebrahimzadeh 2005).

4.1.11 Superoxide dismutase activity (ΔA min^{-1} g^{-1} FW)

Activity of SOD in OL-10 genotype recorded at control, 75mM and 100mM was 108.9, 110.5, 104.1. In Kent it was 143.6 at control, 155.2 at 75mM and 106.7 at 100mM whereas in OL-1862 genotype SOD activity recorded was 110.4, 145.6 and 106.3. When salinity was less than 100mM activity of superoxide dismutase increased steadily with increase in salt stress, and under 75mM salt concentration, activity of superoxide dismutase remained at a level higher than in control, but at 100mM salt

concentration, activity of superoxide dismutase decreased and the difference was narrowed gradually (Table 15). This showed that low salt concentration can cause the increasing of activity of superoxide dismutase in oat seedlings, it can clear free radical and prevent the peroxidation and membrane damage. When the concentration was over a certain range, the activity of Superoxide dismutase decreased and this showed that exorbitant concentration can slow down the activity of superoxide dismutase.

Table 15: Effect of salt stress on superoxide dismutase activity (ΔA min^{-1} g^{-1} FW) of oat (*Avena* sativa L.)

Genotypes / Salt Conc.	OL-10	Kent	OL-1862
Control	108.9±2.2	143.6±2.7	110.4±1.1
75mM	110.5±2.3	155.2±0.5	145.6±3.8
100mM	104.1±1.1	106.7±2.2	106.3±2.2
CD at 5%	A=2.1	B=2.1	AB=3.7

A=Genotype

B=Salt concentration

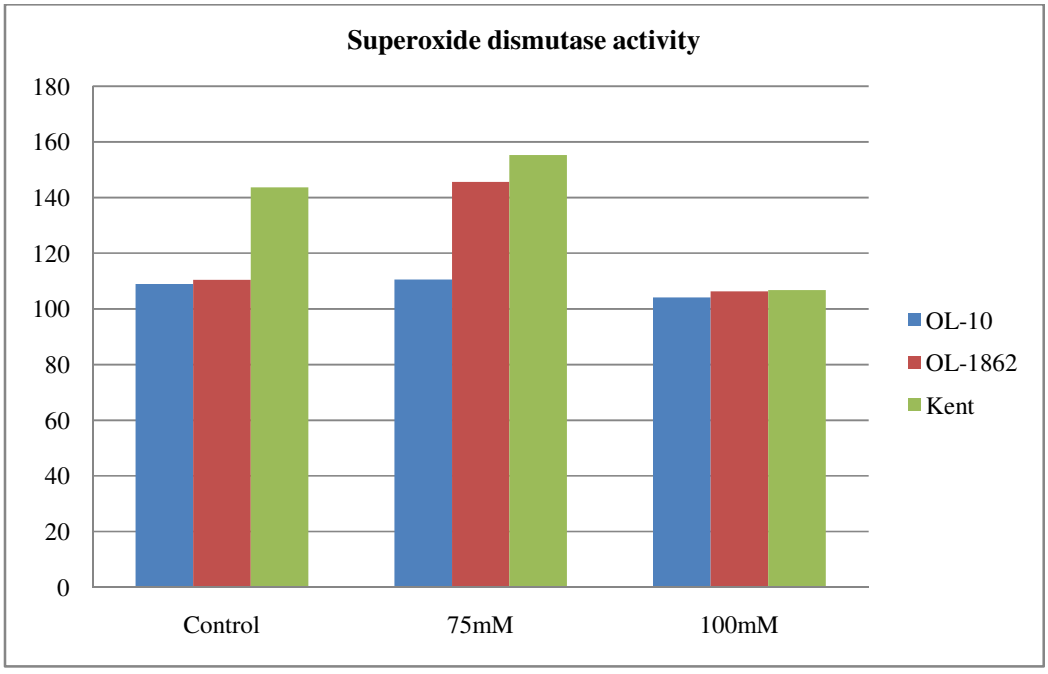

Fig. 12: Effect of salt stress on superoxide dismutase activity (ΔA min^{-1} g^{-1} FW) in oat (*Avena sativa* L.).

Activity of superoxide dismutase showed a change trend of high-low- high, with the increase in stress time. It showed that only under low salt stress (less than 100mM) and higher concentration stress in a short time could increase the activity of

superoxide dismutase and protect oat plants. Superoxide dismutase catalyzed the disproportion of superoxide O2- to molecular oxygen and hydrogen peroxide (Cristina *et al* 2008). Plants grown under saline conditions tend to have greater activity of SOD (Ashraf *et al* 2009). High SOD levels have been found in salt-resistant plants (Gosset *et al* 1994 and Hernandez *et al* 1995) and constitutive high levels of this enzyme are correlated with salt tolerance (Acar *et al* 2001).

Effect of salt stress on molecular parameters

Two genotypes one comparatively salt sensitive (OL-10) and one comparatively salt tolerant (Kent) were selected for transcript profiling. As, differential response was observed in physiological parameters were seen at 75mM and 100mM salt concentration, so 100mM concentration was selected for genes expression studies.

To check RNA integrity and its quantification

- The integrity of RNA was checked on 1.2 percent denaturing agarose gel.
- Total RNA was resolved into three separate rRNAs i.e 28S rRNA, 18S rRNA and 5s rRNA on the basis of their molecular weight (Plate 2).
- RNA was quantified using Thermo Scientific NanodropTM1000 Spectrophotometer, values ranged from 364.5 - 454 ng/µl (Table 16).
- RNA quality was also checked on the basis of Absorbance ratio (A_{260}/A_{280})
- Absorbance greater than 1.8 indicates the presence of RNA in all samples (Plate 2a).

Table 16: RNA quantification by using Nanodrop spectrophotometer

	Kent		**OL-10**	
	Control	**75mM**	**Control**	**75mM**
A260/A280	2.09	2.07	2.09	2.06
RNA(ng/µl)	364.5	430.6	433.9	454

cDNA synthesis and Confirmation

Cdna was quantified by nanodrop spectrophotometer and ranged from 1957ng/µl to 2070ng/µl. The Cdna Samples were normalized at working conc. of 30 ng/µl for synthesis of cDNA. The cDNA synthesis was confirmed by PCR amplification using 26S rRNA primers. Amplicons were resolved on 3% agarose gel and an amplicon corresponding to 534 bp was amplified in all samples which revealed the confirmation of cDNA synthesis in all samples (Plate 3). Then, cDNA was used as template for standardizing the PCR amplification of genes expressed under salt stress.

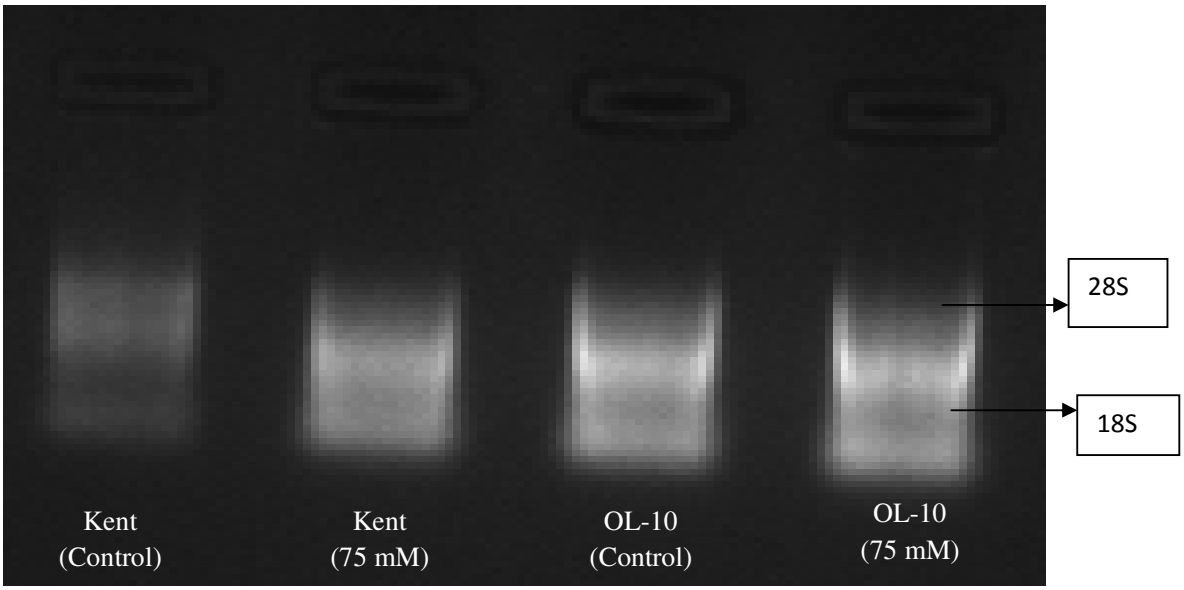

Plate 2: RNA Integrity on denaturing Agarose gel electrophoresis

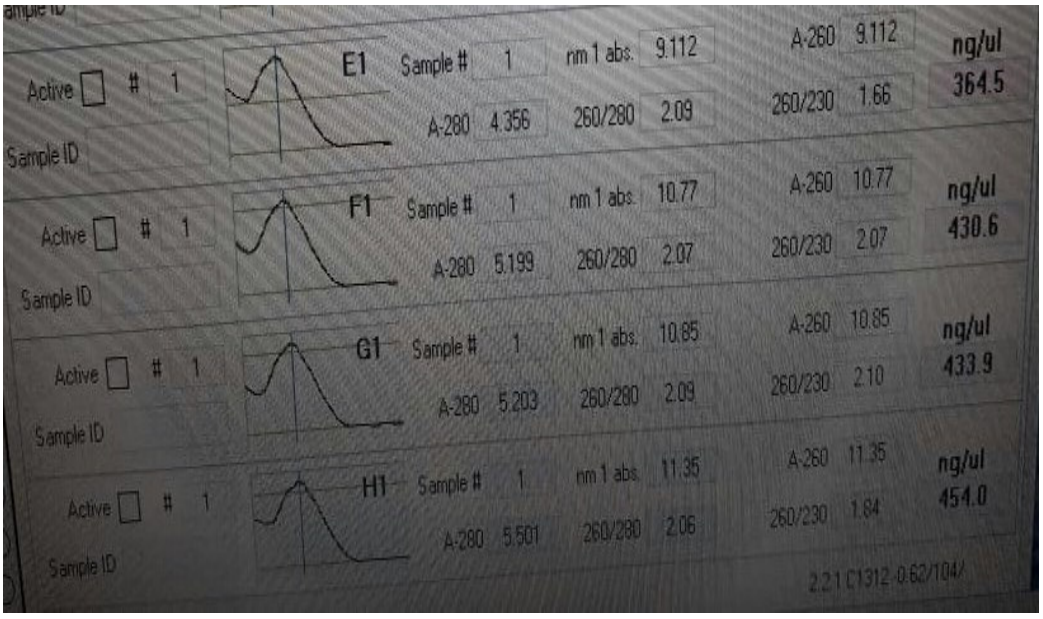

Plate 2a: RNA quantification by using Nanodrop spectrophotometer

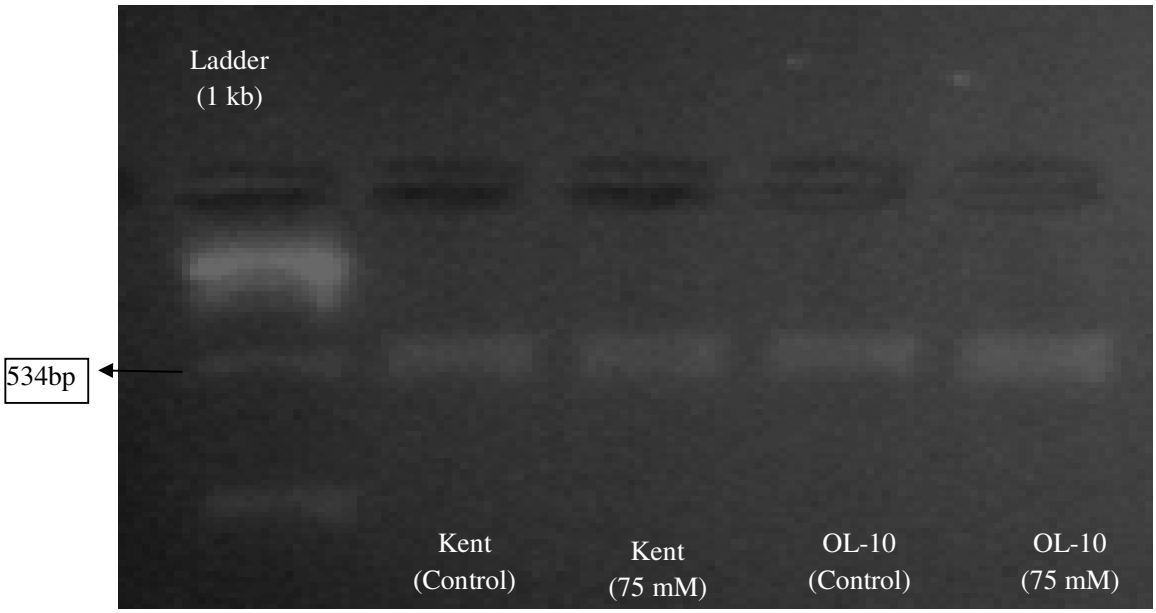

Plate 3: cDNA confirmation on 3% Agarose gel using 26S ribosomal RNA.

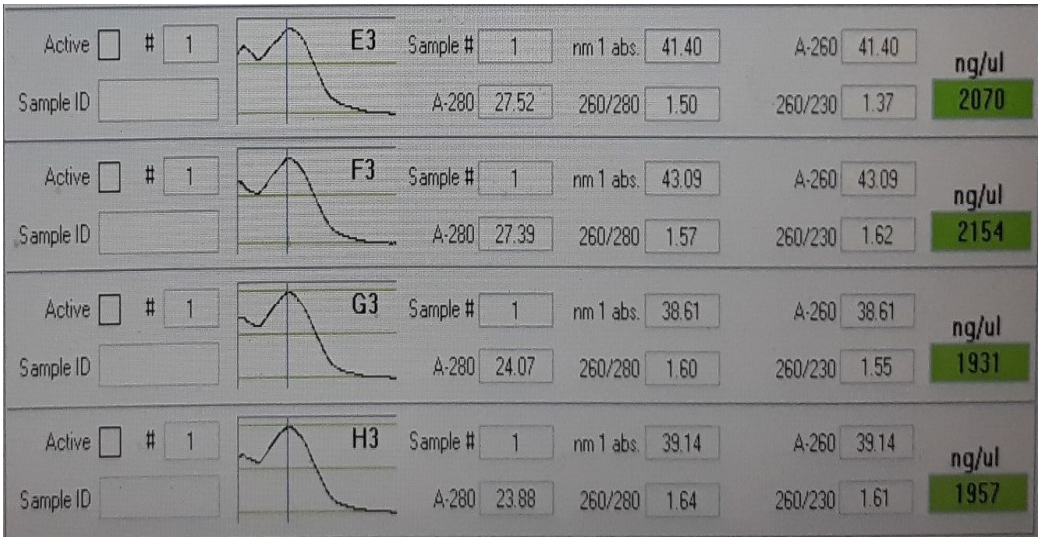

Plate 3a: cDNA confirmation on nanodrop spectrophotometer

Table 17: cDNA confirmation on nanodrop spectrophotometer

	Kent		OL-10	
	Control	**75mM**	**Control**	**75mM**
RNA(ng/µl)	2070	2154	1931	1957

CHAPTER V

SUMMARY

Oat is a cereal crop having global significance and is used for food, feed and forage. This contains soluble fibres which help in prevention of heart disease and make it a popular part of human diet. Salt stress is main abiotic stress that reduces productivity of crop. The present study was planned to see the effect of salt stress on physiological, biochemical and molecular parameters in different genotypes of oats (*Avena sativa* L.). Seeds of OL-10, OL-1862, OL-1895, OL-1966, OL-125, OL-1869, OL-1876, Kent genotypes were subjected to different salt stress concentrations (25mM, 50mM, 75mM and 100mM, 125mM, 150mM, 175mM and 200mM NaCl) and different physiological, biochemical and molecular parameters were recorded. Differential response was observed at 75mM and 100mM NaCl with respect to the physiological parameters. So the biochemical parameters of the selected tolerant, moderate and sensitive genotypes were evaluated at these concentrations.

A summary of the salient findings is given below:

- Percentage germination decreased significantly with increase in salt concentration in all the genotypes of oat. Genotypes OL-1895, Kent, OL-1876, OL-125, OL-1862 recorded a less reduction in percent germination indicating these to be comparatively salt tolerant as compared to the genotypes OL-10, OL-1869, OL-1966 which recorded more reduction indicating their comparative sensitivity to salt stress. In OL-1895 genotype, maximum percentage germination was seen. In OL-10 genotype, minimum percentage germination was seen.
- Shoot length decreased with increase in salt concentration in all the genotypes of oat. The maximum shoot length was shown by genotype Kent. Minimum shoot length was shown by genotype OL-1966. Genotypes Kent, OL-1876, OL-1895, OL-10, OL-1862 recorded a less reduction in percent germination indicating these to be comparatively salt tolerant as compared to the genotypes OL-1966, OL-1869, OL-125 which recorded more reduction indicating their comparative sensitivity to salt stress.
- Lamina length decreased gradually as concentration of NaCl was increased. Genotypes OL-1876, Kent, OL-1862, OL-10, OL-1869 recorded a less reduction in percent germination indicating these to be comparatively salt tolerant as compared to the genotypes OL-1966, OL-1895, OL-125 which recorded more reduction indicating their comparative sensitivity to salt stress. The maximum lamina length was recorded in OL-1876 genotype. Minimum lamina length was seen in OL-1966 genotype. Sheath length decreased significantly with increase in salt concentration

in all the genotypes of oat. There was not much difference in sheath length at 75mM and 100mM. Genotypes OL-1895, Kent, OL-1876, OL-1862, Kent, OL-1966 recorded a less reduction indicating comparatively salt tolerance as compared to the genotypes OL-125, OL-1869, OL-10 which recorded more reduction indicating their comparative sensitivity to salt stress.

- Similarly in root length, genotypes OL-1869, OL-1876, Kent, OL-10, OL-1862 recorded a less reduction, were comparatively salt tolerant than the genotypes OL-125, OL-1865, OL-1966 which showed more reduction indicating comparative sensitive. The maximum root length was recorded in genotype OL-1869. Minimum root length was seen in genotype OL-125.
- Fresh and Dry weight decreased significantly with increase in salt concentration in all the genotypes of oat. Genotypes OL-1876, OL-1869, OL-125, OL-1966, OL-1895 recorded a less reduction in fresh weight indicating these to be comparatively salt tolerant as compared to the genotypes OL-10, OL-1862, Kent which recorded more reduction indicating their comparative sensitivity to salt stress. The maximum fresh weight was shown by genotype OL-1876 whereas the minimum fresh weight was shown by genotype OL-10. Similarly in dry weight, genotypes OL-1869, OL-1966, OL-125, OL-1876, OL-1895 recorded a less reduction in dry weight, were comparatively salt tolerant as compared to the genotypes OL-1862, OL-10, Kent which recorded more reduction indicating their comparative sensitivity to salt stress. The maximum dry weight was recorded in genotype OL-1869 whereas minimum dry weight was seen in genotype OL-1862.
- Seed vigour I and Seed vigour II decreased significantly with increase in salt concentration in all the genotypes of oat. Genotypes Kent, OL-1876, OL-1895, OL-10, OL-1862 recorded a less reduction in seed vigour I indicating these to be comparatively salt tolerant as compared to the genotypes OL-1966, OL-125, OL-1869 which recorded more reduction indicating their comparative sensitivity to salt stress. The maximum seed vigour I was shown by genotype Kent. Minimum seed vigour I was shown by genotype OL-1966. Similarly Genotypes OL-1869,OL-125, OL-1895, OL-1876, OL-1966 recorded a less reduction in seed vigour II indicating these to be comparatively salt tolerant as compared to the genotypes OL-1862, OL-10, Kent which recorded more reduction indicating their comparative sensitivity to salt stress.
- Total soluble sugars were recorded to increase in the stressed seedlings as compared to the control in all the genotypes. The increase in the soluble sugar content was more in the comparatively tolerant genotype (Kent) and moderate genotype (OL-

1862) as compared to the sensitive genotype (OL-10). Similarly total soluble proteins were recorded to increase in tolerant genotype as compared to sensitive genotype.

- Antioxidant enzymes (peroxidase, superoxide dismutase and catalse) are very important for protecting plants from oxidative stress. Catalase activity was recorded to increase in the stressed seedlings as compared to the control in all the genotypes. Similarly peroxidase activity was more in the comparatively tolerant genotype as compared to the sensitive genotype. Superoxide activity was increased by salt concentrations at control and 75mM salinity levels and decreased at 100mM salinity levels.
- Molecular studies were conducted on one comparatively salt sensitive and one comparatively salt-tolerant genotype under selected salt concentration level (75mM). Data compilation of the previous physiological and biochemical entities, screening of two genotypes was done to test their molecular potential for salt stress. The confirmation of tissues was done at RNA level with Reverse-transcriptase PCR.

REFERENCES

Abbasi F M and Komatsu S (2004) A proteomic approach to analyze salt-responsive proteins in rice leaf sheath. *Proteomics* **4**: 2072-81.

Abdul-Baki A A and Anderson J D (1973) Vigor determination in soybean seed by multiple criteria. *Crop Sci* **13**: 630-33.

Abul-Naas A A and Omran M S (1974) Salt tolerance of seventeen cotton cultivars during germination and early seedling development. *AGRIS* **140**: 229-36.

Acar O, Turkan I and Ozdemir F (2001) Superoxide dismutase and peroxidase activities in drought sensitive and resistant barley (*Hordeum vulgare* L.) varieties. *Acta Physiol Plant* **3**:351-56.

Acosta-Motos J R, Ortuno M F, Bernal-Vicente A, Diaz-Vivancos P, Sanchez-Blanco M J and Hernandez J A (2017) Plant Responses to Salt Stress: Adaptive Mechanisms. *Agron Res* **7**:1-38.

Afzal I, Maqsood S, Basra A and Cheema M (2013) Seed priming: A shotgun approach for alleviation of salt stress in wheat. *Int J Agric Biol* **15**: 1199-1203.

Agami R A (2014) Applications of ascorbic acid or proline increase resistance to salt stress in barley seedlings. *Biol Plantarum* **58**: 341-47.

Agarwal S and Pandey V (2004) Antioxidant enzyme responses to NaCl stress in *Cassia angustifolia*. *Biol Plantarum* **48**: 555-60.

Agastian P, Kingsley S J and Vivekanandan M (2000) Effect of salinity on photosynthesis and biochemical characteristics in mulberry genotypes. *Photosynthetica* **38**: 287-90.

Ahmad P and Prasad M N V (2012) Environmental Adaptations and Stress Tolerance of Plants in the Era of Climate Change. New York, NY: Springer 297-324.

Ahmed I M, Cao F, Zhang M, Chen X and Zhang G (2013) Difference in yield and physiological features in response to drought and salinity combined stress during anthesis in Tibetan wild and cultivated barleys. *PLoS ONE* **8:**e77869.

Alkaraki G N (2001) Germination, sodium and potassium concentrations of barley seeds as influenced by salinity. *J Plant Nutr* **24**: 511-22.

Al-Mutawa M (2003) Effects of salinity on germination and seedling growth of chick pea (*Cicer arietinum* L.) genotypes. *Intl J Agro Biol* **5:** 227-29.

Amirjani M R (2010) Effect of salinity stress on growth, mineral composition, proline content, antioxidant enzymes of soyabean. *Am J Plant Physiol* **5**: 350-60.

Anbumalarmathi J and Mehta P (2013) Effect of salt stress on germination of Indica rice varieties. *Eur J Biol Sci* **1**: 1- 6.

Ashraf M A, Basra S M A and Shahbaz M (2012) Growth stage-based modulation in antioxidant defense system and proline accumulation in two hexaploid wheat (*Triticum aestivum* L.) cultivars differing in salinity tolerance. *Flora - Morphology Distribution Functional Ecology of Plants* **207**: 388-97.

Ashraf M and Fatima H (1995) Responses of some salt tolerant and salt sensitive lines of safflower (*Carthamus tinctorius* L.). *Acta Physiol Plant* **17**: 61-71.

Ashraf M (2004) Some important physiological selection criteria for salt tolerance in plants. *Flora* **199**: 361-76.

Ashraf M and Akram N A (2009) Improving salinity tolerance of plants through conventional breeding and genetic engineering: an analytical comparison. *Biotechnol Adv* **27**: 744-52.

Ashraf M and Harris P J C (2013) Photosyntheis under stressful environments: An overview. *Photosynthetica* **51**: 163-90.

Ashraf M and Tufail M (2008) Variation in Salinity Tolerance in Sunflower (*Helianthus annum* L.). *J Agron Crop Sci* **174**: 351-62.

Ashraf M, Shagufta S, Sobia and Nudrat Aisha Akramb (2012) Salt stress affects water relations, photosynthesis, and oxidative defense mechanisms in (*Solanum melongena* L.). *J Plant Interact* **1**: 85-96.

Athar H, Khan A and Asharf M (2008) Exogenously applied ascorbic acid alleviates salt-induced oxidative stress in wheat. *Environ Exp Bot* **63**: 224-31.

Azevedo Neto A D, Prico J T, Eneas-Filho J, Braga de Abreu C E and Gomes-Filho E (2006) Effect of salt stress on antioxidative enzymes and lipid peroxidation in leaves and roots of salt-tolerant and salt-sensitive maize genotypes. *Environ Exp Bot* **56**: 235-41.

Azevedo Neto A D, Prisco J T, Eneas-Filho J, Medeiros J V R and Gomes-Filho E (2005) Hydrogen peroxide pre-treatment induces stress acclimation in maize plants. *J Plant Physiol* **162**: 1114-22.

Balal R M, Khan M, Shahid M A and Mattson N S (2012) Comparative studies on the physio biochemical, enzymatic, and ionic modifications in salt-tolerant and salt-sensitive citrus rootstocks under NaCl Stress. *J Am Soc Horti Sci* **137**: 86-95.

Bao A K, Guo Z G, Zhang H F and Wang S M (2009) A procedure for assessing the salt tolerance of Lucerne (*Medicago sativa* L.) cultivar seedlings by combining agronomic and physiological indicators. *New Zeal J Agr Res* **52**: 435-42.

Baskin C C and Baskin J M (2014) *Seeds:* Ecology, Biogeography, and Evolution of Dormancy and Germination 2nd (edn.) San Diego, CA Academic/Elsevier.

Berk and Ozkan (2016) Determination of responses of some oat cultivars (*Avena sativa* L.) to salt and drought stress at the germination period. *Department of Field Crops*, Agriculture Faculty, Ankara University **31**: 254-23.

Bewley J D (1997) Seed germination and dormancy. *The Plant Cell* **9:** 1055-66.

Billah M (2018) Omics-Based strategies for improving salt tolerance in maize (*Zea mays* L.) abiotic stress-mediated sensing and signaling in plants. *An Omics Perspective* **10**: 243-46.

Blumwald E, Grover A and Good A G (2004) Breeding For Abiotic Stress Resistance: Challenges and Opportunities New directions for a Diverse Planet, 4th International Crop Science Congress, Brisbane, Australia.

Botia P and Martinez V (2005) Yield and fruit quality of two melon cultivars irrigated with saline water at different stages of development. *Eur J Agron* **23**: 243-53.

Bruce R H (2008) Health benefits of fruits and vegetables are from additive and synergistic combinations of phytochemicals. *Am J Clin Nutr* **78**: 517-20.

Bustin S A (2000) Absolute quantification of mRNA using real-time reverse transcription polymerase chain reaction assays. *J Mol Endocrinol* **25**:169-93.

Bybordi A and Tabatabaei S J (2009) Effect of salinity stress on germination and seedling properties in canola cultivars (*Brassica napus* L.). *Not Bot Horti Agrobot Cluj-Napoca* **37**: 71-76.

Carding S R, Lu D and Bottomly K A (1992) Polymerase chain reaction assay for the detection and quantification of cytokine gene expression in small number of cells. *J Immunol* **151**: 277-87.

Chance B and Maehly A C (1955) Assay of catalases and peroxidases. *Methods Enzymol* **2**: 764-77.

Chauhan A (2019) Influence of gibberellic acid and different salt concentrations on germination percentage and physiological parameters of oat cultivars. S*audi J Biol Sci* **10**:1319-62.

Chauhan A, Rajput N, Kumar D, Kumar A and Chaudhary A K (2016) Effect of different salt concentration on seed germination and seedling growth of different varieties of oat (*Avena sativa* L.). *Int J Inf Res Rev* **3**: 2627-32.

Cha-um S, Supaibulwatana K and Kirdmanee C (2004) Physiological responses of Thai neem (*Azadirachta siamensis* Val.) to salt stress for salt-tolerance screening program. *Sci Asia* **30**: 17-23.

Chinnusamy V, Jagendorf A and Zhu J K (2005) Understanding improving salt tolerance in a plants. *Crop Sci* **45**: 437-48.

Couee I, Sulmon C, Gouesbet G and El Amrani A (2006) Involvement of soluble sugars in reactive oxygen species balance and responses to oxidative stress in plants. *J Exp Bot* **57**: 449-59.

Cristina B, Paunescu G, Popa D and Badita A (2008) Changes of some antioxidant enzyme activities in leaves of drought tolerant varieties of wheat from oltenia during vegetation stages. *Bulletin UASVM Agriculture* **65**:1843-46.

Cristina S, Zuzana K, Alberto K, Flavia N I and Riccardo I (2008) Irrigation with diluted seawater improves the nutritional value of cherry tomatoes. *J Agric Food Chem* **56**: 3391-97.

Dadar A, Asgharzade A and Nazari M (2014) Investigation effects of different salinity levels on sorghum Bicolor seed germination characters. *Indian J Sci Res* **7**: 1031-34.

Dadkhah A and Grrifiths H (2005) The effect of salinity on growth, inorganic ions and dry matter partitioning in sugar beet cultivars. *J Agr Sci Tech* **8**: 199-210.

Dash M and Panda S K (2001) Salt stress induced changes in growth and enzyme

actives in geminating *Phaseolus mungo* seeds. *Plant Mol Biol* **44**: 587-89.

Deinlein U, Stephan A B, Horie T, Luo W and Xu G (2014) Plant salt-tolerance mechanisms. *Trends Plant Sci* **19**: 371-79.

Del-Amor F M, Martinez V and Cerda A (2001) Salt tolerance of tomato plants as affected by stage of plant development. *Hortscience* **36**: 1260-63.

Demir M and Arif I P E K (2003) Effects of different soil salinity levels on germination and seedling growth of safflower (*Carthamus tinctorius* L.). *Turk J Agric* **27**: 221-27.

Doganlar Z B, Demir K, Basak H and Gul I (2010) Effects of salt stress on pigment and total soluble protein contents of three different tomato cultivars. *Afr J Agric Res* **15**: 525-645.

Dolatabadian A and Saleh R J (2009) Impact of exogenous ascorbic acid on antioxidant activity and some physiological traits of common been subjected to salinity stress. *Not Bot Horti Agrobo* **37**: 165-72.

Dubois M, Gilles K A, Hamilton J K, Rebers P A and Smit F (1956) Colorimetric methods for determination of sugars and related substrates. *Anal Chem* **28**: 350-56.

El Sabagh A, Hossain A, Islam M S, Barutçular C, Fahad S, Ratnasekera D, Kumar N, Meena R S, Vera P and Saneoka H (2018) Role of osmoprotectants and soil amendments for sustainable soybean (*Glycine max* L.) production under drought condition: A review. *J Exp Biol Agric Sci* **6**: 32-41.

El-Mashad and Kamel (2001) Amelioration of NaCl stress in (*Pisum sativum* L.). *Indian J Exp Biol* **39:** 469-75.

El-Sabagh A, Omar A E, Saneoka H and Barutcular C (2016) Physiological performance of soybean germination and seedling growth under salinity stress. *Dicle Univ J Inst Nat Appl Sci* **4**: 6-15.

El-Shaieny (2015) Seed germination percentage and early seedling establishment of five (*Vigna unguiculata* L. (Walp) genotypes under salt stress. *Euro J Exp Bio* **5**: 22-32.

Erdal S (2012) Alleviation of salt stress in wheat seedlings by mammalian sex hormones. *J Sci Food Agric* **92**: 1411-16.

Ferreira-Silva S L, Voigt E L, Silva E N, Maia J M and Silveria J A G (2012) Partial oxidative protection by enzymatic and non enzymatic components in cashew leaves under high salinity. *Biol Plantarum* **56**: 172-76.

Flowers T J and Colmer T D (2015) Plant salt tolerance: adaptations in halophytes. *Ann Bot* **115**: 327-31.

Fu Q, Liu C, Ding N, Lin Y and Guo B (2010) Ameliorative effects of inoculation with the plant growth-promoting rhizobacterium Pseudomonas sp. DW1 on growth of eggplant (*Solanum melongena* L.) seedlings under salt stress. *Agric Water Manag* **97**: 1994-2000.

Gao R, Duan K, Guo G, Du Z, Chen Z, Li L, He T, Lu R and Huang J (2013) Comparative transcriptional profiling of two contrasting barley genotypes under salinity stress during the seedling stage. *Int J Genomics* **5**: 1-19.

Gao S, Ouyang C, Wang S, Xu Y, Tang L and Chen F (2008) Effects of salt stress on growth, antioxidant enzyme and phenyalanine ammonia-lyase activities in (*Jatropha curcas* L.) seedlings. *Plant Soil Environ* **54**: 374-81.

Gao Y, Lu Y, Wu M, Liang Y, Li Y and Zhang D (2016) Ability to remove Na^+ and retain K^+ correlates with salt tolerance in two maize inbred lines seedlings. *Front Plant Sci* 7:16-17.

Genc Y, Oldach K, Taylor J and Lyons G H (2015) Uncoupling of sodium and chloride to assist breeding for salinity tolerance in crops. *New Phytol* **210**:145-56.

Ghorbani A, Razavi S M, Ghasemi V O, Pirdashti H (2018) *Piriformospora indica* inoculation alleviates the adverse effect of NaCl stress on growth, gas exchange and chlorophyll fluorescence in tomato (*Solanum lycopersicum* L.). *Plant Biol* **20**:729-36.

Ghoulam C and Fares K (2001) Effect of salinity on seed germination and early seedling growth of sugar beat (*Beta vulgaris* L.). *Seed Sci Technol* **29**: 357-64.

Gill P K, Sharma A D, Singh P and Bhullar S S (2003) Changes in germination growth and soluble sugar contents of (*Sorghum bicolor* L.) Moench seeds under various abiotic stresses. *Plant Growth Regul* **40**:157-62.

Gill S S and Tuteja N (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol Biochem* **48**: 909-30.

Gioria M and Pysek P (2016) Timing is everything: does early and late germination favor invasions by herbaceous alien plants. *J Plant Ecol* **11**: 4-16.

Gorham J, Hughes L Y and Wynjones R G (1981) Low molecular weight carbohydrates in some salt stressed plants. *Plant Physiol* **53**: 27-33.

Gosset D G, Millhollon E P, Lucas M C, Banks S W and Marney M M (1994) The effects of NaCl on antioxidant enzyme activities in callus tissue of salt-tolerant and salt-sensitive cultivars (*Gossypium hirsutum* L.). *Plant Cell Reports* **13**:498-503.

Gupta S C and Srivastava C P (1989) Effects of salt stress on morphological parameters in wheat (*Triticus aestivum* L.). *Indian J Plant Physiol* **32**: 169-71.

Ha E, Ikhajiagba B, Bamidele J F and Ogic-odia E (2008) Salinity effects on young healthy seedling of kyllingia peruviana collected from escravos, Delta state. *Int J Environ Res* **2**: 74-88.

Han X, Yang Y, Wu Y, Liu X, Lei X and Guo Y (2017) A bioassay-guided fractionation system to identify endogenous small molecules that activate plasma membrane H^+-ATPase activity in Arabidopsis. *J Exp Bot* **68**: 2951- 62.

Han Y and Zhao Q (2013) The E3 ligase AtRDUF1 positively regulates salt stress responses in *Arabidopsis thaliana. PLOS Comput Biol* **10**:1371-1476.

Han Y, Yin S and Huang L (2014) Towards plant salinity tolerance-implications from ion transporters and biochemical regulation. *Plant Growth Regul* **10**: 99-107.

Hasegawa P M, Bressan R A, Zhu J K and Bohnert H J (2000) Plant cellular and molecular responses to high salinity. *Ann Rev Plant Phyiol Plant Mol Biol* **51**: 463-99.

Hernandez J A, Aguilar A B, Portillo B, Lopez-Gomez E, Beneyto J M and Garcia-Legaz M F (2003) The effect of calcium on the antioxidant enzymes from salt-treated loquat and anger plants. *Funct Plant Biol* **30**: 1127-37.

Hernandez J A, Corpas F J, Gomez M, del Rio L A and Sevilla F (1995) Salt induced oxidative stress mediated by activated oxygen species in pea leaf mitochondria. *Plant Physiol* **89**:103-10.

Hoekstra F A, Golovina E A and Buitin J (2001) Mechanisms of plant desiccation tolerance. *Trends Plant Sci* **6**: 431-38.

Holmstrom K O, Somersalo S, Mandal A, Palva T E and Welin B (2000) Improvement tolerance to salinity and low temperature in transgenic tobacco producing glycine betaine. *J Exp Bot* **51**: 177-85.

Hoque M M I, Jun Z and Guoying W (2015) Evaluation of salinity tolerance in maize (*Zea mays* L.) genotypes at seedling stage. *J Bio Sci Biot* **4**: 39-49.

Hsiao T C and Xu L K (2000) Sensitivity of growth of roots versus leaves to water stress: biophysical analysis and relation to water transport. *J Exp Bot* **51**:1595-1616.

Hu Y, Zhang Q, Chen X and Huo P (2017) Transcriptome analysis of hexaploid hulless oat in response to salinity stress. *PLoS One* **12:** 161-451.

Isayenkov S V (2012) Physiological and molecular aspects of salt stress in plants. *Cytol Genet* **46**: 302-18.

Jafari M H S, Kafi M and Astaraie A (2009) Interactive effects of NaCl induced salinity, calcium and potassium on physio morphological traits of sorghum (*Sorghum bicolor* L.). *Pak J Bot* **41**: 3053-63.

Jaleel C A, Sankar B, Sridharan R and Panneereselvam R (2008) Soil salinity alters growth, chlorophyll content, and secondary metabolite accumulation in *Catharanthus roseus. Turk J Biol* **32:** 79-83.

Jamil A, Anwar F and Ashraf M (2005) Plant Tolerance to Biotic and Abiotic Stresses through Modern Genetic Engineering Techniques. In: Dris R (ed) Crops: Growth, Quality & Biotechnology 1276-1299. WFL Publisher, Helsinki, Finland.

Jamil M and Rha E S (2013) NaCl stress-induced reduction in growth, photosynthesis and protein in mustard. *J Agric Sci* **5**: 114-27.

Jamil M, Bashir S, Anwar S, Bibi S, Bangash A, Ullah F, Rha ES (2012) Effect of salinity on physiological and biochemical characteristics of different varieties of rice. *Pak J Bot* **44**: 7-13.

Jamil M, Rehman S, Rha E S (2007) Salinity effect on plant growth, ps11 photochemistry and chlorophyll content in sugar beet (*Beta vulgaris* L.) and cabbage (*Brassica oleracea capitata* L.). *Pak J Bot* **39**: 753-60.

Janmohammadi M, Moradi Dezfuli P and Sharifzadeh F (2008) Seed invigoration techniques to improve germination and early growth of inbred line of maize under salinity and drought stress. *Gen Appl Plant Physiol* **34**: 215-26.

Kabar K and Baltepe S (1987) Alleviation of salinity stress on germination of barley seeds by plant growth regulators. *Turk J Biol* **11**: 108-17.

Kapoor K and Srivastava A (2010) Assessment of salinity tolerance of vingamungover. Pu-19 using *exvitro* and *invitro* methods. *Asian J Biotechnol* **2**: 73-25.

Kaveh H, Nemati H, Farsi M and Jartoodeh S V (2011) How salinity affect germination and emergence of tomato lines. *J Biol Environ Sci* **5**: 159-63.

Keshavarzi M H B, Rafsanjani S M, Moussavinik and Lak A P (2011) Effect of salt (NaCl) stress on germination and early seedling growth of Spinach (*Spinacia oleracea* L.). *Ann Biol Res* **2**: 490-97.

Khan M H and Panda S K (2015) Alterations in root lipid peroxidation and antioxidative responses in two rice cultivars under NaCl-salinity stress. *Acta Physiol Plant* **30**: 81-89.

Khosravinejad F, Heydari R and Farboodnia T (2009) Effect of salinity on organic solutes content in barley. *Pak J Biol Sci* **12**: 158-62.

Koca H, Bor M, Ozdemir F and Turkan I (2007) The effect of salt stress on lipid peroxidation, antioxidative enzymes and proline content of sesame cultivars. *Environ Exp Bot* **60**: 344-51.

Krasensky J and Jonak C (2012) Drought, salt, and temperature stress-induced metabolic rearrangements and regulatory networks. *J Exp Bot* **63**:1593-1608.

Krishnamurthy L, Reddy B V S and Serraj R (2003) Screening sorghum germplasm for tolerance to soil salinity. *Int Sorghum Millets Newslett* **44:**155-57.

Kumari S, Sabharwal V P, Kushwaha H R, Sopory S K, Singla-Pareek S L and Pareek A (2009) Transcriptome map for seedling stage specific salinity stress response indicates a specific set of genes as candidate for saline tolerance in (*Oryza sativa L.*). *Funct Integr Genom*ics **9**: 109-23.

Lauchli A and Grattan S (2012) Plant responses to saline and sodic conditions. *Agril Salinity Assess Mgmt* **10**: 169-205.

Li B W, Zhang H J, Qiu Y Z, Xu X W, Li S Y, Zhou H W, Chang Q and Xu W (2011) Effects of saline water irrigation on plants growth of the tarim desert highway shelter belt. *Arid Land Geography* **34**: 215-21.

Lianes A, Reinoso H and Luna M V (2005) Germination and early growth of prosopis strombulifera seedlings in different saline solutions. *WRJS* **1**:120-28.

Liu W, Zhang Y, Yuan X, Xuan X, Gao Y and Yan Y (2016) Exogenous salicylic acid improves salinity tolerance of (*Nitraria tangutorum). Russ J Plant Physiol* **63**:

132-42.

Liu Y L (2007) Roles of *OsCKI1*, a rice casein kinase I, in root development and plant hormone sensitivity. *Plant J* **36:** 189-202.

Lobato A K S, Santos Filho B G, Costa R C L, Gonçalves-Vidigal M C, Moraes E C, Oliveira Neto C F, Rodrigues V L F, Cruz F J R, Ferreira A S, Pita J D and Barreto A G T (2009) Morphological, physiological and biochemical responses during germination of the cowpea (*Vigna unguiculata* Cv. Pitiuba) seeds under salt stress. *J Agric Sci* **5**: 590-96.

Long W, Zou X and Zhang X (2015) Transcriptome analysis of canola (*Brassica napus*) under salt stress at the germination stage. *PLoS One* **10**: 1-18.

Lowry O H, Rosenbrough N J, Farr A L and Randall R J (1951) Protein measurement with the folin phenol reagent. *J Biol Chem* **193**: 265-75.

Maas E V and Poss J A (1989) Salt sensitivity of cowpea at various growth stages. *Irrig Sci* **10**: 313-20.

Maas E V, Hoffman G J, Chaba G D, Poss J A and Shannon M C (1983) Salt sensitivity of corn at various growth stages. *Irrig Sci* **4**: 45-57.

Maas E V, Poss J A and Hoffman G J (1986) Salinity sensitivity of sorghum at three growth stages. *Irrig Sci* **7**: 1-11.

Majeed A, Haq M A, Akhtar J and Basra S M A (2014). Screening of maize genotypes against poor quality water in solution culture. *J Agric Res* **52**(3): 356-68.

Mantri N, Patade V, Peena S and Ford R (2012) Abiotic stress responses in plants: present and future. In: Ahmad P, Prasad M N V (ed) Abiotic Stress Responses in Plants. 1-19. New York, Springer.

Mao G L, Xu X and Xu Z Z (2004) Advances in physiological and biochemical research of salt tolerance in plant. *Chin J Eco-Agric* **12**:43-46.

Marklund S and Marklund G (1974) Involvement of the superoxide anion radical in the autoxidation of pyragallol and a convenient assay for superoxide dismutase. *Eur J Biochem* **47**: 469-74.

Marone M, Mozzetti S and Pierelli L (2001) Semiquantitative RT-PCR analysis to assess the expression levels of multiple transcripts from the same sample. *Biol Proced Online* **3**:19-25.

Marschner H (1995) Adaptation of plants to adverse chemical soil conditions: Mineral nutrition of higher plants. *Ed Acad Pr* **2**: 596-80.

Mauromicale G and Licandro P (2002) Salinity and temperature effects on germination, emergence and seedling growth of globe artichoke. *Agronomie* **22**: 49-61.

Mekawy A M M, Assaha D V M, Yahagi H, Tada Y, Ueda A and Saneoka H (2015) Growth, physiological adaptation and gene expression analysis of two Egyptian rice cultivars under salt stress. *Plant Physiol Biochem* **87**: 17-25.

Memon S A, XiLin H and LiangJu W (2010) Morphological analysis of salt stress

response of pak choi. *EJEAFChe* **9**: 248-54.

Mittler R (2002) Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci* **7**:405-10.

Mittova V and Tal M (2003) Up-regulation of the leaf mitochondrial and peroxisomal antioxidative systems in response to salt-induced oxidative stress in the wild salt-tolerant tomato species (*Lycopersicon pennellii). Plant Cell Environ* **26**: 845-56.

Mittova V, Guy M, Tal M and Volokita M (2004) Salinity upregulates the antioxidative system in root mitochondria and peroxisomes of the wild salt-tolerant tomato species (*Lycopersicon pennellii). J Exp Bot* **55**:1105-13.

Moradi F and Ismail A M (2007) Responses of photosynthesis, chlorophyll fluorescence and ROS-scavenging systems to salt stress during seedling and reproductive stages in rice. *Ann Bot* **99**: 1161-73.

Mostofa M G, Fujita M and Tran L S P (2015) Nitric oxide mediates hydrogen peroxide- and salicylic acid-induced salt tolerance in rice (*Oryza sativa* L.) seedlings. *Plant Growth Regul* **77**: 265-77.

Mozafariyan M, Bayat K S A E and Bakhtiari S (2013) The effects of different sodium Chloride concentrations on the growth and photosynthesis parameters of tomato (*Lycopersicum esculentum* cv. Foria). *Int J Agric Sci* **4**: 203-07.

Munns R (2002) Comparative physiology of salt and water stress. *Plant Cell Environ* **25**: 239-50.

Murshed R, Lopez-Lauri F and Sallanon H (2014) Effect of salt stress on tomato fruit antioxidant systems depends on fruit development stage. *Physiol Mol Biol Plants* **20**:1-15.

Nasibi F, Rahneshan Z and Moghadam A A (2018) Effects of salinity stress on some growth, physiological, biochemical parameters and nutrients in two pistachio (*Pistacia vera* L.) rootstocks. *J Plant Interact* **13**:73-82.

Nasri N, Saidi I, Kaddour R and Lachaal M (2015) Effect of salinity on germination, seedling growth and acid phosphatase activity in lettuce. *Am J Plant Sci* **6**: 57-63.

Nawaz K, Hussain K, Majeed A, Khan F, Ito S and Saito K (2010) Fatality of salt stress to plants: Morphological, physiological and biochemical aspects. *Afr J Biotechnol* **34**:5474-80.

Nedjimi B, Daoud Y and Touati M (2006) Growth, water relation, proline and ion content of invitro cultured subsp. schweinfurthi as affected by CaCl. *Crop Sci* **6***:* 79-89.

Nemati I, Moradi F, Gholizadeh S, Esmaeili M A and Bihamta M R (2011) The effect of salinity stress on ions and soluble sugars distribution in leaves, leaf sheaths and root of rice (*Oryza sativa L.*) seedlings. *Plant Soil Environ* **57**: 226-33.

Oyiga B C, Sharma R C, Shen J, Baum M, Ogbonnaya F C, Leon J and Ballvora A (2016) Identification and characterization of salt tolerance of wheat germplasm

using a multivariable screening approach. *J Agron Crop Sci* **202**: 472-85.

Palma F C, Lluch C, Iribarne J M,Garcia-Garrido and Garcia N A T (2009) Combined effect of salicylic acid and salinity on some antioxidant activities, oxidative stress and metabolite accumulation in *Phaseolus vulgaris*. *Plant Growth Regul* **58**: 307-16

Panuccio M R, Jacobsen S E, Akhtar S S and Muscolo A (2014) Effect of saline water on seed germination and early seedling growth of the halophyte quinoa. *AoB Plants* **6**:1-18.

Parida A K, Das A B and Das P (2002) NaCl stress causes changes in photosynthetic pigments, proteins and other metabolic components in the leaves of a true mangrove, *Bruguiera parviflora*, in hydroponic cultures. *J Plant Biol* **45**: 28-36.

Pearson G A and Ayers A D (1966) Relative salt tolerance of rice during germination and early seedling development. *Soil Sci* **102:**151-56.

Perry D A (1972) Seed vigour and field establishment. *Horti Abstr* **42**: 334-42.

Perry D A (1980) The Concept of Seed Vigour and its Relevance to Seed Production Techniques In: Hebblethwaite PD (ed.) Seed production London Butterworths: 585-91.

Prado F E, Boero C, Gallardo M and Gonzalez J A (2000) Effect of NaCl on germination, growth and soluble sugar content in *Chenopodium quinoa* wild seeds. *Bot Bull Acad Sin* **41**: 27-34.

Prisco J T, Neto A and De C E B (2006) Effect of salt stress on antioxidative enzymes and lipid peroxidation in leaves and roots of salt-tolerant and salt-sensitive maize genotypes. *Environ Exp Bot* **56**: 87-94.

Qasim M, Ashraf M, Ashraf M Y, Rehman S U and Rha E S (2003) Salt-induced changes in two canola cultivars differing in salt tolerance. *Biol Plantrum* **46**: 629-32.

Rahnama H and Ebrahimzadeh H (2005) The effect of NaCl on antioxidant enzyme activities in potato seedlings. *Biol Plantrum* **49**: 93-97.

Rajput N, Kumar A and Chaudhry A K (2015) Effects of NaCl stress on seed germination and early seedling growth of castor bean. *Int J Sci Res* **11**:104-07.

Ramezani E, Sepanlou M G and Badi H A N (2011) The effect of salinity on the growth, morphology and physiology *Echium amoenum* Fisc & Mey. *Afr J Biotechnol* **10**: 8765-74.

Rathinasabapathi B (2000) Metabolic engineering for stress tolerance: installing osmoprotectant synthesis pathways. *Ann Bot* **86**: 709-16.

Rengel Z (1992) The role of calcium in salt toxicity. *Plant Cell Environ* **15**: 625-32.

Rhodes D and Hanson A D (1993) Quaternary ammonium and tertiary sulfonium compounds in higher plants. *Annu Rev Plant Physiol Plant Mol Biol* **44**: 357-84.

Saeedipour S and Moardi F (2011) Comparison of drought stress responses of tolerant and

sensitive wheat cultivars during grain filling: impact of invertase activity on carbon metabolism during kernel development. *J Agric Sci* **3**: 32-44.

Sairam R K, Rao K V and Srivastava G C (2002) Differential response of wheat genotypes to long-term salinity stress in relation to oxidative stress, antioxidant activity and osmolytes concentration. *Plant Sci* **163**: 1037-146.

Sairam R K, Srivastave G C, Agarawal S and Meena R C (2005) Differences in antioxidant activity in response to salinity stress in tolerant and susceptible wheat genotypes. *Biol Plantarum* **49**: 85-91

Scandalios J G (1993) Oxygen stress and superoxide dismutase. *Plant Physiol* **101**: 712-26.

Seckin B, Sekmen A H and Turkan I (2009) An enhancing effect of exogenous mannitol on the antioxidant enzyme activities in root of wheat under salt stress. *J Plant Growth Regul* **6**: 12-20.

Sekmen A H, Turkan I, Tanyolac Z O, Ozfidan C and Dinc A (2012) Different antioxidant defense responses to salt stress during germination and vegetative stages of endemic halophyte *Gypsophila oblanceolata* Bark. *Environ Exp Bot* **77**: 63-76.

Shafi M, Bakht J, Hassan M J and Raziuddin M (2009) Effect of Cadmium and Salinity Stresses on Growth and Antioxidant Enzyme Activities of Wheat (*Triticum aestivum* L.). *Bull Environ Contam Toxicol* **82**: 772-76.

Shahid M A, Balal R M, Pervez M A, Abbas T, Ashfaq M, Ghazanfar U, Afzal M, Rashid A, Garcia Sanchez F and Mattson N S (2012) Differential response of pea (*Pisum sativum* L.) genotypes to salt stress in relation to the growth, physiological attributes antioxidant activity and organic solutes. *Aust J Crop Sci* **6**: 828-38.

Shannon L M, Kay E and Lew J Y (1966) Peroxidase isozymes from horseradish roots.Isolation and physical properties. *J Biol Chem* **241**: 2166-72.

Shukla P S, Agarwal P K and Jha B (2012) Improved salinity tolerance of (*Arachis hypogaea* L.) by the interaction of halotolerant plant-growth-promoting rhizobacteria. *J Plant Growth Regul* **31**: 195-206.

Singh J (2004) An Analysis of Depleting Water Resources of Indian Punjab and policy options. In: Abrol I P, Sharma B R and Sekhon G S (ed) Groundwater use in North-West India-Workshop Papers. Centre for Advancement of Sustainable Agriculture, New Delhi.

Singh N K, Bracken C A, Hasegawa P M, Handa A K, Buckel S, Hermodson M A, Pfankoch F, Regnier F E and Bressan R A (1987) Characterization of osmotin. A thaumatin-like protein associated with osmotic adjustment in plant cells. *Plant Physiol* **85**: 529-36.

Skorupa M, Golebiewski M, Kurnik K, Niedojadlo J, Kesy J, Klamkowski K, Wojcik K, Treder W, Tretyn A and Tyburski J (2019) Salt stress vs. salt shock - the case of sugar beet and its halophytic ancestor. *BMC Plant Biol* **19**: 1471-1529.

Sneha S, Rishi A and Chandra S (2014) Effect of short term salt stress on chlorophyll content, protein and activities of catalase and ascorbate peroxidase enzyme in

pearl millet. *Am J Plant Physiol* **9**: 32-37.

Sofo A, Scopa A, Nuzzaci M and Vitti A (2015) Ascorbate peroxidase and catalase activities and their genetic regulation in plants subjected to drought and salinity stresses. *Int J Mol Sci* **16**:13561-78.

Sousa M F, Campos F A P, Prisco J T, Eneas-Filho J and Gomes-Filho E (2003) Growth and protein pattern in cowpea seedlings subjected to salinity. *Biol Plantrum* **47**: 341-46.

Subedi K D and Ma B L (2005) Seed priming does not improve corn yield in a humid temperature environment. *J Agron* **97**: 211-18.

Sulmon (2006) Involvement of soluble sugars in reactive oxygen species balance and responses to oxidative stress in plants. *J Exp Bot* **3**: 449-59.

Sulmon C, Van Baaren J, Cabello-Hurtado F, Gouesbet G, Hennion F and Mony C (2015) Abiotic stressors and stress responses: what commonalities appear between species across biological organization levels? *Environ Pollut* **202**: 66-77.

Swapna T S (2003) Salt stress induced changes of enzyme activities during different developmental stages of rice (*Oryza sativa* L). *Indian J Biotechnol* **2**: 251-58.

Takahashi M A and Asada K (1983) Superoxide anion permeability of phospholipid membranes and chloroplast thylakoids. *Arch Biochem Biophys* **226**: 558-66.

Tajbakhsh M, Zhou M X, Chen Z H and Mendham N H (2006) Physiological and cytological response of salt-tolerant and non-tolerant barley to salinity during germination and early growth. *Aust J Exp Agric* **46**: 555-62.

Tejovathi G, Khadeer M A and Anwer S Y (1988) Studies on certain enzymes in salt tolerant and sensitive varieties of sunflower. *Indian J Bot* **11**:113-17.

Tilaki G A D, Shakarami B and Tabari M (2011) Alleviation of salinity stress on the germination and early growth of three fescue species with seed priming treatments. *Propag Ornam Plants* **11**: 102-08.

Tsugane K, Kobayashi K, Niwa Y, Ohba Y, Wada K and Kobayashi H (1999) A recessive arabidopsis mutant that grows photo autotrophically under salt stress shows enhanced active oxygen detoxification. *Plant Cell* **11**: 1195-1206.

Turan M A, Elkarim A H A, Taban A and Taban S (2010) Effect of salt stress on growth and ion distribution and accumulation in shoot and root of maize plant. *Afr J Agric Res* **5**: 584-88.

Ueda A, Kathiresan A, Inada M, Narita Y, Nakamura T, Shi W, Takabe T and Bennett J (2004) Osmotic stress in barley regulates expression of a different set of genes than salt stress does. *J Exp Bot* **55**: 2213-18.

Vaidyanathan H P, Sivakumar R, Chakarbarti and Thomas G (2003) Scavenging of reactive oxygen species in NaCl stressed rice (*Oryza sativa* L.) differential response in salt tolerant and sensitive varieties. *Plant Sci* **165**: 1411-18.

Vaughan L V, Smith S E, Dudley L M and Macadam J (2002) Root growth and yield of differing alfalfa rooting populations under increasing salinity and zero leaching. *Crop Sci* **42**: 2064-71.

Verma J S, Chauhan A, Rajput N, Kumar A and Chaudhry A K (2017) Interactive effects of gibberellic acid and salt stress on growth parameters and chlorophyll content in oat cultivars. *J Environ Biol* **25**: 79-94.

Verma O P S and Yadava R V R (1986) Salt tolerance of some oats (*Avena sativa* L.) varieties at germination and seedling stage. *J Agron Crop Sci* **156**: 123-27.

Wahid A (2004) Analysis of toxic and osmotic effects of sodium chloride on leaf growth and economic yield of sugarcane. *Bot Bull Acad Sinica* **45**: 1-18.

Wahid A, Perveen M S, Gelani and Basra S M A (2007) Pretreatment of seed with H_2O_2 improves salt tolerance of wheat seedlings by alleviation of oxidative damage and expression of stress proteins. *J Plant Physiol* **164**: 283-94.

Wang B, Zhang J C, Song F B, Zhao M and Han X Y (2006) The effects of saline-alkali stress on photosynthetic characteristics in oats. *Chinese Agril Sci Bull* **23**: 35-238.

Wang Y and Nii N (2000) Changes in chlorophyll, ribulose biphosphate carboxylase-oxygenase, glycine betaine content, photosynthesis and transpiration in Amaranthus tricolor leaves during salt stress. *J Horti Sci Biotech* **75**: 623-27**.**

Wang Z, Quebedeaux B and Stutte G B (1996) Partitioning of (14C) glucose into sorbitol and other carbohydrates in apple under water stress. *Aust J Plant Physiol* **23**: 245-51.

Watanabe S, Kojima K, Ide Y and Sasaki S (2000) Effects of saline and osmotic stress on proline and sugar accumulation in *Populus euphratica* in vitro. *Plant Cell Tiss Org* **63**: 199-206.

Wei L M, Guo L C, Sha L and Deng L G (2007) Breeding report on oat new varieties: No.2 white oat. *Field Crops Res* **3**:341-42.

Weisany W, Sohrabi Y, Heidari G, Siosemardeh A and Golezani K (2012) Changes in antioxidant enzymes activity and plant performance by salinity stress and zinc application in soybean (*Glycine max* L.). *Plant Omics J* **5**: 60-67.

Willekens H, Chamnongpol S, Davey M, Schraudner M, Langebartels C and Van Montagu M (1997) Catalase is a sink for H_2O_2 and is indispensable for stress defense in C3 plants. *EMBO J* **16**: 4806-16.

Willenborg C J, Wildeman J C and Miller A K (2005) Oat germination characteristics differ among genotypes, seed sizes, and osmotic potentials. *Crop Sci* **45:** 2023-29.

Wilson C, Lesch S M and Grieve C M (2000) Growth stage modulates salinity tolerance of new zealand spinach (*Tetragonia tetragonioides,* Pall) and red orach (*Atriplex hortensis* L.). *Ann Bot* **85**: 501-09.

Xu H, Jiang X, Zhan K, Cheng X, Chen X, Pardo J M and Cui D (2008) Functional characterization of a wheat plasma membrane Na^+/H^+ antiporter in yeast. *Arch Biochem Biophys* **473**: 8-15.

Yadev S, Irfan F, Hayat S and Ahmad A (2011) Causes of salinity and plant manifestations to salt stress: A review. *J Environ Biol* **32**: 667-85.

Yousofinia M, Ghassemian A, Sofalian O, and Khomari S (2012) Effects of salinity stress on barley (*Hordeum vulgare* L.) germination and seedling growth. *Int J Agri Crop Sci* **4**: 1353-57.

Zhang G, Chen H W, Mu R L, Zhang W K, Zhao M Y and Wei W (2011) Evaluation of salinity tolerance and analysis of allelic function of HvHKT1 and HvHKT2 in Tibetan wild barley. *Ther Appl Genet* **122**: 695-03.

Zhang H and Zhou D (2010) The effects of salinity and osmotic stress on barley germination rate: sodium as an osmotic regulator. *Ann Bot* **106**: 1027-35.

Zhang H, Li W, Mao X, Jing R and Jia H (2016) Differential activation of the wheat SnRK2 family by abiotic stresses. *Front Plant Sci* **7**: 420-28.

Zhang J R, Shan C, Rengel Z, Song Z B and Chen Q (2018) Phytomelatonin receptor PMTR1-mediated signaling regulates stomatal closure in *Arabidopsis thaliana. J Pineal Res* **65**: 12500.

Zhang Z, Wu B, Hu Y, Huo P, Zhang Q and Chen X (2017) Transcriptome analysis of hexaploid hulless oat in response to salinity stress. *PLoS One* **12**: 1-18.

Zhao G Q, Ma B L and Ren C Z (2017) Growth, gas exchange, chlorophyll fluorescence, and ion content of naked oat in response to salinity. *Crop Sci* **47**: 123-31.

Zheng L, Liu G, Meng X, Liu Y, Ji X and Li Y (2013) A WRKY gene from *Tamarix hispida*, ThWRKY4, mediates abiotic stress responses by modulating reactive oxygen species and expression of stress-responsive genes. *Plant Mol Biol* **82**: 303-20.

Zhou L, Liu Y, Liu Z, Kong D, Duan M and Luo L (2010) Genome-wide identification and analysis of drought-responsive microRNAs in *Oryza sativa*. *J Exp Bot* **61**: 4157-68.

VITA

Name	**:**	Kamaljeet Kaur
Father's Name	**:**	S. Ajaib Singh
Mother's Name	**:**	Smt. Manjeet Kaur
Nationality	**:**	Indian
Date of Birth	**:**	20th August 1996
Permanent Address	**:**	Bhai Aachal Singh Colony, Street no-13 Talwandi Sabo, District Bathinda-151302 Punjab, India

EDUCATIONAL QUALIFICATION

Bachelor's Degree	**:**	B.Sc. (Medical)
University	**:**	Panjab University, Chandigarh
Year of award	**:**	2017
%age of Marks	**:**	73.7%
Master's Degree	**:**	M.Sc. (Botany)
University	**:**	Punjab Agricultural University, Ludhiana
Year of Award	**:**	2019
OCPA	**:**	6.78/10.00
Title of Master's Thesis	**:**	"Physiological, Biochemical and Molecular Parameters of salt tolerance in different genotypes of oats (*Avena sativa* L.)"