Exogenous application of auxin to ameliorate adverse saline and drought conditions in wheat.

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DEPARTMENT OF PLANT SCIENCES

FACULTY OF BIOLOGICAL SCIENCES

QUAID-I-AZAM UNIVERSITY

ISLAMABAD

2013

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A dissertation submitted in the partial fulfillment of the requirements for the degree of Master of Philosophy

IN

PLANT SCIENCES

(AGRICULTURE BIOTECHNOLOGY)

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God does not charge a soul with more than it can

bear

It shall be requited for whatever good and

whatever evil it has done.

Our Lord!

Take us not to task

if we forget, or Lapse into error
our Lord!

Charge us not with the burden

You laid upon those before us.
Our Lord!

do not burden us

beyond what we have the strength to bear

and pardon us

and forgive our Sins,

and have mercy on us

You alone are our protector

and help us against people

who deny the Truth.

(Ameen)

Dedicated
To

My Parents

At this day,

this moment,

What I am is

because of my Parents.

If I want to thank them,

I could not,

as they deserve to be thanked,

But my whole

life

is at their service.

DECLARATION

The whole of experiment work including lab described in this thesis was carried out by me in the Laboratory, Department of Plant Sciences, Quaid-i-Azam University Islamabad, Pakistan. The findings and conclusions are of my own investigation with discussion of my supervisor **Dr.. M. Farooq Hussain Munis.** No part of this work has been presented for any other degree.

Noor Muhammad

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LIST OF ABRREVIATIONS

IAA	Indole-3-acetic acid
GA3	Gibberellic acid 3
NAA	Naphthalene acetic acid
GH3	Gretchen Hagen 3
RNA	Ribo nucleic acid
Aux/IAA	Auxin/ Indole-3-acetic acid
ARF	Auxin response factor
ATP	Adenosine tri phosphate
ABP1	Auxin Binding Protein 1
ROS	Reactive Oxygen Species
Pb	*Plumbum* and it's the chemical symbol for lead.
Cd	Cadmium
Cr	Chromium
Ni	Nickel
SOD	Superoxide Dismutase
CAT	Catalase
POD	Preoxide Dismutase
DHAR	Dehydroascorbate reductase
GR	*Glutathione Reductase*
NARC	National Agriculture Research Council
NaCl	Sodium Chloride
RWC	Relative Water Contents
FW	Fresh Weight
DW	Dry Weight
FTW	Fresh Turgid Weight
Rpm	Revolution per Minute
BSA	Bovine serum albumin
SPSS	Statistical package for social study

ACKNOWLEDGEMENTS

All praises for Almighty Allah the most compassionate, the most beneficent and ever merciful, who gives me the power to do, the sight to observe and mind to think and judge. Peace and blessings of Almighty Allah be upon His Prophet Hazrat Muhammad (P.B.U.H) and Ahel Bait (a.s), source of knowledge and blessings for entire creation, who guided Ummah to seek knowledge from cradle to grave and enabled me to win honor of life.

I would like to record my sincerest thanks and grateful appreciation to Professor **Dr. Asghari Bano,** Chairperson of Plant Sciences Department and Dean Faculty of Biological Sciences for providing all the existing research and learning facilities to complete this task.

I feel a deep sense of gratitude and indeptness to my respected and dignified supervisor, **Dr. M. Farooq Hussain Munis**, Assistant Professor, Department of Plant Sciences, Quaid-i-Azam University, Islamabad, for his kind supervision, useful suggestions, consistent encouragement, friendly behavior and dynamic supervision, which enabled me to complete this task successfully. I am truly inspired by him. His energy, optimism, intelligence and continuous encouragement at every step during the course of this whole project enabled me to achieve my goals.

I would also like to thank Dr. Hassaan Javed from Plant Microbe Interaction Lab, Dr. Abdul Samad from Plant Genetics and Genomics Lab, Dr. Tariq Mehmood from Plant Molecular Biology and Biochemistry Lab, Dr. Fariha Hasan and Dr. Imran (Microbiology), Dr. Salman Malik (Biochemistry), Dr. Muhammad Zafar, Dr. Mushtaq, Dr. M Umar, Dr. Sajid Masood and Dr. Sajid Rasheed (Bioinformatics), for provision of equipment facility.I would like to acknowledge all the clerical staff of Department of Plant Sciences. The staff members were very facilitating. Mr. Tanveer (Scientific officer of General Lab) and other administrative staff always provided me technical guidance and required resources.

Words are lacking to express my thanks to Syed Ali Hasan Naqvi and Tahira Ibrahim for support, cooperation and consolatory behaviour during the whole time period of this study.

I am indeed humbly grateful to my Uncles Azhar Hussain khan, Mubark Khan, Ghulam Qasim khan, Ghulam Hassan khan**,** Ghulam Hussain khan, Ghulam Akbar khan, Ghulam Asghar Khan, Sohrab khan, Abid Hussain khan, Mazhar Hussain khan Muhsin, M Hussain Khan and my senior friends Shajar Abbas, Asmat Abbas, Muhib Hussain and Muhammad Abbas for their encouragement, valuable suggestions, most cooperative affectionate behaviour, inspiring and impetuous guidance and moral help for the completion of this task.

Much thanks to my loving friends and colleagues Imran Hussain, Jabir Hussain Syed, Saghir Abbas, Dr. Saghir, Asad Mustafa , Ali Atif Naqvi, Aqeel Kamran, M Asad Ali, Saleem Abbas, Safdar Abbas, Abid Raza, syed M Tahir, M Taqi Sherazi, M Tahir, Sabir Hussain, Munawar Ali, Mirza Hussain and Tauqir Nizami for their nice and sweet company and for their constructive advices and encouragement. The good time spent with them can never be erased from my memories.

I would like to acknowledge my heart-felt appreciation to my seniors colleagues Mr. Tamoor-ul-Hassan**,** Mr. Motsim, Mr. Nadeem, Mr. Samiullah, Mr. Faizan and Mr. Asim for their instigating cooperation, valuable suggestions, most cooperative affectionate behaviour, inspiring and impetuous guidance and moral help at each step, in the completion of this task.

Furthermore, I am deeply obliged and thankful to my class mates and lab fellows Mr. Asadullah, Mr. Ishtiaq, Nasrullah, Izhar, Shehzad, Naeem, Naeem Ahmad, Mr. Mujeeb, Mr. Aamir, Mr. Maqsood, Nazish, Mehwish, Younis, Hafsa, Saba, Faiz-ul-saba, Sadia, Bushra, Muhammadi, Amna, Rabia, Shumaila, Urooj, Razia, Zainab, and Mashal for their co-operation, valuable suggestions, extensive help, critical and constructive discussions, and sympathetic attitude during my study.

Finally, my warmest appreciation is for my dear brothers Atta Hussain, Ali Hussain, M Hassan Khan, Ali Hassan khan, my sweet sisters Sakina Bibi, Kaneez Zainab, Tahira batool, Ghulam Kubra, Safia Bibi, and Asma Batool my lovely cousins Shahnawaz, Zawar Hussain Muhammad Baqir, Muhammad Abbas, M Raza, Iqbal Hussain, M jaffar, M Ali, Ejaz Hussain, Ali Abbas, M Yaqoob, Nasir Abbas, M Naqi, Ali Imran, M Hassnain Abbas and my beloved nephews M yousif, M Asif, M Younis,

Khadija Kubra, Sehrish Fatima, Zeenat Fatima, M Zorieen Abbas, Qurat-ul-Ain, Emman Zahra, Ali Haider, Zaman Mehdi and Abuzar Khan.

No acknowledgement could ever adequately express my feelings to my affectionate and loving **Parents and sweet brother Mr. Midah Hussain Khan**. I am proud of them for their love and care who provided me all what I needed. I can never forget the prayers and untiring efforts of them and brothers and sisters who inspired me to higher ideas of life and sacrificed their comfort for my brilliant future. My words will fail to give them credit. Their uninterrupted support in various aspects of my life and studies, kept me going with grace and honor under the shades of their prayers. I can never repay their unlimited love and precious prayers.

May Almighty Allah shower His blessings and prosperity on all those who assisted me in any way during my research work.

Noor Muhammad.

Abstract

Adverse environmental conditions such as drought and saline conditions are the principal factors that restrict plants growth and production. Plant growth regulators play key role to alleviate the abiotic stress in plants.Present study was aimed to investigate the physiological response of four wheat cultivar (Aari, Baras, Sahar and Aas) under saline and moisture deficit and the role of exogenous application of IAA to ameliorate adverse conditions in these varieties. The response of four varieties under saline and drought stress and exogenous application of indole-3-acetic acid (IAA) was studied in pot experiment. Experiment was conducted in growth chamber in Quaid-i-Azam University Islamabad. For saline stress, NaCl solution (100mM) was prepared and applied at the time of sowing while drought stress was applied after two weeks by withholding water. Foliar application of IAA (80 ppm solution) was prepared and applied after three weeks.After four weeks plants were uprooted and preserved at -20°C. Variation in relative water contents along with sugar contents, protein contents, proline contents and anti-oxidants enzyme activities were measured. Decrease in relative water contents were associated with increase in sugar contents, protein contents, proline contents and anti-oxidants enzyme activities, observed under saline and drought conditions. On the basis of these parameters, Sahar is the most tolerant to drought followed by Baras, Aari and Aas. Similarly under saline stress,Sahar is the most tolerant followed by Aas, Aari and Baras. Adverse effect of drought and salt were ameliorated by the foliar application of IAA in four studied varieties and tolerance in each variety increased significantly. Response of each variety to IAA remained similar.

Key Words: Sahar, Aas, Aari, Baras, Indole-3-acetic acid, drought stress, salt stress.

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Key Words: Sahar, Aas, Aari, Baras, Indole-3-acetic acid, drought stress, salt stress.

Chapter 1

Introduction

1.1 Phytohormone

Plant growth regulators are chemical messengers those are generated in one part of plant and transported to other part of plant, where they play crucial role to govern plant response to adverse condition at very little amount. Plant growth regulators are natural harvest of plants and when they are synthesized chemically, they are termed as plant growth regulators. Plants naturally or artificially face adverse environmental condition such as salinity condition, water deficit condition, high temperature, chilling condition, and heavy metal stress. Plants counteract against these adverse effect by physiological, chemical, metabolic, and byphytohormone. There are five main classes of plant growth regulators.

1.1.1. Abscisic acid

Abscisic acid acts as a mediator in various types of stresses mainly saline and drought stress and also provides internal sign to plants that facilitate plants to stay alive under adverse environmental conditions (Keskin*et al*., 2010)

1.1.2. Cytokinins (CKs)

Cytokininsare involved in several plant processes such as growth and development, cell division, nutrients mobilization, apical dominance, roots and shoots differentiation (Mok and Mok, 2001; Davies, 2004)

1.1.3. Gibberellins

Gibberellins generally control germination, stem elongation, leaf expansion, and flowering (Magome*et al*., 2004)

1.1.4. Ethylene

Ethylene is a gaseous hormone that control several physiological and developmental phenomena in plants, like ripening of fruits and florescence, shedding of leaves, seedling emergence and organ abscission. It is also involved in the response to environmental and biotic stresses in plants (Abeles *et al*., 1992).

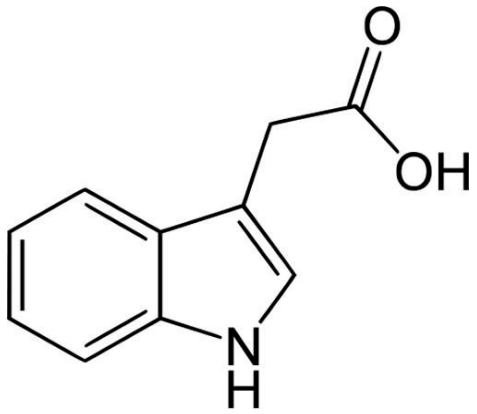
1.1.5. Auxin

Auxin is general group of molecules with an influential capability to stimulate growth function in plants. Irreversible increase in the size and volume is termed as plant growth. It is driven by uptake of water; as a result, individual cells are enlarged. Auxin is a vital group of plant growth regulators that are being responsible for the growth variationstakes place during life cycle of plants. Our research focuses on exogenous application of auxin to ameliorate adverse saline and drought conditions in wheat.

1.2. Auxin and its effect on plants

Julius von Sachs (1887) gave the idea of plant growth regulators for the first time and after that numerous phytohormones have been indentified. These plant's growth regulators have a special effect on plant growth and these are active in a minute concentration in plants. Mechanism of transport of above mentioned five classes of phytohormone is not significantly understood but the action and translocation of auxin in plants is very interesting. Charles and Darwin (1880) investigated the effect of auxin in the book entitles "*The power of movement in plants*". They observed that perception of light to one part of grass coleoptiles, an effect was transported to other part of grass that compels bending of grass towards light.

In 1926, the messenger had been separated simply through diffusion into agar block, from plants tissue, and it has the growth promoting activity (Cholodny*et al.,* 1925; Went *et al.*,1926). Initially three types of auxins were indentified in plants and one of them also found in human urine. Therefore, the first available description began to publish on the crystallization and structural feature of auxin. The structure of indole-3-acetic acid (IAA) has been correctly identified, so far.



Structure of Indole-3-Acetic Acid

Now auxin is a common name for very important class of molecules that are found in plants. These molecules are also present in microbes, animals and human. Indole-3-acetic acid (IAA) is the major auxin in plants and is an important plant growth regulator with corroborate capability to control many processes of plant growth and development. Artificially prepared auxins are used as herbicide, such as 2, 4-dicholorophenoxyacetic acid is a world's popular frequently used herbicide.

Auxin effect depends on the concentration and kind of auxin that have been applied on the growing plants. Internal level of IAA has been concerned in embryonic and post embryonic development, and tropism like motion of plants towards light and away from gravity. Auxin shows response to a large numbers of plant processes e.g.cell division, cell differentiation and cell elongation.Role of auxin to control each process yet not known. It has a wide spectrum of uses in plants as well as in animals. Photo activated auxin are used as cytotoxin in animals as a treatment of cancer (Folkesand Wardman, 2001).

The reason auxins have drawn more interest for nearly a century because it is not only having the ability to effect the growth, but have other long lasting effects on the life cycle of plants. Recent evidences indicate that, using a single sensing mechanism and obtaining, given physiological responses that auxin govern are central for plant structure and functioning.

1.3. Indole-3-Acetic Acid (IAA) and salt stress

Saline condition is one of the abiotic causes that are restricting plant growth and production (Allakhverdiev*et al.,* 2000; Ashraf *et al.,* 2008). A higher concentration of salt particularly Na^+, deposited in the soil, brings variation in the properties of soil by reducing soil porosity and water conductance.Currently there are no reasonably feasible technological resources to make good progress of crop management in adverse environmental situation. Therefore, the production of resistant crops under abiotic condition is thought the best management practice to meet the increasing food demands of developing and under-developed countries. Resistance of crops to face abiotic stresses can be increased by a variety of sources, like selection and breeding, genetic modification, use of osmoprotectants and growth regulators (Parida and Das, 2005). Salty conditions change shoot to root hormonal balance e.g. decreasecytokinin and gibberellin contents and increase the abscisic acid level(Zhang and Zhang, 1994).

IAA plays a key function in regulating plant growth.It controls cell elongation, tissue differentiation, tissue development and apical dominance **(**Wang *et al*., 2001**)**. IAA also performs the function under saline condition in plants but less reports are described that explain correlation between salinity and auxin level in plants and also the mechanism of auxin action to ameliorate salinity conditions. Variation in the level of abscisic acid and IAA contents is also similar in the plants(Ribaut and Pilet, 1991).Higher concentration of IAA has been reported to reduce growth (Ribaut and Pilet, 1994). Hence, hormonal imbalance results in reduction of plant growth under adverse environmental conditions. So, external or foliar treatment of phytohormones is the best approach to alleviate the effect ofadverseenvironmental conditions. Saline stress such as NaCl causes a significant reduction in IAA concentration in rice leaves (Prakash and Prathapasenan, 1990). A noteworthy decline has also been reported in IAA contents in rice after a few days of saline stress (Nilsen and Orcutt, 1996).

In another study, saline condition reduced 75% of IAA contents in tomato (Dunlap and Binzel, 1996). Salinity is the source of continuous decline in the IAA contents of root system of plants (Sakhabutdinova*et al*., 2003). Before sowing application of wheat seeds with plant growth regulators promotes plant growth and development under abiotic stresses (Sastry and Shekhawa, 2001; Afzal*et al*., 2005). Seeds germination,

in saline treated seeds reduced under saline condition, it can be overcome by application of IAA or NAA to seeds (Balki and Padole, 1982; Gulnaz*et al*., 1999). Foliar application of auxin improves the growth parameters such as root length, hypocotyls length, seedlings fresh and dry weight under saline stress (Akbari*et al*., 2007).

Auxin promotes the transcription of many genes termed as primary auxin receptive genes. A large number of auxin-responsive genes have been recognized and characterized from diverse plant species including soybean, *Arabidopsis* and rice (Hagen and Guilfoyle, 2002). These auxin-responsive genes are divided into three gene families, auxin/indole-3-acetic (Aux/IAA), GH3 and small auxin-up RNA(SAUR) genes (Guilfoyle, 1993). Auxin prevents the outgrowth of tiller buds in rice (*Oryza sativa* L.) by down-regulating the expression of *OsIPT genes* and cytokinin biosynthesis in nodes (Liu *et al.*, 2011).

1.4. IAA-mediated regulation of genes expression

It was thought for a long time, like steroid receptors in animals, plants also have special receptor proteins that start signal transduction of auxin in numerous physiological functions. Ultimately, after decades of scientists work on auxin, two different optionsprovided considerable information on the nature of auxin responses. The first option is depending on observations made over 20 years ago, which show that gene expression altered by auxin in a short time in a selective and remarkable way (Key *et al.*, 1967; Theologis*et al.*, 1982;Theologis*et al.*, 1985; Abeland Theologis, 1996).

The second option depends on analysis of a series of mutations resistant to auxin. Many of these mutant plants lacked various functional components of the ubiquitin-mediated proteolytic pathway, illustrating that the selective breakdown of proteins is a main regulator of many aspects of auxin response (Leyser*et al.*, 1993; del Pozo and Estelle, 1999; del Pozo*et al.*, 1998). The levels of some mRNAs decreased in large proportions in response to auxin, while other mRNAs increase in great proportions, for example, Aux/IAA Gretchen Hagen-3 (GH3) and small auxin members RNA gene family (SAUR) (McClure and Guilfoyle, 1987; Abeland Theologis, 1996; Hagen and Guilfoyle, 1985). Furthermore,auxin activate theregulons of genes directly and quickly.

Genes that are activated or suppressed during this process are finally dependable for many physiological actions of auxin. The complex auxin responses are mediated by two well-studied groups of genes: genes of Aux/IAA, consisting of 29 members, and the genes of the auxin response factor (ARF) with 23 members in *Arabidopsis thaliana* (Hagenand Guilfoyle, 2004;Okushima*et al*., 2005; Overvoorde*et al*.,2005).

1.5. Alternative signaling pathways

Aux/IAA-mediated signaling perhaps not only way through which the role of auxin functions but also the result of different scientists, that impermeable auxin shows its effect without entering into cell (Venis*et al.,* 1990) or the auxin functions that are very rapid to control genes transcription, for example, membrane depolarization (Felle*et al.*, 1991).Decades of research on auxin have shown that auxin controls many cellular phenomena, although the expression of some kinds of response indicates that it can be subject to transcriptional control. The effect of auxin on the abundance and activity of the plasma membrane situated enzyme such as H^{+-} ATPase enzymes are particularly well studied (Senn and Goldsmith, 1988; Hager*et al.*, 1991; Re, 1993;Frias*et al*., 1996). Other targets for the regulation responded by auxin are potassium and chloride channels and transport absorptions of chloride ions (Martene*t al*., 1991; Blatt and Thiel, 1994; Zimmermann*et al.*, 1994;Philippar*et al*., 1999). Auxin mediated induction of ions uptake, correlates either the control or maintenance of intracellular turgor pressure which is essential for growth of plant cells (Evans, 1985).

1.6. Downstream auxin signaling

The auxin effects are wide in range, a large in numbers and hardly too separate. Therefore, its effects can be divided into two main categories: effects on cell division and effects on cell enlargement. Research on auxin shows that it also have the morphological properties, that are similar to the chemicals found in animal kingdom, but the mechanisms of auxin action in developmental processes of plants cell are still not understand.

1.6.1. Auxin and cell expansion

Consequences of ABP1 auxin mediated signaling such as expansions of cells and membrane hyper polarization is evident at the periphery of the cell. However, another part, the genes encoding extracellular proteins, for example, those involved in cell wall breakdown, expansions and arabinogalactans (which are proteins of the cell wall) extensions, pro-rich proteins (that function as links between the cell wall and the plasma membrane), and class III peroxidases (which are involved in pathogen defense lignifications, and wound healing) are affected by a gain of function of indole-3-acetic acid (IAA) mutation (Overvoorde*et al*., 2005). This involved auxin at least two distinct signaling pathways in plants via structure and function of the variation in the cell wall and the plasma membrane

Less obvious, but perhaps only considerable, the answers that could be performed by others, unless to be studied first enzyme gene clusters auxin response. Two groups of these enzymes are identified glutathione-S-transferase and quinine reductases. Glutathione-S-transferase refers to the metabolism and detoxification of xenobiotics and quinine reductases protect cells against oxidative stress due to the decreased formation of reactive oxygen species (ROS) (Laskowski*et al*., 2002).

ROS species cause cell wall loosening and growth extension in a process that is connected with auxin for a long time (Foreman*et al*., 2003; Schopfer, 2001). The action of ROS could be caused either directly as a result of the oxidative effects of ROS on cell wall proteins and structures (Schopfer, 2001) or indirectly through the activation of signaling pathways and intermediates kinases and phosphatases that involved in regulating gene expression (Hancock*et al.,* 2001). It has been suggested auxin induced the enzymes such as quinonereductases that protects the cells against damage caused by oxidative stress (Laskowski*et al*., 2002).

1.6.2. Auxin and cell division

It is well known that Auxin also induces cell division, but the accurate molecular mechanism is not known. Not yet known how auxin is intimately connected to the sequence of cell cycle, although the expression of many cell cycle genes are promoted by

auxin (Richard*et al.,* 2002;Vannest*et al.,* 2005). But ithas been confirmed that this stimulation mediated through both proteasome-dependent pathways and ABP1-dependent(delPozo*et al.,* 2002). These targets include proteins that involved in cell cycle transitions, for example, the S phase entry (delPozo*et al.,* 2002) and the G2-M transition (Blilou*et al*., 2002).

1.7. Auxin and heavy metal stress

Sewage water problem is a common in the industrialization, urbanization and growing population, utilization of sewage water is escalating day by day. Farmers use sewage water not only as a source of water but also as a supplement of nutrients. Hence, high yields are reported with the use of sewage water than of the fresh water (Pradhan et *al.,* 2001). Sewage water is a mixture of various organic and inorganic elements, which contain essential nutrients of plants as well as toxic elements for plant growth. Too much deposition of heavy metals like Pb, Cd, Cr and Ni in the soil due to excessive utilizations of sewage water is a source of phytotoxicity (Tsakou*et al.,* 2001; Peralta *et al.,* 2001). Therefore, plant must acclimatize definite mechanism to scavenge these heavy metal stresses.

Tolerance to adverse conditions in plants has been reported to be connected with an increase in antioxidant activity. Heavy metals such as Cd, Pb and Cr ions induced super oxide generation and inactivation of mitochondrial electron transport has been reported in higher plants (Dixit *et al.,* 2002). Higher concentrations of ROS like hydroxyl ions, singlet oxygen, and hydrogen peroxide at cellular levels are the basis of oxidative stress and toxicity symptoms observed at whole plant due to heavy metals (Cr, Pb) (Hauschild, 1993).

ROS may play two different roles: exacerbating damage or signaling the activation of defense responses. Such a dual function has recently been reported for a number of abiotic stress responses (Dat*et al.,* 2000). The simultaneous action of a number of antioxidant enzymes viz. CAT, SOD enzymes synchronized with thiol DHAR and GR pathway, ascorbate glutathione is a major mechanism of ROS scavenging under heavy metal stress (Clijster*set al.*, 1999).

Plant growth regulators especially IAA is an active members of the signal cascade induction in plants against stress responses (Pedranzani*et al.,* 2003).IAA in exogenous treatment to plant, modulate expression of antioxidant enzymes and increase tolerance to oxidative stress (Barna*et al.,* 1997). Similarly exogenous application of IAA reported to alter in activity and peroxidase isoenzyme pattern in pea (Birecka and Galston, 1970). Grain yield increased in wheat by application of IAA (Kamboj and Kler, 2007).

1.8. Auxin and drought stress

Plants naturally subjected, to face adverse environmental conditions and plants react to avoid the deleterious effect by implication and activation of protective mechanisms. Moisture stress, the main environmental stress, affects every aspect of plant growth and is a principal source for limiting crop yield (Golbashy*et al*., 2010).

Drought stress in wheat provides variation in the pattern of plant growth and development. Low water potential suppresses cell division net photosynthesis, protein synthesis and growth hormones imbalance in major plant tissues (like and Chen, 1987). Moisture stress is a constant problem that reduced global crops production and is the main source of agricultural losses in the arid and semi-arid areas (Boyer, 1982). The percentage of dry land areas affected doubled from 1970 to the 2000s in the world (Isendahl and Schmidt, 2006). Farmers in these areas depend on drought tolerant varieties that give better performance. Grain filling is a sensitive stage in the arid and semi-arid Mediterranean climates. The quality of wheat was controlled not only by hereditary factors but also by environmental conditions, primarily the water and soil fertility can affect the quality of wheat under favorable conditions (Triboi*et al*., 2003). Protein level increased under water stress (Dadashi, 2002). The total protein contents in seeds increased by 5 to 13% in different drying treated plants as compared to the control plants (Jinyin*et al*., 2002). Grain yield was reduced dramatically in the grain filling stage under water deficit condition (Ehdaie and Waines, 1996).

Plants tend to acclimatize to drought by accumulation of compatible organic osmolytes like glycine betaine, proline and polyols(Rhodes and Hanson, 1993). Seed treatment or foliar application of chemicals like glycinebetaine, kinetin, salicylic acid may increase yield of different crops due to acceleration plants growth and development,

enhanced photosynthetic rates, leaf area and plant dry matter production (Gunes*et al*., 2007; Karlidag*et al*., 2009;Elwana and El-Hamahmyb, 2009;Khan *et al*., 2003). Under adverse environmental conditions the concentration of IAA in plants is decreased. Reduction of IAA amount in unfavorable environment conditions might be due to the inhibition of IAA synthesis or acceleration in the degradation of IAA. However foliar application is best option to overcome adverse environmental conditions.

L-Tryptophan is known to be a physiological precursor of auxin in higher plants. It is investigated that L-Tryptophan has more positive effect on plant growth and yield as compared to pure auxin (Zahir*et al*., 1999). L-Tryptophan is an amazing amino acid. It may act as an osmolyte, ion transport regulator, modulates stomata opening and detoxify harmful effects of heavy metals (Rai, 2002). Several studies have been conducted to evaluate the influence of exogenous application of salicylic acid and L-Tryptophan on plant growth, development and stress tolerance. Nonetheless, less attention has been paid to exogenous application of auxin such as indole-3-acetic acid in drought tolerance. Application of IAA increase stress tolerance of plants by positively altering physiological phenomena in plants. Hence, the current study was undertaken to explore the defensive role of IAA to counter drought and saline stress in wheat.

1.9 Aims and objectives of Study

Experiment was conducted to gain the following aims and objectives.

Present investigation was aimed to determine the physiological response of four wheat cultivar in drought stress

To determining the variability in four cultivarsfor evaluate the resistance against salt stress

Foliar application of auxin (IAA) with perspective improving tolerance to water stress in studied varieties

Application of auxin (IAA) to ameliorate adverse saline condition in local grown four wheat varieties

Chapter 2

Materials and Methods

Effect of abiotic stresses (drought and salt stress) was estimated on four wheat genotypes. Experiment was conducted at Department of Plant Sciences, Quaid-i-Azam University, Islamabad, Pakistan. Seeds of four varieties (Ari, Baras, Sahar, and Aas) were collected from NARC Islamabad and surface sterilized with 75% ethanol and rinsed three times with distilled water. Plants were grown in growth chamber and maintained the connditions 15/20°C day/night temperature and 12 h photoperiod. The light intensity was maintained at 800μmole quantum s^{-1}, throughout the experimental period.

Forty small pots, eight for the control, sixteen for drought and sixteen for salt stress treatments, were prepared and filled with 500 g of soil sand mixture for each variety. In each pot, eight seeds were sown and thinned after one week to maintain four uniform seedlings per pot. Moisture level was maintained by adding nano-pure water daily, in the morning at field capacity, after weighting the pots. Salt solution of NaCl(100mM)was prepared and 35 ml solution was given to each pot, at the time of sowing and after two week of sowing, 20 ml salt solution was given again. After two weeks of sowing, drought stress was applied by withholding water. After three weeks of sowing, eight pots from drought stress treatment and eight pots from salt stress treatment were selected for foliar application of 80 ppm IAA solution. After four week of sowing, the plants were uprooted and preserved in the freezer for further analysis of growth and physiological parameters.

2.1. Relative Water Contents

Relative water content of leaves was measured by using the protocol of Arnon (1949). The leaves were harvested, weighed and immersed in distilled water for 24 hrs. The weight of fully turgid leaves was again measured. After that, leaves were dried in an oven for 72 hrs at 70 ºC. Relative water content was determined by using the following protocol of Weatherley(1950).

$$RWC\% = \frac{FW - DW}{FTW - DW} \times 100$$

RWC = Relative water contents

FW = Fresh weight

DW = Dry weight

FTW = Fully turgid weight

2.2. Soluble Sugar Estimation

Estimation of sugar contents in fresh leaves was done according to the protocol determined by Dube*et al*. (1956). Fresh plant material (0.5 g) was homogenized with 10 ml of distilled water in a clean mortar and pestle and centrifuged at 3000 rpm for 5 min. In 0.1 ml of supernatant, 1 ml of 80% (w / v) phenol was added and incubated at room temperature. 5 ml of concentrated sulfuric acid was added to this sample and incubated for 4 hours. Then the absorbance of each sample was recorded at 420 nm. The concentration of the unknown sample was calculated with reference to the standard curve, made by glucose.

2.3. Leaf Protein Contents

The protein content of the leaves was determined following the method of Lowery *et al*. (1951) using BSA as standard.

Phosphate buffer (stock solution)

a. Monobasic Sodium phosphate:

27.6 g was dissolved in distilled water (1000 ml)

b. Dibasic sodium phosphate:

53.6g was dissolved in distilled water (1000 ml)

Sodium phosphate monobasic (16 ml) and sodium phosphate dibasic (84ml) were mixed together to obtain the desired pH (7.5) phosphate buffer.

Reagent A: 2 g of sodium carbonate (Na_2CO_3)

0.4 g of NaOH (0.1 N) and 1 g of Na-K tartrate was dissolved in 100ml of distilled water.

Reagent B: $CuSO_4.5H_2O$ (0.5 g) was dissolved in 100 ml of distilled water.

Reagent C: Solution A (50 ml) and solution B (1 ml) were mixed together.

Reagent D: Folin phenol reagent diluted with distilled water in a 1:1 ratio.

Procedure:

Fresh leaves (0.1 g) were ground with the aid of mortar and pestle in 1 ml of phosphate buffer (pH 7.5) and centrifuged for 10 min at 3000 rpm. The supernatant (0.1 ml) of given sample containing an unknown amount of the protein was poured into the test tubes and 1 ml total volume was made by adding distilled water and 1 ml of reagent C was added therein. After stirring for 10 min, 0.1 ml of reagent D was added. The absorbance of each sample was recorded at 650 nm after 30 min of incubation. The concentration of the protein content was determined with reference to the standard curve made using standard BSA (bovine serum albumin). The different concentrations of BSA 20,40,60,80,320 and 640 mg were prepared. BSA absorbance was recordedat 650 nm.

2.4.Proline contents of leaves:

Proline content of the leaves was measured by the method of Bates *et al*. (1973). Fully expanded fresh leaves were sampled. Purified proline was used to standardize the procedure for the quantification of sample values. Reagent (ninhydrin Acid) was prepared by heating 1.25 g of ninhydrin in 30 ml of glacial acetic acid and 20 ml of 6 M phosphoric acid, with stirring, until dissolved. Solution kept cool and fresh so stored at 4 ° C. The reagent is stable for 24 hours. Approximately 0.5 g of plant material was homogenized in 10 ml of aqueous solution of 3% sulfosalicylic acid and homogenate was filtered with Whatman # 42 filter paper. 2 ml of the filtrate is reacted with ninhydrin acid 2 ml and 2 ml of glacial acetic acid in a test tube for 1 hour at 100 ° C and the reaction was terminated on ice bath. The reaction mixture was extracted with 4 ml of toluene with vigorous mixing with a test tube shaker for 15-20 sec. The chromophore containing toluene was aspirated from the aqueous phase and warmed to room temperature and the absorbance was read at 520 nm against toluene used as a blank. Proline concentration was determined from the standard curve and was calculated on the fresh weight basis as follows:

[(ugproline / ml x ml of toluene) / 115.5ug/umol] / [(sample G) / 5] = μ molproline / g fresh weight of material.

2.5. Extraction for antioxidant enzyme

Fresh leaves (5 g) were homogenized with 15 ml of 0.05 N phosphate buffer (pH 7.0) containing 10% poly vinyl poly pyrrolidore (PVPP) and 0.1 M ethylene diamine tetra acetate (EDTA). Homogenate was centrifuged at 15,000 rpm for 15 min at 4°C. Supernatant was used for superoxide dismutase (SOD) and the assay of peroxidase (POD).

2.5.1. Assay for Superoxide Dismutase Activity (SOD)

SOD activity was determined by measuring the inhibition of the photochemical reduction of nitrobluetetrazolium (NBT) using the method of Beauchamp and Fridovich (1971). The reaction mixture (3 ml) consisting of 13 mm methionine, 0.075 mMNBT, 0.1 mM EDTA, 0.002 mm riboflavin and 0.1 ml enzyme extract in 50 mM phosphate buffer (pH 7.8). The mixture was taken in test tubes placed below the light chamber for 15 min. The absorbance was recorded at 560 nm with a spectrophotometer. One unit of enzyme activity was taken as the amount of enzyme that reduces the absorbance reading of 50unit in comparison with the enzyme activity in test tube.

2.5.2. Assay for Peroxide Dismutase Activity (POD)

POD activity was measured following the assay mixture contained 0.1ml enzyme extract, 1.35ml of 100mM MES buffer (PH 5.5), 0.05% H_2O_2 and 0.1% phenylenediamine. Change was recorded in the absorption at 485 nm for 3min with the spectrophotometer. The POD activity was measured as protinedegration in mg.

2.6.Statistical Analysis

All results were compiled to form a database using the multi-elemental Excel software. Basic descriptive statistics i.e. mean minimum, maximum and standard deviation of varieties and physicochemical parameters of the samples of all varieties were calculated.All statistical analysiswas performed using the Statistica software package (version 8.0) and the statistical package for social study (SPSS).

Chapter 3

Results

3.1. Relative Water Contents

3.1.1. Relative water contents under salt stress

Analysis of variance showed variation in relative water contents (RWC) under abiotic stress conditions. RWC changes in tested varieties under salt stress (Fig.3.1). RWC significantly reduced in Aari (89.6%) and Baras (85.8%) while insignificant reduction was observed in Sahar (85.8%) and Aas (87.9%) under salt stress, in comparison to control.

3.1.2. Relative Water Contents under Drought Stress

Relative water contents of studied varieties were significantly decreased under water stress. RWC significantly decreased in Aari (84.66%), Aas(79%), Baras (82.12%) and Sahar (81.64%) under moisture stress (Fig. 3.2).

3.1.3. Variation in Relative Water contents by Indole-3-aceticAcid under Salt Stress

Foliar application of indolAcetic Acid (IAA) did not significantly affect RWC in tested varieties (Fig.3.3). RWC remained unchanged inIAA treated plants ofAari, Baras and Saharas compared to untreated plants. However in Aas (81.94%), RWC reduced significantly in IAA treated, as compared to normal salt stressedplants (87.91%).

3.1.4. Variation in Relative Water contents due Indole-3-aceticAcid under Drought Stress

Indole-3-acetic acid significantly (IAA) affected RWC of drought stressed plants. RWC of untreated drought stressed genotypes, Baras (82.12%) and Aas (79%) were raised due to IAA treatment to 84.24% and 81.52% respectively. RWC insignificantly increased in Sahar and remain unchanged in Aariby IAA application as compared to untreated drought stressed plants (Fig.3.4).

3.2. Soluble Sugar Contents

3.2.1. Sugar Contents under Salt Stress

Sugar contents of tested varieties changed under salt stress condition as compared to control. Significant increase in sugar contents was observed in Aari (296.4µg/g) under salt stress as compared to control (250.2 ug/g) While Baras, Sahar and Aas exhibited insignificant increase (Fig. 3.5).

3.2.2. Sugar Contents under Drought Stress

Imposition of drought stress resulted in considerable increases in sugar contents(Fig. 3.6). There was a large increase in total soluble sugars contents in Sahar (289.6µg/g), as compared to control (262.2µg/g). Sugar contents increased significantly in Aari (275.2 µg/g) and Baras (270.2 µg/g), as compared to control (250.2 µg/g) and (254µg/g), respectively. While in Aas, non-significant reduction in sugar contents was observed under drought stress (255.8µg/g), as compared to control (258.8µg/g).

3.2.3. Changes in Sugar Contents due to IAA treatment under Salt Stress

Indolaceticacid treated plants could not show significant change in total sugar contents of studied varieties as compared salt stressedplants. Due to IAA treatments, Sugar contents slightly increased in Baras (282 µg/g) and Sahar (279.2 µg/g), as compared to normal salt stress while in Aari and Aas, the sugar contents slightly decreased by IAA traetment as compared to salt stress plants (Fig. 3.7).

3.2.4. Variation in Total Soluble Sugar Contents by IAA treatment under Drought Stress

Indolacetic acid treatment showed peculiar effect on sugar contents in plants of studied varieties under drought stress condition. IAA treated plants exhibited significantly increased sugar contents in two tested varieties;Sahar (341.6 µg/g) and Aas (293 µg/g) while in Aari (256 µg/g), the sugar contents significantly decreased as compared to non-treateddrought stressed genotypes (289.6 µg/g), (255 µg/g) and (275 µg/g), respectively. However in Baras the sugar contents slightly decreased inIAA treated plants as compared to drought stressed plants (Fig.3.8).

3.3. Total Protein Contents

3.3.1. Total Protein Contents under Salt Stress

There was no significant variation observed in protein contents, except Baras (115.66µg/g), in which the protein contents reduced significantly under salt stress as compared to control (167.79µg/g). In Aari, protein contents slightly increased while in Sahar, protein contents were decreased in salt stressed plants, as compared to control but in Aas protein contents remained unchanged (Fig. 3.9).

3.3.2. Protein contents under drought stress

Protein contents of studied varieties under drought stress were changed significantly (Fig.3.10). Protein contents increased in varieties Aas (171.77µg/g), Sahar (168.37µg/g) and Aari (122.62µg/g) as compared to control (150.46µg/g), (140.69) and (95.48µg/g), respectively. However, in Baras (124.54µg/g), the protein contents reduced under drought stress as compared to control (167.79µg/g).

3.3.3. Changes in protein contents by indole-3-acetic acid treatments under salt stress

Indolacetic acid treatment did not exhibit significant change in protein contents of saline stressed plants (Fig. 3.11). Due to IAA treatment the protein contents increased in Baras (145.4µg/g) and Aari (120µg/g) as compared to salinity stressed plants (115.6µg/g) and (116µg/g), respectively. In variety Aas (132.71µg/g), the protein contents decreased in IAA treated plants as compared to saline stressed (150.3µg/g) but in Sahar, protein contents remained unchanged (132µg/g).

3.3.4. Variation in protein contents under drought stress by Indol Acetic Acid treatments

Protein contents did not vary significantly by IAA treatment under drought stress condition in studied varieties (Fig 3.12). Protein contents of varieties Aas (150µg/g) and Sahar (129µg/g) decreased by IAA treatments as compared to drought stressed plants (171.77µg/g) and (168.37µg/g), respectively. Protein contents in Baras (148µg/g) and Aari (127µg/g) increased non-significantly by IAA treatments in comparison to drought stressed plants.

3.4.Proline Contents

3.4.1.Proline contents under salt stress

Proline accumulates in wheat under aboitic stresses like salinity and drought. The concentration of proline is an important indicator for tolerance of abiotic stress in wheat. In the present study, an increase in proline accumulation was observed in four varieties of wheat at seedlings stage under salinity (Fig.3.13). Statistical data showed significant increase of proline contents in Aas (300.3µg/g), Sahar (247.19µg/g) and Baras (239.95µg/g) while in Aari (183.61µg/g), insignificant increase under salt stress was observed.

3.4.2.Proline contents under drought stress

Under drought stress proline contents significantly increased in all varieties (Fig 3.14). Maximum proline contents were observed in Aas (329.11µg/g), followed by Baras (320.33µg/g), Sahar (265.13µg/g) and Aari (211.38µg/g) under moisture limiting condition as compared to their control plants (Fig. 3.26).

3.4.3. Effect of IAA treatments on Proline contents under salt stress

IAA application showed stimulatory effect on proline contents accumulation in salt stressed plants (Fig. 3.15). Maximum increase was observed in Aari (336.54µg/g), followed by Aas (324.18µg/g), and Sahar (289.62µg/g) due IAA treatment as compared to untreated salt stressed plants (183.61µg/g), (300.3µg/g) and (247.19µg/g), respectively. In Baras, proline contents significantly reduced (213.90µg/g) by IAA treatments as compared to normal salt stress condition (239.95µg/g).

3.4.4. Influence of IAA treatment on proline accumulation under drought stress

Foliar application of IAA treatment on proline contents was significant in drought stressed plants (Fig 3.16). After IAA application on drought stressed plants, highest proline content was observed in Aas (545.05µg/g), followed by Sahar (410.36µg/g), Baras (401.63µg/g) and Aari (389.10µg/g) as compared to normal drought stressed plants (Fig.3.16).

3.5. Superoxide Dismutase Activity:

3.5.1. Superoxide Dismutase Activity under Salt stress

Superoxide dismutase (SOD) activity also increased under salt stress as compared to control in all four tested varieties (Fig.3.17). SOD activity under controlled condition (without stress) was higher in Baras (1.23 g^{-1}fr.wt) followed by Aari (1.11g^{-1}.fr.wt), Aas (0.53 g-1.fr.wt) and Sahar (0.35 g^{-1}.fr.wt). However under salinity conditions, the SOD activity was highest in Sahar (1.455 g^{-1}.fr.wt), followed by Aas (1.145 g^{-1}.fr.wt), Baras (1.35 g-1.fr.wt), and Aari (1.095 g^{-1}.fr.wt).

3.5.2. Superoxide dismutase activity under drought stress

SOD activity in two varieties, Aas (1.33g^{-1}.fr.wt) and Sahar (1.22g^{-1}.fr.wt) increased significantly under moisture stress as compared to control (0.53g^{-1}.fr.wt) and (0.35g^{-1}.fr.wt), respectively (Fig.3.18). In Aari (1.11g^{-1}.fr.wt), non-significant increase was noted as compared to control (1.11g^{-1}.fr.wt), while in Baras (1.23g^{-1}.fr.wt), non-significant reduction in SOD activity was observed than control (1.20g^{-1}fr.wt).

3.5.3. Deviation in SOD activity due to Indol Acetic Acid application under salt stress

Foliar application of IAA changed Superoxide dismutase activity in all four tested wheat varieties. These variations were non-significant in all varieties except Aari (Fig.3.19). In three varieties Sahar, Aas and Baras, the SOD activity increased non-significantly by IAA treatment as compared to normal stressed plants but in Aari, the SOD activity increased significantly by IAA application.

3.5.4. Variation in SOD activity by IAA treatment under moisture stress

Superoxide dismutase activity altered significantly in tested varieties by foliar application of IAA in water limiting condition as compared to normal drought stressed plants (Fig.3.20). SOD activity significantly raised in Sahar (1.58 g^{-1}.fr.wt) by IAA treatment as compare to normal moisture stress condition (1.22g^{-1}.fr.wt). Similar significant increase was observed in Aari (1.55g^{-1}.fr.wt). In Aas variety, increase in SOD activity was non-significant while Baras exhibited non-significant reduction in SOD activity.

3.6. Peroxide Dismutase Activity

3.6.1. Peroxide dismutase activity under salt stress

Peroxide dismutase (POD) activity showed peculiar behavior in tested varieties. In general, the POD activity increased under saline condition (Fig.3.21). POD activity increased significantly in Sahar ($0.024g^{-1}$.fr.wt) under salt stress as compared to control ($0.0092g^{-1}$.fr.wt). Variety Aas ($0.0113g^{-1}$.fr.wt) showed non-significant increase in SOD activity whereas Aari ($0.0103g^{-1}$.fr.wt) and Baras ($0.0108g^{-1}$.fr.wt) revealed non-significant reduction in POD activity.

3.6.2. Peroxide activity under drought stress condition

Peroxide dismutase (POD) activity increased in three varieties under drought stress except Aari, in which POD activity was decreased (Fig.3.22). The POD activity significantly rose up in Baras ($0.0232\ g^{-1}$.fr.wt), as compared to control ($0.0123g^{-1}$.fr.wt). Two other varieties under study, Sahar ($0.0162g^{-1}$.fr.wt) and Aas ($0.0159g^{-1}$.fr.wt) exhibited insignificant increase in POD activity.

3.6.3. Variation in Peroxide Dismutase activity by IAA treatment in salt stress

There was no significant effect of IAA treatment on POD activity of salt stressed varieties (Fig. 3.23). The POD activity decreased non-significantly due to IAA treatment in Sahar ($0.0193g^{-1}$.fr.wt) and Aas ($0.0055g^{-1}$.fr.wt) as compared to salt stressed normal plants ($0.024g^{-1}$.fr.wt) and ($0.0113g^{-1}$.fr.wt), respectively, while the treatment of IAA in Aari (0.0133 g-1.fr.wt) and Baras ($0.0148g^{-1}$.fr.wt) increased SOD activity non-significantly.

3.6.4. Variation in Peroxide Activity by IAA treatments in drought stress

Peroxide activity did not vary significantly by IAA treatment in drought stressed plantsas compared to non-treated drought plants. The POD activity slightly reduced in Baras and Sahar while it increased in Aas and Aari, non-significantly (Fig.3.24)

Plate 3.1: Plants of variety Aari without any treatment serve as a control

Plate 3.2. Plants of Aari grown under salts stress condition

Plate 3.3. Plants grown under moisture stress condition of Aari

Plate 3.4. Plants of cultivar Baras grown under normal condition

Plate3.5. Variety Baras grown under saline condition

Plate3.6. Wheat variety Baras grown under moisture stress

Plate3.7. Plants of variety sahar grown under control condition

Plate3. 8. Plants grown under salt stress of variety Sahar.

Plate3.9. Showing the plants of variety Sahar that are grown under water deficit

Plate 3.10. Plants of variety Aas grown under normal condition

Plate 3.11. Plants of variety Aas grown under salinity stress

Plate 3.12. Plants of variety Aas grown under moisture stress

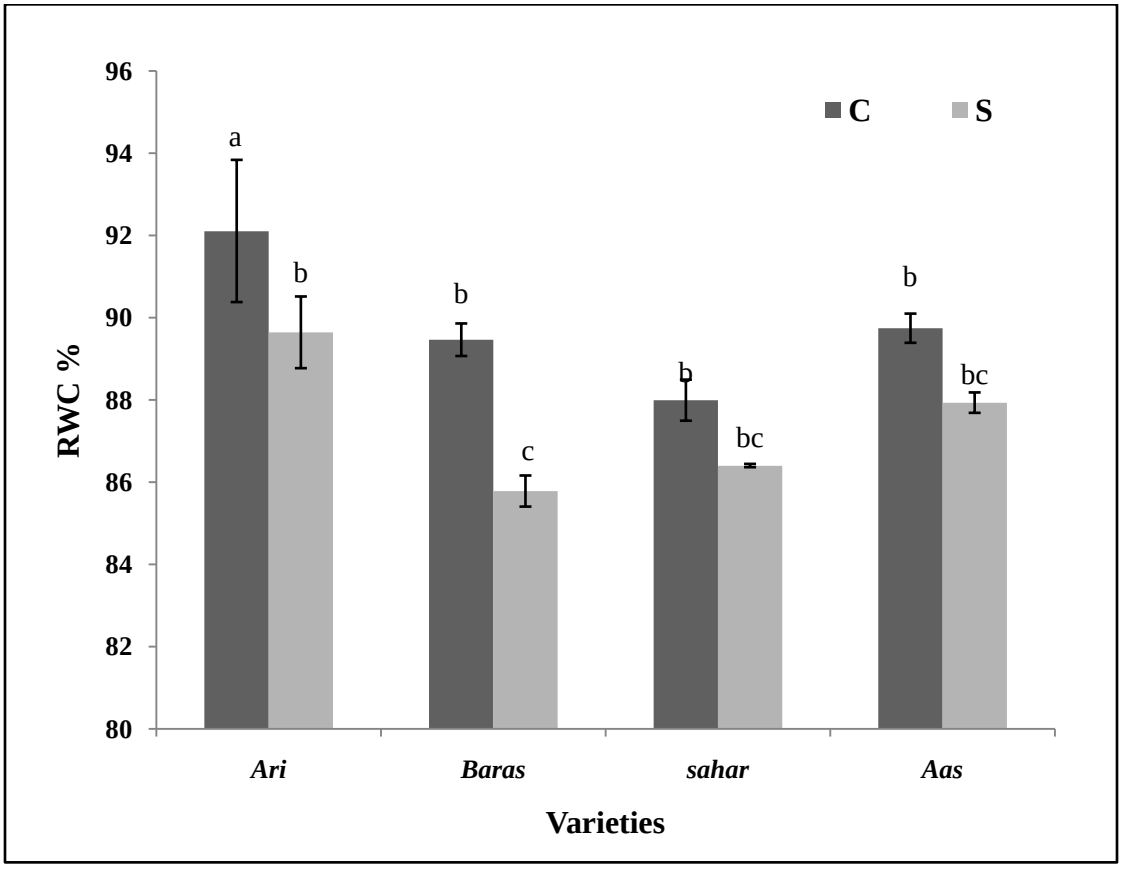


Fig. 3.1:Variation in relative water contents under 100 mMNaCl stress in four wheat varieties. Same letter (a, b, c) indicate non-significant difference at p< 0.5 level while vertical barsrepresent standard error of means (n=3).

C= Control

S= Salt Stress

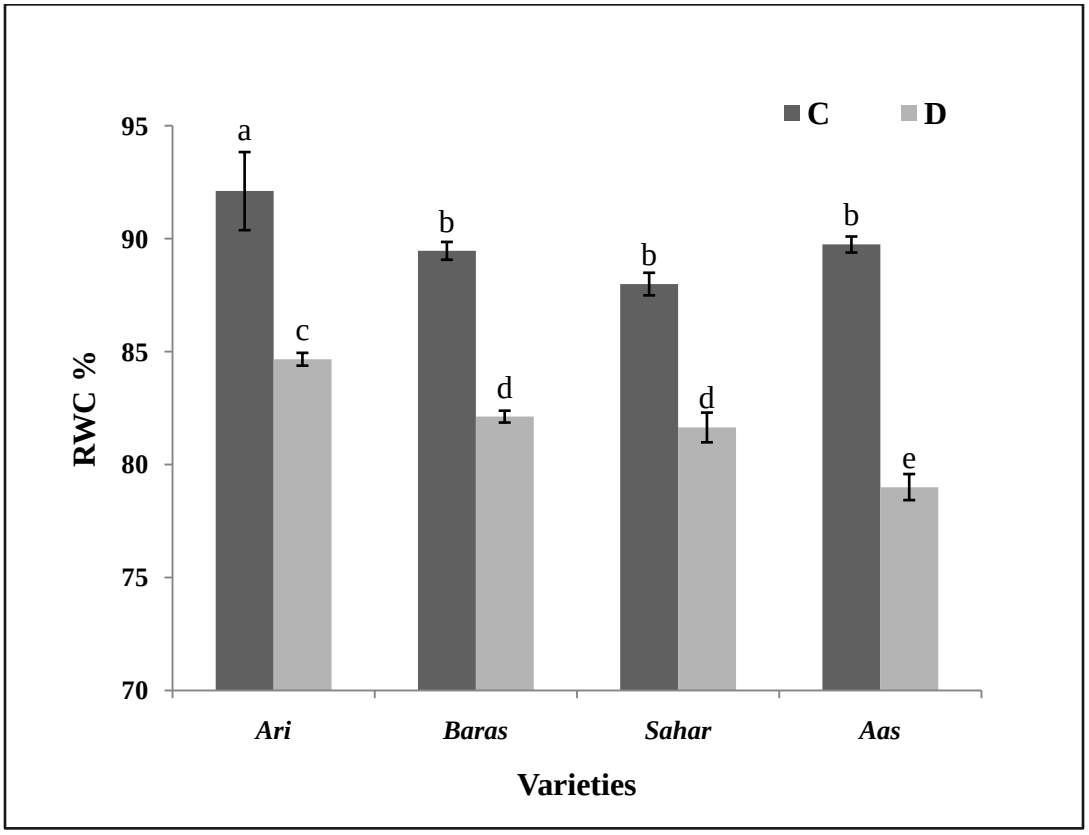


Fig. 3.2.Changes in RWC under water stress in four wheat varieties. Same letter show no significant difference p< 0.5 level while vertical bars represent standard error of means (n=3).

C= Control

D= Drought Stress

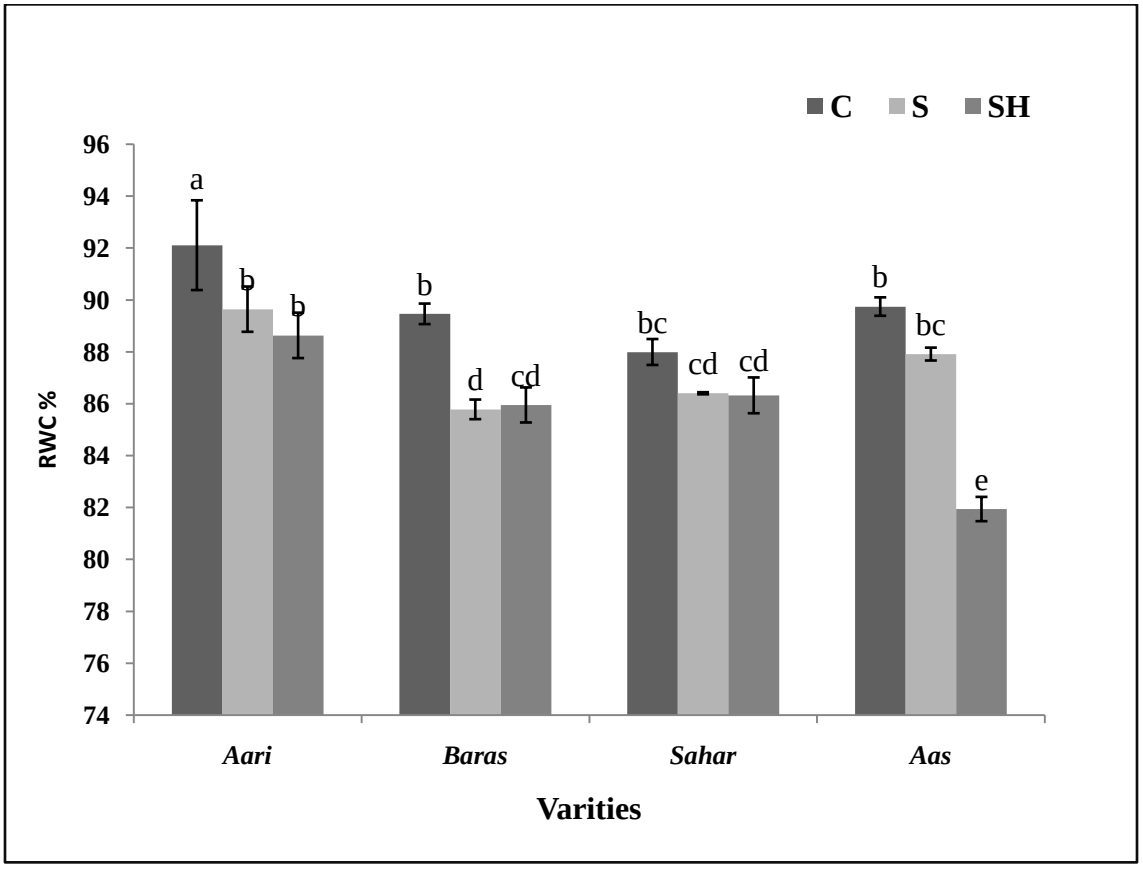


Fig. 3.3. Variation of RWCin four wheat varieties bytreatment of indole-3-aceticacid under salt stress. Same letter (a, b, c, d ,e) illustrate no significant variation at $p < 0.5$ level while vertical bars show standard error of means (n=3).

C= Control

S= Salt Stress

SH= Foliar application of IAA under salt stress

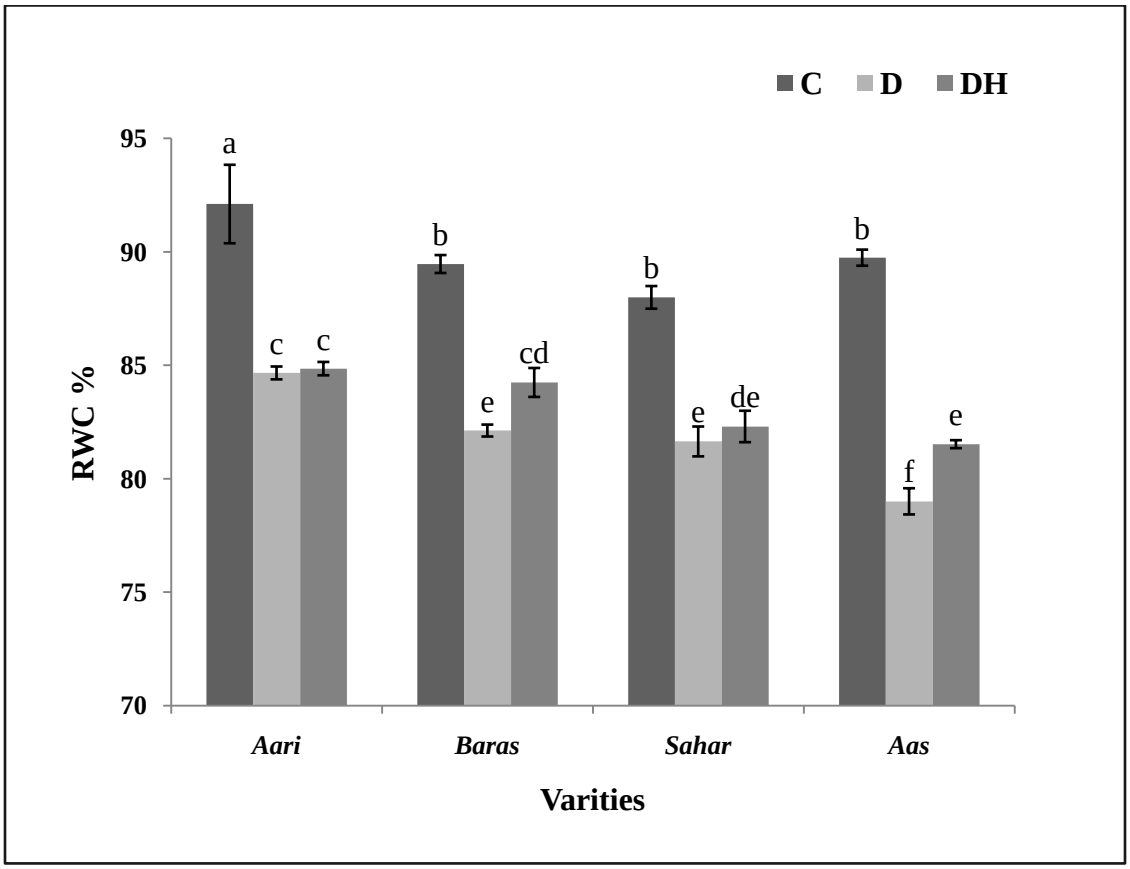


Fig. 3.4: Variation in RWC by indole-3-acetic acid application in four wheat varieties under drought stress. Same letter indicate no significant variation at $p < 0.5$ level while vertical bars represent standard error of means (n= 3).

C= Control

D= drought stress

DH= IAA application in drought stress

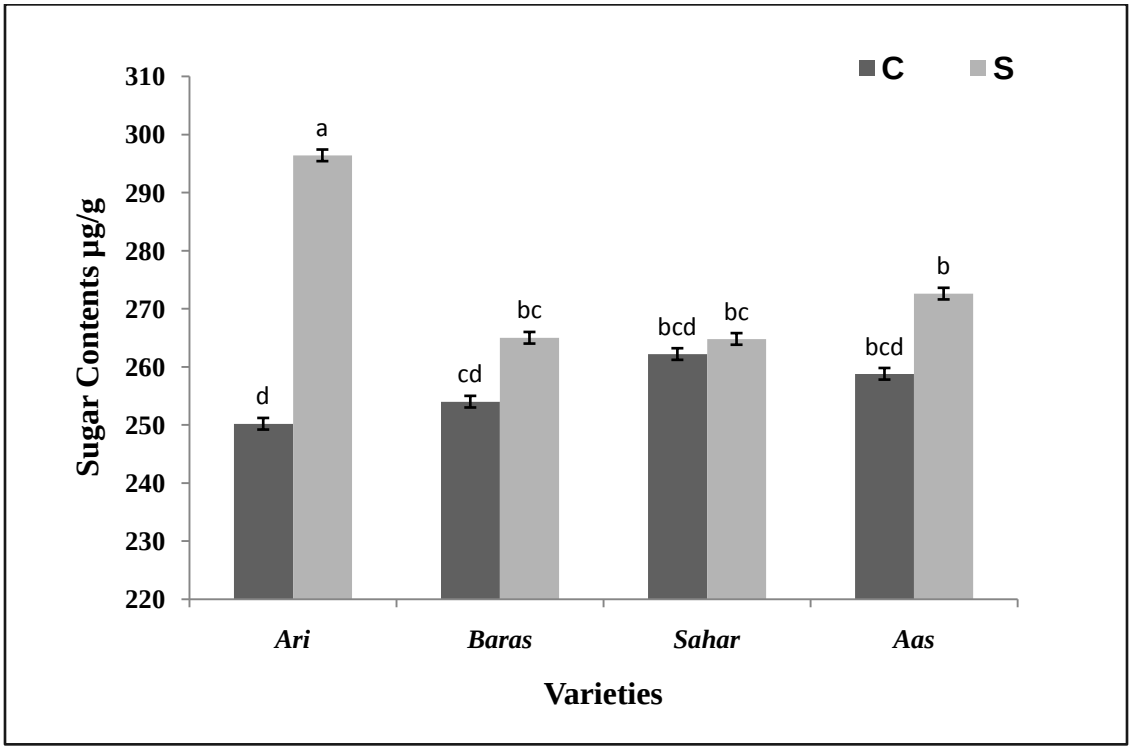


Fig. 3.5. Variation in sugar content offour wheat varieties by 100 mM saltstress. Same letter (a, b, c, d) represents no significant variation at $p < 0.5$ levelwhile vertical bars show standard error of means (n= 3).

C= Control

D= Drought Stress

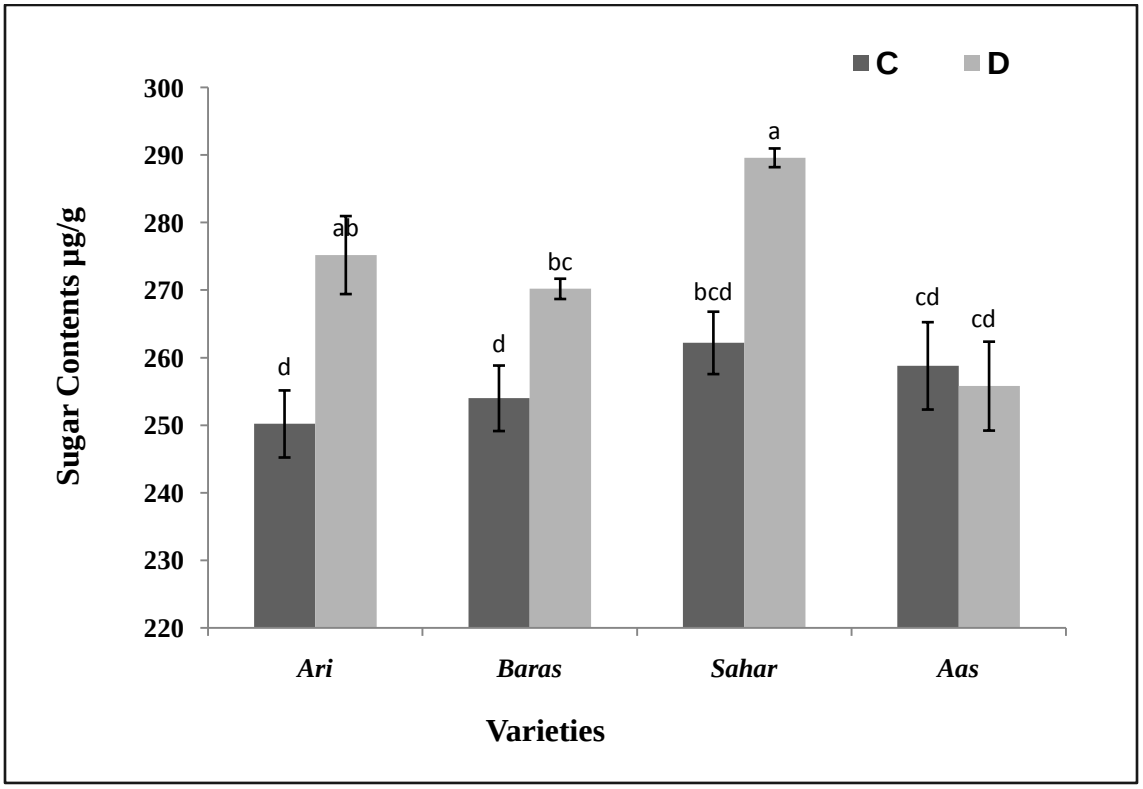


Fig 3.6. Sugar contents in four wheat varieties under moisture stress. Same letter (a, b, c, d) represent no significant variation at $p < 0.5$ level while vertical bars show standard error of means (n= 3).

C= Control

D= Drought Stress

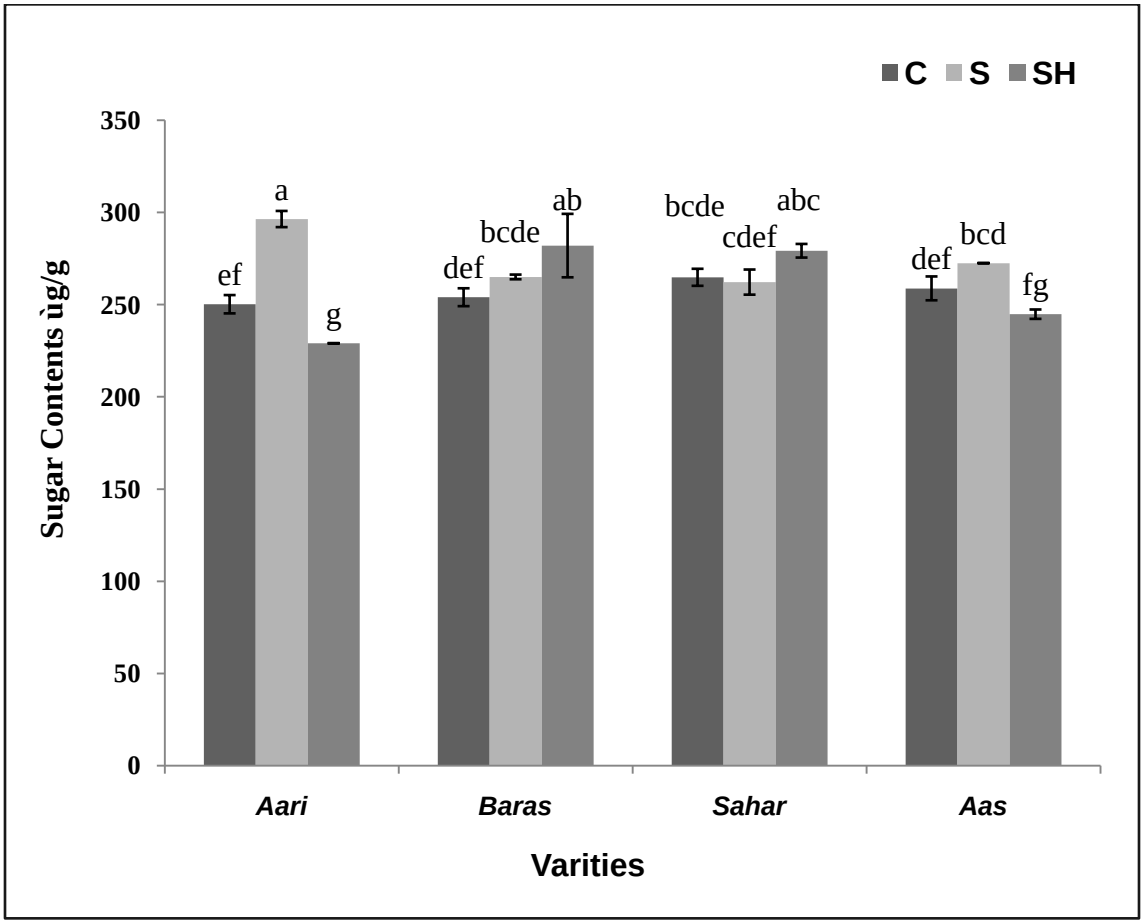


Fig. 3.7. Variation in sugar contents by foliar application of indole-3-acetic acid on saline stressed plants in four wheat varieties. Same letter (a, b, c, d, e, f, g) represent no significant variation at $p < 0.5$ level while vertical bars show standard error of means (n= 3).

C=Control

S= Saline Stress

SH= Foliar application of IAA on salt stressed plants

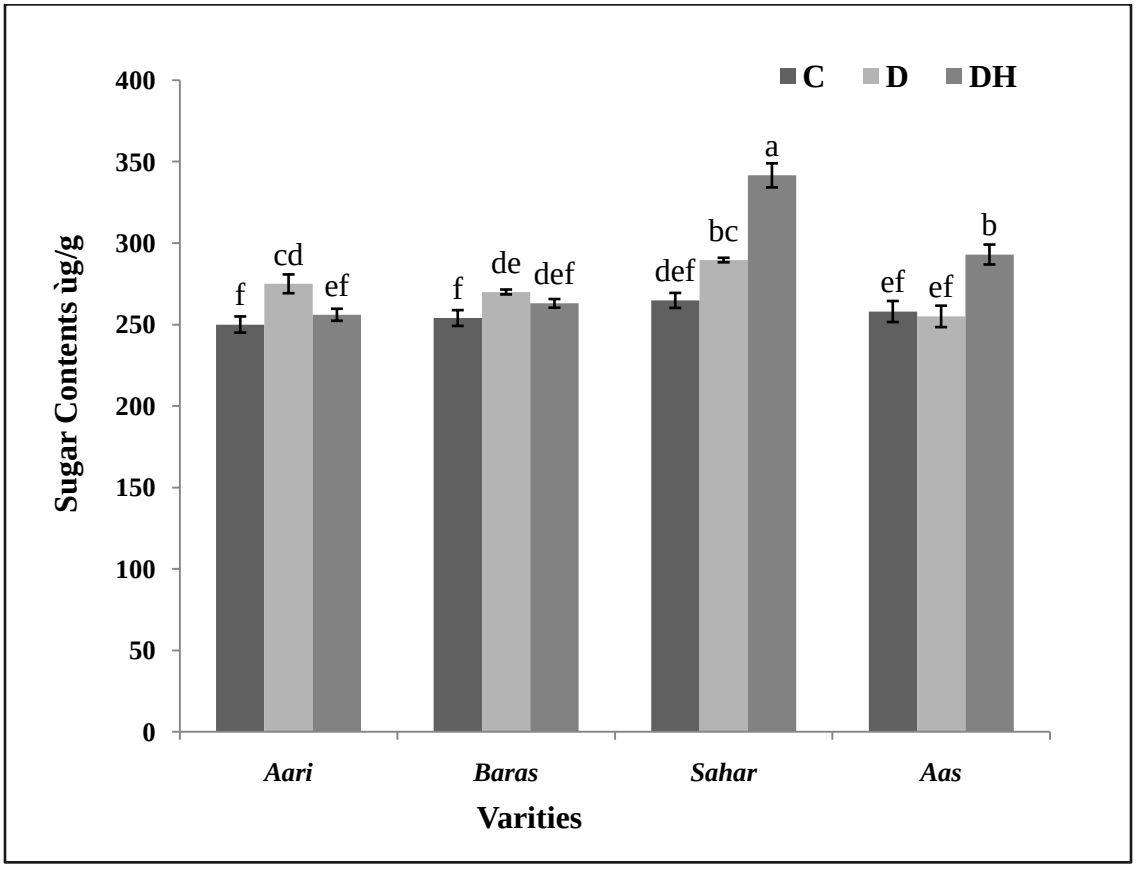


Fig 3.8. Changes in sugar contents by IAA treatments in four wheat varieties. Same letter (a, b, c, d, e, f) represent no significant variation at $p < 0.5$ level while vertical bars show standard error of means (n= 3).

C= Control

D= Drought Stress

DH= Foliar application of IAA on drought stressed plants

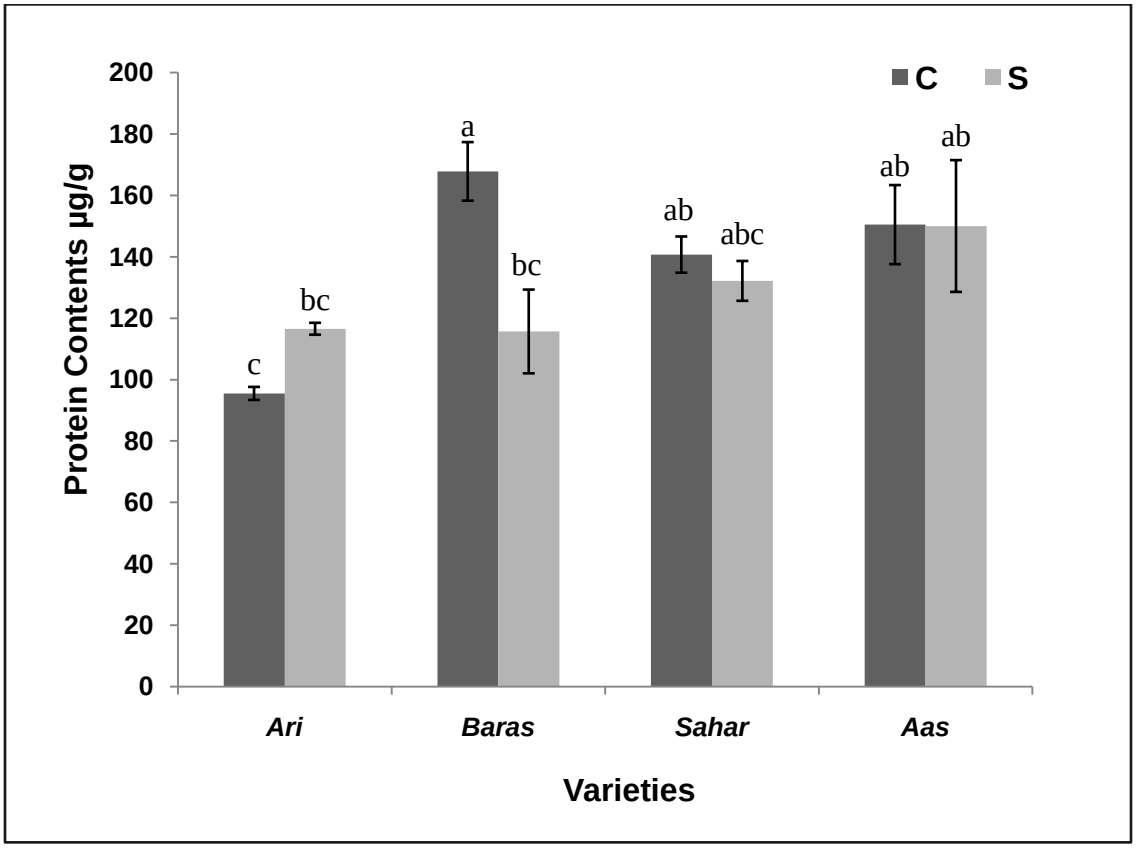


Fig 3.9. Protein contents variation under salt stress in four wheat varieties. Same letter (a, b, c) represent no significant variation at $p < 0.5$ level while vertical bars show standard error of means (n= 3).

C= Control

S= Saline Stress

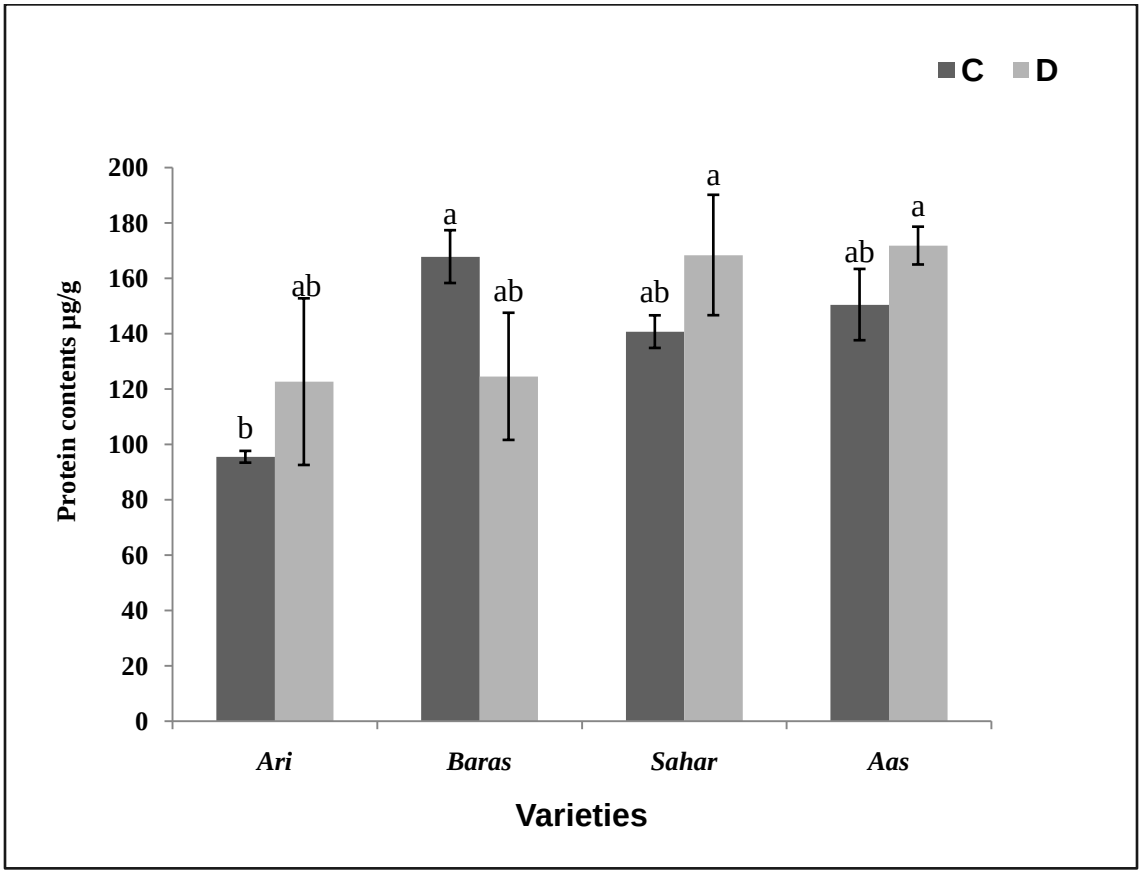


Figure 3.10. Protein contents variation in four wheat varieties under moisture stress. Same letter (a, b) represent no significant variation at $p < 0.5$ level while vertical bars show standard error of means (n= 3).

C= Control

D= Drought Stress

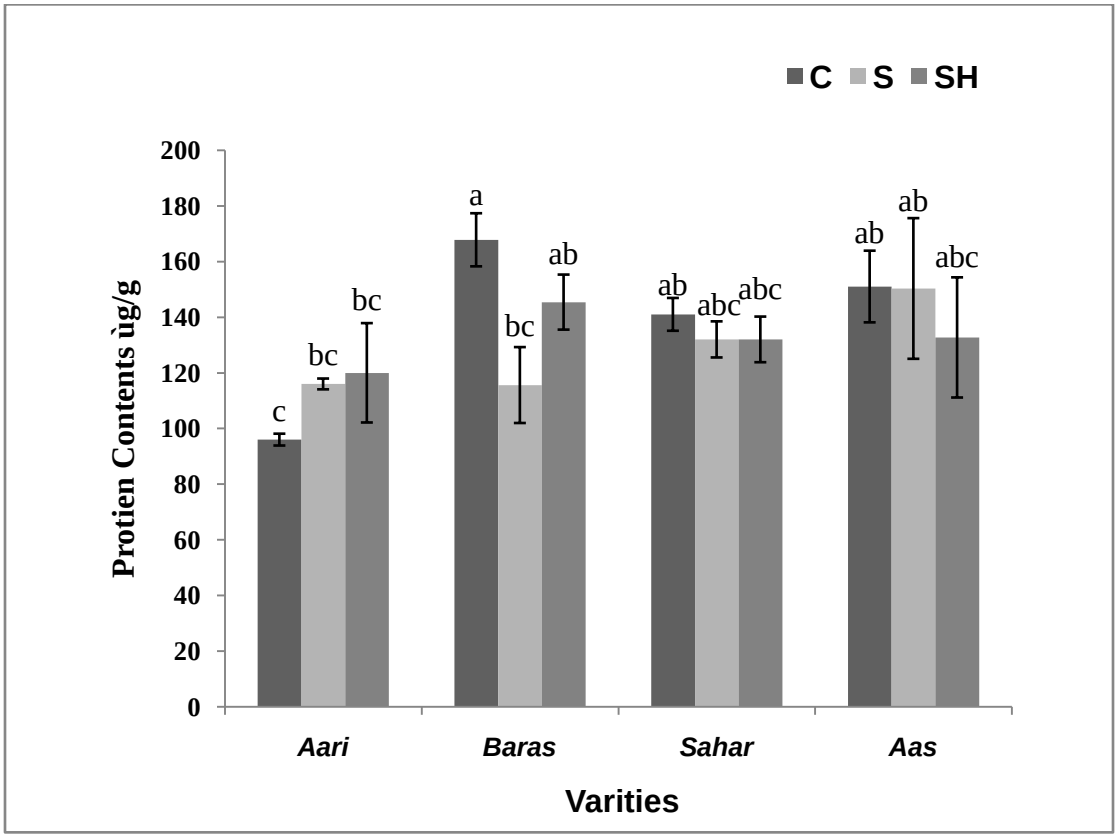


Fig. 3.11. Variation in protein contents by indole-3-acetic acid treatmentunder salt stress in four wheat varieties. Same letter (a, b, c) represent no significant variation at $p < 0.5$ level while vertical bars show standard error of means (n= 3).

C= Control

S= Salt stress

SH= Foliar application of IAA under salt stress

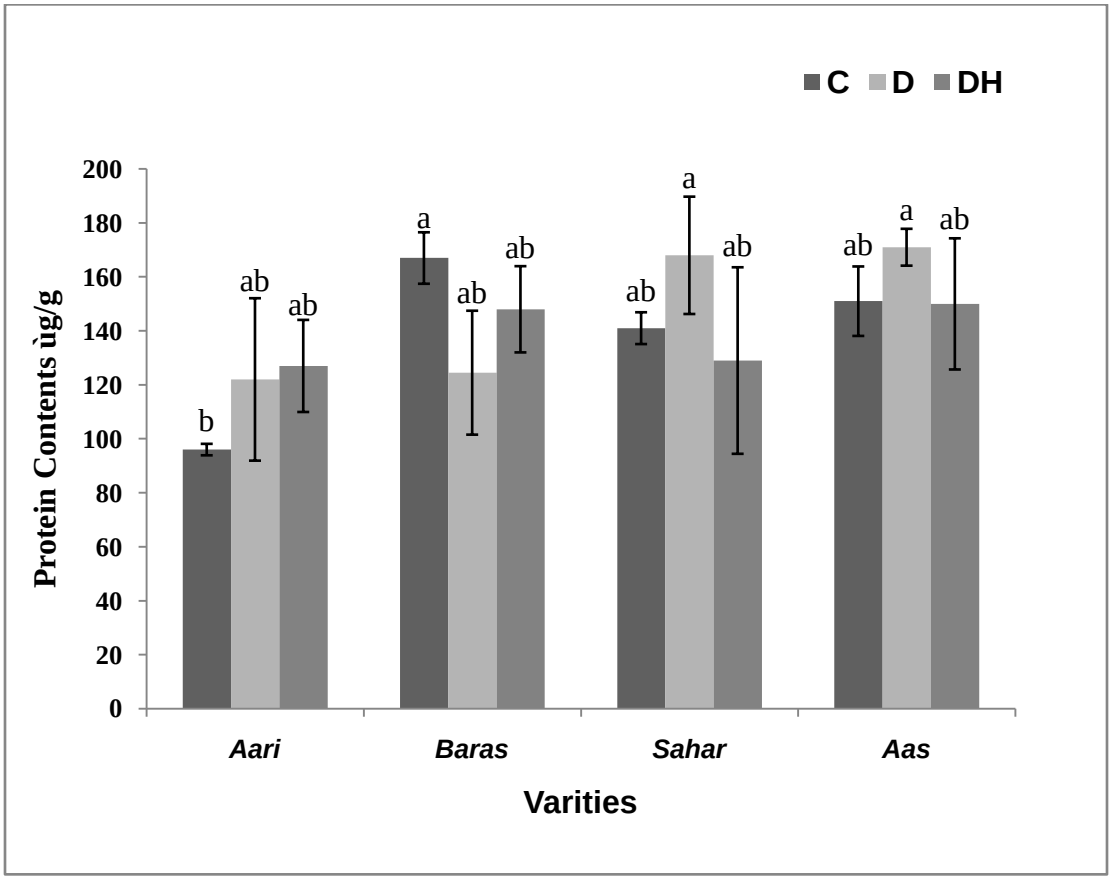


Fig. 3.12.Protein contents changesin four wheat varieties by iodol-3-acetic acid application under drought stress. Same letter (a, b) represent no significant variation at p < 0.5 level while vertical bars show standared error of means (n= 3).

C= Control

D= drought Stress

SH= IAA application under drought stress

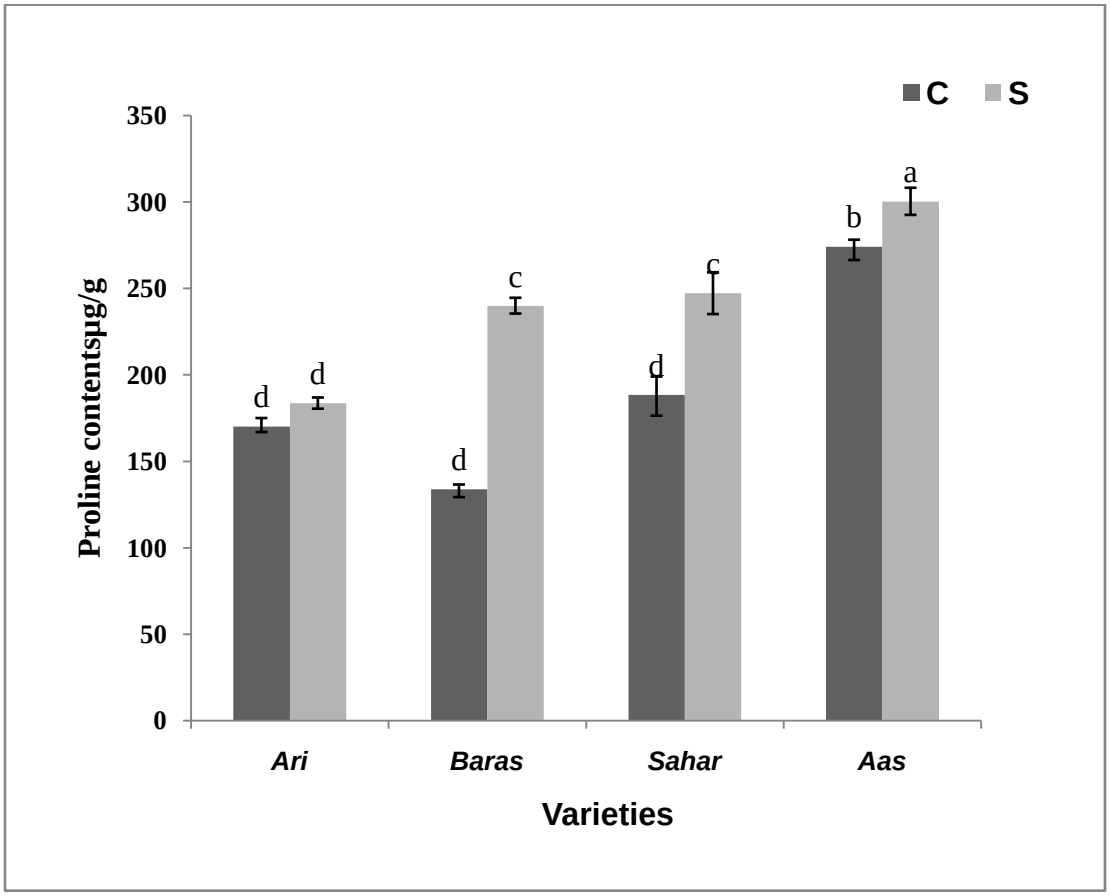


Fig. 3.13. Proline contentsin four wheat varieties under salt stress. Same letter (a, b, c, d) represent no significant variation at $p < 0.5$ level while vertical bars show standard error of means (n= 3).

C= Control

S= Salt Stress

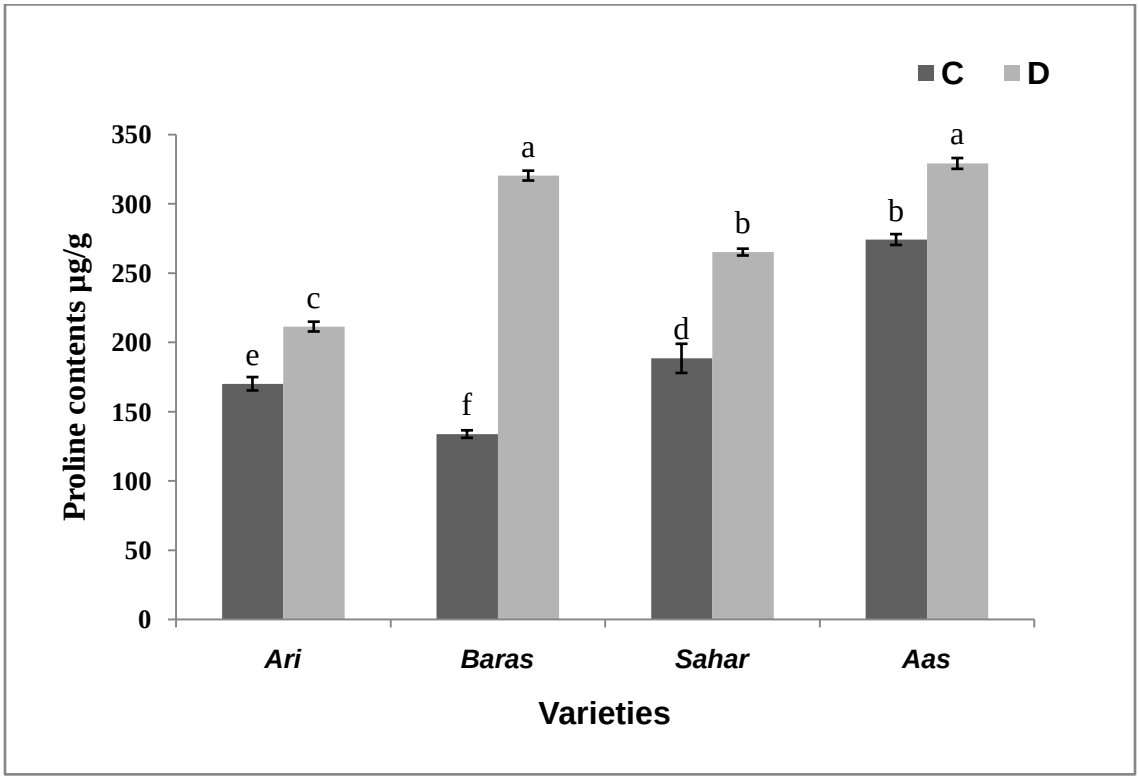


Fig. 3.14. Proline contentsin four wheat varieties under drought stress. Same letter (a, b, c, d, e, f) represent no significant variation at $p < 0.5$ level while vertical bars show standard error of means (n= 3).

C= Control

D= Drought Stress

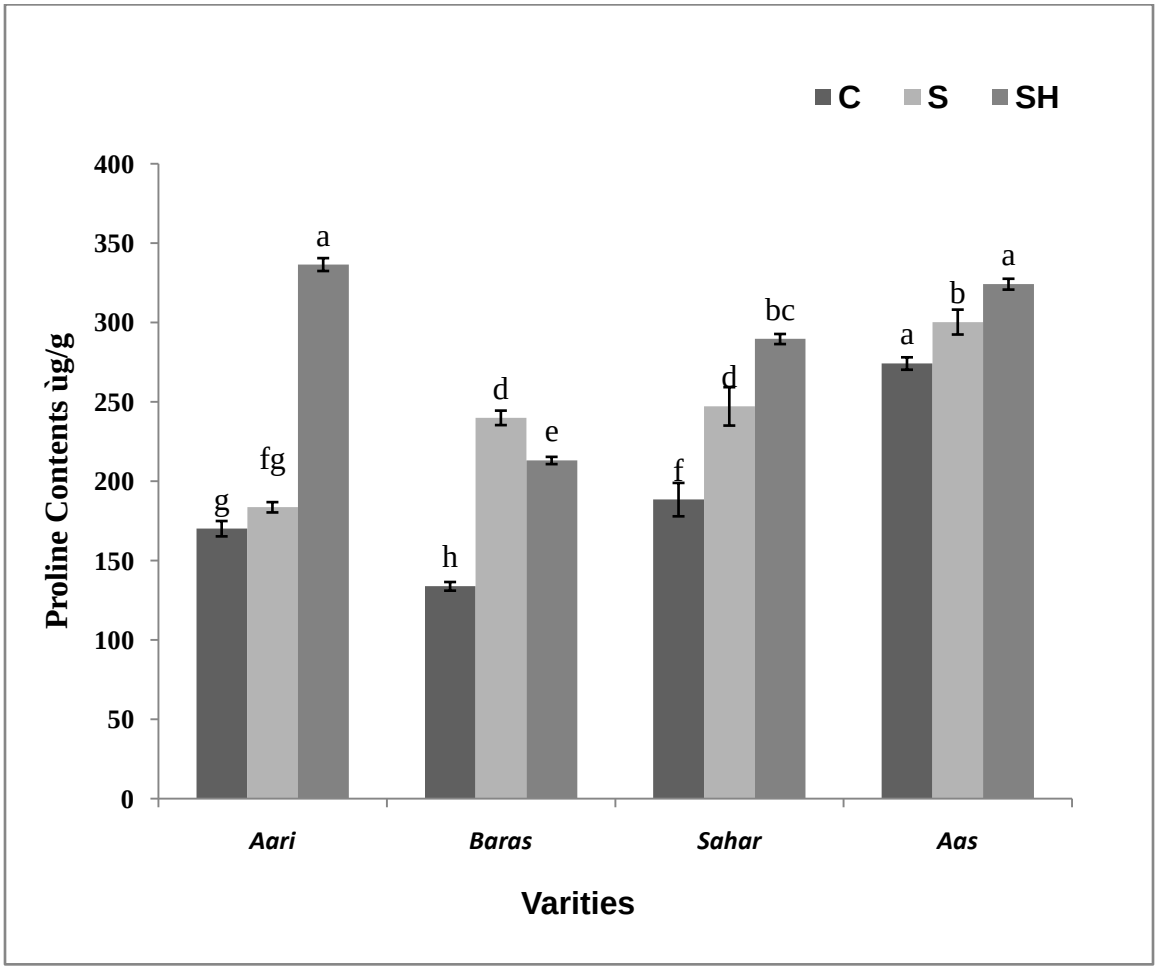


Fig. 3.15. Proline variation by foliar spry of indole-3-acetic acid under salinity stressin four wheat varieties. Same letter (a, b, c, d, e, f, g, h) represent no significant variation at $p < 0.5$ level while vertical bars show standard error of means (n= 3).

C= control

S= Salt stress

SH= Foliar application of IAAon salt stressed plants

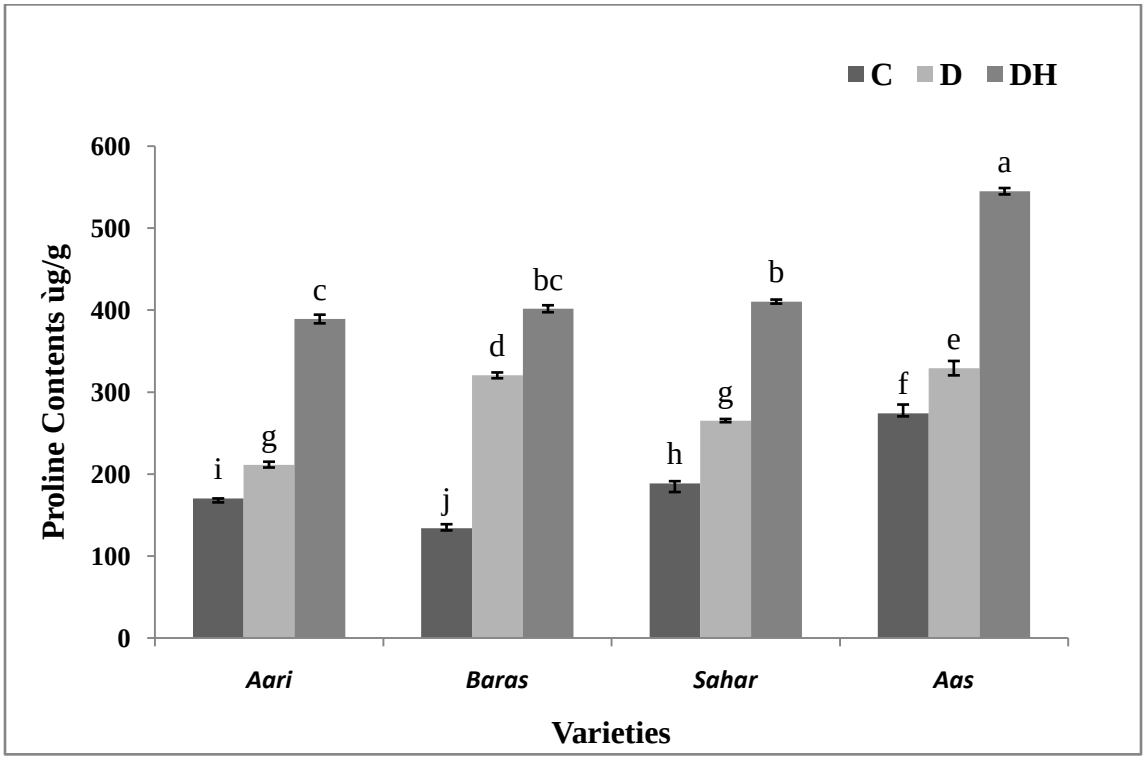


Fig. 3.16. Changes in proline contents in four wheat varieties due to indole-3-acetic acid application under drought stress. Same letter (a, b, c, d, e, f, g, h, i, j) represent no significant variation at $p < 0.5$ level while vertical bars show standard error of means (n= 3).

C= Control

D= Drought stress

DH= Foliar application of IAAon drought stressed plants

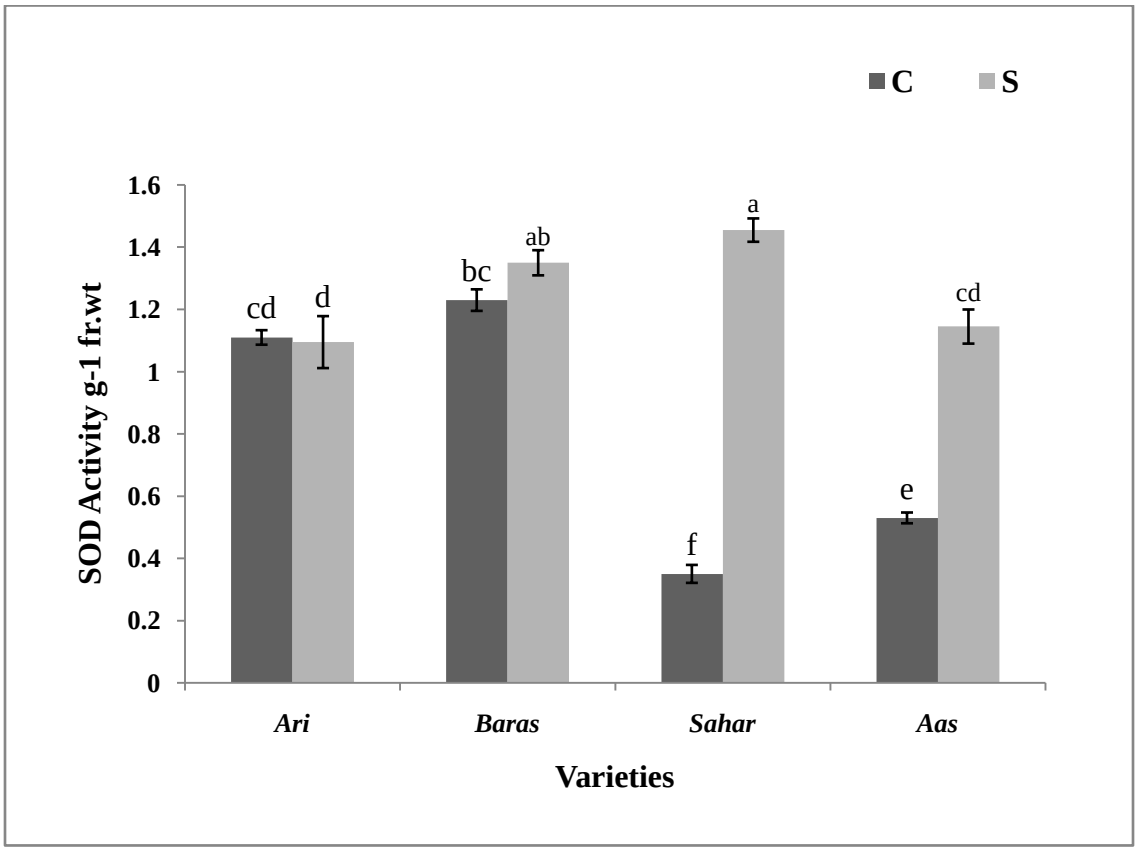


Fig. 3.17. Superoxide dismutase activity in four wheat varieties under salt stress. Same letter (a, b, c, d, e, f) represent no significant variation at $p < 0.5$ level while vertical bars show standard error of means (n= 3).

C= Control

S= Saline Stress

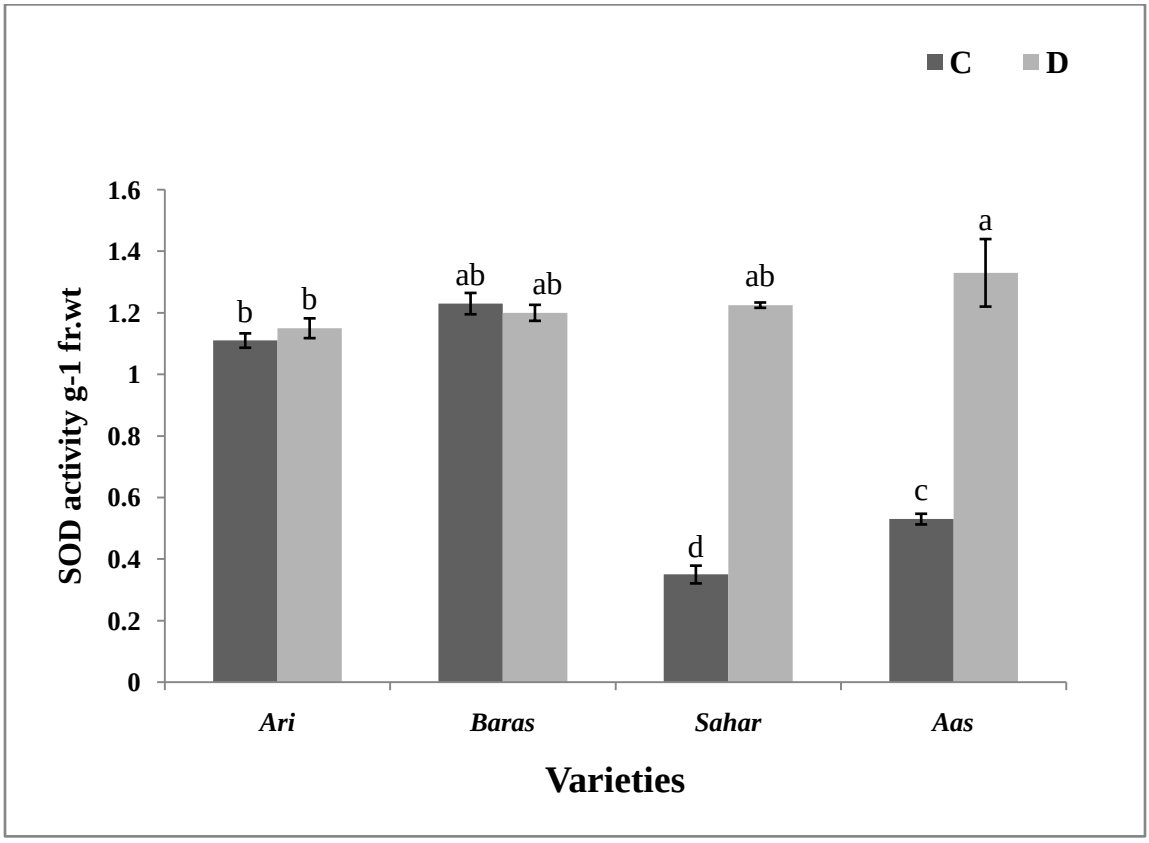


Fig. 3.18.SOD activities under drought stressin four wheat varieties. Same letter (a, b, c, d) represent no significant variation at $p < 0.5$ level while vertical bars show standard error of means (n= 3).

C= Control

D= Drought Stress

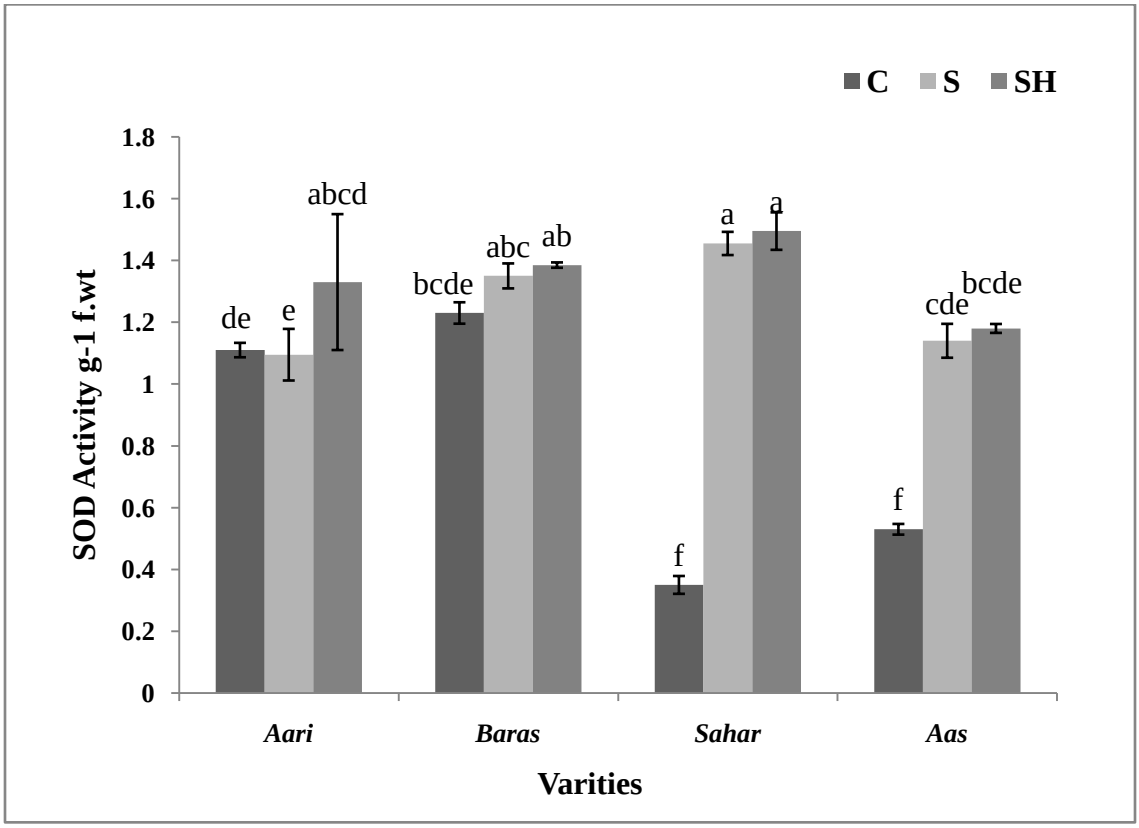


Fig 3.19. Change in SOD activity in four wheat varieties by indole-3-acetic acid application under salt stress.Same letter (a, b, c, d, e, f) represent no significant variation at $p < 0.5$ level while vertical bars show standard error of means (n= 3).

C= Control

S= Salt Stress

SH= Foliar application of IAA on salt stressed plants

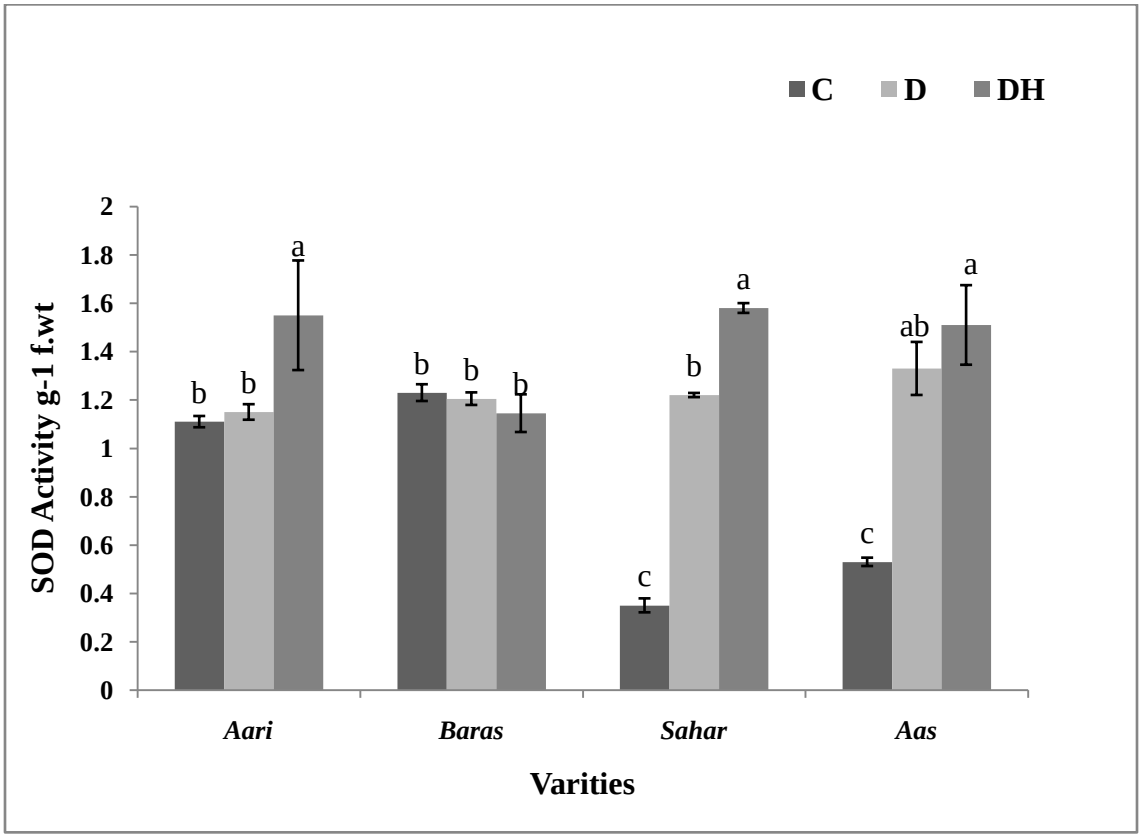


Fig. 3.20. Change in SOD activity under drought stress by indole-3-acetic acid treatment in four wheat varieties. Same letter (a, b, c) represent no significant variation at $p < 0.5$ level while vertical bars show standard error of means (n= 3).

C= Control

D= Drought Stress

DH= IAA application on drought stressed plants

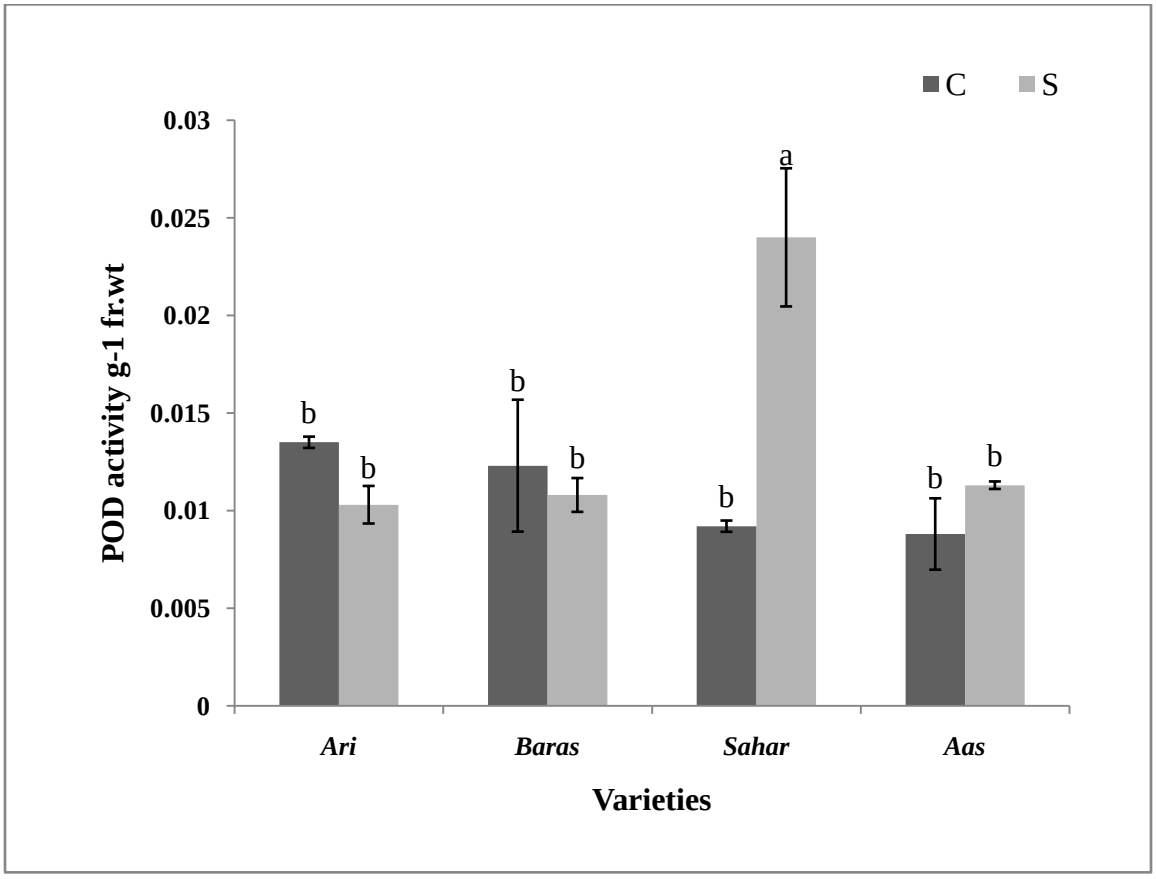


Fig. 3.21.POD activity of four wheat varieties under salt stress. Same letter (a, b) represent no significant variation at $p < 0.5$ level while vertical bars show standard error of means (n= 3).

C= Control

S= Saline Stress

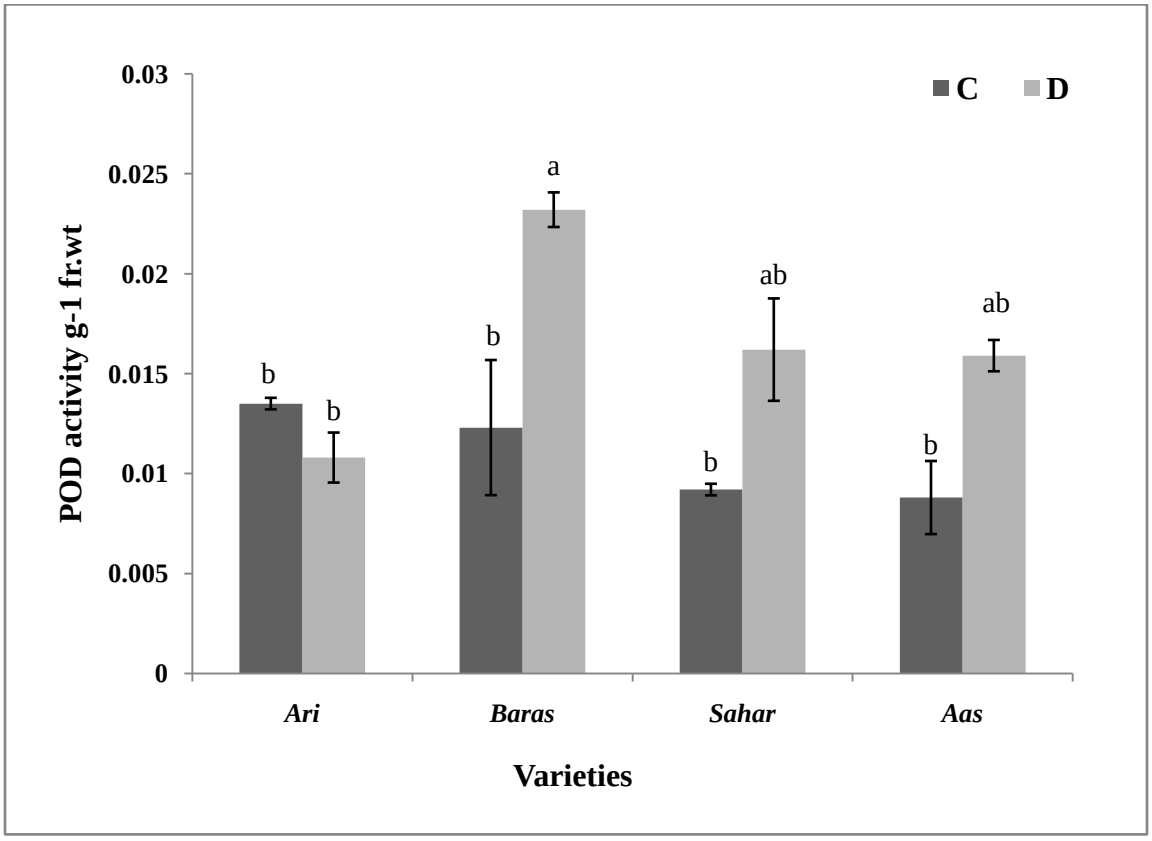


Fig. 3.22. POD activity of four wheat varieties under drought stress. Same letter (a, b) represent no significant variation at $p < 0.5$ level while vertical bars show standard error of means (n= 3).

C= control

D= Drought Stress

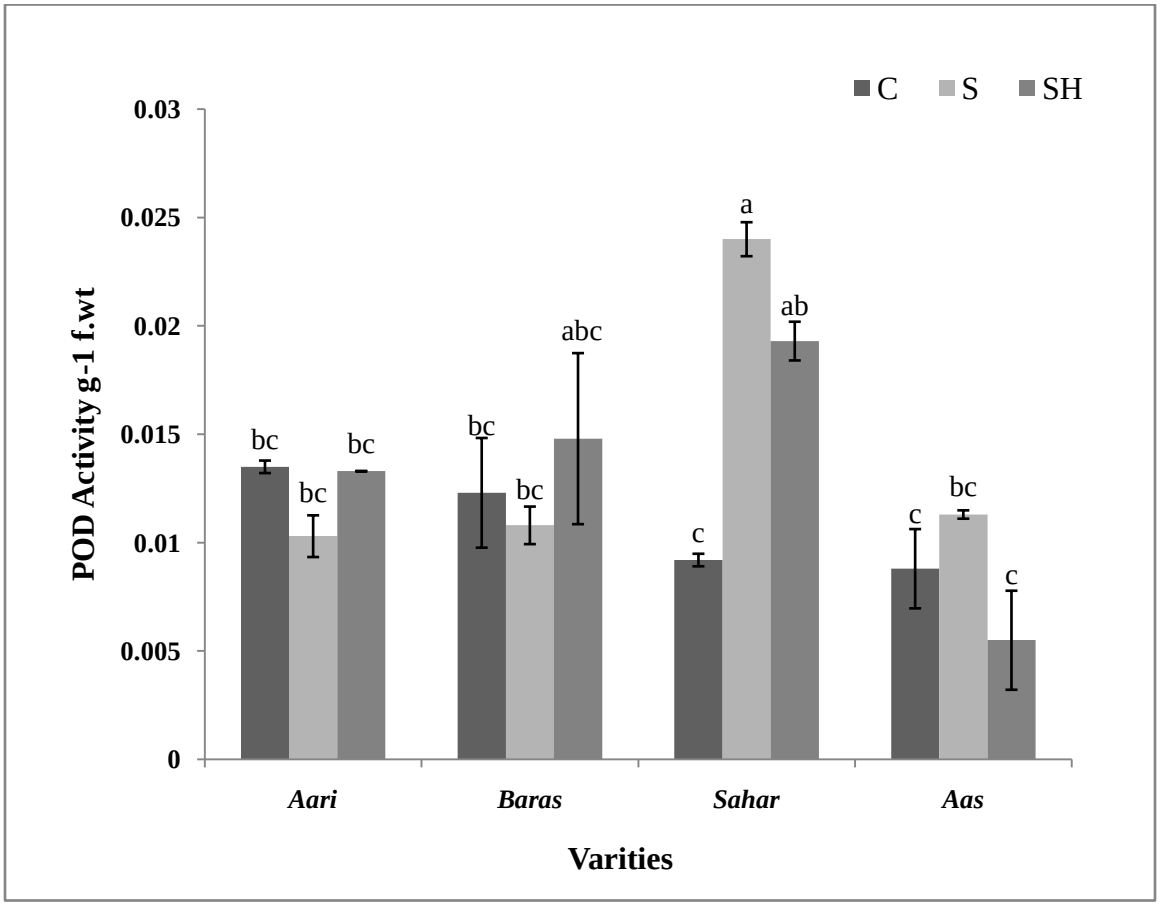


Fig.3.23. Variations in POD activity by indole-3-acetic acid treatment in four different wheat varieties Same letter (a, b, c) represent no significant variation at $p < 0.5$ level while vertical bars show standard error of means (n= 3).

C= Control

S= Salt stress

SH= Foliar application of IAA on salt stressed plants

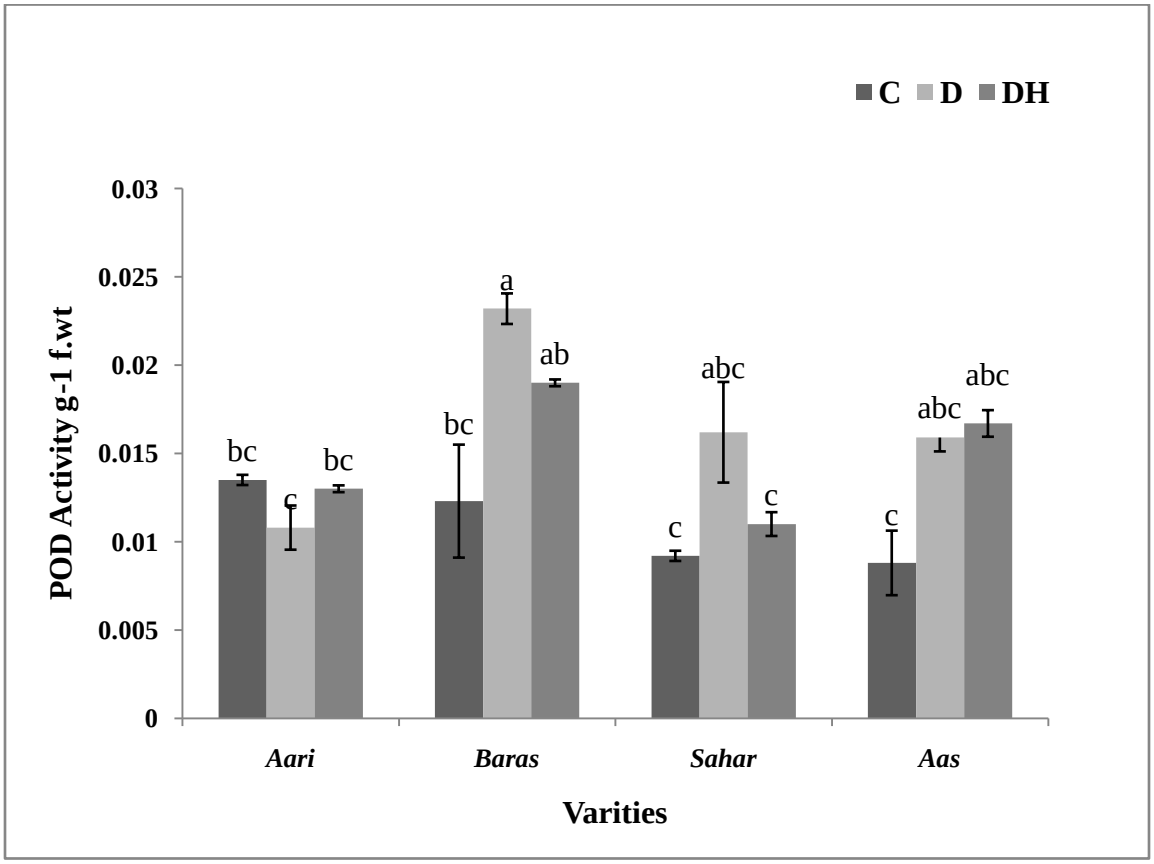


Fig.3.24. POD activities in four wheat varieties by indole-3-acetic acid treatments under drought stress. Same letter (a, b, c) represent no significant variation at $p < 0.5$ level while vertical bars show standard error of means (n= 3).

C= Control

D= Drought Stress

DH= Foliar application of IAA on drought stressed plants

Table 3.1: Basic statistics for variety Aari showing results of all physiological parameters

Treatments	Control		Drought Stress		Drought + Hormone		Salt stress		Salt + Hormone	
Parameters	**Mean**	**S.D**	**Mean**	**S.D**	**Mean**	**S.D**	**Mean**	**S.D**	**Mean**	**S.D**
Sugar contents (µg/g)	250	8.6	275	10	256	6.4	296	7.6	229	0.2
Total Protein Contents (µg/g)	95.5	3.6	123	52.1	127	29.6	116	3.4	120	31.0
RWC %	92.1	2.99	84.7	0.5	84.8	0.5	90	1.5	88.6	1.5
Proline Contents(µg/g)	170	8.37	211	6.1	389	9.1	184	5.6	336	7.03
SOD Activity (g-1fr. Wt.)	1.1	0.04	1.15	0.05	1.5	0.07	1.09	0.14	1.3	0.38
POD Activity (g-1fr. Wt.)	0.01	0.0	0.01	0.0	0.01	0.0	0.01	0.0	0.01	0.0

Table 3.2: Basic statistics for variety Baras showing results of all physiological parameters

Treatments	Control		Drought Stress		Drought + Hormone		Salt stress		Salt + Hormone	
Parameters	**Mean**	**S.D**	**Mean**	**S.D**	**Mean**	**S.D**	**Mean**	**S.D**	**Mean**	**S.D**
Sugar contents (µg/g)	254	8.4	270	2.6	263	4.6	265	2.2	282.6	20.1
Protein Contents (µg/g)	168	16	124	39.8	148	27.7	116	23.2	145.4	17.1
RWC %	89	0.68	82	0.45	84.2	1.10	85.8	0.65	85.95	1.17
Proline Contents (µg/g)	139	5	362	6.14	401	7.35	240	7.9	213.09	3.95
SOD Activity (g^{-1}fr.wt)	1.23	0.06	1.21	0.05	1.15	0.135	1.35	0.07	1.385	0.015
POD Activity (g^{-1}fr.wt)	0.01	0.00	0.023	0.00	0.02	0.00	0.011	0.00	0.0148	0.00

Table 3. 3: Basic statistics for variety Sahar showing results of all physiological parameters

Treatments	**Control**		**Drought Stress**		**Drought + Hormone**		**Salt stress**		**Salt + Hormone**	
Parameters	**Mean**	**S.D**	**Mean**	**S.D**	**Mean**	**S.D**	**Mean**	**S.D**	**Mean**	**S.D**
Sugar contents(µg/g)	265	8	289	2.4	341	12.8	262	11.8	279.2	6.4
Total Protein Contents(µg/g)	141	10.2	168	37.7	129	59.87	132	11.2	131.59	14.2
RWC %	88	0.86	82	1.14	82.3	1.2	86.4	0.07	86.32	1.19
Proline Contents(µg/g)	188	18.2	410	3.43	209	4.25	247	20.9	289.62	5.5
SOD Activity (g-1 fr.wt)	0.35	0.05	1.26	0.02	1.58	0.04	1.46	0.06	1.495	0.1
POD Activity (g-1 fr.wt)	0.01	0.00	0.02	0.00	0.008	0.00	0.02	0.00	0.02	0.00

Table 3. 4: Basic statistics for variety Aas showing results of all physiological parameters

Treatments	**Control**		**Drought Stress**		**Drought + Hormone**		**Salt stress**		**Salt + Hormone**	
Parameters	**Mean**	**S.D**	**Mean**	**S.D**	**Mean**	**S.D**	**Mean**	**S.D**	**Mean**	**S.D**
Sugar contents (µg/g)	258	11.2	256	11.4	293	10.6	272.6	.2	244.4	4.4
Protein Contents (µg/g)	150	22.4	172	11.8	150	42.102	150.36	55	132.71	39
RWC %	89.7	0.06	79	1	81.5	0.3	87.93	0.43	81.94	0.81
Proline Contents (µg/g)	274	6.77	329	6.75	545	15.2	300.3	13.58	324.18	5.9
SOD Activity (g-1 fr.wt)	0.53	0.03	1.33	0.19	1.52	0.28	1.145	.09	1.18	0.02
POD Activity (g-1 fr.wt)	0.008	0.001	0.0159	0.00	0.016	0.00	0.0113	0.0001	0.005	0.00

Chapter 4

Discussion

4.1. Relative water contents

Our results indicate that the each variety responds differently for their relative water contents (RWC) under drought and salt stresses. Relative water contents decrease in plants when these are subjected to abiotic stresses. Results represent significant variation in RWC in different wheat varieties under salt and drought stress (Fig. 3.1, 3.2). Data indicates that Sahar and Aas genotype performed better than Aari and Baras. This reduction in RWC under drought and salt stress is due to decrease in water uptake under salt stress and increase rate of transpiration than absorption rate under drought stress as reported by earlier studies(Balouchi, 2010). Salt stress also caused ions homeostatic imbalance, resulted in hindrance of water uptake in studied varieties as reported by Khan and Panda, (2008).When plants face water deficit condition the rate of water absorption decreased than the transpiration rate, due to which water potential decreased in cells and turgor preasure decrease in plants and plants leaves become loosed (Plates 3.3, 3.6, 3.9, 3.12) match with the findings of (Sairam and Saxena, 2000;Al-HakimiMannoveux, 1993; Tambussi, 2000).

Our study showed that foliar application of indole-3-acetic acid gave positive relationship in Sahar, Baras and Aas under abiotic stresses. In these varieties by IAA treatment the RWC could not reduce significantly as compared to untreated plants (Fig. 3.3, 3.4). Prakash and Prathapasenan, (1990) reported decreased IAA concentration under environmental stress. Therefore, exogenous application of IAA provided an attractive approach to counter the stress conditions. There was a little information available about the mechanism of IAA to alleviate the abiotic stress. Sadeghipour and Aghaei, (2012) stated that abiotic stress inhibit the synthesis of growth promoting phytoharmone and accelerate the degradation of IAA. The best option to overcome the deficiency of IAA is exogenous treatment of IAA. Our results also indicate that Sahar and Aas are tolerant cultivar.

4.2. Soluble Sugar Contents

Sugars are vital for plants growth and metabolism, as source of energy and structural components. Many genes expression are affected by sugar contents such as photosynthesis, glycolysis, nitrogen metabolism, cell cycle and defense mechanism. In four tested varieties, the soluble sugar accumulation increased and this increase indicates environmental stress (Fig. 3.5, 3.6). Increase in sugar contents is an adoptive mechanism in plants to minimize the deleterious effect of environmental stress. Increased in sugar contents under salt and drought stress due to activation of α-amylase which breakdown starch into soluble sugars because sugar molecules act as osmolytes to maintain water as well as homeostatic balance in plant cells and in tolerant varieties the soluble sugar contents accumulation more as compared to susceptible.Our results are in line with the finding of (Keyvan, 2010; Jalal *et al*, 2012; Bolarin*et al*, 1995; Kerepesi and Galiba, 2000).

In current study foliar application of indole-3-acetic acid (IAA) significantly increased the soluble sugar contents in Sahar, Baras and Aas because IAA application enhanced the activity ofα-amylase as well as photosynthesis also enhanced as compared to normal stressed plants (Fig. 3.7, 3.8). By foliar application of IAA, sugar contents increased of SaharandAas, IAA treatment also increased soluble sugar contents in treated plants ofBaras varietyas compared tonon-treated salinity stressed plants. Under adverse environmental condition, IAA treatment increased sugar contents in varieties Sahar and Aas to overcome deleterious effects of abiotic stresses. Similar results have been reported earlier(Devi *et al*, 2012;Sivakumar, 2002).

4.3. Total Protein Contents

In current research, all four varieties demonstrate variation in soluble protein contents (Fig. 3.9, 3.10). Maximum decrease in protein contents was observed in Baras under saline and moisture stress while total proteins contents in Aari, Sahar and Aas were increased. These results revealed that Aari, Sahar and Aas are tolerant varieties while Baras is susceptible to abiotic stresses. Increase in protein contents signifies activation of plant defense mechanism and synthesised protein to maintain and stiblized metabolic,

chemical, and physioloical processes to counteradverse conditions. These findings match with the earlier research (Mishra *et al.*,2011; Sock*et al.*, 1990).

Foliar application of IAA exhibited peculiar influence on protein contents (Fig. 3.11, 3.12). In our investigation, reduction in protein contents specify increased enzymatic activity under unfavorable environmental condition that degrades protein into peptides and amino acids to provide energy and maintain the osmotic adjustment in tolrants varieties same result obtained earlier(Bakrim*et al*,2007; Ramakrishna, 2005;Shutov and Vaintraub, 1987; Müntz*et al*., 2001). Exogeniousapplication of IAA has a direct effect on the enzymes activity.This mightactivates those genes that control the enzymaticfunctioning.

4.4.Proline Contents

Accumulation of proline under salt and moisture limitation is the first response of plants (Ashraf and Foolad, 2007). Proline contents significantly rose up in four tested varieties in environmental stress such as salt and water stress (Fig. 3.25,26). Accumulation of proline contents enhanced in all tested varieties, under salt as well as moisture stress (Fig. 3.13, 3.14). Increasing trend in proline contents is an adoptive mechanism in wheat to maintain the osmotic adjustment in cytoplasm under saline and drought stresses and scavenging the free radicals that are generated under adverse environmental condition. Current research showed similar trend with the finding of Ashraf and Foolad,(2007). Proline is an osmolyte as well as it is involved in stabilizing sub-cellular structures (e.g. proteins and membranes), buffering cellular redox potential, and scavenging free radical (Ashraf and Foolad, 2007). Prolineis also involved in provoking expression of salt stress responsive genes which acquire proline receptive elements in their promoters (Satoh *et al*., 2002;Oono*et al* 2003;Chinnumsamv*et al.*, 2005). Our findings are in confirmation with previous research(Srinivas and Balasubramanian, 1995; Aziz *et al*., 1999;Munns and Tester, 2008). Accumulation of proline is a mechanism of protection to counteract osmotic potential created by the salt stress (Chen *et al*., 2007; Hoque*et al*., 2008). Through our findings we conclude that proline content is an important indicator to distinguish the stress tolerant and stress susceptible varieties. Misraand Gupta (2005)reported higher prolineaccumulation in

tolerant plants than sensitive plants. Increase in proline contents reflect environmental stress on plants.

Foliar application of IAA significantly influenced proline synthesis in studied varieties (Fig. 3.15, 16). In current study, data indicate that IAA treatment increased proline contents in Aari, Sahar and Aas as compared to untreated plants whereas in Baras IAA treatment decreasedproline contents under salt stress and increased in drought stress as compared to untreated plants (Fig. 3.27, 3.28). Exogenous application of IAA play important role to alleviate abiotic stress by increasing proline contents. Environmental stress such as drought and salt stress altered hormonal balance in plants. Moisture and salinity cause a significant reduction in IAA concentration in plants (Xie*et al*., 2003Prakash and Prathapasenan, 1990). Exogenous application of IAA ehanced the synthesis of proline contents or retards the activity of those enzymes that degrade proline contents as reported earlier(Bano and Yasmeen,2010;Vardhini, 2012; Ozdemir*et al*, 2004).

4.5. Superoxide Dismutase (SOD)

In present study, our findings show increased SOD activity under environmental stress conditions (Fig. 3.17, 3.18). Under unfavorable environmental condition ROS are generated and SOD activity increases to scavenge properly and protect plants against ROS damage to various membranes, especially superoxide radical. Similar results reported by numerous scientists(Esfandiari*et al.,* 2007; Zhao *et al.,* 2006;Candan and Tarhan, 2003; Martinez *et al.,* 2001;Mittler, 2000;Scandalios, 1993 and Sen Gupta *et al.,* 1993).

Results of foliar spray of plant growth regulator IAA under 100 mMNaCl treatment and drought stress showed (Fig. 3.31, 3.32). The IAA treatment insignificantly increased the SODs activity in Sahar,Aas and Baras under 100 mMNaCl stress while in Aari, SODs activity decreased. Under drought stress IAA application gave positive results. SODs activity increased in Aari, Sahar, and Aas as compared to untreated plants while in Baras insignificant decreased was observed (Fig. 3.19, 3.20). Unfavorable environmental stress alters ions homeostasis in wheat cultivar, resulting in ion toxicity and osmotic stress that generate ROS in plants that cause oxidative stress. Exogenous

applications of phytoharmones are the best option to alleviate oxidative stress by increasing the activity of SODs in plants. ROS productions more under adverse conditions, IAA treatment accelerate the defensemechanisms of plant especiallyenzymes activity that protect plant from the damage of free radicals our investigations are in confirmation with earlier research (Arfan,2009; Chen and Li, 2001;Mittler, 2002).

4.6. Peroxide Dismutase Activity

In current study, results show that POD activity increased inSahar and Aas under saline and drought stress. While inBarasthe POD activity increased under moisture stress and decreased under salt stress but in Aari POD activity reduced under both stresses (Fig. 3.21, 3.22). Adverse environmental stress causes osmotic and homeostatic imbalance in tested varieties, resulting oxidative stress. Under oxidative stress activity of antioxidant enzymes e.g. SOD, POD and catalase is more in tolerant varieties than susceptible varieties (Misan*et al*., 2006; Csiszá*et al.*, 2008; Bano*et al.*, 2012).

4.7.Conclusions

Experiment was conducted to determine tolerance to saline and drought conditions and also response of auxin in ameliorating adverse environmental conditions in four wheat varieties. Different physiological parameters like relative water contents, sugar contents, leaf protein contents, proline contents and antioxidant enzyme essayhave been measured. Considerable variation exists among these parameters. On the basis of results of these parameters,we concluded that

- Saharis the most tolerant to drought stress followed by Baras, Aari and Aas.
- Under saline stress,Sahar is the most tolerant to salinity followed by Aas, Aari and Baras.
- Foliar application of auxinenhanced tolerance by positively affecting four tested varieties.

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