



Plant-Environment Interaction

Responses and Approaches to Mitigate Stress

Edited by
Mohamed Mahgoub Azooz • Parvaiz Ahmad



WILEY Blackwell

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Mohamed Mahgoub Azooz

South Valley University, Egypt

Parvaiz Ahmad

S.P. College, Srinagar, India

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This edition first published 2016 © 2016 by John Wiley & Sons, Ltd.

Registered Office

John Wiley & Sons, Ltd, The Atrium, Southern Gate, Chichester, West Sussex, PO19 8SQ, UK

Editorial Offices

9600 Garsington Road, Oxford, OX4 2DQ, UK

The Atrium, Southern Gate, Chichester, West Sussex, PO19 8SQ, UK

111 River Street, Hoboken, NJ 07030-5774, USA

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Library of Congress Cataloging-in-Publication Data

Names: Azooz, M. M., editor. | Ahmad, Parvaiz, editor.

Title: Plant-environment interaction : responses and approaches to mitigate stress / edited by Mohamed Mahgoub Azooz, South Valley University, Egypt; Parvaiz Ahmad, S.P. College, Srinagar, India.

Description: Hoboken : John Wiley & Sons, 2016. | Includes index.

Identifiers: LCCN 2015037818 | ISBN 9781119080992 (cloth)

Subjects: LCSH: Plants--Effect of stress on. | Plant ecophysiology.

Classification: LCC SB112.5.P533 2016 | DDC 632/.1--dc23 LC record available at <http://lccn.loc.gov/2015037818>

A catalogue record for this book is available from the British Library.

Wiley also publishes its books in a variety of electronic formats. Some content that appears in print may not be available in electronic books.

Cover image: ©iStockphoto.com/artisteer

Set in 8.5/12pt Meridien by SPi Global, Pondicherry, India

Contents

- List of contributors, vii
- Preface, x
- About the editors, xii
- 1** Biotechnological applications to improve salinity stress in wheat, 1
Sami ullah Jan, Ghulam Kubra, Mehreen Naz, Ifrah Shafqat, Muhammad Asif Shahzad, Fakiha Afzal and Alvina Gul Kazi
 - 2** Soybean under abiotic stress: Proteomic approach, 28
Arafat Abdel Hamed Abdel Latef, Sumaira Jan, Elsayed Fathi Abd-Allah, Bushra Rashid, Riffat John and Parvaiz Ahmad
 - 3** Proteomic analysis of food crops under abiotic stresses in the context of climate change, 43
P. S. Sha Valli Khan, P. Osman Basha, G. Vijaya Lakshmi, M. Muniraja, K. Sergeant and J. F. Hausman
 - 4** Transcriptome modulation in rice under abiotic stress, 70
Smita Kumar and Prabodh Kumar Trivedi
 - 5** Sulphur: Role in alleviation of environmental stress in crop plants, 84
Dagmar Procházková, Daniela Pavlíková and Milan Pavlík
 - 6** Proline and glycine betaine modulate cadmium-induced oxidative stress tolerance in plants: Possible biochemical and molecular mechanisms, 97
Mohammad Anwar Hossain, David J. Burritt and Masayuki Fujita
 - 7** Enhancement of vegetables and fruits growth and yield by application of brassinosteroids under abiotic stresses: A review, 124
Bojjam Vidya Vardhini
 - 8** Physiological mechanisms of salt stress tolerance in plants: An overview, 141
Hadi Pirasteh-Anosheh, Gholamhassan Ranjbar, Hassan Pakniyat and Yahya Emam
 - 9** Heat stress in wheat and interdisciplinary approaches for yield maximization, 161
Sajjad Hussain, Muhammad Jamil, Abdul Aziz Napar, Rida Rahman, Asghari Bano, Fakiha Afzal, Alvina Gul Kazi and Abdul Mujeeb-Kazi
 - 10** Effect of elevated CO₂ and temperature stress on cereal crops, 184
Ashutosh Tripathi, Devendra Kumar Chauhan, Gopal S. Singh and Niraj Kumar
 - 11** Lipid metabolism and oxidation in plants subjected to abiotic stresses, 205
Adriano Sofo, Antonio Scopa, Abeer Hashem and Elsayed Fathi Abd-Allah
 - 12** Physiological response of mycorrhizal symbiosis to soil pollutants, 214
Mercedes García-Sánchez, I. García-Romera, J. A. Ocampo and E. Aranda
 - 13** Microbially derived phytohormones in plant adaptation against abiotic stress, 234
Dilfuza Egamberdieva
 - 14** Synergistic interactions among root-associated bacteria, rhizobia and chickpea under stress conditions, 250
Dilfuza Egamberdieva, Anvar Abdiev and Botir Khaitov
 - 15** Plant secondary metabolites: From molecular biology to health products, 263
L. F. De Filippis
 - 16** Medicinal plants under abiotic stress: An overview, 300
Sameen Ruqia Imadi, Alvina Gul Kazi, Abeer Hashem, Elsayed Fathi Abd-Allah, A. A. Alqarawi and Parvaiz Ahmad

- 17** Signalling roles of methylglyoxal and the involvement of the glyoxalase system in plant abiotic stress responses and tolerance, 311
Tahsina Sharmin Hoque, Mohammad Anwar Hossain, Mohammad Golam Mostofa, David J. Burritt and Masayuki Fujita
- 18** Role of sedges (Cyperaceae) in wetlands, environmental cleaning and as food material: Possibilities and future perspectives, 327
Sanjay Mishra, Ashutosh Tripathi, Durgesh Kumar Tripathi and Devendra Kumar Chauhan
Index, 339

List of contributors

Elsayed Fathi Abd-Allah

Department of Plant Production
College of Food and Agricultural Sciences
King Saud University
Riyadh, Saudi Arabia

Anvar Abdiev

Karshi Institute of Agricultural Engineering and Economics
Uzbekistan

Fakiha Afzal

Atta-ur-Rahman School of Applied Biosciences (ASAB)
National University of Sciences and Technology (NUST)
Islamabad, Pakistan

Parvaiz Ahmad

Department of Botany
S.P. College
Srinagar, Jammu and Kashmir, India

A. A. Alqarawi

Department of Plant Production
College of Food and Agricultural Sciences
King Saud University
Riyadh, Saudi Arabia

E. Aranda

Department of Soil Microbiology and Symbiotic Systems
Estación Experimental del Zaidín (CSIC)
Granada, Spain

Asghari Bano

Department of Plant Science
Faculty of Biological Sciences
Quaid-i-Azam University
Islamabad, Pakistan

P. Osman Basha

Department of Genetics and Genomics
Yogi Vemana University
Vemanapuram, Kadapa, India

David J. Burritt

Department of Botany
University of Otago
Dunedin, New Zealand

Devendra Kumar Chauhan

D D Pant Interdisciplinary Research Laboratory
Department of Botany
University of Allahabad
Allahabad, India

L. F. De Filippis

School of the Environment, Faculty of Science
University of Technology
Sydney, Australia

Dilfuza Egamberdieva

Institute for Landscape Biogeochemistry
Leibniz Centre for Agricultural Landscape Research (ZALF)
Müncheberg, Germany

Yahya Emam

College of Agriculture
Shiraz University
Shiraz, Iran

Masayuki Fujita

Laboratory of Plant Stress Responses
Department of Applied Biological Science
Faculty of Agriculture
Kagawa University
Kagawa, Japan

I. García-Romera

Department of Soil Microbiology
and Symbiotic Systems
Estación Experimental del Zaidín (CSIC)
Granada, Spain

Mercedes García-Sánchez

Department of Agro-Environmental Chemistry
and Plant Nutrition
Faculty of Agrobiological, Food and Natural Resources
Prague, Czech Republic

Abeer Hashem

Department of Botany and Microbiology,
Faculty of Science
King Saud University
Riyadh, Saudi Arabia

J. F. Hausman

Environment Research and Innovation Department (ERIN)
Luxembourg Institute of Science and Technology
Luxembourg

Tahsina Sharmin Hoque

Department of Soil Science
Bangladesh Agricultural University
Mymensingh, Bangladesh

Mohammad Anwar Hossain

Department of Genetics and Plant Breeding
Bangladesh Agricultural University
Mymensingh, Bangladesh

Sajjad Hussain

Wheat Wide Crosses Program
National Agricultural Research Center (NARC)
Islamabad, Pakistan

Sameen Ruqia Imadi

Atta-ur-Rahman School of Applied Biosciences (ASAB)
National University of Sciences and Technology (NUST)
Islamabad, Pakistan

Muhammad Jamil

Department of Botany
University of Sargodha
Sargodha, Pakistan

Sami ullah Jan

Atta-ur-Rahman School of Applied Biosciences (ASAB)
National University of Sciences and Technology (NUST)
Islamabad, Pakistan

Sumaira Jan

Center for Research and Development (CORD)
University of Kashmir
Srinagar, Jammu and Kashmir, India

Riffat John

Department of Botany
University of Kashmir
Srinagar, Jammu and Kashmir, India

Alvina Gul Kazi

Atta-ur-Rahman School of Applied Biosciences (ASAB)
National University of Sciences and Technology (NUST)
Islamabad, Pakistan

Botir Khaitov

Faculty of Agronomy
Tashkent State University of Agriculture
Tashkent, Uzbekistan

P. S. Sha Valli Khan

Department of Botany
Yogi Vemana University
Vemanapuram, Kadapa, India

Ghulam Kubra

Atta-ur-Rahman School of Applied Biosciences (ASAB)
National University of Sciences and Technology (NUST)
Islamabad, Pakistan

Niraj Kumar

National Academy of Sciences
Allahabad, India

Smita Kumar

CSIR-National Botanical Research Institute (CSIR-NBRI)
Rana Pratap Marg, Lucknow, India
and
Department of Biochemistry
University of Lucknow
Lucknow, India

G. Vijaya Lakshmi

Department of Botany
Yogi Vemana University
Vemanapuram, Kadapa, India

Arafat Abdel Hamed Abdel Latef

Department of Botany
Faculty of Science at Qena
South Valley University
Qena, Egypt

Sanjay Mishra

D D Pant Interdisciplinary Research Laboratory
Department of Botany
University of Allahabad
Allahabad, India

Mohammad Golam Mostofa

Laboratory of Plant Stress Responses
Department of Applied Biological Science
Faculty of Agriculture
Kagawa University
Kagawa, Japan

Abdul Mujeeb-Kazi

Department of Botany
University of Sargodha
Sargodha, Pakistan

M. Muniraja

Department of Botany
Yogi Vemana University
Vemanapuram, Kadapa, India

Abdul Aziz Napar

Department of Plant Science
Faculty of Biological Sciences
Quaid-i-Azam University
Islamabad, Pakistan

Mehreen Naz

Department of Bioinformatics and Biotechnology
International Islamic University
Islamabad, Pakistan

J. A. Ocampo

Department of Soil Microbiology and Symbiotic Systems
Estación Experimental del Zadín (CSIC)
Granada, Spain

Hassan Pakniyat

College of Agriculture
Shiraz University
Shiraz, Iran

Milan Pavlík

Institute of Experimental Botany
Academy of Sciences of the Czech Republic
Prague, Czech Republic

Daniela Pavlíková

Faculty of Agrobiological, Food and Natural Sources
Czech University of Life Sciences
Prague, Czech Republic

Hadi Pirasteh-Anosheh

National Salinity Research Center
Yazd, Iran

Dagmar Procházková

Institute of Experimental Botany
Academy of Sciences of the Czech Republic
Prague, Czech Republic

Gholamhassan Ranjbar

National Salinity Research Center
Yazd, Iran

Bushra Rashid

Center of Excellence in Molecular Biology
University of Punjab
Lahore, Pakistan

Rida Rahman

Atta-ur-Rahman School of Applied Biosciences (ASAB)
National University of Sciences and Technology (NUST)
Islamabad, Pakistan

Antonio Scopa

School of Agricultural, Forestry, Food and
Environmental Sciences
University of Basilicata
Potenza, Italy

K. Sergeant

Environment Research and Innovation Department (ERIN)
Luxembourg Institute of Science and Technology
Luxembourg

Ifrah Shafqat

Department of Bioinformatics and Biotechnology
International Islamic University
Islamabad, Pakistan

Muhammad Asif Shahzad

Atta-ur-Rahman School of Applied Biosciences (ASAB)
National University of Sciences and Technology (NUST)
Islamabad, Pakistan

Gopal S. Singh

Institute of Environment and Sustainable Development
Banaras Hindu University
Varanasi, India

Adriano Sofo

School of Agricultural, Forestry, Food and Environmental
Sciences
University of Basilicata
Potenza, Italy

Ashutosh Tripathi

D D Pant Interdisciplinary Research Laboratory
Department of Botany
University of Allahabad
Allahabad, India
and
National Academy of Sciences
Allahabad, India

Durgesh Kumar Tripathi

Centre of Advanced Study in Botany
Banaras Hindu University
Varanasi, India

Prabodh Kumar Trivedi

CSIR-National Botanical Research Institute (CSIR-NBRI)
Rana Pratap Marg, Lucknow, India

Bojjam Vidya Vardhini

Department of Botany
Telangana University
Nizamabad, India

Preface

As we know, several thousand years ago man was a hunter, struggling for his daily needs. Today, human-kind has created every comfort for life. However, as we have progressed, our environment has been diminished and is still deteriorating at an alarming rate. Industrialization, urbanization and changing lifestyles have created more severe problems for the environment. Many disasters, including cyclones, floods, tornadoes, drought, etc. are due to changes in the environment and man is directly responsible for these disasters because of overexploitation of natural resources. Due to this degradation of the environment, crop production is decreasing and it will be very difficult to feed this growing population in the near future.

So our concern is to obtain maximum food production from limited resources. This book summarizes the pros and cons of these environmental effects on crop production. It will also discuss how to get maximum yield from limited resources.

The book is composed of 18 chapters. Chapter 1 deals with biotechnological applications to improve salinity stress in wheat. Molecular markers and defence responses of wheat plants against salinity are also discussed. Chapters 2 and 3 provide insights into proteomic approaches in soybean and other food crops under abiotic stress. Proteomics under various abiotic stresses are very well explained in these two chapters. Chapter 4 discusses the transcriptome modulation in rice under abiotic stress. Chapter 5 explains the role of sulphur in mitigating different stresses in crop plants. Here the authors have described sulphur assimilation and its role under heavy metal stress, salinity, drought, etc. Chapter 6 throws light on the modulation of oxidative stress induced by cadmium through the use of proline and glycinebetaine. Chapter 7 deals with the role of brassinosteroids as potential enhancers of growth and yield of fruits and vegetables. The authors also highlight the impact of brassinosteroids on vegetables and fruits under environmental stresses. Chapter 8 is about

physiological mechanisms of salt stress tolerance in plants. Here the author explains the adverse impact of salinity on plants, mechanisms of salinity tolerance, seed priming for higher salinity tolerance and foliar application of salicylic acid. Chapter 9 deals with the effect of heat stress on performance of wheat plants. Mineral activity during heat stress and interdisciplinary approaches to improve heat tolerance in wheat are also discussed. Chapter 10 is about the effect of elevated CO₂ and temperature stress on cereal crops. Stress responses, tolerance and molecular approaches for high yield and gene expression are also explained. Chapter 11 discusses lipid metabolism and oxidation in plants under abiotic stress. Chapter 12 covers the role of mycorrhizal symbiosis in heavy metal phytoremediation. Chapter 13 deals with microbially derived phytohormones in plant adaptation against abiotic stress. Chapter 14 is about synergistic interactions among root-associated bacteria, rhizobia and chickpea under stress conditions. Chapter 15 deals with plant secondary metabolites, their effect on growth and development of plants, molecular genetics and impact on humans. Chapter 16 discusses the effect of abiotic stresses on different medicinal plants. Chapter 17 throws light on signalling roles of methylglyoxal and the involvement of the glyoxalase system in plant abiotic stress responses and tolerance. Chapter 18 deals with the role of sedges in wetlands, environmental cleaning and as food material.

This volume presents extensive information regarding crop plants, their growth and development, physiological and molecular responses, and adaptability to different environmental stresses. Chapters contributed in this book have been published keeping the author's justifications intact; however, suitable editorial changes were made where considered necessary. We have tried our best to gather information on different aspects of this extensive topic but there is a possibility that errors have still crept in, for which we seek the reader's indulgence and feedback. We are extremely thankful to

contributors because without their contribution this volume would have not been possible. We are also grateful to John Wiley and Sons, particularly Gudrun Walter (Editorial Director, Natural Sciences), Audrie Tan (Project Editor), Laura Bell (Assistant Editor), and all the other staff members of Wiley who were directly or

indirectly associated with us in this project, for their constant help, valuable suggestions and efforts in bringing about the timely publication of this volume.

M.M. Azooz
Parvaiz Ahmad

About the editors



Professor Dr Mohamed Mahgoub Azooz

Prof. Dr Mohamed Mahgoub Azooz is a Professor of Plant Physiology in the Department of Botany, Faculty of Science, South Valley University, Qena, Egypt. He received his BSc in 1984 and MSc in 1990 from Assiut University, Egypt; and a PhD in 1997 from South Valley University, Qena, Egypt, in collaboration with Tübingen University, Germany. He has been seconded to King Faisal University, Saudi Arabia, to act as full-time faculty member (from 2005 to present) in the Department of Biological Sciences, College of Science. Prof. Azooz has published more than 90 research articles in peer-reviewed journals and contributed 13 chapters in internationally published books. He has also authored three books and edited five volumes with international publishers. He has supervised and approved many MSc students and PhD scholars, and is a member of the editorial boards and reviewer of many international journals. Prof. Azooz has participated in varying capacities as principal investigator, co-investigator, consultant and collaborator in more than 20 research projects. He has been awarded scientific publication prizes 2012, 2013 and 2014 from both King Faisal University and South Valley University for his scientific articles published in international scientific journals with high impact factors. Prof. Azooz has been included in Marquis *Who's Who in the World* (28th edition, 2011), and in the 'Top 100 Scientists' list published by the International Biographical Centre (Cambridge, UK, 2011). His current research is on the

physiological, biochemical and molecular responses of plants against various biotic and abiotic stresses and their mechanisms of tolerance, with the main focus on medicinal and economic plants, aiming to improve their growth and productivity using some growth stimulators.



Dr Parvaiz Ahmad

Dr Parvaiz is Senior Assistant Professor in the Department of Botany at Sri Pratap College, Srinagar, Jammu and Kashmir, India. In 2000 he completed his postgraduate studies in botany at Jamia Hamdard, New Delhi, India. After receiving a Doctorate from the Indian Institute of Technology (IIT), Delhi, India, he joined the International Centre for Genetic Engineering and Biotechnology, New Delhi, in 2007. His main research areas are stress physiology and molecular biology. He has published more than 35 research papers in peer-reviewed journals and 29 book chapters. He is also an editor of 13 volumes (one with Studium Press Pvt. India Ltd., New Delhi, India; nine with Springer USA; and three with Elsevier USA). He is a recipient of the Junior Research Fellowship and Senior Research Fellowship from the Council of Scientific and Industrial Research (CSIR), New Delhi, India. In 2007 Dr Parvaiz was awarded the Young Scientist Award under a Fast Track scheme by the Indian Department of Science and Technology (DST). Dr Parvaiz is actively engaged in studying the molecular and physio-biochemical responses of different agricultural and horticultural plants under environmental stress.

CHAPTER 1

Biotechnological applications to improve salinity stress in wheat

Sami ullah Jan¹, Ghulam Kubra¹, Mehreen Naz², Ifrah Shafqat², Muhammad Asif Shahzad¹, Fakiha Afzal¹ and Alvina Gul Kazi¹

¹Atta-ur-Rahman School of Applied Biosciences (ASAB), National University of Sciences and Technology (NUST), Islamabad, Pakistan

²Department of Bioinformatics and Biotechnology, International Islamic University, Islamabad, Pakistan

1.1 Introduction

For food, humans rely on approximately 275 crops (Tilman *et al.*, 2011). Out of these, three crops, wheat, maize and rice, are significant cereal crops that contribute to major dietary requirements as staple foods for humans – a reason why they are collectively termed the ‘big three cereal crops’ (Shewry, 2009). Comparatively, wheat is the most important cereal crop that contributes a major portion of the daily diet for humans (Slade *et al.*, 2012). It is estimated that wheat is a source for one-fifth of total calories utilized by humans globally (Waines & Ehdaie, 2007). Wheat grains contain vital constituents such as carbohydrates, including 60–70% starch (Slade *et al.*, 2012) and 8–15% protein such as glutenin (Shewry *et al.*, 1995) and gliadin (D’Ovidio & Masci, 2004). From the total wheat grain produced globally, 65% is utilized as food by humans while the remaining 35% is distributed among livestock feed (21%), seed material (8%) and raw material (6%) in industries such as the production of vitamins and antibiotics, manufacturing of paper; it is also used as a fermentation substrate or as adhesives in various products (Shewry & Jones, 2005).

1.1.1 History of wheat: from domestication to revolutions

In ancient times, wheat was a product of the activities of hunter-gatherers but about 10,000 years ago, the Neolithic Revolution laid the basis for domestication of various crops (Waines & Ehdaie, 2007). This domestication process focused mainly upon cereal crops, and

wheat is considered the originator of domesticated crops (Peleg *et al.*, 2011). With the passing of time, problems arising in the domestication process compelled scientists to analyse and study various concerns such as local conditions, yield maximization, development of improved cultivars and storage techniques (Cavanagh *et al.*, 2013). Eventually, these findings resulted in major events such as the Agricultural Revolution in the 19th century (Godfray *et al.*, 2010) and the Green Revolution in the 20th century (Waines & Ehdaie, 2007).

Wheat domestication followed by major revolutions and scientific achievements contributed to speciation and initiation of new varieties (Shewry, 2009). The factors involved in such speciation primarily include adaptations to the ecology of an area as soon as wild-type wheat cultivars were moved for domestication purposes (Chaudhary, 2013). These adaptations under the influence of epigenetics offered the opportunity to select the desired traits in wheat such as yield, grain quality, grain size and many other phenotypic attributes (Burger *et al.*, 2008). Thus, wheat evolved into many varieties in response to human cultivation practices, selection procedures and the phenomena of epigenetics (Fuller, 2007).

Since the Green Revolution, technologies have been incorporated into crop improvement practices, specifically wheat, in various ways (Schmidhuber & Tubiello, 2007). These include successful development of hybrids with enhanced desired traits, development of pathogen-resistant plants, enhanced yield, improved nutrient contents, affordable fertilizer requirements and improved irrigation systems (Godfray *et al.*, 2010).

The consequences of all aspects of the Green Revolution increased yield to fulfil the world's food requirements (Tilman *et al.*, 2011).

1.1.2 Wheat genome

Modern wheat includes six sets of genomes, called hexaploidy, and is a result of domestication and scientific processes practised by man. Polyploid genomes of wheat cultivars evolved after crossing or hybridization, selection procedures and cultivation practices in domestication. The wild wheat ancestor *Triticum turgidum* sp. *dicoccoides* is considered as the first domesticated wheat species in the Near East region (Maier, 1996). This wheat species was spread across Europe and gave rise to new varieties like *Triticum turgidum* sp. *dicoccum* and *Triticum turgidum* sp. *durum* (Buckler *et al.*, 2001). Durum wheat is still widely grown in the Near East crescent around the Mediterranean Sea (Thuillet *et al.*, 2005).

In reference to common bread wheat, this is an allopolyploid consisting of three genomes designated as A, B and D originating from wild wheat grasses of the genera *Triticum* and *Aegilops* (Zohary *et al.*, 1969). Modern wheat is hexaploid, existing in three sets, A-Genome, B-Genome and D-Genome. The ancestor of A-genome wheat *Triticum urartu* contained 14 chromosomes in two sets, and was crossed with *Aegilops speltoides* (B-genome) that resulted in a hybrid which contained both genomes (AB) which after doubling yielded a viable tetraploid containing 28 chromosomes (AABB). This hybrid, known as wild emmer (Chen *et al.*, 2013), upon further crossing with *Aegilops squarrosa* (a diploid grass), produced a new hybrid with 21 chromosomes (42 chromosomes in diploid form). The later hybrid produced is the hexaploid wheat utilized today and contains genomes from three ancestors (AABBDD) (Levy & Feldman, 2002).

1.1.3 Wheat production and concerns

During the past 50 years, research and technological applications in the cultivation of wheat have increased its yield to a rate of 41 kg per hectare (Ewert *et al.*, 2005). But the world's population is increasing all the time (Godfray *et al.*, 2010). If this continues, by the mid-century, the world's population is estimated to be 9–10 billion (DeLong *et al.*, 2010). Simultaneously, demands for more food and energy resources will also be raised such that, by the middle of the century, necessary food production will be double that of the present (Ray *et al.*,

2013). Numerically, the required rate of increase in food production by the year 2050 is 100–110% compared to the current rate of production (Tilman *et al.*, 2001). About 600 million metric tons of wheat is produced per year worldwide but with the increment in population, by 2020 we would require an estimated yield of 1 billion metric tons (Shewry, 2009). In 2005, calculated yield per hectare of wheat was 2.5 tons which was forecasted to reach a figure of 4.0 t/ha by 2020 (Rajaram, 2005).

Despite these important facts, only 3 billion hectares of land out of 13.4 billion hectares is available for crop cultivation (Smith *et al.*, 2010). One solution to overcoming the world's food requirements is to turn more land over to arable in order to increase wheat global production (Gregory *et al.*, 2002). It has been estimated that by utilizing only 20% of untilled land, we could increase crop yields up to 67% (Bruinsma, 2003). In 2007, total yield of cereal crop was 3.23 tons per hectare which could be increased to 4.34 tons per hectare by increasing land under cultivation to 25% (Bruinsma, 2009). The actual figure for per capita arable land is continuously decreasing due to industrialization, housing and deforestation as well as some environmental concerns (Gregory & George, 2011). However, environmental concerns are among the major problems that cause the loss of yield such that only 50–80% yields are achieved (Lobell *et al.*, 2009). Various scientific communities contribute to minimize the gap between actual and potential yields (Jaggard *et al.*, 2010) but the problems remain the same and the environmental concerns are important, such as abiotic (salinity, drought, temperature) and biotic stresses (Atkinson & Urwin, 2012).

1.2 Salinity stress is a striking environmental threat to plants

Agricultural production all over the world is constrained by salinity stress and it is becoming a growing universal issue that affects almost 20% of cultivated land globally (Flowers & Yeo, 1995). From the agricultural point of view, salinity is the aggregation of dissolved salts within soil or agricultural water to an extent which adversely affects plant growth (Gorham, 1992). High salinity influences the physiological mechanism that adversely affects plant growth and development which necessitates detailed investigation of tolerance mechanisms in salinity (Abogadallah, 2010).

Salinity-induced stress increases the accumulation of salts in plant roots (Zhang *et al.*, 2014). Such hyperaccumulation of salts in roots restricts water absorption from the soil surface and thus also causes water stress, in spite of available water at the root zone. Water absorption from saline soils requires extra energy expenditure. Thus, higher salinity will always lead to decreased levels of water as well as inducing analogous stresses like water and osmotic stress (Bauder & Brock, 1992).

1.2.1 Statistics of salinity stress-affected land

Saline soils are widespread in arid and semiarid regions, especially in areas where heavy irrigation or overfertilization is common (Reynolds *et al.*, 2005). It is estimated that 800–930 million hectares (7%) of the world's total arable land is influenced by salt stress (Shannon, 1997; Szabolcs, 1994) while 230 million hectares of irrigated land are affected by salts (Oldeman *et al.*, 1991). Extensive salts in soil arise due to natural processes such as rainfall containing salts as well as irrigation practices such as the use of fertilizers, resulting in poor water quality (Reynolds *et al.*, 2005).

1.2.2 Causes of salinity stress

Salinity is a primary abiotic stress that hinders plant growth. Numerous causes are responsible for salinity. Some prominent causes include extensively irrigated lands, use of improper waters, inefficient drainage, practising inappropriate irrigation, standing water for prolonged time and water seepage from reservoirs. Underground leakage or seepage from water reservoirs tends to raise the water table which mobilizes salt and thus causes salinity stress (Awad, 1984). Further, increment of the saline water table to 2 meters speeds up the evaporation process, leaving excessive salt in the soil, resulting in waterlogged soil. Due to the limited oxygen in water-logged soils, the survival of vegetation is endangered. Another important reason causing salinity is heavy rainfall which drains salts away with it and may saturate cultivated land.

Despite such diverse causes, the main reasons for salinity are (1) natural (primary) salinity, and (2) human-induced (secondary) salinity.

Primary salinity or natural salinity is a result of a prolonged natural build-up of salt in water or soil which occurs by the natural process of breakdown of rocks containing SO_4^{2-} , Ca^{2+} , CO_3^{2-} , Cl^- , Na^+ and Mg^{2+} .

Furthermore, evidence of ocean salt conveyed by wind and downpour is likewise a reason, which changes with the types of soil.

More specifically, the main reason for natural salinity is the excess accumulation of salts at the soil surface. Among all these causes, studies have revealed that those salts which accumulate and cause salinity stress are composed of a particular set of ions, including Na^+ , K^+ , Ca^{2+} , Mg^{2+} and Cl^- . However, among these, Na^+ is more dominant and its excess makes soil sodic. Sodic soil is more challenging because it has a poor architecture, thus limiting or preventing infiltration of water as well as drainage. Some soils harbour large amounts of salt which are flushed away along with rain or irrigation water drainage. Salt accumulation in the form of precipitation or resulting from weather changes as well as mineral degradations also leads to salinity. Salt accumulation in dry lands is very common and rapidly causes salinity conditions (Brinkman, 1980).

Secondary salinity or human-induced salinity is induced because of anthropogenic activities that disturb the hydrological stability of the soil between water connected (watering system or precipitation) and water utilized by plants (transpiration) (Garg & Manchanda, 2008). The water table is increased because of the large quantity of water supplied to the irrigated fields with poor drainage systems. Globally all these watering systems may result in secondary salt stress and saturation of soil with water (Garg & Manchanda, 2008).

1.2.3 Types of salinity stress

Salinity stress can be classified on the basis of its initiation process, mobilization means and mode as well as their impacts. Regardless of the sources or types of salinity, the effects of salinity on plant development remain constant but these effects are variable in terms of salt concentration encountered by plants. A comprehensive classification of salinity is presented as follows.

1.2.3.1 Dry land salinity stress

Dry land salinity refers to a type of salinity that occurs in unirrigated land. Such land is usually colonized by shallow-rooted plants instead of deep-rooted crops because the shallow-rooted plants can withstand less water and their growth cycle is short. Dry land, as compared to normal or moist land, increases seepage rates to ground water and induces mobilization of salts pre-stored in soil. In low areas or slopes, ground water along

with its salts is exposed to evaporation, leaving salts at the soil surface and thus increasing salinity-plant interactions. Likewise, ground water may also finally be added to streams and rivers, in which case, salt concentration in water resources is increased and when these resources are utilized for irrigation purposes, they will cause salinity stress to plants (Dias & Thomas, 1997).

Dry land salinity is dependent upon type and composition of soil when subjected to irrigation. Predominantly sodic or naturally saline clays fall under the category of dry land salinity which may also spread if such soil is transported by any means and/or mixed with other soils. However, when sodic soil becomes wet, it disperses and causes blockage of pores within the soil which are common routes of water seepage towards ground water. In other cases, when sodic soil is dry, it becomes dense and hard and forms a crust on the soil surface. In the latter case, soil structure loses its capacity for infiltration of water, leaving no or little salt at the root. In some conditions, subsodic soils can produce a perched water table which creates water logging at the root zone. In view of such conditions, if large amounts of water are applied in irrigation, it will increase the ground water level which eventually causes irrigation salinity. This problem will tend to increase if such soils are accompanied with crops that utilize lower amounts of water. The worst condition of irrigated salinity can occur if water for irrigation purposes is obtained from salty ground water or salty rivers (Dumsday *et al.*, 1983).

1.2.3.2 Urban salinity stress

Urban salinity includes the activities involved in urbanization and its development as well as excessive use of water for gardens and other low-level cultivation practices. Other phenomena include leakage from pipelines, tanks and drainage systems or changing water flow direction from its normal routes. All these factors support the rise of the water column in the ground water table. Sources of salt arising from these processes include material used in buildings, waste from industries, use of chemicals and fertilizers, salt effluents as well as naturally occurring salt.

Industrial waste and effluents are excreted from industrial and some domestic areas; those which have high salt concentrations have proved more dangerous compared to other categories in urban salinity. Industries using coal for fire also use huge amounts of water for cooling purposes which results in evaporation of water

while salts are deposited and finally added to soil. Similarly, mining activities also play a role in causing urban salinity (Murray, 1999).

1.2.3.3 River salinity stress

Rivers are the major source of irrigation but this source is very much contaminated with salts. All the drainage from domestic and industrial systems, as well as affected dry lands, finally end in a river. The same water is recycled for irrigation purpose which creates a stress environment for plants. With the passage of time, the quality of river water becomes more saturated as water level decreases in saline rivers which in turn irrigates plants, resulting in impaired plant survival (Awang *et al.*, 1993).

1.2.3.4 Irrigation salinity stress

When the source of water used for plants is a saline water reservoir, a condition arises known as irrigation salinity. It is different from river salinity because river salinity refers to salinity caused by river water used for irrigation purposes while irrigation salinity encompasses the salinity caused by any water source for plants (Ayres & Westcot, 1985). Sources of salts in such conditions are mostly similar to those mentioned in rivers and dry land salinity. However, in addition, climate and routes of irrigation system also determine the irrigation salinity levels (Bauder & Brock, 2001).

1.3 Effects of salinity stress on wheat

Wheat can tolerate salt to some extent but as the salinity concentration increases, more serious risks of damage are likely to occur. The vast dimensions of threats to wheat and other plants by salinity stress range from its physiological characteristics to vital biochemical pathways and its genetic make-up. These effects include hindrance of seed germination and seedling growth and dry mass accumulation in wheat (Shirazi *et al.*, 2001; Sourour *et al.*, 2014), disturbed biochemical pathways (Radi *et al.*, 2013) and disorganized patterns of gene expression. Many investigators have screened numerous wheat cultivars, including salt-tolerant varieties, to check the effects and variations due to salinity on various growth levels (Munns & Termaat, 1986). Some of the patterns of salt effects on numerous attributes of wheat are discussed below.

1.3.1 Effects of salinity stress on phenological attributes of wheat

To assess the impact of salinity on some physiological (Yang *et al.*, 2014) and phenological qualities in durum wheat, a factorial investigation was directed at time of stem lengthening, time to heading and development, chlorophyll content and chlorophyll fluorescence of leaf. Results demonstrated that the relation between salinity and cultivars (61130 and PGS) causes expansion in time to heading (Kahirizi *et al.*, 2014). Distinctive attributes like grain number obtained on a meter square area, grain yield, 1000 grains weight, leaf's Na⁺ and K⁺ ion contents and plant's height had been measured in field analyses for bread wheat. Numerous quantitative trait loci (QTL) were determined by ecological contacts which demonstrate several phenological characteristics for salt resistance. Numerous QTL for biomass of seedlings and end of Na⁺ particles were seen in hydroponic and field tests (Genc *et al.*, 2013).

Excessive salinity has always diminished development, yield and related yield characteristics, photosynthetic traits (Mathur *et al.*, 2013), ionic substances and biochemical activities in all wheat cultivars. Nonetheless, application of potash composts in soil and also in foliar application counteracts the unfavourable impacts of salt stress on all wheat cultivars and the application of sulphate of potash (SOP) in soil and as a foliar splash was more compelling in triggering salt stress tolerance. No unfavourable impact of chloride on plant development was noticed. Among the cultivars, 'S-24' and 'Sehar' demonstrated remarkable development, yield and biochemical matter and subsequently could be utilized as parental material for achieving better yield under saline conditions (Ashraf *et al.*, 2013).

1.3.2 Effects of salinity stress on morphological attributes of wheat

Morphologically, salt stress causes reduction in overall plant growth but shoots are seen to be more affected than roots and the ratio between them is increased (Allen, 1995). Salt stress also reduces the total dry matter yield and number plus appearance of wheat tiller (Mass & Poss, 1989). Salt stress also causes stunting of shoots (Walker *et al.*, 1981). Two reasons why increased salt levels found in soil water cause reduction of plant growth are (1) plants' ability to take up water from soil is decreased due to ionic imbalance which decelerates natural growth processes, and (2) when salts get into a

plant through any possible route, such as transpiration stream into the transpiring leaves, this eventually injures cells which also restricts growth (Munns, 2005).

Initial contact with salinity causes water stress in plants, resulting in reduction of leaf expansion ability. Another associated problem, osmotic stress, also arises in plants on initial exposure to salt stress and can lead to inhibition of cell division, expansion and function of stomata (Munns, 2002).

Soil salinity causes formation of Na⁺ and Cl⁻ that affects the ionic composition taken up by plants (Rengasamy, 2006). Salt stress directly affects plant growth through osmotic stress and ionic toxicity caused by Na⁺ and Cl⁻ ions which promote imbalance in plant nutrient metabolism (Rowell, 1994). Adverse effects of salt stress on cell morphology include accumulation of toxic ions that disrupts intra- and extracellular components like DNA, enzymes, membranes, mitochondria, chloroplasts and many more by the development of reactive oxygen species (ROS) (Allen, 1995; Saqib *et al.*, 2012).

1.3.3 Effects of salinity stress on physiological attributes of wheat

Salt stress has numerous consequences for germination methods. Germination is delayed due to high salt concentrations in soil which create high osmotic pressure, thus reducing water intake by seeds (Khan & Weber, 2008), that may cause the metabolism of nucleic acid digestion (Gomes-Filho *et al.*, 2008), changes in metabolism of protein (Dantas *et al.*, 2007) and aggravation of hormonal offset (Khan & Rizvi, 1994), as well as lessening the ability to utilize seed stores (Othman *et al.*, 2006). It might likewise influence the fine structure of cell, tissues and organs (Al-Maskri *et al.*, 2014; Rasheed, 2009). However, there are different intramural (plant) as well as external (natural) aspects that influence seed germination under saline conditions which incorporate nature of seed layer, seed torpidity, seedling power, seed polymorphism, seed age, water, gases (Mguis *et al.*, 2013), light and temperature (Wahid *et al.*, 2011).

Death of the plant occurs at higher concentrations of the salt as a result of hyperionic and hyperosmotic stress. The result of these impacts may cause membrane layer harm, nutrient unevenness, distorted levels of enzymatic hindrance, developmental regulators and metabolic abnormality, including photosynthesis which at last prompts plant demise (Hasanuzzaman *et al.*, 2012;

Mahajan & Tuteja, 2005). Similar consequences have been observed in various varieties globally, including *Triticum aestivum* (Akbarimoghaddam *et al.*, 2011), *Brassica* spp. (Ulfat *et al.*, 2007), *Zeamays* (Khodarahmpour *et al.*, 2012), *Oryza sativa* (Xu *et al.*, 2011), *Vigna* spp. (Jabeen *et al.*, 2003), *Helianthus annuus* (Mutlu & Buzcuk, 2007) and *Glycine max* (Essa, 2002). It was observed that germination of seeds has a negative relationship with salinity (Rehman *et al.*, 2000). Higher salinity represses the germination of seeds while seed dormancy is incited by lower levels of salt stress (Khan & Weber, 2008).

The most harmful ions which cause several physiological impacts on plants are Na^+ and Cl^- (Tavakkoli *et al.*, 2010). Many stresses, abiotic and biotic, affect photosynthesis which is a complex process involving many components such as photosynthetic pigments, photosystems, the electron transport system, CO_2 reduction pathways, etc. Stress of any kind can affect any of these components, reducing the photosynthetic capacity of plants. To combat this harm, harvested plants typically utilize protein kinases, for example, MAPKs and transcription factors (Ashraf *et al.*, 2013; Saad *et al.*, 2013; Zhang L *et al.*, 2012).

Research undertaken by Rajendran *et al.* (2009) showed the impact of ion exchange under salt stress in advanced stages of plant development. They observed that hazardous ions accumulated after 2–4 weeks of exposure of salt stress. The stress caused by ions (Na^+ and/or Cl^-) overlaps with the osmotic impacts and demonstrates more hereditary variety than osmotic impacts (Munns *et al.*, 2002).

1.3.4 Effects of salinity stress on biochemical attributes of wheat

From the biochemical point of view, life-sustaining pathways like respiration (Shekoofoa *et al.*, 2013) and photosynthesis as well as their associated enzymes are affected by high salt levels while responses to these salts by cellular machinery mostly use enzymes (Walker *et al.*, 1981). In such conditions, salt stress triggers further phenomena such as hyperosmotic shocks, cell turgidity is lost, ROS are formed and stomatal size is minimized (Price & Hendry, 1991). Eventually, these conditions collectively or individually may restrict plant growth.

As NaCl is an actual component of saline soil, plants gather Na^+ and Cl^- particles up to levels that are

detrimental (Cuddy *et al.*, 2013). Shoot Na^+ poisonous quality is connected with the decline of stomatal conductance while high shoot Cl^- levels immediately influence chlorophyll and repress photosystem II (Ashraf & Ashraf, 2012; Tavakkoli *et al.*, 2011).

Higher Na^+ and Cl^- in plant cells are seen as the key components of ionic damage (Cuin *et al.*, 2009; Munns & Tester, 2008; Rajendran *et al.*, 2009). There are various studies and evaluations that discuss the connection of Na^+ , K^+ and K^+/Na^+ homeostasis with salt stress tolerance in harvest plants (Horie *et al.*, 2009). The effect of Cl^- homeostasis in salt tolerance is little understood (Teakle & Tyerman, 2010). The elevated levels of Cl^- that congregate in the plant leaves developed under saline conditions will affect the whole plant (White & Broadley, 2001). Hence, it is striking that minimal investigation has been undertaken into the impacts of Cl^- content in connection to salt tolerance; however, there are no reports on the hereditary control of this attribute. Just a few studies showed that treatment of Cl^- may be essential for salt tolerance in a few products including grain (Tavakkoli *et al.*, 2010a; Tavakkoli *et al.*, 2010b; Teakle & Tyerman, 2010).

Metabolically harmful quantities of Na^+ to a greater extent are a consequence of its capability to contend with K^+ for binding components vital for cell capacity. High Na^+/K^+ proportions can disturb different enzymatic courses of action in the cytoplasm (Tester & Davenport, 2003). Stress caused by ions is connected with a decline in chlorophyll content and restrains photosynthesis, impelling leaf senescence and early leaf fall. Ionic stress consequently diminishes photosynthesis limit, biomass and yield (Tester & Davenport, 2003).

In one study, the impact of salicylic acid or indoleacetic acid (IAA) was tested by spraying them on *Triticum aestivum* genotypes which were subjected to different saline levels in order to check their impact on growth of different plant organs. It was concluded that under such circumstances, cell enzymes having antioxidant properties like reducing sugars, catalase, peroxidase, ascorbate peroxidase, photosynthetic shades, amino acid, proline in shoot and root were enhanced (Nassar *et al.*, 2013).

Salinity has been reported to induce oxidative stress as the ROS are enhanced by producing superoxide (O_2^-), hydroxyl (OH) and hydrogen peroxide (H_2O_2) radicals. As the accumulation of ROS increases, scavengers initiated protection mechanisms in plants from

salinity-induced damage (Al-Quraan *et al.*, 2013; Hayat *et al.*, 2014). Plants harbour an antioxidative defence mechanism, activated in salinity to overcome oxidative stress mediated by ROS, which is composed of ions (Asgari *et al.*, 2012), antioxidant enzymes and osmotic homeostatic conditions. Ionic homeostasis under salinity stress is to be maintained for physiological and biochemical conditions of the plant because such ionic balance regulates the essential ion concentration rather than toxic ions (Gupta & Huang, 2014; Hajiboland, 2012). Certain plants possess a potential to maximize the cellular level of osmotically compatible solute concentration. Such osmotically compatible solutes can mediate ionic homeostasis related to water concentration, eventually leading to minimized impact of ionic concentrations upon cell proteome including enzymes, protein complexes plus membrane proteins and other proteins required in cell stability under stress (Ma *et al.*, 2012). The role of antioxidative enzymes, for instance APX (ascorbate peroxidase), SOD (superoxide dismutase), CAT (catalase) and POD (peroxidase), was important in minimizing oxidative stress/damage induced during salinity stress (Ahmad *et al.*, 2010a).

Under saline conditions, the activities of the cell antioxidant system, for example, SOD (superoxide dismutase) and CAT (catalase), in susceptible cultivars were lower as compared to controls. With respect to APX, there was no significant difference between saline and control conditions. Under salt stress, the MSI (membrane stability index) of two tested cultivars was adversely affected. Hydrogen peroxide (H_2O_2) content of salinity susceptible cultivars was higher than controls. Salt-tolerant mixtures had more K^+ levels and Na^+ ratio; relative water substance, yield and chlorophyll under saline condition and susceptible cultivars accumulate higher Na^+ content at the tillering stage. The method of salt stress may be accomplished because of low lipid peroxidation, presumably, fewer changes in MSI, evasion of Na^+ quantity and release of antioxidant enzymes (Rao *et al.*, 2013).

Salt tolerance in plants is dependent on their ability to alter osmotically to decreased soil water potential as well as keeping intracellular levels of ROS under control. Both of these techniques are accepted to depend on accumulation of natural osmolytes. In one study, outside NaCl complex was connected by the dynamic congregation in leaf Na^+ . This aggregation was much higher in old leaves and juvenile ones. In old leaves, three inorganic ions (Cl^- , K^+ and Na^+) contributed 70.4%

and 67.7% of leaf osmotic potential in wheat and grain individually when presented to 200 mm NaCl treatment, while in junior leaves their assertion was just 46.8% and 43.9% separately. It was suggested that salinity prompted increase of natural osmolytes in wheat grain and leaves corresponding with exaggerated oxidative stress tolerance and provides the confirmation of a system of cross-tolerance between these two stresses (Puniran-Hartley *et al.*, 2014).

So as to overcome perverse intercellular ROS, plants have created cell antioxidant guard frameworks assisting them in management of ROS levels. Plants utilize different components to protect themselves from the distant impacts of salinity (Zhang Y *et al.*, 2012). Plants react to excessive salinity by accretion of osmoprotectants including proline and sugar (Gurmani *et al.*, 2013).

Plant hormones have outstanding effects on the framework of cell division and development of a whole plant. Salt stress-induced decline in general development signifies endogenous irregular hormonal levels (Iqbal & Ashraf, 2013).

1.4 Wheat natural tolerance and defence against salinity

Wheat also possesses particular mechanisms that help it to combat harmful effects of primary as well as secondary stress with the accretion of osmolytes and antioxidants production (Ahmad *et al.*, 2010a; Ahmad *et al.*, 2010b; Ashraf & Foolad, 2007; Devi & Prasad, 1998; Foyer *et al.*, 1994). The principal antioxidant enzymes that assist plants to withstand numerous environmental stresses include peroxidases, superoxide dismutases, catalases and glutathione reductases. These enzymes co-ordinate in orchestra; for instance, superoxide dismutase converts superoxide anions to H_2O_2 (Alscher *et al.*, 2002). Similarly, catalase stands as a second line of defence against varied stresses by converting H_2O_2 to molecular oxygen and water. With reference to their potential of free radical quenching, quantization and other patterns of analysing antioxidant enzymes and non-enzymatic antioxidants levels are utilized to determine the effectiveness of oxidative defence mechanisms in plants (Geebelen *et al.*, 2002).

Tolerance to salinity stress is generally evaluated from the biomass produced under saline compared to controlled conditions grown over a convenient time period

(Munns, 2002). Tolerance levels in plants also vary depending upon genetic potential plus biochemical and physiological characteristics. A halophytic salt-tolerant plant upon abrupt exposure to prevailing salinity or salinity shock will develop diverse strategies that could lead to gradual acclimation, depending on developmental stages and sensitivity of plants. Conversely, tolerance mechanism is determined by interaction between environment and particular plant species. Some plant species may demonstrate stage-specific sensitivity, exhibiting greater susceptibility to salt in the germination phase while others may be highly sensitive in the reproduction stage (Mahmoodzadeh *et al.*, 2013). Evolution is also important in this regard and numerous mechanisms are evolved in plants to defend against salinity stress.

1.4.1 Mechanisms of salt tolerance in wheat

Salinity in plants is counteracted by diverse components (Maas, 1986). Agricultural productivity of certain plants appears to be more sensitive to high levels of salt concentrations, such as glycophytes. While halophytic species are exceedingly salt tolerant and can survive and maintain development even at saline levels much higher than that of seawater, none of the radical examples can tolerate more than 25% salt levels without yield loss and development impairment. The high salt tolerance of halophytes is ascribed to rare anatomical and morphological adjustments, or avoidance tools (Greenway & Munns, 1980). However, halophytes are exceptional among the 250,000 types of promising plants (Flowers & Flowers, 2005). Past studies have grouped plants into two classifications: salt includers and salt excluders. Sodium ions (Na^+) are transported to shoots with the help of salt includers where it is used in vacuole-mediated osmoticum-intended tolerance followed by salt excluders which adjust these ions to saline conditions by eluding uptake of Na^+ ions (Mian *et al.*, 2011a). Overall, plant response to salinity is categorized in three classes: (1) osmotic stress tolerance; (2) excluding Na^+ from leaves; and (3) tolerance at tissue level (Munns & Tester, 2008).

Halophytic species form systems like proficient Na^+ sequestration into the vacuole, which preserve low cytosolic Na^+ , as well as allowing these plants to utilize Na^+ as an osmoticum to maintain cell turgor and development. Accordingly higher Na^+ uptake can lead

to rapid development of halophytes (Blumwald, 2000). Change of salt tolerance in harvested glycophytes like rice and durum wheat has been attained by the advancement of cultivars with low Na^+ in shoot or high K^+/Na^+ proportion (Munns *et al.*, 2012; Thomson *et al.*, 2010).

Plants use three normal instruments of salt tolerance (Rajendran *et al.*, 2009): osmotic change; suitable confirmation for Na^+ uptake via roots and restricting its entry into susceptible tissue; and tissue tolerance (Na^+ incorporation, Na^+ compartmentation). These systems are controlled by integrated physiological, biochemical pathways (Zhou *et al.*, 2012). Osmotic alteration includes the combination and aggregation of perfect solutes inside the cytoplasm. Compatible solutes are smaller water-solvent particles that contain nitrogen-holding blends, for example, betains, amino acids, additionally natural sugars, polyols and acids (Chen *et al.*, 2007). The ability of the compatible solutes is not constrained to maintaining osmotic balance. Compatible solutes are usually hydrophilic as well as they may have the capacity to displace water at the protein surface advancing towards low subatomic weight chaperones (Carillo *et al.*, 2011). Furthermore, these solutes have the ability to maintain cell structure through ROS scavenging (Hasegawa *et al.*, 2000).

High quantities of Na^+ and Cl^- are lethal to all plant cells. The ability of plants to keep up a high K^+/Na^+ proportion in the cytosol is a contributory element of plant salt resistance. A few genes and transporters that plants utilize to maintain high K^+/Na^+ proportion have been described (Jamil *et al.*, 2011). These include the following.

1 Na^+/H^+ antiporters in plasma layers that expel Na^+ from the cytosol as a major aspect of the administrative SOS pathway (Zhu, 2001). Three obviously subtle (SOS) proteins (SOS 1, 2 and 3) suggest an organizational fraction in the communication of particle transporters to maintain small cytoplasmic amounts of Na^+ under salt stress (Lu *et al.*, 2014). Zhu (2003) proposed that a protein kinase complex composed of calcium-bound protein (SOS3) and serine/threonine protein kinase (SOS2) is activated by stress induced in salinity-mediated calcium signals. Subsequently, transporter particles such as Na^+/H^+ plasma layer and SOS1 antiporters are phosphorylated by protein kinases, as shown in Figure 1.1.

2 Vacuolar Na^+/H^+ antiporters (NHXs) and energy suppliers of these NHXs (like H^+ pumps: HVA/68 and

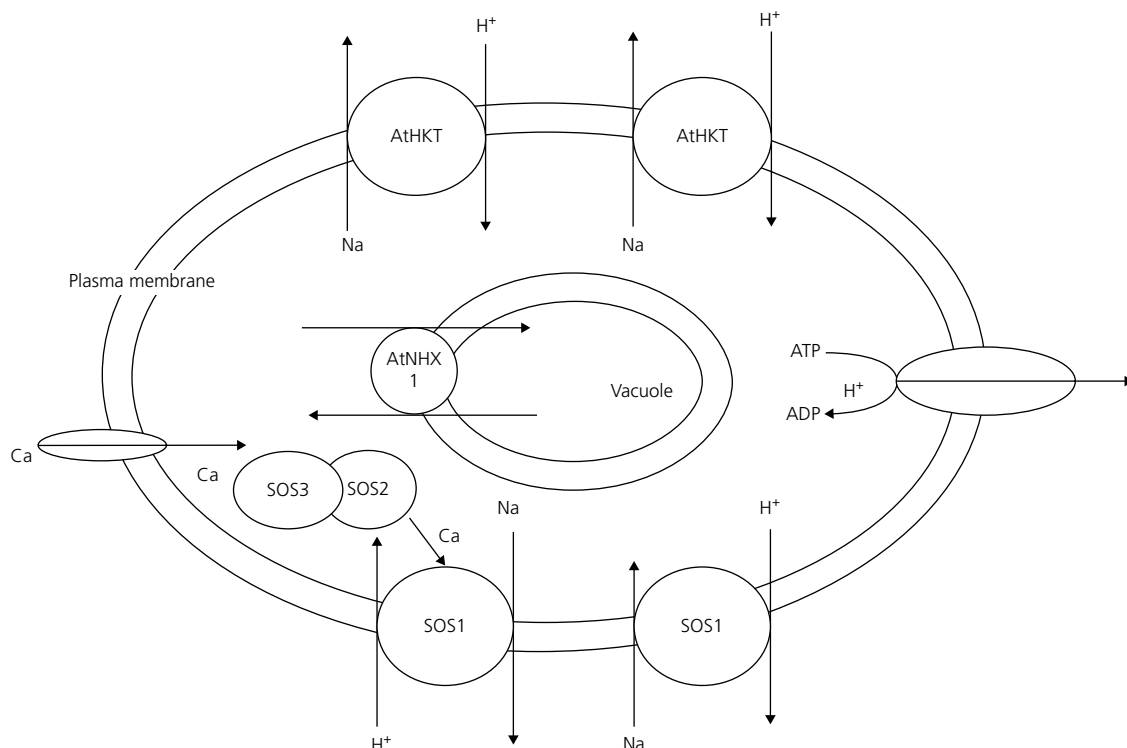


Figure 1.1 SOS pathway indicating Na⁺/H⁺ antiporters in plasma layers that expel Na⁺ from the cytosol.

Hvp1) (Blumwald *et al.*, 2000; Ligaba & Katsuhara, 2010). NHX proteins sequester Na⁺ in the vacuoles and provide an effective component to avoid the harmful impacts of Na⁺ in the cytosol and sustain osmotic equivalence (Glenn *et al.*, 1999). Thus, Cl⁻ is likely transported into the vacuole by anion transporters, for example, CLC proteins (Teakle & Tyerman, 2010; Zifarelli & Pusch, 2010).

3 High- and low-partiality K⁺ transporters (HKT).

The HKT family comprises two classes which work either as particular Na⁺ transporters or Na⁺ and K⁺ co-transporters (Hauser & Horie, 2010; Shabala *et al.*, 2010). HKT21 was demonstrated to improve Na⁺ uptake and higher Na⁺ levels in xylem sap (salt including conduct) which are associated with prolonged salt tolerance (Mian *et al.*, 2011a). Numerous researchers proposed that Na⁺ avoidance from the shoot is connected with salt tolerance and that genes from the HKT1 subfamily, for example, HKT1;4 and HKT1;5, are included (James *et al.*, 2011; Munns *et al.*, 2012). Shabala *et al.* (2010) indicated that both salt

exclusion and deliberation are vital for grain salt tolerance. In fact, grain is an upright illustration of a harvest that links halophytic and glycophytic properties, and accordingly may be an outstanding model to study both the glycophytic and halophytic components that might be used to adapt to salt stress (Mian *et al.*, 2011b).

1.4.2 Conventional strategies to enhance salinity stress tolerance

Various tools and techniques have been devised by many researchers around the world to support plants in acquiring tolerance against salinity and producing higher yields. The most common strategy to enhance induction of salinity tolerance in plants is seed priming which requires less energy and is quite successful in minimizing the harmful effects of salinity on plants (Fercha *et al.*, 2014; Hou *et al.*, 2013). Speed and rate of germination in equipped seeds under salt stress have reportedly increased. This pre-sowing priming technique with different growth regulators, osmoprotectants and halotolerant non-pathogenic micro-organisms

(Ramadoss *et al.*, 2013) as well as water has also proved supportive in developing field-level salinity tolerance in plants because it establishes an aid to germination (Qiu *et al.*, 2014). Seeds initially primed with CaCl_2 followed by KCl and NaCl remained effective in minimizing adverse effects of salinity of wheat plants as they change levels of numerous phytohormones. Physiological attributes of plants have also shown improvement after priming seeds at both laboratory and field level. This technique also illustrates the complete representation of salinity tolerance mechanisms in plants (Cantliffe, 2003).

Some other strategies utilized to minimize salinity-induced effects on plants, especially in wheat, include removal of excessive salts from soil surface or harvesting aerial parts containing accumulated salt in areas where low rainfall occurs, and utilizing practices that aid in saline soil amelioration (Bacilio *et al.*, 2004).

1.5 Biotechnological applications to improve salinity stress in wheat

Advancements in agricultural biotechnology are employed to overcome several problems related to plants, including salinity. The primary goals of agricultural biotechnology include minimizing production cost by derogating inputs like pesticides and nutrient requirements grown in diverse climates. The main goal is to enhance plant quality by producing strains which have better yield with low inputs, can also yield good crop rotation for conservation of natural resources and also obtain more nutritious products that are suitable for long-term storage or travel and low purchase costs for consumers.

After years of expensive and intensive studies, today agricultural biotechnology has made it possible to produce transgenic plants commercially. In 1990, it was speculated that 40 million hectares of land had been utilized in the growth of 20 important species including corn, rapeseed, soybean and cotton (ISAAA 1999). But due to widespread criticism and opposition at the beginning of the 21st century, transgenic wheat was not accepted to be cultivated and commercialized. However, in 2009 the NAWG (National Association of Wheat Growers) conducted a survey which revealed that 76% of the growers supported the cultivation of transgenic wheat for large commercial-scale purposes.

Over the previous decades, diverse methodologies have been used to enhance salt resistance in plant harvests (Munns *et al.*, 2006). New salt-tolerant hybrids, including tobacco (Hu *et al.*, 2012), rice and wheat, have been utilized in nations far and wide like Pakistan, India and the Philippines (Bennett & Khush, 2003). Screening of a substantial accumulation (~5000 increases) of common wheat in Australia and 400 Iranian wheat mixed bags in California for salt tolerance has recognized lines that delivered seeds under high salt focus (half seawater) or provided exceptional profits on saline soil. So far no new cultivar has been developed from these tolerant lines (Munns *et al.*, 2006).

Two broad types of approaches can be employed to overcome the problems caused by salt stress. Soil affected with salt can be managed and brought under cultivation by reclamation, or alternatively, modern biotechnological techniques have been utilized to exploit the germplasm for the development of salinity tolerant varieties (Farshadfar *et al.*, 2008).

Among conventional germplasms, an Indian cultivar/landrace named *Kharchia 65* was tested against salt stress and was found to be salt resistant up to a certain level (Mujeeb-Kazi *et al.*, 2008), making plant breeders select this variety over others for cultivation. Numerous examples/landraces of wheat with the potential for salt tolerance have been reported from several areas of Pakistan, Iran and Nepal (Martin *et al.*, 1994). In addition to these, several other wheat varieties, for instance KRL1-4 and KRL 19 (India), LU26 S and SARC-1 (Pakistan) and Sakha 8 (Egypt), are also considered as salt-tolerant cultivars (Munns *et al.*, 2006).

Numerous genes control the characteristics that may be included in salt tolerance. These genes are communicated diversely throughout the plant lifespan and are affected by numerous ecological variables (Roy *et al.*, 2011). Plant agriculturists are searching for more authentic methodologies with the assistance of molecular markers or transgenic methodologies (Arzani, 2008).

The vast majority of the genes that may help salt tolerance still remain a mystery, even in model plants like *Arabidopsis* and rice. Likewise, salt tolerance is a multi-genic attribute; consequently, extensive change focused around alteration of a single gene is not likely to occur (Colmer *et al.*, 2005). Distinctive evidence of new attributes aiding in salt tolerance is possible through immediate traditional selection in stress situations or focused around mapping investigations of QTL (Holland, 2007).

At present, association mapping seems an alluring and effective methodology to distinguish extra genes serving the regular occurrence of changes for salt tolerance in varieties, landraces and wild relatives of yields. Previously the molecular foundation of the characters that aid in salt tolerance has been determined: marker-aided selection (Munns *et al.*, 2012). This might be utilized to productively exploit the new qualities and genes or to induce hereditary adjustment which could produce genetically engineered crops with new genes exhibiting enhanced levels of salt tolerance.

The relevant selection approach to screen expansive mapping populations and produce precise data on attributes is crucial for recognizing the characteristics and genes for assisting salt tolerance (Ramezani *et al.*, 2013). This will provide understanding on the vicinity/ extent of the heritable variety for tolerance attributes, their inheritance and the magnitude of genotype and environmental co-operation.

1.5.1 Plant phenotyping

To reveal the hereditary premise of complex qualities like salt tolerance, it is important to assist genotypic marker data with the relating phenotypic information. The precise phenotyping is a basis to ascertain and present new genes for salt tolerance into productive plants (Munns *et al.*, 2006). Recently, advancement in DNA marker and sequencing advances has allowed high-throughput genotyping of numerous individual plants with moderately minimal effort. Rapid strategies to assess huge amounts of genotypes are critical to completely exploit the immediate improvement of biotechnological systems and to encourage hereditary analysis of complex qualities.

Traditional selection for execution and yield under saline field conditions has different impediments identified by natural variables, for example, soil heterogeneity and climate conditions (Chen *et al.*, 2005; Isla *et al.*, 1998; Munns *et al.*, 2006). The supportive physiological characteristics serving salt tolerance and the genes underlying these qualities could be distinguished more proficiently under natural conditions (Cuin *et al.*, 2008). Effective screening systems that were used recently to assess the response of grains to salinity were plasticized on hydroponics (Chen *et al.*, 2005; Munns & James, 2003) or on sand as well as soil-based substrates (Munns *et al.*, 2002; Tavakkoli *et al.*, 2010b). The shoot Na⁺ (Cl⁻) content and K⁺/Na⁺ degree have been recommended as

dependable characteristics for salt tolerance determination in products (Munns & Tester, 2008; Munns *et al.*, 2002; Tester & Davenport 2003). Hereditary investigations employing traits that influence particle homeostasis have distinguished QTLs characterized by Na⁺ and K⁺ transporters which facilitate salt tolerance in rice (Bonilla *et al.*, 2002; Ren *et al.*, 2005) and in wheat (Munns *et al.*, 2012). Comparative studies in barley have not yet unravelled genes for salt tolerance, despite the fact that it is the most salt-tolerant oat crop. Shabala *et al.* (2010) and Mian *et al.* (2011a) demonstrated that both particle exclusion and deliberation assist grain salt tolerance. Further precise and appropriate screening strategies may be required that permit numerous stage estimations of salt stress throughout the life cycle of barley. Also, the processes ought to allow examination of the synthesis and collaborative impacts between distinctive qualities and incorporate Cl⁻ risk as Cl⁻ is an 'overlooked adversary' for salt tolerance research (Munns & Tester, 2008; Teakle & Tyerman, 2010).

1.5.2 QTL mapping

QTL mapping has been a key apparatus in studying the genetic structure for engineering of complex characteristics in plants (Kearsey, 1998). Most agronomically significant characteristics, for example yield, grain quality and tolerance to biotic and abiotic stresses, are indistinct (Azadi *et al.*, 2014). Genetic assembly modelling alludes to various genome areas with genes that influence the attributes, the magnitude of the impact, and the relative assurance, prevailing and epistatic impacts (Holland, 2007). The discovery of QTLs of agronomical significance and the underlying genes has significantly extended our understanding of the intricacy of characteristics (Salvi & Tuberosa, 2005).

Advancement in distinguished QTLs that trigger the characteristics will fundamentally assist breeding through marker-aided selection (Collard & Mackill, 2008) and pyramiding of numerous suitable alleles (Yang *et al.*, 2012). Biparental (customary) QTL mapping focused around a single dividing population inferred from two homozygous parental genotypes has been the basic methodology for genetic investigation of salt tolerance in rice (Lee *et al.*, 2006), wheat (Genc *et al.*, 2010) and grain (Xue *et al.*, 2009). A few loci were found to encode parts of the HKT group of particle transporters which together

enhance salt tolerance like the *Kna1* locus in common wheat (Dubcovsky *et al.*, 1996) as well as *Nax1* plus *Nax2* in durum wheat (Munns *et al.*, 2012). In the meantime, biparental QTL mapping has constraints identified with the reduced investigation of allelic variety exhibited in the gene pool for each of the loci influencing the qualities, absence of isolation for some characteristics, and poor determination (Rock Garcia, 2003). Biparental QTL mapping discovers genomic areas connected with characteristics with precision successively in normal range from 10 to 30 centimorgan (cm) (Bernardo, 2008). Such chromosomal locales could harbour up to a few thousand genes (Ingvarsson *et al.*, 2010), thus proving the fact that effective QTLs which are currently being cloned are underlined by more than one gene (Mackay & Powell, 2007). Further research is required to determine the mapping requisite in order to overcome the utilization of the hereditary variety for salt tolerance in barley germplasm.

1.5.3 Association mapping

Association mapping, also known as linkage disequilibrium mapping, is a system utilized for mapping QTLs which interprets outstanding disequilibrium linkage with phenotype connection (perceptible characters) to genotypes. Recently, association mapping has been supported as the technique for distinguishing loci included in the inheritance of complex characters in genetics. This technique includes distinctive markers connected with the phenotypes of interest found among a group of irrelevant individuals (Pritchard *et al.*, 2000). Association mapping has recently been presented in plant genetics (Kloth *et al.*, 2012) and demonstrated promise to utilize the exact capacity of novel molecular markers and sequencing progress (Zhu *et al.*, 2008).

Association mapping depends on the local characteristics related to linkage disequilibria in accrual of normally different germplasms (Mackay & Powell, 2007). It provides dynamic utilization of all the recombination incidents that have occurred throughout the long evolutionary history of a crop species, distributing meagre linkage obstructions than those found in biparental QTL mapping studies (Nordborg & Tavare, 2002). Furthermore, association mapping deals with all actual allelic variants of QTLs, influencing the attributes of study when performed with an adequate association

mapping group dialogue to the majority of the crop gene pool (Figure 1.2).

In association mapping, linkage disequilibrium (LD) is a central element. LD is a population detail for non-arbitrary relationship between alleles of distinctive polymorphic loci. The destruction in LD among adjacent markers determines the marker thickness and trial outline required to perform association mapping effectively. Linkage, choice, transformation and assortment all influence the level of LD. Furthermore, LD depends upon the mating framework and hence fluctuates from species to species among populations (Rostoks *et al.*, 2006).

An association mapping group includes vast land regions, areas of adjustment with an upright representation of its evolutionary history typically non-arbitrary because of familial relatedness and may indicate distinctive sorts of structure (Pritchard *et al.*, 2000). This may suggest counterfeit marker quality affiliations (Zhao *et al.*, 2007). In this way it is essential to have appropriate statistical procedures and methods to elucidate such complexities (Patterson *et al.*, 2006). The most prevalent course is to assemble the constituents affiliated with mapping section and consolidate the data in measurable models in which markers are being discovered within the familiar subpopulations (Balding *et al.*, 2006).

Other important approaches to control population structure are the utilization of intermingled models to document contrast in genetic relatedness between section representatives (kinship matrix) (Malosetti *et al.*, 2007). While evaluating, population structure is an important computational demand; Patterson *et al.* (2006) presented a methodology utilizing principal component analysis (PCA) to manage the issue of counterfeit associations. This is quick and simple and works well with extensive information sets.

1.5.4 Proteomic approach

Proteomics is the most advanced approach to categorization of diverse proteins that are included in unique and/or distorted structures (Maleki *et al.*, 2014). Particular genes or families of genes might regulate a few protein types to control specific characteristics. Advancement in proteomics has been used to focus information on instruments that manage complex inherent characteristics (Capriotti *et al.*, 2014). Peng *et al.* (2009)

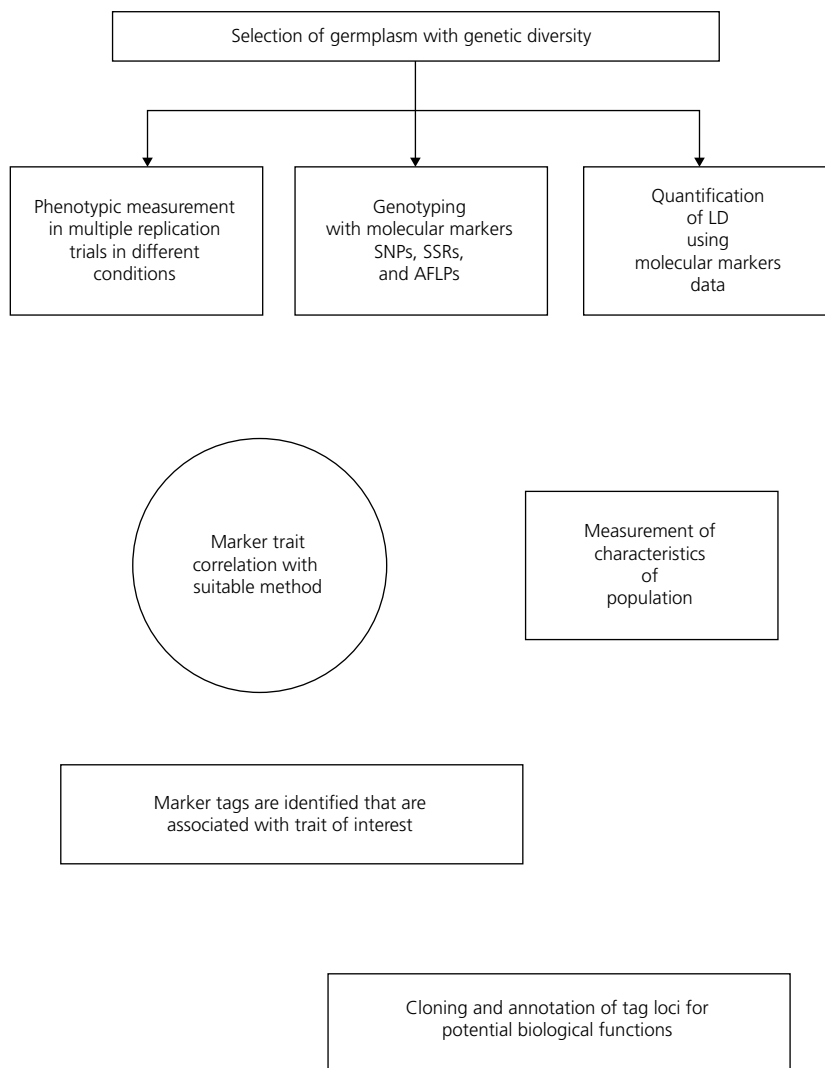


Figure 1.2 Schematic representation of association mapping.

assessed that cultivars of wheat Shanrong 3 and progenitor cultivar Jinan 177 are utilized in two-dimensional gel electrophoresis and mass spectroscopy for protein profiling. Consequences revealed that 6 and 34 protein conserved differentially in leaves (Maleki *et al.*, 2014) and roots, respectively.

A few uniquely conserved proteins could further be described in terms of their capacities in metabolomics or other indicators for transduction cross-communication in salt tolerance systems in plants. This was also the

case with seedlings of wheat when treated with salicylic acid (0.5 mM) and sodium chloride NaCl (250 mM) for 3 days. In both salicylic acid and salt analysis, 39 proteins are demonstrated by 2d PAGE and MALDI-TOF/TOF-MS is used to control 38 protein (Kang *et al.*, 2012). The research proposed that communicated proteins are being included in diverse cells along with metabolic methodologies, for example metabolism, stress safeguard, signal transduction and photosynthesis.

1.5.5 Salt tolerance-related genes

Enhanced salt tolerance is controlled by genes (Table 1.1) that can be classified into three categories (Munns, 2005) as discussed below.

1.5.5.1 Genes for salt uptake and transport

A considerable amount of research is dedicated globally to investigating the processes and understanding the interactions occurring among the genome and proteome within a plant cell during salt stress (Hirayama & Shinozaki, 2010). One of these processes is the primary phase of gene expression, transcription, which is a vital process because the production of a vigorous transcriptome is a promising feature for better protein yield and stabilized cellular activities (Chew & Halliday, 2011; Christov *et al.*, 2014). Salt stress influences the genome and gene expression patterns, thus resulting in a diverse proteome (Nakashima *et al.*, 2009).

Researchers from the Commonwealth Scientific and Industrial Research Organization (CSIRO) isolated two salt-tolerant wheat genes, *Nax1* and *Nax2*, evolved from an old relative of wheat, *Triticum monococcum*. Both genes were responsible for limiting toxic sodium passage from root to shoot, thus causing inhibition of toxic sodium. In one study evaluating the *Nax2* gene in field trials, a variety with the *Nax2* gene showed a 25% greater yield than without *Nax2* under saline environment (James *et al.*, 2006).

Proteins implanted within the membrane lipid bilayer are involved in controlling Na^+ ion uptake from soil and in transport across the whole plant body. The Na^+ ion may be regulated by the K^+ ion transporter and channels directly since they are not completely selective for K^+ ions as it buffers the cell for uptake of Na^+ ions by

rigorous K^+ homeostasis (Munns, 2005). Ion selective channels are involved in transportation of ions passively under electrochemical gradient. Non-selective channels allow transport of Na^+/K^+ (Demidchik *et al.*, 2002). Active particle transport occurs through symporters and antiporters. Transport occurs under conditions of electrochemical potential distinction of a conjugated solute, generally H^+ . Numerous genes significantly maintain Na^+ or K^+ homeostasis in higher plants while possibly being promising for hereditary controls (Munns, 2005). Different genes like *AKT1*, *AKT2* and *KAT1* encode for K^+ ion channels such as *AKT1*, *AKT2*, *KAT1* (which encode for shaker-type inward channels having a single pore), *KCOI* (two-pore channel of KCO family); K^+ antiporters like *KEA*, *SKOR* (shaker-type outward channel), *CPA* (KVIf antiporter). K^+ transporters like *HAK1*, *KUPI 4* and *SOS 1*, *HKT1*; Na^+ antiporters like *NHX1-5* and proton pumps like *AVPI* code for H^+ -ATPase. *AHA2* plus H^+ -PPase (Mäser *et al.*, 2002) can be employed to enhance salt tolerance.

1.5.5.2 Genes for osmotic function

Solutes that show a protective or osmotic effect are classified into four categories: (1) N-containing compounds like glycine betaine and proline; (2) sugars including sucrose and raffinose; (3) straight-chain polyhydric alcohols like mannitol and sorbitol; and (4) cyclic polyhydric alcohols such as myoinositol, pinitol and ononitol. Numerous genes have been recognized that code for such osmotically important compounds, such as *P5CS* gene coding for proline (Hong *et al.*, 2000), *mtID* for mannitol, *otsA* and *S6PDH* for sorbitol (Gao *et al.*, 2001), *codA* for glycine betaine, *ots8* for tetrahalose (Gong *et al.*, 2002) and *imtl* for myoinositol. These genes are

Table 1.1 Genes for salinity tolerance in wheat.

Serial no.	Gene category	Genes	References
1	Genes encoding for K^+ channels	<i>AKT1</i> , <i>AKT2</i> , <i>KAT1</i> (encoding for shaker-type single-pore inward channels), <i>SKOR</i> (shaker-type outward channel), <i>KCOI</i> (two-pore channel of KCO family), K^+ antiporters like <i>KEA</i> , <i>CPA</i> (KVIf antiporter), K^+ transporters like <i>HAK1-IO</i> , <i>KUPI-4</i> and <i>HKT1</i>	Mäser <i>et al.</i> 2002
2	Na^+ antiporters	<i>NHX1-5</i> and <i>SOS 1</i> and proton pumps like <i>AHA2</i> and <i>AVPI</i> encoding for H^+ -ATPase and H^+ -PPase, genes for Na^+ exclusion, named <i>Nax1</i> and <i>Nax2</i>	Mäser <i>et al.</i> 2002 Munns and James 2003
3	HKT gene family	<i>HKT7</i> (<i>HKT1;4</i>), <i>HKT8</i> (<i>HKT1;5</i>), <i>TmHKT7</i> (<i>TmHKT1;4-A2</i>), <i>TmHKT8</i> (<i>TmHKT1;5-A</i>) (origin in <i>T. monococcum</i>)	Huang <i>et al.</i> 2006 Byrt <i>et al.</i> 2007
4	HKT gene family	<i>TaHKT8</i> (<i>TaHKT1;5-D</i>), <i>AtHKT1;1</i> (origin in <i>T. aestivum</i>)	Byrt <i>et al.</i> 2007

mainly present in model cash crops as well as other plants required to be transformed followed by field trials to produce and commercialize easy, cost-effective and high-yield varieties (Yamaguchi & Blumwald, 2005).

1.5.5.3 Genes for cell growth

Certain genes have also been identified that play vital roles in plant growth such as development of new roots or leaves, or may also be involved in life-sustaining biochemical pathways like photosynthesis. Once such genes are well characterized and transformed efficiently, this can lead to mediating cell division, growth rate and other measurements under varied environmental conditions. Similarly rate of photosynthesis, stomatal closure and opening or measuring mesophyll cells, signalling pathways (Choi *et al.*, 2014; Schmidt *et al.*, 2014) and co-workers like hormones, a variety of proteins and enzymes (Kahrizi *et al.*, 2012) like kinases and phosphatases are easily studied and regulated via detailed assessment of such genes (Zhang *et al.*, 2004). It is proposed that such genes can simultaneously also be instructive in water stress (Chaves *et al.*, 2003). Some other factors like CBFs (C-repeat binding factors) and ABFs (ABRE binding factors) have also proved enhancement in tolerance against varied abiotic stresses (Ozturk *et al.*, 2002).

1.5.5.4 Genes for reducing Na⁺ accumulation

Cereal crops like durum wheat (Cuin *et al.*, 2010; Munns & James, 2003), rice (Haq *et al.*, 2010), pearl millet (Krishnamurthy *et al.*, 2007), *Medicago sativa* (Castroluna *et al.*, 2014) and grain (Shavrukov *et al.*, 2010) all possess the pattern of salinity tolerance with exclusion of Na⁺ from plant leaves. On the other hand, wheat wild progenitors like *Triticum tauschii* (Schachtman *et al.*, 1991), durum (pasta) wheat (*Triticum turgidum*), tall wheatgrass (Colmer *et al.*, 2006) and hordeum species are more susceptible to salinity stress as compared to *Triticum aestivum* (bread wheat) due to its low Na⁺ exclusion potential (Munns & James, 2003).

In order to obtain salinity-tolerant durum wheat, durum or durum-associated wheat, genotypes were gathered across the world. Initial studies revealed 'Line 149 genotype' among gathered genotypes which surprisingly possessed the property to avoid Na⁺. Following detailed investigations, it was found that two genes, Nax1 and Nax2 from Line 149, are both responsible for Na⁺ exclusion (James *et al.*, 2011). QTL analysis revealed

that Nax1 is located on chromosome 2A, distinguished by mapping as Na⁺ ion transporter of HKT7 (HKT1:4) HKT gene family (Huang *et al.*, 2006). Nax2 is located on chromosome 5A with HKT8 (HKT1:5) (Byrt *et al.*, 2007). Currently wheat does not exhibit Nax genes but both Nax1 and Nax2 genes were coincidentally shifted into Line 149 after crossing *Triticum monococcum* (C68 101) with durum wheat with the intention to exchange rust resistance genes (James *et al.*, 2006). Following that, these genes were named as TnHKT8 (TmHKT1: 5-A) and TmHKT7 (TmHKT1: 4-A2) in *Triticum monococcum*, respectively.

These genes in durum wheat play their role in exclusion of Na⁺ from xylem so that leaves may receive lesser amounts of Na⁺ ions (James *et al.*, 2006). More specifically, Nax1 ejects Na⁺ ions from roots, lower parts of leaves and xylem, while Nax2 plays the same role in root xylems. Nax2 bears a phenotype for expulsion of Na⁺ and enhances K⁺/Na⁺ selection in *Triticum aestivum* (bread wheat) while Nax1 has a phenotype of high sheath-blade proportion of Na⁺ ion concentration (Dvořák *et al.*, 2004). It was demonstrated that, in *Triticum aestivum*, Nax2 is homologous to Kna1, especially Tahkt8 (Tahkt1:5-D) (Byrt *et al.*, 2007). The HKT gene family also encodes transporters in plasma membrane which mediate Na⁺ or K⁺ uptake from apoplast (Hauser & Horie, 2010). These are crucial for cell homeostasis in terms of Na⁺ and K⁺, and if carried to stele, more specifically the parenchymatous lining in xylem, they recover Na⁺ ions from the transpiration path and thus protect leaves from Na⁺ ions (Hauser & Horie, 2010; Munns & Tester, 2008). Transportation of Na⁺ to leaves is decreased if stele-specific supporting genes are upregulated, thus increasing salt tolerance to *Arabidopsis* (Moller *et al.*, 2009). These Nax genes are associated with *Triticum monococcum*, a diploid wheat progenitor, while they have vanished from advanced wheat cultivars (Huang *et al.*, 2008).

These genes are brought into bread wheat by the usual combination of tetraploid wheat, i.e. durum being crossed with hexaploid wheat and resultant F1 generation, i.e. pentaploid was again backcrossed to bread wheat. Offspring of hexaploid wheat that contain one or both Nax genes were selected as four Australian cultivars of wheat. Genes near Nax genes were examined for their ability to promote Na⁺ exclusion and division of Na⁺ ions between the sharpened and sheath stele as well as their photosynthetic implementation in 150 mM

NaCl. Saline soils were frequently wet throughout the time in advance of planned vegetative development (Colmer *et al.*, 2005).

1.5.6 Molecular markers

Molecular markers are principally related to evaluating polymorphisms in DNA arrangements (i.e. base pair cancellations, substitutions, augmentations or patterns). Molecular markers are amongst the most effective machinery for the assessment of genomes and allow the relationship of heritable qualities with underlying genomic diversity to be determined (Table 1.2).

The most widely used DNA marker systems for assessment of genetic diversity in wheat are SNPs (single nucleotide polymorphisms), SSRs (simple sequence repeats), ISSRs (inter-simple sequence repeats), AFLP (amplified fragment length polymorphism), RFLP (restriction fragment length polymorphism), RAPD (random amplified polymorphic DNA), ESTs (expressed sequence tags) and microarray technology. All molecular marker methods can be utilized for diverse applications including germplasm characterization, hereditary diagnostics, characterization of transformants, investigation of

genome association, marker-assisted selection (MAS) and phylogenic dissection (Mishra *et al.*, 2014).

1.5.6.1 Single nucleotide polymorphisms

Single nucleotide polymorphisms (SNPs) are a single base-change or small insertions or terminations in homologous sections of DNA. In human genome sequencing, 10–30 million SNPs were discovered and were the greatest source of polymorphisms (Collins *et al.*, 1998), present both in coding and non-coding locales (Aerts *et al.*, 2002). As markers, SNPs are favoured over other marker frameworks owing to their more continuous, co-dominant nature and occasional connection with morphological progressions (Lindblad-Toh *et al.*, 2000). Genomes of higher plants such as barley (Kanazin *et al.*, 2002), soybean (Choi *et al.*, 2007), maize (Tenaillon *et al.*, 2001), sunflower (Lai *et al.*, 2005), sugar beet (Schneider *et al.*, 2001), rye (Varshney *et al.*, 2007) and cotton (Ahmad *et al.*, 2007; Lu *et al.*, 2005; Shaheen *et al.*, 2006) have furthermore been studied for SNP revelation and characterization. Since SNPs are exceptionally polymorphic, each gene must contain few SNPs even among strains (Cho *et al.*, 1999).

Table 1.2 Advantages and disadvantages of some commonly used DNA markers.

Serial no.	Molecular marker	Advantages	Disadvantages	References
1	RFLP	Robust Reliable Transferable across population	Time consuming Laborious Expensive Large amount of DNA required Limited polymorphism	Beckman and Soller 1986 Kockert 1991
2	RAPD	Quick and simple Inexpensive Multiple loci from single primer possible Small amount of DNA required	Problems with reproducibility Generally non-transferable	Penner 1996 Williams <i>et al.</i> 1990
3	SSR	Technically simple Robust and reliable Transferable between populations	Large amount of time and labour required for production of primers Usually require PAGE	McCouch <i>et al.</i> 1997 Rodcr <i>et al.</i> 1995
4	AFLP	Multiple loci High level of polymorphism generated	Large amount of DNA required Complicated methodology	Vos <i>et al.</i> 1995
5	SNP	High abundance Cross-study comparisons are easy Low mutation rate Easy to type	Expensive to isolate Low information content of the single SNP Substantial rate of heterogeneity among sites	Christian 2004

AFLP, amplified fragment length polymorphism; RAPD, random amplified polymorphic DNA; RFLP, restriction fragment length polymorphism; SNP, single nucleotide polymorphism; SSR, simple sequence repeat.