

Energy, Environment, and Sustainability  
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Sudhir P. Singh  
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Ashutosh Pandey  
Sunil Kumar *Editors*

# Molecular Approaches in Plant Biology and Environmental Challenges



 Springer

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Sudhir P. Singh · Santosh Kumar Upadhyay ·  
Ashutosh Pandey · Sunil Kumar  
Editors

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*Editors*

Sudhir P. Singh  
Center of Innovative and Applied  
Bioprocessing (CIAB)  
Mohali, India

Santosh Kumar Upadhyay  
Department of Botany  
Panjab University  
Chandigarh, India

Ashutosh Pandey  
National Institute of Plant Genome  
Research (NIPGR)  
New Delhi, India

Sunil Kumar  
Technology Development Centre  
National Environmental Engineering  
Research Institute  
Nagpur, India

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# Preface

Energy demand has been rising remarkably due to increasing population and urbanization. Global economy and society are significantly dependent on the energy availability because it touches every facet of human life and activities. Transportation and power generation are two major examples. Without the transportation by millions of personalized and mass transport vehicles and availability of 24×7 power, human civilization would not have reached contemporary living standards.

The International Society for Energy, Environment and Sustainability (ISEES) was founded at Indian Institute of Technology Kanpur (IIT Kanpur), India in January 2014 with an aim to spread knowledge/awareness and catalyze research activities in the fields of energy, environment, sustainability, and combustion. The Society's goal is to contribute to the development of clean, affordable, and secure energy resources and a sustainable environment for the society and to spread knowledge in the above-mentioned areas and create awareness about the environmental challenges, which the world is facing today. The unique way adopted by the society was to break the conventional silos of specializations (engineering, science, environment, agriculture, biotechnology, materials, fuels, etc.) to tackle the problems related to energy, environment, and sustainability in a holistic manner. This is quite evident by the participation of experts from all fields to resolve these issues. The ISEES is involved in various activities such as conducting workshops, seminars, and conferences in the domains of its interests. The society also recognizes the outstanding works done by the young scientists and engineers for their contributions in these fields by conferring them awards under various categories.

Third International Conference on “Sustainable Energy and Environmental Challenges” (III-SEEC) was organized under the auspices of ISEES from December 18 to 21, 2018, at Indian Institute of Technology Roorkee. This conference provided a platform for discussions between eminent scientists and engineers from various countries including India, USA, Norway, Finland, Sweden, Malaysia, Austria, Hong Kong, Bangladesh, and Australia. In this conference, eminent speakers from all over the world presented their views related to different aspects of energy, combustion, emissions, and alternative energy resource for sustainable development and cleaner

environment. The conference presented five high-voltage plenary talks from globally renowned experts on topical themes, namely “The Evolution of Laser Ignition Over more than Four Decades” by Prof. Ernst Wintner, Technical University of Vienna, Austria; “Transition to Low Carbon Energy Mix for India” by Dr. Bharat Bhargava, ONGC Energy Center; “Energy Future of India” by Dr. Vijay Kumar Saraswat, Hon. Member (S&T) NITI Ayog, Government of India; “Air Quality Monitoring and Assessment in India” by Dr. Gurfan Beig, Safar; and “Managing Large Technical Institutions and Assessment Criterion for Talent Recruitment and Retention” by Prof. Ajit Chaturvedi, Director, IIT Roorkee.

The conference included 24 technical sessions on topics related to energy and environmental sustainability including 5 plenary talks, 27 keynote talks, and 15 invited talks from prominent scientists, in addition to 84 contributed talks and 50 poster presentation by students and researchers. The technical sessions in the conference included Advances in IC Engines, Solar Energy, Environmental Biotechnology, Combustion, Environmental Sustainability, Coal and Biomass Combustion/Gasification, Air and Water Pollution, Biomass to Fuels/Chemicals, Combustion/Gas Turbines/Fluid Flow/Sprays, Energy and Environmental Sustainability, Atomization and Sprays, Sustainable Transportation and Environmental Issues, New Concepts in Energy Conservation, and Waste to Wealth. One of the highlights of the conference was the Rapid-Fire Poster Sessions in (i) Engine/Fuels/Emissions, (ii) Renewable and Sustainable Energy, and (iii) Biotechnology, where 50 students participated with great enthusiasm and won many prizes in a fiercely competitive environment. 200+ participants and speakers attended this four-day conference, which also hosted Dr. Vijay Kumar Saraswat, Hon. Member (S&T) NITI Ayog, Government of India, as the chief guest for the book release ceremony, where 14 ISEES books published by Springer, Singapore, under a special dedicated series “Energy, Environment and Sustainability” were released. This was second time in a row that such significant and high-quality outcome has been achieved by any society in India. The conference concluded with a panel discussion on “Challenges, Opportunities and Directions for National Energy Security,” where the panelists were Prof. Ernst Wintner, Technical University of Vienna; Prof. Vinod Garg, Central University of Punjab, Bhatinda; Prof. Avinash Kumar Agarwal, IIT Kanpur; and Dr. Michael Sauer, Boku University of Natural Resources, Austria. The panel discussion was moderated by Prof. Ashok Pandey, Chairman, ISEES. This conference laid out the roadmap for technology development, opportunities and challenges in energy, environment, and sustainability domain. All these topics are very relevant to the country and the world in present context. We acknowledge the support received from various funding agencies and organizations for the successful conduct of the Third ISEES Conference (III-SEEC), where these books germinated. We would, therefore, like to acknowledge NIT Srinagar, Uttarakhand (TEQIP) (special thanks to Prof. S. Soni, Director, NIT, UK), SERB, Government of India (special thanks to Dr. Rajeev Sharma, Secretary); UP Bioenergy Development Board, Lucknow (special thanks

to Sh. P. S. Ojha), CSIR, and our publishing partner Springer (special thanks to Swati Mehershi).

The increasing global population, urbanization, and industrialization are generating a plethora of unfavorable and adverse environmental factors for the living organisms in the ecosystem. The environmental factors, such as temperature variations, drought, salinity, flood, metal concentration, are the major stresses to the plants which are negatively affecting the plant's physiology, like photosynthetic capacity, plant's growth and biomass, sustainability of the crop yield, and nutrient quantity and quality. These environmental stresses pose higher mutation rates, metabolomic changes, and epigenetic modifications leading to the genotypic and phenotypic alterations in plants. Many transcription factors have been described to play a key role in developing tolerance mechanism against a variety of environmental challenges. Secondary metabolites may not be essential for growth and development of plants; however, they play important role in plant survival under certain sets of environmental conditions, such as biotic and abiotic stresses. Therefore, these compounds may lead to many agronomic traits including quality, yield, resistance, and stress tolerance. Further, secondary metabolites constitute important dietary components as well as phytomedicines. Cellular events, such as autophagy and apoptosis, play an important role in conferring stress tolerance in plants. Numerous defense-related proteins, including receptor-like kinases, facilitate stress signaling for adaptation under various abiotic and biotic stress conditions in plants. Further, the environmental stress increases reactive oxygen species (ROS), which are useful in signaling at low concentration but toxic at a higher level. Several protein families, such as superoxide dismutase, ascorbate peroxidase, glutathione peroxidases, are known to perform the homeostasis of ROS in the plant cell to maintain optimal concentration. This book intends to compile a comprehensive knowledge about the plant molecular approaches to take up the environmental challenges for productivity and sustainability of agricultural crops. The book will also cover the natural tolerance mechanism, which the plants adopt to cope with the adverse environmental factors, as well as the novel molecular strategies to engineer the plants in human interest.

The editors would like to express their sincere gratitude to large number of authors from all over the world for submitting their high-quality work in a timely manner and revising it appropriately at a short notice. We would like to express our special thanks to Dr. Martin Sagasser, Dr. Rakesh K. Upadhyay, Dr. Prashant Mishra, Dr. Smita Kumari, Dr. Rahul Singh, Dr. Jitendra Kumar, Dr. Lokesh Narnoliya, Dr. Jyoti Singh, and all others, who reviewed various chapters of this monograph and provided their valuable suggestions to improve the manuscripts.

Mohali, India  
Chandigarh, India  
New Delhi, India  
Nagpur, India

Sudhir P. Singh  
Santosh Kumar Upadhyay  
Ashutosh Pandey  
Sunil Kumar



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 and Praveen Chandra Verma

# Editors and Contributors

## About the Editors



**Dr. Sudhir P. Singh** is currently Scientist at the Center of Innovative and Applied Bioprocessing, Mohali, India. He has been working in the area of molecular biology and biotechnology for more than a decade. He developed novel stringently regulated gene expression systems for inducing male sterility and fertility restoration in plants. He revealed tissue-specific distribution pattern of mineral nutrients in grains and its probable impacts on mineral bioavailability. He provided the first molecular insights into fruit and seed development in custard apple and litchi by analyzing de novo transcriptome of developing fruits and ovules of contrasting genotypes. His de novo transcriptomic study provided the first molecular details of the specialized metabolic pathways in rose-scented geranium. Currently, his main focus of research is gene mining and biocatalyst engineering for development of approaches for transformation of agro-industrial residues and under- or un-utilized side-stream biomass into value-added bio-products. His group has discovered novel genes for D-allulose 3-epimerase, amylosucrase, xylanase, and cellulase enzymes from the extreme habitat metagenomes. Dr. Singh has over 50 scientific publications and 11 patents (10 filed, and 01 granted) to his credit. He has been conferred International Bioprocessing Association-Young Scientist Award-2017, School of

Biosciences-Madurai Kamraj University (SBS-MKU) Genomics Award-2017, and Professor Hira Lal Chakravarty Award, Indian Science Congress Association (ISCA, DST)- 2018. He has also been the recipient of Early Career Research Award, SERB, 2016.



**Dr. Santosh Kumar Upadhyay** is currently Assistant Professor in the Department of Botany, Panjab University, Chandigarh, India. He has been working in the field of Plant Biotechnology for more than 12 years. His present research focuses in the area of functional genomics. He is involved in the characterization of various insect toxic proteins from plant biodiversity, and defense and stress signaling genes in bread wheat. He has played significant role in identification of whitefly toxic proteins from lower plants and involved in the development of whitefly resistant cotton plants. His research group at PU has characterized numerous important gene families related to the abiotic and biotic stress tolerance and signaling in bread wheat. He has also established the method for genome editing in bread wheat using CRISPR-Cas system. His research contribution led publication of more than 40 research papers in leading journals of international repute. Further, there are more than five national and international patents and five book chapters in his credit. In recognition of his strong research record he has been awarded NAAS Young scientist award 2017-18, NAAS-Associate (2018), INSA Medal for Young Scientist (2013), NASI- Young Scientist Platinum Jubilee Award (2012) and Altech Young Scientist Award (2011). He has also been the recipient of the prestigious DST-INSPIRE Faculty Fellowship (2012), and Early Career Research Award, SERB (2016) from Ministry of Science and Technology, Government of India.



**Dr. Ashutosh Pandey** is currently Staff Scientist at National Institute of Plant Genome Research, New Delhi, India. His research area is metabolic engineering of flavonoid biosynthesis in plants. He has overexpressed AtMYB12 and AtMYB11 in tomato, which led large-scale differential modulation in transcriptome and flavonoid content in leaf and fruit tissues, and it conferred insect attack tolerance. Further, he coexpressed Arabidopsis transcription factor, AtMYB12, and soybean isoflavone synthase, GmIFS1, genes in tobacco, which enhanced biosynthesis of isoflavones and flavonols resulting in osteoprotective activity. He has characterized isoflavone biosynthesis in *Psoralea corylifolia*. He developed callus culture for large scale production of rutin with biopesticidal potential. He has performed genome-wide analysis of carotenoid biosynthesis pathway in banana which facilitates metabolic engineering for enhance provitamin A biosynthesis and bioavailability in biofortified banana cell lines. Further, he achieved several folds increase in  $\beta$ -carotene content by overexpressing phytoene synthase1 of Nendran (NEN-PSY1) in banana cell lines. He has been conferred membership of Indian National Young Academy of Sciences (IN-YAS) in 2018 (Plant Sciences), INSA Medal for Young Scientist (2017), and the Alexander von Humboldt Post Doctoral Research Fellowship (2016) from Alexander von Humboldt Foundation, Germany.



**Dr. Sunil Kumar** is currently Principal Scientist and Head, Technology Development Centre, Council of Scientific and Industrial Research-National Environmental Engineering Research Institute (CSIR-NEERI), Nagpur, India. Dr. Kumar has carried out several national and internationally sponsored projects. Most of the projects are related to solid and hazardous waste management and environmental impact and risk assessment related aspects. He has authored 117 research articles, 3 books, edited 8 book volumes, 25 book chapters, 35 technical reports, and 25 conference presentations/publications. He has been conferred CSIR-NEERI

Foundation Day Award (2009), CSIR-NEERI Outstanding Scientist Award (2015), and Alexander von Humboldt-Stiftung Jean-Paul-Str.12 D-53173 Bonn, Germany (2018).

## Contributors

**Aditya Vikram Agarwal** DST—Centre for Policy Research, Babasaheb Bhimrao Ambedkar University, Lucknow, India

**Parul Agarwal** Council of Scientific and Industrial Research—National Botanical Research Institute (CSIR-NBRI), Lucknow, Uttar Pradesh, India

**Anshu Alok** Department of Biotechnology, Panjab University, Chandigarh, India

**S. Anil Kumar** Department of Biotechnology, Vignan's Foundation for Science, Technology and Research, Vadlamudi, Guntur, Andhra Pradesh, India

**Surjeet Kumar Arya** Council of Scientific and Industrial Research—National Botanical Research Institute (CSIR-NBRI), Lucknow, Uttar Pradesh, India; Academy of Scientific and Innovative Research (AcSIR), Ghaziabad, India

**Monika Bhuria** Department of Biotechnology, CSIR—Institute of Himalayan Bioresource Technology, Palampur, India

**Subhankar Biswas** Department of Botany, Centre of Advanced Study in Botany, Institute of Science, Banaras Hindu University, Varanasi, India

**Amit Choudhary** Council of Scientific and Industrial Research—National Botanical Research Institute (CSIR-NBRI), Lucknow, Uttar Pradesh, India; Academy of Scientific and Innovative Research (AcSIR), Ghaziabad, Uttar Pradesh, India

**Aksar Ali Chowdhary** Department of Botany, Central University of Jammu, Samba, India

**Sameer Dixit** Council of Scientific and Industrial Research—National Botanical Research Institute (CSIR-NBRI), Lucknow, Uttar Pradesh, India; Department of Biology, University of Western Ontario, London, ON, Canada

**Umar Gani** Plant Biotechnology Division, CSIR—Indian Institute of Integrative Medicine, Jammu, India; Academy of Scientific and Innovative Research (AcSIR), New Delhi, India

**Deepak Ganjewala** Amity Institute of Biotechnology, Amity University Uttar Pradesh, Noida, Uttar Pradesh, India

**Devina Ghai** Department of Botany, Panjab University, Chandigarh, India

**Parul Goel** Department of Biotechnology, CSIR—Institute of Himalayan Bioresource Technology, Palampur, India

**Parul Gupta** Department of Botany and Plant Pathology, Oregon State University, Corvallis, USA

**P. Hima Kumari** Department of Genetics, Osmania University, Hyderabad, India

**Shweta Jha** Plant Functional Genomics Lab, Biotechnology Unit, Department of Botany (UGC-CAS), J.N.V. University, Jodhpur, Rajasthan, India

**Shourabh Joshi** Department of Plant Biotechnology, Agriculture University, Jodhpur, India

**Gurminder Kaur** Faculty of Bioscience, Institute of Bioscience and Technology, Shri Ramswaroop Memorial University, Barabanki, Uttar Pradesh, India

**S. Krupanidhi** Department of Biotechnology, Vignana's Foundation for Science, Technology and Research, Vadlamudi, Guntur, Andhra Pradesh, India

**Sunil Kumar** CSIR—National Environmental Engineering Research Institute, Nagpur, India

**Vinod Kumar** Biochemistry Laboratory, Forage Section, Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar, India

**Maridul Kundan** Plant Biotechnology Division, CSIR—Indian Institute of Integrative Medicine, Jammu, India;  
Academy of Scientific and Innovative Research (AcSIR), New Delhi, India

**Charu Lata** Council of Scientific and Industrial Research—National Botanical Research Institute (CSIR-NBRI), Lucknow, Uttar Pradesh, India;  
CSIR—National Institute of Science Communication and Information Resources, New Delhi, India

**Shakti Mehrotra** Department of Biotechnology, Institute of Engineering and Technology, Lucknow, India

**Sonal Mishra** School of Biotechnology, University of Jammu, Jammu, India

**Yogesh Mishra** Department of Botany, Centre of Advanced Study in Botany, Institute of Science, Banaras Hindu University, Varanasi, India

**Prashant Misra** Plant Biotechnology Division, CSIR—Indian Institute of Integrative Medicine, Jammu, India;  
Academy of Scientific and Innovative Research (AcSIR), New Delhi, India

**Akshay Nag** Department of Biotechnology, Panjab University, Chandigarh, India

**Abhishek Kumar Nautiyal** Plant Biotechnology Division, CSIR—Indian Institute of Integrative Medicine, Jammu, India;  
Academy of Scientific and Innovative Research (AcSIR), New Delhi, India



**Ashutosh Pandey** National Institute of Plant Genome Research, New Delhi, India

**Naveen C. Pant** Department of Plant Biochemistry, SKN Agriculture University, Jobner, India

**Sumya Pathak** DST—Centre for Policy Research, Babasaheb Bhimrao Ambedkar University, Lucknow, India

**Boas Pucker** Genetics and Genomics of Plants, Center for Biotechnology (CeBiTec), Bielefeld University, Bielefeld, Germany

**Punesh Sangwan** Department of Biochemistry, Eternal University, Baru Sahib, India

**Abhishake Saxena** Department of Biotechnology, Eternal University, Baru Sahib, India

**Gauri Saxena** Department of Botany, University of Lucknow, Lucknow, Uttar Pradesh, India

**Hanna Marie Schilbert** Genetics and Genomics of Plants, Center for Biotechnology (CeBiTec), Bielefeld University, Bielefeld, Germany

**Jaspreet K. Sembi** Department of Botany, Panjab University, Chandigarh, India

**Tilak Raj Sharma** ICAR—Indian Institute of Agricultural Biotechnology, Ranchi, India

**Akanchha Shukla** Department of Biology, University of Western Ontario, London, ON, Canada

**Shumayla** Department of Botany and Department of Biotechnology, Panjab University, Chandigarh, India

**Anil Kumar Singh** Department of Biotechnology, CSIR—Institute of Himalayan Bioresource Technology, Palampur, India;  
ICAR-Indian Institute of Agricultural Biotechnology, Ranchi, India

**Dharmendra Singh** Department of Biotechnology, Eternal University, Baru Sahib, India

**Kashmir Singh** Department of Biotechnology, Panjab University, Chandigarh, India

**Sanchita Singh** Department of Botany, University of Lucknow, Lucknow, Uttar Pradesh, India;

Council of Scientific and Industrial Research—National Botanical Research Institute (CSIR-NBRI), Lucknow, Uttar Pradesh, India

**Sudhir P. Singh** Center of Innovative and Applied Bioprocessing, Mohali, India

**Sunil Kumar Singh** Department of Biology, University of North Dakota, Grand Forks, USA

**Ragini Sinha** ICAR—Indian Institute of Agricultural Biotechnology, Ranchi, India

**Akanksha Srivastava** Department of Botany, Centre of Advanced Study in Botany, Institute of Science, Banaras Hindu University, Varanasi, India

**Nidhi Srivastava** Amity Institute of Biotechnology, Amity University Uttar Pradesh, Noida, Uttar Pradesh, India

**Vikas Srivastava** Department of Botany, Central University of Jammu, Samba, India

**Sushmita** Council of Scientific and Industrial Research—National Botanical Research Institute (CSIR-NBRI), Lucknow, Uttar Pradesh, India; Academy of Scientific and Innovative Research (AcSIR), Ghaziabad, India

**Manish Tiwari** Council of Scientific and Industrial Research—National Botanical Research Institute (CSIR-NBRI), Lucknow, Uttar Pradesh, India

**Shalini Tiwari** Council of Scientific and Industrial Research—National Botanical Research Institute (CSIR-NBRI), Lucknow, Uttar Pradesh, India

**Prabodh Kumar Trivedi** Council of Scientific and Industrial Research—National Botanical Research Institute (CSIR-NBRI), Lucknow, Uttar Pradesh, India

**Shivi Tyagi** Department of Botany, Panjab University, Chandigarh, India

**Santosh Kumar Upadhyay** Department of Botany, Panjab University, Chandigarh, India

**K. Venkatesh** Department of Genetics, Osmania University, Hyderabad, India

**Jagdeep Verma** Department of Botany, Government College, Rajgarh, Sirmaur, Himachal Pradesh, India

**Praveen Chandra Verma** Council of Scientific and Industrial Research—National Botanical Research Institute (CSIR-NBRI), Lucknow, Uttar Pradesh, India;

Academy of Scientific and Innovative Research (AcSIR), Ghaziabad, Uttar Pradesh, India

**Ajar Nath Yadav** Department of Biotechnology, Eternal University, Baru Sahib, India

**Sandhya Yadav** Department of Botany, Centre of Advanced Study in Botany, Institute of Science, Banaras Hindu University, Varanasi, India

# Chapter 1

## Molecular Approaches in Plant Biology and Environmental Challenges



**Sudhir P. Singh, Santosh Kumar Upadhyay, Ashutosh Pandey and Sunil Kumar**

**Abstract** Global development has generated a plethora of changes in the ecosystem, which directly or indirectly affect living organisms in the biosphere. Plants are crucial to sustaining life on this planet. Therefore, it is desirable to understand manifold biological processes in plants at molecular levels in response to the environmental challenges. The development in molecular technologies worldwide provides a great hope to evolve approaches for augmenting stress tolerance in plants, keeping in mind the extreme environmental conditions in different parts of the world. Research strategies need to be designed in executing the favorable genetic manipulations in the crucial molecular components of the plants such as transcription factors, superoxide dismutases, receptor kinases, histone acetyltransferases and histone deacetylases, signaling pathways, secondary metabolic pathways, etc.

**Keywords** Environmental challenges · Transcription factors · Stress tolerance · Histone acetyltransferases · Superoxide dismutases · CRISPR-Cas9

Plants are sessile organisms, and they face enormous environmental challenges, related to abiotic and biotic stresses, which can induce numerous molecular and physiological changes in the biology of a plant. Abiotic stresses such as heat, drought, salinity, UV, metal stress, etc., and biotic stresses like fungal, bacterial and viral pathogens, and insect pests are the major challenges to the various grain and horticultural crops, negatively influencing the yield and quality of agricultural produce

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S. P. Singh (✉)

Center of Innovative and Applied Bioprocessing, Mohali, India  
e-mail: [sudhirsingh@ciab.res.in](mailto:sudhirsingh@ciab.res.in)

S. K. Upadhyay

Department of Botany, Panjab University, Chandigarh, India

A. Pandey

National Institute of Plant Genome Research, New Delhi, India

S. Kumar

CSIR—National Environmental Engineering Research Institute, Nagpur, India

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(Mittler and Blumwald 2010). However, the environmental challenges in the biosphere should be investigated, keeping in mind the evolutionary processes like adaptation, dispersal and genetic drift, which are crucial in shaping the ecological habitat for organisms and the species.

Plants have evolved various molecular mechanisms to resist these environmental challenges. They have a system of communication through numerous signaling molecules and molecular processes that function in response to various abiotic and biotic inducers (Saijo and Loo 2019). The emitter plants produce signals in the form of common or specific molecules that are perceived by the plants in the vicinity called as receiver plants. The receiver plants then enable themselves for a better adaptation to the changing conditions by producing the relevant proteins. The communication among plants can occur either with the help of a biological or chemical mediator or sometimes without any mediator. Usually, the emitters could not receive benefits in this communication process, but it provides significant benefits to the other plant species. This type of communication among plants is beyond the boundary of species, genus, and family (Heil and Karban 2010). This communication phenomenon in plants can be very useful in future crop designing.

Activation of a cell signaling pathway is one of the most efficient and primary responses of plants and the abiotic and biotic stresses that ultimately produce cellular responses like the expression of stress proteins and/or gathering of compatible solutes to cope up with the changing conditions. Receptor-like kinases (RLKs) are important and stress-specific defense-related proteins that play vital functions stress conditions by activating the signaling cascade. They are not only involved in stress signaling but also play significant roles in plants growth and developments (Liang and Zhou 2018). Structurally, plants have an extracellular domain to perceive the signal, a transmembrane domain to anchor the protein into the membrane, and a cytoplasmic serine/threonine kinase domain to stimulate the signaling for immunity development in plants. Till date, 15 different classes of RLKs are identified on the basis of variation in the extracellular domain in plants. These extracellular domains are diverse in their structural organization as well as signal perception. The detail about structural and functional divergence is explained inside the chapter.

Further, variation in environmental conditions is responsible for overproduction of reactive oxygen species (ROS) such as superoxide ion ( $O_2^-$ ), hydrogen peroxide ( $H_2O_2$ ), singlet oxygen ( $^1O_2$ ), etc. in plants. The limited production of ROS is useful to the plants as some of them act as signaling molecules, but a higher amount of ROS produces a negative impact on plants growth and development. To overcome the effect of ROS, various enzymatic and non-enzymatic antioxidant systems have been involved in plants. The superoxide dismutases (SODs) are a major class of antioxidant proteins that provide the first line of defense. SODs are metalloenzymes that perform dismutation of superoxide radicals into molecular oxygen and hydrogen peroxide. There are groups of SODs have been identified in plants based on metal cofactor, i.e., Cu/ZnSODs, FeSODs, and MnSODs. They are known to play vital roles in plant development and combating oxidative stress (Alscher et al. 2002).

Researches on transcription factors (TFs) have revealed the involvement of several TFs belonging to the families such as NAC, MYB, WRKY, AP2/EREBP, bHLH,

and bZIP, etc., in conferring multiple stress tolerance to plants (Lan Thi Hoang et al. 2017). The gene regulatory networks is a complex molecular mechanism to manage the cellular physiology in response to biotic and abiotic stresses. Furthermore, the potential of specific transcription factors in developing stress tolerance in plants has been discussed. Heat shock transcription factors (Hsfs), and histone acetyltransferases (HATs) and histone deacetylases (HDACs) also perform vital functions for adaptation in the presence of abiotic stresses by different mechanisms (Priya et al. 2019; Luo et al. 2017). HsFs exhibited stress response by regulating the expression of heat shock proteins (Hsps). However, HATs and HDACs are involved in epigenetic modifications by controlling the level of histone acetylation. There are four different HATs families, i.e., CBP/p300, GNAT, MYST, and TAF<sub>II</sub>250, in which GNAT/MYST is associated with UV-B induced DNA damage repair and various other stress responses. Apart from the staple food crops like rice and wheat, this book has also covered the molecular interventions in the flowering plant like orchid, and lower plants like bryophytes. These plants can be used as marker plants to address various environmental challenges, keeping in mind the global climate dynamics. The adaptation mechanism of the lower plants can be a role model for molecular engineering of the environmental robustness in economically important crops.

Recent progress in sequencing technologies and bioinformatics aspects has generated enormous genomic resource, facilitating the engineering and editing of plant genetic material. Numerous genetic engineering strategies have been used for the development of superior varieties concerning stress tolerance and yield. Now a days, targeted genome editing using artificial nucleases such as Zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated protein (Cas) have been used in various crop plants like rice, wheat, maize, tomato, etc. to successfully edit or modulate the target genes as crop improvement strategies. These methods are useful in developing improved plant lines by adding important traits or by removing undesirable traits (Jaganathan et al. 2018). Herein, the recent development and application of the CRISPR/Cas9 system and its advancement in comparison to the ZFNs and TALENs, and its application in the agricultural crops like rice and tomato have been discussed. These technologies have paved ways to engineer biosynthesis of secondary metabolites, which play essential roles in adaptation to changing the environment, management of abiotic stresses, including drought, temperature, salinity, etc. Several specialized secondary metabolites such as isoprenoids, carotenoids, and flavonoids have been established as important stress protectant biomolecules, biosynthesis of which should be taken into consideration for the development of designer crops to face the environmental challenges.

This monograph presents the dynamics of molecular responses, and molecular approaches to combat the environmental challenges in plants. Specific topics covered in the monograph include:

- Molecular Approaches in Plant Biology and Environmental Challenges
- Promising Transcription Factors for Salt and Drought Tolerance in Plants
- Role of Superoxide Dismutases (SODs) in Stress Tolerance in Plants

- Receptor-like Kinases and Environmental Stress in Plants
- Role of Histone Acetyltransferases in Plant Abiotic Stress
- Function of Plant Heat Shock Transcription Factors in Abiotic Stress
- Mode of Communication Among Plants During Environmental Stress
- Molecular Approaches for Combating Multiple Abiotic Stresses in Crops of Arid and Semi-arid Region
- Applications of Landscape Genetics to Study the Effect of Varying Landscapes and Environmental Challenges in Plant Populations
- Arsenic in Rice Grain: Role of Transporters in Arsenic Accumulation
- Metabolic Engineering of Stress Protectant Secondary Metabolites to Confer Abiotic Stress Tolerance in Plants
- An update on molecular strategies of transgenic rice tolerance to abiotic stresses
- An Update on the Applications of CRISPR/Cas9 Technology in Tomato
- Transgenic Approaches for Enhancement of Salinity Stress Tolerance in Plants
- Genome Engineering in Rice: Applications, Advancements and Future Perspectives
- Secondary Metabolite Pathways in Medicinal plants: Approaches in Reconstruction and Analysis
- Molecular Biology of Glandular Trichomes and Their Functions in Environmental Stresses
- Gene Regulatory Networks: Current Updates and Applications in Plant Biology
- Genomics and Transcriptomics Advance in Plant Sciences
- Molecular Interventions to Ameliorate Environmental Stresses in Orchids
- Development of Bryophytes as A New Model System to Understand the Phenomenon of Terrestrialization with Environmental Changes
- Role of Endosymbionts in Nutritional Uptake of Sap Sucking Insects.

## References

- Alscher RG, Erturk N, Heath LS (2002) Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. *J Exp Bot* 53(372):1331–1341
- Heil M, Karban R (2010) Explaining evolution of plant communication by airborne signals. *Trends Ecol Evol* 25(3):137–144
- Jaganathan D, Ramasamy K, Sellamuthu G, Jayabalan S, Venkataraman G (2018) CRISPR for crop improvement: an update review. *Front Plant Sci* 9
- Lan Thi Hoang X, Du Nhi NH, Binh Anh Thu N, Phuong Thao N, Phan Tran LS (2017) Transcription factors and their roles in signal transduction in plants under abiotic stresses. *Curr Genomics* 18(6):483–497
- Liang X, Zhou JM (2018) Receptor-like cytoplasmic kinases: central players in plant receptor kinase-mediated signaling. *Annu Rev Plant Biol* 69:267–299
- Luo M, Cheng K, Xu Y, Yang S, Wu K (2017) Plant responses to abiotic stress regulated by histone deacetylases. *Front Plant Sci* 8:2147
- Mittler R, Blumwald E (2010) Genetic engineering for modern agriculture: challenges and perspectives. *Annu Rev Plant Biol* 61:443–462. <https://doi.org/10.1146/annurev-arplant-042809-112116>

Priya M, Dhanker OP, Siddique KH, HanumanthaRao B, Nair RM et al (2019) Drought and heat stress-related proteins: an update about their functional relevance in imparting stress tolerance in agricultural crops. *Theor Appl Genet* 1–32

Saijo Y, Loo EP (2019) Plant immunity in signal integration between biotic and abiotic stress responses. *New Phytol.* <https://doi.org/10.1111/nph.15989>

# Chapter 2

## Promising Transcription Factors for Salt and Drought Tolerance in Plants



Parul Goel, Monika Bhuria, Ragini Sinha, Tilak Raj Sharma  
and Anil Kumar Singh

**Abstract** Drought and salinity are the most common environmental stresses that cause major loss to crop productivity. To combat such stresses and to satisfy the world food demand, it is desired to develop stress tolerant crop plants. To date, transgenic approach, mainly by overexpressing stress-responsive transcription factors (TFs) is highly appreciated. TFs can activate large set of genes that participate towards multiple stress response. TFs are thus considered as potential candidates for developing stress tolerant plants. Most of the TFs, which play important role in abiotic stress tolerance in plants, fall into major TF families namely AP2/EREBP, NAC, MYB/MYC, WRKY, bZIP, bHLH and ZFP etc. In the current chapter, we have discussed the role of major TF families in enhancing salt and/or drought tolerance in plants. Additionally, we have also discussed the importance of multiple-stress responsive promoters in engineering plants with sustainable abiotic stress tolerance.

**Keywords** Abiotic stress · Drought stress · Salinity stress · Stress signaling · Stress tolerant plants · Transcription factor · Transgenic plants

### 2.1 Introduction

Being sessile, plants have to continuously face several biotic and abiotic stresses throughout their life cycle. Abiotic stresses such as extreme temperature (high or low), salinity, drought, UV-radiation, flooding and submergence are detrimental to plants as they negatively influence their survival and yield. In field conditions, plants may encounter a combination of these stresses together, that leads to extensive losses in agriculture productivity (Mittler and Blumwald 2010). It has been estimated that abiotic stresses cause 50–70% loss to the world crop productivity (Wang et al. 2003).

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P. Goel · M. Bhuria · A. K. Singh  
Department of Biotechnology, CSIR—Institute of Himalayan Bioresource Technology,  
Palampur 176061, India

R. Sinha · T. R. Sharma · A. K. Singh (✉)  
ICAR—Indian Institute of Agricultural Biotechnology, Ranchi 834010, India  
e-mail: [anils13@gmail.com](mailto:anils13@gmail.com)

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It is predicted that, the world population will reach 9 billion by 2050; and to satisfy their food demand, agriculture productivity should be enhanced approximately by 70% (Bruinsma 2009).

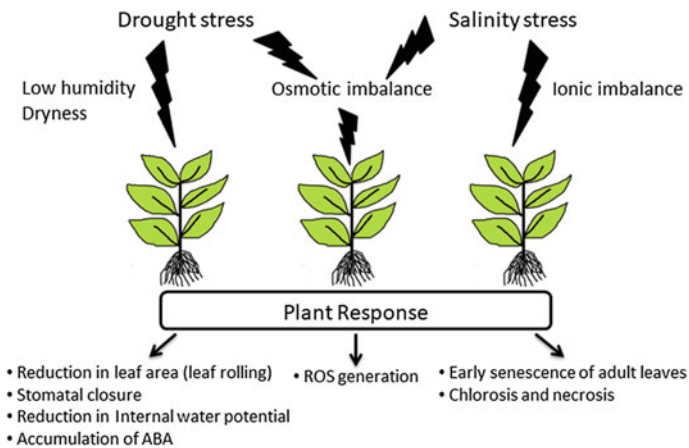
Among abiotic stresses, salinity and drought are considered as major abiotic stresses, which commonly trigger osmotic and oxidative stresses in plants (Reynolds and Tuberosa 2008; Landi et al. 2017). In addition, salinity stress also leads to ionic stress due to high proportion of  $\text{Na}^+$ ,  $\text{Cl}^-$ ,  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  ions (Hasegawa et al. 2000). Salinity stress is one of the major abiotic stresses affecting more than 45 million hectare of irrigated land, worldwide (Munns and Tester 2008). In comparison to the salt stress, the consequence of drought stress is more widespread and devastating (Boyer 1982). Drought stress is the condition of low water availability in the soil. In general, plants experiences drought stress, when soil water potential ranges  $-0.5$  to  $-1.5$  megapascal (MPa) (Onaga and Wydra 2016). In 21st century, approximately 30% of the lands will face extreme drought condition due to climate changes (Burke et al. 2006). Several factors such as low rainfall; high evaporation rate and low water retention capacity of soil contribute to drought stress in crops plants (Wery et al. 1994). However, plant response towards salinity and drought is quite similar as both these stresses lead to the osmotic stress in plants and also trigger ABA accumulation that induces several adaptive responses.

The abiotic stress tolerance is a highly complex trait in plants, and governed by various mechanisms at physiological, biochemical and molecular levels. However, the advancements in various omics (transcriptomics, proteomics and metabolomics) approaches has made feasible to understand these complex mechanisms, comprehensively. In order to cope up with a stress or combination of stresses, plants have evolved various strategies. Better understanding of these strategies and availability of natural genetic variability within crop species have provided opportunities to introgress desirable traits in important crops through breeding approaches. Traditional breeding approaches have been extremely successful in developing various crop varieties with desirable traits. However, development of abiotic stress tolerant crops through breeding has achieved low success, due to low selection efficiency using agronomic characters, complexity of phenotyping methods and limited availability of natural genetic resource with inherent stress tolerance trait (Richards 1996; Singh et al. 2008). Transgenic approach has emerged as an alternative to breeding with several advantages, such as, transgenic approach is faster than conventional breeding; it can transfer desirable gene isolated from any organism; the expression of transgene can be controlled spatio-temporally. During recent past, availability of whole genome sequence of several organisms has made it possible to identify novel genes associated with stress response and deploy them in economically important plants. However, abiotic stress tolerance in plants is a multigenic trait, thus, targeting a single gene may not be very efficient in imparting desired level of stress tolerance. Therefore, targeting a transcription factor (TF) gene that can regulate expression of genes involved in multiple stress responsive pathways seems an interesting alternative. Functional characterization of several members of TF families such as NAC, MYB, WRKY, AP2/EREBP, bHLH and bZIP etc., has revealed their role in multiple stress tolerance. In the present chapter, we firstly discussed the effect of salinity and

drought on plant growth and development and how plants respond to these stresses. Secondly, we discussed the potential of transcription factor genes in developing salt and drought tolerant transgenic plants.

## 2.2 Plant Growth During Drought and/or Salinity Stress

The intensity and the duration of stresses are the two major factors that determine the degree of damage on plant growth and development under unfavorable conditions (Dolferus 2014). Plants can utilize escape, tolerance and avoidance strategies in order to counter the abiotic stress conditions (Touchette et al. 2009). On the basis of tolerance or sensitivity towards salt stress, plants are commonly categorized as glycophytes and halophytes (Flowers et al. 1977). Glycophytes are salt sensitive plants that cannot tolerate high concentration of salts. Unfortunately, most of the agricultural crops are glycophytes. The halophytes can grow and complete their life cycle under relatively high concentration of salt. Halophytes can be further classified as euhalophytes (true halophytes), pseudohalophytes (salt-avoider) and crinohalophytes (salt excretors). Consequences of drought and salinity stress on plant growth are depicted in Fig. 2.1. Inhibition of plant growth during salt stress is a consequence of osmotic stress, ionic stress and nutritional imbalance. Two-phase effect of salinity is observed on plant growth (Munns and Termaat 1986). In first phase (osmotic phase), reduction in soil water potential leads to stomatal closure and inhibition of leaf expansion. The second phase (ionic phase) leads to premature senescence resulting in cell death due to increases cytotoxic ion level. In general, plants face drought stress, when transpiration rate exceeds the root water absorption rate. In case of drought stress, reduced plant growth is mainly due to loss of turgor pressure that impairs cell elongation and



**Fig. 2.1** Consequence of drought and salinity stress on plant growth and development

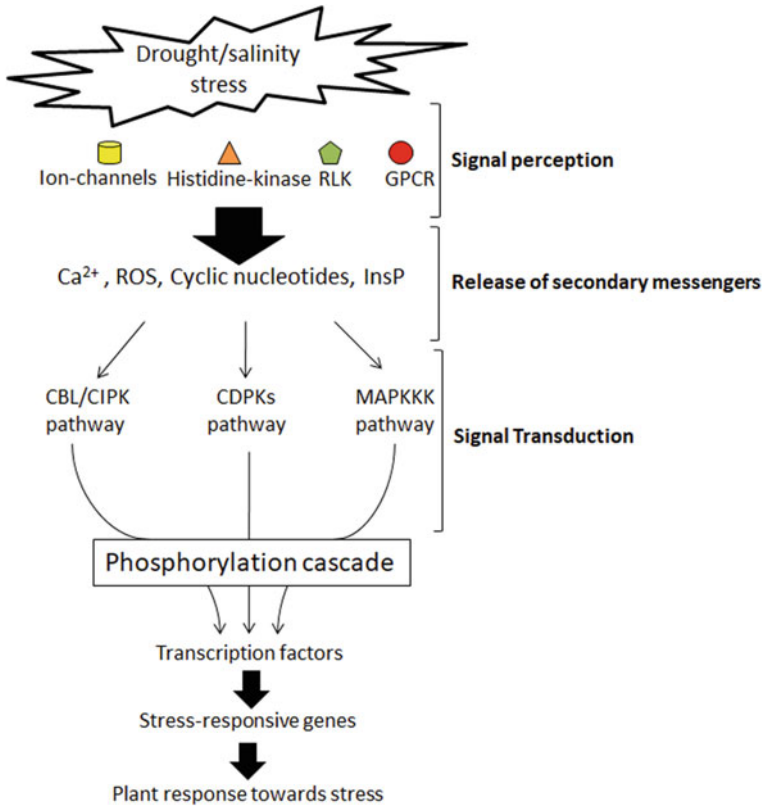
expansion (Farooq et al. 2009). Shoot growth is affected more under both the stresses, while, root elongation in search of groundwater could be observed in plants facing drought stress (Brunner et al. 2015). Whereas, reduced leaf size, early senescence and abscission of older leaves are observed in above ground parts (Forni et al. 2017), similarly salinity stress reduces shoot growth more severely as compared to the root growth (De Oliveira et al. 2013).

## 2.3 Plant Response Towards Stress

Plant response towards abiotic stresses is highly complex that involves modulation in expression of several genes and also diverse changes at physiological and biochemical levels. The following sub-section describes the mechanism of signal perception and also the changes that take place in plants upon stress exposure.

### 2.3.1 Stress Perception and Signal Transduction

Plant has to first perceive the outside stress signal by means of stress sensors or receptors localized on the cell surface. These sensors include histidine kinases, receptor like kinases and G-protein coupled receptors (Fig. 2.2). The signal perception is followed by signal transduction that leads to the generation of second messenger molecules mainly  $\text{Ca}^{2+}$ , ROS, cyclic nucleotides and inositol phosphate (Jain et al. 2018). These messenger molecules activate various signal transduction pathways that ultimately phosphorylate and activate transcription factors regulating the expression of several downstream genes involved in plant stress adaptation. Three most common signal transduction pathways are (i) calcium dependent SOS pathway; (ii) calcium dependent signaling; (iii) MAP Kinase pathway. The calcium dependent SOS pathway is the first identified CBL-CIPK pathway that is activated during ionic stress. The calcineurin B-like proteins (CBLs) are the  $\text{Ca}^{2+}$  sensor proteins that interact and activate CIPKs (CBL-interacting protein kinase), forming the CBL-CIPK complex. As a consequence of stress,  $\text{Ca}^{2+}$  level rises transiently, which is perceived by SOS3/CBL4 (Kolukisaoglu et al. 2004). In the presence of  $\text{Ca}^{2+}$ , the SOS3 physically interacts with SOS2/CIPK24, which encodes a serine/threonine protein kinase (Gao et al. 2008). The SOS2 phosphorylates and activates SOS1 in plasma membrane that acts as a  $\text{Na}^+/\text{H}^+$  antiporter. *Arabidopsis* CBL1 and CBL5 act as positive regulators of drought stress (Cheong et al. 2003, 2010). The calcium dependent signaling pathway activates specialized  $\text{Ca}^{2+}$  sensors known as CDPKs ( $\text{Ca}^{2+}$  dependent protein kinases). The CDPKs are one of the largest subfamilies of protein kinases (Ludwig et al. 2004) and considered as positive regulators of abiotic stress tolerance (Schulz et al. 2013). The CDPKs have calmodulin  $\text{Ca}^{2+}$  binding domain and a Ser/Thr kinase domain for transmitting calcium signals. CDPKs are activated by  $\text{Ca}^{2+}$  binding and then transfer the signal via phosphorylation of downstream target genes. The downstream targets



**Fig. 2.2 Mechanism of abiotic stress signaling in plants** Perceiving stress signal from the outside environment is the very first step initiated by receptors, such as ion channels, histidine kinases, receptor like kinase (RLK) and G-protein coupled receptors (GPCR). Signal perception is then followed by release of secondary messengers, like  $Ca^{2+}$ , ROS, cyclic nucleotides and inositol phosphate (InsP). These secondary messengers in turn activate major signal transduction pathways namely CBL-CIPK, CDPK, and MAPK, which lead to phosphorylation and activation of TFs These TFs bind to the promoter regions of stress-responsive genes and regulate their expression to bring about morphological, physiological, biochemical and molecular response

of CDPKs are ABA response factors, ion channels or transporters. The TFs activated via  $Ca^{2+}$  mediated signaling pathways are reported to contain ABA-responsive element (ABRE; CACGTG[T/C/G]) and its coupling element ([C/A]ACGCG[T/C/G]) in their upstream promoter regions (Kaplan et al. 2006). Transcriptomic study of Arabidopsis seedlings, subjected to specific  $[Ca^{2+}]_{cyt}$  transients has categorized TFs families mainly MYB, bZIP, bHLH and zinc finger protein as early  $Ca^{2+}$ -responsive genes (Kaplan et al. 2006). Another signaling pathway activated mainly in response to osmotic stress is Mitogen-activated protein kinase (MAPK) pathway (Sinha et al. 2011). ROS are considered as central players in activating this pathway. MAPK pathway is comprised of three-components, namely a MAPK kinase kinase

(MAPKKK), a MAPK kinase kinase (MAPKK), and MAP kinase (MAPK). Activation of MAPKKK upon signal perception activates MAPKK by activating two serine or threonine residues. The activated MAPKK then phosphorylates MAPK on its threonine and tyrosine residues. The MAPK then finally activates various downstream genes. Two MAPK pathways, namely MPK4 and MPK6 have been found to be involved in salinity stress response in plants (Ichimura et al. 2000). TFs mainly MYB/MYC and WRKY are known to be phosphorylated via MAPK pathway (Li et al. 2017).

### ***2.3.2 Physiological, Biochemical and Molecular Responses to Drought and Salinity Stress***

In plants, drought stress leads to water deficit condition that trigger reduction in cell volume and turgor pressure, which ultimately reduce the cell wall extensibility (De Oliveira et al. 2013). Drought stress also reduces leaf water potential and stomatal conductance. Plant photosynthetic efficiency also declines due to decrease in Rubisco activity (Bota et al. 2004). Decline in net photosynthetic rate, chlorophyll content and reduction in water potential are also observed in plants facing salinity stress (Forni et al. 2017). During salt and drought stress, plants also produce wide spectrum of metabolites that include amino acids, sugars and amines. The most important amino acid, accumulated under such stresses is proline. As a compatible solute, proline protects plants via osmotic adjustment and ROS detoxification (Hayat et al. 2012). Another compatible solute is non-reducing disaccharide named as trehalose. Transgenic plants overexpressing trehalose biosynthetic genes confer tolerance towards drought and salinity (Iordachescu and Imai 2008). Sugar alcohols mainly mannitol and sorbitol also serve as compatible solutes during osmotic stress. Some plants also produce group of aliphatic amines mainly polyamines and glycine-betaine, which act as osmo-protectants. The major polyamines imparting stress tolerance are, putrescine, spermidine and spermine. Beside these metabolites, various classes of defense proteins mainly osmotins, dehydrins, and late embryogenesis abundant proteins (LEA) are also produced by plants in response to drought and salinity. Osmotins and dehydrins are mainly involved in cellular protection against osmotic shocks (Liu et al. 2017). LEAs are group of low-molecular weight hydrophilic proteins that contribute in stabilizing structural integrity of cell (Hand et al. 2011). Reactive oxygen species (ROS), such as hydrogen peroxide ( $H_2O_2$ ), superoxide radical ( $O_2^{\cdot-}$ ) and hydroxyl radical ( $OH^{\cdot}$ ) etc. generated under abiotic stress leads to the oxidative damage to cell membrane, DNA, RNA and proteins (You and Chan 2015). To protect cell from such damage, plants produce antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) (Kusvuran 2015). SOD converts superoxide radicals to  $H_2O_2$ , whereas APX, GPX and CAT detoxify  $H_2O_2$  (Choudhury et al. 2013). Molecular responses include transcriptional changes in hundreds of stress responsive genes that ultimately help

plant to tolerate such adversities (Fraire-Velazquez S Balderas-Hernandez 2013). Transcriptional regulators mainly transcription factors are the major component of the plant molecular response towards drought and salinity. As a regulator, the TF protein binds to the *cis*-acting element in the promoter region of genes that are responsive to various stresses. The downstream targets of these TFs are discussed in detail in sub Sect. 4.2. Majority of transcription factors have positive regulatory effect on stress responsive genes. For instance, enhanced expression of antioxidant genes (SOD, CAT etc.) under drought stress has been observed in transgenic cotton overexpressing potato DREB2 TF (El-Esawi and Alayafi 2019).

## 2.4 Transcription Factors: Potential Candidates for Enhancing Multiple Stress Tolerance

Transcription factors (TFs) are the regulatory proteins that can turn specific genes on (activate) or off (repress) by binding to the *cis*-acting sequence in the promoter region of their target genes. So far, a total of 58 TF families have been identified in higher plants (Jin et al. 2017). Moreover, whole genome sequencing of majority of plants revealed that approximately 7% of the coding sequences, code for TFs (Udvardi et al. 2007). Stress tolerance mechanism in plants is controlled by complex transcriptional network (Umezawa et al. 2006) and TFs are the major player in this network. In general, the TFs involved in plant abiotic or biotic stress response are mainly referred as stress-responsive transcription factors. These TFs are induced early in response to various stresses and in turn regulate the expression of several downstream target genes. The induction of these TFs is regulated by the presence of conserved *cis*-elements present in their promoter regions. For e.g. the induction of TFs like AP2/EREBP, MYB/MYC whose promoter contains ABA-responsive element (ABRE) is mediated by binding of ABRE-binding proteins (Sah et al. 2016). Additionally, histone modification such as phosphorylation mediated by protein kinases can also activate TFs expression (Pfluger and Wagner 2007). Developing stress tolerant plants by overexpressing TFs is much promising approach due to the reason that a single TF can regulate expression of vast array of stress-responsive genes (Goel and Singh 2018). The following sub-section covers major TF families which have been targeted to develop salt and/or drought tolerant transgenic plants and their downstream targets.

### 2.4.1 *Transcriptional Regulatory Network Under Drought and Salinity Stress*

The phytohormone ABA plays a central role in abiotic stress response especially under drought and salinity (Zhang et al. 2006). Water deficit resulting from drought