Sustainable Development and Biodiversity 38

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Sustainable Utilisation and Bioengineering of Halophytes

Springer

Sustainable Development and Biodiversity

Volume 38

Series Editor

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ISSN 2352-474X ISSN 2352-4758 (electronic)
Sustainable Development and Biodiversity
ISBN 978-981-97-9818-6 ISBN 978-981-97-9819-3 (eBook)
https://doi.org/10.1007/978-981-97-9819-3

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Preface

The global population has increased from 1 billion in 1800 to 7.9 billion in 2020. The United Nations anticipates that the population will continue to expand, with estimates indicating that the total will reach 8.6 billion by mid-2030, 9.8 billion by mid-2050, and 11.2 billion by 2100. Nevertheless, the global population could exceed 12 billion by the end of the century if fertility declines less than anticipated. Sadly, according to FAO data, there are more than 800 million individuals worldwide who are routinely malnourished and go to bed hungry. Chronic hunger has an irreversible impact, accounting for nearly half of child deaths worldwide.

Unfortunately, this gray scenario is intimately correlated with climate change, the effects of which have become increasingly visible in recent decades. Broadly speaking, the impact of climate change translates into a wide variety of threats to humanity: extreme high temperatures (global warming), alternating with episodes of atmospheric instability, floods, water scarcity throughout the planet, increasing aridification and desertification, in parallel with massive and uncontrolled deforestation, especially in the tropics.

The converging effect of these threats is the growing pressure on agriculture, the role of which is to provide food for a population, which is, as we have seen, constantly growing. Now, agriculture is facing two major problems: salinization and aridification of cultivated areas. Salinization is a concern on all continents, with the exception of the South Pole. The most severe salinity issues are found in arid and semi-arid regions of the Globe. The salinity issues that are affecting large portions of the Middle East and Northern Africa are also causing a decline in yields in Australia. Although there is currently no uniform data on the total area of land affected by salinization, there are some general principles that can be applied to the salinization approach in agriculture. First, salinization of agricultural areas must be considered, for the most part, an irreversible process, which is valid at least for natural (primary) salinization; also, within this principle, it is, it turned out that secondary salinization also became an irreversible and difficult to manage phenomenon. Second, it has become clear that attempts to improve these salinized areas by physical, technical, and mechanical means represents a limited strategy, which is expensive and requires massive investment. Third, after the accumulation of an important volume of information on plant-soil interactions, it brought to the fore the idea that the approach of agricultural areas affected by salinity must be done with the help of halophytes and salinity-tolerant plants.

Halophytes are a unique group of plants that have adapted to grow in extreme environmental conditions of high salt, low water and sometimes, high temperature. Halophytes have been known for a very long time, since ancient times, from the era of Theophrastus; however, scientific interest in them arose much later, in the eighteenth century, in the days of Goethe and the French Enlightenment. It has been a while before halophytes are brought to the fore in the discussion of the exploitation of salinity-affected lands, using their cultivation with salinity-tolerant plant species; this happened sometime in the late nineteenth and early twentieth centuries.

Surprisingly at first glance, although halophytes seem to have a long history, some aspects of their delicate mechanisms of salt tolerance are limited, even today, even after accumulating an impressive amount of data. Thus, we can explain, on the one hand, the existence of a large number of publications on this subject, up to this point and, on the other hand, the need to make further contributions to the possibility of using halophytes in the improvement of salinity-affected areas. In desertic conditions these plants are life supporting species for animals and humans. Overexploitation of these plants degrades the ecosystem. Sustainable utilization of resources and improvement through modern tools will help in sustainability of plants and environment. These plants possess unique metabolic and physiological features, making them an attractive target for bioengineering. In recent years, the field of bioengineering has witnessed significant advancements in the study and manipulation of halophytes.

This book aims to provide a comprehensive overview of the cutting-edge research in the bioengineering of halophytes, with a particular focus on the advancements in biotechnology, transcriptomics, proteomics, metabolomics, and genomics background. By exploring the intricacies of these technologies, this book seeks to address key questions and challenges in harnessing the potential of halophytes for sustainable plant resources, environmental remediation, and the development of novel biotechnological applications. There are 17 chapters in the book, divided into three parts. Part I "Biology and Biotechnology of Halophytes" comprises nine chapters covering biology of halophytes, their microbiome including beneficial endophytes, adaptations to cope with saline environment along with halophyte bioengineering for environmental restoration, crop improvement, bioactive molecules production, and bioenergy generation including case studies of Leguminous Halophytes of Türkiye and Romanian tree species. Part II "Genomics of Halophytes" comprises three chapters covering molecular networking, signaling pathways, and genetic manipulations in non-halophytes crop to increase salinity tolerance with a special chapter dedicated to Mangroves Biodiversity and Omics Insights, and Part III "Metabolomics, Proteomics and Nanomaterials of Halophytes" comprises five chapters covering metabolomics to unravel complex metabolic responses in halophytes under salt stress, proteomic analysis, nanomaterial synthesis, and role of halophytes in bioeconomy, which has been described very well with latest reference citations.

Editors wish to thank all the international contributors who have put their time at our disposal and made serious efforts to put all the latest information in one place for this book. We are thankful to Prof. K.G. Ramawat, Series editor for accepting the book proposal in the "Sustainable Development and Biodiversity" book series. We are also thankful to the staff at Springer editorial and production house for their professional support.

Udaipur, Rajasthan, India — Jaya Arora
Iași, Romania — Marius-Nicusor Grigore
Udaipur, Rajasthan, India — Abhishek Joshi
July 2024

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Marius-Nicusor Grigore is an editor at Alexandru Ioan Cuza University Press and an Adjunct Professor at the Doctoral School of Faculty of Biology. He received his B.Sc. and Ph.D. in Biology from Alexandru Ioan Cuza University in Iași, Romania. He was subsequently a postdoctoral fellow in the field of halophytes' ecophysiology at the same university, as well as an invited researcher at the Polytechnic University of Valencia, Spain. His primary research interests are in the ecology, anatomy, and physiology of halophytes and plant responses to abiotic (salt, drought) stress. He has published 11 books, 9 book chapters, and over 80 peer-reviewed papers on halophytes. In 2016, he was awarded the Romanian Academy's "Emil Racoviță" prize for the book *Halophytes: An Integrative Anatomical Study*, published with Springer in 2014; *Handbook of halophytes: From Molecules to Ecosystems Towards Biosaline Agriculture*, Springer, was awarded the scientific merit "Nicolae Giosan" by Academy of Agricultural and Forest Sciences Gheorghe Ionescu-Șișești București, Romania, in November 2022. He coordinated national projects and conducted many scientific missions abroad, at Polytechnic University of Valencia, Spain, in the field of biochemistry and ecophysiology of halophytes and plant responses to salt stress.

Abhishek Joshi is currently associated with research and teaching at the Department of Botany, Mohanlal Sukhadia University, Udaipur, India. He received his M.Sc. and Ph.D. in Botany with a specialization in plant tissue culture and secondary metabolite production from the Department of Botany, Mohanlal Sukhadia University. Joshi's doctoral research was focused on unraveling the biochemical and molecular mechanisms that enable halophytes to thrive in saline conditions. He has 10 years of research experience on halophytes occurring in Southern Rajasthan of Indian subcontinent, with 1 e-book and 34 publications in the international repute journals and books, with one latest edited book published with prestigious publishing house.

Chapter 10
Genetic Manipulation in Halophytes for Abiotic Stress Tolerance: An Overview

Sanjana Suresh, C. Akshaya Prakash, Sarath G. Nair, A. M. Shackira, P. Faseela, Delse P. Sebastian, and Jos T. Puthur

Abstract In the current scenario, one of the major challenges we are addressing is various kinds of stress conditions which create huge repercussions and drastically change the normal balanced ecosystem to an unbalanced one. Plants react to these challenging environments in different ways and certain plants are born to withstand these unwanted stresses. Besides, some sensitive plants witness heavy damages or even death in response to adverse environmental conditions ultimately leading to loss in crop yield and impinge on food security. In this juncture, advanced modern technologies can play a fundamental role in enhancing plants' survival rate as well as reducing the severity of impacts. Among plants, halophytes are able to cope with multiple stress conditions including salinity, oxygen deficiency, toxic metal ions, etc. Halophytes have served as excellent germplasm reserve to unravel the source for salinity, drought as well as heavy metal tolerant genes. Utilizing this proficiency as a criterion along with the aid of modern genome engineering tools, it has been possible to burgeon new relevant stress-resistant transgenic lines. Meganucleases, clustered regularly interspaced short palindromic repeat (CRISPR)-Cas 9, zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), RNA interference (RNAi), etc. are the widely used gene editing tools for the purpose

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J. Arora et al. (eds.), *Sustainable Utilisation and Bioengineering of Halophytes*,
Sustainable Development and Biodiversity 38,
https://doi.org/10.1007/978-981-97-9819-3_10

of halophyte genome engineering. This chapter focuses on various gene editing tools and the significance of genome engineering in the development of stress-tolerant varieties using halophytes as a source of multiple abiotic stress-tolerant genes.

Keywords CRISPR-Cas 9 · Gene editing · Halophyte · Salinity

10.1 Introduction

Halophytes are plants that grow vigorously under saline conditions. Recent reports have suggested that the halophytic response to salinity might impart tolerance to halophytes toward other stresses as well. It has been found that halophytes have better strategies for cross-stress tolerance compared to glycophytes (Hamed et al. 2013; Liu et al. 2022). That is, halophytes can thrive under salinity as well as other unfavorable environmental conditions like drought, flood, or cold (Leng et al. 2018). This indirectly points out that halophytes are important sources of stress-responsive genes (Hafeez et al. 2021). Stress in halophytes induces the activity of various stress tolerance mechanisms that consist of different signaling proteins and upregulation or downregulation of stress-responsive genes leading to the respective stress response in plants (Mishra and Tanna 2017).

Nowadays, various abiotic factors limit crop productivity by causing severe damage to plants. Therefore, it is necessary to develop abiotic stress-tolerant varieties in order to adjust toward the prevailing environmental conditions and this goal is mainly achieved with the help of genome editing techniques. Genome editing is a precise technique of adding, deleting, or altering the genetic composition by targeting a specific gene in the DNA through the employment of advanced molecular techniques (Zhang et al. 2018). The success of achieving the desired trait mainly depends on the proper selection of the candidate gene. Among the genome editing tools, clustered regularly interspaced short palindromic repeat (CRISPR)/cas 9 (CRISPR-associated nuclease 9) is the most efficient and promising approach for targeting structural, regulatory, as well as cis-regulatory genes which play a vital role in achieving stress tolerance (Zafar et al. 2020). The recent gene editing tools are able to address many of the regulatory issues concerned with the traditional transgenic approaches such as public concern and lesser consumer acceptance of transgenic crops, etc. (Zhang et al. 2018; Kumar et al. 2020). In agriculture, genome editing is a potential tool for creating and correcting mutations, regulation of desired gene expression, swapping and editing of regulatory elements, epigenome editing, and site-specific insertion of genes. Gene editing also promotes hybrid breeding thereby enhancing crop productivity (Kumar et al. 2020).

The mechanism of salinity tolerance in halophytes is complicated and involves pathways that are linked. A number of salt-responsive genes from different halophytes have been identified by various authors, and scientists are still in search of stress-responsive genes that can impart tolerance toward salt stress. The possible

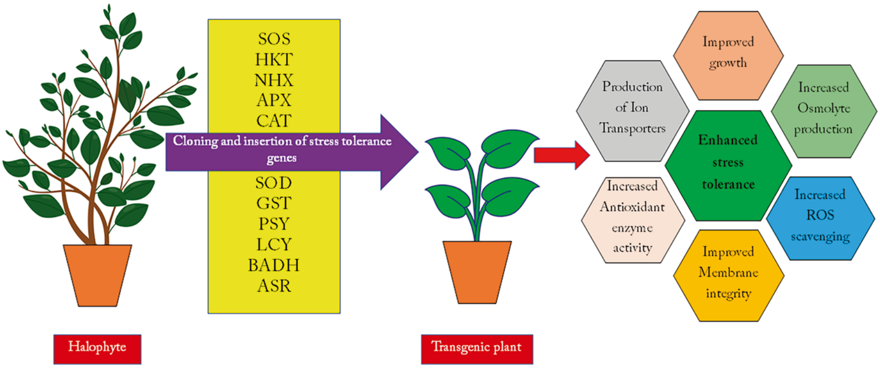

Fig. 10.1 Imparting stress tolerance through halophyte genetic engineering

candidates for the salt-responsive genes and promoters comprise halophytes including *Aeluropus, Suaeda, Mesembryanthemum, Atriplex, Salicornia, Cakile*, and *Thellungiella* (Mishra and Tanna 2017). Various genomic and transcriptomics techniques have been employed to identify genes related to salt response in some halophytes, following which their functional confirmation has been achieved by transgenic methods. In order to investigate the development of stress tolerance in glycophytes, a number of genes like *NHX, HKT, SOS, APX, GST, CAT, USP, SRP*, etc. were extracted from halophytes and studied (Fig. 10.1).

10.2 Gene Editing Tools Employed in Halophytes

The whacking advancements in technology bring solutions to the majority of snags that we are addressing today. Among these gene editing holds greater attention and the meganucleases, clustered regularly interspaced short palindromic repeat (CRISPR)-Cas9 (CRISPR-associated nuclease 9), zinc finger nucleases (ZFNs), and transcription activator-like effector nucleases (TALENs) are most commonly known genome editing tools across various plants to build up stress tolerance (Das et al. 2023). In addition to this, salt-tolerant plants can be generated with the aid of the emerging technology of RNA interference (RNAi) which is based on gene silencing mechanisms (Wani et al. 2020). But at present CRISPR/Cas technology has greater heed because it is a low-cost, simple, and highly efficient tool (Ai et al. 2023; Liu et al. 2022) even though the increased number of choices for the selection of target locus makes TALENs genome editing tool advantageous (Wani et al. 2020; Abbas et al. 2022; Das et al. 2023). miRNA fine-tuning through nucleases such as ZFNs, TALENs, CRISPR/Cas 9, and RNA interference (RNAi) helps to improve stress tolerance efficiency in plants (Abbas et al. 2022).

10.2.1 ZFN (Zinc-Finger Nucleases)

ZFNs are small-sized endonucleases with zinc finger proteins having the capability to recognize specific triplet nucleotides (Şenödeyici et al. 2021; Das et al. 2023). ZFN induces double-stranded breaks in the genome. Basically, ZFN has two domains: the first is a nuclease domain which is made up of a Flavobacterium okeanokoites I (FokI) restriction enzyme, and the second domain is the DNA binding domain which has a zinc finger repeats (recognizes the nucleotide sequences of 9–18 bp) (Urnov et al. 2010). ZFN facilitated gene knockout and designed gene edits in bread wheat which are heritable and exhibited the expected Mendelian segregation (Ran et al. 2018).

The insertion of PAT gene cassettes in maize plants using the ZFN tool altered the inositol phosphate pathway which imparted herbicide tolerance (Shukla et al. 2009). Low success rate, time consumption, and high cytotoxicity make ZFNs disadvantageous over other gene editing tools (Das et al. 2023). In another study, the viability of utilizing a ZFN-based method for site-directed mutagenesis within an endogenous gene of *Arabidopsis*, with ABA-INSENSITIVE4 (*ABI4*) as the designated target gene, was demonstrated and the mutants exhibited ABA accumulation as well as abiotic stress tolerance (Osakabe et al. 2010). ZFN can be used to modify endogenous loci in *Zea mays* and the plants modified with ZFNs reliably pass on these genetic alterations to their offspring (Shukla et al. 2009).

10.2.2 TALENs (Transcription Activator-Like Effector Nucleases)

Compared with ZFNs, TALENs are highly specific, accurate, and a better choice for crop improvement along with the generation of marker-free plants (Khan et al. 2017). TALENs exhibit the property of endogenous gene editing in plants which gives it a versatility nature (Şenödeyici et al. 2021). Along with the transcription activator-like (TAL) effector DNA-binding domain, TALENs also possess a nonspecific DNA-cleavage domain (Das et al. 2023). Unlike ZFNs, TALENs are also based on protein-DNA interactions and play a significant role in gene activation, repression, disruption, deletion, correction, addition, Tag ligation, and Obligate Ligation-Gated Recombination (ObLiGaRe) (Khan et al. 2017). TALEN method was performed to knock out the herbicide-resistant gene *bar* from the herbicide-resistant rice line, and it became evident that these mutations had little effect on the whole transcriptome profile of rice (Qin et al. 2021).

TALEN-based targeting of *OsSWEET13*and *Os09g29100* in rice crops resulted in enhanced resistance to bacterial leaf blight (Blanvillain-Baufume et al. 2017; Cai et al. 2017). Using TALEN-mediated knockout mutagenesis on the *TaMLO* gene in bread wheat led to the identification of resistant plants against various stresses, including salinity, with heritable traits (Wang et al. 2014). TALENs are a potential tool to achieve a high level of somatic mutagenesis and these mutations are transmitted to the next generation. In Arabidopsis, the genes ADH1, TT4, MAPKKK1, DSK2B, and NATA2 were targeted via TALENs (Christian et al. 2013).

10.2.3 RNA Interference (RNAi)

RNA interference has been found to be an easy and effective mechanism of gene silencing in a variety of organisms. Gene silencing occurs as a result of RNA degradation. The short RNAs produced as a result of RNA degradation activate ribonucleases that target mRNAs that are homologous. The mechanism of RNA-induced silencing of genes mainly involves two steps: (i) generation of siRNAs or small interfering RNAs by the degradation of dsRNAs and (ii) degradation of target mRNA by the activity of RNA-induced silencing complex (RISC). The siRNAs bind to the RISC for carrying out this activity. The involvement of various components like dicer, RNA-dependent RNA polymerase, dsRNA endonucleases, helicases, etc., has been reported in RNA interference mechanism (Agrawal et al. 2003; Ullah et al. 2022). Due to the efficiency and precision of this mechanism, RNAi has been utilized for gene silencing and crop improvement.

Oh et al. (2007) reported that the downregulation of the *ThSOS1* gene in the halophyte *Thellungiella halophila* by RNAi led to increased salt sensitivity in the plant. The study pointed out the role of the *ThSOS1* gene in providing salinity tolerance to the plant. RNAi technique is performed in the halophyte *Karelinia caspica* in order to identify the role of two vacuolar Na^+/H^+ antiporter (NHX) genes (KcNHX1 and KcNHX2) in salt tolerance, and the results showed that KcNHX1 gene silencing reduces salt tolerance, whereas silencing of KcNHX2 does not have any obvious effect on salt tolerance in *Karelinia caspica* (Liu et al. 2012). The xerophytic plant, *Zygophyllum xanthoxylum*, has been found to be endowed with salt tolerance potential due to the presence of the *ZxNHX1* gene that is involved in Na+ compartmentation. The silencing of this gene by RNAi led to the reduction of salt tolerance in *Z. xanthoxylum* (Liu et al. 2022). The advantages of the RNAi technique encompass precise sequence specificity, the capacity to target multiple genes simultaneously, and control over the degree of gene silencing in critical tissues and developmental stages (Ullah et al. 2022).

10.2.4 CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeat)

CRISPR/Cas method is considered one of the large-scale gene editing tools for crop improvement and paves a base for functional genomics (Liu et al. 2022). Among the CRISPR/Cas 9 genome editing, the YAO-promoter driven CRISPR/Cas 9 system is more advantageous because of its high homozygous rate and high editing efficiency and is successfully performed in the xero-halophytic plant *Lycium ruthenicum* (Ai et al. 2023). Similarly, the CRISPR/Cas 9 system is effectively established in the recretohalophyte *Limonium bicolor* (Li et al. 2023). The ability of CRISPR/Cas technology to induce site-specific genome editing makes it beneficial in food crop production to develop new varieties of rice with improved tolerance as well as yield under salinity stress conditions (Khan et al. 2021).

CRISPR-Cas 9 technology was used to determine the role of rice NAC transcription factor (TF) under salinity stress conditions by mutating the *OsNAC041* gene that encodes NAC TF (Bo et al. 2019). CRISPR/Cas9-mediated genome editing target for drought and salinity tolerance in *Oryza sativa* is *OsmiR535* (Yue et al. 2020). Another approach to enhanced salt tolerance in rice plants was by knockout that negatively regulated *OsbHLH024* gene, which belonged to bHLH subfamily U. This was accomplished through the CRISPR/Cas 9 method by generating a loss of function mutant, *OsbHLH024* mutant/A91 (Alam et al. 2022). One major limitation of CRISPR/Cas gene editing is the high risk of off-target effects (Das et al. 2023).

10.3 Halophytic Genes as a Source of Abiotic Stress Tolerance

10.3.1 Salt-Tolerant Genes

The key genes involved in the salt stress tolerance mechanism in halophytes which include changes in ion homeostasis (both influx and efflux), the formation of osmoprotectants, activation of crosstalk genes, and induction of antioxidants are intrinsically more active than the corresponding glycophytic genes and enable them to be explored deeply up to the gene and gene network level. Various genomic and transcriptomics strategies, like genetic engineering and molecular breeding by marker-assisted breeding, phenotyping technologies, genome-wide association studies, etc., have been made to isolate salt-responsive genes from some halophytes and their functional validation through different transgenic approaches. Genetic engineering can achieve a higher level of abiotic stress tolerance efficiency in transgenic plants and represents the best method of studying the function of various genes related to antioxidant enzymes and stress-tolerant metabolism in plants (Afzal et al. 2022; Villalobos-López et al. 2022; Mann et al. 2023).

It will also help to elucidate the gene networking process behind the effective salinity response in halophytes. Several known genes, like salt overly sensitive system (SOS)-related cation/proton antiporters of plasma (*NHX/SOS1*) and vacuolar membranes (*NHX*), energy-related pumps, such as plasma membrane and vacuolar H^+ adenosine triphosphatase (PM & V-H^+ ATPase), vacuolar H^+ pyrophosphatases (V-H^+ PPase), high-affinity potassium transporters, calcineurin B-like (CBL) proteins and CBL-interacting protein kinases (CIPK) and genes encoding metallothioneins were isolated from various dicotyledonous and monocotyledonous halophytes, and it was explored for developing stress tolerance in glycophytes (Table 10.1).

Table 10.1 Halophytic genes as sources for enhanced abiotic stress tolerance in various plants

S. No	Source of the gene	Gene	Host plant	Abiotic stress	Reference
1.	*Thellungiella halophila*	*TsVP*	Cotton	Salt stress	Lv et al. (2008)
2.	*Suaeda corniculata*	*ScVP*	*Arabidopsis*	Salt, saline-alkali, and drought stress	Liu et al. (2011)
3.	*Spartina alterniflora*	*SaVHAc1*	Rice	Salt stress	Baisakh et al. (2012)
4.	*Suaeda liaotungensis*	*SlNAC1*	Arabidopsis	Drought, salt, and cold stress tolerance	Li et al. (2014)
5.	*Salicornia brachiata*	*SbMT-2*	Tobacco	Salt stress	Chaturvedi et al. (2014)
6.	*Atriplex hortensis*	*AhAL1*	*Arabidopsis*	Drought and salt stress	Tao et al. (2018)
7.	*Lobularia maritima*	*LmSAP*	Tobacco	Cd, Cu, Mn, and Zn stress	Saad et al. (2018)
8.	*Suaeda liaotungensis*	*SINAC8*	*Arabidopsis*	Drought and salt stress	Wu et al. (2018)
9.	Nitraria tangutorum	NtCIPK9	*Arabidopsis*	Salt stress	Lu et al. (2020)
10.	*Hordeum brevisubulatum*	*HbMBF1a*	*Arabidopsis*	Salt stress	Zhang et al. (2020)
11.	*Nitraria sibirica*	*NsNHX1*	*Poplar* species	Salt stress	Geng et al. (2020)
12.	*Nitraria tangutorum*	*NtCIPK11*	*Arabidopsis*	Drought and salt stress	Lu et al. (2021)
13.	*Zoysia matrella*	*ZmGnTL*	*Arabidopsis*	Salt stress	Zheng et al. (2022)
14.	*Thellungiella halophila*	*ThST5*	Cotton	Salt stress	Tahmina et al. (2022)
15.	*Nitraria billardieri*	*NbCIPK25*	*Arabidopsis*	Salt stress	Lu et al. (2022a, b)
16.	*Lobularia maritima*	*LmTrxh2*	Tobacco	Cd, Cu, Mn and Zn stress	Saad et al. (2023)
17.	*Atriplex hortensis*	*AhBADH*	Soybean	Salt stress	Yu et al. (2023)
18.	*Halostachys caspica*	*HcNFYA1*	*Arabidopsis*	Drought and salt stress	Ji et al. (2023)
19.	*Glycyrrhiza inflata*	*GiLEA5–2.1*	Tobacco	Drought and salt stress	Zhang et al. (2024a)
20.	*Canavalia rosea*	*CrPIP2;3*	*Arabidopsis*	Drought and salt-alkali stress	Zheng et al. (2021)

(continued)

Table 10.1 (continued)

S. No	Source of the gene	Gene	Host plant	Abiotic stress	Reference
21.	*Nitraria sibirica*	*NsSOS1*	Transgenic 84 K poplar (*Populus alba* × *P. glandulosa*)	Salt stress	Chen et al. (2024)
22.	*Nitraria billardieri*	*CIPK25*	*Arabidopsis*	Salt stress	Lu et al. (2022)
23.	*Puccinellia tenuiflora*	*NTN6*	Maize	Salt stress	Guo et al. (2024)
24.	*Salicornia brachiata*	*SbCPN10L*	Tobacco	Heat stress	Kumari et al. (2024)
25.	*Haloxylon ammodendron*	*HaPEPC1*	*Arabidopsis thaliana*	Drought stress	Zhang et al. (2024b)
26.	*Suaeda corniculata*	*ScABI3*	*Medicago sativa*	Salt stress	Zhang et al. (2024c)

Among them, the membrane and vacuolar Na^+/H^+ antiporters provide the best mechanism for ionic homeostasis in plants under salt stress, and a large number of transgenic plants with variable salt tolerance have been produced on the basis of antiporter genes (Rahman et al. 2021; Mansour 2022; Mansour and Hassan 2022).

Sengupta et al. (2022) characterized the promoter region of a stress-responsive gene *SaAsr1* from monocot halophyte cordgrass, *Spartina alterniflora*, and salt tolerance contributed by higher relative water content and membrane stability. Zheng et al. (2022) studied the salt-tolerant genes in the salt-tolerant turfgrass *Zoysia matrella* using a highly efficient method named FOX (full-length cDNA overexpression) hunting system and a novel salt-inducible candidate gene *ZmGnTL* was transformed into *Arabidopsis* and the transformed plants exhibited improved salt tolerance by regulating ion homeostasis, reactive oxygen species scavenging, and osmotic adjustment. Time-course transcriptome analysis under salt treatments revealed detailed responses of *Suaeda glauca* and the enrichment of the transition-upregulated genes in the leaves associated with DNA repair and chromosome stability, lipid biosynthetic process, and isoprenoid metabolic process (Cheng et al. 2023).

Overexpression of a tonoplast Na+/H+ antiporter from the halophytic shrub *Nitraria sibirica* in transgenic poplar tree species has been used for increased biomass, survival rate, and plant height through improved ion homeostasis, osmoregulation, and increased photosynthetic efficiency under salt stress conditions (Geng et al. 2020). The overexpression of salt stress-related genes isolated from a euhalophyte herb *Suaeda* to various glycophytic plant species enhanced the expression of stress response genes and salt tolerance of transgenic plants (Yu et al. 2022). Moreover, transgenic *Arabidopsis* plants overexpressing *SlNAC8* from *Suaeda liaotungensis* had a significantly higher proline concentration, antioxidant enzyme activity (superoxide dismutase, peroxidase, and catalase), and level of chlorophyll fluorescence than wild-type, and a significantly lower malondialdehyde (MDA) concentration and electrolyte leakage under drought and salt stress. The overexpression of *SlNAC8* in *Arabidopsis* plants also enhanced the expression of stress-responsive genes such as *RD20*, *GSTF6*, *COR47*, *RD29A*, *RD29B*, and *NYC1* (Wu et al. 2018).

Recently, the abiotic stress tolerance of transgenic plants was analyzed by field experiments rather than laboratory or greenhouse experiments. *AhBADH* from *Atriplex hortensis* was successfully introduced into soybean by *Agrobacterium*-mediated transformation, and the plant exhibited stable enhanced salt tolerance and improved agronomic traits when subjected to 300 mM NaCl treatment (Yu et al. 2023). *BBADH* gene that encodes for betaine aldehyde dehydrogenase is a key enzyme involved in the biosynthesis of the osmoprotectant, glycine betaine (GB), and maintaining osmotic balance in plants under abiotic stress. Overexpression of *PvLBD12* in switchgrass (*Panicum virgatum* L.) enhanced salt tolerance by modulating a wide range of physiological responses like reduced Na^+ absorption, increased proline accumulation, reduced malondialdehyde accumulation, and improved K^+ accumulation. Some stress response genes such as proline biosynthesis gene *PvP5CS1*, vacuolar Na+(K+)/H+ antiporter gene *PvNHX1*, and two key ROS-scavenging enzyme genes *PvCAT* and *PvSOD* were also upregulated in *PvLBD12* overexpression lines (Guan et al. 2023).

Lu et al. (2022b) identified *NbCIPK25* in the halophyte *Nitraria billardieri* as a salt stress-responsive gene and the overexpression of *NbCIPK25* gene in salt-stressed transgenic *Arabidopsis* seedlings resulted in enhanced tolerance to salinity by mediating scavenging of reactive oxygen species, thereby protecting cells from oxidation and maintaining plant development under salt stress. Furthermore, their proline content and the transcription of genes related to proline accumulation were positively regulated in transgenic plants under salt conditions. In addition to this, they also identified *NbCIPK25* gene overexpressing *Arabidopsis* seedlings under salt stress exhibited more efficient photosynthesis and soluble sugar and proteins, accordingly photosynthesis products accumulated at a higher level in transgenic plants. This provided molecular insight into how *NbCIPK25* promotes the expression of genes involved in photosynthesis, thereby maintaining plant growth under salt stress (Lu et al. 2022a).

Using recombinant DNA techniques, a large number of halophytic genes that are either upregulated or downregulated in response to multiple abiotic stress factors have also been discovered and characterized. Overexpression of the *SlNAC1* gene from *Suaeda liaotungensis* in *Arabidopsis* plants provided resistance toward drought, high-salt, and cold (4 °C) stresses (Li et al. 2014). Overexpression of *AhCMO*—choline monooxygenase gene from *Atriplex hortensis*—improved drought tolerance in transgenic tobacco (Shen et al. 2002). Tao et al. (2018) introduced the *AhAL1* gene from *Atriplex hortensis* in *Arabidopsis*, which enhanced salt and drought tolerance in transgenic *Arabidopsis* as reflected by decreased MDA content and reduced water loss by abscisic acid-mediated stomatal closure. Inhibition of seed germination and primary root elongation and the expression levels of positive stress regulator genes *DREB1A*, *DREB2A*, and three *ABFs* were also increased in *AhAL1*-expressing plants. *NtCIPK11* gene expression in *Arabidopsis* from the halophyte *Nitraria tangutorum* promotes plant growth and mitigates damage associated with drought and salt stress by regulating the expression of genes controlling proline accumulation, and the transgenic plants grew more vigorously under salt stress and developed longer roots under salt or drought conditions (Lu et al. 2021).

When *Suaeda salsa* plants were grown under 500 mM NaCl treatment, the upregulation of the gene *SsNHX1* was observed. This gene is supposed to have a role in the production of Na+/H+ antiporter, which is involved in providing salt tolerance to the plant (Chen et al. 2010). Plasma membrane H+-ATPase has also been found to have a role in salinity stress tolerance. The increased activity of the plasma membrane H+-ATPase under NaCl stress was found to be due to the increased transcriptional and protein levels of plasma membrane H+-ATPase (Chen et al. 2010; Shao et al. 2014). Another gene called Salt-Overly Sensitive1 (*SOS1*) which encodes Na+/H+ antiporter was also found to have increased activity and role in Na^+ efflux across the plasma membrane of *S. salsa* under NaCl stress (Shao et al. 2014). Under increased saline conditions, the uptake of K^+ ions occurred by the upregulation of the activity of the gene *SsHKT1*. The upregulated activity of this gene in *Arabidopsis* enhanced its salinity stress tolerance (Shao et al. 2014). Another gene, the S-adenosylmethionine synthetase (SAMS) gene, when incorporated in tobacco, could enhance the salt tolerance of this plant by enhancing the rate of photosynthesis and also by delimiting the physiological drought by increasing the water retention capacity of the cells (Qi et al. 2010). SAMS is involved in the biosynthesis of the hormone ethylene and also acts as a methyl donor for the transmethylation of nucleic acids, proteins, fatty acids, and polysaccharides which help the plants to cope with the adverse environmental conditions.

Dehydration-responsive element-binding (*DREB*) transcription factor (TF) is an important transcription factor that has a role in abiotic stress tolerance and was found in *S. salsa*. When compared with wild-type controls, transgenic tobacco plants transformed with *SsDREB* demonstrated enhanced resistance to salt and drought (Zhang et al. 2015). Similarly, the drought tolerance of the transgenic *Arabidopsis* plants having *SsVP* (*S. salsa* vacuolar H+-pyrophosphatase gene) was found to be enhanced compared with wild plants (Guo et al. 2006). The genes of *S. salsa* encoding glycine betaine (glycine betaine aldehyde dehydrogenase (*BADH*), choline monooxygenase (*CMO*), and pyrroline-5-carboxylate synthetase (*P5CS*) when cloned and inserted into tobacco plants resulted in increased salinity tolerance in these plants (Su et al. 2018). Moreover, the genes from *S. salsa* like *SsAPX, SsCAT2, SsPrxQ, SsGST, SsCAT1, SsAPX*, and *SsTypA1* that are important in providing oxidative stress tolerance were overexpressed in transgenic *Arabidopsis* plants grown under stress (Li et al. 2012; Pang et al. 2011). When *Arabidopsis* plants were transformed with the gene phytoene synthase (*SePSY*) and beta lycopene synthase gene (*SeLCY*) of *S. europaea,* it was found that the plants exhibited increased growth, oxidative stress tolerance, and salt tolerance due to the overexpression of this genes (Han et al. 2008; Fan 2020). Glutathione S-transferases (GST) gene of *Limonium bicolor*, *LbGST1*, was inserted into tobacco plants, whereby the plants overexpressed this gene under salt stress. This resulted in increased activity of GST, glutathione peroxidase, catalase, and superoxide dismutase compared with the wild-type tobacco plants thereby providing stress tolerance (Diao et al. 2011). *Arabidopsis* plants transformed with the genes NHX1 and HKT1 of *Reaumuria trigyna* (*RtNHX1* and *RtHKT1*) exhibited improved salinity tolerance (Li et al. 2017, 2019). Several genetic transformation techniques have been used to create salt stress-resistant transgenic crops, including castor, jatropha, cumin, and peanuts, utilizing the salt-responsive genes from *Salicornia brachiata* (Joshi et al. 2012; Singh et al. 2014; Patel et al. 2015; Tiwari et al. 2015; Jha et al. 2011).

Transgenic *Arabidopsis thaliana* with the *KfVP1* gene was found to have vigorous growth under saline and drought conditions. These findings suggested that *KfVP1*, a tonoplast H+-pyrophosphatase from the plant *Kalidium foliatum*, may have a role in salt and drought tolerance (Yao et al. 2012). A gene called Alfin-like (AL) has been found to play a role in abiotic stress response. When *Arabidopsis* was transformed using this gene from *Atriplex hortensis* (*AhAL*), *Arabidopsis* exhibited increased drought tolerance (Tao et al. 2018). Likewise, the abscisic acid stress ripening gene (*ASR*) induced by the abiotic stress factors is responsible for the regulation of glucose metabolism by controlling the glucose transporters and also turns on the antioxidant machinery thereby preventing the oxidative stress in plants. The abscisic acid stress ripening gene (*SbASR-1*) from *Salicornia brachiata* was cloned and successfully transformed in *Arachis hypogaea* plants, and it was observed that transgenic plants exhibited enhanced endurance to salinity and drought tolerance due to the overexpression of this gene (Tiwari et al. 2015). Transformation of *Arabidopsis* by the insertion of the gene *SpBADH* involved in glycine betaine synthesis provided drought tolerance to the transgenic plants due to enhanced scavenging of ROS (Yang et al. 2015). The gene *AlTMP2* from *Aeluropus littoralis*, when inserted into tobacco plants, resulted in enhanced tolerance to various abiotic stresses like salinity, heat, and freezing due to improved membrane integrity and downregulation of several stress-related genes (Ben-Romdhane et al. 2018).

10.3.2 Drought-Tolerant Genes

Halophytes can be endowed with the capacity to drought tolerance through the regulation of various drought-related genes. With the aid of genetic engineering tools, drought-tolerant transgenic lines can be developed using halophytic genes as a good source of drought tolerance. The abscisic acid, Stress- and; Ripening-induced (ASR) genes, aquaporin class of genes, as well as ascorbate peroxidase (APX) genes are known to exhibit significant role in drought stress responses and sequentially lend solutions to overcome the drought stress-induced ruins (Singh et al. 2014; Zheng et al. 2021; Cao et al. 2023). The overexpression of HaASR2 gene isolated from the xerohalophyte *Haloxylon ammodendron* showed improved drought as well as salt tolerance in the transgenic *Arabidopsis* plant. These transgenic lines had enhanced capacity of water retention, photosynthesis, and ROS-scavenging system; on the other hand, the electrolyte leakage, H_2O_2 content, and genes involved in the biosynthesis of ABA were downregulated particularly under drought stress conditions (Cao et al. 2023). The highly salt-tolerant, sand-fixing *Sophora alopecuroides* is a suitable candidate for drought stress tolerance along with the expression of 17,479 differentially expressed genes (DEGs) under these conditions. Among these, four PYL-related genes in ABA signaling (SaPYL4–1, SaPYL4–2, SaPYL5, and SaPYL9) and genes involved in ROS-scavenging mechanism were upregulated, whereas nine genes corresponding to CTR1 (serine/threonine-protein kinase) got downregulated under drought stress in *S. alopecuroides* (Yan et al. 2020).

Plant aquaporins (AQPs) contribution in various abiotic stress amelioration, particularly under drought stress, is well studied. In mangrove-accompanied halophyte *Canavalia rosea*, a drought-tolerant PIP2 gene (plasma membrane intrinsic proteins), CrPIP2;3 belongs to the largest group of AQPs were isolated and characterized. CrPIP2;3 overexpression in *Arabidopsis* helped to increase drought tolerance by facilitating water transport rather than ROS signaling (Zheng et al. 2021). Another method to improve drought tolerance in *Arabidopsis* through the overexpression of SaBADH gene was derived from the well-known natural glycine betaine (GB) accumulating halophyte *Sesuvium portulacastrum*. Its mode of action is in such a way that it increases GB synthesis which in turn helps to protect cell membranes from stress-induced damage by lowering the level of MDA content. Moreover, it maintains a higher level of proline and antioxidant enzymes in the transgenic plant thereby improving ROS scavenging, which contributes to enhanced drought tolerance (Yang et al. 2015). Peroxisomal Ascorbate peroxidase (SbpAPX) gene is a potential candidate to aggrandize drought as well as salt tolerance in transgenic tobacco, and this gene was isolated from extreme halophyte *Salicornia brachiata.* In addition, its overexpression also positively influenced the vegetative growth and germination rate of tobacco even under stress conditions (Singh et al. 2014).

10.3.3 Heavy Metal Tolerant Genes

Overexpression of *LmSAP*, a member of the stress-associated protein (SAP) gene family isolated from *Lobularia maritima*, in transgenic tobacco led to enhanced tolerance toward heavy metals such as Cd, Cu, Mn, and Zn. Activities of the antioxidant enzymes SOD, CAT, and POD were significantly higher in *LmSAP* overexpressing tobacco seedlings. Moreover, transcription of several genes involved in metal tolerance in tobacco such as genes encoding metallothioneins (*Met1*, *Met2*, *Met3*, *Met4*, and *Met5*), a copper transport protein *CCH*, a Cys and His-rich domain-containing protein RAR1 (*Rar1*), and a ubiquitin-like protein 5 (*PUB1*) were highly enhanced and the concentration of intracellular free heavy metals were decreased by the activity of metal-binding proteins in the cytosol (Saad et al. 2018). Recently, Saad et al. (2023) produced *LmTrxh2*-overexpressing tobacco plants from *Lobularia maritima* and its overexpression-conferred tolerance to heavy metals such as Cd, Cu, Mn, and Zn by stimulating antioxidant capacities and the expression of several stress-responsive genes in plants (genes encoding metallothioneins—*Met-1*, *Met-2*, *Met-3*, and *Met-4*, a copper transport protein, a Snakin/GASA protein—*Snakin-2*, and ROS-scavenging enzymes—*SOD*, *APX1*, and *CAT*). Thus, halophytes can be explored for genetically modifying the glycophytes for better abiotic stress adaptation and enhanced stress tolerance in a broader way. Similarly, when the DREB gene of *L. bicolor* was incorporated into tobacco plants, the plants showed increased tolerance to $CuSO_4$ stress (Ban et al. 2011).

The insertion of phytochelatin synthase (*PCS*) genes from *Phragmites australis* to tall fescue plants enhanced their Cd tolerating potential (Zhao et al. 2014). The expression of the c-GCS glutamyl cysteine synthase gene (*PaGCS*) in the rapidly growing species *Agrostis palustris* has been shown to significantly increase resistance to Cd toxicity. This gene has been found to have a crucial role in providing heavy metal tolerance to *P. australis* (Zhao et al. 2010). *SbMT2*, a metallothionein gene that provides tolerance to heavy metal stress in *Salicornia brachiata*, was used to transform tobacco plants. The transgenic tobacco plants thus created were found to have enhanced tolerance to heavy metal stress (Chaturvedi et al. 2014).

10.4 Future Perspectives

The stress-responsive genes in halophytes can play a significant role in the mitigation of various kinds of stress factors. The expression of halophyte-derived salt-tolerant, drought-tolerant, and heavy metal-tolerant genes in glycophytes is successfully established with the help of various genome editing tools. The genome editing tools comprise of meganucleases, CRISPR-Cas9, ZFNs, TALENs, RNAi, etc., and each tool has both advantages and disadvantages when it comes to application. Transgenic lines with halophyte genes can induce antioxidant machinery, downregulate ROS, and induce other stress tolerance mechanisms, which can eventually maintain or regain plant growth and health under stress conditions. However, it is crucial to evaluate these transgenic systems in open fields where crops are subjected to a range of challenges simultaneously or at distinct phases of plant growth. Furthermore, these plants are subjected to a variety of biotic and abiotic stresses, thus a full investigation of the effectiveness of transgenic plants in these circumstances is necessary.

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