Sustainable Agriculture Reviews 16

Eric Lichtfouse
Aakash Goyal *Editors*

Sustainable Agriculture Reviews

Cereals

Springer

Sustainable Agriculture Reviews

Volume 16

Series editor
Eric Lichtfouse

Other Publications by Dr. Eric Lichtfouse

Books
Scientific Writing for Impact Factor Journals
Nova Publishers 2013

Sustainable Agriculture
Springer 2009

Sustainable Agriculture Volume 2
Springer 2011

Environmental Chemistry. Green Chemistry and Pollutants in Ecosystems
Springer 2005

Rédiger pour être publié ! Conseils pratiques pour les scientifiques
Springer 2012, 2^{e} édition.

Journals and Series
Agronomy for Sustainable Development
www.springer.com/journal/13593

Sustainable Agriculture Reviews
www.springer.com/series/8380

Environmental Chemistry Letters
www.springer.com/journal/10311

Environmental Chemistry for a Sustainable World
www.springer.com/series/11480

Blog
Agronomy blog
http://www1.montpellier.inra.fr/agronomy-blog

Magazine
Publier La Science
https://listes.inra.fr/sympa/d_read/veillecaps/

More information about this series at http://www.springer.com/series/8380

Eric Lichtfouse • Aakash Goyal
Editors

Sustainable Agriculture Reviews

Cereals

Springer

Editors
Eric Lichtfouse
INRA-CMSE-PME
Dijon cedex, France

Aakash Goyal
Bayer Crop Science
Bayer Inc
Saskatoon, SK, Canada

ISSN 2210-4410 ISSN 2210-4429 (electronic)
Sustainable Agriculture Reviews
ISBN 978-3-319-16987-3 ISBN 978-3-319-16988-0 (eBook)
DOI 10.1007/978-3-319-16988-0

Library of Congress Control Number: 2015944114

Springer Cham Heidelberg New York Dordrecht London
© Springer International Publishing Switzerland 2015
Chapter 2 is published with kind permission of the Her Majesty the Queen in Right of Canada
This work is subject to copyright. All rights are reserved by the Publisher, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other physical way, and transmission or information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed.
The use of general descriptive names, registered names, trademarks, service marks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.
The publisher, the authors and the editors are safe to assume that the advice and information in this book are believed to be true and accurate at the date of publication. Neither the publisher nor the authors or the editors give a warranty, express or implied, with respect to the material contained herein or for any errors or omissions that may have been made.

Printed on acid-free paper

Springer International Publishing AG Switzerland is part of Springer Science+Business Media (www.springer.com)

Contents

Chapter 1
Drought and Salt Stress in Cereals

Linda Mondini and Mario Augusto Pagnotta

Abstract Abiotic stresses, such as salinity, drought, extreme temperatures, chemical toxicity and oxidative stress represent a grave threat to agriculture dramatically affecting the crop production around the world. Climate changes are projected to have a significant impact on temperature and precipitation profiles increasing the incidence and severity of climate changes-related stresses and reducing in particular the productivity of rain-fed crops. In fact, drought and salinity stresses determine the primary cause of worldwide crop loss. Plant adaptation to environmental stresses is based on the activation of molecular networks involved in stress perception, signal transduction, and expression of specific stress-related genes and metabolites. Plants respond to the stresses in part by modulating gene expression in order to restore cellular homeostasis, detoxifying the toxins present into the cells and through the recovery of growth.

In present chapter the physiological and biochemical aspects of plant response to water stresses are reviewed together with the new frontiers studies on the genetic tools on stress tolerance. The recent exploitation of next generation resources applied to the functional genomics combined with a gradual increasing in transformation frequencies for many grasses, is supporting the study and the manipulation of abiotic stresses in grasses, notably increasing the plant tolerance. Mutational analysis and microarrays have led to the identification of numerous candidate genes involved in a series of stresses comprising drought, salt, freezing, and heat. The variability found in the genetic traits related with abiotic stress tolerance has permitted to identify and mapping several candidate genes and has confirmed the importance of wild relatives to identify the traits that domestication has canceled in the selected lines. The recent knowledge on candidate genes organization has led to the identification of promising allelic variants that, through Marker Assisted Selection (MAS), can be easily transferred into the susceptible commercial lines. Thence, the advent and development of molecular markers in quantitative genetics have greatly facilitated the study of complex quantitatively inherited traits by the construction of high density genome linkage maps for crops such as wheat. The

---

L. Mondini (✉) • M.A. Pagnotta
Department of Science and Technologies for Agriculture, Forestry, Nature and Energy (DAFNE), Tuscia University, via S. C. de Lellis, Viterbo 01100, Italy
e-mail: linda.mondini@unitus.it; pagnotta@unitus.it

E. Lichtfouse, A. Goyal (eds.), *Sustainable Agriculture Reviews*,
Sustainable Agriculture Reviews 16, DOI 10.1007/978-3-319-16988-0_1

identification of Quantitative Trait Loci (QTLs) ruling the genetic variability of the traits controlling such tolerance and the consequent manipulation to use in MAS is of crucial importance. The knowledge of the number and effects of QTLs can help breeders to understand the genetic control of these traits and to design more efficient selection strategies for improvement. To date, the modern commercial cultivars, able to survive to severe abiotic stresses regimes performing a good level of productivity, are the result of this activity.

Keywords Abiotic stresses • Ion homeostasis • Signal transduction • Transcription factors • Drought • Salinity • Cereals

Abbreviations

ABA	Abscisic acid
ALDH	Aldehyde Dehydrogenase
HSPs	Heat Shock proteins
LEA	Late Embryogenesis Abundant
MAS	Marker Assisted Selection
NILs	Near Isogenic Lines
NSCs	Non Selective Cation Channels
QTLs	Quantitative Traits Loci
RILs	Recombinant Inbred Lines
ROS	Reactive Oxygen Species
TFBSs	Transcription Factors Binding Sites.
TFs	Transcription Factors

1.1 Introduction

Despite a global scarcity of water resources and the increased salinization of soils and water, abiotic stresses are, at present, the main limiting factors in plant growth and crop cultivation and are bound to soon become even more severe as desertification covers most of the world's terrestrial area. Drought and salinity are actually widespread in many regions around the world and are expected to increase rapidly reaching the salinization of more than 50 % of the arable lands by the 2050 (Fig. 1.1). Moreover, the frequency and duration of dry spells and heat waves are likely to increase, especially in the dryland areas. Extremes, variability, and rates of change are all key features in addressing vulnerability and adaptation to climate changes. The combination of larger human population pressure, which is increasing at a rate of about 1.3 % per year, and climate uncertainty will expose millions of people to severe water shortages and this is expected to have a proportionally high impact on social, economic, and ecological aspects.

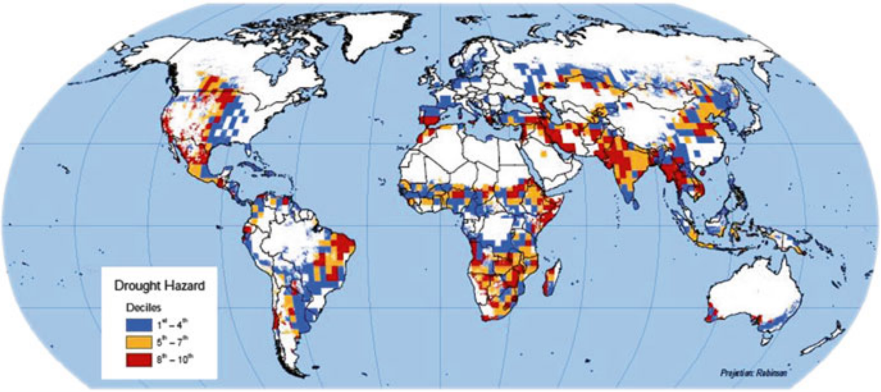

Fig. 1.1 Global drought hazard distribution. Drought periods were defined using an index known as the Weighted Anomaly of Standardized precipitation (WASP). The WASP index assesses the precipitation deficit or surplus over a three-month running average for the 21-year period from 1980–2000. Findings show that about 38 % of the world's land area has some level of drought exposure

Fig. 1.2 Wheat cultivation and depleted soil under drought conditions

In particular, abiotic stresses to which currently plants are exposed, include a decreased availability of water, extreme peaks of temperature including freezing, decreased availability of essential nutrients from the soil, poisoning of the plant due to the build-up of toxic ions during the stress, excessive irradiation, and increased hardness of the soil that limits root penetration (Fig. 1.2). Most abiotic stresses are interconnected by the fact that their disruptive effect on plant *equilibrium* determining cellular damages, are due to the alteration and interference with the plant water regime. In particular, this effect occurs in conjunction with water scarcity or altered ion content with a consequent water loss for osmosis caused by salinity or for dehydration due to the formation of extracellular ice during freezing stress. It must be considered that water is required as a medium for biochemical activities and in particular for plant cells, water creates the turgor pressure which represents the driving force for cell expansion and growth. Therefore, through the maintenance of a positive turgor pressure, cells can regulate the osmotic potential in order to balance their water content. Weighty changes in water potentials in the soil can carry osmotic stress to plant compromising normal cellular activities so much to cause plant death.

Abiotic stresses determine, in plants, a series of morphological, biochemical, physiological and genetic changes causing a critical decreasing in growth and productivity. Moreover, oxidative stress frequently consequent to high temperature, salinity, and drought stresses often can cause denaturation of functional and structural proteins (Smirnoff 1998). Thence, these stresses even if diverse, can activate parallel cell signaling pathways (Shinozaki and Yamaguchi-Shinozaki 2000; Zhu 2002) and cellular responses comprising the up-regulation of anti-oxidants, the production of stress proteins, and the accumulation of solutes (Vierling and Kimpel 1992; Cushman and Bohnert 2000). The plant response to abiotic stresses involves so a complex of genes and mediator regulated by biochemical and genetic interlinked mechanisms. These genes include three major categories distinguished for their function: (i) genes involved in transcriptional control and signaling cascade; (ii) genes involved in the protection of membranes and proteins and finally (iii) genes involved in water and ion uptake and transport. In this perspective, the strategy for enhance abiotic stress tolerance in plants aims to manipulate genes involved in the protection and maintaining of the functions and the structures of cellular compounds. Nevertheless, on the contrary to the genes involved in resistance to biotic stresses which are constituted by monogenic traits, the gene network that controls the tolerance to abiotic stresses, is very complex and thence very difficult to understand and engineer.

Higher plants, have still developed different but interlinked stratagems to survive and to get over the stress. On the contrary, these stratagems are not equally present in the majority of crops. In addition, for crops, the traditional breeding programs involving the transfer of desirable genetic traits from wild relatives, are ineffective due to the objective difficulty to manipulate and breeding for tolerance traits. In particular for cereals, most of the abiotic stress tolerance traits are localized in landraces and related wild species, nevertheless most of these traits have been not yet identified in grass. In fact, although wheat, rye and barley possess a good level of abiotic stress tolerance respect to maize and rice, information about genes and mechanisms involved are very scarce or sometimes lacking making impossible the improvement of commercial lines for the stress tolerance traits. So the future challenge of abiotic stress research consists in to plug the gap between the knowledge and the transfer of related traits into promising material.

1.2 Strategies for Stress Tolerance: Drought Stress

1.2.1 Water Potential

One of the components that cause the occurrence of a stress condition is the decrease in the availability of water in soil. This reduced water availability are quantified as a reduction in water potential (ψ_w, Kramer and Boyer 1995), defined as the chemical potential of water divided for the partial molar volume. In this perspective, the free energy of water with the cell turgor are expressed in units of pressure and as a direct consequence of the water movement in the plant/soil system. A reduction of the ψ_w, increases the difficulty for the plant to take up water touching off a cascade of responses that help the plant to avoid the water loss. The avoidance strategies can include changes in water fluxes starting from the plant *in toto* up to the cellular level and consist in: i) the implementation of stratagems for the continuing water up taking at reduced ψ_w and ii) the capability of plant to tolerate a decreased tissue water content (Fig. 1.3).

1.2.2 Tolerance of Low ψ_w

The first response of plants to a reduced ψ_w is to avoid it. Tissue ψ_w and water content are constantly maintained by the plant at the *equilibrium* condition and when a decrease in water supply occurs, these are retained close to the unstressed condition

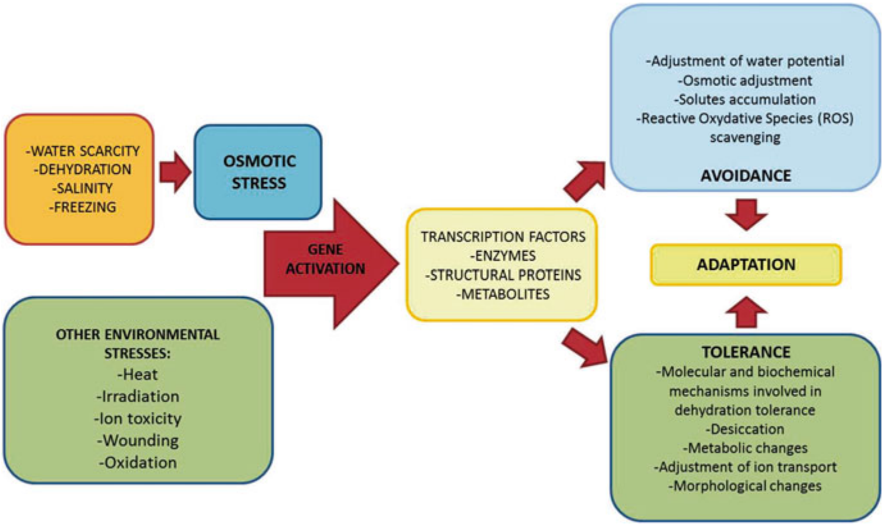

Fig. 1.3 Chart of the different typology of abiotic stresses activating different metabolic pathways which confer adaptation either to avoidance or tolerance strategies

for the time being, by limiting the water loss through a fast stomatal closure and increasing water uptake. Long term effects comprise the increase of water capability and water permeability in tissues, the expansion of cuticle volume and thickness and, finally the alteration of growth ranking of sprouts and roots. In particular the modification of growth ranking of sprouts and roots permits to increase the sprout/root ratio that represents the main strategy of drought avoidance. Nevertheless, these mechanisms of low ψ_w avoidance are useful and efficient only for limited time periods, due to a reduction of photosynthesis process for the scarcity of CO_2 caused by the stomatal closure. If long term drought stress occurs plants must resort to further mechanisms.

1.2.3 Dehydration Avoidance

The dehydration avoidance is a stratagem consisting in the adjustment made by the plant of ψ_w as a consequence of a reduced level of ψ_w in the soil. This modification consists in a decrease of ψ_w of the plant to avoid the water loss from roots through the accumulation of solutes and cell wall reinforcement. The movement of water into or out the plant, strictly depends on ψ_w gradient created between the cell and the environment. Thence, a plant, extracting water from the soil, must possess a ψ_w lower than that. However the ψ_w of a cell provided of a cell wall, includes two different components: the osmotic potential (ψ_s) and the turgor pressure (ψ_p) and is represented from this equation: $\psi_w = \psi_s {}^+ \psi_p$. The maintenance of equilibrium between the two components is crucial, in fact at a given ψ_w to a higher level of ψ_p due to accumulation of solutes inside the cell must correspond a lower level of ψ_s. The accumulation of solutes as a consequence of a reduced ψ_w is known as "osmotic adjustment" (Zhang et al. 1999). It is crucial for a correct cell functioning, that the accumulated solutes should not affect the cellular function. Low ψ_w is due to freezing or salinity or other type of abiotic stresses. Generally, plants tend to contrast low ψ_w accumulating compatible solutes unaffecting their metabolism and having also protective function (Yancey et al. 1982). Another protective mechanisms includes the maintenance of cell turgor adjusting osmotic pressure into the cell. There are several specialized osmolytes such as proline, mannitol, glycine betaine, and ectoine, synthetized by the cell in order to readjust the osmotic potential. Among these, proline has received much attention due to the fact that its accumulation has been observed in several plant species upon exposure to abiotic stresses (Delauney and Verma 1993). Recently, genetic and transgenic studies have confirmed the capability of proline to increase the abiotic stress tolerance in plants (Xin and Browse 1998; Hong et al. 2000; Ronde et al. 2000). The possibility of plants to accumulate compatible solutes is crucial to adverse drought stress in field (Morgan 1984, 1991). For this reason the genetic research aims to engineer transgenic plants to increase the synthesis of compatible solutes and consequently, the tolerance to abiotic stresses (Bohnert and Shen 1999). Nevertheless, several works have demonstrated that the increase of proline production, under abiotic stresses, is not enough considerable for overall osmotic adjustment (Zhu 2001) suggesting that probably the osmolytes work for osmotic adjustment, mainly scavenging reactive oxygen species and so maintaining the natural status of macromolecules (Smirnoff and Cumbes 1989; Hong et al. 2000). Moreover, damaged proteins are immediately eliminated by the cell to prevent their aggregation. The elimination allows the directly degradation of damaged proteins up to the destruction of the entire cell through apoptosis. Both of these events have been identified in plant cells under abiotic stresses (Katsuhara 1997). Considering that proteolytic systems occur through an ubiquitin-mediated degradation into the proteasomes, the expression of genes encoding ubiquitin-related genes has been deeply studied (Ciechanover et al. 2000). In confirmation, it has been demonstrated that under abiotic stresses condition and in particular under drought stress, the expression of these genes is consistently enhanced proportionally to the protein degradation requirement (Ingram and Bartels 1996) (Fig. 1.4). Finally, the cell wall itself, thanks to its deformability, works to plant's advantage promoting the dehydration avoidance.

1.2.4 Dehydration Tolerance

When the exposure to abiotic stresses becomes severe and continuous, the ψ_w starts to decrease until for plants it is impossible to avoid dehydration. At this stage, becomes crucial to carry out all the molecular and biochemical mechanisms involved in dehydration tolerance. Some plants are able to escape to dehydration through "desiccation" recurring to a fully air-dried state (Olive et al. 2000; Vicré et al. 2004). During this phase, the plant is in a dormant state where the metabolism is temporarily reduced as happens in seed dormancy. Nevertheless, most plants tends to carry out tolerance mechanisms exhibiting a wide range of responses at the molecular, cellular and whole-plant levels (Greenway and Munns 1980; Bohnert and Shen 1999; Hasegawa et al. 2000). These mechanisms, tending to mainly protect the cellular structures from the dehydration damages and controlling the level of reactive oxygen species (ROS), involve a series of changes. These includes: (i) morphological and developmental changes; (ii) adjustment in ion transport (uptake, extrusion and sequestration of ions) and (iii) metabolic changes (synthesis of compatible solutes and carbon metabolism) (Figs. 1.4 and 1.5). The different typologies of responses to the stress are activated by the primary osmotic stress signals instead others can be triggered by secondary stresses through a chain reaction sparked by the primary signals. The secondary signals comprise phytormones (e.g. Ethylene, abscisic acid ABA), reactive oxygen species (ROS) and intracellular second messengers (e.g. phospholipids). Some of them are not restricted in the sites where the primary signal occurs but can move to other parts of the whole plant contributing to the network and organization of total plant response (Fig. 1.5).

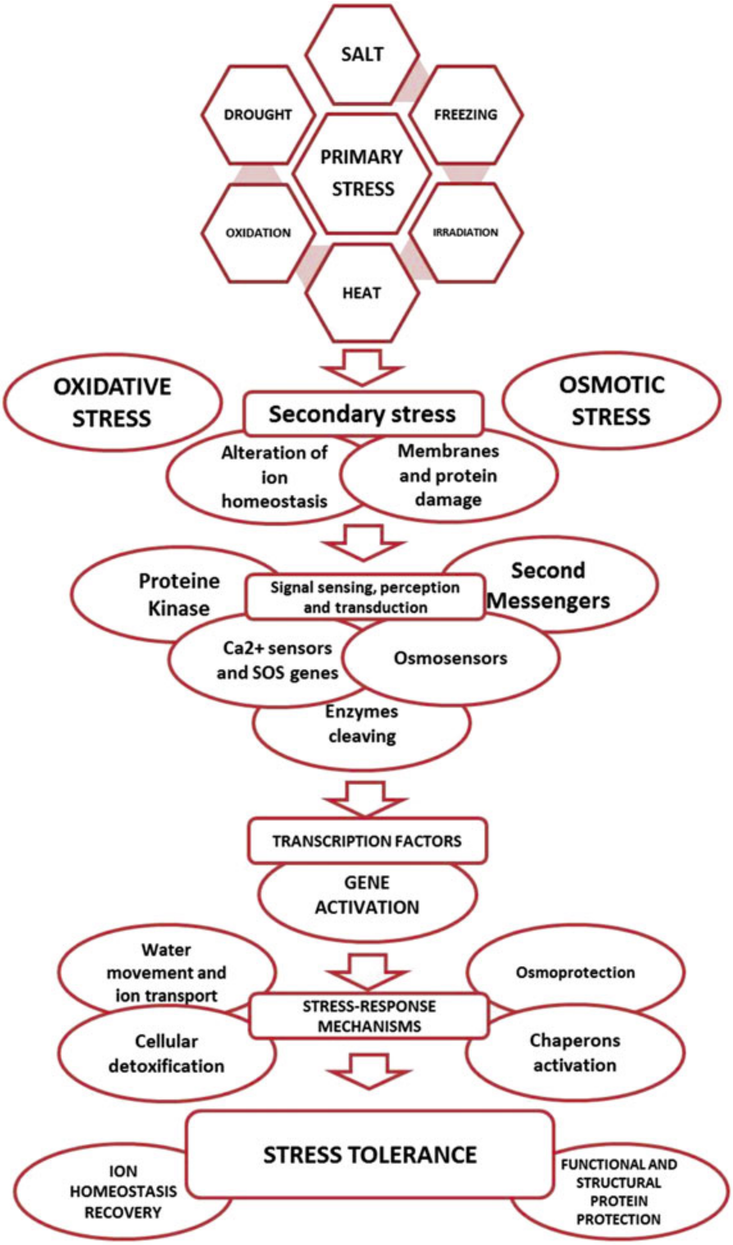

Fig. 1.4 Cascade of biochemical, physiological and genetic mechanisms and interacting factors occurring under abiotic stress

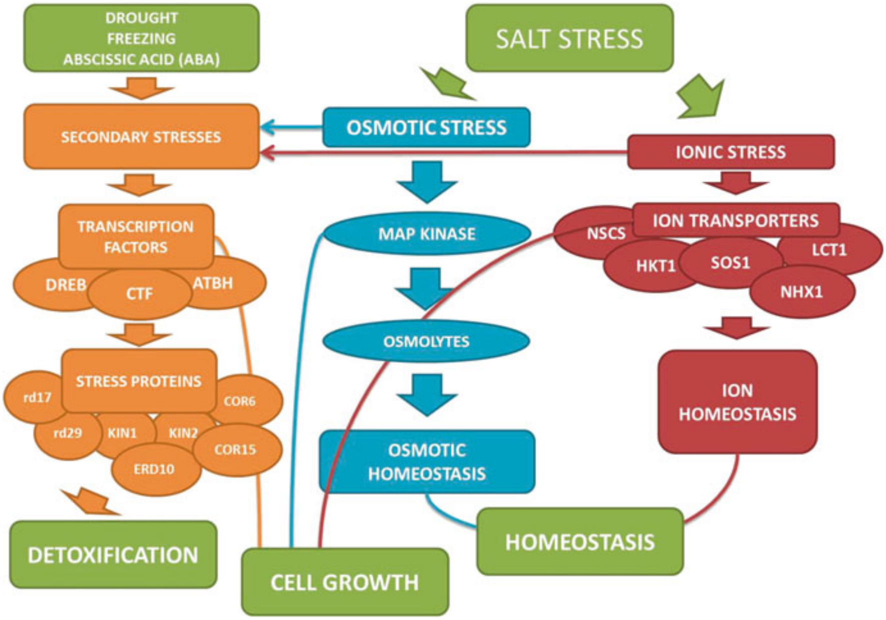

Fig. 1.5 The three different components of plant tolerance to salinity stress (detoxification, cell growth control and homeostasis)

1.2.5 Cell Protection

Plant cells, to avoid damages, make use of systems assigned to protect protein structures. Among these, Heat-Shock Proteins (Hsps), molecular chaperones and Late Embryogenesis Abundant (LEA) proteins are mostly involved in cells protection (Wang and Luthe 2003, Wang et al. 2004, Goyal et al. 2005). The protection function comprises the control of folding and conformation of both functional (such as enzymes) and structural proteins (i.e. proteins constituting cell membrane and complexes). Some functional genomics and proteomics studies have confirmed an increasing in Hsps expression occurring under stress conditions (Sun et al. 2002; Wang and Luthe 2003; Sung and Guy 2003). Crop plants that grow under drought condition are particularly subjected to other different stresses caused by high temperatures, soil resistance to root penetration, excessive irradiance and low water potential. These stresses can produce protein denaturation and dysfunction. Despite the presence of photo-protective mechanisms able to dissipate excess light energy, the persistent exposure to extreme excitation energy can cause photo-reduction of oxygen molecules generating highly toxic reactive oxygen species (ROS). ROS, comprising super-oxides and peroxides, are usually produced by normal cellular activity occurring during photorespiration and beta oxidation of fatty acids and so their level increases under abiotic and biotic stress conditions. These dangerous molecules, causing chemical damage to DNA and proteins, bring to grave or even fatal effects on cellular metabolism. Reactive species can be neutralized by antioxidants and ROS scavengers and improving this capacity through manipulation, can represent an important goal. ROS elimination is mainly performed by antioxidant such as carotenoids, glutathione, ascorbic acid, and thioredoxin and by ROS scavenger enzymes such as catalase, superoxide dismutase, glutathione peroxidase. Bowler et al. (1992) demonstrated that under drought conditions the production of these components increases dramatically with a consequent higher scavenger activity enhancing plant drought tolerance. Aldehyde dehydrogenase (ALDH), known as one of the most effective ROS scavenger, catalyzes the oxidation of toxic aldehydes accumulated from the side reactions among ROS, lipids and proteins (Kirch et al. 2004). Transgenic plants overexpressing aldehyde dehydrogenase showed improved tolerance to dehydration, heavy metals, hydrogen peroxide and salt stresses (Sunkar et al. 2003). Moreover, as previously described, there are several osmolytes that can protect the cell maintaining the turgor pressure. Among these, proline and some sugars can protect the proteins covering their surfaces by the solutes in the medium and reducing so their unfolding (Hoekstra et al. 2001). LEA proteins and dehydrins seem to work when the desiccation rate becomes more severe, substituting the water through the constitution of hydrogen bonds for polar amino acid residues on the protein surface and thence decreasing the rate of unfolding (Crowe et al. 1997).

1.3 Salt Stress

High concentrations of salts in the soil, particularly NaCl, can cause ion imbalance and hyperosmotic stress in the plant. The consequence of these events is a secondary stress consisting in the oxidative damage previously described. Damages due to the salt presence can be prevented re-establishing homeostatic conditions and resuming the growth also at a reduced rate. The homeostasis re-establishment in stressful plants requires both ionic and osmotic homeostasis recovering. Ionic homeostasis is maintained by several ion transporters that work avoiding the accumulation to high levels of Na^+ into the cytosol, in the organelles and in the vacuole. Na^+ inhibits many enzymes, thence the prevention of its accumulation is fundamental. The transport of Na^+ into the cell is usually mediated by non-selective cation channels (NSCs), although other mediators have been recently identified such as LCT1 and HKT1 transporters which were originally identified as K transporters. Some works on the identification of mutations in the genes for HKT1 and LCT1 have demonstrated an increased salt tolerance in plants (Amtmann et al. 2001; Mondini et al. 2011). Nevertheless, the main aim of the studies focusing on salt tolerance consists in the identification of all the transporters involved in Na^+ entry into the plant. A consequent targeted mutagenization of these transporters could stop the Na^+ entry and increase the salt tolerance. Recently, it was reported in *Arabidopsis* that plants overexpressing AtNHX1 or SOS1 have significantly increased salt tolerance. In

Arabidopsis, ion homeostasis is mediated mainly by the SOS signal pathway, which consists of three main components. SOS1 encodes a plasmamembrane Na^+/H^+ antiporter that plays a critical role in sodium extrusion and in controlling long-distance Na^+ transport from the root to shoot (Shi et al. 2002). SOS2 encodes a Ser/Thr protein kinase (Liu et al. 2000) and finally SOS3 encodes an EF-hand Ca^{2+}-binding protein that functions as a calcium sensor for salt tolerance (Liu and Zhu 1998). AtNHX1 is the first studied plant vacuolar protein that can mediate Na^+ transport into vacuoles (Apse et al. 1999). Overexpression of SOS1 or AtNHX1 has been reported to deeply improve the plant salt tolerance (Apse et al. 1999; Zhang and Blumwald 2001; Zhang et al. 2001; Shi et al. 2003). This kind of transporter is very important due to its capability to store and remove from the cytosol the Na^+ in excess making the vacuolar compartmentation of Na^+ an efficient method to sequester Na^+. Moreover, it must be considered that plants, unlike animals, fungi and algal cells, do not contain Na^+-K^+ ATP-ases for Na efflux, anyway the same function of antiporter activity has been identified in plasma membrane-enriched membrane vesicles and in particular in the product of SOS1 gene (Blumwald et al. 2000; Shi et al. 2000). Another component of salt tolerance is the growth regulation. Salt stress inhibits growth, thence the slowing down of growth represents an important adaptive stratagem for plant survival allowing a more efficient resources exploitation. Salt stress acts directly on cell growth (i) reducing the photosynthesis efficiency caused by the stomatal closure and thence by the decrease of carbon dioxide uptake, (ii) inhibiting cell division and finally (iii) reducing the cell expansion. Several studies have been confirmed the connection between salt stress and growth rate decreasing (Holmström et al. 1996; Liu et al. 1998; Soderman et al. 2000). Considering that currently the overexpression of transgenes does not compromise or alter the plant cell growth, the overexpression of genes, known to be involved in salt tolerance, causes a slow growth of transgenic plants. Among these, DREB1 (Dehydration Responsive Binding elements-1) (Riechmann and Meyerowitz 1998), CBF1 (CRT/DRE binding factor 1) (Zhang et al. 2011) and ATBH7 (lipocalin-like protein) (Soderman et al. 2000) are cold and drought stresses-inducible genes and are not expressed in normal growth conditions. DREB genes family plays an important role in salt, drought and freezing stress tolerance even if their gene products or downstream target molecules act with different and sometimes contrasting actions which contribute to hinder the comprehension of their action mechanisms (Shen et al. 2003; Egawa et al. 2006; Mondini et al. 2012).

1.4 Stress Networks

The response of a plant to "environmental injury" and in particular to abiotic stresses aggression comprises multiple, complex and connected networks (Fig. 1.5). Stress tolerance is realized by the plant with the contemporary action of physical and molecular changes activated through a cascade of reactions by the stress itself (Fig. 1.4). The first limit to unravel the mechanism of action of plant response consists in the difficulty to assign to a specific stress typology the corresponding response. In fact, different stresses can jointly act through the same network and with the same cascade of reactions, for example drought, salt, and freezing stresses can all determine a decrease in cellular osmotic potential going to activate the same cellular response. At the same time, also abiotic stresses and nutrient deficiency can act with the same mechanisms, for instance drought stress and boron toxicity are strictly related in cereals causing both a decreasing in root development. Moreover, stresses causing an osmotic unbalancing can act producing two different type of responses, following two different ways operating through abscisic acid ABA-dependent and ABA-independent via. In addition, this primary response can activate a second ionic response where ion transporters confine ions in excess into specific cellular compartments.

1.4.1 Signaling Pathway

The starting perception of the stress immediately activates a downstream signal transduction pathway acting through the simultaneous induction and repression of different genes with the consequent activation of key enzymes. This cascade of reactions comprises molecular and biochemical changes which represent the entire network of abiotic stress responses. The transduction of the signal immediately generates an exchange of information within and between the cells and the induction of both regulatory and functional sets of genes (Ingram and Bartels 1996; Ramanjulu and Bartels 2002; Bartels and Sunkar 2005). Very little is known about the early events in the signaling pathway (Urao et al. 1999; Ueguchi et al. 2001; Wohlbach et al. 2008). Nevertheless, at large it can been distinguished into abscisic acid (ABA) dependent and ABA independent (Shinozaki and Yamaguchi-Shinozaki 1997, 2000). Among the key genes, transcription factors belonging to the class of DRE-binding protein (DREB), C-repeat-binding factor (CBF), ABA-binding factor (ABF), MYC, and MYB (Abe et al. 1997; Bartels and Sunkar 2005; Sakuma et al. 2006), including the identification of the stress-responsive cis-elements ABA-responsive element (ABRE) and dehydration responsive element (DRE; Yamaguchi-Shinozaki and Shinozaki 1994; Ramanjulu and Bartels 2002; Bartels and Sunkar 2005) play a key role. The presence of these different classes of elements in the promoters of drought, salt and freezing signal transduction suggests that these probably represent a point of convergence among the different pathways that permit a coordinated response to different stresses (Fig. 1.4). In this perspective, also the oxidative stress pathway seems to be connected with Ca^{2+} mediated response. In fact, among the early events in abiotic stress perception, the downstream signaling includes genes and molecules acting as secondary messengers, in particular the elevation of cytoplasmic Ca^{2+} levels, reveals the role of Ca^{2+} as secondary messenger (Bartels and Sunkar 2005). The elevation of Ca^{2+} concentration leads to the activation of signal transduction pathway involving Ca^{2+}-regulated protein phosphatases (Knight and Knight 2001). In particular, Urao et al. (1999) have demonstrated that when an osmotic stress occurs, the perception of the stress involves a plasma membrane histidine-kinase.

On this base, in transgenic plants, the engineering of the tolerance through the up regulation of transcription factors or other regulatory elements acting in the signal-transduction pathway represents a powerful and promising approach (Cushman and Bohnert 2000; Umezawa et al. 2004). Studying the different pathways, an important factor must be considered: the simultaneous exposure of a plant to different abiotic stresses results in a contemporary co-activation of different pathways acting with synergic or antagonistic mechanisms. Nevertheless, a dissection of the dedicated pathway specific for the particular stress is very difficult. The cross-talk among different pathways can occur at different levels: (i) integrations of networks of calcium-ROS signaling (Bowler and Fluhr 2000; Mittler et al. 2004); (ii) integrations of transcription factors-MAP Kinases (Cardinale et al. 2002; Xiong and Yang 2003); (iii) integrations among hormones (ABA, ethylene, jasmonic acid, etc.) (Anderson et al. 2004); (iv) integrations of receptors and signaling complexes (Casal 2002). For instance, Suzuki et al. (2006) and Kotak et al. (2007) have confirmed the involvement of factors other than classical heat-stress responsive genes in thermotolerance. In fact both plant hormones such as ethylene and reactive oxygen species also contribute to heat-stress signaling. The expression of co-activator MBF1c in *Arabidopsis* enhances at the same time, the tolerance to heat and osmotic stresses activating or disturbing the ethylene response signal transduction pathway.

1.5 Transcription Factors

Transcription factors are modular proteins that often contain separate domains that participate in DNA binding, protein/protein interactions, and transcriptional activation or repression. Transcription factors work largely by interacting with the basal transcriptional machinery and/or chromatin modifying proteins, thereby altering the rate of gene transcription (Latchman 1998; Lee and Young 2000). Transcription factors physically interact with genomic cis-regulatory DNA elements, referred to as transcription factor-binding sites (TFBSs), through a specific DNA-binding domain. Numerous types of DNA-binding domains have been identified in plant genomes that contain a large number of transcription factors such as *Arabidopsis* that contains more than 1,500 transcription factors covering a portion of about 5.9% of its genome (Riechmann et al. 2000). The most important transcription factor families are represented by MYB, AP2/EREBP, ZIP and WRKY elements (Table 1.1 and Fig. 1.6). These are large and multi-gene families where different members belonging to the same gene family respond to different stress typology. At the same time, it has been observed that different stresses can induce the expression of the same genes, as demonstrated by similar expression profiles indicating that some stress responsive genes can share the same transcription factors (Seki et al. 2001; Chen et al. 2002, Kreps et al. 2002). C-repeat binding factors (CBF) and dehydration responsive binding elements (DREB) specifically recognize CRT and DRE

Table 1.1 Main Transcription factors involved in plant abiotic stress tolerance

Gene family	Functional domain	ABA induced	Gene	Stress susceptible	Species investigated
DREB/ CBF	AP2/EREBP	No	AtDREB1A	Cold	*Arabidopsis thaliana*
		No	AtDREB2A	Drought, salinity	*Arabidopsis thaliana*
		No	AtDREB12C	Cold, salt, Mannitol	*Arabidopsis thaliana*
		No	OsDREB1A	Salt, Cold, wounding	*Oryza sativa*
		No	OsDREB1B	Cold	*Oryza sativa*
		Yes	OsDREB1C	Salt, drought, cold, wounding	*Oryza sativa*
		No	OsDREB1D	–	*Oryza sativa*
		No	OsDREB1F	Salt, drought, cold	*Oryza sativa*
		No	OsDREB1G	–	*Oryza sativa*
		No	OsDREB2A	Salt, drought, cold	*Oryza sativa*
		No	OsDREB2B	Cold, heat	*Oryza sativa*
		No	OsDREB2C	–	*Oryza sativa*
		No	OsDREB2E	–	*Oryza sativa*
		Yes	HvDRF1	Salt, drought	*Hordeum vulgare*
		No	ZmDREB2A	Salt, drought, heat, cold	*Zea mays*
		No	PgDREB2A	Salt, drought, cold	*Pennisetum glaucum*
		No	SbDREB2	Drought	*Sorghum bicolor*
		No	SiDREB2	Salt, drought	*Setaria italica*
		No	CaDREB-LP1	Wounding, salt, drought	*Capsicum annum*
		No	TaDREB1	Drought, cold	*Triticum aestivum*
		Yes	TaDREB2A	Salt, drought, cold	*Triticum aestivum*
		No	TaDREB3	–	*Triticum aestivum*
		No	TaDREB4	–	*Triticum aestivum*
		No	TaDREB5	–	*Triticum aestivum*
		No	GmDREBa	Salt, drought, cold	*Glycine max*
		Yes	GmDREBb	Salt, drought, cold	*Glycine max*
		No	GmDREBc	Salt, drought	*Glycine max*
		No	GmDREB2	Salt, drought	*Glycine max*
		No	CBF1	Cold	*Arabidopsis thaliana*
		No	CBF2	Cold	*Arabidopsis thaliana*
		No	CBF3	Cold	*Arabidopsis thaliana*
		Yes	CBF4	Drought	*Arabidopsis thaliana*
WRKY	WRKY	–	TaWRKY1	Drought	*Triticum aestivum*
		–	Os WRKY45	–	*Oryza sativa*
		–	Gm WRKY21	–	*Glycine max*
		–	Gm WRKY54	–	*Glycine max*
		–	Gm WRKY13	–	*Glycine max*
		–	Nb WRKY	–	*Nicotiana benthamiana*

(continued)

Table 1.1 (continued)

Gene family	Functional domain	ABA induced	Gene	Stress susceptible	Species investigated
bZIP	ABREs	Yes	ABF1	Cold	*Arabidopsis thaliana*
		Yes	ABF2	Drought, salt	*Arabidopsis thaliana*
		Yes	ABF3	Salt	*Arabidopsis thaliana*
		Yes	ABF4	Drought, salt, cold	*Arabidopsis thaliana*
		Yes	OsAREB1	Heat, drought	*Oryza sativa*
		Yes	Wlip19	Drought, cold	*Triticum aestivum*
		Yes	OsbZIP23	PEG, salt, drought	*Oryza sativa*
		Yes	GmbZIP44	Salt, drought	*Glycine max*
		Yes	GmbZIP62	Drought, cold, salt	*Glycine max*
		Yes	GmbZIP78	Salt, drought	*Glycine max*
		Yes	GmbZIP132	Salt, drought, cold	*Glycine max*
		Yes	OsABI5	Salt	*Oryza sativa*
MYB	MYB	Yes	MYB15	Salt, cold, drought	*Arabidopsis thaliana*
		–	OsMYB3R-2	Salt, cold, drought	*Oryza sativa*
		Yes	AtMYB2	Salt, drought	*Arabidopsis thaliana*
		Yes	AtMYB4	Ethylene, Jasmonic acid, Salt, SA	*Arabidopsis thaliana*
		Yes	AtMYB6	Ethylene, Jasmonic acid, salt, SA	*Arabidopsis thaliana*
		Yes	AtMYB7	Ethylene, Jasmonic acid, salt, SA	*Arabidopsis thaliana*
		Yes	AtMYB44	Ethylene, Jasmonic acid, salt, SA	*Arabidopsis thaliana*
		Yes	AtMYB73	Ethylene, Jasmonic acid, salt, SA	*Arabidopsis thaliana*
		No	GmMYB76	Salt	*Glycine max*
		No	GmMYB92	Salt, cold	*Glycine max*
		No	GmMYB177	Salt, drought	*Glycine max*
MYC	bHLH	Yes	AtMYC2	Salt, cold, drought	*Arabidopsis thaliana*
ZFP	Zinc finger binding domain	–	ZPT2-3	–	*Arabidopsis thaliana*
		–	Zat12	Oxidative, irradiation	*Arabidopsis thaliana*
		–	CaZF	Salt	*Nicotiana benthamiana*
		–	Alfin	Salt	*Medicago sativa*
		–	SCOF-1	Cold	*Nicotiana benthamiana*
		–	ZPT-2	Drought	*Arabidopsis thaliana*
		–	OSISAP1	Cold, salt, drought	*Nicotiana benthamiana*
		–	OSISAP2	Salt	*Nicotiana benthamiana*
		–	Zat7	Salt	*Arabidopsis thaliana*

(continued)