Maryam Sarwat · Altaf Ahmad
M.Z. Abdin · Mohamed M. Ibrahim
Editors

Stress Signaling in Plants: Genomics and Proteomics Perspective, Volume 2

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Maryam Sarwat obtained her Ph.D. 2007 from Jamia Hamdard (Hamdard University). She then carried out postdoctoral research at the International Centre for Genetic Engineering and Biotechnology (ICGEB), New Delhi Chapter. Presently, she is working as a Senior Assistant Professor at Amity Institute of Pharmacy, Amity University, Noida, India. She has guided 15 master's research projects for her students, who are also awarded at various conferences and workshops. Dr. Sarwat has successfully handled three research projects funded by the Government of India and received extramural grants of US $15,980, US $31,961 and US $36,755, respectively, from D.S.T., D.B.T. and C.C.R.U.M., Government of India. She has presented her research work in various conferences nationally as well as internationally (France, Prague, and Germany). Based on her research findings, she has also filed two patents. Dr. Sarwat has 25 international publications with a cumulative impact factor of more than 65. Additional publications are in various stages of publication. Considering her achievements and outstanding contributions, the International Board of Awards of the National Academy of Environmental Sciences has bestowed her with the Scientist of the Year Award for 2015.

Chapter 1
Physiological, Metabolic, and Molecular Responses of Plants to Abiotic Stress

Vicent Arbona, Matías Manzi, Sara I. Zandalinas, Vicente Vives-Peris, Rosa M. Pérez-Clemente, and Aurelio Gómez-Cadenas

Abstract Plants respond to environmental challenges inducing several physiological, metabolic, and molecular responses. These responses are oriented to avoid or endure the adverse environmental condition in non-adapted plant genotypes. Under abiotic stress conditions, plants trigger mechanisms to minimize water loss through stomata; this affects photosynthetic ability of plants by reducing CO_2 intake and fixation, therefore favoring the production of ROS and the incidence of oxidative damage. Therefore, the main metabolic responses of plants to abiotic stress will be oriented to cope with water loss (inducing compatible osmolyte biosynthesis) and oxidative stress (inducing biosynthesis of antioxidant compounds). Integration of environmental stimuli and adequate modulation of the physiological response is achieved by synthesizing plant hormones (ABA, JA, SA, ET, PAs, CKs, or GAs), metabolites that act as endogenous regulators of different plant processes. Plant hormones usually act in cross talk so that different signaling pathways contribute to fine-tune specific stress and developmental responses. At the molecular level, this cross talk implies interaction with different transcription factors that bind to common and specific *cis*-acting elements in promoter regions of stress and hormone-inducible genes. Fundamental physiological and molecular information is essential to build up models and design strategies to improve or confer abiotic stress tolerance to elite crops. Based on this knowledge, different strategies are used to introgress these tolerance traits into cultivated species: marker-assisted selection of genotypes (QTLs, MABC, MARS, or GWAS), induction of polyploidy and mutagenesis followed by variant selection, and, finally, plant genetic transformation. Strategies used for crop improvement are discussed in detail, the physiological and molecular basis explained, and the potential advantages and drawbacks highlighted.

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Abbreviations

3-PGA	3-Phosphoglycerate
ABA	Abscisic acid
AB-QTL	Advanced backcross QTL
ABRE	ABA-responsive element
ADC	Arginine decarboxylase
APX	Ascorbate peroxidase
BABA	β-amino butyric acid
CAT	Catalase
CE	Coupling element
CK	Cytokinin
DHAR	Dehydroascorbate reductase
DRE	Dehydration-responsive element
EMS	Ethylmethanesulfate
ET	Ethylene
GA	Gibberellin
GR	Glutathione reductase
GWAS	Genome-wide association studies
JA	Jasmonic acid
MABC	Marker-assisted backcrossing
MARS	Marker-assisted recurrent selection
MDHAR	Monodehydroascorbate reductase
MeJA	Methyl jasmonate
NO	Nitric oxide
Pro	Proline
PSII	Photosystem II
ROS	Reactive oxygen species
SA	Salicylic acid
SAMDC	*S*-adenosyl methionine decarboxylase
SOD	Superoxide dismutase
SPDS	Spermidine decarboxylase synthase
SPMS	Spermine synthase

Plant Responses to Abiotic Stress

Introduction

Climate change represents one of the major challenges to cope with feeding an increasing world population. In this context, plants will be affected by adverse environmental conditions, as increasing temperatures might affect the crop cycle, metabolic processes such as photosynthesis and respiration, and finally affecting yield.

In addition, alterations in temperature distribution could be especially harmful during crops reproductive periods inducing gamete sterility, lower yields, and even complete crop failure (Teixeira et al. 2013). Increasing temperature would raise evapotranspiration and intensify the hydrological cycle (frequent flooding and run-off increasing drought conditions) resulting in changes in soil moisture. Increments in air pollutants such as CO_2 or ozone (O_3) are also expected and might affect plant performance. Most of the plant responses to individual abiotic stresses are well known. However, the interactions between various simultaneous stress factors are scarcely studied due to the complexity of the approach (Lobell and Gourdji 2012). In this chapter, some of these interactions and their implications to plant physiology will be discussed.

So far, most of the physiological information on the stress response has been generated using artificial approaches and model plants. Therefore, these approaches do not completely correlate with real field conditions: plant acclimation, age of plant material, and biased plant phenotyping could lead to misinterpretation of results, and only the plant response that takes place under severe stress conditions is clearly identified (Claeys et al. 2014a).

Tolerance and Adaptation

Abiotic stress is defined as the effect of an adverse environmental condition that limits plant growth and productivity (Boyer 1982). Therefore, this concept implies economic aspects and differs from those usually employed in controlled experimental conditions, where parameters such as plant survival or seed germination are usually used (Dolferus 2014). From the productive point of view, the gap between yields under suboptimal conditions and those achieved by unstressed plants is used as a magnitude of the stress indicator. However, irrespective of yield, plants are able to acclimate to diverse environmental conditions triggering different mechanisms to cope with the stressful situation (Gepstein and Glick 2013). All plant species have evolved mechanisms to cope with stressful situations, referred to as adaptation (involving genetic changes followed by selection over many generations, e.g., desiccation-tolerant seeds, pollen grains) (Minocha et al. 2014). Genetic and physiological traits conferring resistance to abiotic stresses are difficult to target at the organ or tissue level; however, physiological and molecular mechanisms leading to stress tolerance are usually restricted to particular tissues and organs (Minocha et al. 2014). Firstly, it is necessary to clarify several concepts regarding the strategy developed by plants to cope with abiotic stress factors (Verslues et al. 2006): *stress resistant* is an ambiguous term to refer to stress-tolerant plants for which the specific tolerance mechanism is not known, *stress avoidance* includes a number of strategies aimed to minimize the damaging effects of stress to tissues (e.g., stomatal closure to reduce transpiration and, hence, water requirements preventing a severe reduction in tissue ψ_w), and when avoidance mechanisms are not enough to keep plant tissues from experiencing stress *stress tolerance* mechanisms are then induced (e.g., synthesis of compatible osmolytes and protective effector proteins, changes in metabolite composition, and induction of ROS detoxification mechanism). When tolerance mechanisms are induced and adverse conditions persist, plants reach a physiological "steady state" known as *acclimation* (also known as *hardening*) in which plants become less sensitive to the adverse conditions. It is important to note that whereas adaptation implies changes in the plant genome aimed to colonize a particular ecological niche, acclimation only involves physiological and biochemical changes orchestrated at the molecular level and modulated by plant hormones. As a general trait, crops do not possess any specific adaptation to adverse environmental conditions. Therefore, physiological, metabolic, and molecular mechanisms involved in stress resistance in crops are referred to as acclimation or tolerance.

The effects of the stress on plant growth and gene expression are dose-responsive, evidencing a fine-tuning machinery that allows sensing the stress level and adjustment of specific responses (Claeys et al. 2014a). A common response to different abiotic stress conditions is the downregulation of photosynthesis that is associated to several impairments to metabolism and growth. Under stress conditions, several metabolites are induced to cope with stress as osmoprotectants, antioxidants, or others that respond to specific stresses such as phytochelatins (see "Biochemical Responses of Plants to Stress: Basal Tolerance and Induced Tolerance" section). In addition, integration of environmental stimuli and physiological responses is mediated by an intricate network of plant hormones: ABA, jasmonates, SA, or ET that modulate stress responses (Peleg and Blumwald 2011).

Photosynthesis as a Central Process in the Response to Abiotic Stress

Abiotic stress conditions (drought, salinity, soil flooding, extreme temperatures, UV light, or O_3) reduce stomatal conductance restricting CO_2 diffusion to the substomatal chamber and limiting carbon assimilation required to maintain plant growth and development (Roy et al. 2014). Photosystem II (PSII) is particularly sensitive to CO_2 limitations, and the induction of photoinhibition reduces its efficiency enhancing ROS production (Noctor et al. 2014). Additionally, the combined effect of continuous light flux and abiotic stress accelerates the production of ROS having a synergistic impact on photoinhibition. The CO_2 limitation is linked to a reduction in the utilization of ribulose-1,5-bisphosphate by the Rubisco negatively affecting the synthesis of 3-PGA. This process uses NADPH generated in the photosynthetic electron transport chain. Increases in the $NADP^+$/NADPH ratio accelerate the reduction of O_2 to O_2^- and, subsequently, H_2O_2, derived from dismutation catalyzed by SOD. At the reaction centers, $^1O_2$ is generated after dissipation of excitation energy from chlorophylls (Asada 1999). ROS production, especially H_2O_2 and $^1O_2$, inactivates the translation of a pre-D1 protein, essential for D1 protein repair at the core of PSII reaction center (Nishiyama et al. 2004).

Plants have evolved diverse mechanisms to keep the photosynthetic apparatus functional, and photosynthetic rate is usually used as an indicator of plant/cultivar tolerance to abiotic stress. Nevertheless, there are certain plant species that show the opposite response to stress such as the NaCl-tolerant citrus rootstock Cleopatra mandarin that shows an early and strong repression of the photosynthetic machinery. Salt stress-induced stomatal closure impacts photosynthetic activity but also decreases transpiration hence reducing uptake of toxic saline ions (López-Climent et al. 2008; Brumós et al. 2009). In unstressed plants, ROS levels are tightly controlled by antioxidant systems. However, as stress conditions usually increase ROS production exceeding the cell antioxidant capacity, oxidative stress is likely to occur (Barcia et al. 2014). The cell antioxidant enzymatic system includes the enzymes SOD and CAT, operating in chloroplasts and peroxisomes; the enzymes of the ascorbate–glutathione cycle are found in chloroplasts and the cytosol: APX, MDHAR, DHAR, and GR (Asada 2006). An increase in antioxidant activity has been traditionally linked to stress tolerance (Arbona et al. 2009). Similarly, treatments that stimulate antioxidant activity have been correlated with enhanced photosystem protection and increased stress tolerance (Bandurska and Cieślak 2013).

Cross-tolerance, Memory, and Combined Stress Effects

Plants are frequently exposed to multiple stress conditions leading to synergistic, antagonistic, and sometimes neutral effects on plants. Evidently, the agronomical and physiological responses of plants exposed to several stressful factors can differ depending on the stress intensity or duration (Syvertsen and Garcia-Sanchez 2014). Literature describing the effects of combined stress conditions in plants is scarce. However, evidences point towards a negative effect of the individual stress conditions when applied together. For instance, in *Lotus japonicus*, combination of heat and drought induces the degradation of chloroplastic Cu/Zn SOD leading to increased O_2^- production, D1 protein degradation, and a decrease in PSII activity, therefore promoting photoinhibition (Sainz et al. 2010), although positive synergistic effects have also been observed (Colmenero-Flores and Rosales 2014). Environmental factors that reduce the transpiration rate such as high CO_2 concentration, low temperature, and high relative humidity could help to improve salt tolerance. In this sense, adequate stress management could result in important benefits (Syvertsen and Garcia-Sanchez 2014). Similarly, the application of heat stress could potentiate citrus and tomato fruit tolerance to other abiotic stresses such as cold damage during postharvest storage (Lu et al. 2010; Bassal and El-Hamahmy 2011). However, to be able to develop these strategies of stress management, more knowledge needs to be generated, since the specific responses to certain concurring stress conditions are not yet known.

Cross-stress tolerance is defined as physiological changes in response to previous stress conditions that protect plants from future stress events (Suzuki et al. 2012). This phenomenon is frequently linked to enhanced production of ROS such as H_2O_2 together with the associated regulation of the expression of antioxidant and defense genes, also integrating the action of several plant hormones (reviewed in Bartoli et al. 2013 and references therein). In several research works, it was demonstrated that an increase in SA levels in response to UV-B radiation protects from drought-induced oxidative damage (Bandurska and Cieślak 2013). Similarly, S- and Cd-induced oxidative damage also promotes metabolic changes and accumulation of Pro contributing to stress damage mitigation (Bashir et al. 2013).

The "memory" concept (classically known as hardening) implies that a previous stress exposure makes the plant less susceptible to possible future stress onset. Abiotic stress conditions occur repeatedly throughout a plant life span. Therefore, plants "remember" past events and use the previous experience to build a robust response (Kinoshita and Seki 2014). This mechanism of acclimation could involve several hormone signaling pathways and the antioxidant system (Asensi-Fabado et al. 2012) triggering adaptive changes not present in relatives not previously exposed to such stress (Ding et al. 2012). Moreover, epigenetic DNA methylation appears as a plausible mechanisms underlying this "memory" effect that could be inherited (Bruce et al. 2007), representing an important mechanism driving adaptation (Munné-Bosch and Alegre 2013).

Biochemical Responses of Plants to Stress: Basal Tolerance and Induced Tolerance

Plant Tolerance to Abiotic Stress

Plants responses to abiotic stress conditions are oriented to the activation of several biochemical pathways leading to the production of defensive compounds and enhanced tolerance. These responses are referred to as "induced tolerance." However, when innate physiological status of the plant allows stress tolerance without significant induction of further defenses, it is known as "basal tolerance." Generalist species are able to induce stress tolerance to a more or less wide range, whereas basal tolerance is a result of an adaptation to harsh environmental conditions often restricted geographically and temporally.

Basal Tolerance

In basal tolerance, stressed plants have a genetically innate defense against the stress that helps them to tolerate it without any previous stimulus. Plants have developed this defense mechanism as a result of a constant and repeated exposure to the stress factors. Therefore, this has driven the conservation of certain genome changes resulting in improved physiological and metabolic defenses and adaptation to the stress conditions (Clarke et al. 2004). This is of especial relevance regarding temperature stress (Hong and Vierling 2000) or metal toxicity (Clemens 2006).

Induced Tolerance

In Induced tolerance, plants are acclimated to the adverse conditions by a gradual exposure to stress or by chemical treatments. Plant hormones such as ABA, MeJA, or SA and many other different chemical compounds can act promoting tolerance to abiotic stress. Exposure of whole plants or plant tissues in vitro to sublethal stress doses (salinity, temperature, drought, etc…) triggers progressive acclimation (or hardening) of plants to the adverse conditions (Janská et al. 2010; Mittler and Blumwald 2015). This has been traditionally used to generate stress-tolerant lines in vitro employing polyethylene glycol, mannitol, or hydroxyproline to simulate different abiotic stress conditions (Rai et al. 2011). Moreover, exogenous application of compatible osmolytes Pro and GB also induced tolerance to salinity and drought in tobacco BY-2 cell cultures and *Phaseolus vulgaris*, respectively (Hoque et al. 2007; Xing and Rajashekar 1999). In *Vicia faba*, the plant hormone SA induced tolerance to high salinity by inducing antioxidant defenses therefore reducing the impact of stress-induced oxidative damage (Orabi et al. 2013). In *Cucumis sativus*, the exogenous application of 24-epibrassinolide increased systemic photooxidative stress tolerance associated to overproduction of H_2O_2 and enhancing its signaling role (Xia et al. 2011). This response appeared also to be mediated by NO downstream the brassinosteroid signal (Cui et al. 2011). Treatment with plant hormones and other chemical compounds (such as BABA) act as *priming* agents inducing different defense mechanisms and pathways whereas progressive exposure to the stress agents increases the deleterious threshold, making plants less susceptible to stress.

Biochemical Responses to Abiotic Stress

Plants have evolved a compendium of biochemical resources to survive to stressful environmental conditions. Abiotic stress induces changes in primary and secondary metabolite composition. Regarding the primary metabolism, variations in the accumulation of carbohydrates, amino acids, and polyamines are the main responses observed in plants subjected to different abiotic stresses. The myriad of compounds produced in minute amounts and that are not essential for plant survival are referred to as secondary metabolites. These compounds have several functions depending on their chemical structure and properties: antioxidant, antimicrobial, signaling, etc. (Fig. 1.1).

Primary Metabolism

Carbohydrates

Under stress conditions, carbohydrates metabolism and accumulation is directly linked to photosynthetic performance. Plants mobilize starch and fructans from storage organs (roots, stems, amyloplasts in leaves) to obtain glucose and fructose

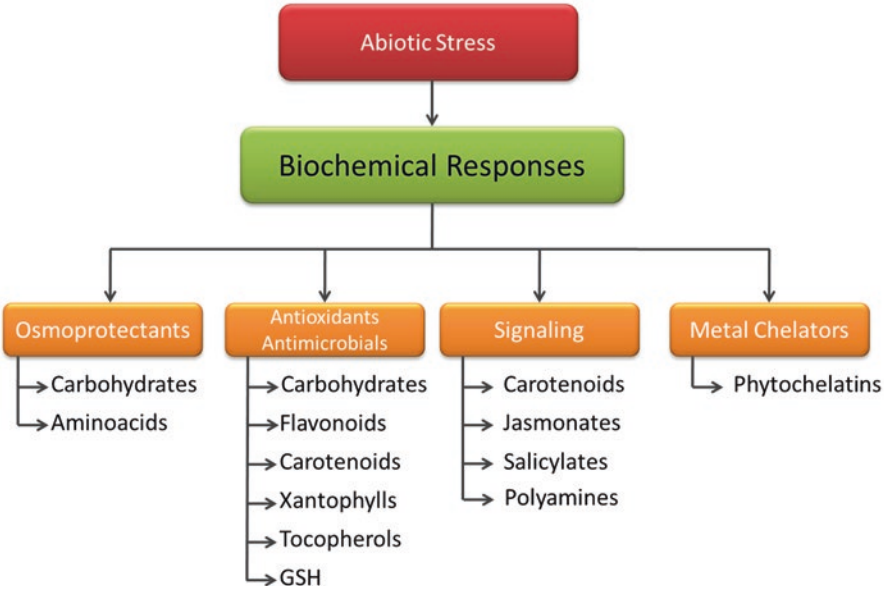

Fig. 1.1 Compound classes and roles exerted in response to abiotic stress

equivalents by increasing β-amylase activity. Besides being an important energy source, carbohydrates could act as osmoprotectants to maintain cell turgor, stabilize cell membranes, and avoid protein degradation (Sicher et al. 2012). Moreover, under stress conditions, several carbohydrates could interact with ROS, having an antioxidant activity and preventing oxidative damage (Keunen et al. 2013).

Several carbohydrate families have been shown to improve tolerance against different kinds of abiotic stress in several plant species through their action as osmoprotectants or antioxidants. Disaccharides such as trehalose are thought to have a protective role in plants subjected to drought, salinity, high or low temperatures, and oxidative stress. In transgenic tomato, overexpression the trehalose 6-phosphate 1 gene (TPS1) altered carbohydrate metabolism and improved stress tolerance (Cortina and Culiáñez-Macià 2005). Results obtained from transgenic *Arabidopsis thaliana* overexpressing a yeast TPS1-TPS2 gene fusion, suggested that trehalose was involved in altering sugar sensing and carbohydrate metabolism resulting in improved tolerance to multiple abiotic stresses (Miranda et al. 2007). Recently, trehalose has arisen as an important carbohydrate involved in the responses to heat and salt stress combination (Rivero et al. 2014). Raffinose family of oligosaccharides (RFOs) including galactinol and raffinose act as protecting macromolecules from drought, salinity, chilling, freezing, and oxidative stress (Nishizawa et al. 2008; Pennycooke et al. 2003). Overexpression of sucrose 1-fructosyltransferase gene under the control of a constitutive promoter increased oligo- and polysaccharide contents and improved tolerance to freezing in tobacco (Li et al. 2007) and chilling in rice (Kawakami et al. 2008). Moreover, transgenic plants with an enhanced ability to accumulate sugar alcohols such as sorbitol and mannitol showed improved cell membrane stability and increased tolerance to drought, salinity, chilling, and oxidative stress (Pujni et al. 2007; Chiang et al. 2005).

Amino Acids

Amino acids are primarily present as constituents of proteins and peptides. However, this is not their only function in the plant, since some amino acids are accumulated under abiotic stress conditions in a great variety of plant species, potentially acting as osmoprotectants, antioxidants, and cell membrane stabilizers. Among these, Pro and the betaine conjugate GB are thought to play a role as compatible osmolytes, and the tripeptide glutathione (GSH, γ-L-Glutamyl-L-cysteinylglycine) is known for acting as the redox exchange molecule and also for being the precursor of PCs (Noctor et al. 2011).

Pro is synthesized from L-glutamic acid by the action of the enzymes P5CS (^{1}-pyrroline-5-carboxylate synthase) and P5CR (^{1}-pyrroline-5-carboxylate reductase) that has several roles in abiotic stress conditions, including osmotic adjustment, stabilizing subcellular structures, scavenging free radicals, and buffering cellular redox potential (Ashraf and Foolad 2007). Under drought and salt stress conditions, there is an accumulation of this amino acid in the cytosol, contributing to the osmotic adjustment. In addition, there is also an increase in Pro concentration in other abiotic stresses, as flooding or extreme temperatures (Kaplan and Guy 2004). Under stress conditions, Pro concentration is usually higher in stress-tolerant plants than in sensitive ones, contributing to plant resistance to stress. For example, in salt-tolerant alfalfa plants (*Medicago sativa*), Pro concentration under stressed conditions doubles that found in sensitive plants (Petrusa and Winicov 1997). Despite all these evidences, Pro accumulation is not a universal response associated to tolerance. In response to salt stress, Pro accumulation in the *Arabidopsis* mutant *eskimo-1* was nearly ten times higher than in wild type and 3.1-fold higher than in the halophyte *Thellungiella halophila*. Nevertheless, this mutant exhibited higher salt stress sensitivity than Thellungiella or even wild-type plants (Ghars et al. 2008).

Another molecule with a protective role to abiotic stress is the amino acid derivative GB, a quaternary ammonium compound synthesized in chloroplasts from ethanolamine, choline, and betaine aldehyde, and it is accumulated in plants in response to stress. Although its distribution among plant species is not universal, *Arabidopsis* and many crop plants do not accumulate GB (Krasensky and Jonak 2012). Like Pro, GB accumulation is usually higher in tolerant plants than in sensitive ones (Ashraf and Foolad 2007). In plants that do not produce GB naturally, introduction of biosynthetic genes improved their ability to tolerate abiotic stress, pointing to the beneficial effect of this metabolite in stress tolerance (Krasensky and Jonak 2012; Chen and Murata 2008). It has been shown that, under stressful conditions, GB protects vegetative as well as reproductive organs. Exogenously applied GB is translocated via phloem to actively growing and expanding tissues and organs where it can act as osmoprotectant and as ROS-scavenging enzymes stabilizer (Chen and Murata 2008). It has been indicated that in salt-tolerant plant genotypes, GB accumulates to osmotically significant levels protecting PSII, stabilizing membranes and reducing the incidence of oxidative damage (Krasensky and Jonak 2012). Contrastingly, in tomato subjected to a combination of abiotic stress conditions, GB and the choline precursor did not show any significant accumulation in response to salt stress. Upon imposition of heat stress, GB and choline increased their tissue concentration about fourfold, but the combined imposition of salinity and heat reduced levels of both metabolites. In these experiments, Pro showed a significant accumulation only in response to salt stress and not to heat or combined abiotic stress conditions (Rivero et al. 2014). Taken together, these results indicate that the accumulation of osmoprotectants could be a species- and stress-specific response, probably related to the particular basal tolerance of the plant species.

As mentioned above, PCs are γGlu-Cys oligomers derived from glutathione act as intracellular chelators of toxic heavy metal ions, including As, Cd, Cu, Zn, and Mn in different plant species, such as *A. thaliana*, *O. sativa*, and *V. vinifera* (Huang et al. 2012; Dave et al. 2013; Yao et al. 2012). Nevertheless, PCs also might overaccumulate in response to other abiotic stresses.

Polyamines

PAs are low molecular weight nitrogenous aliphatic molecules derived from arginine that are involved in abiotic stress responses (Alet et al. 2012). The most widespread PAs are putrescine (Put), spermidine (Spd), and spermine (Spm) and can be present in both free and conjugated forms. The key biosynthetic genes are ADC, SPDS, SPMS, and SAMDC (Alcázar et al. 2010). Accumulation of PAs has been associated to the reduction of H_2O_2 by increasing peroxidase and CAT activities as well as Pro concentration. Abiotic stress conditions generally increase PAs concentration, and drought induces *ADC2*, *SPDS1*, and *SPMS* gene expression leading to Put accumulation but not Spd and Spm (Alcázar et al. 2006) probably as a result of efficient conjugation or removal. Salt stress also induces the expression of *ADC2* and *SPMS* subsequently inducing higher levels of Put and Spm (Urano et al. 2003). Cold stress also induces *ADC1*, *ADC2*, and *SAMDC2* gene expression therefore increasing Put concentration but, again, no effect on Spd and Spm levels is observed (Cuevas et al. 2008). In the constitutive halophyte *Prosopis strombulifera* (a native habitant of saline arid lands in Argentina), NaCl stress induces the accumulation of free Put in leaves, but not in roots, whereas the rest of polyamines reduce their concentration with respect to control value, probably as a result of NaCl-induced SAMDC inhibition (Reginato et al. 2012).

Secondary Metabolism

Phenylpropanoids

Phenolic compounds are secondary metabolites that have one or more phenolic rings, for example, flavonoids or coumarins. These compounds are derived from phenylalanine through reaction catalyzed by phenylalanine ammonia lyase (PAL) rendering cinnamic acid, the first precursor of phenylpropanoids. Phenylpropanoids constitute a highly diverse chemical class and several metabolites play important roles in abiotic stress tolerance: as important structural constituents of the secondary cell wall, ROS scavengers, protectants against UV radiation, signaling molecules and also modulators of auxin transport, etc. (Cheynier et al. 2013).

Flavonoids

Flavonoids are polyphenolic compounds derived from chalcone. According to their chemical structure, they can be classified into flavones, flavanones, flavonols, flavans, isoflavones, and anthocyanins (Djoukeng et al. 2008). In several plant species, abiotic stress induces the expression of flavonoid biosynthetic genes and the subsequent accumulation of flavonoids. However, it seems that this accumulation is not progressive and as the stress pressure increases the ability to synthesize flavonoids decreases (Bettaieb et al. 2011; Ithal and Reddy 2004). In response to soil flooding, tolerant citrus rootstocks accumulated more flavonoids than sensitive ones (Djoukeng et al. 2008), apparently constituting an adaptive response. As mentioned above, flavonoids are excellent protectants against UV radiation (Agati et al. 2011; Schenke et al. 2011).

Phenolic Acids and Other Non-flavonoid Phenylpropanoids

Phenolic compounds have several roles in plant cells: constituents of secondary cell walls (Moura et al. 2010), antioxidants (e.g., gallic and tannic acids, hydroxycinnamates and derivatives, etc.), signaling (e.g., salicylic acid), and also as phytoalexins (Arbona and Gómez-Cadenas 2015). In poplar, cadmium induced lignification of root secondary cell walls and reduced root expansion, in order to protect cells from heavy metal toxicity (Elobeid et al. 2012). On the contrary, lignification was reduced in maize roots subjected to water stress. Continuous accumulation of lignin in the absence of growth would lead to the lignification of the root elongation zone; therefore, this could be interpreted as an adaptive response to water stress allowing growth recovery after rehydration (Vincent et al. 2005). Phenolic compounds have shown an activity against high light intensities and UV-B radiation. Excess light acts as an important inducer of lignin biosynthesis in plants aiming to provide a barrier against high irradiation, and UV-B radiation induces the production of flavonoids and tannins (Moura et al. 2010). Moreover, phenolics were also induced in *Brassica* *rapa* grown under elevated CO_2 (Karowe and Grubb 2011). In response to ozone stress, hydroxycinnamates can be conjugated to apoplastic polyamines acting as powerful ROS scavengers (Iriti and Faoro 2009). The role of SA in the regulation of responses to abiotic stress is not clear, and low or high levels of this compound can increase the susceptibility to abiotic stresses, being the optimal levels from 0.1 to 0.5 mM for most plant species (Yuan and Lin 2008). Furthermore, it has been demonstrated that exogenous applications of SA at low concentration induce tolerance to multiple abiotic stress conditions such as low temperatures or heavy metal toxicity. SA-induced acclimation to these adverse environmental conditions is thought to occur through the induction of ROS production as well as upregulation of the antioxidant machinery (Horváth et al. 2007).

Carotenoids and Other Terpenoids

These metabolites are generally overproduced in several abiotic stress conditions (Espinoza et al. 2013; Kim et al. 2012) and might have a protective role stabilizing the lipid phase of thylakoid membranes of chloroplasts (Volkova et al. 2009). Moreover, carotenoids act as absorbing excess light or UV radiation and also as powerful antioxidants (α-tocopherol) (Binder et al. 2009; Pateraki and Kanellis 2010). Besides, carotenoids are also sources of volatile (Beck et al. 2014) and nonvolatile (Hauser et al. 2011) signaling compounds. This is the case of ABA, a sesquiterpene derived from lycopene with several functions in abiotic stress responses.

Molecular Responses to Abiotic Stress: Hormonal Regulation and Cross talk, Target Genes, and Gene Products

Regulators of Abiotic Stress Signaling: Signal Perception

Environmental signals are perceived by receptor proteins and sequentially transmitted to target signaling elements in order to respond to various external and developmental cues in a suitable and integrated manner. Receptor-like kinases (RLKs) constitute a large gene family in plants characterized by the presence of a cytosolic Ser/Thr kinase domain involved in the signal transduction to their target proteins by direct phosphorylation (Osakabe et al. 2013). RLKs control several plant mechanisms of plant growth and development as well as homeostatic processes underlying abiotic stress responses. Additionally, RLKs have been reported to have a key role in integrating environmental and plant hormone signaling (Shiu and Bleecker 2001; Diévart and Clark 2004) and can be considered as important regulators in growth and developmental processes in several environmental stress responses in the resulting adaptive mechanisms (Marshall et al. 2012). RLKs, such as RPK1, CYSTEINE-RICH RLK (CRK36), PROLINE-RICH-EXTENSIN-LIKE RLK4 (PERK4), and

GHR1 (GUARD CELL HYDROGEN PEROXIDE-RESISTANT1), have been reported to control water stress signaling directly in *Arabidopsis thaliana* (Tanaka et al. 2012; Hua et al. 2012; Osakabe et al. 2010).

Apart from RLKs, histidine kinases (HKs) are histidine-to-aspartate (His–Asp) phosphorelays similar to bacterial two-component signal transduction mechanisms involving a phosphotransferase activity that transfers a phosphate group from the sensor to the target protein. These receptors are plasma membrane- or endoplasmic reticulum membrane-bound and are involved in the control of different biological processes, including responses to different abiotic stresses such as drought, high salinity, and cold (Tran et al. 2010; Wohlbach et al. 2008; Jeon et al. 2010; Pham et al. 2012) and the perception of plant hormones. Specifically, five HK members (ETR1, ERS1, AHK2, AHK3, and AHK4) are implicated in the perception of the plant hormones ET and CK (Schaller et al. 2008). In addition, AHK2, AHK3, and AHK4 have been shown to negatively regulate ABA and stress signaling (Tran et al. 2007, 2010; Jeon et al. 2010). Moreover, AHK1 has been identified as a unique osmosensor with positive regulatory function in the expression of genes in both ABA-dependent and ABA-independent manner (Tran et al. 2007; Wohlbach et al. 2008).

Regulators of Abiotic Stress Signaling: Signal Transduction

Mitogen-activated protein kinase (MAPK) cascades constitute one of the most studied signaling mechanisms in plants. These comprise a group of highly conserved proteins with essential roles mediating perception of external stimuli and the induction of adaptive responses in all eukaryotic organisms (Hamel et al. 2012). MAPK cascades are composed of MAP kinase kinase kinases (MAP3Ks/MAPKKKs/MEKKs), MAP kinase kinases (MAP2Ks/MAPKKs/MEKs/MKKs), and MAP kinases (MAPKs/MPKs) sequentially activated by phosphorylation and have been involved in abiotic stresses responses (Rodriguez et al. 2010; Mittler 2002). The *A. thaliana* genome contains approximately 80 MAPKKKs, 10 MAPKKs, and 20 MAPKs that are activated by diverse stress signals, offering the possibility of cross talk between diverse stress signals. For instance, AtMPK6 is involved in O_3, H_2O_2, ET, ABA, and JA signaling pathways, and also in several developmental processes (such as epidermal cell patterning and anther and embryo development), probably acting as points of convergence of different stress and hormonal signaling pathways (Sinha et al. 2011). In *Arabidopsis*, the best characterized MAPK cascade in abiotic stresses is the MEKK1-MKK2-MPK4/MPK6 module. Mutants impaired in MKK2 activity also exhibited a deficient MPK4 and MPK6 activation and showed salt and cold hypersensitivity. Moreover, abolition of MKK2 activity altered expression of 152 genes involved in transcriptional activation in the *Arabidopsis* genome (Teige et al. 2004).

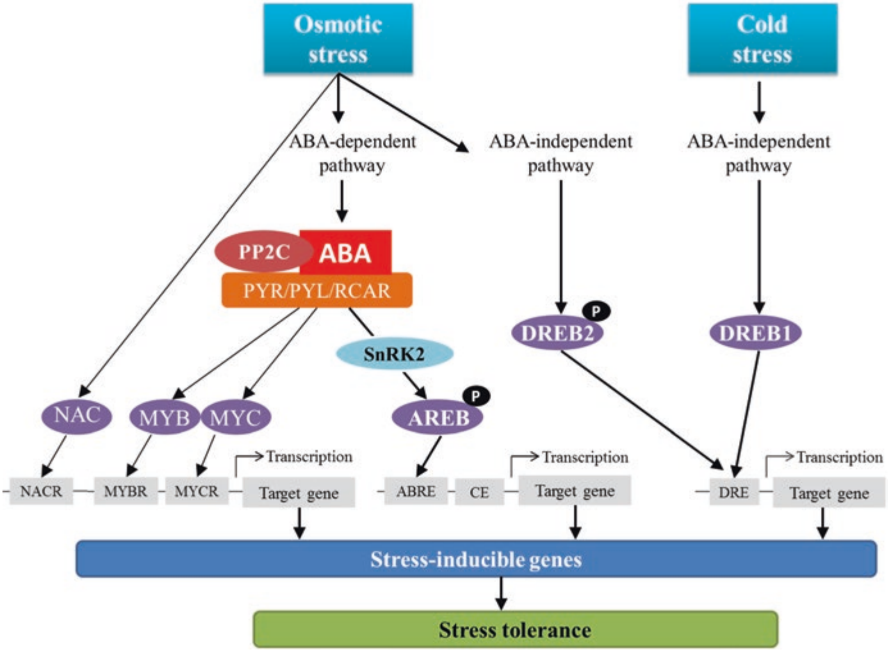

Fig. 1.2 Hormone and stress-dependent signaling pathways leading to stress-inducible gene expression

Hormonal Regulation of Abiotic Stress Responses

Plant hormones play central roles in the ability of plants to adapt to changing environments by mediating growth, development, and/or nutrient allocation (Peleg and Blumwald 2011). To this respect, ABA is a key regulator of many plant responses to environmental stresses, particularly osmotic stress (Hubbard et al. 2010). Recent studies have identified the components of the ABA signaling pathway (Fig. 1.2). Briefly, the core pathway consists of ABA receptors (PYR/PYL/RCAR), clade A protein phosphatases 2C (PP2C) such as ABI1 which act as negative regulators, and SNF-related protein kinases (SnRK2) which are positive regulators that phosphorylate several TFs and effectors, like the guard-cell SLAC-family anion channels, which mediate fast stomatal closure. Besides, other kinase families participate in ABA signal transduction leading to stomatal closure, such as Ca^{2+}-dependent CPK/CDPKs and the CBL-interacting CIPKs (Geiger et al. 2011). In the presence of ABA, PYR/PYL/RCAR inhibit PP2C activity resulting in release of SnRK2 proteins enabling ABA-mediated responses and the transcription of ABA-responsive genes (Santiago et al. 2012).

Experimental results have clearly shown that cross talk among different plant hormones is essential in integrating different environmental signals and readjusting growth as well as acquiring stress tolerance. Other plant hormones have important roles in abiotic stress responses: GAs, ET, SA, JA, and auxin have recently been found to interplay with ABA at different levels. Vegetative and reproductive growth and development are regulated by GAs throughout the plant life cycle (Claeys et al. 2014b; Schwechheimer and Willige 2009; Wang and Irving 2011). DELLA (Asp-Glu-Leu-Leu-Ala) proteins act as repressors of GA-dependent gene expression, and their degradation results in GA response in plants (Hauvermale et al. 2012). Cross talk between GAs and ABA occurs at the biosynthetic level via the putative early DELLA target gene XERICO whose expression is induced by salt and osmotic stress and causes hypersensitivity to ABA. Moreover, overexpression of this gene results in elevated ABA content (Ko et al. 2006). ET/ABA cross talk regulates stomatal opening (Wilkinson and Davies 2010). Auxins have an epistatic role over ET/ABA cross talk in controlling root hair elongation and root branching under abiotic stress (Wang et al. 2013). Besides, ET induces the expression of ERF TFs that are also responsive to JA (Wu et al. 2009) and regulate a diverse range of processes associated to environmental cues (Wang et al. 2013).

The vast majority of studies on SA mode of action have predominantly been oriented toward its role in plant defense responses. However, recent reports have demonstrated that SA also plays an important role in modulating the plant response to many abiotic stresses (Kang et al. 2014; Miura and Tada 2014). SA interacts with GAs at different levels: SA application partially rescues seed germination in *ga1-3* mutant, and GA_3 application improved performance of SA-deficient *sid2* under salt stress (Alonso-Ramírez et al. 2009). Cross talk between ABA and SA signaling pathways was evidenced by the effect of SA on the synthesis of ABA-regulated proteins in *Arabidopsis* (Rajjou et al. 2006). Under stress, cross talk between jasmonates and ABA at the biosynthetic levels has been also observed in Arabidopsis and Citrus (Oa et al. 2009; De Ollas et al. 2013). In *Arabidopsis thaliana*, the TF MYC2 has been proposed to regulate the interaction between ABA and JA signaling pathways acting as a master switch between the two signaling pathways (Dombrecht et al. 2007; Kazan and Manners 2013). Auxins have also been shown to participate in the positive regulation of drought stress tolerance through regulation of root architecture, expression of ABA-responsive genes, ROS metabolism, and metabolic homeostasis (Shi et al. 2014). Under moderate drought, ABA accumulation modulates auxin transport in the root tip, which enhances proton secretion necessary to maintain root growth (Xu et al. 2013).

Transcriptional Factors Involved in Hormonal and Abiotic Stress-Associated Gene Expression

TFs interact with *cis*-elements in the promoter regions of several stress-inducible genes to regulate the expression of many stress-inducible genes involved in stress acclimation and tolerance (Agarwal et al. 2006). Studies of the transcriptional regulation under drought and salinity have revealed the existence of ABA-dependent and -independent pathways (Yoshida et al. 2014). The ABA-dependent signaling system induces a family of TFs ABF/AREB/ABI5 (ABA-binding factor/ABA-responsive element-binding protein/ABA Insensitive 5) constituted by bZIP TFs that control ABA-mediated gene expression under abiotic stress conditions and developmental processes. These TFs are activated through multiple-site phosphorylation of their conserved domains by SnRK2s (Fujita et al. 2011, 2013; Furihata et al. 2006). Some of them also respond to specific environmental challenges; for instance, ABF1 is significantly induced by cold (Kim 2006) but not by osmotic stress (Fujita et al. 2004), whereas AREB1/ABF2, AREB2/ABF4, and ABF3 are induced both by ABA and high osmoticum (Yoshida et al. 2014). Besides ABF/AREB/ABI5, MYB2 TF belonging to the R2R3-type MYB family has been shown to be an important mediator of ABA-mediated gene expression under adverse conditions in *Arabidopsis* (Stracke et al. 2001). Other MYB TFs participate in ABA-mediated gene expression, including MYB102 that has been reported to respond to ABA, JA, salt stress, and wounding (Denekamp and Smeekens 2003); MYB41 that responds to drought, ABA, and salt treatments (Cominelli et al. 2008); MYB108 that functions as a positive transcriptional regulator of JA- and ABA-inducible genes, therefore playing an important role in abiotic and biotic stress tolerance (Mengiste et al. 2003); MYB44 which is activated by several hormone treatments (ABA, auxins, ET, JA, and GA) as well as by environmental conditions such as drought, high salinity, and low temperature (Jung et al. 2008; Persak and Pitzschke 2014).

On the other hand, AP2/ERF (reviewed in Lata and Prasad 2011) are a large family of plant-specific TFs that share a well-conserved DNA-binding domain. This family includes DRE-Binding proteins that activate the expression of stress-responsive genes, independent of ABA. This group includes CBF/DREB1 (cold-binding factor/dehydration responsive element binding 1) TF whose expression is strongly and transiently induced by low temperature stresses (Fowler et al. 2005) and DREB2 (e.g., DREB2A and DREB2B) that are induced by drought, high salinity, and heat stress but not by cold stress or exogenous ABA (Sakuma et al. 2006a, b).

There are other TFs involved in abiotic stress tolerance responses such as NACs or WRKYs (Chen et al. 2012; Nakashima et al. 2012). The encoded proteins show a conserved N-terminus region possessing five DNA-binding motifs, whereas the more divergent C-terminus contains domains that function as transcription activators (as extracted from yeast and plant assays) (Kleinow et al. 2009; Chen et al. 2014; Puranik et al. 2012). NAC TFs have been identified in Arabidopsis (ANACs) (Wang and Dane 2013) and crops such as banana (Cenci et al. 2014), cotton (Huang et al. 2013), tomato (Yang et al. 2011a, b, c), rice (Chen et al. 2014), poplar (Hu et al. 2005), and citrus (Puranik et al. 2012). Promoters in NAC genes contain domains known to be responsive to ABA (ABREs), JA, SA, drought (DREs), and low temperature (LTREs) as well as sites of recognition for MYB and MYC TFs (Puranik et al. 2012). In addition, in Arabidopsis, NAC factors such as ATAF1 have been recently shown to regulate expression of NCED3 and subsequent ABA accumulation (Jensen et al. 2013) and, at the same time, this TF is able to physically interact with SnRK2, involved in ABA signaling (Kleinow et al. 2009).

Cis-*Acting Elements Involved in ABA-Dependent and ABA-Independent Gene Expression*

Promoter sequences of stress- and hormone-inducible genes contain conserved boxes (or *cis*-acting elements) that allow binding of specific TFs acting as downstream stress or hormone signaling pathways (Shinozaki et al. 2003). Hence, promoter regions of ABA-inducible genes contain multiple conserved *cis*-elements globally named ABREs (PyACGTGGC) or combinations of ABREs and CE (Shinozaki et al. 2003). These are similar to ABREs and contain A/GCGT motif (Hobo et al. 1999). Genes containing ABREs in their promoter regions include several LEA class proteins, clade A PP2Cs, and diverse types of TF (Nakashima and Yamaguchi-Shinozaki 2013). In *Arabidopsis*, plant dehydrins RD29A and B are upregulated by stress and ABA, respectively. RD29B has two ABRE domains in its promoter region (Uno et al. 2000), whereas RD29A contains ABREs and also DREs. The existence of different *cis*-acting elements allows cross talk between different signaling pathways. For instance, DRE and ABRE motifs in RD29A promoter have been reported to act independently but, under certain circumstances, DRE could function cooperatively with ABRE (Narusaka et al. 2003). In addition, DREBs can physically interact with AREBs (Lee et al. 2010) suggesting potential cross talk between stress- and hormone-signaling pathways.

Stress-Responsive Effector Proteins

Stress-regulated genes and their products have important roles in stress response and tolerance. There are several groups of proteins that are induced in response to abiotic stress and are involved in stress acclimation mechanisms. For instance, hydrophilins are a group of proteins (including LEA proteins) which possess a 6 % Gly content and hydrophilicity index higher than 1 (Battaglia et al. 2008). Other proteins induced by stress are small heat shock proteins (HSP) that are primarily induced upon heat stress (Liu et al. 2012). Small HSPs are molecular chaperones that assist correct protein folding. Under heat stress, mitochondrial HSP22 and chloroplast HSP21 were highly expressed in grapevine (Liu et al. 2012). Heat stress can also alter plasma membrane fluidity activating certain HSP genes encoding for proteins that act in preventing membrane disintegration (e.g., hspA) (Allakhverdiev et al. 2008). As mentioned above, plants alter their cell wall composition and stiffness in response to abiotic stress by inducing the phenylpropanoid pathway. In this process, there are a group of proteins called hydroxyproline-rich glycoproteins or HRGPs that are an integral part of cell walls and have functions in several aspects of plant growth and development. These can be divided into hyperglycosylated arabinogalactan proteins, moderately glycosylated extensins, and lightly glycosylated proline-rich proteins (Showalter et al. 2010). Although it is not absolutely clear, available evidence suggests a role of HGRPs in heavy metal stress tolerance (Yang et al. 2011a, b, c).

In addition, environmental stress also increases ROS production; hence, a myriad of proteins aimed to alleviate oxidative stress are induced: SOD, APX, CAT, GR, etc… Most of these enzyme activities have been targets of breeding programs aimed to improve abiotic stress tolerance (Gill and Tuteja 2010).

ABA- and stress-responsive genes, such as LEA class genes (RD29B, RAB18), cell cycle regulator genes (ICK1), PP2Cs (ABI1 and ABI2), and RD22 and RD26, have received special attention as potential targets for improvement of stress tolerance. The genes encoding for these proteins have conserved ABREs and DREs motifs in their promoter regions. RD22 gene expression is cooperatively mediated by ABA, MYC2, and MYB2 TFs (Shinozaki and Yamaguchi-Shinozaki 2007). In *Arabidopsis*, RD26 encodes a NAC protein induced by dehydration and ABA (Fujita et al. 2004). On the other hand, promoter of ERD1 gene (encoding a Clp protease regulatory subunit, ClpD) contains a MYC-like sequence (CATGTG) and a 14-bp *rps1* site 1-like sequence involved in dehydration response (Simpson et al. 2003). This aspect is reviewed in detail in the next Section of this Chapter.

Strategies for Engineering Abiotic Stress Tolerance: From Genotype Selection to Genetic Transformation and Genome Editing

A current challenge in applied plant physiology is the production of abiotic stress-tolerant crops able to endure water shortage, raising ambient temperature and CO_2 and other environmental cues (Suzuki et al. 2014). This aspect is becoming critical as world population, and food demand is steadily increasing while the land available for agriculture is being progressively reduced. Currently, more than one billion people have insufficient food to sustain life, and it is expected that by 2050 food supply would have to double to satisfy demand, as calculated on the basis of staple grain crops (maize, wheat, and rice, Reynolds et al. 2012). The four major strategies to induce stress tolerance in crops are: (1) identification of variability into wild populations relatives to the crops of interest and use them as rootstocks or parentals for breeding and transfer stress tolerance traits to crops, (2) polyploidization of crops of interest, (3) in vitro random mutagenesis and selection of variants, and (4) identification of particular genes from related or other plant species that might confer stress tolerance and transfer them into crops by genetic transformation.

Identification of Variability in Stress Tolerance from Natural Populations

Stress tolerance, high yield, and quality features do not come often together and, as a result, it is necessary to introduce these traits into economically valuable crops. Most of the stress tolerance traits observed in crops are controlled by a complex