13
Activation of the Jasmonate Biosynthesis Pathway in Roots in Drought Stress

Palmiro Poltronieri, Marco Taurino, Stefania De Domenico, Stefania Bonsegna, and Angelo Santino

Abstract

Roots are the primary organs that first sense the soil environment. Plant growth and development are largely dependent on the plant root system, due to its crucial role in water and mineral uptake. Symbiotic microorganisms affect and improve the root response to stresses. Root endophytes and bacteria synthesize a wide array of plant-protecting chemicals, hormones, and compounds acting on hormone degradation. Since hormonal homeostasis is tightly regulated, the effects of abiotic factors may translate to specific molecular mechanisms though hormone cross-talk. Abiotic below-ground stresses are early signals affecting root growth regulation, resource acquisition, and root–shoot communication. Abiotic stresses elicit early signals that need to be transduced at distance to affect protection mechanisms, such as growth regulation, resource acquisition, synthesis of osmoprotectants, change in water potential, and regulation of stomatal closure, among others. The oxylipin family of signals represents one of the main mechanisms employed by plants. This family comprises fatty acid hydroperoxides, hydroxy-, keto- or oxo-fatty acids, volatile aldehydes, divinyl ethers, and jasmonic acid. Most of them are volatile compounds participating in several physiological processes, defense mechanisms, stress adaptation, and communication with other organisms. This chapter reports on new insights into the role of the activation of jasmonic acid biosynthesis during abiotic stresses in plant roots, and on the importance of earlier and stronger jasmonic acid induction as a trait conferring better drought tolerance in legume varieties able to cope with water stress.

13.1
Background and Introduction

Abiotic stress is a primary cause of crop loss worldwide, causing average yield losses in major crops. Tolerance and susceptibility to abiotic stresses are very complex. Plants can resist abiotic stresses by activating different distinct

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mechanisms, whose traits are multigenic, often converging on genes shared by different stresses. Drought is one of the major constraints in agriculture. Therefore, improving water availability and drought stress tolerance are of great importance for future breeding strategies. Extreme environmental conditions are expected to become more frequent in many European regions in the near future. This will require new cultivars with high resilience that make good use of favorable conditions while withstanding periods of drought, cold, or heat.

Molecular genetics and genomics studies of stress responses in model plants such as *Arabidopsis* and *Medicago* revealed that abiotic stresses such as drought, salinity, and cold stress are characterized by ionic and osmotic disequilibrium components, eliciting general as well as specific responses and mechanisms of stress protection [1]. These studies underpinned the importance of early responses to the various stresses for plant survival [2]. Drought stress induces a range of physiological and biochemical responses in plants, such as stomatal closure, reduction of water evaporation, repression of growth and photosynthesis, and activation of respiration. Many of the drought-inducible genes identified can be classified into two major groups: proteins that function directly in abiotic stress tolerance and regulatory proteins that are involved in stress signal transduction or activation of stress-responsive gene expression.

The physiological mechanisms governing plant responses to salinity and drought show high similarity, suggesting that both stresses are perceived by plant cells as deprivation of water [3]. High salt concentrations (NaCl) in the soil lead to a decrease of water potential, which affects water availability. In addition to the hyperosmotic shock and the subsequent oxidative stress [4], deleterious consequences of high NaCl concentration in the apoplast also include ion toxicity and nutrient imbalance [5–7].

13.2
Plant Growth Factors: Key Role in Biotic and Abiotic Stress Signaling

In the case of biotic stress responses, a correct and proper response is important for plant fitness, while enhancing disease resistance against pathogens. Plants have evolved sophisticated defense systems to cope with a multitude of harmful environmental conditions. Resistance strategies of plants against biotic threats are very diverse, including constitutive defenses and induced responses. Hormones such as abscisic acid (ABA), salicylic acid, jasmonic acid, and ethylene are important players both in the biotic and abiotic stress response of plants and in plant–microbe interactions, regulating the fine-tuning of plant defense mechanisms, and in the establishment of the hypersensitive response and systemic acquired resistance.

PAMP-triggered immunity (PTI) involves a bacterial compound-sensing, receptor-mediated mechanism that protects plants from non-pathogenic microbes. Plants perceive such pathogen-derived effector molecules via disease resistance proteins, invoking effector-triggered immunity (ETI). ETI is a more rapid and

stronger type of response than PTI and it often results in the so-called hypersensitive response. The salicylic acid response stalls plant growth and stimulates an accompanying immune response. Owing to its growth-inhibitory effects, plants gradually stop salicylic acid signaling via a salicylic acid glucosylation, that transforms salicylic acid into the inactive derivative salicylic acid-2-*O*-β-D-glucoside, or through salicylic acid hydrolysation. Most salicylic acid-inducible genes are controlled by the transcriptional activator NPR1. NPR1 proteins are normally present as cytosolic oligomers linked by intramolecular disulfide bonds. Upon salicylic acid treatment, NPR1 oligomers are monomerized due to a change in the intracellular redox status. NPR1 monomers are translocated to the nucleus where they activate gene expression [8]. Recently NPR1 was shown to bind directly to salicylic acid through a metal (probably copper) via two cysteine residues. NPR1 is also a protein target for nitric oxide (NO)-mediated cysteine nitrosylation.

Jasmonates are produced by plant tissues, such as leaves, in response to environment and biotic stresses. When the plants sense the presence of pathogens, jasmonic acid regulates subsets of genes involved in the induction of a necrotic cell death as a defense mechanism against the spreading of microorganisms. In defense against necrotrophic pathogens, jasmonic acid and ethylene signaling pathways synergize, converging on the AP2 (apetala 2)/ERF (ethylene response factor) family of genes (i.e., AP2, ERF, and DREB (dehydration-responsive element-binding) transcription factors) controlling the expression of genes synergistically induced by jasmonates and ethylene [9]. The GCCGCC motif is commonly found in promoters activated synergistically by jasmonate and ethylene [10]. Other jasmonic acid-responsive transcription factors, such as MYC, bind to the G-box sequence, specific for promoters activated by jasmonates and repressed by ethylene [11].

In plants insensitive to jasmonic acid, such as the jasmonate-resistant 1 (*jar1*) mutants, jasmonic acid is essential for the resistance to the necrotrophic fungus *Botrytis cinerea*. *Botrytis* infection triggers the synthesis of jasmonic acid, which induces the expression of *Botrytis* susceptible 1 (BOS1), a MYB transcription factor that mediates both biotic and abiotic stress signaling via reactive oxygen species (ROS) production [12].

Dehydration-responsive NAC transcription factors, such as RD26 and RD22, are induced by jasmonic acid, hydrogen peroxide, pathogens, drought, salinity, and ABA. ABA is a hormone involved in senescence, seed dormancy, plant development, and stress response. In the aerial parts of the plant ABA regulates stomatal movement and the activity of shoot meristems. ABA can flow in the root cortex across apoplastic barriers in the form of ABA-glucose ester (GE), a stress signal stored in microsomes and released into xylem by the activity of β-glucosidases in mesophyll cells. A β-glucosidase gene was found upregulated in water stress in roots [13]. At the initial stages of water stress, the amount of ABA-GE stored in roots is too low to produce the high ABA increase observed during water stress. Sulfate, mobilized by the action of an early-overexpressed root sulfate transporter, acts as a long-distance signal moving through the sap to induce ABA biosynthesis in leaves. ABA then is transported to roots via the phloem where it induces water uptake from soil and expression of stress-resistant genes. Subsequently, ABA is

cycled back to leaves via the xylem to close the stomata and reduce the transpiration rate, an action mediated by the release of NO. The costimulation with ABA, ethylene, and sulfate produces an additive increase in stomata closure reinforcing the block of transpiration for an extended period of drought persistence.

The hormone cytokinin regulates growth and development, and also influences root elongation. Diverse activities of cytokinin have been elucidated, including cross-talk with other hormones in response to different environmental stimuli. AP2/ERF transcription factors were identified in particular as cytokinin-responsive, involved in translational control of cytokinin-induced changes.

13.3 Jasmonate Biosynthesis Pathway

The oxygenation of polyunsaturated fatty acids (PUFAs) gives rise to a variety of oxylipins, such as fatty acids hydroperoxides, hydroxy-, keto- or oxo- fatty acids, aldehydes, divinyl ethers, green leaf volatiles (a series of chemicals belonging to the volatile organic compounds), and jasmonates. These bioactive compounds participate in several physiological processes, such as defense mechanisms (sensing herbivores, insects, and pathogens), adaptation to abiotic stress, and communication with other organisms [14,15].

In the jasmonic acid biosynthesis pathway, linolenic acid (18 : 3) is used as substrate for the sequential action of lipoxygenases (LOXs). A cytosolic 9-LOX produces 9(*S*)-hydroperoxy fatty acids, while a plastidial 13-LOX produces 13(*S*)-hydroperoxy fatty acids. In chloroplasts, in addition to 13-LOX, allene oxide synthase (13-AOS) and allene oxide cyclase (AOC) act in concert to produce 12-oxophytodienoic acid (OPDA) or dinor-OPDA (Figure 13.1).

Two oxylipin branches diverge from the main jasmonic acid synthesis pathway. In the first pathway, divinyl ether synthases (DESs) convert hydroperoxides to divinyl ethers; in the second branch, hydroperoxide lyases (HPLs) produce short-lived hemiacetals that decompose to aldehydes and *n*-fatty acids ($n = 6, 9$) [16]. These reactive oxylipins (RES) are formed under a variety of biotic and abiotic stress conditions [17].

A crucial step in jasmonate biosynthesis is catalyzed by AOC, an enzyme shown to be active in oligomer forms, such as homodimers and heterodimers [18]. A central role of AOC oligomerization in jasmonate production in *Arabidopsis thaliana* has been demonstrated. This research led to detailed information on the role of the four AOCs in plant, indicating redundant and non-redundant functions during development. AOC promoter activities corresponded to expression of jasmonate-responsive genes in distinct tissues, and suggested a potential cross-talk between jasmonates and auxins in the regulation of root growth.

Nitrosylation of cysteines in the enzymes of salicylic acid/jasmonic acid synthesis has been found to be important in regulating and controlling jasmonate production [19]. AOC has been found *S*-nitrosylated in a cysteine proximal to the catalytic site by NO during the hypersensitive response [20].

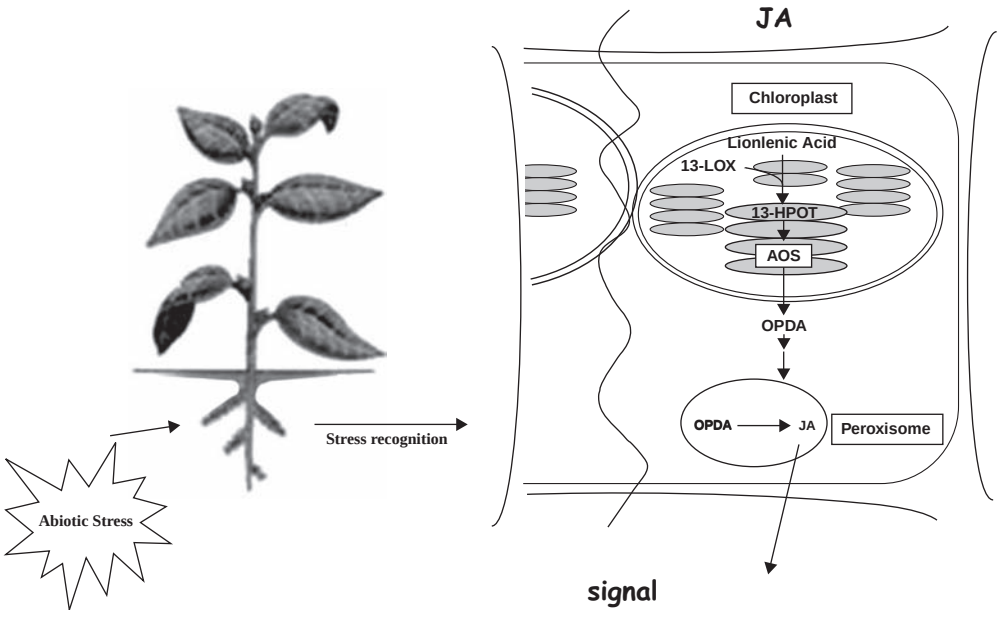

Figure 13.1 The jasmonic acid biosynthesis pathway necessitates the subsequent involvement of plastidial enzymes and peroxisomal enzymes. Inside the chloroplast, three enzymes (13-LOX, AOS, and AOC) cooperate in the production of OPDA, that moves into the peroxisome where it undergoes three cycles of β-oxidation.

In *Arabidopsis* plants treated with NO, NO was found to induce key enzymes of jasmonic acid biosynthesis such as AOS and LOX2 [21]. NO induction of jasmonic acid biosynthesis enzymes did not result in elevated levels of jasmonic acid and jasmonic acid-responsive genes such as defensin (PDF1.2) are not induced. This finding supports the hypothesis that the level of expressed genes needs to be paralleled by increased levels of translated proteins and by the correct assemblage of active enzyme oligomers. The intracellular production/release and containment of jasmonic acid intermediates is conducted in specific and often strictly localized reactions, to allow for spatially and timely regulated signaling events.

The next step in jasmonic acid synthesis is the import of OPDA into peroxisomes, where it is reduced by 12-oxophytodienoate reductase 3 (OPR) to 3-oxo-2(2′-pentenyl)-cyclopentane-1-octanoic acid, which undergoes three cycles of β-oxidation by the activity of an acyl-CoA oxidase (ACX), that produces OPC:6, a multifunctional protein involved in the synthesis of OPC:4CoA, a ketoacyl-CoA thiolase (KAT2) to produce jasmonic acid-CoA and finally jasmonic acid (Figure 13.1).

Peroxisomes are ubiquitous organelles that are essential in plants, fungi, yeasts, and animals, but their importance is underestimated. Recent identification of several novel peroxisome functions, related to resistance to various stresses, revealed yet-unknown mechanisms that allow plants to adapt to adverse environmental conditions. Unexpected enzyme activities, novel metabolic pathways, and

unknown non-metabolic peroxisome functions have been recently found, such as production of secondary metabolites and the role of glutathione as a major antioxidant [22]. For instance, glutathione reductase as well as other additional proteins were found to be specific to peroxisome variants from abiotically stressed plants [23].

jasmonic acid can be methylated by a jasmonic acid-methyltransferase to form the volatile compound methyl-jasmonate (Me-JA), freely diffusing across biological membranes and acting at short distances. When jasmonic acid is converted to 12-hydroxy-JA (12-OH-JA) and 12-OH-JA sulfated forms, its bioactivity is reduced and does not inhibit root growth [24,69].

Jasmonic acid is modified by the action of the JAR amino acid synthetase to form jasmonoyl derivatives (JA-Ile, JA-Val, JA-Leu) that may be stored in glycosylated form in the vacuoles. JA-Ile is able to move through the xylem from roots to leaves and backwards [25]. JA-Ile is the active hormone derivative responsible for jasmonic acid biological activity mediated by jasmonic acid receptors [26]. Coronatine, a compound synthesized by *Pseudomonas syringae*, is a JA-Ile mimic that affects the regulation of plant defense responses [27,28]. Coronatine insensitive 1 (COI1) was identified as the receptor for JA-Ile in a study of mutants of the ubiquitin proteasome components [29,30].

JA-Ile response is controlled by a group of nuclear proteins called jasmonate ZIM domain (JAZ) repressors that interact with COI1 [25]. AtMYC2 interacts with JAZ proteins, until JA-Ile binds to COI1. The F-box protein COI is involved in the SCF (Skp/Cullin/F-box) ubiquitin ligase complex. Upon JA-Ile–COI interaction, it promotes the ubiquitinylation of JAZ proteins, thereby liberating AtMYC2 from repression. Then MYC2 binds to the G-box region of promoters inducing the expression of jasmonic acid-induced genes [11].

13.4 Roots as the Primary Organ Sensing the Soil Environment

Plant growth and development are largely dependent on the plant root system, due to its crucial role in water and mineral uptake. A deep and well-developed root system that is both larger in length and in volume is an important character that confers better drought tolerance.

Improved uptake for water and nutrients requires root systems that either have more adequate root geometry to tap into the soil-based resources or have strong, active uptake mechanisms to acquire nutrients. Up to present, root traits have hardly been used by breeders due to the limited information on the suitability of root traits and their heritability. The application of root traits into performance in the field is dependent on the specific environmental conditions of each country. For example, a deep root system might be highly beneficial in seasonal rainfed agriculture with deep soils.

Root growth is tightly regulated and controlled by plant growth factors, such as root growth factor (RGF) [31], a small sulfated peptide with similarity to CLE, a

peptide transported via the xylem from root to shoot to regulate nodulation and suppression of arbuscular mycorrhizal colonization. CLE-like (CLEL) small sulfated peptides, unlike RGF, function in the regulation of the direction of root growth and promotion of lateral root development. Phytosulfokin (PSK) is a sulfated pentapeptide that enhances root elongation by controlling cell size. PKS is a ligand for the PSK-R, a member of the leucine-rich repeat receptor-like kinase (LLR-RLK) family of receptors, such as SYS, CLV3, and PSK [32,33]. These receptors possess cytosolic domains for the translation of the signal. A first domain has kinase activity, while a second domain is supposed to have guanylate cyclase activity [34]. CTG134 is a peach 18.5-kDa sulfated peptide [35], showing at its C-terminal domain a highly conserved motif present in RGF and other sulfated peptide hormones [36]. Tobacco transgenic 35S::CTG134 plants displayed enhanced growth of root hairs [35]. When the mature peptide was exogenously added to the growth medium, it induced the formation of supernumerary roots [37].

13.5
Symbiotic Microorganisms Affect Root Growth and Plant Performance

Numerous microorganisms contribute to the rhizosphere and often are beneficial to the crop, acting as crop-protecting agents against root pathogens. Plant–microorganism interactions produce benefits for both the partners. Legume–rhizobia symbiosis occurs between *Rhizobium* species and legumes [38]. Actinorhiza-based symbiosis occurs between actinobacteria of the genus *Frankia* and plants of *Fagales*, *Cucurbitales*, and *Rosales* [39]. Arbuscular mycorrhizal fungi are the most extensively studied fungal symbionts, which are associated with approximately 90% of all land plants and contribute multiple benefits to their host plants. Endophytic fungi also are fungal symbionts associated with plants. Endophytic fungi, such as *Piriformospora indica* [40–43], reside entirely within plant tissues and may be associated with roots, stems, and/or leaves, and also extend out into the rhizosphere, able to colonize a wide range of monocot and dicot plants [44]. Interactions among *Paenibacillus lentimorbus* NRRL B-30488, *P. indica* DSM 11827, and chickpea enhance root nodulation and plant growth, evidenced by higher N, P, and K uptake [42]. Fungal symbionts express a variety of symbiotic lifestyles including mutualism, commensalism, and parasitism. Mutualistic symbioses confer host fitness benefits that can result in drought tolerance, growth enhancement, and enhanced nutrient acquisition. Mutualistic benefits for endophytes may involve acquiring nutrients from hosts, abiotic and biotic stress avoidance, and dissemination by seed transmission. Endosymbiotic bacteria hosted by arbuscular mycorrhiza provide beneficial properties such as protection from pests and functions for the growth of plants and trees, such as plant growth promotion, plant elicitation, nutrient acquisition, competition for pathogens, priming, and preconditioning of induced systemic resistance [45]. The potential of the microorganisms hindered by the huge diversity of soil microbes can be translated in the development of mycorrhizal fungal establishment methods for soil improvement [44].

Bacteria promote plant growth through the activity of proteins involved in survival in the rhizosphere (to cope with oxidative stress or uptake of nutrients released by plant roots), in root adhesion (pili, adhesion, cellulose biosynthesis), in colonization/establishment inside the plant (chemiotaxis, siderophore production), and compounds affecting plant protection against fungal and bacterial infections (antimicrobial compounds 4-hydroxybenzoate and 2-phenylethanol) [46]. In addition to these species, *Pseudomonas fluorescens* WCS417r produces plant-stimulating compounds.

Among the compounds synthesized by bacteria that are beneficial to plants are the gasotransmitters NO, carbon monoxide (CO), and hydrogen sulfide (H_2S), implicated in the communication between bacteria and roots, and regulating root growth. Nitrate reductase-dependent production of NO either in plant roots and in soil bacteria has been implicated in the control of root growth [47,48]. NO is able to decrease primary root growth and promote auxin-induced adventitious lateral root development, supporting auxin activity [49,50], as shown in tomato.

Endophytic fungi inside plant roots and rhizosphere fungi near plant roots can benefit plants either by the production of phytohormones such as indole acetic acid (auxin), ABA (soil fungi) [51], acetoin, and 2,3-butanediol, or by modulating hormone activity [46]. In *P. indica*, a component in the exudates of the fungal hyphae was found to induce root growth. ethylene-responsive genes are repressed in *P. indica*-colonized barley roots [52]. Considering that some rhizobacteria produce enzymes that degrade ethylene, the *P. indica* compound seems to inhibit ethylene signaling, thus contributing to plant growth promotion. Additional phytohormones synthesized or manipulated by the root endophyte include cytokinins, gibberellins, and brassinosteroids [52]. Two oxylipin biosynthesis genes, OPR3 and LOX2, were found negatively regulated by brassinosteroids under specific conditions. In fact, brassinosteroids negatively regulate jasmonic acid-induced inhibition of root growth. Strigolactones are a group of carotenoid-derived signaling molecules that are exuded by the roots during phosphate starvation that promote arbuscular mycorrhizal hyphal branching and mycorrhiza establishment. Once the symbiosis is well established, strigolactone production decreases. Strigolactones play roles in signaling within the plant by acting on the regulation of shoot and root architecture. Strigolactones, together with auxins, favor lateral root development, enabling the root system to reach new areas in the soil with available phosphate.

13.6
Symbiotic Organisms Alleviate and Improve Abiotic Stress Tolerance of Host Plants

Plant growth-promoting (PGP) fungi and rhizobacteria are both able to elicit "induced systemic tolerance" to salt and drought in plant roots. PGP endophytes induce root biomass, counteract salt-induced increases in heat efflux, produce changes in fatty acid composition, increase antioxidant enzyme activities, and enable roots to maintain ascorbate in its reduced state under salt stress [53].

During water stress, the nodules sense drought and respond by activating stress protecting mechanisms. Once the soil-derived signals (mechanical and osmotic stress) and signals originated from the stressed ectomycorrhiza are sensed by the root, they are rapidly translated into specific signals [42]. The relevance of an early and immediate response in stress-tolerant varieties is essential, while in unresponsive varieties there is a delayed and reduced response [54]. Thus, immediate and early genes are expressed and produce signals that are transported to surrounding cells and, at distance, through the xylem.

13.7
Role of Jasmonates in Roots

High levels of Me-JA have been detected in germinating soybeans and in root tips [55,56]. Jasmonates directly induce *nod* gene expression in rhizobia and indirectly promote bacterial Nod factor production by inducing (iso)flavonoid biosynthesis genes [57]. As a feedback, Nod factor induces Ca^{2+} spiking in root hairs and inhibition of jasmonic acid synthesis [55]. Jasmonic acid, in the form of Me-JA, is involved in the growth inhibition of lateral roots. CLEL peptides have an effect opposite to jasmonic acid on lateral root inhibition. Regulation of the nutrient redistribution is one of roles of jasmonates in arbuscular mycorrhizal roots. In plants such as *Medicago truncatula* and barley, developing a mutualistic symbiosis that ultimately leads to a promoted growth, jasmonates might help to regulate the nutrient exchange between both partners. However, root-produced jasmonic acid and Me-JA perform important roles also in plants devoid of symbiotic relationships.

The involvement of oxylipins in root growth has been recently shown by Velosillo *et al*. [58]. The 9-hydroperoxy-derivative of linolenic acid (9-HPOT) produced by the activity of specific 9-LOXs expressed in lateral root primordials was shown to be involved in lateral root growth in *Arabidopsis*. 9-HPOT was shown to modulate root development through cell wall modification (stimulating callose and pectin deposition) and ROS accumulation. Other oxylipins affect root growth, such as oxoacids, produced by the HPL pathway, which were reported to arrest root growth and can determine the loss of apical dominance. In *M. truncatula*, a 9/13-HPL is expressed in *Sinorhizobium meliloti* (formerly *Rhizobium meliloti*) inoculated roots and nodules, indicating a role in interaction with microorganisms [16].

13.8
Jasmonic Acid Signal Transduction in Roots and Jasmonic Acid Involvement in Abiotic Stress Response

Abiotic below-ground stresses are early signals affecting root growth regulation, resource acquisition, and root–shoot communication [54,59]. Abiotic stresses elicit early signals that need to be transported at distance to affect protection

mechanisms, such as growth regulation, resource acquisition, synthesis of osmoprotectants, water potential, and stomatal closure, among others. There are several signaling compounds (RNAs, lipids, PGPs, and peptide factors) involved in root–shoot communication [54,59,60].

Early synthesis of jasmonic acid has a crucial role in local and systemic response to abiotic (salt, drought) stresses. Since specific events are triggered locally, molecular analyses in stress-perceiving roots have been the object of most studies. In roots, activation of the jasmonate biosynthetic pathway in drought stress has been elucidated through a series of studies relating identification of transcripts with alternative splicing and enzyme activity, through quantification of metabolites and hormones, in chickpea [61,62,65], *Medicago* [64,67], *Arabidopsis*, tomato [66], and other plant species. High expression of the main structural genes of the jasmonate pathway in root tissues of various plant species under different physiological conditions has been shown. In some cases, transcript upregulation was supported also by measuring higher levels of jasmonic acid, JA-Ile, and OPDA [66].

In tomato, Abdala *et al.* [66] examined the saline stress response of hairy roots from tomato cultivars with different sensitivity to NaCl. The results suggested that changes in endogenous jasmonic acids were different in genotypes of contrasting salt tolerance. A jasmonic acid increase was observed in salt-sensitive varieties with the time of salinization, whereas the salt-tolerant cultivar showed a higher endogenous content of jasmonic acid and related compounds, which diminished to the basal level of the control at 72 h of salt treatment.

13.9 Jasmonate in Root Response to Abiotic Stresses: Model Legumes and Chickpea Tolerant Varieties Showing Differential Transcript Expression During Salt and Drought Stress

In chickpea, around 7580 chickpea expressed sequence tags (ESTs) are public and available at the National Centre of Biotechnology Information (NCBI). SuperSAGE studies carried out by Molina *et al.* analyzed drought response in the drought-tolerant ILC588 chickpea variety [61]. Seedlings grown for 28 days were removed, carefully preventing mechanical damage, and subjected to dehydration for 6 h at room temperature. After the desiccation period, the plants showed wilting symptoms (turgor loss), and the roots were separated from the shoots and shock-frozen in liquid nitrogen. Twenty different LOX UniTags in chickpea roots under drought stress were identified, corresponding to 11 SNP-associated alternative tags (SAATs). Two LOX sequences were highly regulated in drought stress (ILC588) as well as in salt stress (INRAT-93), of which STCa-24417 was 25-fold upregulated [62]. AOC also was represented by five UniTags, varying in expression from down-regulation to 20-fold upregulation. This finding supports the need to also measure enzyme activity, with a requirement of AOC oligomerization in the synthesis of jasmonic acid [63]. TaqMan probes for specific isoforms of several genes in the

jasmonic acid synthesis pathway were designed, based on differently spliced isoforms and SAAT sequences, selective enough to discriminate different LOX, AOC, and HLP isoforms and spliced variants. The probes were used to confirm the SuperSAGE data on transcript induction in the roots of the ILC588 chickpea drought-tolerant variety, as well of salt-induced transcripts in roots and nodules [62]. This study monitored the response to salt stress (at 2, 8, 24, and 72 h) in roots and nodules, using the salt-tolerant chickpea INRAT-93 variety, the salt-sensitive Amdoun control, the ICC4958 salt-sensitive variety, and the ICC6098 weakly tolerant variety. Seedlings with a minimum root length of 5 cm were inoculated with *Mesorhizobium ciceri* strains by dipping each seedling into growing media for 10 s and packages of 15 individuals were transferred to 12 40-l hydroaeroponics buckets. Three-week-old chickpea plants were transferred to new buckets with freshly prepared medium containing 25 mM NaCl while control plants were placed into buckets with new nutrition medium. The RNA extracted from roots and nodules was retrotranscribed for transcript profiling using deepSuperSAGE and quantitative reverse transcription-polymerase chain reaction (qRT-PCR) [62]. Comparative qRT-PCR assays from chickpea confirmed the deepSuperSAGE data on the identified and sequence-specific UniTags.

Upregulated transcripts in salt-stressed chickpea nodules and common nodule–root responses in legumes led to the identification of several upregulated genes, such as LOX, MAPK, cytochrome *c* oxidases, agglutinins, alternative oxidases (AOX), as well as genes coding for phosphatidylinositol transfer proteins. The results showed a strong activation of ROS-scavenging mechanisms, a well-known event in stressed plant tissues, and oxylipin synthesis as prime responses in the stressed roots.

In the model legume *M. truncatula*, transcriptome analysis based on 16K+ microarrays (Mt16KOLI1) using salt-treated root apexes was performed to compare the data in this model legume to those found in chickpea [64]. The hormonal response to salt stress of *M. truncatula* roots was monitored in different tissues (roots, stem, and leaves) at different timepoints from stress onset. Four key genes involved in oxylipin metabolism (i.e., LOX, HPL, AOS, and AOC) were upregulated in the salt-tolerant genotype Jemalong A17, under salt stress conditions. Comparison of transcription profiles from desiccated young roots using the *Medicago* 16K microarray [67] with SuperSAGE data from drought-stressed chickpea roots showed differences in drought response in tolerant varieties in the two species [61].

Among the chickpea-specific TaqMan probes, we conducted additional studies using LOX1, LOX2, AOS, AOC, HLP1, HPL2, and OPR primers. The chickpea drought-tolerant ICC4958 and the drought-sensitive ICC1882 variety were cultivated in pots, then subjected to water stress, maintaining them under the same conditions for 72 h [65]. The drought resistance of ICC4958 is known to be associated with its root system, that is both larger in length and in volume than that of non-tolerant varieties such as Annigeri or ICC1882, while the accumulation of seed mass, after flowering starts, is faster in ICC4958. This trait permits ICC4958 to accumulate a large seed mass before the soil moisture recedes and drought becomes increasingly severe.

The expression of key genes and specific isoforms involved in oxylipin metabolism by qRT-PCR on individual roots (in triplicate) showed an earlier timing and higher expression intensity in the drought-tolerant ICC4958 variety [65]. *AOS* and *HPL* were found rapidly (already 2 h after the onset of stress) and highly (up to 19-fold) induced by drought stress in the tolerant ICC4958 chickpea variety. The role of the jasmonate pathway in the early signaling of drought stress and jasmonic acid involvement in drought tolerance in chickpea roots was thus confirmed in different varieties. The results showed a sustained and earlier activation of a root-specific LOX (*lox1)* isoform, two HPLs (*hpl1* and *hpl2*), an AOS (*aos*), and an OPR (*opr*) gene in the drought-tolerant varieties. In roots of ICC4958, *LOX2*, *OPR*, and *AOS* were found several fold overexpressed already after 2 h of water stress and remained overexpressed during the time course of the experiment. HPL1 was expressed during the initial phase, while HPL2 expression increased at the 72-h stress point.

To confirm the significance of expression induction of different LOX isoforms, high-performance liquid chromatography (HPLC) quantification of the main oxylipins in root tissues was performed. Higher levels of oxylipins produced by the AOS branch (i.e., jasmonic acid, its precursor OPDA, and the active hormone, JA-Ile) were detected in root tissues of the tolerant variety. Increased levels of OPDA, jasmonic acid, and JA-Ile were found already at 2 h after stress onset [65]. The rapid rise of OPDA and JA-Ile levels concomitant to the induction of *AOS* and *OPR* gene expression in drought-stressed roots in ICC4958 suggests that JA-Ile and OPDA may act coordinately for the full activation of root responses to stress in the drought-tolerant variety.

Semeraro [68] performed oxylipin extraction in drought-stressed chickpea roots using the ICC4958 variety followed by HPLC analysis, with enantiomer separation using a chiral column for the quantification of two different stereospecific hydroperoxy fatty acids. The auto-oxidation of PUFA can give rise non-enzymatically to the *R*-enantiomer of hydroperoxy fatty acids. Starting from the second hour of water stress in ICC4958, the 13(*S*)-hydroperoxy fatty acid, specific substrate for 13-AOS, started to increase in level in the presence of the *R*-enantiomer (Figure 13.2). On the other side, the *S*-enantiomer of 9-HPOD, specific substrate for 9-HPL, accumulated at high levels (Figure 13.3). These findings taken together support the involvement of jasmonic acid and other oxylipins already at 2 h of stress response. The total hydroperoxy fatty acids produced in ICC4958 during the time course of water stress has been quantified, with a profile that is different from that observed in the drought sensitive variety [65].

13.10 Role of Transcription Factors and MicroRNAs in the Regulation of Jasmonic Acid Signaling

Long-distance signaling is a fundamental mechanism in plants for the regulation of several processes including leaf development, flowering, and pathogen defense.

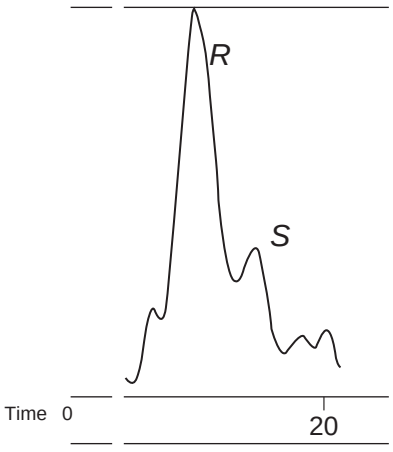

Figure 13.2 HPLC separation of the *R*- and *S*-enantiomers of 13-HPOD hydroperoxy fatty acids extracted from chickpea roots after 2 h of water stress.

Small RNAs have been detected in the phloem sap of plant species, including microRNA (miRNA). Recently, several works pointed to the importance of small RNAs in the maintenance of memory in jasmonic acid-mediated response [69].

A large number of miRNAs target transcription factors with a role in development, and in environmental and hormone responses. Thus, miRNAs are important in plant stress response to abiotic stresses and nutrient deprivation. The *miR319* signaling molecule moving through the phloem to the roots targets transcription factors of the MYB and TCP families. TCP4 regulates several genes of the LOX pathway in *Arabidopsis*, based on a conserved nucleotide sequences in their promoters [70]. It is proposed that an early activation by TCP4 of jasmonic

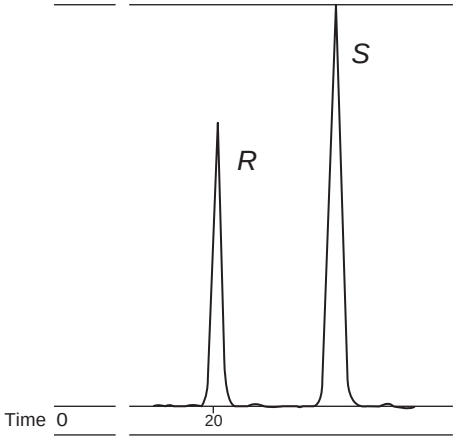

Figure 13.3 HPLC separation of the *R*- and *S*-enantiomers of 9-HPOD hydroperoxy fatty acids extracted from chickpea roots after 2 h of water stress.

acid biosynthesis pathway genes may be contained in the case of high levels of circulating *miR319*, through its binding and inhibition of TCP4. This coordinated activity may orchestrate timely and localized differential gene expression of LOX, OPR, and AOS in roots responding to different stresses.

13.11 Conclusion

Several different sets of findings point to the importance of an early activation of jasmonic acid synthesis in roots responding to abiotic stress, and in particular in legume varieties more tolerant to drought and salt stresses. The molecular analysis of stress-induced signaling pathways that lead to plant adaptation constitutes a major research area in biotic and abiotic stress fields. These studies may lead to new and specific assays and phenotyping techniques to evaluate a species rootstock in order to choose hybrids better suited to respond to abiotic stress with an optimization of available phenotyping techniques.

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