



From environmental responses to adaptation: the roles of plant lncRNAs

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Abstract

As sessile organisms, plants are continuously exposed to heterogeneous and changing environments and constantly need to adapt their growth strategies. They have evolved complex mechanisms to recognize various stress factors, activate appropriate signaling pathways, and respond accordingly by reprogramming the expression of multiple genes at the transcriptional, post-transcriptional, and even epigenome levels to tolerate stressful conditions such as drought, high temperature, nutrient deficiency, and pathogenic interactions. Apart from protein-coding genes, long non-coding RNAs (lncRNAs) have emerged as key players in plant adaptation to environmental stresses. They are transcripts larger than 200 nucleotides without protein-coding potential. Still, they appear to regulate a wide range of processes, including epigenetic modifications and chromatin reorganization, as well as transcriptional and post-transcriptional modulation of gene expression, allowing plant adaptation to various environmental stresses. lncRNAs can positively or negatively modulate stress responses, affecting processes such as hormone signaling, temperature tolerance, and nutrient deficiency adaptation. Moreover, they also seem to play a role in stress memory, wherein prior exposure to mild stress enhances plant ability to adapt to subsequent stressful conditions. In this review, we summarize the contribution of lncRNAs in plant adaptation to biotic and abiotic stresses, as well as stress memory. The complex evolutionary conservation of lncRNAs is also discussed and provides insights into future research directions in this field.

Introduction

As sessile organisms, plants must constantly adapt to their heterogeneous and fluctuating environment to ensure their growth, survival, and reproduction. As a result, plants are highly plastic, enabling them, within the same genotype, to grow in various environments and face diverse stresses. Plants have evolved several mechanisms that allow them to recognize stress, integrate information, and modulate the expression of multiple genes to respond to new environmental conditions. These gene expression regulations occur at all possible levels of gene expression: epigenetic, transcriptional, and post-transcriptional. Among the various molecular mechanisms underlying this adaptive plasticity, one of the most intriguing areas of study focuses on the role of non-coding RNA molecules in mediating environmental responses. It is now accepted that a major part of eukaryotic

genomes is transcribed into RNA even outside protein-coding genes. First thought to be simple transcriptional noise, growing evidence has since shown that some of these new RNAs have functional roles, suggesting that the genome is a source not only of protein-coding genes, but also of a myriad of non-coding RNA molecules (discussed in Palazzo and Gregory 2014). However, the amount of non-coding RNA molecules that are actually functional is still highly debated in the community (discussed in Ponting and Haerty 2022). Non-coding RNAs (ncRNAs) comprise a heterogeneous group of transcripts, including housekeeping ncRNAs (such as small nuclear and nucleolar RNAs, transfer RNAs, ribosomal RNAs, and telomerase RNAs) and regulatory ncRNAs. This latest subgroup can be further divided into two main categories, with discernible distinctions: small ncRNAs, spanning 21 to 24 nucleotides, and their larger counterparts, extending from 200 nucleotides to several

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- Plants have remarkable developmental plasticity that allows them to adapt to changing conditions, and lncRNAs have appeared as key mediators of plant adaptation to their environment.
- The regulatory contributions of lncRNAs to plant adaptation include responses to abiotic and biotic stresses, unraveling a web of regulatory networks that are critical for enhancing plant resilience and survival.
- lncRNAs are subject to evolutionary selection to a higher extent than nonfunctional elements, exhibiting specificity in expression patterns among different plant species and natural accessions, pointing to their potential involvement in local environmental adaptations.

kilobases, referred to as long ncRNAs (lncRNAs) (reviewed in Wierzbicki et al. 2021). According to their genomic location relative to nearby protein-coding genes, lncRNAs can be broadly classified as long intergenic ncRNAs (lincRNAs) that are transcribed from intergenic regions and have normally a *trans*-regulatory function; natural antisense transcripts (NATs) that are transcribed from complementary DNA strands of sense coding regions and display *cis*- or *trans*-regulatory action; intronic ncRNAs (incRNAs) that originate from intronic regions; sense lncRNA, transcribed from the sense strand of a protein-coding gene, overlapping with a part of it or spanning the entire sequence through an intron; or circular lncRNAs (circRNAs) that are generated by nonsequential back-splicing of exons and/or introns (reviewed in Ma et al. 2013). Each lncRNA generally originates from polymerase II transcription but has distinct regulatory features; they usually exhibit limited protein-coding potential and modest expression levels (reviewed in Yang et al. 2023). The roles attributed to lncRNAs extend far beyond the conventional gene-to-protein paradigm, highlighting their involvement in fine-tuning gene expression independently of their protein-coding potential. Notably, lncRNAs have been implicated in various processes, including epigenetic modifications, chromatin organization, transcriptional regulation, and post-transcriptional processing (reviewed in Yang et al. 2023), and have emerged as pivotal regulators of gene expression that can modulate plant plasticity in response to environmental fluctuations. Thanks to the advent of RNA-seq technology popularized in the 2010s, numerous genome-wide analyses have been conducted in different plant species, at various developmental stages, or in response to different stresses, thus identifying lncRNAs (reviewed in Palos et al. 2023). These analyses have revealed that lncRNAs exhibit a selective pattern of expression, often in specific cell types and in response to environmental

stimuli. For example, one of the first studies combined tiling array and RNA-seq to annotate around 6,500 lincRNAs (Liu et al. 2012). More recently, a meta-analysis using more than 16,000 publicly available RNA-seq experiments from various tissues, mutants, and conditions annotated around 130,000 lincRNAs in 4 Brassicaceae species (Palos et al. 2022). Although genome-wide analyses have helped uncover the abundance and specificity of lncRNAs in different plant species, a significant portion of these molecules remains functionally uncharacterized, leaving a wealth of potential roles waiting to be uncovered. In light of these recent findings, the role of lncRNAs in shaping the adaptation of plants to their environment becomes even more relevant. In this review, we aim to provide an overview of the current state of knowledge regarding the functional implications of lncRNAs in environmental adaptation. More precisely, we summarize recent progress related to the function of lncRNAs in response to abiotic and biotic stresses, and how lncRNAs may play a role in the evolutionary adaptation of species or particular ecotypes to their local environment.

Abiotic stress responses

Abiotic stresses encompass diverse factors such as high soil salinity, extreme temperatures, inadequate amount of water, presence of toxic heavy metals, or nutrient deficiencies, and they have notable impacts on both plant metabolism and gene expression. Different abiotic stresses have been shown to upregulate or downregulate the expression of specific lncRNAs, suggesting that some of them may have evolved as key players to respond to particular stress factors and contribute to plant adaptation strategies (Fig. 1).

lncRNAs in drought stress tolerance

The *DROUGHT-INDUCED LNCRNA (DRIR)* is an example of positive modulation of resistance to both water deficiency and salt stress. *DRIR* is a nucleus-located lincRNA expressed at a minimal level under normal conditions, but it is markedly enhanced under osmotic stress and by the stress hormone abscisic acid (ABA). At the same time, the enhanced level of *DRIR* increases plant sensitivity to ABA by mediating the expression of genes involved in ABA signaling and ABA-regulated stress tolerance, suggesting a potential positive feed-forward mechanism (Qin et al. 2017). Also in *Arabidopsis thaliana*, the lncRNA *asDOG1 (1GOD)* is transcribed from the antisense strand of the regulator of salt stress responses and seed germination *DELAY OF GERMINATION 1 (DOG1)*, suppressing *DOG1* expression under standard growth conditions. Drought stress and ABA signaling repress *1GOD* transcription, resulting in a constitutive high-level *DOG1* expression increasing drought tolerance (Yatusevich et al. 2017). In response to salt stress, several lncRNAs (named *PUPPIES*) are also co-directionally transcribed from the promoter of the *DOG1* gene and

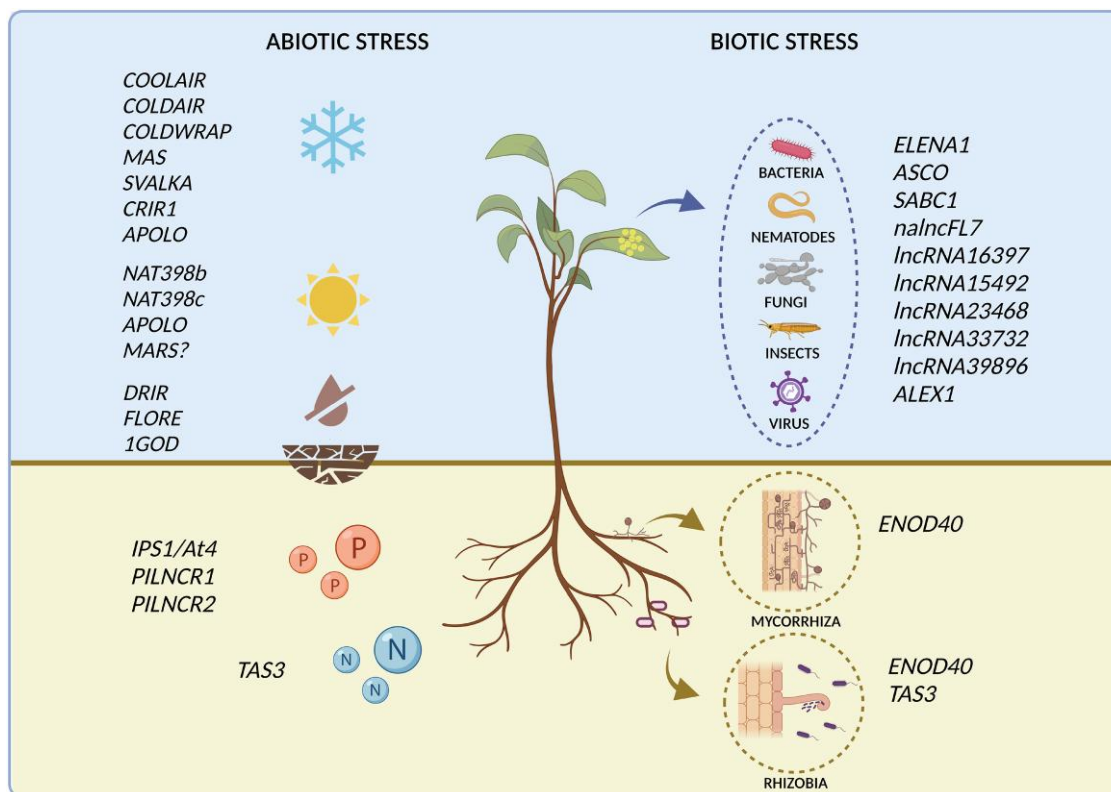


Figure 1. lncRNAs regulate adaptation to abiotic and biotic stresses in plants. Response to abiotic stress (left): *COOLAIR*, *COLDAIR*, *COLDWRAP*, *MAS*, *SVALKA*, and *CRIR1* respond to cold. *APOLO* responds to both cold and heat stress. *NAT398b* and *NAT398c* regulate heat stress adaptation. *MARS* is induced by ABA and may have a role in adaptation to heat stress. *DRIR* enhances resistance to drought and salt stress. *FLORE* regulates water loss and increases drought tolerance. *1GOD* responds to drought stress and ABA signaling. *IPS1*, *PILNCR1*, and *PILNCR2* are induced in response to phosphate (P) starvation. *TAS3* is a nitrogen (N)-responsive lncRNA involved in N transport. Response to biotic stress (right): *ELENA1*, *ASCO*, *nalncFL7*, *lncRNA16397*, *lncRNA15492*, *lncRNA23468*, *lncRNA33732*, *lncRNA39896*, and *ALEX1* are lncRNAs involved in plant responses to pathogen attacks. *TAS3* and *ENOD40* are involved in beneficial plant–microbial interactions. Created with Biorender.com.

extend into the *DOG1* coding region. *PUPPIES* boost *DOG1* expression to delay germination by altering Pol II dynamics on the gene (Montez et al. 2023). Another lncRNA involved in drought tolerance is the *CDF5* LONG NONCODING RNA (*FLORE*), a circadian-regulated lncRNA identified in *Arabidopsis* as a NAT of the *CYCLING DOF FACTOR 5* (*CDF5*) gene and promoting flowering. *FLORE* and *CDF5* show an antiphasic pattern of expression with mutual inhibitory regulation, directly impacting flowering time. Furthermore, *FLORE* represses not only *CDF5* expression in *cis*, but also the expression of other *CDFs* such as *CDF1* and *CDF3* in *trans*, fine-tuning flowering time to fit with environmental conditions (Henriques et al. 2017). In *Solanum tuberosum* (potato), *StCDF1*, together with its NAT lncRNA *StFLORE*, regulates water loss by regulating stomatal growth and diurnal opening in an ABA-dependent manner. Enhanced expression of *StFLORE* increases drought tolerance, and CRISPR-Cas9 mutations produce plants with high sensitivity to water-limiting conditions, suggesting that the *StCDF1/StFLORE* locus regulates water homeostasis in potato (Ramírez Gonzales et al. 2021).

lncRNAs in thermotolerance

Several lncRNAs have also been associated with plant responses to heat stress. One example is the *MARNER* SILENCING (*MARS*) lncRNA, which is embedded within the cluster of marneral biosynthetic genes in *Arabidopsis*. Basal expression of *MARS* recruits the PRC1-component LIKE HETEROCHROMATIN PROTEIN 1 (LHP1) to the gene cluster, repressing its expression through condensation of the chromatin. Upon ABA treatment, *MARS* expression is strongly activated, decoying LHP1 and allowing the transcription of the genes in the cluster. No phenotype associated with the response to environment has yet been linked to *MARS*; however, its strong induction by ABA and heat points to a potential role during heat stress response (Roulé et al. 2022). In *A. thaliana* and Brassica, two NATs of *MIR398* genes, *NAT398b* and *NAT398c*, were reported to repress the processing of the associated pri-miRNAs. Heat stress was shown to induce *miR398* processing and to reduce the number of transcripts of its target genes, the *COPPER SUPEROXIDE DISMUTASE 1* and *2* (*CSD1* and *CSD2*, respectively), and the *COPPER CHAPERONE* of *CSD1* and *CSD2* (*CCS*). Knockdown of *NAT398b* and *NAT398c* promotes *miR398* processing and

enhances plant tolerance to high temperatures by increasing the silencing of *CSD1*, *CSD2*, and *CCS*, whereas overexpression of *NAT398b* or *NAT398c* results in the opposite response. Moreover, overexpression of *MIR398b* and *MIR398c* activates *NAT398b* and *NAT398c*, suggesting enhanced thermotolerance is regulated by a feedback loop (Li et al. 2020).

Conversely, several lncRNAs have been associated with plant response to cold temperatures. Most of them are related to the *FLOWERING LOCUS C* (*FLC*) gene, a clade of MADS-box transcription factors responsible for vernalization response. The antisense lncRNA *COOLAIR* is induced after several days of cold exposure and mediates chromatin repression of the *FLC* gene during the process of vernalization in *Arabidopsis*. *COOLAIR* is recruited by the POLYCOMB REPRESSIVE COMPLEX 2 (PRC2), a regulatory complex involved in repressing the transcription of diverse developmental genes that guides the *FLC* silencing (Swiezewski et al. 2009). *COLD AIR* is an intronic lncRNA transcribed from *FLC* required for the enrichment of PRC2 at the *FLC* locus, establishing a stable vernalization-mediated epigenetic repression of *FLC* through its interaction with PRC2 (Heo and Sung 2011). *COLDWRAP*, an additional polycomb-binding lncRNA, derived from the repressed promoter of *FLC*, reinforces the *FLC* silencing (Kim et al. 2017). *MAF4 ANTISENSE RNA* (*MAS*) is also a cold-induced lncRNA produced from the *MADS AFFECTING FLOWERING4* (*MAF4*) locus, a member of the *FLC* family that prevents early vernalization response. *MAS* induces *MAF4* transcription by binding to WDR5a, an element of the COMPASS-like complex involved in histone methylation, and recruits it to *MAF4*. This interaction enhances trimethylation of lysine 4 on histone 3 (H3K4me3) of *MAF4* and, consequently, *MAF4* transcription and thereby regulating flowering time (Zhao et al. 2018). Another cold-responsive lncRNA is *SVALK*, identified by transcription start site (TSS)-seq as transcribed by Pol II from the antisense strand downstream of the *C-REPEAT BINDING FACTOR 1* (*CBF1*), a key activator of the cold acclimation response involved in cold tolerance. *SVALK* was shown to negatively control the expression of *CBF1* in *cis*. Indeed, upon cold treatment, a read-through transcript is generated from the *SVALK* locus, continuing after the polyA site of *SVALK* and therefore becoming antisense to *CBF1*. This nascent readthrough transcript was named *asCBF1*. The transcription at the same time of *CBF1* and of *asCBF1* results in collisions of Pol II, preventing the transcription of full-length *CBF1* and therefore downregulating the expression level of *CBF1*. This *SVALK*-*asCBF1* cascade provides a mechanism to regulate *CBF1* expression and cold acclimation response (Kindgren et al. 2018; Zacharaki et al. 2023). Global identification of lncRNAs in cassava also unveils the role of *COLD-RESPONSIVE INTERGENIC LNCRNA 1* (*CRIR1*) in cold stress response. *CRIR1* was shown to interact with COLD SHOCK PROTEIN 5 (*MSCSP5*), which acts as an RNA chaperone to increase its translational yield and cope with cold stress (Li et al. 2022).

In *Arabidopsis*, the *AUXIN-REGULATED PROMOTER LOOP* (*APOLO*) lncRNA has been reported to show enhanced expression either by low or high temperature. *APOLO* is able to recruit LHP1 and DNA methylation regulator VARIANT IN METHYLATION 1 (*VIM1*) at the promoter of *YUCCA2* through the formation of an R-loop, resulting in the repression of *YUCCA2* expression. Upon heat, the expression level of *APOLO* increases and decoys *VIM1* and LHP1 from *YUCCA2* promoter, activating *YUCCA2* expression and auxin synthesis and allowing hypocotyl elongation during the thermomorphogenesis response (Fonouni-Farde et al. 2022). Likewise, the basal level of *APOLO* controls a low level of transcription of *ROOT HAIR DEFECTIVE 6* (*RHD6*) by maintaining a repressive chromatin loop in its promoter through the formation of an R-loop. In response to cold, the transcription level of *APOLO* is increased, decoying LHP1 from the *RHD6* promoter and allowing the deployment of the chromatin loop and activation of *RHD6* transcription. Furthermore, *APOLO* is able to recruit the WRKY42 transcription factor, activating even further the transcription of *RHD6*. This activation of *RHD6* by *APOLO* increases the expansion of root-hair cells (Moison et al. 2021).

lncRNAs in nutrient deficiency

Nutrient deficiency also deeply affects plant growth and development. Several years ago, a lncRNA named *INDUCED BY PHOSPHATE STARVATION1* (*IPS1*) in rice and its At4 homolog in *Arabidopsis* were identified. *IPS1* is activated in response to phosphate (Pi) starvation, acting as a target mimic, capturing miRNA399, therefore impeding the cleavage of its target, the E2 ubiquitin conjugase *PHO2*, and consequently affecting the regulation of Pi-related processes (Franco-Zorrilla et al. 2007). More recently, two more lncRNAs related to Pi deficiency, *Pi-DEFICIENCY-INDUCED LNCRNA 1* (*PILNCR1*) and *Pi-DEFICIENCY-INDUCED LNCRNA 2* (*PILNCR2*), were identified in maize. *PILNCR1* can inhibit the cleavage of *ZmPHO2* by miRNA399 depending on the complementarity regions that exist between *PILNCR1* and miRNA399, in the same way as for the interaction observed between *IPS1/At4* and miRNA399 (Du et al. 2018). While the action on miRNA399 is conserved between *PILNCR1* and *IPS1/At4*, no sequence homology nor synteny exist between these two lncRNAs. Furthermore, based on sequence homology, *IPS1/At4* candidates can be found in the maize genome but are distinct from *PILNCR1* (Du et al. 2018). Furthermore, *PILNCR2* was found to be transcribed from the opposite strand of the *TRANSPORTER GENE 1* (*PHT1*), a direct target of miRNA399 in maize. By opposition to *IPS1/At4* and *PILNCR1* that prevent the action of miRNA399 by capturing it, *PILNCR2* is able to form duplexes with the *PHT1* gene family members, protecting them and inhibiting their cleavage by miRNA399 and regulating molecular and physiological responses to low Pi (Wang et al. 2023).

Thus, many lncRNAs have been identified as expressed in response to various abiotic stresses, and the characterization of some of them clearly showed their regulatory role in

coping with stress, indicating that in such responses, they may act as key players.

Biotic stress responses

Plants are also continuously exposed to a wide range of biotic interactions. Although some biotic interactions are beneficial for the plant (e.g. rhizobia and mycorrhiza), others such as those due to pathogens are detrimental and require fast adaptation.

lncRNAs in pathogenic plant-microbe interactions

Many pathogen-responsive lncRNAs have been identified in plants such as wheat, rice, Kentucky bluegrass, *Arabidopsis*, grapevine, *Hevea brasiliensis*, kiwifruit, and tomato (Xin et al. 2011; Zhang et al. 2013; Zhu et al. 2014; Wang et al. 2015, 2017b; Xing et al. 2019; Yin et al. 2019; Yu et al. 2020; Zhao et al. 2023), but only a few have been functionally characterized (Fig. 1).

In *Arabidopsis*, the lncRNA *ELF18-INDUCED LONG NONCODING RNA1* (*ELENA1*) has been identified as a pathogen-associated molecular pattern (PAMP)-responsive lncRNA, positively regulating the expression of specific immune-responsive genes. *ELENA1* was shown to directly interact with the MEDIATOR SUBUNIT 19a (*MED19a*), modulating the enrichment of *MED19a* on the promoter of *PATHOGENESIS-RELATED GENE1* (*PR1*), a defense-related protein that protects plants against microorganism attack (Seo et al. 2017). *ELENA1* was also shown to interact with another mediator subunit *MED36a/FIBRILLIN2* (*FIB2*), dissociating the *FIB2/MED19a* complex and releasing the repressor *FIB2* from the *PR1* promoter to enhance *PR1* expression (Seo et al. 2019). The *Arabidopsis* SA-inducible lncRNA *AtR8* was also suggested to participate in salicylic acid (SA) response-related defense upon *P. syringae* infection, regulating the expression of the *WRKY53* and *WRKY70* transcription factors (Yu et al. 2020), even if its exact role remains unknown. Another lncRNA shown to modulate plant molecular defense responses is the lncRNA *ALTERNATIVE SPLICING COMPETITOR* (*ASCO*), achieving its role through the alternative splicing regulation of pathogen-related mRNAs. Indeed, *ASCO* physically interacts with the NUCLEAR SPECKLE RNA-BINDING PROTEINS (*NSRs*), which are known to regulate alternative splicing, but also with *PRP8a* and *SmD1b*, two essential components of the spliceosome core, finely regulating the transcriptional response to flagellin22 and impairing root growth sensitivity to this key signaling molecule (Rigo et al. 2020).

Several natural antisense transcripts (NATs) were also characterized as negative regulators of plant immunity. For example, the lncRNA *SALICYLIC ACID BIOGENESIS CONTROLLER 1* (*SABC1*) is a NAT of the transcription factor *NAC3* and functions in *cis* to reduce *NAC3* expression. Thus, upon pathogen infection, *SABC1* expression is reduced, allowing *NAC3* and then *ISOCHORISMATE SYNTHASE2* (*ICS1*) expression, and at last SA biosynthesis activation (Liu et al.

2022). Likewise, the lncRNA *nalncFL7* is a NAT of *FORKED-LIKE7* (*FL7*) and *cis*-downregulates *FL7* expression through its binding to the RNA-binding protein *BPA1-LIKE PROTEIN3* (*BPL3*). As a consequence, since *FL7* can no longer associate with and suppress the activity of the *PP2CA* protein *HAI1*, the phosphorylation levels of *MITOGEN-ACTIVATED PROTEIN KINASE 3* (*MPK3*) and *MPK6* increase, enhancing immunity responses. Thereby *FL7* positively regulates plant immunity to *Phytophthora capsici*, while *nalncFL7* negatively regulates resistance (Ai et al. 2023). Several pathogen-responsive lncRNAs were also characterized in tomato. The lncRNA16397 responsive to *Phytophthora infestans* infection is a NAT of *GLUTAREDOXIN 22* and functions in *cis* to induce *GLUTAREDOXIN 22* expression, resulting in enhanced pathogen resistance (Cui et al. 2017). In the same way, three other positive regulators, the tomato lncRNA15492, the lncRNA23468, and the lncRNA33732, confer resistance to *P. infestans*. lncRNA15492 is a NAT-lncRNA transcribed with-in the antisense sequence of the pri-miR482a, and acts inhibiting the expression of the mature miRNA, a negative regulator of *P. infestans* resistance, by targeting the *NBS-LRR* genes (Jiang et al. 2020). lncRNA23468, in its case, contains target mimic sites for miR482b, thus capturing and suppressing the expression of miR482b and concomitantly enhancing the expression of *NBS-LRR* genes (Jiang et al. 2019). Meanwhile, lncRNA33732 increases the accumulation of H_2O_2 during early defense against *P. infestans* by inducing the expression of the *RESPIRATORY BURST OXIDASE* (*RBOH*) gene, a homolog of NADPH oxidases. lncRNA33732 is located downstream of the *RBOH* gene and is activated by the transcription factor *WRKY1* by recognizing the W-box in its promoter region. Overexpression of lncRNA33732 increases the expression of *RBOH*, whereas lncRNA33732 silencing decreases the expression level of this enzyme (Cui et al. 2019). On the other hand, lncRNA39896 negatively regulates resistance to *P. infestans* in tomato. lncRNA39896 is induced by *P. infestans* infection and acts as an endogenous target mimic of miR166b, increasing transcript levels of the class III homeodomain-leucine zipper *SIHDZ34* and *SIHDZ45* genes and modulating resistance via the ethylene/jasmonic acid pathway (Hong et al. 2022). Even if many lncRNAs were characterized in several plant species in response to biotic stresses, only a few were in monocots. In rice, the lncRNA *AN LEAF EXPRESSED AND Xanthomonas oryzae-INDUCED* (*ALEX1*) has been identified as a pathogen-responsive and jasmonic acid (JA)-associated lncRNA, positively regulating broad-spectrum resistance to rice bacterial pathogens by activating the JA pathway (Yu et al. 2020). Thus, increasing evidence suggests a key role for lncRNAs in plant defense responses and putative connection with the JA pathway in the regulation of plant innate immunity.

lncRNAs in beneficial plant-microbe interactions

lncRNAs were also identified in beneficial plant-microbe interactions and appear to play diverse roles, contributing to communication, coordination, and establishment of

symbiotic relationships between plants and beneficial microbes (Fig. 1). Biological nitrogen (N) fixation is a well-studied biochemical process that involves a symbiotic interaction between legume plants and soil bacteria known as rhizobia. This interaction allows the plant to acquire nitrogen to support its growth and development, in an intricate process regulated by complex signaling mechanisms. In the past few years, using RNA-seq, a multitude of lncRNAs with potential roles in the symbiotic nitrogen fixation process have been discovered in *Medicago truncatula* (Pecrix et al. 2018). Several lncRNAs differentially regulated in early times after rhizobial symbiosis were also shown to display cell-specific regulation (Traubenik et al. 2020). Moreover, 126 lncRNAs were putatively linked with later stages of nodule development, involving lncRNAs with the process of nodule senescence (Yu et al. 2022). *ENOD40* is an old and well-known lncRNA, initially identified in *M. truncatula*, and considered to be a key player in the signaling pathway that leads to nodule formation (Charon et al. 1999). *ENOD40* is classified as a bifunctional lncRNA (Bardou et al. 2011) that plays a role in transporting the RNA-BINDING PROTEIN MtRBP1 between the nucleus and the cytoplasm, while also producing small peptides that contribute to the development of root symbiotic nodule organogenesis. Recently, a new regulatory pathway involving the miR169c-NFYA-C-*ENOD40* module was discovered in soybean. In this module, miR169c integrates the N availability signal to strictly control nodulation via regulating the expression of NUCLEAR FACTOR-Y SUBUNIT A-C (NFYA-C) and its downstream gene *ENOD40*; to date, the molecular role of *ENOD40* remains unknown (Xu et al. 2021). *ENOD40* was also shown to regulate mycorrhizal colonization. Arbuscular mycorrhizal (AM) fungi are soil microorganisms that can form a mutually beneficial symbiosis with the vast majority of terrestrial plants (Wang et al. 2017a). Overexpression of *ENOD40* in *M. truncatula* plants increased the frequency of AM formation, whereas plants with suppressed levels of *ENOD40* transcripts exhibited reduced mycorrhizal colonization (Stahelin et al. 2001). More recently, sequencing approaches revealed 63 and 587 lncRNAs differentially expressed between AM inoculated plants and non-inoculated ones in maize (Han et al. 2019) and tomato (Zeng et al. 2023), respectively, also suggesting the involvement of lncRNAs in AM colonization.

Finally, in a few cases, lncRNAs were found to participate in both biotic and abiotic plant stress responses. That is the case of *TRANS-ACTING siRNA 3* (*TAS3*) first identified in *M. truncatula*. *TAS3* is cleaved by miR390 generating trans-acting small interfering RNAs (ta-siRNAs) mediating the inhibition of the auxin-related transcription factors (ARFs), and positively regulating lateral root growth (Marin et al. 2010). The miR390/*TAS3* pathway has been shown to have detrimental effects on nodulation and the growth of nodules (Hobecker et al. 2017). Two *TAS3* transcript variants produced by alternative polyadenylation were identified, one of them putatively acting as a target mimic capturing miRNA390, impeding its

action on the cleavage of the full *TAS3* variant (Traubenik et al. 2020). *TAS3* was also identified as an N-responsive lncRNA in *A. thaliana*, and the *NITRATE TRANSPORTER2* gene was identified as a potential target of *TAS3*-derived siRNAs, suggesting that *TAS3* regulates not only root nodule development but also N transport (Fukuda et al. 2019).

lncRNAs have definitely emerged as key regulators of plant adaptation to both beneficial and pathogenic microorganisms, and different layers of complexity have been unraveled so far. Understanding the intricate molecular mechanisms that involve lncRNAs in the control of diverse responses to abiotic stress is crucial for refining plant adaptation to diverse types of biotic stresses in the near future.

lncRNAs and their conservation in plants as factors of adaptation

The evolution of lncRNAs remains a complex and poorly understood area of research. Unlike protein-coding genes, which often exhibit high sequence conservation between species, lncRNAs generally display poor sequence conservation or have only small stretches of conserved sequences. Consequently, identifying orthologs is challenging, hampering the transfer of knowledge between orthologous lncRNAs, a process commonly employed for protein-coding genes (Hezroni et al. 2015). As one would expect, with increasing evolutionary distance between compared species, the number of identified sequence-conserved lncRNAs decreases significantly. Only a small fraction of lncRNAs has been identified as conserved throughout the entire plant lineage, whereas the majority of the already characterized lncRNAs appear to be specific to individual species (Sang et al. 2021; Song et al. 2021; Chen and Zhu 2022; Liu et al. 2023). Nonetheless, lncRNA sequences are subject to evolutionary selection, albeit at a lower level compared to protein-coding genes but at a higher level than pseudogenes or transposable elements (Blein et al. 2020; Chen and Zhu 2022; Liu et al. 2023). In addition, intergenic lncRNAs display some partial splicing conservation. Investigation among Brassicaceae revealed that around 20% of intergenic lncRNAs display splicing conservation (Corona-Gomez et al. 2020), an observation also reported for vertebrate lncRNAs (Nitsche et al. 2015). Altogether, this suggests that a significant proportion of these lncRNAs are likely functional and hold relevance for plants despite poor sequence conservation.

In some cases, however, homologous lncRNAs can be identified by sequence conservation. For example, the *CDF5/FLORE NAT* pair appears to be conserved between *Arabidopsis* and potato, and possibly also in other species (Henriques et al. 2017; Ramírez Gonzales et al. 2021). Indeed, sequence comparison revealed that *StCDF1* is the closest homologue of *AtCDF5* in the potato genome. Furthermore, the regulation loop between the CDF and their respective NAT are conserved in *Arabidopsis* and potato, pointing to the conservation of this regulatory module, allowing plants to adapt their flowering time to environmental

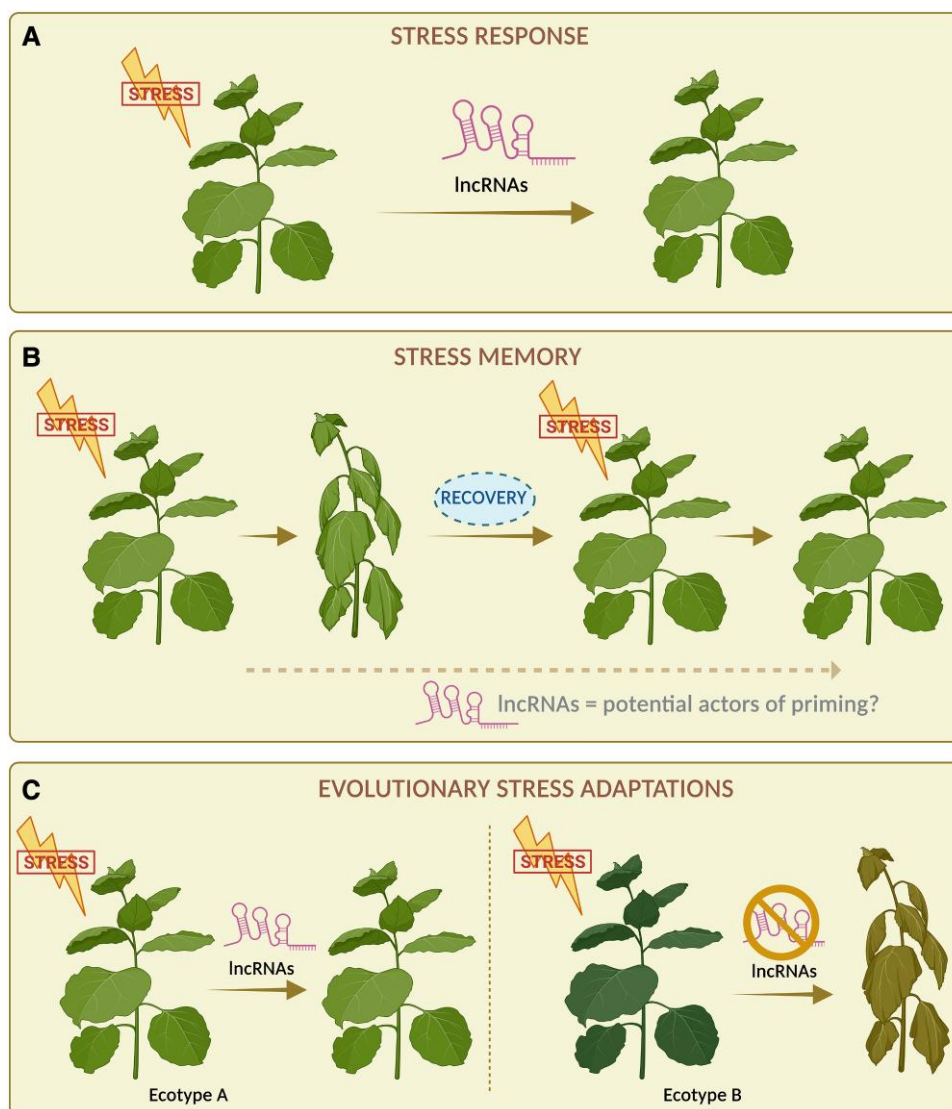


Figure 2. Schematic figure conceptualizing the roles of lncRNAs in stress response in plants. **A)** Response to stress. lncRNAs are key players in plant adaptation to overcome stress factors. **B)** Stress memory. Stress triggers plant defenses that are essential for survival. If the stress is transient and followed by a period of recovery, plants memorize the event. Repeated stress exposure and recovery actions develop stress memory (priming) to overcome repeated exposure to stress and improve plant survival, in a process where lncRNAs may play an important role. **C)** Conservation of lncRNAs in plant stress adaptation. lncRNAs exhibit differences in expression between natural accessions in response to stress, leading to adaptation to different environments. lncRNAs mediate stress response in Ecotype A (left) leading to plant stress tolerance, but they are absent or silenced in Ecotype B (right), leading to plant sensitivity. Created with Biorender.com.

cues but also to resist drought conditions (Henriques et al. 2017; Ramírez Gonzales et al. 2021). Another example is the *IPS1/At4* gene family of target mimicry, whose sequence and function are conserved from rice to *Arabidopsis* (Franco-Zorrilla et al. 2007). Interestingly, the regulation of miR399 by target mimicry can be accomplished by lncRNAs with no homology with the *IPS1/At4* gene family such as for the *PILNCR1* lncRNA in maize, pushing further the question of conservation of function without sequence homology (Du et al. 2018). In the case of *TAS3*, there is an evolutionary conservation of the *miR390/TAS3* pathway across vascular and nonvascular plants (Krasnikova et al.

2013). Despite the nucleotide sequence showing little conservation across different species, it possesses some highly conserved features such as the 5' and 3' binding sites of miR390, the sequences of tasiARFs, and the presence of a small ORF. Although the amino acid sequence of the ORF is not conserved, its length and its position are conserved in different plant species (Bazin et al. 2017).

At the species level, significant differences in expressed lncRNAs were observed between natural accessions adapted to different environments, even when grown under the same conditions (Blein et al. 2020; Kornienko et al. 2023). This finding suggests that the number of lncRNAs might be greater

than previously anticipated. Indeed, a comprehensive investigation of the transcriptomes from hundreds of *Arabidopsis* accessions unveiled numerous new lncRNA loci that were not expressed in the reference accession (Kornienko et al. 2023). Intriguingly, the majority of lncRNAs are actively silenced at the transcriptional level, either by the transposable element (TE) machinery, for the intergenic lncRNAs harboring TE fragments, or by Polycomb Repressive Complex 2 (PRC2). The high epigenetic variability that exists between the natural accessions results in expression patterns that greatly differ between accessions. Consequently, lncRNAs exhibit not only tissue and condition-specific expression but also strong accession-specific expression patterns (Blein et al. 2020; Kornienko et al. 2023). These findings suggest that lncRNAs likely play crucial roles in the specific environmental adaptations of individual accessions, and their regulation might offer a substantial source of transcriptomic plasticity for plants across short and long evolutionary time scales (Fig. 2).

Given the specificity of lncRNA expression among natural accessions, it is expected that there will be differences in expression between natural accessions in response to a given stress. In response to progressive drought treatment, two *Eutrema salsugineum* ecotypes displayed different transcriptomic responses, as expected. However, surprisingly, there was almost no overlap in the drought-associated lncRNAs expressed in these two ecotypes (Simopoulos et al. 2020). This lack of overlap can be attributed to the rapid evolution of lncRNAs and conditions leading to local adaptation in different environments. By studying two *Arabidopsis* accessions in response to phosphate starvation, it was shown that while the number of protein-coding genes differentially expressed was comparable in response to the stress and between the two accessions, there were 10 times more lncRNAs differentially expressed between the two accessions than in response to stress, and very few were present on only one of the genomes (Blein et al. 2020). These observations suggest that lncRNAs might be highly involved in the local adaptation of plants to their environment. Indeed, lncRNAs, as regulators of gene expression level and dynamics, might be better targets of selection for local adaptation of a species compared to protein-coding genes.

COOLAIR, an antisense lncRNA of the *FLOWERING LOCUS C* (*FLC*) gene, exemplifies the potential role of lncRNAs in local environmental adaptation. *FLC* functions as a repressor of the flowering transition and undergoes epigenetic silencing upon prolonged cold exposure (Wu et al. 2020). In response to cold, COOLAIR is induced and contributes to the silencing of *FLC*. Intriguingly, polymorphisms in the *FLC* locus within *Arabidopsis* accessions have been linked to flowering adaptation to local conditions (Hepworth et al. 2020), and one specific single-nucleotide polymorphism (SNP) was shown to affect COOLAIR splicing, correlating with *FLC* transcript accumulation (Li et al. 2015). Traditionally, vernalization studies involve exposing plants to a constant temperature of 5°C, enabling the classification of accessions based on their vernalization speed. To explore vernalization in a more natural context, representative accessions were studied in three field locations with varying

temperature profiles or in laboratory settings mimicking natural temperature fluctuations (Zhao et al. 2021). The level of COOLAIR was higher in slowly vernalizing (SV) accessions compared to the rapidly vernalizing (RV) Col *FRI* accession. Interestingly, while the COOLAIR expression in Edi-0 and Var2-6 RV accessions was comparable to Col *FRI* in England, it showed higher expression levels in Sweden, where the climate is colder than in England, suggesting different temperature sensitivity of COOLAIR expression across accessions. Across the tested accessions, COOLAIR was strongly induced when the temperature dropped below zero, corresponding to the first frost in autumn, which coincided with a decrease in *FLC* transcripts (Zhao et al. 2021). These findings highlight the importance of COOLAIR in vernalization and its role in fine-tuning flowering responses in diverse environments.

Conservation of lncRNAs extends to their secondary structures, which are believed to be more conserved than their nucleotide sequences (Graf and Kretz 2020). For example, COOLAIR lncRNA exhibits wide sequence divergence among different species, even within the Brassicaceae family, making accurate prediction challenging. However, using its secondary structure, Hawkes and colleagues successfully predicted COOLAIR exons in Brassicaceae species with significant evolutionary divergence (Hawkes et al. 2016). Despite relatively low nucleotide sequence identity, the structures, including multi-helix junctions, showed remarkable evolutionary conservation. Notably, the structure conservation occurred through covariation of noncontiguous DNA sequences. The conservation of their secondary structures across evolutionary divergent species emphasizes their likely functional significance. Notably, it was shown that a particular isoform displayed structural variation in a hyper-variable region during cold and warm conditions. Mutations in this region modify COOLAIR association with the chromatin at the *FLC* locus, reduce *FLC* transcription, and cause early flowering, highlighting the role of COOLAIR in modulating *FLC* expression through structural changes (Yang et al. 2022). Conservation of COOLAIR was also reported in the monocot *Brachypodium distachyon*, where two antisense transcripts of *FLC* homologs were identified (Jiao et al. 2019). These two lncRNAs do not share any sequence conservation with AtCOOLAIR but exhibit some characteristics similar to AtCOOLAIR, including being induced by cold and repressing their antisense gene. However, it remains unknown whether COOLAIR structure is conserved between Brassicaceae species and *Brachypodium*, given their divergence originating approximately 150 million years ago. Understanding the structural conservation of COOLAIR in such distant species would provide further insights into its functional relevance across evolutionary time scales.

For the bifunctional *ENOD40* lncRNA, such structural conservation analysis was made possible thanks to the identification of numerous homologous genes. This lncRNA exhibits a highly structured molecule without any long ORF but a series of short ORFs (sORFs) that also play a role in its function (Sousa et al. 2001). Using sequence similarity search and comparative RNA structure analysis, *ENOD40* homologues

were identified in almost all leguminous species and grasses (Gultyaev et al. 2023), as well as in non-leguminous plants (Gultyaev and Roussis 2007). Despite relatively low sequence similarity, even within legumes, all *ENOD40* RNAs hold comparable structure with two sORFs-containing conserved regions (named region I and region II) and a conserved core secondary structure encompassing region II. Surprisingly, stem-loop domains of the secondary structure appeared to be much more conserved than the encoded peptides, and some of them (in the 3' region of the lncRNA) were clearly linked to beneficial plant–microbe interaction capacity, suggesting that some function features of *ENOD40* could be determined by the RNA secondary structure, whereas short peptides may be responsible for more diverse functions found only in specific plant families (Gultyaev and Roussis 2007). Similarities of *ENOD40* domain insertions to repetitive elements and microRNA precursors such as mtr-MIR169d in *M. truncatula* were also found, raising questions given that some microRNAs have been shown to regulate plant symbiosis with bacteria and fungi. As repeated insertions of TE-derived sequences occur at similar locations minimally disturbing the encoded RNA structure, such insertions of new modules may create an opportunity to adapt rather than to destroy the associated function (Gultyaev et al. 2023). Studies deciphering the biological relevance of structured *ENOD40* RNA domains would help to precise how this lncRNA appeared and evolved.

Conservation of lncRNAs extends beyond sequence and secondary structures. In mammals, studies have shown conservation of expression patterns and position (synteny) (Ulitsky 2016). Similar conclusions have been reached in plants, although fewer detailed analyses have been performed (Mohammadin et al. 2015; Deng et al. 2018; Simopoulos et al. 2019; Chen and Zhu 2022). Synteny conservation is particularly relevant for lncRNAs whose genomic localization is crucial for their mode of action. Approximately 17% of *Arabidopsis* intergenic lncRNAs have been found to be syntenically conserved in other Brassicaceae species (Palos et al. 2022). However, to date, with the exception of antisense lncRNAs such as *COOLAIR* or *FLORE*, no functional example of conserved syntenic intergenic lncRNA has been reported.

Future perspectives and conclusion

Prediction of functional lncRNAs and their evolution

The numerous RNA-seq experiments conducted during past years allowed annotation of several hundreds of thousands of lncRNAs in different organisms. However, in comparison, only a few thousand of them have been demonstrated to be functional (reviewed in Zhou et al. 2021). Indeed, it is not easy at the moment to differentiate *bona fide* lncRNAs from transcriptional nonfunctional noise (discussed in Ponting and Haerty 2022). The majority of discovered mechanisms of action for lncRNAs have not been easily generalized, preventing the prediction of the function of the numerous annotated lncRNAs based on the available genomic data as can be

done for a large part of the protein-coding genes. This is linked to the poor sequence conservation of lncRNAs, or perhaps to the lack of understanding of the factors driving the selection of functional lncRNAs, which can be different than for protein-coding genes. The identification of functional domains inside lncRNAs and how they may vary is one of the key challenges in the community that will allow predicting the functions of lncRNAs. Despite the existence of functional analysis demonstrating the role of certain lncRNAs in plant responses to environmental factors, none of them has yet been linked to long-term adaptation processes. For instance, their roles in processes such as population-level adaptation through evolutionary processes remain largely unexplored. A comprehensive functional characterization of lncRNAs implicated in such processes holds the potential to unveil novel regulatory mechanisms that contribute to plant resilience.

lncRNAs in stress memory

Exposing an organism to mild stress conditions results in an enhanced ability to adapt to subsequent stresses through priming. The duration of this stress memory varies, ranging from days to weeks, and may even be transmitted to the next generation (Charng et al. 2023). Importantly, the primed state is not manifest at the phenotypic level, nor does it induce alterations in the DNA sequence; rather, it is mediated by epigenetic mechanisms that regulate gene expression through DNA methylation, histone modifications, and small RNAs (Oberkofler et al. 2021). Since lncRNAs have been demonstrated as potential regulators of epigenetics, they are good candidates to be involved in the control of stress memory. In response to hyperosmotic stress, distinct regions of the *Arabidopsis* genome were found to be susceptible to DNA (de)methylation and transmitted to offspring. Such a differentially methylated region (DMR) was identified downstream of the transmembrane ubiquitin ligase *CARBON/NITROGEN INSENSITIVE 1* (*CNI1*) gene. This DMR was shown to correlate with the expression of an antisense lncRNA to *CNI1* named *CNI1-AS1*, subsequently potentially modulating the activity of *CNI1* (Wibowo et al. 2016). Disrupting the DMR prevented the activation of *CNI1-AS*, and, in consequence, the transcripts of *CNI1* were no longer reduced upon hyperosmotic stress (Wibowo et al. 2016). The downregulation of *CNI1* might increase *Arabidopsis* tolerance to subsequent stress, as its overaccumulation decreases the tolerance to salt stress (Du et al. 2023). Likewise, under repeated mild drought stress in rice, transcriptomic approaches revealed that 17 lncRNAs harbored expression profiles compatible with a role as memory actors. For example, the lncRNA *TCONS_00028567*, located downstream of the *STRESS-ACTIVATED PROTEIN KINASE 10*, was found to be upregulated after drought stress, possibly regulating ABA signaling (Li et al. 2019). However, for the moment, the potential role of lncRNAs in memory stress is only based on the correlation of expression (Fig. 2). Further lncRNA characterization in the context of stress memory will greatly open new perspectives on lncRNA and their functions.

OUTSTANDING QUESTIONS

- How can we exploit the functions of specific lncRNAs in response to abiotic stresses, such as drought and extreme temperatures, to be used to enhance plant resilience and agricultural productivity?
- How does stress memory, particularly the memory acquired through priming, affect the expression and function of lncRNAs in plants across different generations?
- What are the key factors that determine whether a particular lncRNA is retained and functional in specific natural accessions or plant species, contributing to local adaptation?
- How can we quickly and systematically identify and validate the functional roles of the multitude of yet uncharacterized lncRNAs in plant adaptation?

lncRNAs are emerging as key players in plant adaptation to environmental stresses, encompassing both abiotic and biotic factors. They play a pivotal role in modulating gene expression and finely regulating plant responses to a diverse array of stresses. Given that plant lncRNAs are extremely responsive to stresses in their expression and that they evolve rapidly compared with protein-coding genes, they make suitable environmental sensors or effectors to help plants adapt to changing environments. Moreover, the evolutionary conservation of lncRNAs, although challenging, provides evident opportunities for crop improvement.

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