Trends in
Plant Science

CellPress

Review

Plant memory and communication of encounters

Judit Dobránszki [1], Dolores R. Agius [2,3], Margot M.J. Berger [4], Panagiotis N. Moschou [5,6,7], Philippe Gallusci [4], and Federico Martinelli [8,*]

Plants can communicate with each other and other living organisms in a very sophisticated manner. They use biological molecules and even physical cues to establish a molecular dialogue with beneficial organisms as well as with their predators and pathogens. Several studies were recently published that explore how plants communicate with each other about their previous encounters or stressful experiences. However, there is an almost complete lack of knowledge about how these intra- and interspecies communications are directly regulated at the epigenetic level. In this perspective article we provide new hypotheses for the possible epigenetic modifications that regulate plant responses at the communication level.

Highlights

Biocommunication is one of the main players in the evolution of life, and is directly regulated at the epigenetic level by mobile signals.

Epigenetic modifications can act either directly, by modifying the expression of key signaling molecules in the plant or by acting as mobile nucleic acids, or indirectly by modifying other mobile compounds or physical signals between plants or between plant and external organisms, thereby causing modifications in the epigenetic state of the recipient organism.

Mobile signals such as small RNAs (sRNAs), volatile organic compounds (VOCs), and ultrasound emitted by plants are unique depending on the triggering stimuli, suggesting fine regulation of plant communication.

Understanding how plants memorize encounters and stressful events holds potential for agricultural applications, including climate-smart crop production.

Translating communication signals into the language of epigenetics

Plants, as sessile organisms, live closely together with all types of living forms including other plants, either of the same or different species, microorganisms, fungi, and animals. Plants also memorize their interactions with other organisms. Their memory is based on cellular, molecular, and biochemical networks that store, retrieve, recall, and eventually erase information. In contrast to animals their archiving process does not include the presence of specialized sensory organs [1]. For this reason, plants need to communicate with each other through biological molecules (RNAs, DNA, proteins, metabolites, and volatiles) [2]. This communication is important for interactions, and competition for available resources [3]. In addition to the use of chemicals, plants communicate via acoustic, visual, and tactile signals [4,5]. Furthermore, they can modify their color to enter a dialogue with other organisms [5]. However, the mechanisms underlying these communications remain almost completely unknown, although it was suggested that **epigenetic** (see Glossary) mechanisms may play a major role in mediating these events.

There are different types of epigenetic mechanisms: DNA methylation that can also involve short interfering RNAs, siRNAs), histone post-translational modifications (HPTMs), histone variants, and chromatin remodeling [6]. **Epigenetic modifications** play a key role in plant–external organism communications by acting mainly at two levels: (i) directly modifying the expression of key molecules in the plant [7], and (ii) directly modifying their methylation status which regulates the behavior of other plants or organisms communicating with plants (Figure 1, Key figure) [8].

Extracellular DNA and RNA molecules in plant–organism communications

Regarding mobile nucleic acids, it is well known that RNA species function as mobile signals in plants, over both short and long distances, as part of cell-to-cell communication [8]. Recent evidence has demonstrated exchange of RNAs between a host plant and colonizing microbes or pathogens, or even between plants, highlighting that RNA transfer may also occur through

[1]Centre for Agricultural Genomics and Biotechnology, FAFSEM, University of Debrecen, Debrecen, Hungary
[2]Centre for Molecular Medicine and Biobanking, University of Malta, Msida, Malta
[3]Ġ.F. Abela Junior College, University of Malta, Msida, Malta
[4]Ecophysiologie et Génomique Fonctionnelle de la Vigne (EGFV), University of Bordeaux, Bordeaux Sciences Agro, Institut National de la Recherche Agronomique (INRAE), Institut des Sciences de la Vigne et du Vin (ISVV), Villenave d'Ornon, France
[5]Department of Biology, University of Crete, Heraklion, Greece
[6]Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology Hellas, Heraklion, Greece

the extracellular space, possibly involving **exosomes** or similar secreted vesicles [9–11]. **Small RNAs (sRNAs)** and other **non-coding RNAs (ncRNAs)** are not only mobile regulatory molecules within the plant but are also transmitted between plants and interacting organisms, including pathogens, to induce gene silencing. This phenomenon is known as cross-kingdom/organism **RNA interference (RNAi)** [12] (Box 1). These epigenetic mechanisms in plant–organism communication are of central importance because they enable plants to memorize a particular

[7]Molecular Sciences Department, Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden
[8]Department of Biology, University of Firenze, Firenze, Italy

*Correspondence:
federico.martinelli@unifi.it (F. Martinelli).

Key figure

Interspecies communications in plant–plant and plant–organism interactions through different types of nucleic acids

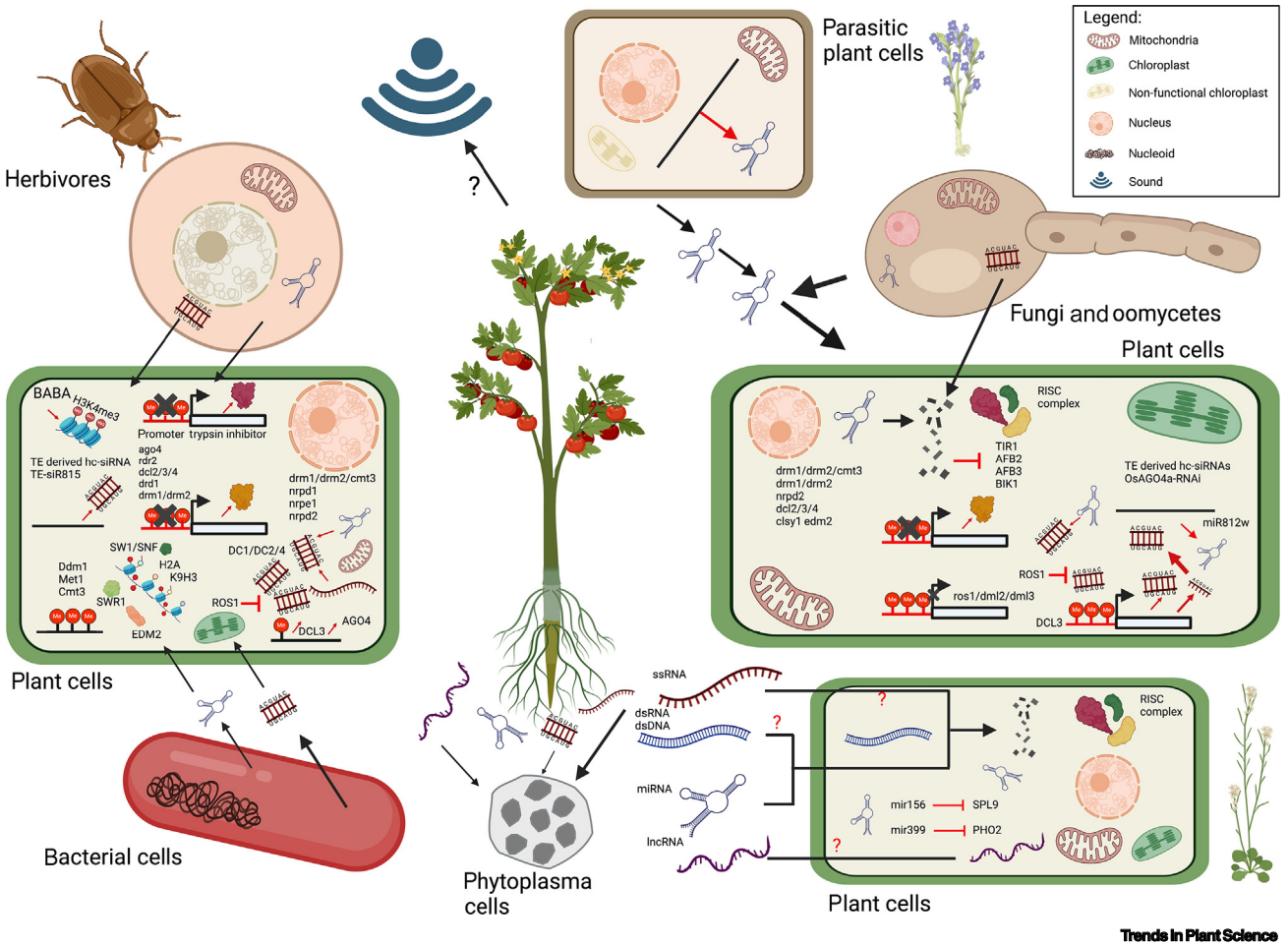

Figure 1. Different miRNAs and host target genes that are known to be exchanged between different organisms are indicated. Abbreviations: AFB1/2/3, AUXIN SIGNALING F-BOX 1, 2 and 3; AGO4, ARGONAUTE 4; BIK1, BOTRYTIS-INDUCED KINASE 1; CLSY1, CHROMATIN REMODELING 38, CLASSY 1; CMT3, CHROMOMETHYLASE 3; DCL2/3/4, DICER-LIKE 2, 3, and 4; DDM1, DECREASED DNA METHYLATION 1; DRD1, DEFECTIVE IN RNA-DIRECTED DNA METHYLATION 1; DRM1/2, DORMANCY-ASSOCIATED PROTEIN 1 and 2; dsDNA/RNA, double-stranded DNA/RNA; Me, methylation; MET1, METHYLTRASFERASE 1; NRPD1/2, NUCLEAR RNA POLYMERASE D1 and D2; NRPD1/NRPE1, NUCLEAR RNA POLYMERASE D1; PHO2, PLUMULAR HOOK OPEN 2; RDR2/RDR6, RNA-DEPENDENT RNA POLYMERASE 2 and 6; RISC, RNA-induced silencing complex; ROS1, REPRESSOR OF SILENCING 1; SPL9, SQUAMOSA PROMOTER BINDING PROTEIN-LIKE 9; SWR1, MYB-LIKE TRANSCRIPTION FACTOR FAMILY PROTEIN OF SWR1 COMPLEX; TE, transposable element; TIR1, TRANSPORT INHIBITOR RESPONSE 1;

Box 1. Extracellular DNA and RNA molecules in plant–organism communications

MicroRNAs (miRNAs) may be secreted by a plant into the external medium to regulate gene expression and the phenotype of nearby recipient plants, thus defining a new concept in plant communication. Exogenous RNA extracts enriched in a specific miRNA or chemically synthesized miRNAs were shown to modulate the expression of target genes in the recipient plant or protoplast [42]. A wide range of RNAs including miRNAs are likely to be involved in the cell-to-cell communications between plants and across kingdoms. Determining which molecular factors are involved in the mobility of RNAs across the boundaries between different plant species will shed light into the dynamic balance of virulence, susceptibility, and compatibility reactions in plant–pathogen interactions [11]. Many aspects of these trans-kingdom silencing phenomena remain poorly understood. These include how specific small RNAs (sRNAs) are selected for transport outside the cell, their recognition and entry into the target cell, and how sRNAs hijack the RNA inhibition (RNAi) machinery to obtain their silencing effect (Figure I).

Evidence has been provided that self-DNA, despite fragmentation, may have modulating effects on litter autotoxicity and plant–soil nitrogen fixation [85]. This evidence was corroborated by the toxic effects on plant growth observed by self-DNA in a size- and concentration-dependent manner. The possible role of DNA as a molecule involved in extracellular communications between plants and organisms is corroborated by the fact that DNA can persist in soil because of its chemical stability and its protection against enzymatic degradation [86] (Figure I). The known capability of plant root cells to take up extracellular DNA [84], together with the discovery that self-DNA is toxic, indicate that self-DNA inhibitory effects should be considered as a further mechanism explaining species-specific negative feedback. These modulating effects of self- and non-self-DNA were observed mostly for smaller fragments (<500 bp) because they can enter the cell and produce their effects. Recently it was shown that external fragmented self-DNA can induce significant plasma membrane depolarization and an increased flux of intracellular calcium in lima bean (*Phaseolus lunatus* L.) and maize (*Zea mays* L.) leaves, whereas non-self-DNA was unable to trigger such signaling events [87]. More recently, treatment of suspension-cultured plant cells with fragmented extracellular self-DNA enhanced H_2O_2 production and activated mitogen-activated protein kinase (MAPK), promoting early immunity-related signaling responses [88].

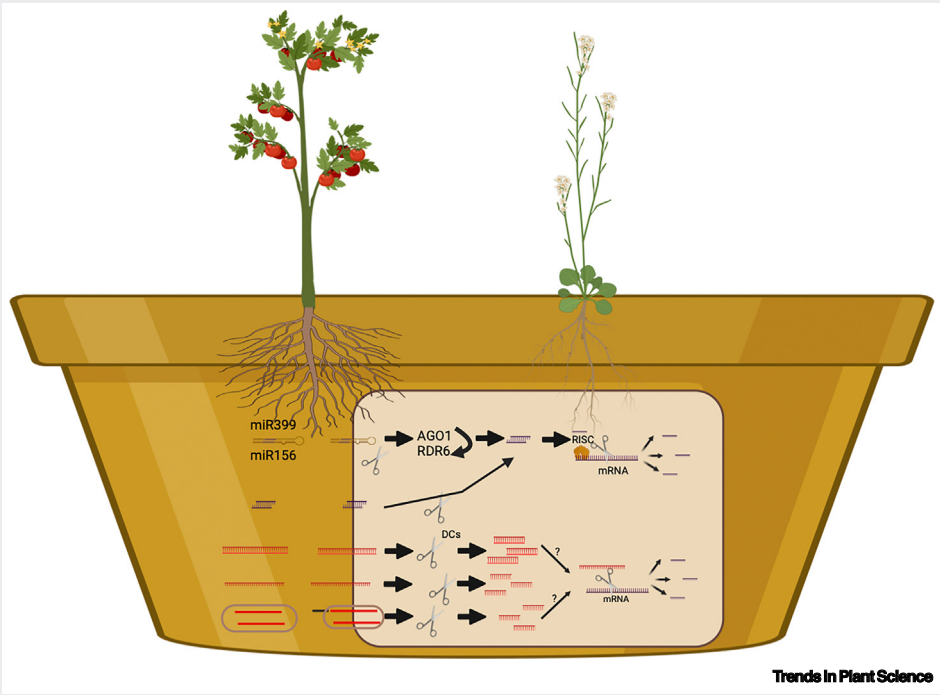

Figure I. RNA and DNA molecules are communication vectors that modulate gene target expression across plant species. Abbreviations: AGO1, ARGONAUTE 1; DC, DICER; RDR6, RNA-DEPENDENT RNA POLYMERASE 6; RISC, RNA-induced silencing complex.

environmental cue. This memory could enable faster acclimation when plants later encounter the same cue. This phenomenon, known as priming, may in some situations be inherited at the transgenerational level [13].

Glossary

Antisense RNA (asRNA): 19–23 nt transcripts that are complementary to mRNA and regulate gene expression at various levels including transcription, translation, and replication.
Chromatin silencing: repression of transcription by modification of chromatin organization, such as through the formation of heterochromatin.
Epigenetic modification: a heritable and stable change in gene expression levels and plant phenotypes without alterations in the underlying DNA sequence.
Epigenetics: a branch of molecular biology that studies any heritable change in chromosome organization that regulates gene activity in the absence of changes in the nucleotide sequence.
Exosomes: membrane-bound extracellular vesicles that are produced in the endosomal compartment of eukaryotic cells.
Grafting: combining the shoot of one plant (scion) with the root system of another one (rootstock) to generate new plants with improved agronomic traits.
Heterografting: grafting between two different genotypes.
Histone modifications: post-translational modifications of histone proteins which affect histone–DNA interactions and DNA accessibility to the transcriptional machinery. Histone tails protruding from the nucleosome core are subjected to different types of post-translational modifications, such as methylation, acetylation, and ubiquitination.
MicroRNAs (miRNAs): highly conserved ncRNA molecules of 18–25 nt that are involved in the regulation of gene expression. miRNAs are transcribed by RNA polymerases II and III, generating precursors that undergo a series of cleavage events to form mature microRNAs. These bind to the 3′-untranslated regions of target mRNAs and cause post-transcriptional repression of gene expression or mRNA degradation.
Morphogen: a signaling molecule that acts directly on cells to produce specific cellular responses depending on its local concentration.
Non-coding RNAs (ncRNAs): a heterogeneous group of RNAs that do not encode proteins but have vital roles in cellular processes. ncRNAs include long non-coding RNAs (lncRNAs), circular RNAs (circRNAs),

In addition to RNA, DNA may also operate as a mobile signal between species. Extrachromosomal DNAs can play a role in genome plasticity, which allows the adaptation of plants to stress conditions. As recently reported, extrachromosomal circular DNA (eccDNA) was responsible for the transfer of glyphosate resistance among *Amarantus* spp. [14] and assisted rice adaptation to intense light conditions [15]. The formation of eccDNAs from active transposable elements (TEs) was epigenetically suppressed in wild-type *Arabidopsis thaliana*. eccDNAs formed from active TEs impacted on genomic structural variations in hypomethylated *A. thaliana* mutants (*ddm1, rdr6, pollV*), which may contribute to environmental adaptation by causing genome instability based on altering DNA repair pathways [16]. Evidence has also shown that external self-DNA and non-self-DNA may modulate plant physiological processes through gene silencing via hybridization between complementary sequences [17] (see Figure I in Box 1). Taken together, these findings let us hypothesize that fragmented DNA may also operate as an extracellular mobile molecule that mediates communications between plants and other organisms. These fragmented DNA (or RNA) molecules may originate from internal extrachromosomal storage sites [18].

Epigenetic mechanisms involvement in communication between plants

Grafting

Grafting has been used for several millennia [19] and is still intensively used in arboriculture (including viticulture), and more recently was also developed in horticulture. Several studies have investigated the exchange of molecules between the rootstock and the scion to investigate the mechanisms responsible for the difference in behavior of the grafted plant. These studies show that, in addition to nutrients and metabolites, mRNAs, proteins, and sRNAs move between the grafted parts of the newly formed plants (reviewed in [20]). The communication between graft partners, which requires generating new epidermis, parenchyma, and vascular bundles, is crucial for the modified performance of the grafted plants (see Figure I in Box 2). There

heterogeneous nuclear RNAs (hnRNAs), microRNAs (miRNAs), ribosomal RNAs (rRNAs), small interfering RNAs (siRNAs), small nuclear RNAs (snRNAs), small nucleolar RNAs (snoRNAs), and transfer RNAs (tRNAs).

Post-transcriptional gene silencing (PTGS): an RNA silencing pathway involving double-stranded RNA. It includes the accumulation of 21–23 nt siRNAs and sequence-specific degradation of target mRNAs.

RNA-directed DNA methylation (RdDM): a biological process, unique to plants, in which ncRNA molecules of 24 nt (24 nt siRNAs) mediate the addition of methyl groups to specific DNA sequences.

RNA interference (RNAi): a biological process in which small ncRNAs selectively downregulate gene expression at the post-transcriptional level.

SET domains: protein domains with methyltransferase activity. They modify chromatin structure by affecting histone methylation.

Small RNAs (sRNAs): ncRNAs <200 nt in length that serve a variety of essential functions within cells. These include miRNAs, siRNAs, and tRNA-derived small RNAs (tsRNAs). siRNAs can be defined based on their function: the 21/22 nt sRNAs are essentially involved in PTGS, whereas the 24 nt sRNAs are essential to transcriptional gene silencing (TGS).

Volatile organic compounds (VOCs): chemicals released by plants that can be detected by neighboring plants to equip them with increased defense pathways against environmental stresses, even before stress exposure, by mimicking the behavior of the emitting plant.

Box 2. Epigenetic dialogue between graft partners

Studies on heterografting have shown both transcriptomic reprogramming [89] and methylome remodeling [90–93] that can be due to a diversity of physiological processes, including the exchange of hormones and/or of other signaling molecules, and modified responses to environment, that also involve epigenetic dialogue between graft partners. Consistent with an exchange of epigenetic information between graft partners, sRNAs were shown to move bidirectionally through the graft junction and direct DNA methylation in distant cells/tissues (reviewed in [94]) (Figure I). sRNAs include miRNAs that are involved in **post-transcriptional gene silencing (PTGS)** and are transcribed from endogenous genes in the form of primary miRNA (pri-miRNAs) and matured. The siRNAs are produced from double-stranded RNAs (dsRNAs) of different origins [95]. Those of 21/22 nt are involved in PTGS, whereas those of 24 nt are essential for transcriptional gene silencing (TGS) and direct the DOMAIN REARRANGED METHYLTRANSFERASES (DRMs) to their targets in a process called RNA-directed DNA methylation (RdDM) [96].

Mobile sRNAs were initially identified in the phloem sap of rapeseed pumpkin and potato [97,98]. Further evidence of sRNA mobility was obtained by grafting transgenic tobacco and potato plants expressing an artificial siRNA, which allowed the silencing of both a transgene and an endogenous gene through the graft junction [99]. The silencing was more efficient from scion to rootstock than the opposite, suggesting more efficient top-down than bottom-up transfer of siRNA [100,101]. The importance of 24 nt siRNA was demonstrated by grafting scions or rootstocks impaired in siRNA-induced gene silencing onto a part of a plant which is unimpaired. The 24 nt siRNA could only be found in *dicer-like 3* mutant roots grafted with a wild-type scion, but not when grafted with a scion carrying *dcl2,3,4* mutations [43]. Recent analysis of sRNA populations in both the rootstocks and scions of grapevine heterografts indicates that some siRNA found in either the rootstock or the scion can only be explained by migrating from one graft partner to the other. Some of these mobile siRNAs were associated with changes in DNA methylation [102]. The number of siRNAs migrating from the scion to the rootstock was an order of magnitude higher than the converse [102], potentially due to the mechanisms involved in the movement of siRNA. Scion to rootstock mobility follows the sieve flux through the phloem and can be enhanced by modulating the sink strength of the scion [100]. The opposite direction from rootstock to scion seems to occur by cell-to-cell communication through plasmodesmata [103].

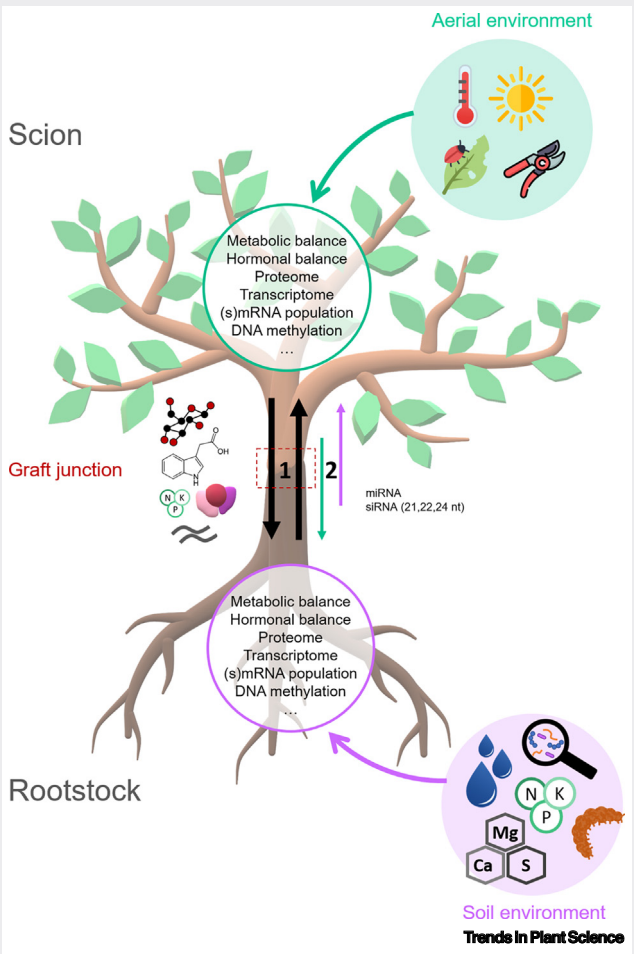

Figure I. Graft junctions allow information exchange between the two graft partners after vascular and cellular reconnection. (i) Molecules including metabolites, hormones, nutrients, proteins, mRNA, and sRNA can pass through the graft junction in a top-down or bottom-up manner. These exchanges can impact on the phenotype, physiology, and metabolism, as well as on the transcriptome, sRNA population, and epigenome, of both scion and rootstock. (ii) Environmental conditions can alter the physiological, metabolic, transcriptomic, and epigenetic state, as well as the sRNA population, of each partner. This might modify the dynamic of exchanges between scion and rootstock, as well as the type of molecules exchanged. Changes in sRNA populations can alter gene expression through the TGS and PTGS pathways, or modify the DNA methylation profiles of the graft partner through the RdDM pathway.

is accumulating evidence of an epigenetic dialogue between the two graft partners, mainly mediated by bidirectional sRNA exchanges, as investigated using **heterografting** and transgenic approaches (Box 2). Because the sRNA populations of both compartments may be influenced by environmental conditions, a better understanding of the mechanisms involved in sRNA mobility and impact on DNA methylation, gene expression, and TE mobility would be of primary importance in the context of plant adaptation to climate change. Further studies will be necessary to investigate the mechanisms involved in the sRNA exchange through the graft junction and to demonstrate links between sRNA mobility, DNA methylation, and plant phenotypes.

Plant–plant communication is epigenetically regulated

Plant-to-plant communication plays a crucial role in the survival, performance, and fitness of plant individuals as well as in the evolution of plant species and communities [21–24]. During plant–plant communication, both chemical (volatiles, nucleic acids, proteins, metabolites) and physical (touching and even sound including ultrasound) signals or cues emitted by neighboring plants may be involved in the transmission of information about environmental threats and opportunities [2,4,5,25]. The threats include parasitic plants, intra- or interspecific

competitors, and resource limitations, whereas possible symbiotic partners in the environment represent opportunities [11]. As emerging evidence suggests [26,27], these communications are epigenetically regulated (Box 3).

Box 3. Different types of epigenetic regulation in plant-to-plant dialogue

In response to herbivore (*Mythimna separation*) attack in maize (cv. Royal Dent), after priming with a specific herbivore-induced plant volatile (HIPV), the perceiving plant was shown to be able to memorize the warning signal of attack via altered DNA methylation. HIPV treatment caused demethylation at selected cytosines located in the promoter region of the *TI* (Bowman–Birl-type trypsin inhibitor) gene, leading to recall of its expression when herbivores fed on the plant later on [26].

In response to the β-ocimene, histone acetylation of H3 and H4 was enriched at genes encoding ethylene response factors (*ERF8, ERF104*) by the histone acetyltransferases HAC1, HAC5, and HAM1. This correlated with increased transcription of *ERF* genes. Anti-herbivore activity sustained for 5–10 days was reversed via histone remodeling by the histone deacetylase HDA6. It is also likely that increased histone acetylation at some defense genes, such as *PDF14* and *TI*, contributes to a defense response memory in plants perceiving β-ocimene [27].

Secreted exogenous miRNAs (*miR399* and *miR156a*) from miRNA-overexpressing *A. thaliana* mutant lines or chemically synthesized miRNAs had an influence on the expression of their target genes (*PHO2* and *SPL*) in neighboring plants. They act via PTGS by inducing RNAi, and, as a result of perceiving *miR156*, the juvenile phase of the perceiving plants was prolonged and they flowered later [42].

Sound-induced (10 kHz, 90 dB, every 3 h for 10 days) histone (H3K27me3) modifications at the promoters of genes related to the aliphatic glucosinolate biosynthesis and cytokinin signaling were detected in *A. thaliana* (Col-0) mutants (*cyp79*, *cyp83*, *sur1*, *ahk2*, *ahk2/3*, and *cre1*). The change in histone methylation led to transcriptional changes in the sound-perceiving plants and resulted in increased root immunity against the pathogenic bacterium *Ralstonia solanacearum*. In addition, decreased expression of *miR397b* via activation of *LACCASE* transcripts caused the accumulation of lignin and thereby cell wall strengthening, which contributed to increased tolerance [4]. Global DNA hypomethylation was detected in 1-week-old winter wheat (*Triticum aestivum* L.) seedlings developed from ultrasonicated (30 kHz, 70 W for 5 minutes) seeds, which altered their transcription pattern and promoted shoot and root growth, suggesting that such physical treatments lead to epigenetic remodeling that is at least in part memorized by the plant [104].

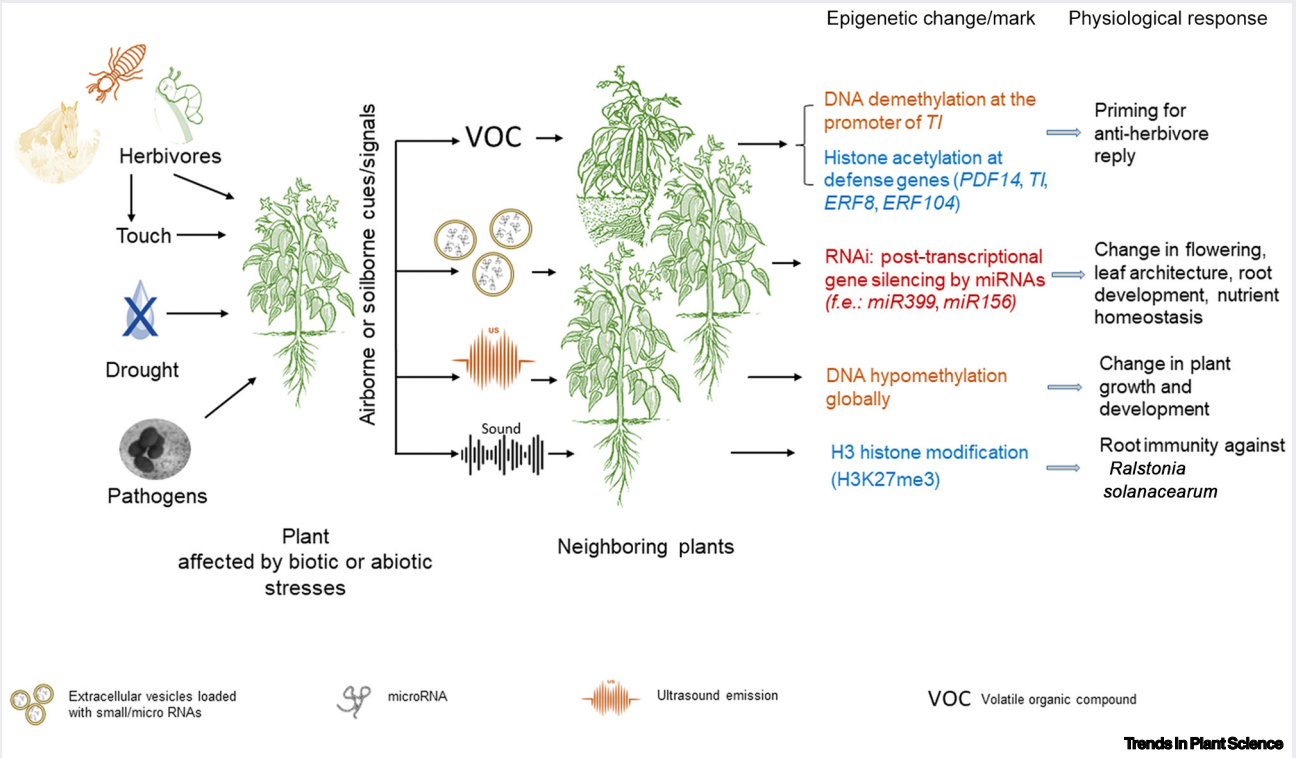

Figure I. Ways of warning neighboring plants during plant–plant communication and the underlying induced epigenetic changes in the neighbors.
Abbreviations: ERF8/104, ETHYLENE RESPONSE FACTOR 8 and 104; PDF14, PATHOGEN-RELATED GENE DEFENSE 14; TI, Bowman–Birl-type trypsin inhibitor; VOC, volatile organic compound;

Physical signals (sound or touch) are a more energy-efficient and faster means of communication owing to their more rapid spread and lower energy costs compared to chemical signals [28]. Plants can detect and emit acoustic vibrations, such as sound or ultrasound [5,29,30], which makes it likely that they use acoustic vibrations for communication. Conversely, plants under stress can emit ultrasounds that are stress- and species-dependent [5,28]. Based on these recent results, even if not yet directly demonstrated, it can be speculated that acoustic vibration plays a role in plant–plant communication and may act on epigenetic regulation (Box 3).

How could this regulation be achieved? As plants lack specialized sensory organs, sensing of sound (and touch) may occur through the extracellular matrix or specialized cell types such as trichomes [30–33]. Next, these sensors may relay mechanical signals such as sound waves or touch stimuli that deform the plasma membrane, which is a highly stretchable structure, and therefore an excellent transducer of physical signals. Higher-frequency stimuli produced by Hemiptera and echolocators such as bats and moths stretch the plasma membrane, resulting in the opening of specific stretch-activated mechanosensitive ion channels or other receptors [30,34]. Mechanosensitive channels such as MECHANOSENSITIVE CHANNEL OF SMALL CONDUCTANCE (MSC)-like, MID1-COMPLEMENTING ACTIVITY (MCA), and PIEZO may promote downstream signaling by modulating Ca^{2+} influx and apoplastic pH. These molecular events culminate in the activation of downstream signaling that involves modulation of redox homeostasis/defense genes, touch-regulated (*TCH*) genes, and various transcription factors [30,33]. Interestingly, these channels can distinguish between sound and touch stimuli, thus providing the necessary specificity.

Accordingly, sound-treated *A. thaliana* plants showed higher resistance to *Ralstonia solanacearum* infection [4]. This enhanced defense was likely a result of epigenetic regulation (histone H3 lysine 27 trimethylation, H3K27me3) at the promoters of defense-related aliphatic glucosinolate and cytokinin signaling genes. Sound treatment induces **histone modifications** of fruit ripening and transcription factor genes associated with ethylene biosynthesis in tomato fruits [35]. Herbivore sound may induce epigenetic changes that prime plants against impending herbivore attacks [36]. Similarly, plants may develop a memory of sound based on epigenetic changes to respond more efficiently to persistent environmental sounds. Nevertheless, how the physical constraints of the plasma membrane are translated to an epigenetic change remains unknown. It is still not known how these physical signals alert the epigenome and whether voltage-dependent transporters or changes of cell charge are involved. As a cautionary note for future studies, whether the amplitude of typical sounds is sufficient to induce the suggested changes is a matter of controversy (e.g., [37]).

Plants emit **volatile organic compounds (VOCs)** which are used for communication not only in plant–environment interactions but also in plant–plant dialogue [38]. Although this phenomenon is unexplored, plants may have evolved epigenetic mechanisms to memorize useful molecular signals emitted by nearby plants to react more promptly to environmental changes. Emitted VOCs from stressed plants can be perceived by neighboring unstressed plants and induce their preparation for active defense against environmental stresses, even before the stresses affect them [39]. VOC-induced alterations in DNA methylation and histone modifications were detected in plants perceiving VOCs emitted by neighboring plants after herbivore attacks. The memory of the attack was short-term and was stored for some days after VOC treatment [26,27] (Box 3).

In response to touch by an herbivore, plants emit VOCs that can be detected by surrounding plants to modulate their growth and interactions with herbivores. The emitted VOC are touch type-specific [34], and induce the same early defense-related genes in the neighboring plants as were induced by touch in the emitter plant, showing that the exposed plants can rapidly and

accurately mirror the specific changes in the emitter plants. Although not investigated in this study, it can only be speculated that the VOCs exerted their effects through epigenetic regulation, given that VOCs can cause both DNA methylation changes and histone modifications, as shown in other studies [26,27]. A genetic mechanism by which airborne plant–plant communication and UV light (UV-C) irradiation activate DNA repetitive elements in *A. thaliana* has been identified [40]. This type of plant–plant communication is well known, but whether emitted volatiles can bring about epigenetic changes in the recipient plant remains unknown.

Extracellular vesicles (EVs) loaded selectively with regulatory sRNA cargoes participate not only in the cell-to-cell and long-distance transport within the plant but also in inter-organismal RNA transport [10,11,41], thus enabling communication between plants using sRNAs as signaling molecules. As recently reported, plants can even take up naked **microRNAs (miRNAs)** from their environment [42]. Recipient plant cells seem to use the miRNA in mechanisms that are already present within the cell, and specific receptors and activation of signaling pathways are not needed [11,42]. In the recipient plant cell, mobile sRNAs can direct DNA methylation [43], mediate **chromatin silencing** [44], and cause gene silencing [45]. Therefore, nucleic acids (e.g., mobile sRNAs) from other plants can be considered as specific, genetic signals for communication between plants. RNAi-derived sRNAs, as mobile signals, may also act as **morphogens** and affect plant development [46] including flowering, leaf architecture, root development, and nutrient homeostasis [47].

Epigenetic modifications in cross-kingdom communication between organisms

Plant–pathogen interactions

Several host–parasite interactions involve cross-kingdom trafficking of sRNA, mainly miRNA and siRNA. These sRNAs, packaged in exosome-like vesicles, induce gene silencing in hosts and interacting organisms [2,12]. The fungal plant pathogen *Botrytis cinerea* counters this by its own sRNA targeted at silencing the host immunity genes. This silencing is achieved by binding to host ARGONAUTE1 (AGO1) and hijacking the RNAi machinery [48] in cross-kingdom RNA interference (ck-RNAi). *A. thaliana* and soybean (*Glycine max* L. Merr.) similarly overcome *Phytophthora* spp. [49,50]. miRNA transfer from cotton (*Gossypium hirsutum* L.) plants to the fungal pathogen *Verticillium dahlia* results in specific silencing of highly conserved genes essential for fungal virulence. This has been interpreted as a finely tuned host–pathogen interaction that balances their coexistence due to a stalemate [51]. EVs, traveling to the fungi along the exosomal pathway, are the main candidates for this sRNA transfer between the plant host and the fungal pathogens because they protect and provide a stable environment [52]. Further protection against uridylation that can trigger degradation of this miRNA *in vivo*, is conferred by methylation of the 3′ terminal nucleotide [53,54].

An *in silico* analysis of miRNAs from EVs revealed that the great majority share a sequence motif formed by a hairpin-loop structure and target biological pathways involved in metabolism and regulation [9]. Research into the role of these different RNA molecules in the EVs and their mode of transport between plant and pathogens is very timely.

The ability of sRNA to control plant pathogens is currently being exploited in host-induced gene silencing (HIGS) and spray-induced gene silencing (SIGS). In the former the pathogen pathogenesis gene is cloned and its RNA is expressed in the host plant. The sRNA derived is taken up by the pathogen, triggering silencing of the target gene. SIGS involves the direct application (e.g., by spraying) of dsRNAs or sRNAs that target pathogen genes to reduce pathogenesis (reviewed in [55]). These technologies might see wider applications in time, although the applicability of HIGS depends on the acceptance of genetically modified organisms. Further developments in the

commercial application of HIGS are expected as transgenic technology is deregulated. In the meantime, a large number of investigations have been carried out worldwide on SIGS for plant disease control, although risk assessment and legislation for this technology should be enhanced. The design of HIGS and SIGS specific for gene targets should be facilitated by the release of more plant and pathogen genome sequences. In addition, CRISPR/Cas9 technology may be combined with HIGS and SIGS to develop crop resistance to disease.

Several observations point to a two-way communication between plants and their prokaryotic pathogens. Bacterial sRNAs include (i) elements present in the 5′-untranslated regions, (ii) those acting in *cis*, termed **antisense RNAs (asRNAs)** [56], and (iii) those acting in *trans* [57]. In addition, both Gram-positive and Gram-negative pathogens secrete EVs [58,59]. These EVs range in size from 20 to 400 nm, are surrounded by a lipid bilayer, and contain various types of biomolecules including nucleic acids, virulence factors, toxins, and lipopolysaccharides [59]. To facilitate the uptake of these EVs by plant cells, bacteria produce cell wall-degrading enzymes such as polygalacturonases, cellulases, amylases, and xylanases [60]. Prokaryotic EVs elicit an immune response via pattern recognition receptor (PRR), BRI1-ASSOCIATED RECEPTOR KINASE (BAK1), and SUPPRESSOR OF BIR1 (SOBIR1) signaling pathways [61]. It has also been observed that EVs proliferate and are secreted by bacterially infected plants. *Pseudomonas syringae*-infected *A. thaliana* cells secrete more EVs than uninfected cells [62]. A mechanism for the eukaryotic miRNA control of bacterial growth has been proposed [62]. These observations suggest two-way communication between plants and their bacterial pathogens, but the exact underlying mechanisms have not yet been experimentally addressed. An epigenetic component on both fronts is likely to be at play.

Plant–pathogen communication can also result in duping the host plant to not react to the infection and instead orchestrate a pathophysiological mechanism to the advantage of the pathogen. *Agrobacterium tumefaciens* is capable of inter-kingdom gene transfer, evades host immune responses, and uses the eukaryotic epigenetic machinery to its advantage. Its highly divergent N-terminal domain of flagellin is not recognized by the receptor kinase FLS2 [63], while the highly conserved microbial peptide elf26, which is recognized by the receptor kinase EFR that is typically associated with triggered innate immunity, has minimal effect in this regard during *A. tumefaciens* infection of *A. thaliana* [64]. The pathogen-encoded oncogenes *iaaH* and *iaaM* are located on the Ti plasmid, and their enzyme activity results in the production of the phytohormone auxin [65] which increases T-DNA transfer efficiency, stable transformation [66], and crown gall growth [67]. Auxin-induced gene expression is modulated by miRNA as well as by other epigenetic factors such as histone modifications and chromatin remodeling [68]. *A. tumefaciens* was also shown to influence the expression and promoter methylation of most DNA repair genes in *A. thaliana*. Moreover, the promoter methylation of at least three genes, namely *CEN2*, *RAD51*, and *LIG4*, showed transgenerational memory [69].

Several plant pathogen (and symbionts) genomes encode proteins with **SET domains**. These domains modify chromatin structure by affecting histone methylation. Some examples of plant pathogens that produce SET domain proteins include *Acidovorax citrulli* AAC00-1, *Xanthomonas oryzae*, and *Ralstonia solanacearum* [70]. The mode of transfer of these proteins between the bacterial pathogen and host plant, as well as the molecular mechanisms for overcoming host defenses, remain elusive.

Plant symbiotic interactions

Epigenetic modifications seem to be important for the interplay between plants and symbionts that can form a super-organism, termed a 'holobiont' or holobiome (Box 4). For example, in

Box 4. The concept of holobiome

Many, if not most, organisms have 'outsourced' some of their functions to a set of symbionts acquired 400 Myr ago [105]. What we usually consider to be an 'individual' may be a multispecies group that is under selection; the combination of the host and symbiotic genomes is called the hologenome, and this assemblage of organisms is the holobiome. It has been demonstrated that epigenetic mechanisms play important roles in the holobiome generated by symbiosis between plants and microorganisms (rhizobia and mycorrhizae) [71,72]. Plant symbionts are responsible for nitrogen and phosphorus fixation, affect plant–plant interactions, and protect against challenges [106]. For example, orchid seeds contain no energy reserves, and only those that find a fungal partner germinate [107], whereas in the ryegrass *Achnatherum robustum* an endophytic fungus produces a compound that prevents insects and nematodes from eating the grass [108]. Symbiotic fungi can be shared (called common mycorrhizal networks) and transfer resources [109]. Hence, holobionts can survive harsh conditions that neither partner could survive alone. However, it is largely unclear which key molecular players bring together the holobiome. Symbionts can be found all over a plant, but roots are the best-studied system. Roots synthesize, accumulate, and secrete a wide range of compounds into the rhizosphere, the 'root exudates', that can help in assembling the holobionts. Root exudates shape the soil bacterial community by attracting for example plant growth-promoting rhizobacteria that produce a complex blend of volatile species-specific substances [110]. The exudates are rich in water, enzymes (e.g., proteases), H^+ ions, mucilage, and primary and secondary metabolites [111,112]. Some of these volatiles stimulate plant growth, suppress disease, or antagonize phytopathogens, nematodes, or insects [113].

nodulation during *Rhizobium* symbiosis in *Medicago truncatula* [71], the demethylase DEMETER (MtDME) is involved in regulating genes that modulate plant and bacterial cell differentiation for nodule organogenesis [71,72]. *MtDME* suppression prevents nodule formation and leads to deregulation of nodulation-related genes. Further genetic studies in poplar (*Populus tremula* × *Populus alba*) and its ectomycorrhizal fungus *Laccaria bicolor* provided links between DNA methylation and symbiosis [73]. In poplar, overexpression of DEMETER-LIKE (DML) or suppression of the chromatin remodeler DECREASED IN DNA METHYLATION 1 (DDM1), that deregulate DNA methylation [74], induced differential gene expression and TE hypomethylation. The deregulated DNA methylation correlated well with reduced mycorrhization rates in hypomethylated poplar lines. Candidate target genes differentially methylated were those involved in root initiation, immunity, hormones (i.e., ethylene and jasmonate), and terpenoid metabolism. In the case of arbuscular mycorrhizal symbiosis, increased DNA methylation levels occur in the seeds of *Geranium sylvaticum*, and in the roots and leaves of *Geranium robertianum*, in symbiosis with *Funneliformis mosseae* [75]. By contrast, arbuscular mycorrhizal fungi colonization in sunflower (*Helianthus annuus*) led to the activation of specific TEs, suggesting loss of their suppressive DNA methylation [76]. Remarkably, in the same study differentially methylated genes and TEs, as well as gene expression data, were identified for the fungus. Similarly, DNA methylation of adenine bases (in GANTC sites) in the root symbiotic bacterium *Mesorhizobium loti*, that is important for nitrogen fixation in legumes, changes the process of symbiosis with their host plants [77]. Although the adenines in most GANTC sites are methylated in *M. loti*, there are sites in the genome that remain unmethylated, and these are known as specifically unmethylated regions (SUMs) [78]. Intriguingly, SUMs become methylated during the process of symbiosis. These epigenetic modifications of the *M. loti* genome promote nodulation, although the pattern of DNA methylation is not essential for the establishment of plant–microbe symbiosis [78].

Apart from its role in symbiosis, the epigenetic status of the host may also influence other types of plant–microbe associations. To date there does not seem to have been any systematic investigation of such associations, but recent studies in this area provide intriguing insights into 'how methylation moulds microbiomes' [79]. Near-isogenic *A. thaliana* regenerated via somatic embryogenesis carrying epialleles related to regeneration of the tissue of origin showed significantly different associations with bacteria [80]. Myo-inositol production is under the antagonistic regulation of *REPRESSOR OF SILENCING 1* (*ROS1*) and **RNA-directed DNA methylation (RdDM)**, and active demethylation of its biosynthesis genes is required for the establishment of

beneficial *Bacillus megaterium* in the rhizosphere of *A. thaliana* [81]. Furthermore, similar genes to those reported for symbiosis were identified in a genome-wide association study (GWAS) in *A. thaliana*, where loci responsible for defense and cell-wall integrity affected the composition of the microbial community [82]. *LONG-CHAIN ACYL-COA SYNTHETASE* (*lacs*) and *PERMEABLE CUTICLE1/ABCG32* (*pec1*) mutants that affect the wax polymer cuticle, as well as the ethylene-signaling regulator *ETHYLENE INSENSITIVE 2* (*ein2*), modulate symbiosis [83].

We know little about histone modifications with relevance to symbiosis in plants. In soybean nodules, the histone modification H3K4me3 associates with gene expression of nodule-specific genes [84]. In addition, the ratio of H3K27me3 to histone H3 lysine 9 acetylation (H3K9ac) levels is associated with gene expression levels [72]. Moreover, four histone marks (H3K9ac, H3K9me2, H3K27me1, and H3K27me3) in roots and nodules highlighted the differential distributions of these marks during nodule development in *Medicago* [75].

Concluding remarks and future perspectives

Understanding how plants memorize encounters and stressful events will have important applications in agriculture such as the development of transgenerational stress priming that will contribute to creating climate-smart crops (see Outstanding questions). At present, epigenetic markers are almost absent from molecular marker selection protocols; however, given that epigenetic mechanisms are well known to be involved in plant priming, it is urgent to implement this aspect. Finally, shedding light on plant epigenetic mechanisms will also clarify the role and function of previously identified genes that modulate stress responses.

Understanding how plants communicate with each other about their previous encounters or stressful experiences seems at first glance to be only a fundamental scientific question. However, this is not true – the identification of both genetic and epigenetic alleles that modulate these plant capabilities will allow selection for crop genotypes with enhanced capability to 'sense' the nearby environment and enable the development of climate-smart crops (see Outstanding questions). The selected genotypes will need less 'care', which means that less chemical inputs (phytochemicals) and less irrigation will be necessary, thereby enhancing agricultural sustainability in terms of environmental protection and human health. In addition, the identification of molecular mechanisms involved in crop stress priming will allow plants to be primed for use as 'inoculating plants' to distribute stress tolerance to unprimed neighboring plants. In particular, future approaches should aim at integrating big data science with artificial intelligence models. These research methodologies will allow us to develop models and future tools to predict plant behaviors in response to climate change for the enhancement of precision agriculture.

Outstanding questions

How widespread is the transfer of nucleic acids and other chemicals that affect the epigenome during communication between plants and between plants and other organisms?

Is communication via physical signals, such as touch, sound, or ultrasound, another common means of biocommunication in plants? Does this form of communication act ultimately on the epigenome?

Is 'conversation or dialogue' via chemical or physical signals a widespread plant physiological process beyond signaling for defense or symbiosis, and, if so, why does it take place?

What is the role of sRNAs, acting across eukaryotic kingdoms, in interactions between plants and microorganisms?

How do plants and microorganisms identify each other as 'friends' or 'foes'?

For how long does a plant remember an inter- or intraspecific communication; in other words, how long do plant epigenetic modifications triggered by biocommunication last?

What is the role of plant communication in acclimatization and even in evolution? Does this depend on the role of the given epigenetic modification and the resulting gene expression change in increasing plant fitness?

How will the identification of epigenetic marks associated with transgenerational transmission of stress experience allow the delivery of future next-generation molecular markers for breeding?

How is the entire crop holobiome modified by plant encounters? How can it be used for creating crops that are more resilient to climate change by optimizing the application of natural microbe-based biostimulants?

Author contributions

F.M.: conceptualization of the study. J.D., F.M., P.G., M.M.J.B., D.R.A., and P.N.M prepared the original draft. J.D.: finalized, reviewed, and edited the submitted version. J.D., F.M., P.G., M.M.J.B., D.R.A., and P.N.M. created the figures.

Acknowledgments

This article is based upon the work of the COST (European Cooperation in Science and Technology; www.cost.eu) action EPI-CATCH (CA19125). Project TKP2021-EGA-20 has been (J.D.) implemented with the support provided by the Ministry of Culture and Innovation of Hungary from the National Research, Development, and Innovation Fund financed under the TKP2021-EGA funding scheme.

Declaration of interests

The authors declare no competing interests.

References

1. Galviz, Y.C.F. *et al.* (2020) Yes, plants do have memory. *Theor. Exp. Plant Physiol.* 32, 195–202
2. Huang, C.-Y. and Jin, H. (2022) Coordinated epigenetic regulation in plants: a potent managerial tool to conquer biotic stress. *Front. Plant Sci.* 12, 795274
3. Tokeshi, M. and Arakaki, S. (2012) Habitat complexity in aquatic systems: fractals and beyond. *Hydrobiologia* 685, 27–47
4. Jung, J. *et al.* (2020) Sound vibration-triggered epigenetic modulation induces plant root immunity against *Ralstonia solanacearum*. *Front. Microbiol.* 11, 1978
5. Khait, I. *et al.* (2023) Sounds emitted by plants under stress are airborne and informative. *Cell* 186, 1328–1336
6. Mladenov, V. *et al.* (2021) Deciphering the epigenetic alphabet involved in transgenerational stress memory in crops. *Int. J. Mol. Sci.* 22, 7118
7. Kakoulidou, I. *et al.* (2022) Epigenetics for crop improvement in times of global change. *Biology* 10, 766
8. Yan, Y. and Ham, B.-K. (2022) The mobile small RNAs: important messengers for long-distance communication in plants. *Front. Plant Sci.* 13, 928729
9. Lang, C. *et al.* (2022) *In silico* analysis of the sequence and structure of plant microRNAs packaged in extracellular vesicles. *Comput. Biol. Chem.* 101, 107771
10. Ruf, L. (2022) Spotlight on plant RNA-containing extracellular vesicles. *Curr. Opin. Plant Biol.* 69, 102272
11. Marzec, M. (2022) MicroRNA: a new signal in plant-to-plant communication. *Trends Plant Sci.* 27, 418–419
12. Cai, Q. *et al.* (2018) Plants send small RNAs in extracellular vesicles to fungal pathogen to silence virulence genes. *Science* 360, 1126–1129
13. Gallusci, P. *et al.* (2023) Deep inside the epigenetic memories of stressed plants. *Trends Plant Sci.* 28, 142–153
14. Koo, D.-H. *et al.* (2023) Extrachromosomal circular DNA-mediated spread of herbicide resistance in interspecific hybrids of pigweed. *Plant Physiol.* 193, 229–233
15. Zhuang, J. *et al.* (2024) Dynamics of extrachromosomal circular DNA in rice. *Nat. Commun.* 15, 2413
16. Zhang, P. *et al.* (2023) Extrachromosomal circular DNA and structural variants highlight genome instability in *Arabidopsis* epigenetic mutants. *Nat. Commun.* 14, 5236
17. Bonanomi, G. *et al.* (2022) Field evidence for litter and self-DNA inhibitory effects on *Alnus glutinosa* roots. *New Phytol.* 236, 399–412
18. Liu, C. *et al.* (2024) A proxitome-RNA-capture approach reveals that processing bodies repress coregulated hub genes. *Plant Cell* 36, 559–584
19. Mudge, K. *et al.* (2009) A history of grafting. *Hortic. Rev.* 35, 437–439
20. Berger, M.M.J. *et al.* (2018) Roles of epigenetic mechanisms in grafting and possible applications. *Adv. Bot. Res.* 88, 203–246
21. Zhang, Z. *et al.* (2016) Vascular-mediated signaling involved in early phosphate stress response in plants. *Nat. Plants* 2, 16033
22. Xia, C. *et al.* (2018) Elucidation of the mechanisms of long-distance mRNA movement in a *Nicotiana benthamiana*/tomato heterograft system. *Plant Physiol.* 177, 745–758
23. Yang, L. *et al.* (2019) m^5C methylation guides systemic transport of messenger RNA over graft junctions in plants. *Curr. Biol.* 29, 2465–2476
24. Heil, M. and Karban, R. (2009) Explaining evolution of plant communication by airborne signals. *Cell* 25, 137–144
25. Wang, N.-Q. *et al.* (2021) Root exudate signals in plant–plant interactions. *Plant Cell Environ.* 44, 1044–1058
26. Ali, M. *et al.* (2013) Memory of plant communications for priming anti-herbivore responses. *Sci. Rep.* 3, 1872
27. Onosatu, H. *et al.* (2022) Sustained defense response via volatile signaling and its epigenetic transcriptional regulation. *Plant Physiol.* 189, 922–933
28. Hussain, M. *et al.* (2023) Plants can talk: a new era in plant acoustics. *Trends Plant Sci.* 28, 987–990
29. Mishra, R.C. *et al.* (2016) Plant acoustics: in the search of a sound mechanism for sound signaling in plants. *J. Exp. Bot.* 67, 4483–4494
30. Dobránszki, J. (2021) Application of naturally occurring mechanical forces in in vitro plant tissue culture and biotechnology. *Plant Signal. Behav.* 16, 1902656
31. Matsumura, M. *et al.* (2022) Mechanosensory trichome cells evoke a mechanical stimuli-induced immune response in *Arabidopsis thaliana*. *Nat. Commun.* 13, 1216
32. Zhou, L.H. *et al.* (2017) The *Arabidopsis* trichome is an active mechanosensory switch. *Plant Cell Environ.* 40, 611–621
33. Demey, M.L. *et al.* (2023) Sound perception in plants: from ecological significance to molecular understanding. *Trends Plant Sci.* 28, 825–840
34. Hamant, O. and Haswell, E.S. (2017) Life behind the wall: sensing mechanical cues in plants. *BMC Biol.* 15, 59
35. Kim, J.Y. *et al.* (2023) Epigenetic regulation for delaying tomato fruit ripening through histone modification by specific sound wave treatment. *Postharvest Biol. Technol.* 197, 112211
36. Espinas, N.A. *et al.* (2016) Epigenetic control of defense signaling and priming in plants. *Front. Plant Sci.* 7, 1201
37. Son, J.-S. *et al.* (2024) Is plant acoustic communication fact or fiction? *New Phytol.* 242, 1876–1880
38. Picazo-Aragonés, J. *et al.* (2020) Plant volatile organic compounds evolution: transcriptional regulation, epigenetics and polyploidy. *Int. J. Mol. Sci.* 21, 8956
39. Baldwin, I.T. *et al.* (2006) Volatile signaling in plant–plant interactions: 'talking trees' in the genomics era. *Science* 311, 812–815
40. Xu, W. *et al.* (2016) UV-C-Induced alleviation of transcriptional gene silencing through plant–plant communication: key roles of jasmonic acid and salicylic acid pathways. *Mutat. Res.* 790, 56–67
41. Loreti, E. and Perata, P. (2022) Mobile plant microRNAs allow communication within and between organisms. *New Phytol.* 235, 2176–2182
42. Betti, F. *et al.* (2021) Exogenous miRNAs induce post-transcriptional gene silencing in plants. *Nat. Plants* 7, 1379–1388
43. Molnar, A. *et al.* (2010) Small silencing RNAs in plants are mobile and direct epigenetic modification in recipient cells. *Science* 328, 872–875
44. Swiezewski, S. *et al.* (2007) Small RNA-mediated chromatin silencing directed to the 3' region of the *Arabidopsis* gene encoding the developmental regulator, FLC. *PNAS* 104, 3633–3638
45. Knip, M. *et al.* (2014) Trans-kingdom cross-talk: small RNAs on the move. *PLoS Genet.* 10, e1004602
46. Skopelitis, D.S. *et al.* (2012) Plant small RNAs as morphogens. *Curr. Opin. Cell Biol.* 24, 217–224
47. Chen, X. (2009) Small, RNAs and their roles in plant development. *Annu. Rev. Cell Dev. Biol.* 25, 21–44
48. Weiberg, A. *et al.* (2013) Fungal small RNAs suppress plant immunity by hijacking host RNA interference pathways. *Science* 342, 118–123
49. Hou, Y. *et al.* (2019) A *Phytophthora* effector suppresses trans-kingdom RNAi to promote disease susceptibility. *Cell Host Microbe* 25, 153–165
50. Xiong, Q. (2014) *Phytophthora* suppressor of RNA silencing 2 is a conserved RxLR effector that promotes infection in soybean and *Arabidopsis thaliana*. *Mol. Plant-Microbe Interact.* 27, 1379–1389
51. Zhang, T. *et al.* (2016) Cotton plants export microRNAs to inhibit virulence gene expression in a fungal pathogen. *Nat. Plants* 2, 16153
52. Rutter, B.D. and Innes, R.W. (2018) Extracellular vesicles as key mediators of plant–microbe interactions. *Curr. Opin. Plant Biol.* 44, 16–22
53. Li, J. *et al.* (2005) Methylation protects miRNAs and siRNAs from a 3′-end uridylation activity in *Arabidopsis*. *Curr. Biol.* 15, 1501–1507
54. Yu, B. *et al.* (2005) Methylation as a crucial step in plant microRNA biogenesis. *Science* 307, 932–935
55. Bilir, Ö. *et al.* (2022) Small RNA-based plant protection against diseases. *Front. Plant Sci.* 13, 951097
56. Lejars, M. *et al.* (2019) Physiological roles of antisense RNAs in prokaryotes. *Biochimie* 164, 3–16

57. Chakravarty, S. and Massé, E. (2019) RNA-dependent regulation of virulence in pathogenic bacteria. *Front. Cell. Infect. Microbiol.* 9, 337
58. Kulp, A. and Kuehn, M.J. (2010) Biological functions and biogenesis of secreted bacterial outer membrane vesicles. *Ann. Rev. Microbiol.* 64, 163–184
59. Bose, S. *et al.* (2020) Extracellular vesicles: an emerging platform in gram-positive bacteria. *Microbial. Cell* 7, 312–322
60. Faulkner, C. and Robatzek, S. (2012) Plants and pathogens: putting infection strategies and defence mechanisms on the map. *Curr. Opin. Plant Biol.* 15, 699–707
61. Bahar, O. *et al.* (2016) Bacterial outer membrane vesicles induce plant immune responses. *Mol. Plant-Microbe Interact.* 29, 374–384
62. Wang, F. *et al.* (2014) *Arabidopsis* LIP5, a positive regulator of multivesicular body biogenesis, is a critical target of pathogen-responsive MAPK cascade in plant basal defense. *PLoS Pathog.* 10, e1004243
63. Felix, G. *et al.* (1999) Plants have a sensitive perception system for the most conserved domain of bacterial flagella. *Plant J.* 18, 265–276
64. Lee, C.W. *et al.* (2009) *Agrobacterium tumefaciens* promotes tumor induction by modulating pathogen defense in *Arabidopsis thaliana*. *Plant Cell* 21, 2948–2962
65. Thomashow, M.F. *et al.* (1986) Molecular basis for the auxin-independent phenotype of crown gall tumor tissues. *Science* 231, 616–618
66. Krens, F.A. *et al.* (1996) The effect of exogenously-applied phytohormones on gene transfer efficiency in sugarbeet (*Beta vulgaris* L.). *Plant Sci.* 116, 97–106
67. Gafni, Y. *et al.* (1995) Stimulation of *Agrobacterium tumefaciens* virulence with indole-3-acetic acid. *Lett. Appl. Microbiol.* 20, 98–101
68. Yamamuro, C. *et al.* (2016) Epigenetic modifications and plant hormone action. *Mol. Plant* 9, 57–70
69. Joseph, T.J. *et al.* (2021) Methylation status of *Arabidopsis* DNA repair gene promoters during *Agrobacterium* infection reveals epigenetic changes in three generations. *Plant Mol. Biol. Report.* 39, 773–791
70. Alvarez-Venegas, R. (2014) Bacterial SET domain proteins and their role in eukaryotic chromatin modification. *Front. Genet.* 5, 65
71. Satgé, C. *et al.* (2016) Reprogramming of DNA methylation is critical for nodule development in *Medicago truncatula*. *Nat. Plants* 2, 16166
72. Ramos-Cruz, D. *et al.* (2021) Epigenetics in plant organismic interactions. *Curr. Opin. Plant Biol.* 61, 102060
73. Vigneaud, J. *et al.* (2023) DNA hypomethylation of the host tree impairs interaction with mutualistic ectomycorrhizal fungus. *New Phytol.* 238, 2561–2577
74. Sow, M.D. *et al.* (2021) RNAi suppression of DNA methylation affects the drought stress response and genome integrity in transgenic poplar. *New Phytol.* 232, 80–97
75. Varga, S. and Soulsbury, C.D. (2019) Arbuscular mycorrhizal fungi change host plant DNA methylation systemically. *Plant Biol. (Stuttg)* 21, 278–283
76. Vangelisti, A. *et al.* (2019) Arbuscular mycorrhizal fungi induce the expression of specific retrotransposons in roots of sunflower (*Helianthus annuus* L.). *PLoS One* 14, e0212371
77. Asgari, S. (2014) Epigenetic modifications underlying symbiont–host interactions. *Adv. Genet.* 86, 253–276
78. Mouammine, A. and Collier, J. (2018) The impact of DNA methylation in Alphaproteobacteria. *Mol. Microbiol.* 110, 1–10
79. Wilkinson, S.W. and Ton, J. (2020) Methylation moulds microbiomes. *Nat. Plants* 6, 910–911
80. Wibowo, A. *et al.* (2018) Partial maintenance of organ-specific epigenetic marks during plant asexual reproduction leads to heritable phenotypic variation. *PNAS* 115, E9145–E9152
81. Vílchez, J.I. *et al.* (2020) DNA demethylases are required for myo-inositol-mediated mutualism between plants and beneficial rhizobacteria. *Nat. Plants* 6, 983–995
82. Horton, M.W. *et al.* (2014) Genome-wide association study of *Arabidopsis thaliana* leaf microbial community. *Nat. Commun.* 5, 5320
83. Bodenhausen, N. *et al.* (2014) A synthetic community approach reveals plant genotypes affecting the phyllosphere microbiota. *PLoS Genet.* 10, e1004283
84. Paungfoo-Lonhienne, C. *et al.* (2010) DNA is taken up by root hairs and pollen, and stimulates root and pollen tube growth. *Plant Physiol.* 153, 799–805
85. Cartenì, F. *et al.* (2016) Self-DNA inhibitory effects: underlying mechanisms and ecological implications. *Plant Signal. Behav.* 11, e1158381
86. Levy-Booth, D.J. *et al.* (2021) Genomics and metatranscriptomics of biogeochemical cycling and degradation of lignin-derived aromatic compounds in thermal swamp sediment. *ISME J.* 15, 879–893
87. Barbero, F. (2016) Extracellular self-DNA (esDNA), but not heterologous plant or insect DNA (etDNA), induces plasma membrane depolarization and calcium signaling in lima bean (*Phaseolus lunatus*) and maize (*Zea mays*). *Int. J. Mol. Sci.* 17, 1659
88. Duran-Fòlores, D. and Heil, M. (2018) Extracellular self-DNA as a damage-associated molecular pattern (DAMP) that triggers self-specific immunity induction in plants. Brain. *Behav. Immun.* 72, 78–88
89. Cookson, S.J. and Ollat, N. (2013) Grafting with rootstocks induces extensive transcriptional re-programming in the shoot apical meristem of grapevine. *BMC Plant Biol.* 13, 147
90. Karaca, M. and Ince, A.G. (2024) Grafting based DNA methylation alteration of snoRNAs in upland cotton (*Gossypium* L.). *Physiol. Mol. Biol. Plant* 30, 893–907
91. Cerruti, E. *et al.* (2021) Grafting vigour is associated with DNA de-methylation in eggplant. *Hortic. Res.* 8, 241
92. Han, Q. *et al.* (2022) Integrated DNA methylation, transcriptome and physiological analyses reveal new insights into superiority of poplars formed by interspecific grafting. *Tree Physiol.* 42, 1481–1500
93. Liu, K. *et al.* (2023) The role of DNA methylation in the maintenance of phenotypic variation induced by grafting chimerism in *Brassica*. *Hortic. Res.* 10, uhad00891
94. Melnyk, C.W. *et al.* (2011) Mobile 24 nt small RNAs direct transcriptional gene silencing in the root meristems of *Arabidopsis thaliana*. *Curr. Biol.* 21, 1678–1683
95. Axtell, M.J. (2013) Classification and comparison of small RNAs from plants. *Annu. Rev. Plant Biol.* 64, 137–159
96. Borges, F. and Martienssen, R.A. (2015) The expanding world of small RNAs in plants. *Nat. Rev. Mol. Cell Biol.* 16, 727–741
97. Ham, B.-K. *et al.* (2014) Systemic delivery of siRNA in pumpkin by a plant PHLOEM SMALL RNA-BINDING PROTEIN 1–ribonucleoprotein complex. *Plant J.* 80, 683–694
98. Lin, T. *et al.* (2015) Transcriptional analysis of phloem-associated cells of potato. *BMC Genomics* 16, 665
99. Kasai, A. *et al.* (2016) Epigenome editing of potato by grafting using transgenic tobacco as siRNA donor. *PLoS One* 11, e0161729
100. Bai, S. *et al.* (2011) A mobile signal transported over a long distance induces systemic transcriptional gene silencing in a grafted partner. *J. Exp. Bot.* 62, 4561–4570
101. Melnyk, C.W. *et al.* (2011) Intercellular and systemic movement of RNA silencing signals. *EMBO J.* 30, 3553–3563
102. Rubio, B. *et al.* (2022) Small RNA populations reflect the complex dialogue established between heterograft partners in grapevine. *Hortic. Res.* 9, uhab067
103. Liang, D. *et al.* (2012) Gene silencing in *Arabidopsis* spreads from the root to the shoot, through a gating barrier, by template-dependent, nonvascular, cell-to-cell movement. *Plant Physiol.* 159, 984–1000
104. Hidvégi, N. *et al.* (2022) Ultrasound, as a hypomethylating agent, remodels DNA methylation and alters mRNA transcription in winter wheat (*Triticum aestivum* L.) seedlings. *Physiol. Plant.* 174, e13777
105. Rodriguez, R. and Redman, R. (2008) More than 400 million years of evolution and some plants still can't make it on their own: plant stress tolerance via fungal symbiosis. *J. Exp. Bot.* 59, 1109–1114
106. Reynolds, H.L. *et al.* (2003) Grassroots ecology: plant–microbe–soil interactions as drivers of plant community structure and dynamics. *Ecology* 84, 2281–2291
107. Waterman, R.J. and Bidartondo, M.I. (2008) Deception above, deception below: linking pollination and mycorrhizal biology of orchids. *J. Exp. Bot.* 59, 1085–1096

108. Faeth, S.H. *et al.* (2006) Temporal and spatial variation in alkaloid levels in *Achnatherum robustum*, a native grass infected with the endophyte Neotyphodium. *J. Chem. Ecol.* 32, 307–324
109. Selosse, M.A. *et al.* (2006) Mycorrhizal networks: des liaisons dangereuses? *Trends Ecol. Evol.* 21, 621–628
110. Backer, R. *et al.* (2018) Plant growth-promoting rhizobacteria: context, mechanisms of action, and roadmap to commercialization of biostimulants for sustainable agriculture. *Front. Plant Sci.* 9, 1473
111. Tornkvist, A. *et al.* (2019) Proteolysis and nitrogen: emerging insights. *J. Exp. Bot.* 70, 2009–2019
112. Upadhyay, S.K. *et al.* (2022) Root exudates: mechanistic insight of plant growth promoting rhizobacteria for sustainable crop production. *Front. Microbiol.* 13, 916488
113. Ho, Y.-N. *et al.* (2017) Plant–microbe ecology: interactions of plants and symbiotic microbial communities. In *Plant Ecology – Traditional Approaches to Recent Trends* (Zubaida, Y., ed.), pp. 000–000, IntechOpen