Trends in Plant Science

CellPress

Opinion

Deep inside the epigenetic memories of stressed plants

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Recent evidence sheds light on the peculiar type of plant intelligence. Plants have developed complex molecular networks that allow them to remember, choose, and make decisions depending on the stress stimulus, although they lack a nervous system. Being sessile, plants can exploit these networks to optimize their resources cost-effectively and maximize their fitness in response to multiple environmental stresses. Even more interesting is the capability to transmit this experience to the next generation(s) through epigenetic modifications that add to the classical genetic inheritance. In this opinion article, we present concepts and perspectives regarding the capabilities of plants to sense, perceive, remember, re-elaborate, respond, and to some extent transmit to their progeny information to adapt more efficiently to climate change.

Highlights

Molecular mechanisms underlying the 'intelligence' of plants are far from being fully understood while they are under a profound debate in the scientific community.

The contribution of the different types of epigenetic machinery (DNA methylation, chromatin remodeling, and RNA-mediated regulation) to data perception, storage, elaboration, and transmission needs to be elucidated.

Epigenetic modifications are inherited through mitosis and in part through meiosis, thereby contributing to the long-term adaptation of plant species to climate change.

Light shed on mechanisms of plant stress memories allows to define, piece by piece, an epigenetic alphabet of plant responses to environmental changes.

The epigenetic 'orchestra' is played by hundreds of molecular players (writers, readers, erasers) that operate in a fine-tuned, coordinated manner to contribute to the symphony of plant intelligence even in the absence of a nervous system.

The 'intelligent' behavior of plants necessitates memory

There is accumulating evidence that plants, although lacking cognitive abilities, can learn, communicate, memorize, and develop decision-making circuits in a stimulus-dependent way [1,2]. As such, they can modify their behavior when facing adverse environments, which led to the idea that plants have developed a specific type of 'intelligence' [3–5]. Plants can **acclimate** (see Glossary) and adapt to an ever-changing environment to optimize their **fitness** [1,5], and may transmit what they have learned to their offspring [6].

However, the ability to learn relies on the development of precise memory mechanisms [2,7]. In contrast to animals, which have cognitive abilities based on neural structures and mechanisms, plant memory is exclusively based on cellular, molecular, and biochemical networks (metabolic, genetic, and **epigenetic** memories; see Box 1) that allow storing, retrieving, recalling, and eventually erasing information [8,9]. Plants possess a **somatic memory** that can last for some time during the life of an individual plant, and is maintained through mitosis [10], but there is also increasing evidence of long-lasting memories with information transmitted to one or more subsequent generations [6,11,12]. In this context, **epigenetic mechanisms** have drawn attention because they can mediate the learncing, storage, and transmission of information without modification in the DNA sequences [13,14]. As such, these modifications, which constitute an **epigenetic alphabet**, orchestrate the response of plants to their environment (Box 2) and are essential actors in the **priming** phenomenon (Box 1). Epigenetic modifications are also key elements of the molecular mechanisms underlying plant memory, as well as of the ability of plants to forget, and therefore appear as an essential component of plant intelligence. The memory of stress starts from sensing and reacting through the activation of both genetic and epigenetic mechanisms that are inherited, to allow **adaptation** to the environmental changes and evolution of the plant species [15]. Among the different mechanisms that have been suggested to be involved in plant cell memories (Box 1), here we focus on those involving epigenetic processes.

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Box 1. Mechanisms underlying priming in plants

As sessile organisms, plants need to cope with environmental stresses that in most cases occur in a recurring way. Plant priming (also referred to as plant hardening or acclimation) describes the capacity of plants to respond in faster and/or more intense ways to biotic [54,59,75,76] and abiotic stresses ([77] and references therein) after a first exposition to a milder stress [78] or to eliciting molecules [79,80]. Priming effects are at the phenotype level and are not associated with changes in DNA sequence, indicating that it is a reversible phenomenon. Typically, priming encompasses (i) the plant response to a first stress or to eliciting molecules that initiates a cellular memory, (ii) a recovery period, and (iii) a second stress that triggers the cell memory and leads to an enhanced/faster stress response. The ability of plant cells to memorize stresses has been associated with different mechanisms, including the accumulation of signaling molecules or metabolites, the modifications of regulatory proteins, and with epigenetic processes such as DNA methylation and histone post-translational modifications (HPTMs) [77,81]. Accumulation of key metabolites that may last after stress recovery has recently led to the idea that metabolite imprints may prepare the plants for subsequent adverse conditions [82]. The phosphorylation of mitosis-activated protein kinases that accumulate together with benzodiadazole was shown to mediate the priming of arabidopsis (*Arabidopsis thaliana*) against *Pseudomonas syringae* [83]. However, other signaling proteins such as leucine-rich receptor kinases, secondary messengers, and the regulation of specific transcription factors or glycosylated hormones such as salicylic acid (SA) glucosides have also been involved [84,85]. More recently, epigenetic mechanisms have emerged as major players contributing to the primed state of plants after stress exposure (reviewed in [77,81]), as they carry important aspects of the plant cell memory [52]. Initially described in the context of vernalization, such memory effects also exist after environmental stresses [86] as well as after pathogen interactions [87]. Yet their duration is variable and depends on the epigenetic mechanisms and type of stress [88]. Although the relative importance of the mechanisms underlying priming may vary with time and type of stress, priming embodies a plant stress memory, which allows plants to better respond to repetitive stresses with an eventual transmission to their progeny and may improve their survival rate or performance at the individual and population level in environmentally changing conditions [88] (Figure I).

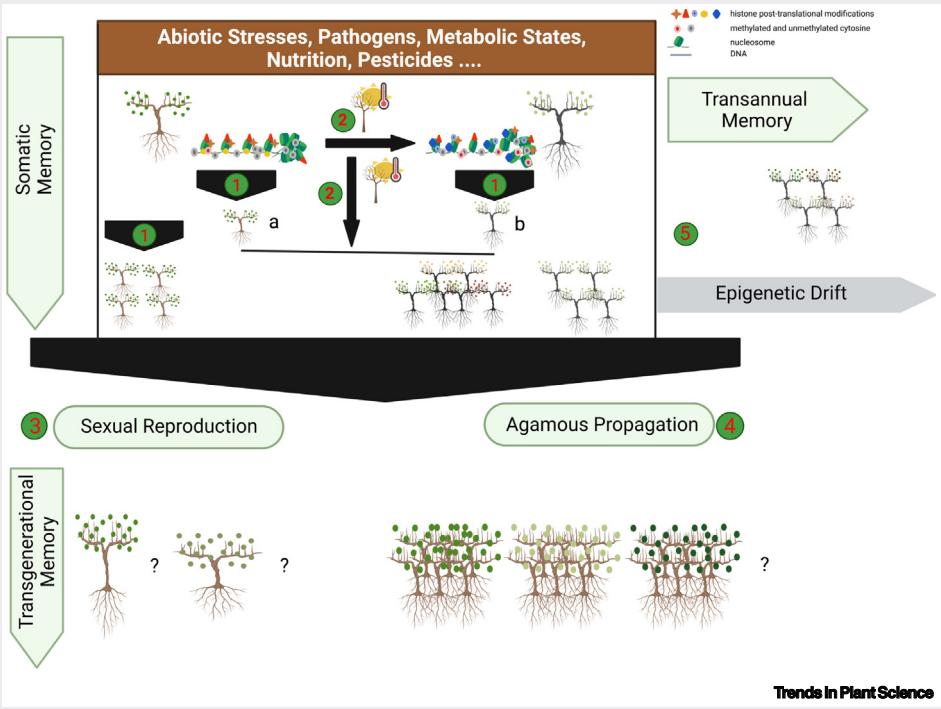

Figure I. The epigenetic landscape of plant organs is determined during plant development and will be maintained during mitosis (1). Environmental cues will impact the distribution of epigenetic marks (2), leading to a primed state of plants (b) that will react in a different way from naive plants (a) to subsequent changes of environmental conditions. Part of these epigenetic/genetic changes may be transmitted to subsequent generations. The transmission of epigenetic imprints depends on the type of reproduction and seems to be more efficient for agamous (4) than for sexual reproduction (3). In addition, perennial plants may maintain over years part of their epigenetic landscape (5) as determined by their growing conditions, which may generate an epigenetic drift. Figure created with BioRender.com.

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Box 2. An epigenetic alphabet of plant responses to stress

Although there has been much progress to unravel the genetic response to environmental stresses such as drought, heat, cold, salt, and pathogen attacks, knowledge of the epigenetic regulatory networks of plant responses to these stresses remains in its infancy. So far, many epigenetic players (proteins, RNA) have been identified that determine elements (histone modifications and DNA methylation), or 'letters' forming the alphabet of the plant epigenetic responses to stresses. A complete alphabet is, however, far from being elucidated. Epigenetic marks are driven by hundreds of key proteins, which like in a piano, constitute an 'epigenetic keyboard' generating a highly complex picture that is further enriched by the presence of histone variants (Figure I). For example, histone deacetylases (HDACs) deacetylate histones and act in coordination with histone acetyltransferases to control the highly dynamic patterns of histone acetylation, and thereby chromatin structure and gene expression to coordinate the multiple cellular processes that underlie plant responses to abiotic stresses (see Figure 1 in main text; [75]). Additional chromatin mark regulators are involved in other HPTMs (methylation, phosphorylation, ubiquitination; see Figure 1 in main text).

Similarly, DNA methylation which occurs essentially at cytosines to form 5-methylcytosine (5mC), the other main epigenetic mark, is controlled by various DNA methyltransferases and demethylases [DNA glycosylase lyase, also called demeter like (DML)] that establish, maintain, and erase cytosine methylation in the CG, CXG, CHH sequence context (where X = A, C, G, T and H = A, T, G). DNA methylation at adenines to form (N6-methyladenine (6mA) has also been described [54]). Among these processes, the RNA-directed DNA methylation (RdDM) pathway is a molecular process that controls *de novo* DNA methylation, in which noncoding RNA directs DNA methylation at specific DNA sequences. Together with DNA demethylation, RdDM plays essential functions in modulating the activity of transposable elements (TEs) and gene expression both in the context of plant development and in response to environmental challenges (reviewed in [59]). The role of TEs in plants under stress seems important in the long-term adaptation of plants (reviewed in [69]). Both pathways are also involved in the response to abiotic and biotic stresses (reviewed in [4,70,89]).

The molecular network underlying the epigenetic responses to environmental stresses (drought, salt, temperature, pathogens) is complex and far to be elucidated. It is almost impossible to fully identify all key players involved in the different molecular 'roads' driving from the signal perception to the physiological effects. Key players are finely interconnected at genetic and epigenetic levels (Figure II). High temperature induced epigenetic responses through different mechanisms: methylations [5-methylcytosine (5mC) in the CG context, N6-methyladenine (6mA)], siRNAs driving RdDM, histone modifications (HD2C), upregulation of gene target expressions driving methylome changes (*DDM1*, *MOM1*, *MSH1*, *RPDs*) [11,55,77]. On the other hand, salt and drought stress (DS) modulate DNA methylations through the actions of key miRNAs [11], while the epigenetic changes in response to pathogen attacks have been widely described due to the activation of several key genes (*AGO4*, *VIM5*, *ROS1*) [54,81,90] (Figure II).

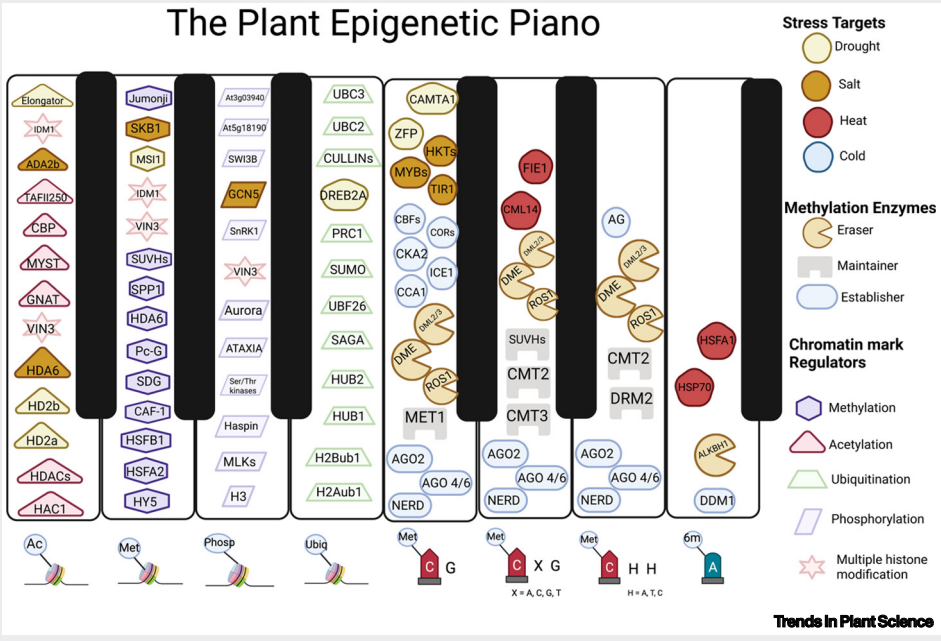

Figure I. Post-transductional modifications of histones and DNA methylation driven by methylation eraser, maintainer, and establisher enzymes in response to abiotic stresses (drought, salt, heat, cold) and biotic stresses. The type of proteins involved in each type of epigenetic modification is indicated. In a metaphoric way, epigenetic modifications are coordinated as music played by piano: fingers are the key proteins and epigenetic mechanisms are piano keys. For an explanation of abbreviations used in the figure please see the supplemental information online. Figure created with BioRender.com.

Glossary

Acclimation: a nonheritable modification for increasing the fitness of the organism.

Adaptation: a heritable modification enabling a species to become better fitted to its environment or mode of existence.

Chromatin remodeling: a change in the nucleosomal packaging, leading to a change in the accessibility of DNA for transcription or recombination.

DNA methylation: an epigenetic mechanism involving the reversible transfer of a methyl group onto the nitrogenous bases of adenine and cytosine, forming 5-methylcytosine (5mC), N^6-methyladenine (6mA), and N^4-methylcytosine. The former is the most common DNA methylation mark in plants. DNA methylation, which depends on the sequence contexts and can have different localization in genes, TEs, or other genomic features, may result in the silencing of transposons and the regulation of gene expression, for example, by interfering with the binding of transcription factors to promoter regions.

DNA methyltransferases: a conserved family of enzymes that mediate the transfer of a methyl residue on the fifth carbon of cytosines (5mC). There are three main types of 5mC methylases in plants depending on the sequence context. Additional methyl transferases have recently been identified that are required for the methylation of adenine. (Refer to Box 1 for further details.)

Epialleles: genomic regions that differ in the distribution and level of epigenetic marks between individuals, most often DNA methylation, and are stably transmitted to the next generations.

Epigenetic alphabet: a 'code' of epigenetic marks underlying modifications in any organism concerning developmental and environmental stimuli.

Epigenetic mechanisms: processes that establish heritable changes in phenotype without altering the underlying nucleotide sequence. These mechanisms include DNA methylation, chromatin remodeling, HPTMs, and regulatory noncoding RNAs.

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.

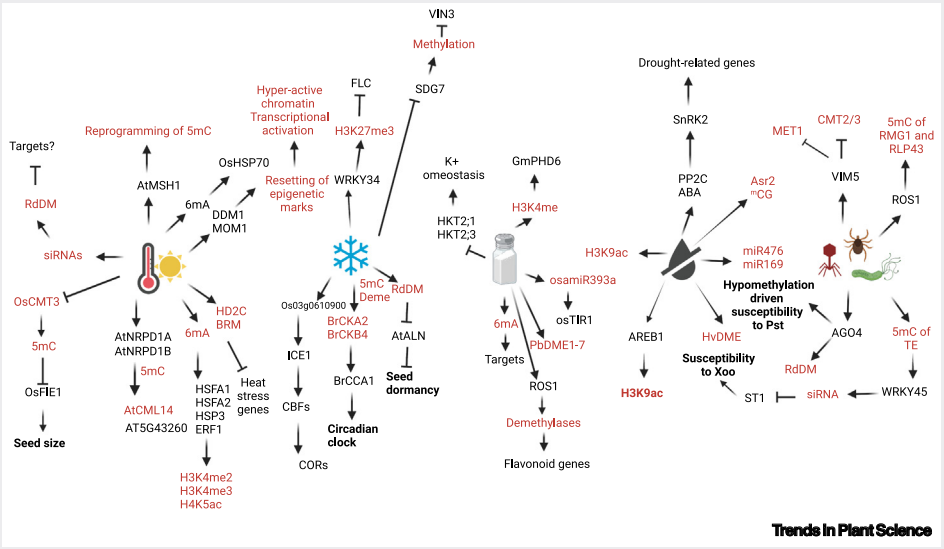

Figure II. Key genes and epigenetic marks involved in plant stress responses to four important abiotic stresses (high temperature, cold, salt, drought) and biotic stress. Some of the key players and networks are transcription factors, proteins, and epigenetic marks. These are shown for each type of stress. For an explanation of abbreviations used in the figure please see the supplemental information online. Figure created with BioRender.com.

Fitness: a measure of the relative breeding success in a given population, in specific conditions at a given time.
Histone post-translational modification: post-translational modification of amino acid residues (e.g., Lys, Tyr, Arg) located on the N-terminal tails of histone proteins that includes acetylation, phosphorylation, methylation, ubiquitylation, ADP-ribosylation, and sumoylation.
Intergenerational epigenetic transmission: the transmission of epigenetic marks from parents to their progeny through one generation only.
Priming: strategy/process whereby a plant is exposed to a single or multiple stresses in preparation for subsequent stress that occurs at later developmental stages.
Retrograde signaling: the process through which a signal travels backward from a target source to its source (i.e., from signaling proteins in the cytoplasm to the cell nucleus instead of the common way from the nucleus to the cytoplasm).
Somatic memory: short-term memory within cells transmitted by mitosis.
Stress memory: the ability of plants to retain changes and store information after stress exposure(s).
Transgenerational epigenetic transmission: the transmission of epigenetic marks for several generations.

Interplay between stress-induced epigenetic changes and stress adaptation

As sessile organisms, plants have evolved sophisticated mechanisms for sensing, responding, and adapting to constantly changing environments including light, temperature, water availability, or mechanical stimuli and pathogens [16]. Sensory systems, which determine the primary contact with the environment, are diverse and utilize various types of macromolecules ranging from specialized receptor proteins (photo- and thermosensors) to nucleic acids (RNA) and subcellular organelles (chloroplasts and mitochondria). Subcellular organelles perceive and transduce environmental (light quality, quantity, and direction, and temperature fluctuations) as well as biochemical signals, such as reactive oxygen species (ROS) and metabolic compounds [17,18].

A direct relationship between the ability to sense the environment and epigenetic remodeling and memory has now been demonstrated in plants. A striking example is provided by analyzing oxidative compounds and antioxidants such as ROS, NADPH, and glutathione in response to environmental stimuli. Stress-induced changes in signaling molecule abundance and distribution can be sensed by plastids and mitochondria that act as key integrating hubs through **retrograde signaling** (Figure 1) and mediate epigenetic changes in the nuclear genome [19]. Changes in ROS levels confer cross-tolerance to stresses by regulating the catalytic activity of histone deacetylases and histone/DNA demethylases, and therefore, the epigenetic landscape and gene expression profile may initiate epigenetic memory events [20]. An excellent example of a nuclear-encoded plastid protein regulating stress-induced transgenerational phenotypic plasticity is the *MUTS HOMOLOGUE 1* (*MSH1*). Abiotic stress factors downregulate the expression levels of *MSH1* that is involved in maintaining plastid genome integrity. Inhibition of *MSH1* leads to the production of plastid-derived stress signals that trigger nuclear events including genome-wide regulation of DNA methylation proximally to transposable elements (TEs) through the action of the *HISTONE DEACETYLASE 6* (*HDA6*) and *METHYLTRANSFERASE 1* (*MET1*) [21]. These events control the expression of clock, hormone, and stress genes, all of which confer transgenerational phenotypic plasticity.

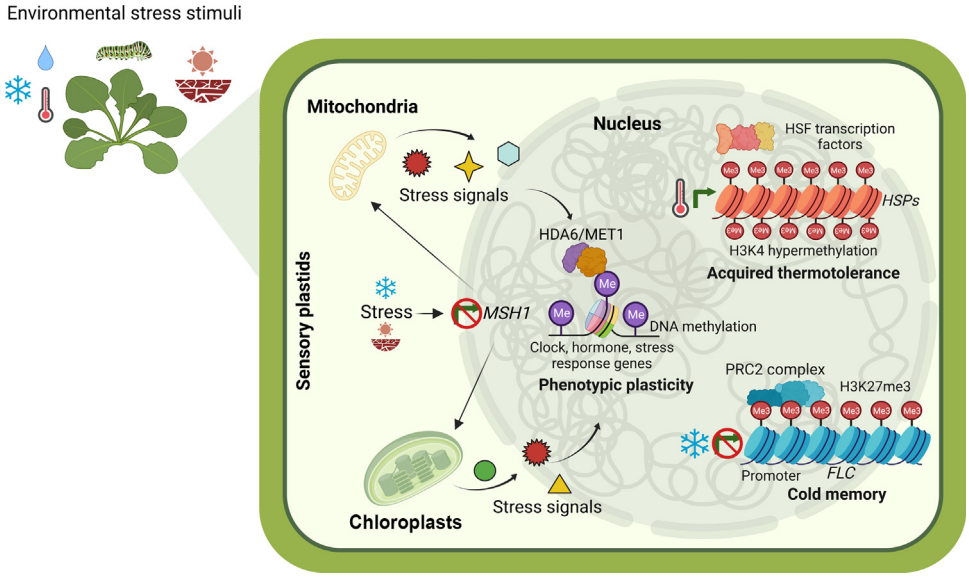

Figure 1. Epigenetic regulation of stress response memory in the plant cell. Abiotic stress factors downregulate the expression levels of the nuclear-encoded *MUTS HOMOLOGUE 1* (*MSH1*) that is involved in maintaining plastid genome integrity. Inhibition of *MSH1* leads to the production of plastid-derived stress signals that trigger nuclear events including genome-wide regulation of DNA methylation proximally to transposable elements (TEs) through the action of *HISTONE DEACETYLASE 6* (*HDA6*) and *METHYLTRANSFERASE 1* (*MET1*), and therefore controlling the expression of clock, hormone, and stress genes. High temperature induces histone H3K4 hypermethylation and *HEAT SHOCK TRANSCRIPTION FACTORS* (*HSFs*) that activate *HEAT SHOCK PROTEIN* (*HSP*) expression leading to acquired thermotolerance. Prolonged cold exposure results in the deposition and gradual spread of histone H3K27 trimethylation (me3) on *FLOWERING LOCUS C* (*FLC*) through the action of the Polycomb repressive protein complex (*PRC2*). *FLC* silencing induces flowering initiation and cold memory. Figure created with BioRender.com.

Plants also sense and respond to changes in light quality, intensity, and periodicity via specialized photoreceptor systems and light signaling components. Some of them (*PHYTOCHROME B, EARLY FLOWERING 3*) also act as thermosensors [22]. Photo- and thermo-adaptation involves major transcriptional reprogramming that is primarily mediated by changes in the nuclear architecture, such as **chromatin remodeling**, involving histone variant deposition (*H2AZ*, [23]); **histone post-transcriptional modifications** (HPTMs), such as H2B monoubiquitination [24] or histone acetylation [25]; and modifications of **DNA methylation** patterns [26]. Thermosensing is one of the best examples of plant acclimation that involves epigenetic regulation of heat shock factors and thermomorphogenesis genes through changes in histone marks and histone variant deposition. More specifically, epigenetic events including RdDM-mediated DNA methylation; histone acetylation on H3K9, H3K14, and H3K56 as well as H3K4 hypomethylation; and H2A.Z nucleosomal eviction contribute to plant adaptation to high temperatures (reviewed in [27]). Prolonged exposure to cold temperature, also referred to as vernalization, provides a direct link between environmental sensing and the establishment and resetting of an epigenetic somatic memory to regulate flowering initiation in arabidopsis (*Arabidopsis thaliana*). Elegant genetic and modeling studies on flowering regulation have uncovered a distinct temperature sensing mechanism where vernalization operates via a digital, polycomb-mediated epigenetic switch leading to the upregulation of *VERNALIZATION INSENSITIVE3 (VIN3)* and silencing of *FLOWERING LOCUS C (FLC)* [28]. In addition, recent reports showed that the epigenetic memory of vernalization is meiotically stable during female gamete formation and is maternally transmitted in arabidopsis before being reset during embryogenesis. This demonstrates an intergenerational transmission of the maternally imprinted *FLC* allele, the function of which remains to be deciphered [29].

Interactions between biotic and abiotic stress were also studied using recombinant inbred arabidopsis lines showing that hypomethylation at specific pericentromeric areas can confer disease resistance in a quantitative manner without impairing plant responses to abiotic stress [30]. In conclusion, multiple specialized, generic, distinct as well as shared sensing mechanisms operate to mediate the integration of environmental signals at the chromatin level and to promote plant adaptation to a constantly changing environment (Figure 1). Further investigation is now necessary for understanding how stress sensing systems interact with chromatin regulators to remodel the epigenetic landscape and initiate an epigenetic memory of the environment. In addition, the impact of epigenetic memory on the plant's ability to sense its environment (i.e., biotic attack) requires better understanding [27].

Transmission of the stress-induced epigenetic state during plant reproduction

The transmission of information from parents to offspring is critical to ensure the proper development of the newly formed organisms and optimize their chances of survival. In addition to the fundamental information provided through genetic inheritance, the question of the **transgenerational** and **intergenerational transmission** of stress-induced epigenetic changes seems essential to allow not only individual plant acclimation, but also adaptation of the plant species [31]. However, as the chromatin is extensively reorganized during mitosis and meiosis [32], the transmission of parental epigenetic states may require specific mechanisms that depend on the type of reproduction.

Is there a long-term stress memory transmitted during agamous propagation?

Epigenetic information may be transmitted during the life of a plant (somatic inheritance), as in priming (Box 1), and through vegetative propagation (clonal reproduction) during mitosis via the stem cells located in meristems. At mitosis, specific HPTMs, such as the condensed chromatin marker histone H3S10p, or the mitosis-specific HPTMs (e.g., H3T3p and H3T11p) are required [33]. Domains enriched in repressive histone marks such as H3K27me3 are stably maintained at mitosis, as, for example, during vernalization. In this case, the *FLC* gene gradually acquires H3K27me3, which is maintained during mitosis by a replication-coupled mechanism [34]. Incorporation of the methylated histone H3.1 into the newly formed chromatin during DNA replication also allows maintaining H3K27me3 at S phase [35]. In addition, it is clearly established that during cell division DNA methylation in symmetrical contexts (Box 2) is maintained through the activity of **DNA methyltransferases** in a postreplicative manner, while DNA methylation at the nonsymmetrical CHH sites depends on the presence of appropriate short interfering RNAs (siRNAs) and on the RdDM pathway [36]. Inheritance of DNA methylation after clonal propagation was demonstrated in arabidopsis plants regenerated *in vitro*, which retained part of the epigenetic landscape and physiological characteristics of the tissue of origin, showing that clonal lineages can retain epigenetic states [37]. Studies in poplar [38], white clover [39,40], and dandelion [41] also show clonal maintenance of stress-induced changes in DNA methylation levels or profiles. Additional studies analyzing the distribution and functional consequences of these clonally inherited epigenetic marks are now required, as well as an evaluation of their long-term stability.

Intergenerational and transgenerational epigenetic inheritance after sexual reproduction

Evidence that epigenetic information can be stably transmitted during sexual reproduction across generations was provided more than 20 years ago [42] and further demonstrated in arabidopsis epigenetic Recombinant Inbred Lines (EpiRILs) [43]. How sexual transmission of newly generated epigenetic imprints works, as those generated by stresses, however remains a matter of debate, because plants have evolved both male- and female-specific germline mechanisms that allow resetting and/or saving selected epigenetic information (reviewed in [44]). Since the early demonstration in arabidopsis of the transgenerational transmission of an epigenetic trait after UVC (ultraviolet C) treatment [45], there have been limited reports concerning the intergenerational or

transgenerational epigenetic memory of (a)biotic stresses. In arabidopsis, DNA methylation changes generated by osmotic stress were shown to be transmitted to the next generation, essentially through the female gamete [46]. Intergenerational but not transgenerational DNA methylation transmission was also observed in another study when a mild drought stress (DS) skipped a generation, in a setting that included four consecutive generations in five accessions and stress was applied before the reproductive stage [47]. Intergenerational epigenetic memory mediated by DNA methylation was further demonstrated in arabidopsis after spaceflight [48] or γ-rays irradiation [49] and in rice (*Oryza sativa*) under heavy-metal treatment [50] and DS [51]. The transmission of phenotypic traits was also observed in the latter case, but the DS was performed during embryo development which makes it difficult to separate an eventual parental transmission of epigenetic information from direct priming of the embryo. Of note, mechanisms involving both *DDM1* (*Decrease in DNA Methylation 1*) and *MOM1* (*Maintenance of Methylation 1*) prevent the transmission of stress-induced methylation imprints [52]. In addition, there is evidence of a parental bias that relies on the DNA (de)methylation machinery which erases methylation from male gametes only [46]. Hence, protective mechanisms may limit transmission of unwanted methylation marks that could, however, be transmitted in some cases depending on the stress organs and developmental stage.

Transgenerational mechanisms driving an increased resistance to pathogen attacks have also been identified. For example, *ddc* mutant has been involved in resistance to *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst* DC3000) but enhanced susceptibility against necrotrophic fungal pathogen *Plectosphaerella cucumerina* (*Pc*) [53]. In addition, *drd1* (AT2G16390) and *npe1* (AT2G40030) genes have been shown to be involved in resistance to *Hyaloperonospora arabidopsidis* [54]. Small RNAs (siRNA and miRNA) and noncoding RNAs are also involved in the memory of stresses. Concerning DS, this was demonstrated in durum wheat [55], with the involvement of miRNAs in the regulation of ROS scavenging, hormone signaling, and carbon fixation in the next generation, whereas a similar work in rice showed that memory genes involved in hormone synthesis are regulated by long noncoding RNAs [51]. Small RNAs, essentially miRNAs, are also participants in the intergenerational inheritance of heat stress in *Brassica napa* [56] and durum wheat [57]. The demonstration that both intergenerational inheritance of DNA methylation and a better adaptation to stresses was dependent on active *DCL* (*Dicer Like*) genes suggested that siRNAs also participate in an epigenetic memory of abiotic stresses [58] or herbivory attacks [59]. Of course, siRNAs play an important role in the programming of DNA methylation patterns in the germ cells, although in a noncell autonomous way. In the male lineage, siRNAs from the vegetative cell reinforce methylation in the male gametes, whereas siRNAs from the central cell are likely playing a similar role in the egg cell (reviewed in [44], and references therein). However, the demonstration that stress-induced changes in siRNA populations contribute to intergenerational epigenetic memory of these stresses has not been investigated. This would require a precise description of the impact of stresses on the small RNA populations in the different cell types of the gametophytes in relation to the DNA methylation profiles of gametes and progeny.

Regarding chromatin modifications, HPTMs also seem to participate in the transgenerational memory of heat stress [60], although the substitution of histones during zygote development [61], together with the loss of H3K27me3 reprogramming that allows differentiating male gametes and reprogramming the paternal HPTM landscape [62], would suggest that HPTMs are not the primary mechanism involved in epigenetic inter/transgenerational memory [44]. As for DNA methylation, much of the 'memory' carried by HPTMs is erased at male meiosis. As pollen can spread over long distances, 'forgetting' the father's environment and remembering the mother's one might be beneficial for the progeny depending on the seed dispersal strategy [62].

So far, there is no comprehensive view on an epigenetic intergenerational and transgenerational memory of stresses after sexual reproduction. Furthermore, such memories may vary as a function of stress type and intensity, duration, and period of stress during the plant life cycle. An overview of some important epigenetic marks transmitted at the filial generation or remaining only at the parental one is shown in Figure 2.

The role of epigenetics in plant transgenerational adaptation

Clearly, epigenetic variations contribute to the phenotypic plasticity of individual plants, and might be transmitted to one or more following generations, thus contributing to the adaptation of populations to variable environments. Over the last decade, several **epialleles** have been described, including both artificially induced [63] and naturally occurring [64] changes. The way epigenetic modifications are under selective pressure, and their contribution to plant evolution remains under debate [65]. It is well-accepted that there is a complex interplay between genetics and epigenetics in determining plant adaptation to the environment. For example, epigenetic diversity might be of primary importance in plant populations with limited genetic diversity, by providing a new source of phenotypic variations [13,66]. Overall, the rate of epigenetic modifications is about five orders of magnitude higher than the rate of genetic mutations [64], which results in a much higher epigenetic than genetic diversity and may therefore be of prime importance also for plants that propagate mainly by sexual reproduction. A critical aspect is, however, to evaluate the extent to which epigenetic variations can be fixed in a population because, as mentioned earlier, epigenetic variation may be erased in some situations, for example, at meiosis, or in the absence of stress. It has also been shown that epigenetic variations, such as those generated by changes in activity of the RdDM pathway [69,70], impact TE distribution, eventually leading to new regulatory networks and stress adaptation. Such an epigenetic/TE interplay may be an important driver to fix the consequences of epigenetic-induced stress response for long-term adaptation of plants [67]. This was nicely illustrated in a recent study using EpiRILs derived from a *ddm1* mutant, which showed enhanced TE transposition at genes involved in environmental changes, a process depending on the histone variant H2A.Z [68].

Taken together, these processes would allow adaptive phenotypes to develop and appear before any genetic change. Indeed, whether these epigenetic changes are fixed in a population and confer a selective advantage depends on their behavior under selection pressure [71]. Epigenetic mechanisms can maintain high phenotypic plasticity, and thereby allow plants to respond faster to environmental challenges than what is achievable via genetic changes [72]. From a practical point of view, this means that challenges triggered by climate change in agriculture may be tackled more efficiently and faster by selecting the important variability generated by the stress-induced epigenetic diversity in addition to genetic variability [73].

Concluding remarks and future perspectives

The definition of the epigenetic alphabet of the plant stress responses is far from completed (see Outstanding questions). Our knowledge of the role of different epigenetic marks in plant responses to environmental stresses is increasing but still fragmented and scattered. For example, the role in stress responses of epigenetic marks such as N^6-methyl-2′-deoxyadenosine remains unknown. It is urgent to elucidate how the different epigenetic mechanisms are activated and coordinated to obtain an integrative view of the functioning of an 'epigenetic orchestra' in response to stresses. To achieve this ambitious aim, some important challenges need to be addressed:

- The specific links between distribution of epigenetic marks, gene expression, activity of TEs, and phenotypes need to be elucidated to enable the development of new plant modeling approaches for stress-induced epigenetic–genetic regulatory networks.

Outstanding questions

How and which epigenetic and genetic processes need to work together to allow the plant to integrate information acquired from previous changes in environmental conditions?

How do the epigenetic and genetic mechanisms integrate information to play the 'epigenetic orchestra' of plant stress responses to environmental changes?

How do epimutations influence plant fitness at a population scale and modulate plant adaptation kinetics?

What are the complementary roles of other factors (i.e., genes, transcripts, proteins, metabolites, and epigenetic changes) in the selection pressure of the environment to define the best adapted (epi)genotypes to environmental stresses?

Which new progress in omic techniques might be exploited to uncover the role of epigenomic features in plant stress response mechanisms at an unprecedented resolution?

How can we exploit long-term epigenetic memory effects as effective applications to crop breeding?

How is the transgenerational (sexual or clonal) memory modulated in plants to optimize a balance between costs and trade-offs?

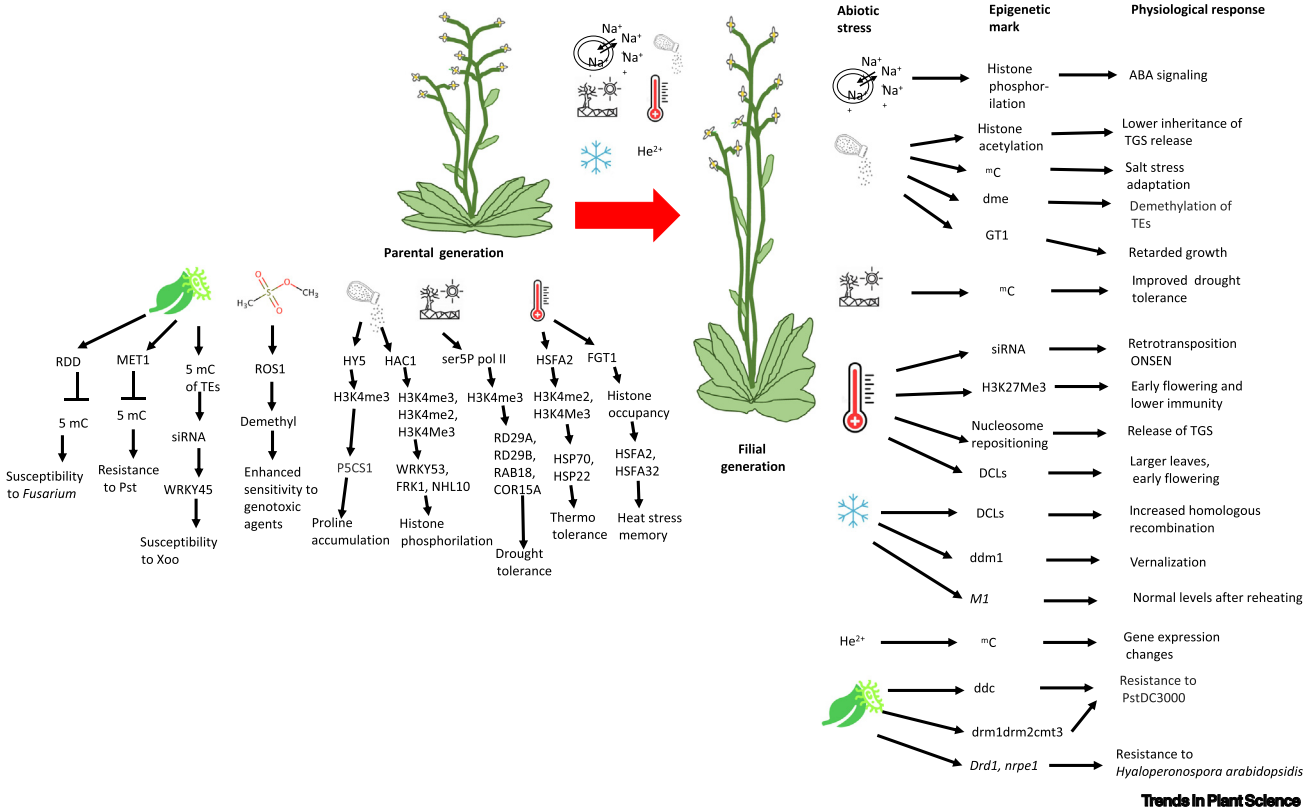

Figure 2. Epigenetic marks and/or genes involved in epigenetic changes at parental and filial stages in response to different abiotic stresses [osmotic, salt, drought, high temperature, cold, heavy metals (He^{2+}) stresses, pathogen attacks]. The physiological stress effects induced by the epigenetic marks and genes are indicated. Abbreviations: COR15A, cold regulated protein 15A; DCL, dicer-like; DDM1, decrease in DNA methylation 1; DME, demeter; DRD1, defective in RNA-directed DNA; FGT1, forgetter 1; FRK1, FLG22-induced receptor-like kinase 1; GT1, glycosyltransferase 1; H3K4Me2, H3 lysine 4 dimethylation; H3K4Me3, H3 lysine 4 trimethylation; HAC1, histone acetyltransferase of the CBP family 1; HSFA2, heat shock factor A2; HSFA32, heat shock transcription factor A32; HSP22, heat shock protein 22; HSP70, heat shock protein 70; HY5, elongated hypocotyl 5; MET1, methyltransferase 1; NHL10, arabidopsis NRD1/HIN1-like 10; NRPE1, defective in meristem silencing 5; RAB18A, Ras superfamily 18A; RD29A, responsive to desiccation 29 A; RD29A, responsive to desiccation 29 B; ROS1, repressor of silencing 1; Ser 5 Pol II, serine polymerase II; TGS, trimethylguanosine synthase.

- Effects of metabolic signaling of stresses on genome-wide epigenetic modifications and memory of these modifications to key phenotypic traits such as primary production need to be investigated.
- The heterogeneity of the epigenome is limiting the study of an epigenetic memory at all levels. There is an important need to use single-cell epigenomic and transcriptomic studies and single-molecule sequencing technologies to uncover epigenomic features at an unprecedented resolution.
- The transfer of epigenetic knowledge to breeding applications is needed to develop smarter crops, more adapted to climate change, pests, and pathogens. Specific quantitative epigenetic approaches, including the mapping of epiloci underlying specific phenotypes, epigenetic quantitative trait loci characterization, or epigenome-wide association studies, depending on population structure have to be implemented.
- How plants regulate the transgenerational and intergenerational memory during sexual reproduction to optimize cost–benefit and trade-offs needs to be deciphered.
- The understanding of the role of small RNA in inter/transgenerational memory of stresses is still not fully understood. Single-cell smRNA transcriptomic studies focusing on gametophytes (male and female) will be necessary to determine their potential role.
- There is a need for a precise analysis of the different types of plant somatic memory to distinguish mechanisms required at the individual level, those necessary for the transmission of epigenetic

information during agamous reproduction, and – for perennials – those involved in the transannual epigenetic memory of stresses.

- Integrative databases including epigenetic modifications are still in their infancy (e.g., iRegNet for arabidopsis [74]). They should integrate data over several generations in various systems to define the part of epigenetic information that is maintained over generations.
- Although the phenomenon of transgenerational **stress memory** protects offspring against the previously occurred stress, it is also likely that inter/transgenerational-induced resistance is associated with an increased susceptibility to other stresses. This antagonism needs to be well investigated to clarify the similar and specific pathways among different environmental stresses and how spatio-temporal patterning of epigenetic responses to each stress affect the specificity of stress memory.

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Declaration of interests

No interests are declared.

Supplemental information

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