The role of epigenetics in natural populations and somaclonal variation

by

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Summary

In nature, a wide variety of species can be found, all uniquely shaped to survive the environment they are subjected to. This variety of organisms owe their phenotypic variation to the genetic information shaping their phenotype. Even within a species or between the cells of one organism, where genetic diversity is limited, can we find phenotypic differences. These phenotypic differences between species and cells with similar genetic information find their origin in how the genetic information present is expressed. Epigenetics is an extra layer of gene regulation influencing gene expression on top of the available genotype. The main role of epigenetics is to regulate the availability of genetic information to transcription machinery, thereby influencing gene expression. Changes in epigenetics enable a plant to rapidly adapt to its environment without the need for genetic mutations, which happen less frequently than epigenetic mutations, thereby increasing its chance of survival. Epigenetics includes changes to DNA methylation, modifications of histones and can also work post-transcriptional. In natural populations, epigenetic changes enable a plant to adapt to stresses like drought and infection with a pathogen. Especially genetically uniform species, like most invasive species, use epigenetics to their advantage by adapting rapidly to their environments.

Genetic uniformity can also be found in some of our crops, like oil palm and bananas. These crops are made with a technique called tissue culture regeneration. With this technique, tissue from high-yielding plants is taken and regrown into multiple genetically identical plants. Because all plants are genetically identical to the high-yielding plant, all offspring possesses the same genetic information to be as high-yielding as the original plant. However, epigenetic changes within the tissue cultures can influence the phenotype of the affected plant and potentially decrease yield. The cause of the altered phenotype has been found to involve targeted differences in DNA methylation at specific sites in the genome and show overlap between differently methylated sites in natural populations. This suggests that these methylation differences might be a stress response of the cells in the tissue cultures to a stress existing in natural populations as well. By taking inspiration from natural populations and their uses for epigenetics, targeted epigenetic changes can be made at specific sites within the DNA and improve stress resistance in our crops.

Abstract

Changes in the epigenome enable a plant to rapidly adapt to its environment without the need for genetic mutations, thereby increasing its chance of survival. The role of epigenetics is even more important in populations with very little genetic diversity, plants that reproduce asexually do not exchange genetic information with other individuals of their species and will create a genetically uniform population. However, the production of genetically uniform crops through tissue cultures is facing problems regarding unwanted epigenetic changes, resulting in somaclonal variation and decreased yield. By taking inspiration from natural populations and identifying differentially methylated loci that have shown to improv stress resistance, targeted epigenetic changes can be made to improve stress resistance in crops. This review aims to discuss the role of epigenetics on the survival of environmental stresses in natural populations and discuss the problems faced with epigenetics in tissue cultures.

Contents

Introduction

In nature, a wide variety of species can be found, all uniquely shaped to survive the environment they are subjected to. This variety of organisms from animals to plants, to something as small as fungi and bacteria owe their phenotypic variation to the genetic information shaping their phenotype. However, even within a species with limited genetic diversity, individuals can show phenotypic differences, usually aimed at adapting the individual to their environment (Mounger et al., z.d.). Cells within an individual, with the same genetic information can form different tissues and have very different functions. These phenotypic differences between species and cells with similar genetic information find their origin in how the genetic information present is expressed (Gibney & Nolan, 2010). Epigenetics is an extra layer of gene regulation influencing gene expression on top of the available genotype (Law & Jacobsen, 2010). The main role of epigenetics is to regulate the availability of genetic information to transcription machinery, thereby influencing gene expression (Zhang et al., 2018). Epigenetics can influence gene expression in multiple ways either at DNA, histone or post-transcriptional levels. In plants, currently, the best-characterised epigenetic changes allowing for phenotypic plasticity involve DNA (de)methylation and histone modifications. These epigenetic changes involving DNA and histones form the plants epigenome (Lu et al., 2023).

Changes in the epigenome enable a plant to rapidly adapt to its environment without the need for genetic mutations, which happen less frequently than epigenetic mutations, thereby increasing its chance of survival (Mounger et al., z.d.). Multiple environmental stresses like drought, cold, humidity and even biotic stresses such as pathogens are found to affect the plants epigenome and induce phenotypical changes favourable for the survival of the stress (Iwasaki & Paszkowski, 2014; López Sánchez et al., 2016; Ramos-Cruz et al., 2021; Tricker et al., 2012; Zheng et al., 2017). The role of epigenetics is even more important in populations with very little genetic diversity, plants that reproduce asexually do not exchange genetic information with other individuals of their species and will create a genetically uniform population. A lack of genetic diversity within a population is generally thought to decrease the population's resilience to environmental stresses (Salgotra & Chauhan, 2023). However, changes in the epigenome can still contribute to phenotypic variation regardless of genetic diversity, increasing the chances of survival. The beneficial role of epigenetic changes in genetically uniform populations is shown by the success of invasive species, which often reproduce asexually, but will still thrive in new environments (Mounger et al., z.d.).

Apart from natural populations, genetic uniformity can be found in crops such as oil palm and banana (Kitavi et al., 2020; Ong-Abdullah et al., 2015). These crops are usually propagated clonally or using tissue culture regeneration, in which material from one parent plant with desirable traits is taken and regenerated into multiple clones genetically identical to the mother plant (Zuzarte et al., 2024). Where epigenetics in natural populations contributes positively to a plant's fitness, some problems are faced with undesired epigenetic changes in tissue cultures (Ong-Abdullah et al., 2015).

The underlying cause of these epigenetic changes is still largely unknown, and more research is needed to prevent undesirable epigenetic changes from happening and ensure stable yield from clonally propagated crops. In this review, I will combine knowledge of the effect of epigenetic changes in natural populations and clonal propagation systems to provide insight in how epigenetics could contribute to increased stress resilience and crops yield, as well as new approaches to the conservation of natural (native) populations.

Chapter 1: An introduction to epigenetics

Changes in a plant's epigenome allow for phenotypic plasticity in response to environmental stresses and are therefore important for survival. Changes in epigenetics influence gene expression and can take place at multiple levels; DNA methylation changes, histone modifications, and post-transcriptional (Lu et al., 2023). The regulation of the three levels of epigenetic changes will be discussed as well as the transgenerational stability of these changes.

1.1 Regulation of DNA methylation and histone modification

DNA methylation

DNA methylation is the addition of one methyl group to the fifth position of certain cytosines (C) in the genome. DNA methylation can only take place in three ways and depends on the nucleotides following the cytosine; CG, CHG and CHH (for which H = A, C or T) both CG and CHG methylation are symmetrical, which means that both DNA strands get methylated, whereas CHH methylation is asymmetric and only one strand gets methylated. DNA methylation is maintained by either METHYLTRANSFERASE1 (MET1), a methyltransferase, CHROMOMETHYLASE 2 or CHROMOMETHYLASE 3, responsible for maintaining CG methylation, CHH and CHG respectively (Law & Jacobsen, 2010). However, these methyltransferases can only maintain already existing methylated cytosines and do not add methyl groups to previously non-methylated cytosines. In plants, one pathway can both maintain and add new DNA methylation, the RNA-directed DNA methylation (RdDM) pathway. The RdDM pathway can work in two ways, canonical and non-canonical, canonical RdDM is mostly responsible for the maintenance of preexisting DNA methylation and non-canonical RdDM for methylation at new sites (see figure 1 for a detailed schematic overview of both RdDM pathways) (Erdmann & Picard, 2020).

Canonical RdDM can be split up into two subsequent processes, starting with the production of small RNAs (sRNA) followed by targeted DNA methylation using these sRNAs. The production of sRNA starts by the recruitment of RNA polymerase IV (POL IV) to silent heterochromatin (a condensed and gene-poor region). Through POL IV, short single-stranded RNAs (ssRNAs) are produced from the targeted region which are then transformed into double-stranded RNAs (dsRNAs) by RNA-directed RNA polymerase 2 (RDR2). The dsRNA is cut into 24 nucleotide sRNA (24nt sRNA) by Dicer-like 3 (DCL3), an endoribonuclease, forming the last step in the production of sRNA. Although this pathway is the biggest contributor of sRNA production, additional pathways can result in the formation of 24nt sRNA. The next step is targeted methylation using the produced sRNAs. One single strand from each sRNA is placed into an Argonaut (AGO) protein (either AGO4, AGO6 or AGO9), these proteins are known to find RNA sequences complementary to the RNA sequence they carry. RNA polymerase V (POL V) produces (together with multiple other compounds) the complementary RNA sequence the AGO-sRNA complex can bind to. Domains Rearranged Methyltransferase 2 (DRM2) gets recruited and then methylated the DNA nearby (Erdmann & Picard, 2020).

The non-canonical RdDM pathway is mostly responsible for the methylation of new regions. And the main difference between canonical and non-canonical is the origin of the sRNAs. In canonical RdDM the sRNAs primarily come from POL IV, while in non-canonical RdDM the sRNAs can originate from multiple sources such as viruses or RNA polymerase II (POL II). Transcripts from these sources get targeted by the post-transcriptional gene silencing (PTGS) pathway and cut and transformed into dsRNA

by RDR6 and in turn cut into sRNAs by DCL2, DCL3 or DCL4. sRNA cut by DCL3 goes directly into the RdDM pathway as described under canonical RdDM, whereas sRNA cut by DCL2 and DCL4 will for the majority associate with AGO1 and go back into the PTGS pathway. Some, however, will associate with AGO6 (note that AGO6 is also present in the canonical RdDM pathway) and go into the RdDM pathway. The non-canonical pathway is still not as well understood as the canonical pathway, so future research will most likely uncover new sRNA-producing sources and add to our understanding of this pathway (Erdmann & Picard, 2020).

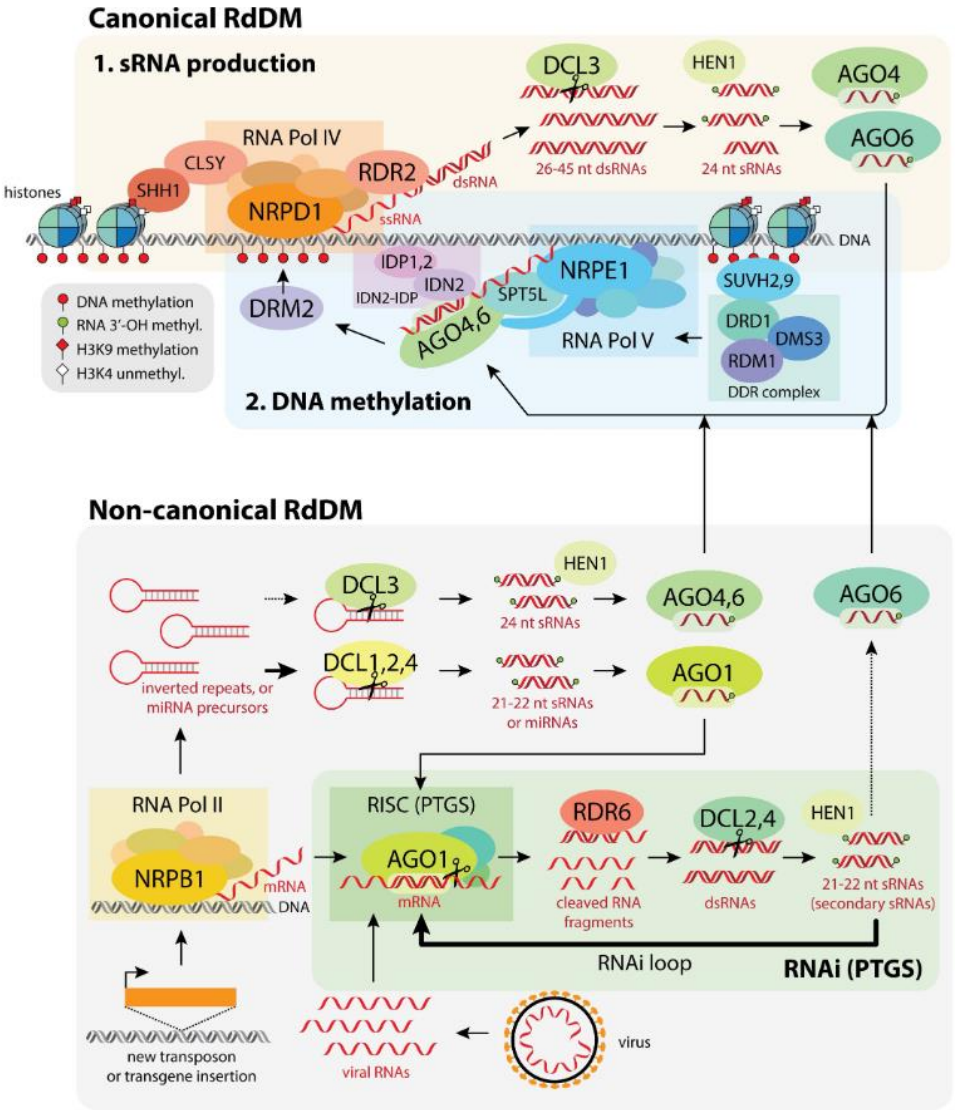

Figure 1: Detailed schematic overview of the canonical RdDM pathway (top) and non-canonical RdDM pathway (bottom). *The RdDM pathway is broken up into (1) sRNA production and (2) targeted DNA methylation. Non-canonnical RdDM differs mainly from canonical RdDM in the origin of the sRNAs. (Figure adapted from Erdmann & Picard, 2020)*

DNA methylation can be removed through demethylation. DNA demethylation can happen in two ways, passive demethylation and active demethylation. Passive demethylation will happen every cell division, as newly synthesised DNA strands lack methylation until one of the DNA methylation maintenance pathways adds methylation.

Active DNA demethylation is carried out by DNA glycosylases through base excision repair. Methylated cytosines are removed and replaced by an unmethylated cytosine. These glycosylases are in balance with the methylation pathways. Repressor of silencing 1 (ROS1) is an example of such a glycosylase that demethylates in balance with the RdDM pathway. The methylation of a transposable element (TE) in the promotor of ROS1 is needed for the expression of ROS1. Plants that are impaired in their RdDM pathway, and thereby lose their ability to methylate the TE in the promotor of ROS1, will also show reduced expression of ROS1 which in turn reduces their capability of demethylation. This feedback loop helps maintain the balance between DNA methylation and DNA demethylation.

Histone modifications

Histones are proteins on which DNA gets coiled to regulate gene expression, this DNA-histone complex is called chromatin (Gentry & Hennig, 2014). Chromatin can either be tightly packed (heterochromatin) or loosely packed (euchromatin), which determines gene expression. Euchromatin is accessible for the cells transcription machinery and leads to gene expression, whereas tightly packed heterochromatin inhibits gene expression (Erdmann & Picard, 2020).

Histones have tails that can be modified, and whether DNA gets tightly packed or loosely packed depends on the type and place of modifications made on these tails. There are multiple types of histone modifications that can alter chromatin density, but for this review the focus will be on histone tail methylation. Histone tails can be methylated at different sites on either arginines or lysines along the tail with either 1, 2 or 3 methyl groups added to a lysine or 1 or 2 methyl groups added to an arginine (Gibney & Nolan, 2010). In literature, this is indicated by histone number, amino acid (and its position along the tail) and the number of methyl groups added. For example, if on histone 3 lysine 4 is trimethylated it is written as: H3K4me3. The placement of these methyl groups along the tail determines whether genes nearby get expressed or repressed. H3K4me3 and H3K36me3 are known to be involved in active transcription of genes, while H3K9me2/3 and H3K27me3 are known to be involved in the repression of genes (Erdmann & Picard, 2020; Lu et al., 2023).

1.2 Transgenerational epigenetics and imprinting

Methylation can be passed on to daughter cells, after every cell division, methylated double-stranded DNA is divided into two new hemimethylated strands, with the original strand still methylated, and the newly synthesized strand unmethylated. Re-methylation of the newly synthesized strand has multiple ways, depending on whether the methylation was on CG, CHG or CHH. CG methylation is symmetrically methylated, MET1 recognizes hemimethylated CG and re-methylates the new, still unmethylated, cytosines (Zhang et al., 2018). CHG methylation is symmetric as well, but its maintenance is done through CMT3 which binds to methylated H3K9 (H3K9me). It has been shown that mutating the H3K9 specific methyltransferase (SUVH4) and thereby inhibiting the addition of a methyl group to H3K9, results in reduced CHG methylation. (Erdmann & Picard, 2020; Zhang et al., 2018) The same methyltransferase is also recruited by methylated CHG, resulting in H3K9 methylation and creating a binding site for CMT3 (Du et al., 2014). So, both pathways form a feedback loop maintaining the

methylation of CHG sites. CHH methylation is asymmetric, during mitosis, only one strand contains the methylated cytosine while the other does not. CHH sites maintain their methylation through the RdDM pathway by being a target region of the pathway and through CMT2 (Saze, 2008; Zhang et al., 2018).

Another important role of epigenetics in plant development is imprinting. Imprinting is the expression or inhibition of an allele depending on which parent it originated from. It is only found in flowering plants and mainly in the endosperm. During imprinting, some maternal genes are demethylated by DME and repression of MET1 in the central cell resulting in the expression of the maternal alleles. Paternal alleles are methylated by MET1 and H3K27 methylation resulting in the silencing of the paternal alleles (Zhang et al., 2013).

The hypomethylated maternal alleles result in the production of siRNAs, these siRNAs are then used by the RdDM pathway to re-methylate these regions. A similar process is involved in the silencing of TEs during fertilization. The pollen nurse cell, surrounding the sperm cells, undergoes reprogramming of heterochromatin and re-activation of TEs. This reactivation of TEs leads to the production of sRNAs against these TEs through the RdDM pathway in the nurse cells. These sRNAs are then transported to the germ cell where they reinforce silencing of the targeted TEs (Martínez et al., 2016).

Chapter 2: The role of epigenetics in natural populations

The ability of a species to adapt its phenotype to the environment is of great importance for its survival. Because epigenetic changes happen more often than genetic mutations, epigenetics provides the plant with a fast way of adapting to its environment without having to undergo generations of genetic selection (Mounger et al., z.d.).

2.1 Epigenetic modifications increase adaptability to environmental stresses

Abiotic stresses

Abiotic stresses like cold and drought are all known to affect the plant's phenotype to increase the chances of surviving the stress (Iwasaki & Paszkowski, 2014; Zheng et al., 2017). Epigenetic changes like changes in DNA methylation or histone modifications can play an important role in adaptation to environmental stresses and allow for phenotypic plasticity (Lu et al., 2023).

Cold does not only form a stress for plants, for some species it is necessary to experience cold in order to bloom. This process is called vernalization and has been studied in thoroughly in *Arabidopsis thaliana (A. thaliana)*. In vernalisation-sensitive Arabidopsis varieties, FLC, a suppressor of flowering, is expressed in the early vegetative stage of development. Only after a time of cold exposure does FLC get silenced and is the plant able to flower in the following spring. Before exposure to cold conditions, multiple complexes and their components (FRI, PAF1, EFS, COMPASS) are responsible for H3K4 tri-methylation and H3K36 dimethylation and resulting in the activation of FLC. When the plant is exposed to prolonged cold conditions, the already present PRC2 complex associates with PHD (a plant homeodomain known to modify chromatin) to form the PRC2-PHD complex and increases H3K27 tri-methylation, resulting in a downregulation of the nearby FLC gene. Additionally, to the trimethylation of H3K27, two long non-coding RNAs, COLDAIR and COOLAIR both aid in silencing FLC expression. COLDAIR does so by interacting with PRC2. COOLAIR is an antisense non-coding RNA which may increase the silencing of FLC. This change is permanent for the individual and is mitotically inherited but is reset every generation. (Iwasaki & Paszkowski, 2014)

Drought is an environmental stress that is expected to occur more frequently due to the changing climate (Intergovernmental Panel On Climate Change, 2022). Drought stress induces epigenetic changes that can be transferred to offspring. Multi-generational drought has been shown to induce non-random epimutations in rice, which resulted in better survival of drought conditions for the later generations. It was shown that after 11 generations of surviving drought conditions, oxidative damage after drought was reduced and reactive oxygen species (ROS) scavenger capacity increased. Moreover, the number of tillers and panicle length on each plant decreased, while the seed setting rate increased. This is thought to be an adaptation to the balance between survival and reproduction. These results together show an increased capacity to survive drought conditions (Zheng et al., 2017).

A seemingly similar stress to drought, but studied in more detail, is low relative humidity. During exposure to low relative humidity, a plant responds by reducing its stomatal density thereby inhibiting excess water loss. The reduction of stomatal density is the result of DNA methylation changes affecting two genes involved in stomatal development, SPEECHLESS (SPCH) and FAMA. Both SPCH and FAMA

show induced methylation for symmetric as well as asymmetric sequence contexts and reduced expression during low relative humidity (figure 2). DMR1 and 2 and MET1 were shown to be involved in the increased DNA methylation of these genes, as mutants of these methyltransferases do not show a reduction in stomatal density under low humidity conditions as wild-type plants do. CMT3 does not play a role in the methylation difference under low relative humidity. When comparing a wild-type plant to a siRNA biogenesis mutant (mutated in RDR2 or DCL3), no increase in methylation at SPCH and FAMA was found in the mutants compared to the wild-type plants, which indicates that both genes are targets of the RdDM pathway (Tricker et al., 2012).

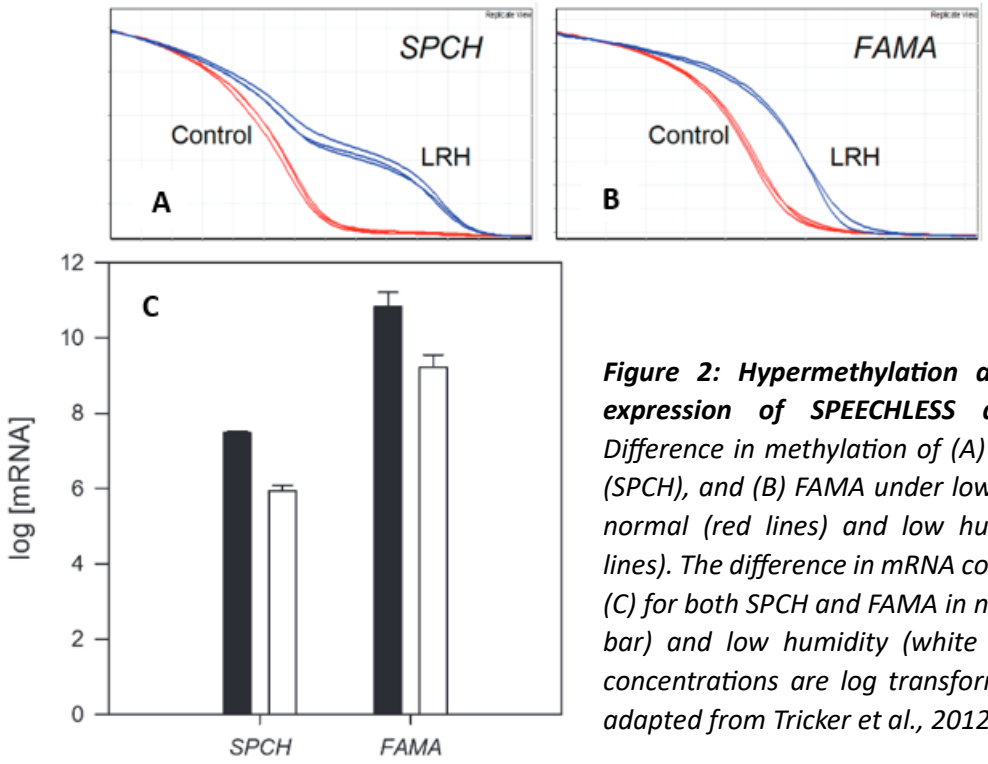

Figure 2: Hypermethylation and reduced expression of SPEECHLESS and FAMA. *Difference in methylation of (A) SPEECHLESS (SPCH), and (B) FAMA under low humidity in normal (red lines) and low humidity (blue lines). The difference in mRNA concentrations (C) for both SPCH and FAMA in normal (black bar) and low humidity (white bar). mRNA concentrations are log transformed. (Figure adapted from Tricker et al., 2012)*

Biotic stresses

Not only does epigenetics play a role in the adaptation to abiotic stresses, but changes in DNA methylation are also involved in a plants ability to defend itself against pathogens (Ramos-Cruz et al., 2021). Hypomethylation of DNA usually leads to increased resistance against pathogens, whereas hypermethylation of DNA usually leads to an increased susceptibility to pathogens. This has been shown for infection with *Hyaloperonospora arabidopsidis* (*Hpa*), an obligate biotrophic oomycete. *A. thaliana* mutants defective in either the RdDM pathway or in their DNA methylation maintenance showed a reduced number of leaves producing HPA spores compared to wild-type plants, and thus showing increased resistance against *Hpa* infection. However, a *ros1* mutant, resulting in DNA hypermethylation, showed increased susceptibility to *Hpa* compared to wild-type plants (López Sánchez et al., 2016). The same results have been shown for the bacterial pathogen *Pseudomonas syringae* (*P. Syringae*), where mutants impaired in their maintenance of DNA methylation showed increased resistance to the pathogen and differentially expressed pathogen-responsive genes (Dowen et al., 2012). These results suggest that DNA hypomethylation might enable genes involved in pathogen defence to be expressed more rapidly when exposed to the same pathogen a second time, thereby 'priming' the plants defence mechanism (Ramos-Cruz et al., 2021).

2.2 Clonally reproducing species benefit from epigenetic changes

Clonally reproducing plants reproduce through mitotic cell divisions rather than meiotic divisions, and therefore do not go through meiotic recombination, meaning they also mis the genetic variation that comes from it. Because of this, genetic diversity within these populations can get very limited (Verhoeven & Preite, 2014). Even though a lack of genetic diversity within a population is generally thought to decrease the population's resilience to environmental stresses, genetic uniformity does not necessarily inhibit the ability to thrive (Mounger et al., z.d.; Salgotra & Chauhan, 2023). This is demonstrated by the success of invasive species, of which the majority (70%) reproduce clonally and suggests that this form of reproduction is not limiting the chances of survival for invasive species (Mounger et al., z.d.; Verhoeven & Preite, 2014).

An explanation for the success of invasive plants could be the fact that epigenetic mutations might play a crucial role in the adaptation of invasive species to their new habitat. As epigenetic mutations are known to happen much more frequently than genetic mutations, the phenotypic plasticity of clonally reproducing plants might be mostly reliant on epigenetic mutations rather than genetic diversity. This would be beneficial considering the high selection pressure invasive species are exposed to when entering a new habitat, and that adapting to the new environment might not happen fast enough when relying only on genetic mutations (Mounger et al., z.d.).

Japanese knotweed is an invasive species and is found in large parts of Europe and the north-eastern USA. Richards et al, studied whether genetic or epigenetic variation is responsible for the phenotypic plasticity of the species. They compared genetic and epigenetic diversity of multiple populations and species of Japanese knotweed from different habitats all from or near Long Island (New York). Some genetic variation was found between species and less genetic diversity among sites within a species. However, epigenetic variation among sites was found to be much higher than genetic variation, which is in line with the idea that epigenetic changes happen more frequently than genetic changes and that a plants environment induces these changes (Richards et al., 2012).

Chapter 3: The role of epigenetics in tissue culture regeneration

Next to genetically uniform populations in natural contexts, genetic uniformity can also be found in crops like oil palm and banana (Kitavi et al., 2020). Oil palm and banana are both mainly produced through tissue culture regeneration, a technique in which a piece of tissue from a high-yielding plant gets taken and regenerated to form new plants under lab conditions, resulting in genetically identical offspring (Zuzarte et al., 2024). However, problems with tissue culture regeneration involve somaclonal variation resulting in decreased yield (Ong-Abdullah et al., 2015).

3.1 Hypomethylation of *Karma* predicts mantled phenotype in oil palm

In oil palm, the problem behind the unwanted phenotype involves epigenetic changes during the tissue culture process and has been researched thoroughly. Some clones produced through tissue cultures exhibited abnormal fruit formation with a 'mantled' phenotype. In the mantled phenotype, pseudocarpel are formed where should have been stamens in male flowers resulting in sterility, and staminodes (sterile stamen) in female flowers resulting in either fertile (figure 3B) or parthenocarpic fruits (figure 3C) (Adam et al., 2007). Previous studies have shown that clones showing mantled fruit have an overall decrease in DNA methylation compared to normal fruit (Ong-Abdullah et al., 2015)

The exact location of demethylation responsible for the mantled phenotype was discovered using a genome wide DNA methylation analysis by Ong-Abdullah et al. They compared DNA methylation maps of ramets (clones) with normal phenotypes, parthenocarpic phenotypes and the ortets (original plants that were cloned to obtain the ramets). A large number of differentially methylated regions (DMRs) were found between normal and mantled phenotypes, but most of these DMRs were only present in one or two of the four populations used for the analysis. However, one DMR was found in all four populations, this region is located within intron 5 of EgDEF1.

Figure 3: Possible phenotypes of oil palm fruits produced through tissue cultures. *Fruit with (a) normal phenotype, (b) fertile mantled phenotype or (c) parthenocarpic phenotype. White arrows indicate kernel, black arrows indicate pseudocarpels. (Figure adapted from Ong-Abdullah et al.,*

Most of the hypomethylated regions were found to be transposons or repeats, so they looked for differentially methylated repetitive regions within intron 5. A not yet described repetitive element, which has a homology to *Karma* in rice was found (Ong-Abdullah et al., 2015). In rice, *Karma* is a retrotransposon and shows hypomethylation in tissue cultures, especially when cultured for longer periods of time as well as in regenerated plants when compared to wild-type plants (Komatsu et al., 2003). To see if *Karma* is hypomethylated in mantled

phenotypes, methylation of EgDEF1 was compared between the normal ortet, the normal ramet and the mantled ramets using genome-wide bisulfite sequencing. Results showed that CG methylation was the same for all groups, whereas CHG and CHH methylation within *Karma* had decreased for the mantled phenotype (figure 4). The hypo- or hypermethylation of *Karma* can therefore predict whether an individual will show a mantled or normal phenotype respectively (Ong-Abdullah et al., 2015).

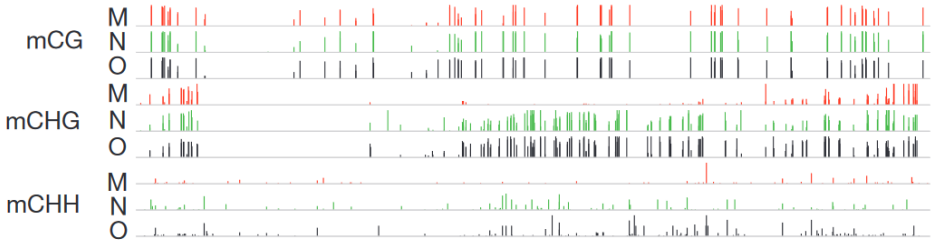

Figure 4: Methylation pattern of Karma in mantled phenotypes. *Whole genome bisulfite sequencing from normal ortet (O), normal ramets (N) and mantled ramets (M). Mean methylation of this region is plotted on a 0-100% scale for each group and for each cytosine context. (Figure adapted from Ong-Abdullah et al., 2015)*

In addition to the find of *Karma*, the mRNA of the gene *EGAD1* has been found to accumulate at higher levels at the callus stage for known mantled tissue cultures, compared to tissue cultures with a known normal phenotype. *EGAD1* encodes a peptide similar to some plant defensin proteins which are involved in pathogen resistance. *EGAD1* transcripts are mainly found in inflorescence tissues, which suggests that this gene protects the inflorescence from pathogen infection. This difference in expression of *EGAD1* between mantled and normal phenotypes at the callus stage has been suggested to also have an epigenetic origin (Tregear et al., 2002).

3.2 Somaclonal variation in maize

Somaclonal variation has also been observed in maize tissue cultures. In the study of Han et al (2018), they found that epigenetic changes in maize in tissue cultures includes both hypomethylation and hypermethylation of DNA. Differentially methylated CHH sites were found to mostly show hypermethylation events, whereas CG and CHG DMRs mostly show hypomethylation events. The DMRs were divided into two types, consistent (present in more than 50% of samples) and rare (present in less than 50% of samples). The frequency of consistent DMRs exceeded the predicted amount expected by random chance, suggesting that some loci may be targets for epigenetic changes in tissue cultures. Most CHH DMRs belonged to the 'rare' category (99%), while CHG and CG DMRs were more often consistent between samples.

Consistent DMRs showed overlap with loci that also show differential methylation in natural populations, while the rare DMRs did not (figure 5). About 30% of DNA methylation changes in the callus tissue were observed in the primary regenerant plant, while 90% of hypermethylation events and 75% of hypomethylation events present in the primary regenerant were passed on to the next generation. This suggests that part of the epigenetic changes happening in tissue cultures are not passed on to the regenerated plants. As no changes in overall DNA methylation were found in recovered plants, the possibility of failing DNA methylation machinery in tissue cultures is unlikely. The possibility of tissue cultures resulting in an overall increased rate of epi-mutations also seems unlikely as loci with methylation changes were affected in both alleles. These observations suggest that

methylation changes in tissue cultures are either a targeted process or an increased sensitivity of certain genes to the tissue culture process (Han et al., 2018).

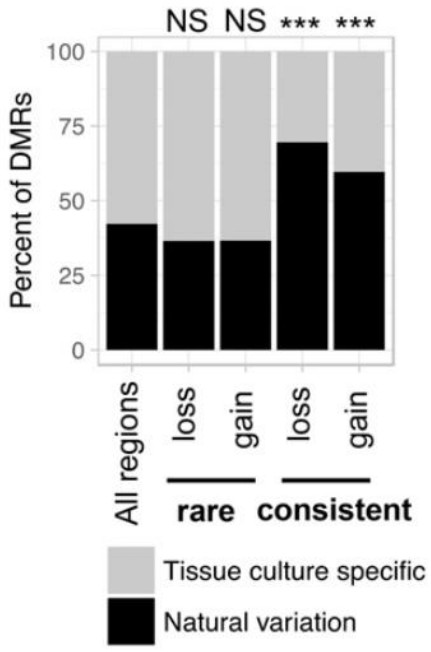

Figure 5: Overlap between tissue culture DMRs and natural variation DMRs. *The percentage of tissue culture DMRs overlapping with DMRs found in natural populations. Significance is shown at the top with * P = 0.001 and NS = not significant, hypergeometric test*

(Figure adapted from Han et al., 2018)

Discussion

The aim of this review was to combine knowledge of the effect of epigenetic changes in natural populations and clonal propagation systems to provide insight into how epigenetics could contribute to increased stress resilience and crop yield, as well as discuss new approaches to the conservation of natural populations. Epigenetic changes play an important role in natural populations to increase survival and adapt to environmental stresses by allowing for phenotypic plasticity without the need for genetic mutations. This is especially important in species that reproduce asexually and therefore often lack genetic diversity (Mounger et al., z.d.). In tissue cultures, epigenetic changes can result in somaclonal variation and may lead to decreased yield in crops (Ong-Abdullah et al., 2015).

In natural populations, epigenetic changes play an important role in a plants phenotypic plasticity and allow the plant to adapt its phenotype and increase its chances of surviving the environmental stresses it is exposed to (Tricker et al., 2012; Zheng et al., 2017). These environmental stresses do not only form a threat to natural populations but form a problem in the agricultural sector as well (Intergovernmental Panel On Climate Change, 2022). Changes in methylation can also increase resistance against pathogens. Hypomethylation on specific genomic regions most likely enables pathogen defence genes to be expressed more rapidly upon infection. Epigenetic changes resulting in improved resistance to these stresses in natural populations could find use in the improvement and stress resistance of crops as well.

The importance of epigenetics in species survival is shown by the success of invasive plants. The majority of invasive species produce asexually and will therefore form mostly genetically uniform populations. However, even with limited genetic variation, they are still very successful in invading new habitats (Richards et al., 2012). Changes in their epigenetics may contribute to their success by decreasing the need for (less frequent) genetic mutations to adapt their phenotype to new environments (Mounger et al., z.d.). Further research is needed to find out if asexually reproducing species may be more flexible in their epigenome and can therefore adapt more quickly to the conditions they are subjected to sexual reproducing species, as they have the most benefit from these epigenetic changes. If so, this would also explain the success of invasive species in new environments even when in competition with native species that are already adapted to the environment. The mechanisms behind the rapid phenotypic adaptations in asexual species can also form inspiration for improving crops by allowing them to adapt their phenotype when needed and making them more resistant to environmental stresses such as high temperatures and drought, expected to happen more frequently due to climate change (Intergovernmental Panel On Climate Change, 2022)

Tissue cultures are known to be prone to DNA methylation changes resulting in somaclonal variation and undesired phenotypes, such as the mantled fruit of the oil palm. Studies have shown that changes in DNA methylation in tissue cultures are most likely targeting specific loci rather than mutate solely by chance. In both oil palm and maize, hypomethylation events were either the cause of the altered phenotype (oil palm) or found to be the most consistent (present in more than 50% of samples) methylation change among samples (maize). In maize the consistent DMRs show overlap between loci known to show methylation changes in natural populations (Han et al., 2018; Ong-Abdullah et al., 2015).

As epigenetic changes in natural populations can benefit a plant to survive an environmental stress, this overlap could suggest a possible connection between the stress experienced in tissue cultures and a stress naturally occurring. The stress tissue cultures face could be triggering differential methylation events at the specific loci needed to survive the similar natural stress. A stress that is present in natural populations and is known to result in hypomethylation, is infection by specific pathogens.

Hypomethylation is thought to aid in a more rapid expression of pathogen defence genes, decreasing susceptibility to the pathogen (Ramos-Cruz et al., 2021). This could suggest that cells in tissue cultures experience a stress mimicking the same stress caused by infection with a pathogen and therefore 'prime' their defence mechanisms against pathogens by hypomethylating defence related loci and would fit the assumption that these methylation changes are targeted to specific loci. This hypothesis could be supported by the findings of Tregear et al (2002), where they found an increased expression of a plant defensin gene in tissue cultures of oil palm. Future research should focus on locating the differential methylated loci and investigate the function of the affected genes to test whether specifically pathogen defence genes are targeted DMRs in tissue cultures.

With the changing climate, resulting in an increase in extreme weather conditions, the ability of crops to survive episodes of environmental stresses such as draught and heat has become an important factor in ensuring food availability. Epigenetics might provide a new way of adapting our crops to their environment, with epigenetic changes in natural populations often enabling a plant to adapt its phenotype rapidly to an environmental stress. By taking inspiration from natural populations and their uses for epigenetics, targeted epigenetic changes can be made at specific loci to improve stress resistance in our crops. Multiple methods to target specific sites and change DNA methylation already exist and have been used for research before (Gardiner et al., 2022; Veley et al., 2023). Therefore, the focus of future research should be on identifying the differentially methylated loci and study their function, so targeted epigenetic changes can be made at the right loci. This is both important for the implementation of epigenetics in agriculture to improve crops and increase their resistance against environmental and pathogenic stresses, as well as for identifying the origin of methylation changes in tissue cultures.

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