Chapter 8
Genetic and Epigenetic Nature of Transgenerational Changes in Pathogen Exposed Plants

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8.1 Introduction

Adverse environmental conditions named stresses are constantly shaping genomes of living organisms. Most of these external stimuli have a negative influence on growth, development, and reproduction (Arnholdt-Schmitt 2004; Madlung and Comai 2004). Avoidance represents the most common response to severe or long lasting environmental conditions. The organisms with a sedentary life style are unable to escape stress, and thus utilize mechanisms of tolerance and resistance. Plants integrate into the environment through the efficient use of adaptation mechanisms that depends on the constant exchange of signaling molecules (reviewed in Cronk 2001).

Plants are the organisms that continue their development throughout the entire life cycle. The germ line in plants is not predetermined but is established during the development. This allows plants to percept stress and integrate the memory of it through multiple feedback mechanisms. The only possible way of transmitting the memory of stress is via the epigenetic regulations involving DNA methylation, histone modifications and chromatin restructuring. These changes lead to the differential gene expression and allow to establish new epialleles, thus resulting in the destabilization of defined loci. The majority of these changes are neutral or deleterious, but in some rare cases they could be beneficial.

Constant exposure to certain stresses should lead to the selection of adaptive traits beneficial to these conditions. The retention and fixation of a necessary trait requires the selection from a number of neutral random changes in the genome.

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Typically, plants don't have time for all these changes to occur. Plants are also capable of acclimation, altering their homeostasis on a reduced time scale (Shinozaki et al. 2003; Sung et al. 2003). The fact that plants similarly respond to unrelated physical, chemical, or temporal environmental factors suggests the existence of complex intercrossing perception and response mechanisms (Shinozaki et al. 2003; Chinnusamy et al. 2004; Ludwig et al. 2004).

In the current chapter we discuss what is known about the stress-induced epigenetic changes resulting in differential genome rearrangements and present several experiments indicating the role of methylation changes and homologous recombination in the plant response to pathogen stress.

8.2 Genome Stability is Regulated via Multiple Pathways

Genome instability is defined by the susceptibility of a genome to mutations and rearrangements, whereby a stable genome impedes these mechanisms. Major mechanisms of the genome protection involve the different arrangement of methylated and unmethylated histones and attachment of chromatin loops to the nuclear matrix. Changes in the chromatin structure represent the natural ability of plants to respond to stress (Takeda et al. 2004; Buchanan et al. 2005). DNA repair mechanisms including two double strand-break repair pathways, non-homologous end-joining (NHEJ) and homologous recombination (HR) represent a more direct way of the genome protection (Jeggo 1998; Hays 2002).

Plants regulate the genome rearrangements by applying different DNA repair mechanisms at different developmental stages. *Arabidopsis* plants have higher HR frequency (HRF) at early developmental stages (Boyko et al. 2006c). In contrast, the frequency of NHEJ employment does not change. It is possible that the older cells suppress the activity of HR in order to decrease the chances of deleterious rearrangements in the polyploidy cells (Boyko et al. 2006c). The younger cells can have an elevated HRF in order to allow the inheritance of genome rearrangements. In this case, the exposure to mutagen early at the developmental stage should result in a higher increase of HR frequency. Recent data indeed showed that the exposure to UVB early in the development resulted in a higher increase in HRF when compared to the exposure at a later stage (Boyko et al. 2006a).

8.3 The Homologous Recombination as a Mechanism Supporting Rearrangements

The homologous recombination is the primary mechanism responsible for crossing over events during meiosis (Gerton and Hawley 2005), and as such could serve as the main mechanism for creating diversity. The HR can prove

dangerous to cells, as it can quickly generate the recessive genotypes from heterozygous loci. The recessive traits, however, are not necessarily deleterious, as they may appear to be useful under certain environmental conditions. The organisms with a large proportion of traits in the heterozygous stage could have the advantage from the evolutionary point of view because of having "evolutionary flexible" genomes. It can be suggested that the genome stability is closely monitored to balance risks of negative events with the need for genome diversity.

The addition of methyl groups to DNA as well as histone deacetylation and specific changes in histone methylation stabilize the genome and prevent the recombination events, whereas the loss of methyl groups, termed hypomethylation and histone acetylation allow such events to occur (Engler et al. 1993; Madlung and Comai 2004).

8.4 Regulation of Gene Expression via Chromatin Modifications

Gene expression is regulated by various mechanisms including kinase-activated transcription factors, RNA turnover, posttranscriptional gene silencing as well as changes in protein half-life (Jover-Gil et al. 2005). One more mechanism involved in the establishment of new chromatin structures is small regulatory RNAs, named short interfering (si)RNAs, involved in targeting specific genome areas and establishing a new chromatin structure (Mathieu and Bender 2004; Steimer et al. 2004; Kapoor et al. 2005). This process starts by the generation of sequence specific siRNAs via the developmental regulation or change in environmental conditions that are capable of spreading to specific tissue or plant organs and promoting changes in a methylation status followed by changes in methylation/acetylation of specific histones (Steimer et al. 2004; Sunkar and Zhu 2004; Borsani et al. 2005). Such changes in chromatin structure reinforced by the small regulatory RNAs allow one to establish and maintain new patterns of chromatin modifications required for the proper leaf development and transition to flowering (Fransz and de Jong 2002; Liu et al. 2004; Peragine et al. 2004; Grigg et al. 2005; Kandasamy et al. 2005).

Gametes are produced in plants from meristemic cells. The ability of these cells to accumulate the information from the developing plant makes it possible to incorporate epigenetic signals into a unique methylation pattern. The newly developed progeny will likely have changes in the pattern of gene expression at differently methylated loci. This variation in the gene expression can be easily found in the population of plants, and it represents a heritable epimutation event. The advantage of having epimutations is that they are often reversible, making the resulting phenotypes more variable and less severe as compared to those resulting from sequence changes (Cronk 2001).

8.5 Exposure to Stress Influences Methylation Status and HRF

Plants – as any other organisms – have the ability to perceive stress and respond to it via differential changes in methylation pattern. Indeed, it has been shown that cold treatment promotes tissue-specific hypomethylation of the particular genome regions, including those specific to retrotransposon sequences (Steward et al. 2002). Similarly, the exposure of tobacco plants to a tobacco mosaic virus (TMV) induces demethylation of the *NtAlix1* stress-responsive gene, resulting in the continuous accumulation of the gene transcript (Wada et al. 2004).

Many experiments have shown that such stresses as changes in growth conditions, exposure to salt, heavy metals, ultraviolet and ionizing radiation, herbicides and even pathogens influence the HR frequency in plants (Kovalchuk et al. 1998, 2000, 2003a,b, 2004; Filkowski et al. 2003; Besplug et al. 2004; Molinier et al. 2005; Boyko et al. 2006a; Boyko et al., unpublished data). Some of these stresses are associated with the changes in a DNA methylation pattern in the progeny (Kovalchuk et al. 2003b, 2004). The association of other stresses with the changes in methylation and genome instability remains to be established.

The most straightforward reliable assay that can be used for the analysis of the HRF in plants is the one that uses visible markers. We have used two reporter genes, either β-glucuronidase (*uid*A, or GUS) or luciferase (LUC), that are integrated into plants as two non-functional overlapping copies (Kovalchuk et al. 1998; Filkowski et al. 2004; Boyko et al. 2006b). The repair of a double strand break in one of the homologous regions (Fig. 8.1) using the second intact homologous copy results in the restoration of the marker gene structure. The activity of these genes is readily visualized either via histochemical staining or observing with a CCD camera for GUS and LUC, respectively (Fig. 8.1).

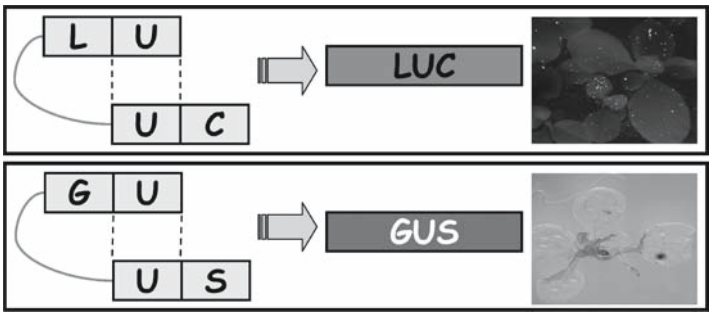

Fig. 8.1 Recombination reporter assays. Homologous recombination reporter lines carry in the genome two non-functional overlapping copies ("GU" and "US" or "LU" and "UC") of the transgene in the genome. The activity of the gene is restored via homologous recombination between the homology regions ("U"). The transgene activation is observed either as shiny sectors (LUC) or grey sectors (GUS) on the transparent background (chlorophyll washed with ethanol)

8.6 Systemic Signaling in Plants

In plants every single cell has the ability to communicate with the adjacent cells through the plasmodesmata openings, and with the distant cells through the phloem. These communication channels allow plants to exchange information about any changes in environmental conditions. Often, local stimuli are integrated by plants into a broad responsive network resulting in a global response like a systemic acquired resistance (SAR) (Dong 2001), systemic wound signaling (Pearce et al. 1991), systemic acquired acclimation to light (Karpinski et al. 1999), systemic post-transcriptional RNA silencing (Mlotshwa et al. 2002; Waterhouse et al. 2001), and the photoperiodic induction of flowering (Colasanti and Sundaresan 2000). It is likely to be a small portion of the great variety of responses that plants are capable of. All these processes depend on the ability of plants to respond to stress and to produce the mobile signals that can activate specific reactions in distant tissues.

8.7 Systemic Recombination Signal Is One of the Mechanisms of Stress Response

Recent experiments in our laboratory suggest that plants respond to local stresses in a systemic way. We found that exposure of a single tobacco leaves to UVC or rose Bengal (singlet oxygen producer) resulted in the HRF increase throughout the whole plant (Filkowski et al. 2004). It should be noted that the pre-treatment of a tissue to be stressed with radical scavenging enzymes decreased but not totally abolished the global HRF increase. This suggests that stress results in the production of a systemic signal that at least is partially dependent on the radical production. The nature of this signal, named the systemic recombination signal (SRS), is still enigmatic. The other questions that still remain to be answered are: whether other stresses are capable to generate the SRS, whether all the plants respond to biotic stress in a similar manner, and what the role of SRS in plants is?

8.8 Can Pathogen Induce Genome Rearrangements?

The result of plant pathogen interaction depends on the presence of avirulence (*Avr*) gene in a pathogen and resistance (*R*) gene in a plant (Dong 2001). The absence of one of the components results in a “compatible” interaction and systemic spread of a pathogen. If both are present, this results in an “incompatible” interaction and leads to a local hypersensitive response, immediately followed by a systemic acquired resistance (SAR) to any future encounter with a similar or different pathogen (Dong 2001). To be a systemic process, SAR has to be dependent on the spread of a signal throughout the plant. The nature of this signal remains enigmatic.

Plants that are not able to mount a hypersensitive response and SAR are not necessarily completely vulnerable. The typical response of such plants is the increase in the level of unspecific resistance/innate immunity. It is believed, however, that the evolution of response to pathogen is a constant arm race, where plants are "trying" to create a new *R* gene, and pathogens are trying to modify the *Avr* gene to escape recognition. This process is constant and thus requires a complex signaling mechanism.

8.9 Compatible Viral Infection Leads to the Production of SRS and Global Increase in HRF

We previously reported that a compatible interaction between the pathogen Tobacco mosaic virus (TMV) and the plant *Nicotiana tabacum* (tobacco) results in the production of a signal that leads to local and systemic changes in the frequency of somatic recombination (Kovalchuk et al. 2003b). We assume that this signal has the same nature as the one produced upon abiotic stress. Since the signal was produced upon the viral infection, it was important to check whether the virus presence was required for the HRF increase. First, we checked how long it takes for a virus to move from the infection site systemically, and we found that cutting leaves at 24 h after infection does not allow the virus to spread. The same experiment showed that cutting the infected leaves as early as 8 h after infection still results in the HRF increase. Thus we concluded that the SRS moves to non-infected leaves faster than the virus (Kovalchuk et al. 2003b).

Next we compared the generation of a signal in tobacco cultivars that do or do not have the gene of resistance to TMV, the *N*-gene. This experiment showed that the infection of SR1 plants that do not have the *N*-gene results in the SRS production, whereas the infection of Big Havana plants that do have the *N*-gene does not. We hypothesized that the absence of *Avr*:*R* gene interaction results in a compatible interaction, and this allows the production of the SRS. We also found that the conditions inactivating the function of the N-gene, like the increase in temperature to over 30 °C, result in a compatible interaction and allows the pathogen to spread. We confirmed that the SRS is indeed generated in such conditions. This suggests that the signal is generated as soon as the resistance gene is either absent (SR1) or inactive ("Havana" at >30 °C).

8.10 SRS Results in Heritable Changes in HRF and Methylation Pattern

We hypothesized that the generation of SRS is a part of a plant adaptive response. Hence, it should lead to the changes that can be observed in the following generation. We call such changes "transgenerational". To check whether these types of events are possible, we collected the seeds from the infected plants and plants

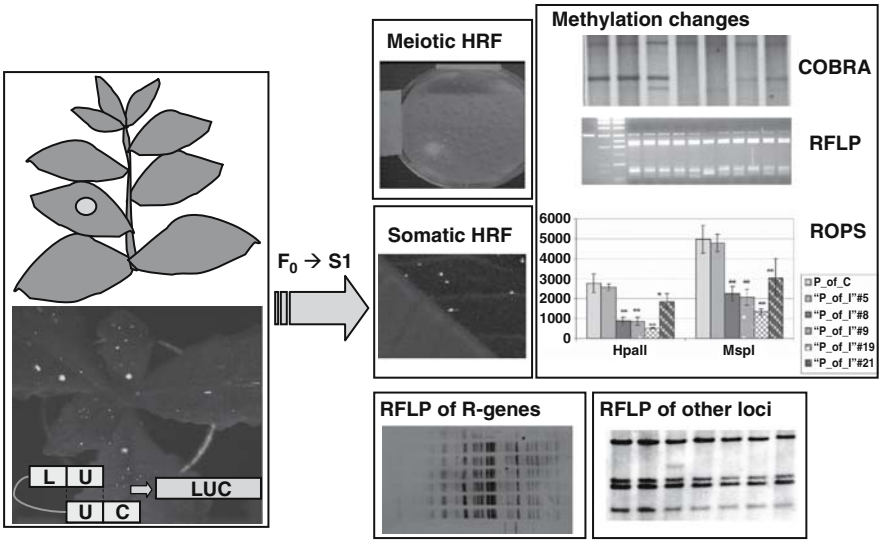

Fig. 8.2 Major changes found in the progeny of infected plants. Schematic representation of the experimental set up. Briefly, in a previous experiment, single leaves of 10-week old SR1 tobacco plants were inoculated with 300 ng of TMV RNA and 24 h after inoculation the infected leaves were removed. Seeds called from these plants were virus free. In the next generation (shown as "S1", or "stressed#1") we analyzed the meiotic and somatic recombination frequency, RFLP of the *N*-gene like and control (actin, 5.8 S and RENT loci) as well as global genome and locus-specific methylation patterns (analyzed with combined bisulfite restriction analysis or COBRA, methylation sensitive RFLP and random oligonucleotide-primed synthesis, or ROPS assays)

treated with buffer (control for wounding stress). First, we checked whether infection leads to the increase in the number of plants with a completely recombined transgene (Fig. 8.2). These plants express the luciferase in all cells. The experiments showed a close to threefold increase in these types of plants (Kovalchuk et al. 2003b). There are two possible reasons for the occurrence of the plants with the completely recombined transgenes. It could be either the somatic recombination event inherited early, or most likely the meiotic recombination event. It is plausible to think that SRS triggers the increase in meiotic recombination frequency, contributing to the diversity in the progeny. The similar increase in the meiotic HRF has been observed previously upon the constant exposure to elevated UVB levels (Ries et al. 2000).

The increase in the meiotic HRF observed in the transgene suggests that there could be similar changes in the other loci. This, however, can be harmful, and plants should employ various strategies to protect the important housekeeping genes essential to the proper plant function. In contrast, rearrangements in the loci carrying the homology to *R* genes might prove to be beneficial. The instability of the transgene could suggest that plants "recognize" the "neutrality" or the "importance" of a gene. It is also possible that the newly formed loci (such as a transgene) do not establish similar levels of chromatin structure, and thus do not follow the same type of the control over the rearrangements. If our hypothesis is correct, an

increase in *R* gene rearrangements triggered by a compatible infection could be seen as an attempt to formulate novel *R* genes for the next generation (Richter et al. 1995; Tornero et al. 2002).

Such a form of adaptive response to stress through the genomic alterations was previously proposed by Barbara McClintock (McClintock 1984). Extensive research has supported this model, showing that salicylic acid, methyl jasmonate, oxidative stress, wounding, pathogen attack, $CuCl_2$, cell subculture, and protoplast isolation activate transposons (reviewed in Arnholdt-Schmitt 2004). Since the activation of transposons is associated with a substantial decrease of the genome stability (Dennis and Brettell 1990; Miura et al. 2001), to regulate their activity is a very important task. The activation of transposons is in part associated with the decrease in DNA methylation (Wang et al. 1996; Neidhart et al. 2000; Cui and Fedoroff 2002). It remains to be established, however, whether there is a link between biotic stress, loci-specific hypomethylation, and changes in the genome stability of pathogen-infected plants.

8.11 Viral Infection Leads to Global Genome Hypermethylation in the Progeny of Infected Plants

The role of epigenetic control in the adaptation and acclimation process is hard to underestimate, as transgenerational changes in DNA methylation, histone modifications patterns and in the regulation of chromatin binding proteins are powerful tools of reversible changes in the gene expression. We have revealed that the progeny of plants that are constantly exposed to ionizing radiation have hypermethylated genomes (Kovalchuk et al. 2003a). Thus changes in DNA methylation patterns could be a part of the plant stress protection mechanism (Rizwana and Hahn 1999).

We have analyzed the global genome methylation status of progenies of infected and buffer-treated plants by digesting their genomic DNA using methylation sensitive enzymes *Hpa*II and *Msp*I (Fig. 8.2; ROPS assay). Four out of five progeny of infected lines showed the significantly increased global genome methylation levels (Boyko et al. 2007). Furthermore, we have found that the changes in DNA methylation were mostly due to the methylation of cytosines at the symmetrical CG and CNG sites rather than at the asymmetrical cytosing methylation (Boyko et al. 2007).

8.12 Viral Infection Results in Loci-Specific Methylation Changes

The high level of genome rearrangements is generally associated with the low level of methylation (Bassing et al. 2002; Bender 1998). The hypermethylation observed in the progeny of infected plants should then be associated with low recombination

frequency. In contrast, we observed a higher somatic and meiotic HRF. Previous reports showed a higher HRF in the progeny of stressed plants (Ries et al. 2000; Kovalchuk et al. 2003b; Boyko et al., unpublished data). The only explanation we could come up with was that there may be a differential pattern of methylation throughout the genome of progeny of infected plants. In this case, the majority of loci have higher methylation levels, while some loci, including the loci containing the transgene, could be hypomethylated.

If the aforementioned hypothesis is right, the *R* gene loci that carry homology to the *N* gene should have a lower methylation level to allow more frequent rearrangements to occur. Hypomethylation of these loci would allow them to have a higher degree of freedom for rearrangements (Bassing et al. 2002; Engler et al. 1993). As a control, we tested the methylation of actin, repetitive elements in *Nicotiana tabacum* (RENT) and 5.8 S rRNA loci in both progenies. The actin and 5.8 S rRNA loci are neutral to stress and thus should not undergo hypomethylation. The RENT loci contain regulatory elements, thus alterations of these loci could potentially change the expression of the neighboring genes (Foster et al. 2003). As such, a decrease in the methylation status at any of these loci could potentially be detrimental to plants.

The methylation sensitive RFLP analysis has indeed shown a differential pattern in the aforementioned loci (Fig. 8.2). Out of 22 loci that carried homology to the *N*-gene visible on the gel, 4 were drastically hypomethylated in the progeny of infected plants; the rest were similarly methylated among the 2 progenies. In contrast, 3 out of 9 actin loci were heavily hypermethylated, and the rest were equally methylated. At the same time, the methylation level of RENT and 5.8 S rRNA was similar in both progenies.

These experiments showed that the methylation status of the progeny of plants infected with a virus was significantly changed, whereby several *N*-gene-like loci were severely hypomethylated, and several actin loci were strongly hypermethylated. This differential methylation could possibly allow more flexibility in rearrangements of *N*-gene-like loci and for less flexibility of actin loci to occur.

8.13 Viral Infection Results in the Destabilization of R-Gene Loci in the Progeny of Infected Plants

The experiments describing a methylation pattern in the resistance gene, actin, RENT and 5.8 S loci represent only a portion of all the changes that occur in various areas of the genome. It is known that the increase in methylation in certain genome loci is correlated with a lower frequency of recombination, while the contrary is true for the loci that have a decrease in methylation (Bassing et al. 2002; Bender 1998; Engler et al. 1993). We hypothesized that the change in the methylation status of the *N*-gene-like *R*-gene loci would change its stability, whereby hypomethylation would lead to more frequent rearrangement events.

A rich variety of polymorphic *R*-gene families apparently have evolved by extensive rearrangement mechanisms such as gene and chromosomal duplications, unequal crossing over, and deletions/insertions incited in plants challenged by pathogens. In fact, all of the above have been shown to exist in various clusters of *R* gene loci (Mauricio et al. 2003; Stahl et al. 1999; Tian et al. 2002; Van der Hoorn et al. 2002). Therefore, in order to survive the constant battle with highly mutable pathogens, plants must continuously modify their *R* genes in order to recognize the pathogen *Avr* genes via gene-for-gene interactions (Madsen et al. 2003).

The RFLP analysis of the *N*-gene like loci in both progenies has indeed revealed a greater than fivefold higher frequency of rearrangements in the progeny of infected plants (Boyko et al. 2007). Most of the rearrangements analyzed by RFLP included the appearance of extra fragment(s) (Fig. 8.2). This is not surprising, as most of the events would be occurring in only one allele, and thus the original fragments would also be retained. In two cases, we observed the disappearance of fragments coupled to the appearance of several others. This suggests that the rearrangements either occur very early in the embryo development, or that the rearrangements occur in both alleles. Taking into consideration the fact that the loci containing the *R*-genes are complex in structure and are often unstable, it is not surprising that changes could be observed in both loci.

8.14 Stability of the Actin, RENT and 5.8 S Loci is not Changed

It is known that *R*-gene loci are generally more unstable than other loci. Spontaneous rearrangements in these loci rather than those induced by pathogen or any other stress, resulting in changes in pathogen resistance, have previously been shown (Sudupak et al. 1993). In contrast, we present the first case when such reshuffling is induced by pathogens. The fact that we observed a significantly high frequency of rearrangements in both transgene (Kovalchuk et al. 2003b) and *R* gene loci (Boyko et al. 2007) does not imply that every genome locus has such a high frequency of reshuffling. In fact, it would be harmful if these events were so frequent throughout the genome. We hypothesized that the majority of loci should have a "normal" frequency of rearrangements in the genome.

The most important task was to show that the genes "neutral" to a pathogen attack but essential for a correct plant function were equally stable in the progeny of both infected and control plants. Our analysis has revealed no difference in the RFLP of actin, 5.8 S rRNA or RENT. The comparable stability of the RENT loci is an important finding. RENT contains cryptic gene regulatory elements that are inactive at their native locations in the genome, but have the capacity to regulate the gene expression when positioned adjacent to genes (Foster et al. 2003). Thus the increased frequency of rearrangements of RENT loci in the genome could lead to

undesired changes in the expression of neighboring genes. Profiling of 5.8 S loci has revealed more rearrangements than profiling of either actin or RENT loci. Higher than normal recombination rates in the clusters of rRNA coding genes is a well-known phenomenon (Kobayashi et al. 2004). Despite the significant variations in the rDNA clusters observed in bacteria and yeasts, these loci have the reasonably uniform expression levels and are not significantly influenced by stress (Brunner et al. 2004). This is the main reason why these loci are frequently used as internal controls (Brunner et al. 2004).

DNA methylation is a well-explored process of genome maintenance, whereby methyl groups tend to make chromatin less accessible to various remodeling processes. It can thus be suggested that hypomethylation is the mechanism that facilitates the rearrangement of *R*-gene loci.

Plant genomes (especially those that are relatively large) are known to be highly repetitive and thus highly methylated. In this case, the methylation could be a mechanism that stabilizes the genome and prevents rearrangements (Puchta and Hohn 1996). The hypermethylation of the rest of the genome, as could be assumed from the global genome methylation data, prevents the deleterious effect of this genome reshuffling at unfavorable loci. Additionally, this can explain why some loci, such as the actin loci, seem to be less duplicated or found in clusters, since these configurations would result in higher conservation (Kroymann et al. 2003; Mauricio et al. 2003).

The chromatin structure of an organism is established after fertilization, and is specific to each individual organism. The changes in DNA methylation observed upon pathogen infection suggest that exposure to a stress apparently "rewrites" the methylation pattern according to the needs of an organism dictated by a particular stress (Wada et al. 2004; Weaver et al. 2004).

The information about pathogen-induced changes in the plant genome is scarce. Similarly, very little is known on how stresses in general influence the stability of the genomes in the progeny. There are only few reports on the changes observed in flax exposed to certain environmental pressures. Heritable changes in inbred flax in response to specific and defined environmental changes, such as nutrients balance and temperature regimes (Schneeberger and Cullis 1991; Cullis et al. 1999) have been reported. These conditions activate a transposon-like sequence, LIS1, that assembles and inserts itself into the genome of stressed plants (Chen et al. 2005). The newly established "genotrophs" appear to be stabilized, as no further changes in LIS1 activity occur in plants upon the exposure to additional stresses (Cullis et al. 1999). No further studies on this phenomenon have been carried out, and thus it is not known whether the transposon activation was methylation dependent.

Recent publication by Molinier et al. (2006) reports the induction of the genome destabilization in the progeny of *Arabidopsis thaliana* plants exposed to UVC or flagellin. The latter is a bacterial elicitor that can mimic a pathogen attack. The fact that flagellin induces both somatic and meiotic recombination frequency is exciting as it supports our finding with TMV-infected tobacco

plants. It is not clear, however, whether flagellin is capable of inducing the SRS as the authors applied the chemical systemically, whereas we performed local TMV infection. The authors were also able to show the epigenetic nature of the phenomenon by crossing the stressed plants to non-stressed plants and showing the increase in HRF in all the progeny. If this is a transgenerational phenomenon (presumably SRS-induced) was a "mendelian event", then the authors would observe either no increase in HRF if this event was recessive (Ns × NN = 0% of ss, where "s" is "stressed" and "N" is non-stressed "allele") or an increase in 50% of the population if the event was dominant (SN × NN = 50% of SN, where "S" is "stressed" and "N" is non-stressed "allele"). It is important to note that both maternal and paternal alleles were able to contribute to the change, since reciprocal crosses resulted in the increase in HRF in the progeny. This means that these transgenerational events were not the result of cytoplasmic (mitochondrial or chloroplast) inheritance but rather of nuclear changes in the chromatin structure or perhaps in the pool of small regulatory RNAs (miRNA, siRNA or others).

There is still a lot to be learned about pathogen-induced changes in the progeny of infected plants. It remains to be determined whether changes observed in the progeny were virus-specific or pathogen-wide. Future studies are needed to show whether the infection with bacterial pathogens leads to rearrangements in *N*-gene-like loci or perhaps any other type of *R*-gene loci, and whether the methylation status and genome stability return to normal in the progeny of infected plants that are grown under normal conditions. It also remains to be established whether plants with higher instabilities and methylation changes acquire any changes in their tolerance to similar (TMV) or different pathogens, or even other stresses.

8.15 Conclusion

We discussed various genetic and epigenetic mechanisms of plant response to stress. We focused mainly on pathogen stress and introduced the phenomenon of SRS-induced somatic and transgenerational changes in genome stability and DNA methylation. We described the existence of a specific, epigenetically controlled mechanism that promotes rearrangements in *R* gene loci in the progeny of plants infected with a compatible pathogen. This phenomenon suggests a second, more flexible level of inheritance regulated by stress that leads to the changes in the progeny of treated plants. Future studies are needed to understand the specificity of such regulation including signal production, maintenance and "inheritance".

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