Review

Epigenetic polymorphisms could contribute to the genomic conflicts and gene flow barriers resulting to plant hybrid necrosis

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The fundamental molecular basis for phenotypic and genetic similarities among many described cases of plant hybrid necrosis has not been fully described. Plants can be good models for studying the basis of such gene flow barriers which occur between species. Many studies in prezygotic barriers like stigma recognition of pollen, environmental adaptation differences and pollinator preferences which can reduce the chances of species mating success have been done. Also studied are post zygotic barriers in gene flow like lack of ecosystem adaptation of hybrids which may include failure of pollinators from being attracted to floral parts due to developmental changes and gene or chromosome incompatibility resulting in genetic isolation. Polyploidy has also been recognized as an isolating force although it might not be the only post zygotic genome isolating force; other forces may also contribute hindrances in the gene flow after zygote formation. Here, papers which have tended to pinpoint the increasing evidence of epigenetic polymorphisms as causes of genomic conflicts which cause barriers to the gene flow resulting in hybrid necrosis in plants were reviewed. Explorations into the mechanisms which unlock hybrid necrosis can help plant breeders to avoid genetic and epigenetic incompatibilities during crop improvement programs.

Key words: Hybrid necrosis, heterosis, epigenetic, hybrid sterility, RNAi.

INTRODUCTION

Little attention has been given to studies on postzygotic barriers in hybrid formation in plants especially hybrid necrosis which is caused by genetic incompatibility (Kirsten and Detlef, 2007a). The current view still holds that negative epistatic interactions of intra and inter genomes of the parents are the main causes of these barriers (Tikhenko et al., 2008; Bomblies and Weigel, 2007). Hybrid necrosis which is sometimes referred to as; firing (Mcmillan, 1936), premature perishing, hybrid weakness (Nishikawa et al, 1974) or often, has had common definitions which include a set of phenotypic characteristics that are similar to those associated with responses to environmental stresses like pathogen attack. But it can be defined as a hybrid formation reproductive barrier that prevents proper exchange of genomic information between well established species or between varieties of one and the same species. This might be due to lack of expression or the action of certain adaptive or regulatory genes, proteins or small RNA (smRNA) fragments. During hybrid formation, necrosis affects the F1 generation but it can also be observed in some F2 generation plants. This phenomenon seems to clearly challenge the most accepted definition of "species" which is referred to as individuals which can mate to produce viable offspring (Weigel and Dangl, 2007) although this reproductive barriers which prevent gene flow might at the same time set the stage for plant speciation (Gross, 2007; Sweigart et al., 2006).

The phenomenon of hybrid necrosis can be compared

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to epigenetic mechanisms in various ways, for example both are common and phenotypically similar in many different species; epialleles or alleles can be recessive or dominant; they are both subjected to environmental selection pressures and are dynamic. They are also involved in plant adaptation mechanisms and eventual evolution. Just as epigenetic mechanisms are complex in their functional interactions also in the same way hybrid formation like in the case of pollen recognition and growth, involves a cumulative action of barriers at multiple stages which may confer many intrinsic prezygotic blocks to plant interspecific hybridization (Kirsten and Detlef, 2007b). Moreover, Hurst and Pomiankowski (1991) and Frank (1991) independently argued of accompanying suppressers in the evolution of hybrid sterility and inviability. Hence, epigenetic mechanisms could be implicated as possible explanations of the divergence of meiotic drive systems.

The formation of hybrid zones often called "tension zones" appears when hybrids have lower fitness than parents (Buggs, 2007; Key, 1968; Barton and Hewitt, 1989) and there is a possibility that in the light of the various mechanisms which implicate epigenetics, reliable explanations can be drawn. Molecular studies have continued to confirm what Johnson, spoke of scant epigenetic evidence to support postzygotic reproductive isolation in hybrid ten years ago (Johnson, 1998). He cited Jablonka and Lamb (1998) and McGrath and Solter (1984) who had studies and postulations that epigenetic divergence in species had a role in hybrid incompatibility. This was not possible ten years ago when only chromatin marks could be considered in detail (McVean, 1998), but now we have powerful molecular tools to enable us to study reproductive isolation (Malone et al., 2007). Old time scientists in hybrids necrosis still state that 'the mechanisms underlying hybrid necrosis are potentially diverse, despite the apparently common involvement of the immune system' (Bomblies et al., 2007). In this review, the necrosis phenomenon with more molecular evidence synthesis and model how it might relate to plant epigenetic mechanisms was revisited. It is proposed here that this gene expression divergence and genome incompatibility may have roots in DNA methylation, histone modification, nucleosome positioning or is greatly influenced by the RNAi pathway mechanism.

EPIGENETICS AND SPECIATION

There is ample evidence that species evolve gradually and usually do not involve a change of the whole genome. Small accumulative changes in genome can eventually separate two former similar species. Recent studies in *Arabidopsis thaliana* have shown that approximately 2% of intraspecific F_1 crosses yield a necrotic progeny even when grown under conditions typical of their natural habitats (Bomblies et al., 2007). Incompatibility

of genomes due to epigenetic changes which might not be reversible as expected might also be involved in necrosis during hybrid genome. If such changes are positive, then a species may eventually be formed which has epigenetic variations and differential adaptations. Epigenetic studies made between established barriers of particular species can aid in evaluating the epigenetic role in creating such barriers.

The spectrum of epigenetic mechanisms in plants are complex although it is an integral part of the plant life cycle; for example cytosine methylation is distributed in symmetric ^{m}CpG and ^{m}CNpG and also in the asymmetric ^{m}CNpN nucleotides unlike in mammals where it is mainly found in ^{m}CpG nucleotides (Zhang et al., 2010; Mathieu et al., 2007). Although the asymmetric is reestablished after each mitotic cell division, the symmetric can be maintained across the mitosis and meiosis cell divisions (Martienssen and Colot, 2001). The ^{m}CpG methylation is crucial in the regulation of gene expression in the promoter regions, although ^{m}CNpG methylation appeared to play a crucial role in regulation of retrotransposons than ^{m}CpG in *Zizania latifolia* (Zhong et al., 2009). Detailed studies showed that methylation of the promoter or part of it was most important in bringing about gene silencing (Cedar et al., 1983; Doerfler et al., 1983; La Volpe at al., 1983).

The cytosine methylation of DNA prevents the binding of key regulatory proteins and could be inherited (Shapiro and Mohandas, 1983). However, molecular characterization of rice revealed that plant genome DNA methylation changes could also be induced by DNA introgression as in the case of rice by *Z. latifolia* (Griseb.) (Liu et al., 1999a,b). Hence, because gene flow barriers act on discrete loci, it means that initial epigenetic influence on those loci can be the triggers of speciation until the gene flow eventually ceases. If the gene flow stops and the established epigenetic variations are stable and adaptive to the environment, then other selective pressures will build on this foundation in the process of speciation. For example loss of DNA methylation has been known to cause abnormal floral development (Finnegan et al., 2000). This difference in the morphology of the flowers could restrict or change the pollinators of the plant or cause other plant pollinators to be able to have access to its stigma hence causing a different gene flow into the species or isolation of the former. Whole-genome scans have provided evidence that gene introgression between largely isolated species routinely takes place in nature (Kirsten and Detlef, 2007a).

EPIGENETICS AND HYBRID STERILITY

In the formation of hybrids there is a mixing of genomes and epigenomic mechanisms which is followed by a new established order of genome structure. This could include formation of highly asymmetric hybrids giving a possibility

of inter genomic exchange (Liu et al., 1999c) during which organizing some of the dominant mechanisms might over rule and silence some crucial development genes or functions causing hybrid sterility in the F1 generation. Studies have shown that sterility does not involve many genes even in cases where multiple loci have been implicated (Moyle et al., 2004; Zhu et al., 2005; Harushima et al., 2002; Burke and Arnold, 2001). This means nucleotide modifications can be enough in causing such drastic changes. These findings still point to what evolutionary biologists have traditionally suggested that the evolution of reproductive isolation is an opportunistic process (Dobzhansky, 1937; Coyne, 1992). Evidence from recent studies on *A. thaliana x A. lyrata* hybrids showed that genetic self incompatibility (SI) breakdown was reversible with no apparent changes in the underlying DNA sequence hence it was concluded that these modifications were mainly epigenetic in their nature (Nasrallah et al., 2007).

EPIGENETICS AND CYTOPLASMIC MALE HYBRID STERILITY

Cytoplasmic male sterility (CMS) occurs when there is no proper coordination between mitochondrial genes inherited from the mother and nuclear genes from the father (Chase, 2007). The causes of this lack of coordination have not so far been elucidated clearly. Hence a revisit using molecular epigenetic tools can aid in unlocking this well studied phenomenon. Combination of polyploidy studies with epigenetic and non-Mendelian genetic changes could aid in precipitating the basis of this barrier especially on the basis of chromosomal and gene incompatibility (Liu et al., 2009). This was first suggested after the first domestication of synthetic allopolyploidy lines of *Brassica* in which, the prominent site for DNA methylation (CCGG) was proposed to underlie some of the eventual genomic changes (Song et al., 1995). Later suggestions and propositions revealed that regions of non-coding DNA, which also regulate post-transcription, but evolve at higher rates than coding, are important in male sterility (Zuckerlandl, 1997). Today we have more evidence that these regions are important in epigenetic regulation. Recent studies imply that hybrid weakness may result from changes in pathogen resistance genes (Rieseberg and Willis, 2007), although the frequency of CMS which lead to necrosis need to be fully determined in order to elucidate the inviability of hybrids (Fishman and Willis, 2006).

EPIGENETIC ABERRATIONS AND HYBRID TUMOUR FORMATIONS IN PLANTS

It has been observed in plants like tobacco, wheat and cotton that some genetically conditioned tumors form during hybrid formation (Joshi, 1972; Phillips and Reid, 1975). This phenomenon has also not been explained to date (Kirsten and Detlef, 2007a). Tumours are due to uncontrolled gene expression which cause abnormal cell divisions especially in animals and have been linked to epigenetic abnormalities. Could this be the case in plants also? It is Interesting to note that the genetic basis of hybrid tumour formation in cotton plants is similar to the genetic basis of the mechanisms that controls necrosis (Hollingshead, 1930).

GENE EXPRESSION AND HORMONAL CHANGES AS RELATED TO NECROSIS AND EPIGENETICS

Gene expression has been known to have a link to hormonal signaling and function. For example studies by Shrivastava and Chawla (1993), showed that gibberellic acid (GA) treatment on *Vigna unguiculata* and *V. mungo* crosses could enhance pollen germination and pollen tube growth by partially overcoming embryo abortion. The same has been connected to hybrid necrosis and epigenetic mechanisms. For example, cytokinin levels have been correlated with the two processes as being positive, while hybrids have been rescued using gibberellic acid (Ladizinsky, 1997). Auxins also have been implicated in the formation of tumour in tobacco (Schaeffer, 1962). After Meyer, 2001 suggestion that chromatin remodeling regulates transcription, recombination, replication and genome organization, Chinnusamy et al, 2008 proposed that abscisic acid (ABA) might also regulate DNA methylation through DEMETER-LIKE PROTEIN (DML3) and siRNAs. Moreover they suggested that ABA downregulates histone deacetylases that is, HDACs (HDA6 and HDA19) together with the inducing of the expression of genes involved in seed maturation and stress induced germination arrest.

HYBRID NECROSIS AND PLANT EPIGENETICS

Epigenetic mutations especially those that involve CpG DNA methylation have been known to be lethal while others have severe developmental consequences (Mathieu et al., 2007). Hybrid necroses have been associated with characteristic phenotypes that deviate greatly from the normal. These include causes of cell death, tissue necrosis, wilting, variegation of leaves or yellowing, chlorosis, stunted growth and reduced plant growth rate. This causes early plant deaths with only a few survivors reaching reproductive maturity. This may have genomic differences from the parents and hence lead to the formation of different species with evolutionary implications. It is possible for hybrid necrosis and epigenetic mechanisms to work together in erecting gene flow barriers in plant natural populations. For example, it has been observed that in crosses between *Arabidopsis*
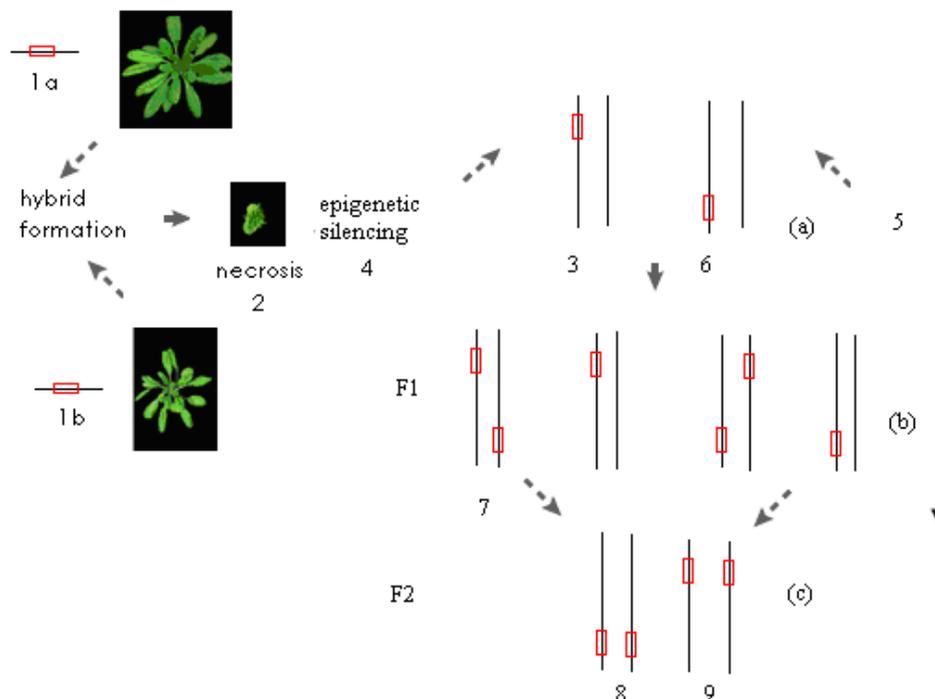
Figure 1. A model showing how levels of silenced genes loci (red boxes) can be brought together after the F1 and F2 generations from different varieties or foreign invaders' sources like pathogenic ones (1a, 1b). The epigenetically silenced genes can survive event 2 and be introduced into other plant genome 3 and 6 in similar mechanisms. After F1, the hybrid necrosis can occur when homologus silenced loci combine as in 7. The rest of the F1 remain normal due to heterozygosis in the loci. In F2 generation, we can expect the necrotic combinations 8 and 9 to occur. Several studies have shown similar trends like a cross between *Crepis tectorum* and *Crepis capillaris* suffer from extensive developmental abnormality. This could be due to the combination of epigenetically silenced loci from *C. tectorum* that found a similar counterpart in *C. capillaries.* This resulted in a suspension of crucial metabolic and developmental processes. This is because epigenetic processes like DNA methylation are transmitted in simple Mendelian fashion. The F2 50% healthy progeny between *Nicotiana sanderae* and *Nicotiana longiflora* crosses could have been be due to the same phenomenal cross outcome. There are implications of formation of former alien dsRNA and triggering of the RNAi pathway followed by PTGS. Even the NB-LRR disease resistance gene homolog in *A. thaliana* is both necessary and sufficient for the induction of hybrid necrosis, when combined with a specific allele at a second locus (photo courtesy of Bomblies et al., 2007).

arenosa and *A. thaliana* individuals of different ploidy levels there was a high seed lethality which correlated with an increase in the relative paternal genome dose. This was in turn suggested to be associated with an increase in epigenetic misregulation (Josefsson et al., 2006). This presented strong evidence that improper imprinting in plants could be the cause of genome dose-dependent seed lethality in hybrids. There are also proposals that specific transposon activation which is parent specific is affected by relative genome dosage of the parents (Kirsten and Detlef, 2007b).

Another interesting study was observed in the wheat leaves of the Kalyansona C306 and WL711 C306 hybrids which showed enhanced generation of a superoxide radical and H_2O_2 than parents before and during progression of necrosis. These higher lipid peroxidations have

been known to trigger epigenetic changes in plants. Surprisingly, they seemed to occur as an early event in hybrid necrosis and were accompanied by a loss in membrane permeability and cell viability and the hybrid necrosis barrier was overcome in some crosses by culturing ears in medium with antioxidants resulting in F_2 seeds being obtained (Sharma et al., 2003).

THE EPIGENETIC DIMENSION OF HYBRID NECROSIS

The basic classic illustration of hybrid necrosis involved the F2 of *Phaseolus vulgaris* in 1926 which had lesions and a lethal wilting. This was attributed with an interaction of two loci in which one could have been recessive. The partner loci of the recessive gene could have epigenetically

Figure 2. Three examples of thale cress (*A. thaliana*) plants showing hybrid necrosis. The left and right plants are the healthy parents, while the middle plants are showing hybrid necrosis by having yellowed misshapen leaves stunted growth and experiencing early death (image used with permission from Kirsten Bomblies-Max Planck Society, 2007, Gross, 2007).

been silenced. Hence the combination of the two genes could have interfered with the normal functioning of the plant development pathways (Figures 1 and 2). Studies have shown that during hybrid formation, there is a disturbance of gene silencing mechanisms in plants as it was observed in the loss of SI in the cross between *Capsella rubella* and *grandiflora* hybrids which were suggested to be connected to epigenetic silencing in the other S locus component, SCR (Nasrallah et al., 2007).

Another example was observed in which the paternal ATHILA transposable elements were specifically activated in hybrids in *A. thaliana* together with a loss of the imprinting of the PHERES1 promoter and the MEDEA gene. The misregulation of PHERES1 was suggested to be the cause of seed lethality because viable hybrids were formed when maternal *A. thaliana* parent had reduced PHERES1 activity during hybridization (Josefsson et al., 2006). Later studies suggested that this type of hybrid failure could surprisingly have been caused by a mismatch in a subset of a few regulatory genes (Kirsten and Detlef, 2007b). What is now becoming clearer is that there is an interactive link between the roles of transposable elements in hybrid dysgenesis together with epigenetic mechanisms and small RNA pathways (Michalak, 2009) were copurification of rasiRNA with piwi but not with dmAGO which binds with miRNA was reported, suggesting their cofunction (Gunawardane et al., 2007). Recent research also had this piwiRNA (piRNA) pathway implicated in maintaining transposon silencing in germ line genome (Chu and Rana, 2007) along side maternal effects (O'Donnell and Boeke, 2007). The possibility of an interactive fine tuning of epigenetic mechanisms can be implied in hybrid formation successes.

The frequency of hybrid necrosis is not a rare event just like the frequency of epigenetic mechanisms in plants. It is possible that silenced interallelic interaction at a single locus can be the gene flow barrier between two different species or even in similar species. The incompatibility between two epigenetic mechanisms can be the hindrance of hybrid formation if the species are from very different genomes. Interestingly, this is the case in hybrid necrosis in which widely homeologues from widely diverged subgenomes are more incompatible as compared to allelic interactions within same subgenome. Earlier evidences had shown that the problem of chromosome pairing and condensation can lead to hybrid sterility (Miklos, 1974; Hale et al., 1993; Forejt, 1996) but at which time the loci could not be characterized at the molecular level. The mixing of divergent genomes in the formation of hybrids can induce many genomic changes like genomic shock, instability, transcriptomic shock and changes in the chromatin structure which can cause gene silencing (Hegarty et al., 2006; Adams and Wendel, 2005;

Chen and Ni, 2006).

AUTOIMMUNITY AND EPIGENETIC MECHANISMS

The model plant *A. thaliana* has been identified as model for studying hybrid necrosis as it has also shown several cases of hybrid necrosis. This can be exciting due to the availability of whole genome sequence. Former studies had presented the hypothesis that autoimmunity is the underlying mechanism of hybrid necrosis (Bomblies and Weigel, 2007). This conclusion was based on results which were obtained after cloning the first gene which was proposed to have caused necrosis. This was a natural variant of a TIR-NB-LRR class pathogen response gene. It was observed that an interaction with another gene at a second locus from another accession TIR-NB-LRR encoded gene triggered necrosis (Bomblies et al., 2007).

Recent research has now shown that a misregulated immune system has the ability of establishing reproductive barriers and this might lead to speciation (Weigel and Dangl, 2007). Their studies showed that the necrotic plants had comparable profiles of gene activity and that the genes that determined autoimmunity were different in most *A. thaliana* crosses. Most strikingly, they discovered that often, only two genes were required to trigger the necrotic hybrid response. They found out that one of the fatal genes was paternal while the other one was maternal but they had normal activity in the parents. How this interaction was achieved could be via a matching and intergenome communication of two epigenetically silenced genes, promoters or enhancers which instead of leading to an unfruitful effort by the plant to activate them, rather led to the necrosis of the hybrid as modeled in Figure 1. Although the Dobzhansky-Muller type incompatibilities via epistatic interactions in plants have proposed it to have arisen via adaptive evolution of pathogen recognition genes (Clauss and Koch, 2006), they might not be the complete picture involved in causing hybrid necrosis without the epigenetic dimension. This can be an area for future verification given the significant large number of necrotic hybrids encountered in plants (Mino et al., 2002; Phillips, 1977; Heyne and Wiebe, 1943). The current use of mutants in the study of changes in gene expression and analysis of increased variation in dosage-regulated gene expression during divergent hybridization (Tikhenko et al., 2008) is showing increasing evidence in epigenetic involvement in elucidating hybrid necrosis.

NECROSIS AND EPIGENETIC IMPRINTING

The parent of origin phenomenon is common in epigenetic studies but in some cases hybrid necrosis has been found to be unidirectional. This suggests that there are interactions between nuclear genes from one parent lie the male parent and cytoplasmic elements from the

female parent (Inai et al., 1993). It is fascinating to note that the 'Spill over' of the cell death response triggered by nuclear and cytoplasmic incompatibility occurs in other tissues other than the anthers, in which cytoplasmic male sterility incompatibility is normally expressed, and hence, this has been suggested as the explanation for the deleterious hybrid phenotypes that are observed in such cases (Frank and Barr, 2003).

THE VIRUS-LIKE SYMPTOMS IN HYBRID NECROSIS AND THE EPIGENETIC RNAi PATHWAY

Virus like symptoms were observed in the F2 hybrids of *Phaseolus vulgaris* in beans which had hybrid necrosis characteristics like wilting, leaf variegation and plant lethality (Burkholder and Muller, 1926; Whitaker and Bemis, 1964). The RNAi pathway which has been shown to be important in plant defense against viral infection was also shown to be triggered by single stranded RNA even without the viruses. It was also shown to be systematic in plants. This implies that the mixing of two hybrids which have many genomic similarities can cause the RNAi pathway to be triggered leading to histone modification, gene silencing or mRNA degradation.

Studies by Weigel and Dangl (2007) showed that the gene which caused hybrid necrosis came from the two different parents (Burkholder and Muller, 1926; Whitaker and Bemis, 1964) and a detailed study of the same found that the gene that caused the hybrids necrosis was normally the one used by the plant to sense the presence of a pathogen. Hence, it is not surprising for us to stipulate that in the evolution of *A. thaliana* and other plants, key genes which pathogens used to invade the plant are again recognized by the RNA interference (RNAi) pathway in this case of hybridization probably due to a formation of dsRNA from the two genomes. This presence of foreign RNA strands is usually cleaved by DICER complex proteins to form siRNA before being incorporated to ARGONAUTE and RNA induced transcriptional gene silencing (RITS) for posttranscriptional gene silencing coupled with mRNAdegradation leading to necrosis. This can cause the appearance of virus symptoms in plants. It can be suggested that the RNAi pathway could be the most likely pathway to fully establish the relationship between hybrid necrosis and epigenetic mechanisms in plants. This is because plants have the advantage of so far not having off target effects of the RNAi. For example a system to identify such possibility using the salicylic acid-binding protein 2 (SABP2) gene found no off target effects (Kumar et al., 2006). This was possible may be due to the miRNA which silences only the sequences with homology causing cleavage although full potential use of RNAi in crop improvement has not yet been realized (Mansoor et al., 2006).

Other epigenetic pointers can be elucidated from

Wheat plants which underwent hybrid necrosis as they were found to have increased levels of superoxide, a molecule that is associated with oxidative stress and a trigger of epigenetic mechanisms (Stephens, 1950). The necrotic tobacco hybrids also showed chromatin condensation and DNA fragmentation (Marubashi et al., 1999). Elevated levels of proteins and genes that are associated with plant pathogen responses (PR proteins) have also been observed in these necrotic hybrids. The role of the RNAi mediated gene silencing could also be envisioned in several cases of hybrid necrosis in *A. thaliana* where different studies show in the absence of pathogen characteristics of pathogen response gene expression in the plants (Kirsten and Detlef, 2007b). Recent studies in wheat, which had classified hybrid necrosis as type I and II; with type I having tissues which had necrosis occurring gradually from old tissues while type II necrosis grew normally until exposed to low temperatures after which necrotic phenotype occurred accompanied with incomplete emergence from the leaf sheath at the tillering stage (Nishikawa, 1962). The type I hybrid necrosis showed limited changes in gene expression which could have induced the phenotypic difference between the WT and the type I necrotic lines (Mizuno and Takumi, 2007). A. *thaliana* strains, Uk-1 and Uk-3 also developed hybrid necrosis at mild temperatures (Bomblies et al., 2007). Is exciting to imagine that the temperature sensitive RNAi pathway could be involved in the type II necrosis, while other epigenetic mechanisms like cytosine DNA methylation be involved in type I hybrid necrosis.

HYBRID NECROSIS, EPIGENETIC MECHANISMS AND ENVIRONMENTAL STRESSES

Due to their sessile nature, plants seem to have a complex epigenetic mechanism which is influenced by environmental changes. This enables the plants to adapt and thrive in many dynamic environmental stresses. Epigenetic studies could aid in understanding the causal molecular mechanism of the lethal mechanism of the F1 hybrid lethality in *Mimulus guttatus*, which could have been a by-product of selection for copper tolerance (Christie and Macnair, 1987; Gepts and Bliss, 1985). The explanation is still unknown, just like the heat shock proteins (HSP) chaperones which have been proposed to be associated with epigenetic mechanisms, the pathogen defense protein called defensins which are associated with zinc tolerance in *A. halleri and A. lyrata* could aid in relating epigenetic mechanisms and hybrid necrosis (Macnair and Christie, 1983). The breakdown of necrosis in wheat after irradiation of wheat C.360 by gamma rays from cobalt-60 which caused NE_1 locus mutant was considered to be due to minute deletion of the NE_1 loci of C.360 (Bhowal et al., 1981). This consideration could not rule out epigenetic gene silencing mechanisms, just like in the reverse when such stresses induce gene silencing.

Accumulation of free radicals in the plant tissue has also been a trigger of epigenetic mechanisms. Earlier studies have shown that in wheat hybrids necrosis there was a rapid accumulation of superoxide anion due to a possible lipid peroxidation which caused an accumulation of free radicals and increased permeability of cell membrane. It was suggested that this could have been due to a breakdown of an ion scavenging mechanism which could have a genetic origin (Dalal and Khanna-Chopra, 1999) and could interfere with gene regulation. Mena et al. 2009, recently reported that reactive-oxygen species (ROS) can cause protein, lipid, and DNA damage in cells (Mena et al., 2009).

CONCLUSION

Environmental stresses whether biotic or abiotic are able to mobilize plant defense genes that can initiate survival mechanisms in plants via physiological responses. Studies in plant pathology proposed a rapid programmed cell death response in case of pathological attacks in order to deter the spread of tissue degradation and help the plant to adapt to changing environment. Plants might keep a memory of that stress via genome epigenetic changes so that it can respond to future stresses (Boyko and Kovalchuk, 2007). In the same thought, it seems that human assisted hybrids breeding and also microbial genomic attacks in plants could at times lead to deleterious epigenetic interactions in plants that can cause hindrances in gene flow in plants. This can also trigger other epigenetically induced regulatory genes and elements like retrotransposons in diverse populations as was observed in *Phragmites australis* populations (Li et al., 2009). Such interactive implications can provide an explanation in the search for full understanding of the molecular basis of hybrid necrosis.

Epigenetic incompatibility can arise and cause pleiotropic effects in hybrid and polyploidy contexts causing a breakdown of the coexistence and co-adaptation between key genes and other genome proteins alongside triggering of the wider epigenetic RNAi pathway mechanisms. Since hybrid necrosis is associated with many interspecific incompatibilities (Phillips, 1977; Krüger et al., 2002; Chisholm et al., 2006; Mondragón-Palomino et al., 2002), there are suggestions that the causal alleles in the genome could, at least often be fixed between populations and act as stress reminders in the genome. Hence there is need for more research to be done in order to establish the correlation between plant species interaction with their biotic and abiotic environments as coupled with the eventual genomic selective outcome amongst conflicting functional mechanisms. For example, the large size and exceptional non synthetic property between maize inbred lines of the maize genome might have been the reason why hybrid necrosis has not been observed in this important crop despite the intensive out crossing

during domestication. This does not mean that hybrid necrosis or silenced interallelic interaction can not be found in the same loci but it just implies that it shall be very rare. This might be one of the explanations of why hybrids heterosis which is the opposite of hybrids necrosis has been difficult to be fixed in maize.

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