Hybrid lethality in plants

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Summary: Plant interspecific F₁ hybrids sometimes are vigorous, but they also reveal severe weakness which has been known as hybrid lethality. Hybrid lethality acts as a major reproductive barrier, eventually blocks gene exchanges between species. Unavailability of interspecific hybrid progenies is an obstacle for wide-crossbreeding, and requiring information on nature of hybrid lethality to improve efficiency of this breeding program. However, little is known about physiology and biochemistry of the hybrid cell death, and the genes involved in hybrid lethality are enigma. Recently, new studies started to uncover the facts about how hybrid cells die and which genes are supposed to control hybrid lethality. This article reviews up-to-date studies on hybrid lethality in plants.

Key words: Reproductive isolation, Wide-cross breeding, Hypersensitive response, Cell death, R gene

Introduction

While acting as a gene-flow barrier and being involved in animal and plant speciation, reproductive isolation is a major obstacle to the wide-crossbreeding of plants. The reduced viability and fertility of interspecific hybrids results from incompatibility of parental genes and chromosomes (Orr and Coyne, 1992; Forsdyke, 2004). Hybrid lethality, an intrinsic postzygotic isolation barrier, appears as developmental defects and has been found in many plant hybrids including Solanum spp. (Valkone and Watanabe, 1999; Krüger et al., 2002), Gossypium spp. (Phillips, 1977), Nicotiana spp. (Marubashi et al., 1999; Mino et al., 2002), and Triticum spp. (Hermsen, 1963). The Dobzhansky-Muller (DM) model predicts that the epistatic interaction of two or more loci among taxa induces sterility and lethality in hybrids, and theoretical and experimental data have been proposed to support this model (Coyne and Orr, 2004). However, information about the cellular features and genic components involved in hybrid lethality is restricted to Drosophila spp. (Orr et al., 1997; Barbash et al., 2003).

Recently, cell death and candidate causal genes in problem hybrids were reported in plants. To tackle difficulties in wide-crossbreeding, it is worth understanding the nature of hybrid lethality. This article briefly reviews the latest advances in study of hybrid lethality in plants, focusing on (1) cell biology and physiology, and (2) plausible genetic components.

Cell biology and physiology of hybrid lethality

Hybrid lethality occurs in several developmental stages, with a complete loss of hybrids observed in Nicotiana and Triticum (Mino et al., 2002; Hermsen, 1963) and a partial loss in Oryza and Hordeum (Chu and Oka, 1972; Konishi, 1985). The morphology and physiology of hybrid lethality has mostly been studied in Nicotiana spp. (Marubashi et al., 1999; Yamada and Marubashi, 2003; Mino et al., 2002; 2005: 2007ab). F₁ hybrid seeds of N. gessei x N. tabacum germinated normally, but cell death initiated at the base of hypocotyls, quickly extended to the entire plant and eventually led to the loss of hybrid plants (Mino et al., 2002). This quick death, a programmed cell death (PCD), was also found in the hybrid of N. glutinosa x N. repanda (Marubashi et al., 1999). The PCD related to temperature, proceeding at 26°C, an impermissive temperature, but not at 37°C, a permissive temperature. Temperature-dependent hybrid lethality is observed in many plant species, e.g., Triticum spp. (Hermsen, 1963), Lactuca spp. (Jeuk et al., 2009) and Arabidopsis (Bomblies et al., 2007). In plants and cultured cells of Nicotiana hybrids, oxidative burst, the quick release of reactive oxygen species (ROS), plays a role in the initiation of cell death, because suppression of the generation of ROS reduces the velocity of cell death (Mino et al., 2002; 2004; 2005). Transcripts of PR1 and PI-II, genes associated with plant pathogen responses, accumulated in hybrid plants grown at 26°C but not at 37°C (Mino et al., 2002). Overproduction of ethylene in a lethal hybrid (N. suaveolens x N. tabacum) was a hallmark of hypersensitive response (HR)-like cell death (Yamada and Marubashi, 2003). These results suggest that proper cellular signaling in the hybrid triggered the generation of ROS, which in turn operated as a signal to initiate HR-like PCD.

Experiments using a mutant form of NtMEK2, an upstream regulator of mitogen-activated protein kinase (MAPK), showed that the MAPK-cascade was involved in the regulation of PCD.
in hybrids (Mino et al., 2007a). Plants and cultured cells of hybrids of \textit{N. gossei} and transgenic \textit{N. tabacum} harboring a dexamethasone-inducible NtMEK2\textsuperscript{RD} or NtMEK2\textsuperscript{RR}, constitutively active and inactive forms of NtMEK2, respectively, were established. NtMEK2\textsuperscript{RD} transmitted a strong signal to a downstream salicylic acid-induced protein kinase, a molecular form of MAPK, and provoked the generation of ROS as well as PCD. By contrast, NtMEK2\textsuperscript{RR} shut down this signal transmission, and suppressed both ROS production and PCD. This result indicated that the MAPK-cascade is the main pathway of signal transduction from problem genes to PCD in this F\textsubscript{1}, hybrid. However, our unpublished results indicated that calcium-dependent signaling to be more important in the PCD of the F\textsubscript{1} hybrid of \textit{N. tabacum} x \textit{N. africana}. Seedlings and cultured cells of this hybrid died quicker than did those of F\textsubscript{1}, hybrid of \textit{N. gossei} x \textit{N. tabacum}. This suggests that the different combinations of species hybrids comply with respective stimuli of hybrid problem genes and cellular signaling pathways that complete hybrid lethality.

Closer inspection of cultured cells of the F\textsubscript{1}, hybrid (\textit{N. gossei} x \textit{N. tabacum}) indicated that disruption of the inner membrane occurred within 60 min after the transition from 37\degree C to 26\degree C (Mino et al., 2005). This suggests that cell death resulted from the collapse of the vacuole. A transmission electron microscopic analysis revealed that tonoplasts in epidermal cells of F\textsubscript{1}, hybrid hypocotyls disintegrated very early after seed germination at 26\degree C (Mino et al., 2007b). This disintegration extended to all cortex cells within 4 days after germination, leaving no cells with normal subcellular organelles. However, no such vacuolar collapse or cell degradation was observed in hybrid seedlings grown at 37\degree C. A biochemical study showed that vacuolar processing enzyme (VPE) is involved in hybrid cell death (Mino et al., 2007b). VPE is a plant leumagin with significant homology in structure and function to animal caspase, and acts as a “death protease” in HR cell death in response to attack by pathogens (Hatugui et al., 2004). Activity of VPE rose significantly higher in hybrid seedlings grown at 27\degree C than at 37\degree C, and specific inhibitor of VPE successfully suppressed PCD. Thus VPE functions not only as an executioner of cell death in the response of plants to several forms of biotic or abiotic stress, but also in the cell death underlying hybrid lethality.

Taken together, studies so far provide evidence that the PCD in hybrid lethality is very similar to the process of HR cell death in plants. There might be some pathogen perception system functioning upstream of the PCD in hybrid lethality.

\textbf{Plausible genetic components involved in hybrid lethality}

The DM model requires epistatic effects of at least two loci differing between the parental species for hybrid incompatibility to occur. Accordingly, in \textit{Drosophila} spp., genetic factors for proteins with MADF DNA-binding domains (Barbash et al., 2003) and nuclear pore proteins (Presgraves et al., 2003) are plausible candidates for hybrid problem genes, each thought to interact with an unknown counterpart in the hybrid genome.

In plants, several genes involved in hybrid lethality have been identified, including \textit{Ne} in \textit{Triticum} spp. (Hermsen, 1963), \textit{Le} in \textit{Gossypium} spp. (Song et al., 2009) and Cf-2/Rcr3 in \textit{Lycopersicon} (Solanum) spp. (Krüger et al., 2002). Cf-2 confers resistance to the fungus \textit{Cladosporium fulvum}, and had been bred from \textit{L. pinninellifolium} into cultivated tomato. Cf- genes encode protein with the leucine-rich repeat (LRR), a common feature of R proteins in plant, and require Rcr3, a papain-like cysteine endoprotease, for proper functioning. The F\textsubscript{1}, progenies of \textit{L. esculentum} (cf2/cf2, rcr3/rcr3) x \textit{L. pinninellifolium} (Cf2/Cf2, Rcr3/Rcr3) gave autoncrotic individuals (Cf2/-, rcr3/rcr3), indicating that Rcr3 acts as suppressor of Cf2-dependent autonecrosis in tomato. Thus to improve resistance to \textit{C. fulvum}, a second \textit{L. pinninellifolium} gene (Rcr3) was also required for introgression of the \textit{R} gene (Cf-2) into \textit{L. esculentum}. Otherwise, no practical cultivar was established. This suggests that aberrant interactions between \textit{R} gene products and associated factors in hybrid cells will result in lethality. Interestingly, recent studies indicate this possibility to be real (Bomblies et al., 2007; Jeucken et al., 2009; Alcázár et al., 2010). In some intraspecific F\textsubscript{1}, hybrids of \textit{A. thaliana}, hybrid necrosis (almost the same as hybrid lethality) was observed at 16\degree C, a common temperature for \textit{Arabidopsis} in nature, but was suppressed at 23\degree C allowing growth to the flowering stage (Bomblies et al., 2007). Through single nucleotide polymorphism scanning and quantitative trait locus mapping of F\textsubscript{1}, plants, the progeny of uncompromised “F\textsubscript{1},” plants grown at 23\degree C, two unlinked loci were verified to be involved in hybrid necrosis. A series of experiments indicated that these loci were alleles of Toll interleukin receptor-LRR proteins. Using artificial micro RNAs which inactivate target genes, it was clarified that these \textit{R} genes were necessary for hybrid necrosis. More recently, it was reported that the extent of the growth defects of \textit{Arabidopsis} recombinant inbred lines, derived from crosses between \textit{Landsberg erecta} and other ecotypes, depends on the number of polymorphic \textit{R} loci in the genome (Alcázár et al., 2010). Epistatic interaction affecting levels of resistance to pathogen might be causally related to levels of hybrid necrosis or lethality. Rin4, another regulatory factor involved in plant resistance against pathogens, was responsible for the lethality of an interspecific lettuce hybrid.
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(Lactuca sativa x L. saligna) (Jeucken et al., 2009). Rin4, RPM-INTERACTING PROTEIN4, is one of the factors associated with R protein, and speculated to 'guard' host proteins and recognize pathogen effectors (Mackey et al., 2002; 2003). Expression of the Rin4 gene of L. saligna (Rin4-sal) in leaves of L. sativa by Agrobacterium tumefaciens-mediated transfer resulted in severe cell death, suggesting that aberrant interaction between the Rin4-sal and R proteins of L. sativa elicits hybrid lethality.

Recent advances in our understanding of plant-pathogen interactions have revealed that multiple factors are orchestrated to recognize pathogen-derived molecules and trigger immune responses in host plants. Since malfunctions would have harmful effects on cells, R proteins must be correctly folded and located in appropriate cellular locations when inactive, but upon pathogen attack, be activated and provoke downstream signaling pathways including HR cell death. The transition from inactive to active forms of R proteins and elimination of used R proteins require molecular chaperone complexes such as SGT1-HSP90 (Shirasu, 2009). Aberrations caused by the mismatched pairing of R proteins with molecular chaperones will induce the inappropriate activation of downstream pathways, leading to unexpected abnormalities including cell death. During host-pathogen co-evolution, strong diversifying selection results in increase and diversification of both R genes and cofactors. Indeed, 150 NB-LRR genes and more than 600 receptor-like kinase genes involved in plant immune systems were reported in A. thaliana (Meyers et al., 2003; Shiu et al., 2004). Mismatches of these parental components in hybrids may lead to the hyperactivation of responses in the absence of pathogen attack, and eventually to death.

Concluding remarks

The mystery behind hybrid lethality is gradually being uncovered. The death of hybrid cells has very similar features to the HR to pathogen attack, and genes involved in plant immune systems might be causative of hybrid lethality. Therefore, R genes and related factors could become a reproductive barrier after the diversification of species under pathogen-related selection pressures. However, genes that exclusively comply with the DM model may not always act as a reproductive barrier. For example, no class DM gene pairs acting as a major driving force of speciation were found in the yeast genome, but more complex interactions of parental factors were discovered instead (Kao et al., 2010). This may explain why and how different levels of reproductive isolation have been evolved among plant taxa. Finally, we have to consider how to use this information to improve the efficiency of wide-crossbreeding. Given that the technology to knock out and/or down causative genes is available, the possibility of obtaining viable F1 hybrids from numerous combinations of species will increase.

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lethality in hybrid seedlings from the cross Nicotiana
death, temperature sensitivity and the genetic control associated
with resistance to cucumber somatic virus (CMV) in diploid

植物の雑種致死
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要旨：植物の雑種F1雑種は、時々強い強勢を示すが、多くは雑種致死として知られるような様々な弱勢を示す。雑種
致死は主要な生殖隔離機構であり、雑種の遺伝子交換を妨げる。致死により雑種雑種で後代が得られないことは遠縁交
雑種種には不利であるため、育種の効率を高める上でも雑種致死について知ることは重要である。しかし、その生化学
や生殖学、また関与する遺伝子については不明なことが多かった。近年、雑種致死の動態や関与する遺伝子についての
新たな研究が始まり、雑種雑種がどのように致死するのかと共に雑種致死発現に関与する可能性の高い遺伝子について
も明らかになってきた。この発見は、雑種致死に関する新しい情報を、(1) 雑種の細胞死の細胞生物および生理学、(2)
遺伝子、の二つの側面からまとめた。

キーワード：生殖隔離、遠縁交雑種種、過敏性反応、細胞死、R 遺伝子

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