Hybrid Necrosis in Wheat—A Genetic System Showing Reduced Capacity to Detoxify Reactive Oxygen Species Leading to Programmed Cell Death

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Hybrid necrosis in wheat is the premature gradual death of leaves and leaf sheath caused by two complementary genes Ne1 and Ne2 when brought together in a hybrid combination. Many promising wheat varieties are carriers of these genes, which limit the parental choice for transfer of desirable traits as the necrotic plants die without producing seeds. The leaves of Kalyansona × C306 and WL711 × C306 hybrids showed enhanced generation of superoxide radical and H$_2$O$_2$ than parents before and during progression of necrosis. Higher lipid peroxidation was an early event in hybrid necrosis and was accompanied by a loss in membrane permeability and cell viability. The anti-oxidant defense in necrotic hybrids was not well coordinated culminating in a persistent oxidative stress in the leaf especially in the chloroplast. The hybrid necrosis barrier was overcome in some crosses by culturing ears in medium containing antioxidants and F2 seeds have been obtained. Hybrid necrosis is a unique genetic system for studying the molecular mechanism of programmed cell death in plants. The cloning and characterization of Nel and Ne2 may provide a tool having wide applications in agriculture. The review compares hybrid necrosis with other PCD phenomenon in plants.

Keywords: hybrid necrosis, programmed cell death, oxidative stress, wheat

Introduction

Hybrid necrosis is the premature gradual death of leaves and leaf sheath (Fig. 1A) caused by two complementary genes Ne1 and Ne2 when brought together in a hybrid combination (Hermsen, 1963). Many promising wheat varieties are carriers of Ne1 or Ne2 genes located on chromosomes 5BL and 2BS, respectively (Nishikawa et al, 1974). This limits the parental choice for transfer of desirable traits at intraspecific i.e. among hexaploids as well as interspecific level (hexaploids × tetraploids). The severity of necrosis varies greatly due to multiple allelism of these necrotic genes. In certain crosses, the F1 shows severe necrosis and dies without producing seeds. Necrosis in the leaves indicates localized death of the cells or tissue (Fig. 1B). Thus, hybrid necrosis appears to be a genetically regulated PCD process.

PCD is a process wherein there is a destruction of unwanted cells through the activation of a genetically controlled cell suicide machinery (Ameisen, 1996). The term programmed cell death is sometimes used synonymously with 'apoptosis.' Apoptosis is a strictly regulated process and is responsible for the ordered removal of superfluous, aged or damaged cells. This form of PCD occurs as part of normal developmental processes and differs from those which occur at the site of environmental stresses such as pathogen infection or physical wounding or in response to low concentration of toxins. These types of cell death appear to occur through a controlled disassembly of the cell (Buckner et al, 1998). The other mode of cell death i.e. necrosis is characterized by cellular swelling (oncosis) and lysis and is the outcome of severe and acute injury such as sudden shortage of nutrients, exposure to a high concentration of a toxin, heating or freezing, etc (Cohen, 1993).

PCD in animal systems is a much researched and reviewed subject (Kroemer et al, 1998). Classical experiments have been conducted on Caenorhabditis elegans on the induction and action of specific genes that bring about the controlled disassembly of the cell (Wadewitz & Lockshin, 1988). The regulation of PCD is conserved in diverse organisms. In plants, the research in PCD has been the focus only in recent years (Lam et al, 1999; Pennell & Lamb, 1997; Richberg et al, 1998). It has been suggested that

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Abbreviations:
APX: Ascorbate peroxidase; CAT: catalase; DAS: days after sowing; GR: glutathione reductase; H$_2$O$_2$: hydrogen peroxide; NADH: nicotinamide adenine dinucleotide (reduced); NADPH: nicotinamide adenine dinucleotide phosphate (reduced); P0X: peroxidase; PCD: programmed cell death; ROS: reactive oxygen species; SA: salicylic acid; SOD: superoxide dismutase; TTC: 2,3,5-triphenyl tetrazolium chloride.
despite some differences both plants and animals could share some common components of a core mechanism to carry out PCD (Danon et al, 2000). Understanding the regulation of PCD in plants may have important applications in agriculture and post-harvest industries helping in prolonging the shelf-life of crops, fruits and vegetables. Also the suppression of PCD could help in minimizing disease symptoms caused by pathogens.

ROS such as superoxide, H$_2$O$_2$ and hydroxyl radicals are key players involved in signaling of PCD (Jabs, 1999; Lamb & Dixon, 1997; VanCamp et al, 1998). Transgenic tobacco plants with reduced activities of H$_2$O$_2$ scavenging enzymes such as APX and CAT had higher rates of PCD following bacterial infection than wild type plants (Chamnongpol et al, 1996; Mittler et al, 1999). Low doses of ROS induces anti-oxidant enzymes, however, when the concentration of ROS reaches a certain threshold, a signal transduction pathway that results in PCD is activated (Levine et al, 1996). Thus, the survival of plants depends on the coordinated effort of the anti-oxidant defense system. Oxidative stress could be a common mechanism involved in PCD. A detailed analysis of the mechanism of hybrid necrosis is required to understand this process of cell death in plants as well as to overcome this barrier to gene transfer in wheat.

This review focuses on the phenomenon of hybrid necrosis as a type of PCD and the different processes associated with PCD.

**Hybrid Necrosis**

Hybrid necrosis is common in wheat as the carriers of the genes Ne$_1$ and Ne$_2$ are present in most of the promising varieties of Triticum aestivum (Linn.). Depending on the nature of the cross, the first necrotic symptoms may appear at any growth stage of the plant from the 2nd leaf onwards. They first appear on the leaves followed by the leaf sheaths. The stems and the ears mostly remain normal green when the leaves and sheaths are already necrotic. In most crosses, the symptoms progress from the tip of the leaf to its base and from the older to the younger leaves. This is called 'progressive necrosis' (Fig. 1B).

The degree of necrosis varies and nine necrosis grades have been distinguished depending on the expressivity of the Ne$_1$ and Ne$_2$ alleles. These alleles could be weak, moderate or strong and their expression is influenced by the environment (Hermson, 1963). When drought tolerant wheat variety C306 was crossed with high yielding varieties such as Kalyansona, WL711, J24, etc of Mexican origin, the F$_1$ progeny exhibited hybrid necrosis and the plants died at various stages of development without producing viable seeds (Khanna-Chopra & Patil, 2002). Kalyansona x C306 hybrid was severely necrotic and died at 6-8 leaf stage while WL711 x C306 hybrid was comparatively less severe and survived up to flag leaf emergence stage (Table 1). The J24 x C306 hybrid survived up to ear emergence stage and exhibited leaf senescence followed by death. Thus, cv. C306 and Kalyansona are carriers of strong Ne$_1$ and Ne$_2$ alleles, while WL711 and J24 are carriers of moderate-strong and moderate Ne$_2$ alleles, respectively. The interaction of strong Ne$_1$ allele with strong or moderate-strong Ne$_2$ alleles produces wheat hybrids, which die without producing grains.

**Oxidative Stress and Hybrid Necrosis**

Generation of ROS has been implicated in various processes of PCD (Inze & Van Montagu, 1995). This increase in ROS leads to lipid peroxidation, which ultimately causes membrane damage and finally leads to cell death. ROS scavenging depends on the detoxification mechanism provided by an integrated system of non-enzymic molecules like ascorbate, glutathione, carotenoids, α-tocopherols and flavonoids as well as enzymatic anti-oxidants. These enzymes include SOD (EC 1.15.1.1), which reacts with superoxide radicals and converts them to O$_2$ and H$_2$O$_2$. H$_2$O$_2$ is then detoxified by CAT (EC 1.11.1.6) and/or APX (EC 1.11.1.11). Glutathione, an
intermediary redox metabolite in the ascorbate-glutathione cycle of scavenging 
$\text{H}_2\text{O}_2$ is maintained in 
the reduced state by GR (EC 1.6.4.2). In addition, 
non-specific (guaiacol) POX (EC 1.11.1.7) also plays 
an important role in the anti-oxidative protection.

Our studies showed that ROS were involved in 
mediating the cell death observed during hybrid 
necrosis in wheat. An increase in superoxide radicals 
was observed even before the onset of necrosis in the 
hybrid leaves as compared to parents. The gradient of 
superoxide levels from tip to the base was parallel 
with the progression of necrosis (Fig. 2A) (Khanna-
Chopra et al, 1998). In‘lesion stimulating disease 
resistance response’ mutant (ild1) of Arabidopsis, 
superoxide radical initiates a runaway cell death 
phenotype (Jabs et al, 1996). Membrane bound NADPH-oxidase is known to be a source of 
superoxide radicals during oxidative burst (Lamb 
& Dixon, 1997). During hybrid necrosis the activity of 
NADPH-oxidase increased significantly only after 
50% necrosis in the hybrid leaf (Fig. 2B). Thus, 
NADPH oxidase may not be responsible for the 
increase in superoxide radicals preceding onset of 
necrosis. A higher $\text{H}_2\text{O}_2$ content was observed even 
before the onset of necrosis in the hybrid leaves as 
compared to the parents and increased several fold 
with the progression of necrosis (Fig. 3A). Thus, the 
early on higher ROS generation in the hybrid leaf 
meditated cell death.

Lipid peroxidation and membrane permeability 
were higher in hybrid leaves than the parents 
throughout the ontogeny (Fig. 2C) (Dalal & Khanna-
Chopra, 1999). Cell viability measured by TTC 
reduction showed significant correlation with 
conductivity. Increase in conductivity and decrease in 
TTC reduction was observed with progression of 
necrosis (Fig. 2D). Thus, in necrotic leaves of hybrid 
wheat, a close relationship was observed between 
lipid peroxidation, membrane permeability and cell 
viability. Loss in chlorophyll and carotenoids was 
observed only after 50% necrosis stage. Similarly, the 
photosynthetic efficiency, measured as Fv/Fm, 
deleed only after the appearance of visible necrosis 
(Dalal & Khanna-Chopra, 1999). The results 
suggested that increased superoxide radical might 
have induced peroxidation of membrane lipids further 
leading to more generation of free radicals. These 
reactions being autocatalytic and non-reversible in 
nature continued and caused membrane damage thus 
disturbing the homeostasis required for the normal 
functioning culminating in the death of cells. Inspite 
of higher lipid peroxidation and membrane damage 
delayed effect on photosynthetic pigments and PSII 
might be due to differential sensitivity of chloroplast 
membrane and PSII to superoxide radical (Shen et al, 
1997).
Study of the anti-oxidant enzyme system in the hybrid leaves at different developmental stages revealed a differential response in necrotic wheat hybrids as compared to their parents (Dalal & Khanna-Chopra, 2001). Kalyansona × C306 hybrid exhibited more severe necrosis than WL711 × C306. In Kalyansona × C306 hybrid, SOD activity showed no increase over the parents whereas WL711 × C306 hybrid showed an early increase, but it was possibly insufficient to scavenge increased superoxide. Activities of APX, GR and POX were enhanced, whereas CAT exhibited a decrease in activity with the appearance of visible necrosis in both the hybrids. The isozyme profile of the anti-oxidant enzymes was similar in the hybrids and their parents. One existing isoform of POX showed an early appearance in the hybrid and increased in intensity with the progression of necrosis thus suggesting a possibility of POX as a source of H₂O₂. Peroxidases can generate H₂O₂ by oxidation of NADH, NADPH, thiols and certain phenols (Pichorner et al, 1992). Thus in necrotic wheat hybrids, the anti-oxidant management was not well co-ordinated leading to a persistent oxidative stress, which caused cell death. The response of necrotic wheat hybrids differed in magnitude at different developmental stages of the leaves, which may be related to the intensity of necrosis expressed by the hybrids.

Subcellular compartmentation of anti-oxidants is necessary for the efficient quenching of ROS at their production sites as chloroplasts, mitochondria and peroxisomes are intracellular generators of ROS. In plants, chloroplasts are a major site of ROS production during photosynthesis (Asada, 1994). Since necrotic lesions appeared mainly on the leaf and

Fig. 3—Changes in (A) H₂O₂ content, (B) chloroplastic SOD activity, (C) chloroplastic ascorbate content, (D) chloroplastic glutathione (reduced) content and (E) chloroplastic SOD isoform at different stages of leaf development in necrotic wheat hybrid WL711 × C306 and its parents. I, II, III and IV represent 0%, 25%, 50% and 75% necrosis in hybrid leaves, respectively. Symbols as in Fig. 2. Vertical bars indicate SE (n=3). In some cases error bars are smaller than the symbols.
leaf sheaths, the anti-oxidant defense in the chloroplasts was investigated. Low ascorbate and glutathione levels were detected in the chloroplasts of the hybrid before the onset of necrosis (Fig. 3 B & C). Chloroplastic SOD activity also followed a similar trend (Fig. 3 D & E). Thus hybrid necrotic leaves exhibited ineffective management of oxidative stress in chloroplasts culminating in cell death. Based on these results a hypothetical model for the involvement of oxidative stress during hybrid necrosis is proposed (Fig. 4). According to the model, enhanced ROS preceding necrosis led to higher lipid peroxidation, loss of membrane permeability and cell viability. Anti-oxidant defense system in the chloroplasts comprising the metabolites, ascorbate and glutathione and enzymes SOD, APX and GR exhibited lower activity even before the onset of necrosis in hybrid leaves as compared to parents. Lack of co-ordination in the anti-oxidant enzyme activities coupled with decline in CAT activity resulted in an increase in superoxide and H$_2$O$_2$ in the cell. Thus, increased ROS along with the inability to manage the oxidative stress signalled PCD resulting in necrotic lesions.

Generation of ROS is a light dependent phenomenon (Foyer et al., 1994). The longevity of the severely necrotic Kalyansona × C 306 hybrid was increased by manipulating the light intensity, temperature and photoperiod in the controlled environment of phytotron. Some hybrids reached flag leaf emergence stage and very few showed ear emergence and grain development (Sharma & Khanna-Chopra, 2001).

**Processes Associated with Other Plant PCD Phenomena**

Signaling processes that activate PCD in plants are as diverse as in animals. Besides ROS, a requirement of calcium signaling has been implicated in the hypersensitive reaction (HR) induced cell death using calcium channel blockers (Pennell & Lamb, 1997). Another positive feedback regulator of cell death during HR is salicylic acid (SA) (Richberg et al., 1998). The conversion of SA into catechol by a gene encoding salicylate hydroxylase (NahG) could suppress HR cell death in different Arabidopsis mutants (Shah et al., 1999). SA has been implicated in ozone-induced cell death as well (Rao & Davis, 1999).

Hormonal balance (auxins and cytokinins) is a critical signaling component in the differentiation of Zinnia tracheary elements (Fukuda, 1996). A requirement of ethylene is necessary in the cell death caused by a fungal toxin ‘victorin’ (Navarre & Wolpert, 1999). In cell death of barley aleurone protoplasts, giberellic acid (GA) stimulates the secretion of hydrolytic enzymes and triggers the onset of PCD, whereas abscisic acid antagonizes the effects of GA and inhibits PCD (Bethke & Jones, 2001).

One of the simplest hallmarks of PCD in plants is the DNA ladder. A DNA ladder is the product of chromatid digestion by nuclease(s) without the concurrent protease activities on histone (Wylie, 1980). Thus, a DNA ladder suggests that cell organelles such as vacuoles or lysosomes are preserved to some extent during cell death process. DNA ladder can be detected using a terminal deoxynucleotidyl transferase-mediated dUTP nick end labelling (TUNEL) reaction. In plants, DNA ladder has been reported in several conditions such as senescence (Yen & Yang, 1998), endosperm development (Young et al., 1997), UV irradiation (Danon et al., 2000), death induced by pathogen toxin (Navarre & Wolpert, 1999), nutrient deprivation (Callard et al., 1996), etc.

Plants undergoing PCD show cytoplasmic shrinkage and condensation but no apoptotic bodies are formed (Lam et al., 1999). This is due to the absence of phagocytosis by the neighbouring cells or macrophages, which engulf the cell corpses due to the presence of a rigid cell wall in plants. Thus, the degenerated cytoplasm and nucleus in plant cells may be eliminated by other processes like autophagy, etc.

The importance of caspases for apoptosis has been evidenced by the fact that caspase activation correlates with the onset of apoptosis. These proteases once activated by apoptotic signal, systematically dismantle and package the cell by cleaving key cellular proteins (Wolf & Green, 1999). In plants, caspase- like proteolytic activities have been observed during HR induced cell death (del Pozo & Lam, 1998). Peptide inhibitors of caspases could block the HR, whereas classical protease inhibitors could not suppress it. Tobacco transgenic plants encoding Ced-9 (a pro-survival cell death regulator) could delay HR induced cell death and death caused by UV-B and paraquat (Mitsuhasha et al., 1999). In addition to caspases, other cysteine proteases have been implicated as having a role in plant PCD (Solomon et al., 1999; Xu & Chye, 1999). Involvement of an extracellular serine protease has also been shown to regulate cell death activation during TE.
cell death and its localization in mitochondria was expressed in tobacco was found to activate HR-like caspases family members in animals. A specific release of cytochrome C from intact mitochondria has through the membrane of the mitochondria (Shimizu et al, 1999). Bcl-2 related proteins can either activate or repress the leakage of cytochrome C activate or repress the leakage of cytochrome C from mammalian systems when

These root show induction of peroxidases and are more resistant to pathogen infection by fungal spores of Phytophthora sojae. Structure of dying cells in r/m roots shows the hallmark events of apoptosis and surprisingly, these cells also exhibited classical features of necrosis. The two morphologies may represent different stages of a common pathway for PCD in plant roots, or two separate pathways could be involved. It has been suggested that the r/m gene could either negatively regulate cell death or may be required for cortical cell survival (Kossak et al, 1997).

**Hypersensitive Response (HR)**

The molecular mechanism for HR involves generation of oxidative burst in infected cells and other events of PCD. Certain genetic mutations are known in maize, barley and Arabidopsis that result in the development of lesions resembling the HR or other disease symptoms in the absence of pathogen infection. In maize, locus rpl1 confers resistance to the maize common rust, Puccinia sorghi (Buckner et al, 1998). Mutations in this locus cause cell death without any infection from outside. The lethal leaf spot (lls 1) mutant of maize is a propagative disease lesion mimic. Homozygous plants for recessive alleles show small chlorotic lesions at 2-4th leaf stage, proceeding from older to younger leaves. Mutant phenotype here is dependent on light and chloroplasts. Bacterial mutants having a mutation in the _hrp_ locus, which is important for the secretion of virulence and avirulence factors, were developed. On using this _hrp_ strain, oxidative burst was detected but cell death did not occur. So, this strain uncoupled the oxidative burst from the cell death. Role of superoxide in executing HR has been studied using the recently developed _lso_ (lesion stimulating disease) mutant of Arabidopsis, which shows an apparent HR after shifting uninfected plants from short-day to long-day growth conditions and accumulates superoxide in leaf tissues (Jabs et al, 1996). Another lesion mimic

**Mutants Showing Different Types of PCD**

**Cortical Cell Death Mutants in Cereal Roots**

Cereals show increasing root cortical cell death (RCD) from the tip and upwards. RCD is likely to play an important role in fungal establishment. Dead cells may serve as a food base for initial colonization of the fungi, which subsequently kill others by toxin production. Certain root necrosis mutants have been obtained in soybean (r/m mutants). These roots show induction of peroxidases and are more resistant to pathogen infection by fungal spores of _Phytophthora sojae_. Structure of dying cells in r/m roots shows the hallmark events of apoptosis and surprisingly, these cells also exhibited classical features of necrosis. The two morphologies may represent different stages of a common pathway for PCD in plant roots, or two separate pathways could be involved. It has been suggested that the r/m gene could either negatively regulate cell death or may be required for cortical cell survival (Kossak et al, 1997).

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**Fig. 4—A proposed hypothetical model to explain the involvement of oxidative stress in the mechanism of hybrid necrosis. Figure not drawn to scale. The dashed arrows indicate impaired pathways.**

- **APX** ascorbate peroxidase; **AsA** Ascorbic acid (reduced);
- **CAT** catalase; **GR** glutathione reductase; **GSH** glutathione (reduced);
- **H₂O₂** hydrogen peroxide; **O₂** molecular oxygen; **O₂⁻** superoxide radical; **PSI** photosystem I; **PSII** photosystem II; **ROS** reactive oxygen species; **SOD** superoxide dismutase.

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differentiation (Groover & Jones, 1999). However, substrates for some of them are not known as they are differentially inhibited. Thus, proteases with different specificities may regulate cell death in different species. In animals, other processes include the cleavage of poly (ADP-ribose) polymerase, the first target proteins to be specifically cleaved by caspases into a 89 kDa apoptotic fragment (Lazebnik et al, 1994). However, abundance of poly (ADP-ribose) polymerase is generally very limited in plants (Krishnamurthy et al, 2000).

The mitochondrion has emerged as a conserved site where cell death signals in the form of ROS and protein factors can be generated. The release of such factors like cytochrome C activates caspases into starting a cascade effect of activations of other caspases family members in animals. A specific release of cytochrome C from intact mitochondria has been shown in cucumber cotyledon undergoing PCD (Balk et al, 1999) and menadione induced cell death (Sun et al, 1999). Bcl-2 related proteins can either activate or repress the leakage of cytochrome C through the membrane of the mitochondria (Shimizu et al, 1999). The gene encoding the pro-apoptotic regulator Bax from mammalian systems when expressed in tobacco was found to activate HR-like cell death and its localization in mitochondria was required (Lancombe & Santa Cruz, 1999).
Table 2—Plant system and lesion mimic transgenes/mutants showing PCD

<table>
<thead>
<tr>
<th>Gene</th>
<th>Source</th>
<th>Function</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Hybrid necrosis</td>
<td>wheat</td>
<td>Not known (Accumulate ROS in leaves)</td>
<td>Hermsen, 1963</td>
</tr>
<tr>
<td>1. Ne1Ne2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. Mutants</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Is</td>
<td>Maize</td>
<td>Suppressor of cell death</td>
<td>Buckner et al., 1998</td>
</tr>
<tr>
<td>2. Isd1</td>
<td>Arabidopsis</td>
<td>Accumulate superoxide on transfer from short-day to long day conditions</td>
<td>Jabs et al., 1996</td>
</tr>
<tr>
<td>3. Less22</td>
<td>Maize</td>
<td>Inhibits porphyrin pathway</td>
<td>Hu et al., 1998</td>
</tr>
<tr>
<td>C. Transgenics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Antisense CAT</td>
<td>Tobacco</td>
<td>Removal of ROS</td>
<td>Channongpol et al., 1996</td>
</tr>
<tr>
<td>3. Antisense PPO</td>
<td>Arabidopsis</td>
<td>Heme biosynthesis</td>
<td>Molina et al., 1999</td>
</tr>
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</table>

Mutant, Less 22 in maize developed necrotic spots on the leaves similar to HR lesions (Table 2). This gene encodes uroporphyrinogen, which produces intermediates for the biosynthesis of chlorophyll and heme-containing enzymes such as catalase. On accumulation in the cells, uroporphyrinogen can become reactive and may transfer its energy to oxygen, thereby forming ROS. These ROS may then kill the cells either by apoptosis or by necrosis directly. The CAT levels also show a decline in these mutants thereby further causing more severe oxidative stress (Hu et al., 1998).

Senescence

It is the final phase in the death of the plant involving active turnover and recapture of cellular material for use in other organs. Increase in ethylene and ROS is seen with the progression of senescence (Inze & Van Montagu, 1995). Mutant studies in Arabidopsis have shown that a dominant mutation in the ethylene responsive (ETR) gene can make it insensitive to ethylene and block natural senescence. Certain senescence upregulated genes (SENSUs) and senescence associated genes (SAGs) encode for cysteine proteases, which may be involved in the PCD phenomenon (Pennell & Lamb, 1997).

Studies in oats revealed that a host selective toxin, victorin produced by Cochliobolus victoriae produced senescence-like symptoms. In addition, DNA from victorin-treated leaves showed a pronounced laddering effect (Navarre & Wolpert, 1999). Thus, senescing parts of a plant may undergo cell death by a series of well-regulated events of an apoptotic pathway.

Conclusions

Studies of PCD reveal some degree of conservation amongst the various PCD processes in plants and animals. Mitochondria are known to be important in execution of animal PCD (Kroemer et al., 1998), but only recently its role in plant PCD has been firmly established. The role of mitochondria during synergid cell death has been highlighted by Christensen et al. (2002). Involvement of ROS signaling in various plant and animal PCD is reported (Jacobsen, 1996). During hybrid necrosis also, higher superoxide levels are observed even before appearance of visible necrosis on hybrid leaves (Khanna-Chopra et al., 1998). Hybrid necrosis barrier in some wheat crosses was overcome by culturing the ears before anthesis in medium containing anti-oxidants and F2 seeds were obtained (Dalal et al., 1999). Currently effort is being made to examine the molecular changes preceding visible necrosis in wheat cross Kalyansona × C306 using subtractive hybridization. A necrotic hybrid (J24 × C306) showed apparent senescence thereby indicating that senescence and necrosis may share a common pathway. Thus, necrotic wheat hybrids provide a unique genetic system to study the mechanism of programmed cell death in plants using modern tools of biotechnology. The following areas of research need attention: (1) the source of ROS generation, (2) identification of the occurrence of various common processes and cellular changes involved in different types of PCD such as presence of caspases, endonucleases and DNA ladder, etc., (3) identification of the genes turned on before the onset or during the visualization of necrotic lesions and compare them with the other plant genes cloned.
that regulate cell death e.g. *lsd1* gene, which encodes a zinc finger protein of a class known in animals to act as transcription factors (Dietrich *et al.*, 1997) and (4) to clone *Ne1* and *Ne2* genes, which may have wider applications in agriculture.

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