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Oxidative burst and plant disease resistance

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1. ABSTRACT

This mini-review summarizes briefly main facts and speculations on roles of reactive oxygen species (ROS) in plant interactions with pathogenic microflora. Examples relating the infection-induced oxidative burst with innate or acquired resistance or susceptibility are provided. Agents triggering ROS production, ROS sources and ROS-involving defense reactions are listed. Special attention has been drawn to the ROS involved in either resistance or compatibility and which are produced by both host and pathogen.

2. INTRODUCTION

Oxidative burst is the, more or less transient, over-production of reactive oxygen species (ROS) by living cells challenged by some endogenous or exogenous stimuli. In plants, many abiotic and biotic extreme factors including infections cause this reaction, usually soon after application. It occurs in viral, bacterial, and fungal diseases as well as in response to nematodes (1,2). In plant-microbe interactions, the term ROS generally refers to hydrogen peroxide (H_2O_2) and the superoxide free radical (O_2^-). The roles of hydroxyl radical ($^{\cdot}OH$) and singlet oxygen ($^1O_2$) are far less studied. Much attention has been paid to nitric oxide (NO), which in a sense is also ROS, and its reactions are tightly coupled with ROS reactions (3).

ROS concentrations in healthy tissues are relatively low. When plants are inoculated with incompatible pathogens a post-inoculation oxidative burst increases the level of ROS several fold (1). This phenomenon is accompanied by a burst of NO production (4).

The oxidative burst is being studied intensively because it appears to be one of the key defense reactions, which determines largely the result of host-parasite interactions.

3. OCCURRENCE OF OXIDATIVE BURST

3.1. Eliciting and suppressing

The direct triggers of the oxidative burst (and other defense responses) are various pathogen-related substances that are referred to as elicitors. These compounds represent all main classes of organic chemistry, namely, proteins, carbohydrates, and lipids (1, 5, 6). Certain metabolites of plants including products of cell wall degradation, which are produced during pathogen invasion, behave as endogenous elicitors with a similar outcome (7).

Some elicitors are specific and trigger their activities only in resistant plants whereas the other elicitors

are active regardless of resistance (8). Nonetheless, responses to nonspecific elicitor may also be specific when it is combined with parasite's specific suppressor. The latter compounds diminish the effects of elicitors only in compatible combinations. These relations are well documented for potato late blight where *Phytophthora infestans* zoospores carry cell wall carbohydrate components as unspecific elicitors and water-soluble glucans as specific suppressors (9). Similar observations were made for interactions of *Mycosphaerella pinodes* with legume plants (10).

It should be noted that pathogen-originated factors other than chemicals may also elicit responses in plants. For example, mechanical irritation of parsley cells by a tungsten needle, which simulated the penetration of a *Phytophthora* hyhpa evoked several but not all the responses associated with fungal attack, including the oxidative burst (11).

3.2. Pathogen originated ROS

The origin of the ROS associated with disease is generally ascribed to the host rather than the pathogen. However, parasitic microbes may not only trigger plant ROS production but may also contribute to the overall oxidative burst. For example, the germinating spores of the blast fungus *Magnaporthe grisea* secrete superoxide or biomolecules capable of its extracellular formation (12, 13). On one day post inoculation, blast spores alone produce this radical and hydrogen peroxide at the same rates as infected leaves or even higher (14). Preliminary evidence suggests hydroxyl radical formation by this fungus also (12). Hydroxyl radical formation has been reported for wood-decomposing fungi (15, 16).

Production of hydrogen peroxide was revealed for the interface of rye tissue and parasitic fungus *Claviceps purpurea* lacking in the transcription factor responsible for the fungal catalase. Here the fungus is suspected to be the peroxide source (17). Similar H_2O_2 secretion (presumably by the fungus) in contact sites between hyphae and plant were observed for *Botrytis cinerea* (18, 19). As well, pathogenic fungi can produce ROS by means of their toxins as described in the Section 4.

Pathogenic microbes, as all other organisms, are most likely capable of nitric oxide production. But it astonishes that possible contribution of pathogen-originated NO and its significance for the interactions with the host are even not discussed so far.

3.3. Relation of oxidative burst to disease resistance

Under normal conditions plants exhibit innate resistance to certain pathogens being susceptible to other pathogens. This ability is determined by combination of host and parasite genes and depends on environmental factors. Diverse physical, chemical, and biological agents applied to susceptible plants prevent diseases or weaken their severity. This phenomenon is termed acquired resistance. It is referred to as local one if it is observed at the sites of inducer application or it is called systemic one if it is seen in the distant parts of the plant (20). The resistance of distant tissues of the same organ is referred to as sub-systemic (21). Non-pathogenic or low aggressive microbes may also induce the acquired resistance (22).

Oxidative burst is often associated with both innate and acquired plant disease resistance so that it occurs stronger in resistant than in susceptible plants.

3.3.1. Innate resistance

Often the pattern of ROS production in infected plants consists of two phases, a short first phase followed by a longer second phase. The latter is more pronounced in incompatible host-parasite interactions than in compatible ones. The first phase is independent of resistance (1, 23). Cultured plant cells retain the capability of post-inoculation oxidative burst with analogous differences between resistant and susceptible cultivars suggesting that this is a cellular phenomenon (24, 25, 26).

Hydrogen peroxide and superoxide radical accumulate in different manners in infected plants. When barley leaves are challenged with the powdery mildew avirulent fungus, the superoxide concentration rises at a slower rate than that of H_2O_2 (27). Cytochemical assays show that the sites of accumulation of these two ROS are different as well (28).

A critical feature of ROS and the oxidative burst in plant disease resistance is its cytotoxicity resulting in suppression of pathogen development. The antidotal effects of antioxidants on this pathogen development indicates ROS involvement (29). While diffusates of healthy rice leaves are weakly toxic to blast fungus spores, the diffusates of leaves inoculated with this fungus have increased levels of toxicity. Diffusates from incompatible combinations are more toxic than in compatible combinations (30). This property was found not only in intact plants but also in rice callus cultures (31).

The most abundant information that links ROS production and innate resistance to diseases corresponds to complete (vertical, monogenic) resistance which prevents disease very effectively but only in specific host-parasite combinations. In contrast, partial (horizontal, general, quantitative, polygenic) resistance is unspecific towards various pathogen races but protects plants to lesser extent than successful complete resistance. Completely resistant cultivars prompt pathogens to evolve virulent races which break the resistance down. Partial resistance is better in this regard as it is not such a strong elective factor and so is more durable (32).

The role of ROS in the partial resistance is poorly investigated. However, rice cultivars partially resistant to blast were found to respond to infection by increased leaf diffusate fungitoxicity like completely resistant cultivars. In both cultivar groups, the effect was diminished by catalase and scavengers of hydroxyl radicals witnessing the involvement of H_2O_2 and $^{\cdot}OH$. Nonetheless, the data on $O_2^{\cdot-}$ were not simple. Superoxide dismutase (SOD) was a good antidote in completely resistant cultivars but was not an antidote at all in partially resistant ones. In cultivars

combining both types of resistance the effect of SOD was intermediate (33). Therefore, partial resistance may involve the oxidative burst as well as complete resistance but the particular ROS involved and mechanisms of their formation may be different in these cultivars.

The non-host resistance is a kind of innate resistance. It is directed against certain taxonomic groups of pathogens to which the given plant species is quite immune even under the most favorable conditions for disease (32). This resistance may also be related to the oxidative burst. For example, such interactions of *Uromyces vignae* with pea or *Erisiphe cichoraceum* with cowpea are accompanied by increased H_2O_2 and O_2^- production (7). Pea leaf inoculation with *Mycosphaerella pinodes* also raises its yield of superoxide (10).

3.3.2. Acquired resistance

In many cases, contact with the inducer or /and inoculation of induced plants causes oxidative burst before the resistance becomes evident (34).

Oxidative bursts prior to detection of disease resistance were found in plants subjected to different agents, for example, high temperature (35), solutions of salicylic or isonicotinic acids (36), digitonin (37), β-aminobutiric acid (38), commercial inducers of resistance such as tricyclazol, fthalide, and probenazol (39). In cucumber, spraying the first leaf with K_2HPO_4 or inoculating with tobacco necrotic virus induces within the leaf the production of H_2O_2 after 3 h hours and O_2^- after 6 h. After one week, the second and third leaves become resistant to *Colletotrichum lagenarium* (40). Treatment of cucumber lower leaves with the ROS-generating herbicides paraquat or actifluoren also renders the upper leaves resistant to the same pathogen. In tobacco plants, paraquat (but not actifluoren) induces systemic resistance to tobacco mosaic virus and the fungus *Peronospora tabacina* (41).

Various pathogen-related elicitors may also induce the resistance. For example, the hyphal wall compounds of *P. infestans* placed locally onto potato leaves or tuber discs initiated their sub-systemic resistance of the tissue to this fungus. Within 10 to 20 min the elicitor stimulates superoxide production at the application sites and gradually propagates to other parts of the organ (21). This treatment of lower leaves of potato can lead to systemic acquired resistance. It stimulates O_2^- production after one day and the resistance to this fungus after three days in the upper leaves (42). N-acetylchitooligosaccharide elicitors induce the burst of H_2O_2 production in rice leaves or suspension cells and render plant resistant to blast (43).

Interestingly, droplets of distilled water, in long contact with rice leaves, stimulate leaf production of superoxide and reduce the severity of blast disease caused by the subsequent challenge with the virulent fungus (44). The effect might be due to the solubilization of endogenous plant elicitors inducing the oxidative burst and, finally, disease resistance. As usual, water film or droplets on plant shoot surface are necessary for pathogen penetration; therefore, high humidity favors most infections (32). The aforementioned opposite effect of water may represent the plant adaptation compensating, although incompletely, the increased risk of diseases under humid conditions.

Inoculation of *Arabidopsis* leaves by avirulent strain of *Pseudomonas syringae* induces a burst of H_2O_2 in the inoculated leaves and smaller oxidative bursts in distant leaves, which then acquire resistance to the virulent strain. Addition of diphenylene iodonium to the inoculum (inhibitor of ROS-producing enzyme) inhibits both primary and secondary oxidative bursts and prevents development of resistance. Application of the H_2O_2-generating system glucose / glucose oxidase in the place of avirulent (first) inoculum leads to the same systemic consequences in the distant leaves (22).

Transfer of genes responsible for ROS hyper-production may render plant resistant. A gene from *Aspergillus niger* that encodes glucose oxidase (yielding hydrogen peroxide from the oxidation of glucose) was inserted into potato plants. As a result, leaves and tubers produced high amounts of H_2O_2 constitutively and acquired resistance to the bacterium *Erwinia carotovora* subsp *carotovora* as well as fungi *P. infestans* and *Verticillium dahliae*. This acquired resistance does not occur if the pathogen inoculum contains catalase indicative the phenomenon really depends on enhanced H_2O_2 level (45).

The over-production of ROS is a scarcely desirable anti-infection measure if it is constitutive. In fact, transgenic tobacco and canola expressing glucose oxidase constantly have fewer numbers of flowers and seeds (46). Similar transgenic rice plants are also partially sterile and their seeds are less viable (47). A more elegant approach was employed on rice plants. The gene of glucose oxidase was inserted under the control of the promoter of phenylalanine ammonia lyase, which inducible only upon wounding or pathogen inoculation. These plants showed no phenotypic distortions but were resistant to the bacterium *Xanthmonas oryzae* and the fungus *M. grisea*. The resistance was accompanied with the activation of the glucose oxidase gene, increased activity of the enzyme, and increased levels of H_2O_2 (47).

4. CHEMICAL SOURCES OF ROS INVOLVED IN OXIDATIVE BURST

Mechanisms of ROS production in plants are diverse (48). Fewer reactions are evidenced in infection-induced oxidative burst (49).

The most important enzymatic sources of superoxide and hydrogen peroxide are NADPH-oxidases of plasma membrane and peroxidases of apoplast (50). The first enzyme is sensitive to diphenilene iodonium (DPI) and the second to cyanide; this is often used to distinguish preliminarily between the two sources. Other considered sources of ROS are amine oxidases and exocellular germin-like oxalate oxidase (51-53) of the apoplast along with xanthine oxidase (49, 54-56). As a rule, these enzymes are activated upon infection and to higher extent in resistant than in susceptible plants.

The products of the chemical systems listed above are mainly extracellular making them easier to detect than those from intracellular sources. ROS produced by organelles such as chloroplasts (yielding superoxide in PSI and singlet oxygen in PSII) (57) and peroxisomes (producing H_2O_2 by glicolate oxidase) (49, 50) are less studied but may contribute to the infection-related oxidative burst. In animal cells the main deal of ROS comes from mitochondria. Contribution of this source is apparently smaller in plants and fungi due to other sources and because of alternative oxidase which reduces mitochondrial superoxide production (50).

Non-enzymatic formation of ROS can occur by autoxidation of various substrates such as hydroquinones and semiquinones, thiols, flavins, etc. (58). Plant photosynthesizers yield ROS at the expense of light energy and participate in plant defense against pathogenic microbes and herbivorous animals (59, 60).

Phytopathogenic fungi also possess ROS-producing enzymes, such as NADPH oxidase (13, 61-63). There is evidence of a fungal oxalate oxidase, which may yield hydrogen peroxide in *Botrytis cinerea* (18). The H_2O_2-generating enzymes glucose oxidase and glyoxal oxidase are specifically peculiar to fungi (64). The fungus *Talaromyces flavus* secretes the first of them (65). Wood-decomposing fungi produce H_2O_2 by cellobiose dehydrogenase or glyoxal oxidase; then hydrogen peroxide may form hydroxyl radicals in the Fenton reaction (15).

Several fungal toxins also generate ROS. For example, *Cercospora* secretes light-activated cercosporin generating singlet oxygen and superoxide (66). The similar properties were found in alterotoxin of *Alternaria* (67). Botrydial, toxin of *Botrytis* yields H_2O_2 under illumination (68). Naphthazarin toxins of *Fusarium* transfer electrons from respiratory and photosynthetic redox systems to dioxygen (67). Several microbial toxins do not produce ROS by themselves but activate plant sources of ROS. For instance, tentoxin of *Alternaria alternata* inhibits photophosphorilation in chloroplasts and closes leaf stomata. This decreases CO_2 fixation and leads to overproduction of photosynthetic electron transport that prompts the ROS formation (69). Tabtoxin of bacteria *Pseudomonas syringae* pv. *tabaci* and isomarticin of fungus *Fusarium solani* inhibit synthesis of glutamine. In chloroplasts, such changes increase concentration of NH_3 which uncouples photophosphorylation and also increases ROS generation in PSI (67).

Main sources of nitric oxide are NO synthases and nitrate reductases. Of other enzymes, xanthine oxidoreductase, peroxidase and cytochrome P450 were found to yield NO. Non-enzymatically, this compound can be formed in nitrite decomposition or in its interaction with ascorbate at low pH, and also in reaction of arginin with H_2O_2. Carotenoids are capable of light-driven conversion of nitrogen dioxide to nitric oxide (70, 71).

5. TARGETS FOR ROS DURING OXIDATIVE BURST

5.1. ROS functions in unstressed organisms

ROS are normal aerobic metabolites of plants and microbes that are indispensable for several functions. They may act as true signal molecules initiating a subsequent cascade of signaling events. As well, they may act as effector molecules altering directly proteins or other structures. In many cases ROS cause reversible oxidation of thiol groups in cysteine or methionine residues of perceiving proteins. This creates disulphide bridges, which modulate protein conformation and activity. In turn, this controls protein kinases and protein phosphotases, ion channels and transcription factors. This gives rise to direct physiological effects or gene expression. ROS can mediate, at least partially, effects of abscisic acid, methyl jasmonate, and auxin since these plant hormones stimulate ROS production. In plants, ROS are involved in cell wall rearrangement during elongation, control of root gravitropism and stomata aperture, etc. (62, 72).

Nitric oxide shares similar signaling work, which is based to large extent on its reaction with –SH groups (S-nitrosylation) of proteins or glutathione. Other pathways include its reactions with iron-containing proteins, activation of MAP kinases and guanylyl cyclases (70). In addition, NO may exert its functions through cyclic ADP-ribose and Ca^{2+} mobilization (73).

In microbes, the regulatory role of ROS is less known. In the filamentous fungus *Aspergillus nidulans*, ROS produced by NADPH oxidase are required for sexual development (61). Neighboring fungal colonies growing on a firm substrate may release H_2O_2 from contacting *hyphae*. The yield of peroxide depends on particular combination of interacting fungal species. Hence, the effect was suggested to participate in the recognition mechanism (74). Hydrogen peroxide produced by glyoxal oxidase is required for filamentous growth and pathogenicity in *Ustilago maydis* (64). Formation of blast fungus appressoria is accompanied by superoxide production in this structure. The necessity of this ROS for the development is demonstrated by the dramatic decrease in spore germination and appressoria formation in the presence of DPI, which inhibits ROS production. ROS scavenging by antioxidants also delays formation of appressoria and alters their morphology (13). Under the other conditions, the early development of this fungus may, on the contrary, be down-regulated by its own ROS. Spore germination is self-inhibited in spore suspensions that are too dense or too dilute. The effect is restored by exogenous SOD, catalase or $^{\cdot}OH$ scavengers (12). This phenomenon may prevent a parasite from switching from a dormant to active state under conditions unfavorable for parasitism.

Obviously, ROS-dependent processes of non-stressed plant will be modified under infection. For example, H_2O_2-dependent stomata closure is induced by elicitors, being one of precautions against infections (75, 76). Symmetrically, influence from the host side would change the ROS turnover and functions in the parasite. It is

easy to assume that the host may discharge ROS in amounts too little for structural injury but sufficient to jam the ROS-dependant signaling system of the parasite. Ultimately this disfunction would alter the parasite's development. Such a possibility does not appear to be discussed yet but consistent with inhibition of spore germination by very low amounts of H_2O_2 (see the section 5.3).

The examples listed and many others show that the pathogenesis-related oxidative burst is a typical response of resistant plants. In many cases it is actually one of causes rather than a consequence of resistance. ROS are involved in resistance mechanisms both directly as toxicants and indirectly as signals or precursors of other products necessary for the defense.

5.2. Post-inoculation events

The contact of pathogens with plant cells induces a chain of events leading ultimately to full-scale resistance or susceptibility depending on the genotypes of each partner and other conditions. Many parts of these chains are common for transduction of any signal. In general, external signals, including elicitors, are perceived by appropriate plant receptors which are usually plasma membrane-bound. The recognition of the signal is followed by G-protein-mediated influx of Ca^{2+} ions to the cytoplasm. This, in turn, initiates multiple phosphorylations by protein kinase cascades. At the last stage, transcription factor is phosphorylated that enables it to move into nucleus and interact with promoter that finally expresses certain genes (77, 78).

The enhanced ROS generation is one of the earliest responses of plant cells to various abiotic stresses such as wounding, low and high temperature, excess irradiation, drought and salinity as well as to biotic stresses caused by parasites (1, 62, 72, 78). In these conditions, one of the first phosphorylations activates the plant NADPH oxidase complex in the plasma membrane that produces superoxide, which is converted to hydrogen peroxide (9). ROS-dependent gene activation may occur through the activation of transcription factors (50, 72). Another ROS-dependent pathway involves peroxidation of lipids to form jasmonate, a known regulator of gene expression (77).

Genes activated with a help of ROS initiate *de novo* syntheses of diverse compounds including pathogenesis-related proteins (78). Syntheses and accumulation of phytoalexins also follow the oxidative burst but do not always resulting from it (9, 79, 80).

One of rapid defense responses in plants resulting from the oxidative burst is cross-linking of hydroxyproline-rich structural proteins of the plant cell wall occurring in incompatible combinations. This reaction involves peroxidase and hydrogen peroxide. As a result, the host's cell wall becomes less permeable to pathogens and their toxins (81). Lignin synthesis in the plant cell wall has similar consequences and also involves hydrogen peroxide and superoxide (53). In the case of blast disease of rice, the induced lignification is indicative of incompatibility as early as 15 h post inoculation (82)

5.3. ROS toxicity to microbes

ROS are universally cytotoxic at high doses and are generally considered to suppress microbial development including bacterial multiplication (22), fungus spore germination (83), appressorium formation, and penetration of plant cells (7, 29), and mycelial growth (84). These toxic effects are ROS-dependent because they diminished or abolished by exogenous antioxidants. Interestingly that hydrogen peroxide may suppress spore germination and appressorium formation of phytopathogenic fungi *M. grisea* and *Cladosporium cucumerinum* at concentrations as low as 10^{-12} M (85) which is likely the result of peroxide signaling interference in fungal metabolism. It is possible that other ROS-driven defense responses may also function at far lower ROS concentrations than it is usually thought.

5.4. ROS phytotoxicity

The best-known ROS-dependent anti-infection phytotoxic effect is the hypersensitive response (HR). It is a rapid death of invaded plant cells resulting in the parasite death or cessation of its development. Consequently, the infection cannot spread from very limited inoculation sites (86). In animals, necrosis and programmed cell death (apoptosis) are quite different phenomena. In plants, HR bears resemblance with both reactions; for example, it manifests such features of the programmed cell death as chromatin condensation, fragmentation of nuclear DNA, activation of nucleases and proteases (87). Very often, HR is associated with the increased ROS production.

The causal role of the oxidative burst in HR follows from the facts that the first phenomenon precedes the second one, and both phenomena are diminished by exogenous antioxidants or inhibitors of ROS-producing enzymes. Another support gives the induction of necrosis may also be caused by plant treatment with excessive ROS by means of exogenous sources applied instead of microbial inocula or elicitors (56, 84, 88). Nonetheless, there is no unequivocal relation between oxidative burst and necrosis because each of the two events may occur solely (89-92). Their interdependency is determined by many factors, for example, where the oxidative burst takes place, inside or outside cells (93).

Nitric oxide, whose production increases during oxidative burst, accelerates reactions of ROS and, in particular, promotes hypersensitive necrosis. For example, inoculation of soy bean cultures with HR-inducing avirulent (but not HR-noninducing virulent) *P. syringae* pv *glycinea* induces rapid concurrent synthesis of H_2O_2 and NO. The bacterially induced cell death is blocked by inhibitors of NO synthase and can be induced, without bacteria, by NO donors (4). One of reasons for the synergism between NO and ROS is the interaction of nontoxic NO with superoxide and hydrogen peroxide yielding peroxinitrite $ONOO^-$ and other species more cytotoxic than those ROS (3, 73). NO is also involved triggering phytoalexin synthesis and other defense responses (69).

5.5. Induction of acquired resistance

Very often but not always the oxidative burst caused by various inducers leads to systemic resistance through the intermediate development of cell necrosis. For example, this sequence of events is observed with the herbicide actifluoren (promoting singlet oxygen formation) applied to cucumber leaves. However, in the case of tobacco, the compound brings about necrosis without induction of disease resistance (41). Solutions of K_2HPO_4 or tobacco necrotic virus inoculum placed onto the lower leaves of cucumber cause necrotic spots in 36-48 h and render the upper leaves resistant to *Colletotrichum lagenariun* in one week. In general, this outcome does not necessarily require oxidative burst and necrotization because the synthetic compound BION does not evoke these effects but protects cucumber better than phosphate does (40). Inoculation of *Arabidopsis* leaves with avirulent bacteria, which induced resistance in these and neighboring leaves also induced local oxidative burst followed by local necrosis. Both sequential events occur in distant leaves especially near vessels, which seem to transport signal molecules (22).

6. AMBIGUITY OF ROS ROLES IN PLANT DISEASE RESISTANCE

Despite numerous reports implicating ROS in plant disease resistance, its role is controversial. As shown in this review, the oxidative burst is not always associated with the resistance and *vice versa*.

The intense delayed ROS production at later stages of compatible interactions is harmful rather than useful because it does not prevent microbial colonization of the plant (94-96). Presumably, the parasite does not suffer from the delayed oxidative stress since it has enough time to adapt its antioxidant systems.

The dual role of oxidative burst is similar to that of its possible result, namely, infection-induced plant cell death. If the latter occurs locally in infected cells simultaneously with or soon after microbial penetration, it provides the barrier for further spread of the parasite and its toxins. But if it is delayed and generalized, it is a mechanism of infective degradation of plant tissue.

Rapid localized death of infected cells is obviously an effective measure against biotrophic pathogens which cannot feed on dead tissues. In contrast, the oxidative burst, either host or pathogen originated, and subsequent cell death may favor tissue colonization by necrotrophs. This idea was supported by experiments on typical necrotroph *Botrytis cinerea* (97). However, it was found that resistance in tomato induced (98) or peculiar to some one mutant (96) or bean innate resistance to this fungus (99) were associated with the earlier and stronger oxidative burst than in susceptible plants. Therefore, this phenomenon may protect from necrotrophic pathogens as well as from biotrophic in some cases.

Several microbial toxins may work as elicitors inducing resistance preceded by stimulation of ROS production in treated plants. Picolinic acid, the toxin of *Magnaporthe* and *Fusarium* fungi, elicits the burst of H_2O_2 production and cell death in leaves and suspension culture of rice. Leaf pretreatment with the toxin diminishes severity of subsequent inoculation with blast (100). Another blast toxin, tenuazonic acid also causes leaf necrosis. Adding the toxin to spore inoculum applied to leaves of susceptible rice cultivar increased the percentage of incompatible-type necrotic spots and decreased that of compatible-type lesions, which also acquired brown margin. In other words, the disease symptoms shifted from compatible to incompatible. In disease-controlling doses, the compound was not toxic to spores but increased the fungitoxicity of diffusates of treated leaves in ROS-dependent manner (101).

The role of fungal produced ROS is not always clear. The hydroxyl radical secretion by wood-decomposing fungi is obviously a mechanism which benefits the pathogen (15). Usage of $^{\cdot}OH$ by other pathogenic fungi for plant cell wall penetration does not seem to be evidenced yet.

However, ROS production of the pathogen is not necessarily a factor of its pathogenicity. For instance, the *Botrytis cinerea* mutant deficit in glucose oxidase and thus lacking in H_2O_2 production is equally aggressive as the wild type (68). In yet another role, pathogen-produced ROS can act as a factor of incompatibility. As mentioned above, in the rice blast pathosystem, H_2O_2 and O_2^- production in the infection droplets appears to originate form fungal spores chiefly and is higher in incompatible combinations (14). The same could be said about *M. grisea* spores at extreme concentrations: their own ROS suppress their germination together with the ability to cause blast disease (12).

ROS are involved in antagonistic interactions not only between plants and microbes but also between different microbes. Thus, the fungus *T. flavus* suppresses the fungus *Verticillium dahliae* by means of hydrogen peroxide secreted. This capability can be used for biocontrol of eggplant wilt caused by the second fungus (65). The ROS involvement in microbe-microbe interactions is suspected for wood-degrading fungi. They were reported to liberate hydroxyl radical in contacts with antagonistic bacteria, probably to digest bacteria (16).

7. PERSPECTIVE

During host-parasite interplay, both partners produce ROS, which participate in miscellaneous reactions to the benefit of both sides of the conflict. At first, the roe of ROS was only considered to toxic and deleterious. However, the ever-growing body of evidence points to their more delicate signaling functions. The regulatory role of ROS seems to be especially interesting and promising for future study.

Despite controversial nature of ROS involvement in host-parasite interactions, the early localized oxidative burst in infected plants is mainly a factor of their disease

resistance. Its investigation may provide information potentially valuable for agriculture. Assays for ROS themselves and accompanying events might find new markers of innate resistance readable in whole plants and cell cultures and notably helpful for *in vitro* breeding for resistance. ROS-generating systems of plants and pathogens may be targets for stimulation by resistance inducers or fungicides. Other directions of chemical attack on microbes are their antioxidants and ROS-depended regulatory systems. Genes involved in the oxidative burst may be used to create resistant transgenic plants. For diseases where ROS favor pathogenicity, artificial induction of antioxidant potential may be used to weaken the disease. In addition to manipulations on hosts and parasites, the usage of the third power, namely, ROS-depended biocontrol microbes and their products, seems to offer another concept for the application of ROS to agriculture.

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Key Words: Reactive Oxygen Species, Plant Disease Resistance, Pathogenicity, Review

Abbreviations: DPI: diphenylene iodonium; H_2O_2: hydrogen peroxide; HR: hypersensitive response; NO: nitric oxide; O_2^-: superoxide anion radical; $^1O_2$: singlet oxygen; $^{\cdot}OH$: hydroxyl radical; ROS: reactive oxygen species; SOD: superoxide dismutase

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