Corrected proof, 2013.09.01., uploaded: 2014.01.24.

Similarities and Differences in Plant and Animal Immune Systems – What is Inhibiting Pathogens?

L. Király*, A. Künstler, R .Bacsó, Y.M. Hafez[1], and Z. Király

Plant Protection Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, H-1525 Budapest, P.O.B. 102, Hungary

[1]Present address of Y.M. Hafez: Department of Agricultural Botany, Plant Pathology Branch, Faculty of Agriculture, Kafr-El-Sheikh University, 33516, Kafr-El-Sheikh, Egypt

*Corresponding author; e-mail: kiraly.lorant@agrar.mta.hu

Similarities and differences in the immune systems of plants and animals are discussed in relation to non-specific and specific immunity (resistance), systemic acquired resistance (immune memory), transgenerational immune memory and gene silencing. Furthermore, we attempt to answer the question "what is inhibiting or killing pathogens during the immune (resistance) process"? Therefore, the possible roles of reactive oxygen species and antioxidants in pathogen inhibition are evaluated in different types of plant disease resistance.

Keywords: plant and animal immunity, immune memory, reactive oxygen species, antioxidants.

If we consider immunity (resistance) to pathogen infections in the case of plants and animals, similarities or analogous mechanisms seem surprising but differences could be also important. The two systems seem to be „so far and yet so close", as was expressed by Maekawa et al. (2011) in an excellent paper. The detailed unraveling of the mechanisms of plant immunity followed vertebrate immunity research with a 40-50 years delay, in spite of the fact that Ward (1902) as early as 110 years ago has shown that some lines of bromes (*Bromus inermis*) react to infection of a rust fungus (*Puccinia dispersa*) with an „immune response".

The essence of both plant and animal immunity is the recognition and protection against the foreign (the non-self). In the course of immune reactions non-specific and specific plant or animal receptors detect non-specific and specific pathogen elicitor molecules also called antigens in animal systems. The first class of plant or animal receptors recognizes the non-specific „pathogen-associated molecular patterns" (PAMP) or „microbe-associated

molecular patterns" (MAMP). These are membrane-resident receptors, also called as pattern-recognition receptors, PRRs. On the other hand, the second class of receptors recognize pathogen effectors (polymorphic strain-specific antigens) usually inside host cells. These host cell receptors belong to the NLR protein family (NLR = Nucleotide-binding oligomerization domain- and leucine-rich repeat-containing proteins). The plant NLR receptors are usually called resistance proteins (R-proteins). Accordingly, one can differentiate between two types of immunity: the non-specific pattern-triggered immunity and the specific effector-triggered immunity (cf. Jones and Dangl, 2006).

As a result of the recognition of non-self molecules in plant and animal organisms, analogous, but not identical, signal responses are initiated. Plants lack mobile immune cells, like lymphocytes (B and T cells) in animals. Lymphocytes that clonally express receptors can detect pathogens in the circulatory system. As a result of the recognition of pathogen antigens by receptors, clonal expansion and differentiation of the receptor-carrying lymphocytes takes place. In addition, both T and B memory cells are formed which produce receptors with the same antigen-specificity and permit subsequent secondary immune responses through highly complex interactions. Because plants lack a circulatory system, different immune strategies are needed to establish specific but less complex immune responses and generate a different type of immune memory. This is indeed a „stress memory" which acts against a secondary infection in remote plant organs.

Non-specific immunity

The first step in the recognition of the non-self is a general response, when non-specific receptors on the surface of plant or animal cells detect non-specific PAMPs. These molecules regularly occur in bacteria, fungi and in several other microbes. Typical examples are: lipopolysaccharides, peptidoglucanes, chitin, bacterial flagellin. PAMP-receptors can be regarded as multidomain proteins with similar biological functions and protein structures (Felix et al., 1999; Ausubel, 2005; Rast et al., 2006; Boller and Felix, 2009; Ronald and Beutler, 2010). When these plant or animal receptors are activated by PAMPs, general reactions are stimulated in infected hosts. Ion fluxes are activated, an oxidative burst is initiated, mitogen-activated protein kinases (MAP kinases) are expressed. Furthermore, a set of transcriptional changes occur, e.g. in plants, so-called pathogenesis-related proteins and phytoalexins are being accumulated. All these alterations may have roles in PAMP-induced immunity (pattern-triggered immunity) (Jones and Dangl, 2006; Boller and Felix, 2009).

Specific immunity

Although PAMP-triggered immunity can be considered as a general response of plant or animal organisms to pathogens, another type of immunity permits the inhibition of specific pathogenic races in resistant hosts. In these cases specific effectors of pathogenic races trigger the immune response (Zipfel and Rathjen, 2008). In both plants and animals, a lipid compound, phosphatidyl-inositol 3-phosphate mediates entry of pathogen effectors into host cells (Kale et al., 2010). Plant or animal NLR receptors that interact with these effectors possess a very specific recognition ability. Interestingly, effector recognition by a receptor is associated with an almost infinite number of effector (antigen)-binding ability of animal receptors. In this case somatic recombination and mutation is generated in the receptor-carrying lymphocytes determining a high degree of immune diversity. This type of specific immunity represents the animal adaptive immune system (Fig.1). Effector recognition by a receptor results in clonal expansion of lymphocytes and formation of memory cells having receptors with effector-binding specificity identical to that of lymphocytes. These procedures allow a secondary immune response against a subsequent infection (cf. Jones and Dangl, 2006).

An adaptive immune system does not exist in plants. Plants have no lymphocytes, immune memory cells are not formed and the phenomenon of somatic recombination has not been unequivocally demonstrated. However, as regards similarity, plant NLR receptors, the R-proteins also have NB and LRR domains like their animal counterparts. In addition, there is a secondary immune response operating in plants that confers inhibition of secondary infections and is triggered by a primary infection that occurred earlier in a distal plant organ. This phenomenon is called systemic acquired resistance (SAR) (Ross, 1961; Durrant and Dong, 2004; Spoel and Dong, 2012).

One can raise the question, how can plants develop specific resistance mechanisms induced by numerous effectors of different pathogenic races? Plants do not have an adaptive immune system, the basis of immune diversity in animals. Only a limited number of specific receptors (R-proteins) exist in plant organisms, and still, immune plants can recognize a high number of effectors of pathogenic races. The “gene-for-gene concept” tried to answer this question (Flor, 1971). According to the original experiments rust-resistant flax strains express different R-genes corresponding to specific avirulence (effector) genes in each pathogenic rust race. In each incompatible (resistant) host/pathogen combination an avirulence gene encodes a specific effector and a plant R-gene encodes a specific receptor. Thus, an effector of a race can activate only the corresponding specific plant receptor. However, it turned out that only a

few hundred R-genes exist in host plants, as compared to the almost infinite number of effectors encoded by pathogen avirulence genes. Therefore, this concept cannot explain the high degree of immune capacity and broad immune diversity of plants (Fig. 2).

Recent investigations on the mechanism of plant non-adaptive immunity point to the possibility that plants may exhibit a different type of immune diversity. Several results have shown that plant R-protein receptors do not directly recognize effectors of pathogens as foreign proteins in most host/pathogen combinations. Rather, pathogen effectors modify target self-proteins in plants in the course of the infection process (Fig. 3). As a result of the proteolytic activity, phosphorylation, acetylation etc. exerted by effectors, the modified self-proteins become "foreign" (non-self) for plant receptors. Thus, the receptor R-protein can recognize the modified target self-protein. This is the essence of the "guard hypothesis". An R-protein is somehow connected to a target self protein(s). After modifications, target proteins are able to initiate recognition processes and an immune response develops (Liu et al., 2011; Chung et al., 2011; Mukhtar et al., 2011).

It seems clear from the "guard hypothesis" that only a limited number of receptor R-proteins will be required to recognize different pathogenic races because the very large number of effectors released by those races may modify the same target protein (albeit in different ways). It also turned out that a large number of effectors can alter only a few conserved target self-proteins, which will be able to activate R-proteins. Thus, immunity will be initiated in a very large number of plant cultivar/pathogenic race combinations. It would seem that immune diversity may exist also in plants, because only a small number of R-proteins can recognize an almost infinite number of race-specific effectors.

As a consequence of the effector-receptor interaction signal transduction chains are activated and, in the end, invading pathogens will be inhibited or killed. A series of genes are activated or inhibited in the resistant plant. However, the role of these genes in the immunity process is not exactly clear so far. Tao et al. (2003) surprisingly demonstrated that similar gene groups are activated in infected hosts whether the plant exhibits susceptibility or resistance. In the case of compatible or incompatible *Arabidopsis-Pseudomonas* combinations one can detect common mRNA expression profiles. If we compare specific and non-specific immune processes, again same or similar gene groups are activated (Navarro et al., 2004; Zipfel et al., 2004). All these facts refer to the possibility that timing of gene activations, rather than gene alteration itself has a pivotal role in disease resistance. It was shown in several experiments that those genes are activated much earlier in resistant plants than in

susceptible ones. Accordingly, it seems reasonable to suppose that different forms of plant immunity have a common basic mechanism.

If an effector protein of a pathogen modifies a plant protein which has no role in non-self recognition, this modified protein will not be foreign therefore will not be recognized by receptor R-proteins. In this case the pathogen effector acts as a virulence factor rather than an avirulence gene product. In fact, the original function of pathogen effectors is to promote pathogenesis as virulence factors (cf. Jones and Dangl, 2006). Therefore, effector proteins of a given pathogen could be regarded as "double agents", as was expressed by Alfano and Collmer (2004), since effectors may behave as avirulence factors in immune processes or virulence factors in reactions of susceptibility.

Systemic acquired resistance (SAR) (Immune memory – Stress memory)

Mobile immune cells and a circulatory system permit diseased animals to exert immunity in the whole body. In addition, immune memory cells are also formed having receptors with antigen-binding ability allowing a secondary immune response to a subsequent infection. This immune memory-based response is a very effective type of adaptive immune response in animals. Interestingly, immune memory operates also in invertebrate animals although they do not have an adaptive immune response system. The mechanism is not well understood at the moment (Netea et al., 2011).

The process of immune memory also exists in plants where signals produced at the site of a primary infection induce a secondary immune response in non-infected distal tissues (systemic acquired resistance, SAR) (Ross, 1961; Balázs et al., 1977; Sziráki et al., 1980; Doss, 1981; Hammerschmidt et al., 1982; Durrant and Dong, 2004; Spoel and Dong, 2012). However, this type of immunity is non-specific, it is effective against symptoms caused by a broad spectrum of pathogens or abiotic stresses usually associated with tissue necroses. It resembles the immune memory of animals although plants do not produce immune cells and memory cells and lack a circulatory system. Interestingly, one theory claims that somatic recombination, a DNA rearrangement analogous to adaptive immunity in animals, occurs in plants. For example, viral infections may induce hypothetical systemic signals which initiate an increase in the rate of somatic recombinations at the site of primary infections as well as in distal tissues (Kovalchuk et al., 2003; Dong, 2004; Boyko et al., 2007; Alvarez et al., 2010; Boyko and Kovalchuk, 2011). Further research is needed to clarify whether somatic recombination is a general mechanism of plant immune memory.

According to another concept the phenomenon of immune memory should rather be regarded as “stress memory”. Primary infection can induce SAR in distal tissues if the pathogen infection or stress is associated with local cell and tissue necrotization. However, there may be certain exceptions to this rule. For example, when a tobacco host plant expresses the *Rx* virus resistance gene and is infected with *Potato virus X* (PVX), a symptomless immunity (extreme resistance) develops, with complete absence of the hypersensitive reaction (i.e. localized necrosis). Liu et al. (2010) have shown that in such cases in spite of the lack of necrotic symptoms SAR is operating in distal leaves. One can suppose that symptomless stress at the site of primary infection is sufficient to induce SAR in other organs. Furthermore, it is important to consider that SAR-induction also occurs if not pathogens but chemicals, such as $HgCl_2$ or liquid N_2, elicit tissue necrotization at the site of primary application. In addition, SAR provides resistance not only against secondary pathogen infections since the rate of necrotization caused by $HgCl_2$, $CuCl_2$ and the herbicide paraquat will also be diminished in distal plant organs following SAR-induction (Sziráki et al., 1980; Doss, 1981; Strobel and Kuć, 1995).

It was shown that salicylic acid (SA) accumulates during SAR (Métraux et al., 1990; Malamy et al., 1990). SA is an essential component of SAR but not the mobile signal itself (Forouhar et al., 2005; Park et al., 2007). Recently, interesting results have been published as regards the mobile signal system which makes it possible to send messages from the site of primary infection to distal plant tissues. These multiple signals can initiate different reactions in systemic tissues and, as a consequence, SA accumulates at the site of secondary infection or stress, where a transcription cofactor, the product of the *NPR1* gene (*Nonexpressor of PR Genes 1*) is activated by SA (Mou et al. 2003; Spoel and Dong, 2012). Following this reaction transcriptional reprogramming cascades are initiated and SAR develops in distal tissues. Furthermore, antioxidants accumulate and production of reactive oxygen species (ROS) is reduced, in accordance with suppression of necrotic symptoms in remote leaves of plants expressing SAR (Fodor et al., 1997; Király et al., 2002; Hafez et al., 2004). It seems important to note that the transcriptional actions of NPR1 are analogous to those induced by the immune regulator nuclear factor kappa B (NF-κB) in mammals.

At the site of primary infection where several immune signals are generated and SA is accumulated, methyl salicylic acid (MeSA), a possible mobile SAR signal, is formed with the aid of methyl transferase. In remote uninfected tissues MeSA will be reformed to SA by MeSA-esterase (Forouhar et al., 2005; Park et al., 2007). According to recent experiments the SA-MeSA transformation may not be a pivotal phenomenon in all plant/pathogen

combinations, its role depends on host plant species, plant/pathogen interactions and environmental conditions. Also, it turned out that during the course of SAR-induction the interactions of several signals are needed. Some of these recently recognized signals are a lipid transfer protein (DIR1), glycerol-3-phosphate (G3P), azelaic acid and pipecolic acid. These compounds, in addition to SA, may take part in the generation and translocation of mobile immune (SAR) signal(s) or function as a mobile immune signal *per se* and induce development of SAR (cf. Dempsey and Klessig, 2012; Shah and Zeier, 2013). However, exact details of the combined actions of these immune (SAR) signals are not clarified so far. In addition, it is known that SA analogs, such as acetyl salicylic acid (aspirin), 2,6-dichloroisonicotinic acid (INA) and benzothiadiazole (BTH) can also act as SAR inducers (White, 1979; Kogel et al., 1994; Görlach et al., 1996). In fact, in the 1990-s BTH was used for a few years as a resistance-inducer pesticide in farming practice.

Transgenerational immune memory

It is known that a transgenerational memory of stress exists in animal systems (Carone et al., 2010). In certain cases plant immune memory can be also transmitted to subsequent generations (Luna et al., 2012). This implies that somatic and/or meiotic recombination may also occur in plants, in response to pathogen infections and abiotic stresses, which could be a cause of transgenerational immune memory (Chiriac et al., 2006; Molinier et al., 2006; Boyko et al., 2007, 2010; Alvarez et al., 2010; Boyko and Kovalchuk, 2011). Furthermore, epigenetic changes may also have a role in the inheritance of plant immune memory. Dowen et al. (2012) emphasize that defense genes can be modulated by DNA methylation. Immunity of *Arabidopsis thaliana* to *Pseudomonas syringae* pv. *tomato* is negatively regulated by DNA methylation and loss of DNA methylation stimulates resistance to bacterial infection. Akimoto et al., (2007) called the attention to the enhanced resistance of globally demethylated rice to infection by a *Xanthomonas* bacterium. Generally, it would seem that some defense genes are controlled by DNA methylation (Dowen et al., 2012). Methylation of histones may also occur. The progeny of salt stressed *Arabidopsis* plants exhibited changes in histones, modifications of DNA methylation and expression of several genes (Bilichak et al., 2012). According to recent research a primary plant infection results in modification of histone methylation and acetylation patterns at promoters of SA-inducible defense (stress)-related genes in systemic tissues. Such changes confer enhanced induction of these genes during a secondary infection and the "primed" state of SA-dependent defenses and SAR is transmitted to subsequent generations. This transgenerational transmission of plant immune memory

requires activity of the transcription cofactor NPR1, a central regulator of SA-dependent defenses and SAR (Jaskiewicz et al., 2011; Luna et al., 2012). However, understanding the exact steps of the development of plant transgenerational immune memory will require further research.

Gene silencing as an immune response

Until the mid 1990-s the generally accepted notion was that the essence of animal and plant immunity is the recognition of non-self proteins. However, it turned out that during certain plant virus infections and in transgenic plants the expression of foreign genes (e.g. viral RNA or transgenes) that enter plant cells may be inhibited (silenced) (cf. van der Krol et al., 1990; Napoli et al., 1990; Baulcombe, 1996, 2004; Voinnet, 2001, 2005). Although transcription of a transgene or virus gene could be normal, the resulting mRNA may be degraded, i.e. post-transcriptional gene silencing occurs. In these cases immunity is expressed on the level of nucleic acids (RNA, DNA), rather than on the level of proteins.

Plants recognize a certain level of transgene mRNA and invading viral RNA as foreign, initiating thereby a specific degradation mechanism. A distinct type of RNA-dependent RNA polymerase(s) is activated and antisense RNA strands are synthesized that are complementary to the transgene and/or virus RNA. This process results in the formation of double-stranded (ds) RNA. In fact, dsRNA is indeed regarded as foreign by plants, since they normally do not encounter these molecules. In virus infections, dsRNA is also formed as an intermediate product of pathogen replication. These foreign dsRNA structures are degraded by dsRNA-specific ribonucleases. This is how the process of post-transcriptional gene silencing is initiated, the end result being the inhibition of expression of all genes that show sequence homology to the foreign gene (transgene or virus gene) (see e.g. Baulcombe, 2004; Eamens et al. 2008; Ghildiyal and Zamore 2009; Ruiz-Ferrer and Voinnet, 2009; Wang et al., 2012).

Artificial production of dsRNA in plants (e.g. via a transgenic approach) may induce strong gene silencing (Hamilton et al. 1998; Waterhouse et al. 1998; Carthew, 2001). Interestingly, it has been also shown that truncated (defective) viral RNA-s, a characteristic of certain plant virus infections, may cause gene silencing and therefore, virus inhibition (Szittya et al., 2002; Silhavy and Burgyán, 2004). Gene silencing is an unwanted phenomenon in plant breeding and biotechnology, because it inhibits effective expression of transgenes. On the other hand, gene silencing may be beneficial for pest management, because it could confer plant resistance (immunity) to e.g. virus infections. Interestingly, gene silencing may also

protect a host crop against a root parasitic weed. Transgenic alfalfa was created that expresses an antisense (i.e. dsRNA-producing) construct based on the acetyl-CoA carboxylase gene of the root parasite *Triphysaria versicolor*. Development of the root parasite on transgenic alfalfa was inhibited by up to 80 % (Bandaranayake and Yoder, 2013). It is likely that gene silencing is also functional during virus infections of humans and animals although there is insufficient proof so far to claim that this mechanism is effective not only in laboratory experiments but is also a pivotal element of immunity under natural conditions.

What is inhibiting or killing pathogens during the immune process?

The interaction between pathogen effectors and resistant host receptors results in inhibition or killing of pathogens. In past years, several theories tried to explain the possible mechanisms of disease resistance in plants, such as accumulation of antimicrobial compounds, cell wall thickening, activities of cell wall degrading enzymes, localized necrosis (hypersensitive response, HR), accumulation of phytoalexins, reactive oxygen species (ROS) etc. (cf. Király et al., 1972; Goodman et al., 1986; Jones and Dangl, 2006; Spoel and Dong, 2012). Although excellent results have been published on the genetics of plant/pathogen interactions (e.g. Staskawicz et al., 1995; Schulze-Lefert and Bieri, 2005; Maekawa et al., 2011; Gassmann and Bhattacharjee, 2012), the direct mechanism of the "killing effect" has remained unknown. Recently, the role that ROS, primarily oxygen free radicals, play in animal and plant immunity has become a pivotal research topic mainly because of two reasons. First, there is a cause-and-effect relationship between animal phagocytosis and the accumulation of ROS (Morel et al., 1991). Second, ROS accumulation has been also detected in the course of several plant immunity events (Doke, 1983; Doke and Ohashi, 1988; Ádám et al., 1989; Levine et al, 1994; Baker and Orlandi, 1995; Delledonne et al., 2001; Apel and Hirt, 2004; Delledonne, 2005; Torres et al., 2006; Shang et al., 2010). Furthermore, it was experienced that the accumulated ROS can protect plants from a late infection. Thus, it was shown that it is possible to "immunize" plants against an expected infection (Hafez and Király, 2003; El-Zahaby et al., 2004; Hafez et al., 2012).

Animal phagocytosis and plant immunity seem to be analogous processes. However, as regards the biochemical mechanisms, certain differences exist. In case of phagocytosis superoxide ($O_2^{\cdot-}$) reacts with nitrogen monoxide ($NO^{\cdot}$) and peroxinitrite ($ONOO^-$) is formed, the latter compound being capable of killing bacterial pathogens. On the other hand, in plants $O_2^{\cdot-}$ is dismutated and hydrogen peroxide (H_2O_2) is produced. In this case H_2O_2 reacts with

$NO^{\cdot}$, as a consequence, the killing action will operate both against pathogens and host plant cells (HR). $ONOO^{-}$ has no killing action in the case of plants (Delledonne, 2005).

Role of reactive oxygen species (ROS) in plant immunity on the basis of recent experiments

About three decades ago Doke (1983) and Doke and Ohashi (1988) demonstrated that in a fungus resistant potato and a virus resistant tobacco exhibiting HR type immune reactions, $O_2^{\cdot -}$ accumulates in contrast to the susceptible infected host plants where there is no change in $O_2^{\cdot -}$ levels. Later, we have characterized a similar phenomenon in tobacco leaves showing an HR-type resistance to bacterial infections (Ádám et al., 1989). In all of these resistant hosts there is a correlation between immunity and accumulation of $O_2^{\cdot -}$. A similar correlation has been demonstrated in rust-resistant wheat cultivars and accumulation of H_2O_2 (Hafez et al., 2009).

Non-host resistance

Fabro et al. (2011) have shown that in the "non-host" plant *Brassica rapa* (turnip) more effectors of *Hyaloperonospora arabidopsidis* are recognized than in *Arabidopsis thaliana* which is a "host" of this oomycete pathogen. This could be a possible cause of the inability of *H. arabidopsidis* to grow in turnip. In other words, the host plant cannot recognize a subset of effectors of its own pathogen which are recognized, and therefore induce an immune reaction in the non-host. However, it is still an unanswered question, how this immune reaction can inhibit pathogens in non-host plants?

Although there is a definite correlation between accumulation of certain reactive oxygen species (ROS) and inhibition of plant pathogens in resistant (immune) plants, the cause-and-effect relationship between these two events (i.e. ROS accumulation and disease resistance) is not entirely clear so far. We have observed in recent unpublished experiments that in a series of plant/pathogen interactions, the "non-host" type of resistance is associated with an early activation of $O_2^{\cdot -}$-accumulation in resistant non-host plants, which could inhibit or kill pathogens early after infection (Fig. 4 and Fig. 5). This may happen in barley plants which have been infected with powdery mildew specialized for infection of wheat, but not barley leaves (*Blumeria graminis* f. sp. *tritici*). Barley is a non-host for this wheat pathogenic fungus, but it is compatible with another, barley-specific powdery mildew, *Blumeria graminis* f. sp. *hordei.* If the infectious pathogen is wheat powdery mildew, barley plants remain symptomless (no HR is produced) and disease development is fully inhibited. If barley is infected with its own powdery mildew, the reaction may result in resistance with inhibition of

the pathogen and production of HR symptoms or in susceptibility with development of typical disease symptoms and unarrested growth of the pathogen. In the former case of "host" type of resistance $O_2^{\cdot-}$ also accumulates, although relatively late after infection, as compared to non-host resistance. However, in infected susceptible barley there is no $O_2^{\cdot-}$ -production. It would seem that this is a general rule based on several experiments. Accordingly, the cause of symptomless non-host resistance could be the early inhibition or killing of infectious agents by reactive oxygen species. However, in host plants exhibiting resistance to infections (host resistance) $O_2^{\cdot-}$-accumulation takes place somewhat later, thus pathogens can grow for a limited time, the HR can develop, although the host remains resistant to powdery mildew. In this case $O_2^{\cdot-}$ may inhibit or kill, not only pathogens but also several host cells causing resistance with HR symptoms. In a compatible plant/pathogen combination, where the host is susceptible to infection, pathogen growth is uninhibited and disease symptoms are produced possibly because there is no $O_2^{\cdot-}$-accumulation after infection which could be due to elevated antioxidant capacity of the host (El-Zahaby et al., 1995; Harrach et al., 2008). This theory is supported by another finding in our laboratory (unpublished results). It was shown that a short heat-shock in the non-host resistant barley causes a partial susceptibility to wheat powdery mildew and, at the same time, inhibition of $O_2^{\cdot-}$- production (see below).

Inhibition of $O_2^{\cdot-}$ and H_2O_2 production in plants increases susceptibility

We have analyzed the effect of inhibition of ROS-accumulation on plant disease resistance in two types of experiments. In one investigation we wanted to see the influence of inhibited or suppressed $O_2^{\cdot-}$- production caused by high temperature on disease resistance of tobacco and barley. In another experiment we have characterized the effect of suppressed ROS-production of a NADPH-oxidase mutant of *Arabidopsis thaliana* on disease resistance.

It is known for a long time that in TMV-infected resistant tobacco kept at a relatively high temperature (30^{o}C) necrotic lesions (HR) are suppressed and, correspondingly, viral replication is released from inhibition in the originally resistant tobacco that expresses the virus-resistance gene *N* (Samuel, 1931). We have shown (Király et al., 2008) that at 30^{o}C not only the resistance-associated HR-type necrosis is inhibited, but $O_2^{\cdot-}$ production is also suppressed, coupled with a decreased activity of NADPH-oxidase and reduced expression of the encoding NADPH-oxidase gene, factors that have a pivotal role in $O_2^{\cdot-}$- production during plant resistance (Doke and Ohashi, 1988; Torres and Dangl, 2005; Proels et al., 2010; Marino et al., 2012). Furthermore, activity of an antioxidant enzyme, dehydro-ascorbate reductase, was also stimulated in TMV-infected, *N* gene-containing tobacco kept at 30^{o}C, indicating an

enhanced “neutralization” of ROS. As a result, virus-resistance was shifted into the direction of susceptibility and virus (TMV) replication was enhanced. On the other hand, external application of ROS-producing chemicals or H_2O_2 to tobacco leaves causes enhanced virus-resistance if applied early, e.g. two hours after inoculation (Bacsó et al., 2011). From these results one can conclude that the action of high temperature on increased virus susceptibility is in a cause-and-effect relationship with suppression of $O_2^{\cdot-}$- production in tobacco leaves.

In another series of experiments we have immersed barley leaves into hot water (49^oC) for 30 sec. and, 2 hours later, leaves were inoculated with the wheat powdery mildew fungus (*Blumeria graminis* f. sp. *tritici*). In response to this short heat-treatment, non-host resistant barley leaves turned partially susceptible to the fungus and levels of $O_2^{\cdot-}$ were significantly reduced. A similar phenomenon was experienced when heat-treated barley was infected with a hemibiotrophic fungus (*Bipolaris sorokiniana*). This pathogen produces necrotic symptoms at the end of its life cycle in susceptible hosts. Multiplication of *B. sorokiniana* was enhanced and the tissue necroses were more pronounced in heat-treated barley, but the stimulated susceptibility was associated with elevated $O_2^{\cdot-}$ production, which is an early signal of necrotization. In this case reduction in $O_2^{\cdot-}$production caused by heat treatment was counteracted and masked by the elevated $O_2^{\cdot-}$ production caused by plant tissue necrotization (Király et al, 2013).

In order to further demonstrate that inhibition of $O_2^{\cdot-}$ and H_2O_2 production in plants increases susceptibility, the mechanism of this process was also investigated by a different experimental approach. We inoculated an NADPH-oxidase mutant of *Arabidopsis thaliana* with viral and bacterial pathogens. In the *rbohD* “knock out” mutant the production of H_2O_2 and supposedly $O_2^{\cdot-}$ production is inhibited because activity of NADPH-oxidase has been knocked out. Pogány et al. (2009) have shown that this mutant turned to be more susceptible to symptoms induced by *Alternaria brassicicola*. According to recent results this mutant is also susceptible to bacterial infections (cf. Marino et al., 2012). Our unpublished experiments also demonstrated that the *rbohD* mutant *Arabidopsis,* which is inhibited in ROS-production and NADPH oxidase enzyme activity, is more susceptible to infections by both a viral and a bacterial pathogen. Preliminary experiments have shown that the mutant *Arabidopsis* plants regained their original resistance if leaves were externally treated with chemicals that generate ROS (e.g. riboflavine-methionin and glucose-glucose oxidase) or with H_2O_2.

In summary, through genetic and biochemical inhibition of ROS accumulation it has been demonstrated by us and others that several ROS may have a central role in plant resistance against viral, bacterial and fungus infections.

Induction of disease resistance in susceptible plants by external application of ROS

Our earlier publications have shown that it is possible to induce resistance in susceptible plants if we externally apply 50 mM H_2O_2 to leaves or treat susceptible leaves with chemicals (riboflavin-methionine, xanthine-xanthine oxidase) that induce ROS-production (Hafez and Király, 2003; El-Zahaby et al., 2004). As a result of the action of ROS, pathogens are inhibited or killed, therefore typical disease symptoms cannot develop, however HR-type tissue necroses associated with the immune reaction are produced. If the originally susceptible plants (e.g. powdery mildewed barley) receive not only H_2O_2 or other ROS treatments that are able to cause resistance to the fungus but also antioxidants that can neutralize the actions of ROS, the plants remain susceptible. These results demonstrate that plant disease resistance may indeed depend on the killing action of certain ROS.

When we applied H_2O_2 or several other ROS-producing compounds to leaves of *Arabidopsis thaliana* inoculated with *Pseudomonas syringae* pv. *tomato* DC3000, the number of pathogenic bacteria was significantly reduced, as compared to untreated but infected control plants. It was also shown (Bacsó et al., 2011) that externally applied H_2O_2 or ROS-producing compounds can inhibit replication of TMV in a virus-susceptible tobacco (cv. Samsun *nn*) or decrease the number of local necrotic lesions (HR) in the tobacco cultivar Samsun *NN* that is resistant to TMV. It is important to note that external ROS can induce resistance only if it is applied not later than a few hours after inoculation. If reactive oxygen species are applied three days after virus-inoculation, they cannot induce resistance (Király et al, 2008; Bacsó et al. 2011).

Our unpublished experiments demonstrated that if certain ROS are produced or accumulated in due time in plants, e.g. pepper, this may cause resistance to infection. We have shown that it is possible to transfer powdery mildew resistance of cherry pepper (*Capsicum annuum* var. *cerasiforme*) to sweet pepper (*Capsicum annuum*) by grafting. Several cultivars of cherry pepper exhibit almost full resistance to the pepper powdery mildew fungus (*Leveillula taurica*). If cherry pepper is the rootstock, it can transfer resistance to the grafted and originally susceptible sweet pepper scion. It was shown that the level of $O_2^{\cdot-}$, determined by nitroblue-tetrazolium chloride (NBT), is much higher in resistant cherry pepper than in susceptible sweet pepper plants. Following grafting and powdery mildew infection, $O_2^{\cdot-}$ accumulated to high levels also in the originally susceptible scion, and leaves of the scion turned to be powdery mildew resistant. Interestingly, the grafting procedure stimulates $O_2^{\cdot-}$-accumulation in leaves of the scion even if the grafted plant is not infected with the fungus. In

correlation with these events, activity of NADPH-oxidase, the enzyme mainly responsible for $O_2^{\cdot-}$-production caused by abiotic and biotic plant stresses (cf. Torres and Dangl, 2005; Marino et al., 2012), is also stimulated. We can conclude that $O_2^{\cdot-}$ has a central role in this graft-transmissible resistance of cherry pepper to powdery mildew.

Only a few cases are mentioned in the literature when disease resistance has been transferred from the rootstock to scion by grafting (Šutić, 1965; Vulić et al., 2013; Al-Mawaali et al., 2013), however, the mechanisms were not described in any case. On the other hand, Molnar et al. (2010) and Dunoyer et al. (2010) reported that small interfering RNAs (siRNAs) that are mobile elements, could be responsible for transfer of genetic information from the rootstock to scion or vice versa. Whether or not siRNAs are responsible for the stimulated $O_2^{\cdot-}$ production and activated NADPH-oxidase in the grafted pepper scion, remains to be seen in the future.

Symptom resistance caused by stimulated antioxidants

Elevation of plant antioxidant capacity increases resistance to symptoms caused by necrotrophic pathogens (Waller et al., 2005; Barna et al., 2012; Harrach et al., 2013). Symptom resistance means that although disease symptoms are suppressed in the host, the infecting pathogen is not inhibited or killed after infection. This type of immunity may be useful in commercial farming because yield damage could be reduced. Symptom resistance can be induced by a mild ROS-treatment to the host. This causes a mild damage to tissues and, as a response, antioxidant activities will be stimulated (Halliwell and Gutteridge, 1999). Such a stimulated antioxidant capacity can diminish or inhibit ROS-induced necrotization associated with pathogen infections. The phenomenon of symptom resistance is analogous to animal vaccination, and could be regarded as “plant immunization”. However, we have shown that viral, bacterial or fungal pathogens are indeed not damaged in symptom-resistant plants only the development of necrotic symptoms is inhibited or suppressed (Hafez et al., 2012).

Earlier, we experienced that tobacco cells selected *in vitro* for ROS resistance and later induced to produce callus tissues and regenerated to full plants, exhibit resistance to pathogens, toxins and abiotic stresses that cause necrotic symptoms in different plants (Barna et al., 1993; Darkó et al., 2009, 2011). Also in these cases stimulated antioxidants seem to be responsible for the inhibition of necrotic symptoms in the stressed or infected ROS-resistant plants.

A different approach to create ROS-resistance in plants is overexpression of the iron-binding protein ferritin. We have shown that in such tobacco plants generation of the

hydroxyl radical ($OH^{\cdot}$) is inhibited due to the unavailability of free Fe resulting in enhanced resistance to pathogen-induced necrotic symptoms (Deák et al., 1999). Breeding plants for symptom (ROS)-resistance to cell and tissue necrotization by *in vitro* selection could be a commercially useful resistance breeding method in the future.

Acknowledgements

We appreciate the valuable comments of Professors Béla Nagy and Balázs Barna. This research was supported by grants of the Hungarian Scientific Research Fund (OTKA K77705, K84002 and PD83831).

Note added in proof

Recently it was shown that a new type of plant disease resistance mechanism may revolutionize plant protection (Koch et al., 2013: Host-induced gene silencing of cytochrome P450 lanosterol C14α-de-methylase encoding genes confers strong resistance to Fusarium species. PNAS doi: 10.1073/pnas.1306373110).

Literature

Ádám, A., Farkas, T., Somlyai, G., Hevesi, M. and Király, Z. (1989): Consequence of $O_2^{\bullet-}$ generation during a bacterially induced hypersensitive reaction in tobacco: deterioration of membrane lipids. Physiol. Molec. Plant Pathol. 34, 13-26.

Akimoto, K., Katakami, H., Kim, H-J., Ogawa, E., Sano, C. M., Wada, Y. and Sano, H. (2007): Epigenetic inheritance in rice plants. Ann. Bot. 100, 205-217.

Alfano, J. R. and Collmer, A. (2004): Type III secretion system effector proteins: double agents in bacterial disease and plant defense. Annu. Rev. Phytopathol. 42, 385-414.

Al-Mawaali, Q. S., Al-Sadi, A. M. and Deadman, M. K. (2013): Management of cucumber damping-off and vine decline by grafting on resistant rootstocks. Intern. Conf. Plant Diseases and Resistance Mechan. Vienna, Austria. Abstract, p. 76, N 80.

Alvarez, M. E., Nota, F. and Cambiagno, D. A. (2010): Epigenetic control of plant immunity. Mol. Plant Pathol. 11, 563-576.

Apel, K. and Hirt, H. (2004): Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu. Rev. Plant Biol. 55, 373-399.

Ausubel, F. M. (2005): Are immune signaling pathways in plants and animals conserved? Nature Immunol. 6, 973-979.

Bacsó, R., Hafez, Y. M., Király, Z. and Király, L. (2011): Inhibition of virus replication and symptom expression by reactive oxygen species in tobacco infected with *Tobacco mosaic virus*. Acta Phytopathol. Entomol. Hung. 46, 1-10.

Baker, J. C. and Orlandi, E. W. (1995): Active oxygen in plant pathogenesis. Annu. Rev. Phytopathol. 33, 299-321.

Balázs, E., Sziráki, I. and Király, Z. 1977): The role of cytokinins in systemic acquired resistance of tobacco hypersensitive to tobacco mosaic virus. Physiol. Plant Pathol. 11, 29-37.

Bandaranayake, P. C. G. and Yoder, J. I. (2013): Trans-specific gene silencing of acetyl-coa carboxylase in a root-parasitic plant. Mol. Plant-Microbe Interactions 26, 575-584.

Barna, B., Ádám, A., Király, Z. (1993): Juvenility and resistance of a superoxide-tolerant plant to diseases and other stresses. Naturwissenschaften 80, 420-422.

Barna, B., Fodor, J., Harrach, B., Pogány, M., Király, Z. (2012): The Janus face of reactive oxygen species in resistance and susceptibility of plants to necrotrophic and biotrophic pathogens. Plant Physiol. Biochem. 59, 37-43.

Baulcombe, D. C. (1996): Mechanisms of pathogen-derived resistance to viruses in transgenic plants. Plant Cell 8, 1833-1844.

Baulcombe, D. (2004): RNA silencing in plants. Nature 431, 356-363.

Bilichak, A., Ilnystkyy, Y., Hollunder, J. and Kovalchuk, I. (2012): The progeny of *Arabidopsis thaliana* plants exposed to salt exhibit changes in DNA methylation, histone modifications and gene expression. PLoS One 7, e30515.

Boller, T. and Felix, G. A. (2009): A renaissance of elicitors: perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. Annu. Rev. Plant Biol. 60, 379-406.

Boyko, A. and Kovalchuk, I. (2011): Genetic and epigenetic effects of plant–pathogen interactions: an evolutionary perspective. Mol. Plant 4, 1014–1023.

Boyko, A., Kathiria, P., Zemp, F. J., Yao, Y., Pogribny, I. and Kovalchuk, I. (2007): Transgenerational changes in the genome stability and methylation in pathogen-infected plants (virus-induced plant genome instability). Nucleic Acids Res. 35, 1714-1725.

Boyko, A., Blevins, T., Yao, Y., Golubov, A., Bilichak, A., Ilnytskyy, Y., Hollander, J., Meins, F. Jr. and Kovalchuk, I. (2010): Transgenerational adaptation of Arabidopsis to stress requires DNA methylation and the function of Dicer-like proteins. PLoS One 5, e9514.

Carone, B. R., Fauquire, L., Habib, N., Shea, J. M. et al. (2010): Paternally induced transgenerational environmental reprogramming of metabolic gene expression in mammals. Cell 143, 1084-1096.

Carthew, R.W. (2001): Gene silencing by double-stranded RNA. Curr. Opin. Cell Biol. 13, 244-248.

Chiriac, G. I., Andronic, L. I., Bujoreanu, V. V., and Marii, L.I. (2006): Features of crossing-over in virus-infected tomato. Centr. Eur. J. Biol. 1, 386-398.

Chung, E. H., da Cunha, L., Wu, A. J., Gao, Z., Cherkis, K., Afzal, A. J., Mackey, D. and Dangl, J. L. (2011): Specific threonine phosphorylation of a host target by two unrelated type III effectors activates a host innate immune receptor in plants. Cell Host Microbe 9, 125-136.

Darkó, É., Ambrus, H., Szabó, I., Fodor J., Király Z., and Barnabás B. (2009): Enhanced tolerance to oxidative stress with elevated antioxidant capacity in doubled haploid maize derived from microspores exposed to paraquat. Crop Sci. 49, 628-636.

Darkó, É., Fodor, J., Dulai, S., Ambrus, H., Szenzenstein, A., Király, Z., Barnabás, B. (2011): Improved cold and drought tolerance of doubled haploid maize plants selected for resistance to prooxidant tert-butyl hydroperoxide. J. Agron. Crop Sci. 197, 454-465.

Deák, M., Horváth, G. V., Davletova, S., Török, K., Sass, L., Vass, I., Barna, B., Király, Z. and Dudits, D. (1999): Plants ectopically expressing the iron-binding protein, ferritin, are tolerant to oxidative damage and pathogens. Nature Biotech. 17, 192-198.

Delledonne, M. (2005): NO news is good news for plants. Curr Opin Plant Biol 8, 390-396.

Delledonne, M., Zeier, J., Marocco, A. and Lamb, C. (2001): Signal interactions between nitric oxide and reactive oxygen intermediates in the plant hypersensitive disease resistance response. Proc. Natl. Acad. Sci. USA 98, 13454-13459.

Dempsey, D. A. and Klessig, D. F. (2012): SOS – too many signals for systemic acquired resistance? Trends Plant Sci. 17, 538-545.

Doke, N. (1983): Involvement of a superoxide anion generation in the hypersensitive response of potato tube tissues to infection with an incompatible race of Phytophthora infestans and to the hyphal wall components. Physiol. Plant Pathol. 23, 345-357.

Doke, N. and Ohashi, Y. (1988): Involvement of an $O_2^{\bullet -}$ - generating system in the induction of necrotic lesions on tobacco leaves infected with *Tobacco mosaic virus.* Physiol. Molec. Plant Pathol. 32, 163-175.

Dong, X. (2004): Pathogen-induced systemic DNA rearrangement in Plants. Trends Plant Sci. 9, 60-61.

Doss, M. M. (1981): Mechanism of systemic acquired resistance in cucumber caused by pathogenic and non-pathogenic agents. Ph.D. Diss. Res. Inst. Plant Protection, Budapest, 122 p.

Dowen, R. H., Pelizzola, M., Schmitza, R. J., Lister, R., Dowen, J. M., Nery, J. R., Dixon, J. E. and Ecker, J. R. (2012): Widespread dynamic DNA methylation in response to biotic stress. Proc. Natl. Acad. Sci. USA 109, E2183-E2191.

Dunoyer, P., Schott, G., Himber, C., Meyer, D., Takeda, A., Carrington, J. C. and Voinnet, O. (2010): Small RNA duplexes function as mobile silencing signals between plant cells. Science 328, 912-916.

Durrant, W. E. and Dong, X. (2004): Systemic acquired resistance. Annu. Rev. Phytopathol. 42, 185-209.

Eamens, A., Wang, M.-B., Smith, N. A., and Waterhouse, P. M. (2008): RNA silencing in plants: Yesterday, today, and tomorrow. Plant Physiol. 147, 456-468.

El-Zahaby, H. M., Gullner, G. and Király, Z. (1995): Effects of powdery mildew infection of barley on the ascorbate-glutathione cycle and other antioxidants in different host-pathogen interactions. Phytopathology 85, 1225-1230.

El-Zahaby, H. M., Hafez, Y. M. and Király, Z. (2004): Effect of reactive oxygen species on plant pathogens *in planta* and on disease symptoms. Acta Phytopathol. Entomol. Hung. 39, 325-345.

Fabro, G., Steinbrenner, J., Coates, M., Ishaque, N., Baxter, L. et al., (2011): Multiple candidate effectors from the oomycete pathogen *Hyaloperonospora arabidopsidis* suppress host plant immunity. PLoS Pathog. 7, e1002348. doi:10.1371/journal.ppat.1002348.

Felix, G., Duran, J. D., Volko, S. and Boller, T. (1999): Plants have a sensitive perception system for the most conserved domain of bacterial flagellin. Plant J. 18, 265-276.

Flor, H. H. (1971): Current status of the gene for gene concept. Annu. Rev. Phytopatol. 9, 275-296.

Fodor, J., Gullner, G., Ádám, A. L., Barna, B., Kőmíves, T. and Király, Z. (1997): Local and systemic responses of antioxidants to tobacco mosaic virus infection and to salicylic acid. Plant Physiol. 114, 1443-1451.

Forouhar, F., Y. Yang, D. Kumar, Y. Chen, E. Fridman, S. W. Park, Y. Chiang, Acton, T. B., Montelione, G. T., Pichersky, E., Klessig, D. F. and Tong, L. (2005): Crystal structure and biochemical studies identify tobacco SABP2 as a methylsalicylate esterase and

further implicate it in plant innate immunity. Proc. Natl. Acad. Sci. USA 102, 1773-1778.

Gassmann, W. and Bhattacharjee, S. (2012): Effector-triggered immunity signaling: from gene-for-gene pathways to protein-protein interaction networks. Mol. Plant-Microbe Interact. 25, 862-868.

Ghildiyal, M. and Zamore, P. (2009): Small silencing RNAs: An expanding universe. Nature Rev. Genet. 10, 94-108.

Goodman, R. N., Király, Z. and Wood, K. R. (1986): The biochemistry and physiology of plant disease. Univ. Missouri Press, Columbia, MO. 433 p.

Görlach, J., Volrath, S., Knauf-Beiter, G., Hengy, G., Beckhove, U., Kogel, K. H., Oostendorp, M., Staub, T., Ward, E., Kessmann, H. and Ryals, J. (1996): Benzothiadiazole, a novel class of inducers of systemic acquired resistance, activates gene expression and disease resistance in wheat. Plant Cell 8, 629-643.

Hafez, Y. M. and Király, Z. (2003): Role of hydrogen peroxide in symptom expression of barley susceptible and resistant to powdery mildew. Acta Phytopathol. Entomol. Hung. 38, 227-236.

Hafez, Y. M., Fodor, J. and Király, Z. (2004): Establishment of acquired resistance confers reduced levels of superoxide and hydrogen-peroxide in TMV-infected tobacco leaves. Acta Phytopathol. Entomol. Hung. 39, 347-359.

Hafez, Y. M., Király, Z. and Manninger, K. (2009): Hydrogen peroxide has a key role in resistance to leaf rust (*Puccinia triticina*) in several Egyptian and other wheat cultivars. Cereal Res. Commun. Suppl. 37, 161-164.

Hafez, Y. M., Bacsó, R., Király, Z., Künstler, A. and Király, L. (2012): Up-regulation of antioxidants in tobacco by low concentrations of H_2O_2 suppresses necrotic disease symptoms**.** Phytopathology 102, 848-856.

Halliwell, B. and Gutteridge, J. M. C. (1999): Free Radicals in Biology and Medicine. Oxford University Press, Inc., New York.

Hamilton, A. J., Brown, S., Yuanhai, H., Ishiuka, M., Lowe, A., Alpuche Solis, A.-G. and Grierson, D. (1998): A transgene with repeated DNA causes high frequency, post-transcriptional suppression of ACC-oxidase gene expression in tomato. Plant J. 15, 737-746.

Hammerschmidt, R., Nuckles, E. M. and Kuć, J. (1982): Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium*. Physiol. Plant Pathol. 20, 73-82.

Harrach, B. D, Fodor, J., Pogány, M., Preuss, J. and Barna, B. (2008): Antioxidant, ethylene and membrane leakage responses to powdery mildew infection of near-isogenic barley lines with various types of resistance. Eur. J. Plant Pathol. 121, 21-33.

Harrach, B. D, Baltruschat, H., Barna, B., Fodor, J. and Kogel, K. H. (2013): The mutualistic fungus *Piriformospora indica* protects barley roots from a loss of antioxidant capacity caused by the necrotrophic pathogen *Fusarium culmorum*. Mol. Plant-Microbe Interact. 26, 599-605.

Jaskiewicz, M., Conrath, U. and Peterhänsel, C. (2011): Chromatin modification acts as a memory for systemic acquired resistance in the plant stress response. EMBO Rep. 12, 50-55.

Jones, A. M. and Dangl, J. L. (2006): The plant immune system. Nature 444, 323-329.

Kale, S. D., Gu, B., Capelluto, D. G., Dou, D. et al. (2010): External lipid PI3P mediates entry of eukaryotic pathogen effectors into plant and animal host cells. Cell 142, 284-295.

Király, L., Hafez, Y. M., Fodor, J. and Király, Z. (2008): Suppression of tobacco mosaic virus-induced hypersensitive-type necrotisation in tobacco at high temperature is associated with down-regulation of NADPH oxidase and superoxide and stimulation of dehydroascorbate reductase. J. Gen. Virol. 89, 799-808.

Király, L., Künstler, A., Hafez, Y. M., Király, Z. and Bacsó, R. (2013): Heat-induced susceptibility of barley to a biotrophic and a hemibiotrophic pathogen - differential changes in superoxide levels. 11th International Conference on Reactive Oxygen and Nitrogen Species in Plants, Warsaw, Poland. Abstract, in press.

Király, Z., Barna, B. and Érsek, T. (1972): Hypersensitivity as a consequence, not the cause, of plant disease resistance to infection. Nature 239, 215-219.

Király, Z., Barna, B., Kecskés, A. and Fodor, J. (2002): Down-regulation of antioxidative capacity in a transgenic tobacco which fails to develop acquired resistance to necrotization caused by tobacco mosaic virus. Free Rad. Res. 36, 981-991.

Kogel, K.-H., Beckhove, U., Dreschers, J., Munch, S. and Romme, Y. (1994): Acquired resistance in barley. The resistance mechanism induced by 2,6-dichloroisonicotinic acid is a phenocopy of a genetically based mechanism governing race-specific powdery mildew resistance. Plant Physiol. 106, 1269-1277.

Kovalchuk, I., Kovalchuk, O., Kalck, V., Boyko, V., Filkowski, J., Heinlein, M. and Hohn, B. (2003): Pathogen-induced systemic plant signal triggers DNA rearrangements. Nature 423, 760-762.

Levine, A., Tenhaken, R., Dixon, R. and Lamb, C. (1994): H_2O_2 from the oxidative burst orchestrated the plant hypersensitive disease resistance response. Cell 79, 583-593.

Liu, P-P., Bhattacharjee, S., Klessig, D. F. and Moffett, P. (2010): Systemic acquired resistance is induced by R gene-mediated responses independent of cell death. Molec. Plant Pathol. 11, 155-160.

Liu, J., Elmore, J. M., Lin, Z. J. D. and Coaker, G. (2011): A receptor-like cytoplasmic kinase phosphorylates the host target RIN4, leading to the activation of a plant innate immune receptor. Cell Host Microbe 9, 137-146.

Luna, E., Bruce, T. J., Roberts, M. R., Flors, V. and Ton, J. (2012): Next generation systemic acquired resistance. Plant Physiol. 158, 844-853.

Maekawa, T., Kufer, T. A. and Schulze-Lefert, P. (2011): NLR functions in plant and animal immune systems: so far and yet so close. Nature Immunol. 12, 818-826.

Malamy, J., Carr, J. P. Classig, D. F. and Raskin, I. (1990): A likely endogenous signal in the resistance response of tobacco to viral infection. Science, 250, 1002-1004.

Marino, D., Dunand, C., Puppo, A. and Pauly, N. (2012): A burst of plant NADPH oxidases. Trends Plant Sci. 17, 9-15.

Métraux, J-P., Singer, H., Rials, J. et al. (1990): Increase in salicylic acid at the onset of systemic acquired resistance in cucumber. Science 250, 1004-1006.

Molinier, J., Ries, G., Zipfel, C. and Hohn, B. (2006): Transgeneration memory of stress in plants. Nature 442, 1046-1049.

Molnar, A., Melnyk, C. W., Bassett, A., Hardcastle, T. J., Dunn, R. and Baulcombe, D. C. (2010): Small silencing RNAs in plants are mobile and direct epigenetic modification in recipient cells. Science 328, 872-875.

Morel, F., Doussiere, J. and Vignais P. V. (1991): The superoxide-generating oxidase of phagocytic cells. Physiological, molecular and pathological aspects. Eur. J. Biochem. 201, 523-546.

Mou, Z., Fan, W. and Dong, X. (2003): Inducers of plant systemic acquired resistance regulate NPR1 function through redox changes. Cell 113, 935-944.

Mukhtar, M. S., Carvunis, A. R., Dreze, M. N., Epple, P. et. al. (2011): Independently evolved virulence effectors converge onto hubs in a plant immune system network. Science, 333, 596-601.

Napoli, C., Lemieux, C., and Jorgensen, R (1990): Introduction of a chimeric chalcone synthase gene into Petunia results in reversible cosuppression of homologous genes in trans. Plant Cell 2, 279-289.

Navarro, L., Zipfel, C., Rowland, O., Keller, I., Robatzek, S., Boller, T. and Jones, J. D. G. (2004): The transcriptional innate immune response to flg22. Interplay and overlap with Avr gene-dependent defense responses and bacterial pathogenesis. Plant Physiol. 135, 1113-1128.

Netea, M. G., Quintin, J., van der Meer, J. W. (2011): Trained immunity: a memory for innate host defense. Cell Host Microbe 9, 355-361.

Park, S-W., Kaimoyo, E., Kumar, D., Mosher, S. and Klessig, D. F. (2007): Methyl salicylate is a critical mobile signal for plant systemic acquired resistance. Science 318, 113-116.

Pogány, M., von Rad, U., Grün, S. Dongó, A., Pintye, A., Simoneau, P., Bahnweg, G., Kiss, L., Barna, B. and Durner, J. (2009): Dual roles of reactive oxygen species and NADPH oxidase RBOHD in an Arabidopsis-*Alternaria* pathosystem. Plant Physiol. 151, 1459-1475.

Proels, R. K., Oberhollenzer, K., Pathuri, I. P., Hensel, G., Kumlehn, J., Hückelhoven, R. (2010): RBOHF2 of barley is required for normal development of penetration resistance to the parasitic fungus *Blumeria graminis* f. sp. *hordei*. Molec. Plant- Microbe Interact. 23, 1143-1150.

Rast, J. P., Smith, L. C., Loza-Coll, M., Hibino, T. and Litman, G. W. (2006): Genomic insights into the immune system of the sea urchin. Science 314, 952-956.

Ronald, P. C. and Beutler, B. (2010): Plant and animal sensors of conserved microbial signatures. Science 330, 1061-1064.

Ross, A. F. (1961): Systemic acquired resistance induced by localized virus infections in plants. Virology 14, 340-358.

Ruiz-Ferrer, V., and Voinnet, O. (2009): Role of plant small RNAs in biotic stress responses. Annu. Rev. Plant Biol. 60, 485-510.

Samuel, G. (1931): Some experiments on inoculating methods with plant viruses and on local lesions. Ann. Appl. Biol. 18, 494-507.

Schulze-Lefert, P. and Bieri, S. (2005): Recognition at a distance. Science 308, 506-508.

Shah, J. and Zeier, J. (2013): Long-distance communication and signal amplification in systemic acquired resistance. Frontiers Plant Sci. 4, Art. 30.

Shang, J., Xi, D.-H., Yuan, S. et al. (2010): Difference of physiological characters in dark green islands and yellow leaf tissue of *Cucumber mosaic virus* (CMV)-infected *Nicotiana tabacum* leaves. Z. Naturforsch. 65c, 73-78.

Silhavy, D. and Burgyán, J. (2004): Effects and side-effects of viral RNA silencing suppressors on short RNAs. Trends Plant Sci. 9, 57-104.

Spoel, S. H. and Dong, X. (2012): How do plants achieve immunity? Defence without specialized immune cells. Nature Rev. Immunol. 12, 89-100.

Staskawicz, B. J., Ausubel, F. F., Baker, B. J., Ellis, J. G. and Jones, J. D. G. (1995): Molecular genetics of plant disease resistance. Science 268, 661-667.

Strobel, N. E., and Kuć, J. (1995): Chemical and biological inducers of systemic resistance to pathogens protect cucumber and tobacco plants from damage caused by paraquat and cupric chloride. Phytopathology 85, 1306-1310.

Šutić, D. (1965): Vegetative effect of some plants on the curing of plum infected with Sharka (Plum Pox) virus. Zaštita bilja 88, 347-351.

Sziráki, I., Balázs, E. and Király, Z. (1980): Role of different stresses in inducing systemic acquired resistance to TMV and increasing cytokinin level in tobacco. Physiol. Plant Pathol. 16, 277-284.

Szittya, Gy., Molnár, A., Silhavy, D., Hornyik, Cs. and Burgyán, J. (2002): Short defective interfering RNAs of tombusviruses are not targeted but trigger post-transcriptional gene silencing against their helper virus. Plant Cell 14, 359-372.

Tao, Y., Xie, Z., Chen, W., Glazebrook, J., Chang, H-S., Han, B., Zhu, T., Zou, G. and Katagiri, F. (2003): Quantitative nature of Arabidopsis responses during compatible and incompatible interactions with the bacterial pathogen *Pseudomonas syringae*. Plant Cell 15, 317-330.

Torres, M. A., Dangl, J. L. (2005): Functions of the respiratory burst oxidase in biotic interactions, abiotic stress and development. Curr. Opin. Plant Biol. 8, 397-403.

Torres, M. A., Jones, J. D. G. and Dangl, J. L. (2006): Reactive oxygen species signaling in response to pathogens. Plant Physiol. 141, 373-378.

van der Krol, A. R., Mur, L. A., Beld, M., Mol, J. and Stuitje, A. R. (1990): Flavonoid genes in petunia: Addition of a limited number of copies may lead to a suppression of gene expression. Plant Cell 2, 291-299.

Voinnet, O. (2001): RNA silencing as a plant immune system against viruses. Trends Genet. 17, 449-459.

Voinnet, O. (2005): Induction and suppression of RNA silencing: insights from viral infections. Nat. Rev. Gen. 6, 206-220.

Vulić, T., Oparnica, C., Djordjević, B., Kuzmanović, S., Starović, M., Ford, E. and Tošić, M. (2013): Plum sectorial resistance to *Plum pox virus* is graft transmissible. Acta Phytopathol. Entomol. Hung. 48, in press.

Waller, F., Achatz , B., Baltruschat, H., Fodor, J. et al. (2005): The endophytic fungus *Piriformospora indica* reprograms barley to salt-stress tolerance, disease resistance, and higher yield. Proc. Natl. Acad. Sci. USA 102, 13386-13391.

Wang, M-B., Masuta, C.,. Smith, N. A. and Shimura, H. (2012): RNA silencing and plant viral diseases. Mol. Plant-Microbe Interact. 25, 1275-1285.

Ward, H. M. (1902): On the relations between host and parasite in the bromes and their brown rust, *Puccinia dispersa* (Erikss.). Ann. Bot. 16, 233-315.

Waterhouse, P. M., Graham, H. W. and Wang, M. B. (1998): Virus resistance and gene silencing in plants can be induced by simultaneous expression of sense and antisense RNA. Proc. Natl. Acad. Sci. USA 95, 13959-13964.

White, R. F. (1979): Acetylsalicylic acid (aspirin) induces resistance to tobacco mosaic virus in tobacco. Virology 99, 410-412.

Zipfel, C. and Rathjen, J. P. (2008): Plant immunity: AvrPto targets the frontline. Curr. Biol. 18, R218-R220.

Zipfel, C., Robatzek, S., Navarro, L., Oakeley, E. J., Jones, J. D. G., Felix, G and Boller, T. (2004): Bacterial disease resistance in *Arabidopsis* through flagellin perception. Nature 428, 764-767.

Figure Legends

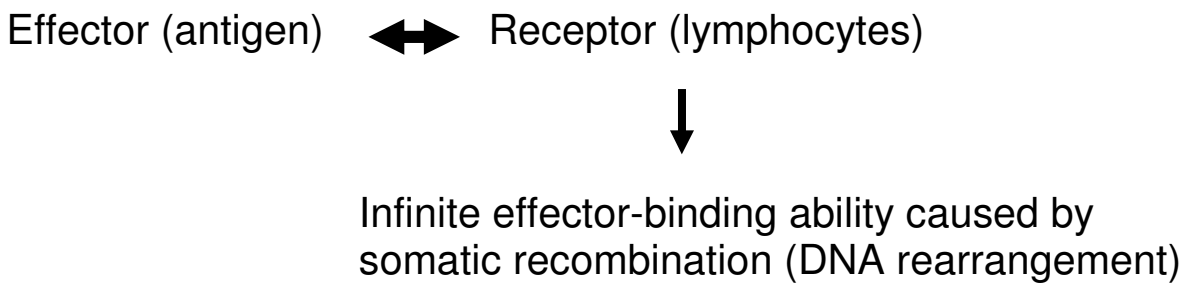
Fig. l. A simplified scheme of animal (human) adaptive immunity.

Fig. 2 The concept of "gene-for-gene resistance" cannot explain the immune diversity of plants.

Fig. 3 Explanation of plant immune diversity: plant R-protein receptors do not directly recognize pathogen effectors as foreign proteins in most host/pathogen combinations that result in resistance.

Fig. 4a, b Association of the "non-host" type of resistance with early superoxide ($O^{2.-}$) accumulation in barley leaves (cv. Botond) and cucumber leaves (cv. Budai csemege) as visualized by nitroblue-tetrazolium chloride (NBT) staining. (a) Left two leaves: inoculated with barley powdery mildew (*Blumeria graminis* f. sp. *hordei*) (susceptible reaction). Right two leaves: inoculated with wheat rust (*Puccinia recondita* f. sp. *tritici*) (non-host resistance). (b) Left two leaves: inoculated with cucumber powdery mildew (*Podosphaera xanthii*) (susceptible reaction). Right two leaves: inoculated with tomato powdery mildew (*Oidium neolycopersici*) (non-host resistance). NBT staining was applied 24 hours after inoculation.

Animal (human) adaptive immunity



Immune diversity

Plant non-adaptive immunity

Effector protein (pathogen) ⟷ Receptor protein (plant)

Concept of “gene-for-gene resistance”

Many effector proteins ——— Only a limited number of R-protein receptors

Immune diversity ???

Indirect recognition of non-self (effector) proteins in plants

Effector: Induces modifications in certain self proteins (phosphorylation, proteolysis)

Receptor (R-protein): Recognizes the effector-modified self protein (not the effector itself)

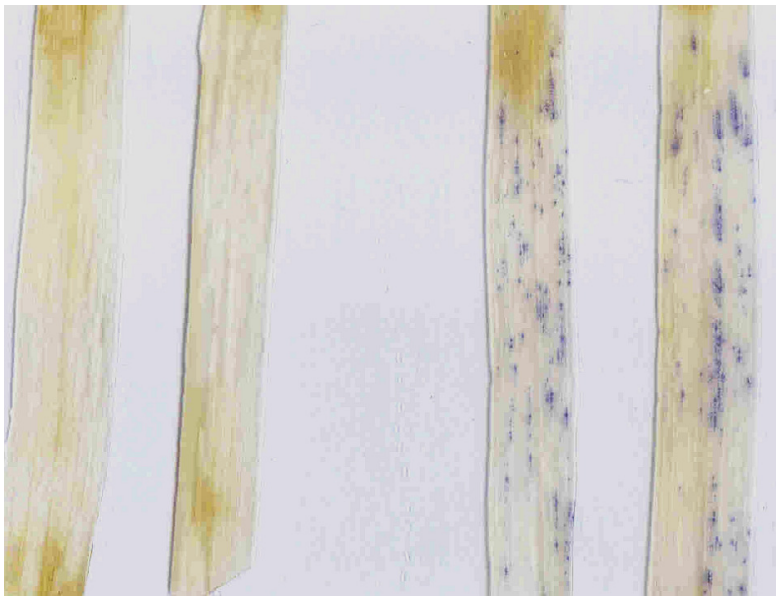
Different effectors can modify the same self protein

One R-protein receptor can indirectly recognize different effectors

↓

Immune diversity

(a)



(b)