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A novel strategy for plant protection: Induced resistance

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Abstract

Most plant protection methods currently applied use toxic chemicals noxious to the environment for pathogen and pest control. Induced resistance exploiting natural defense machinery of plants could be proposed as an alternative, non-conventional and ecologically-friendly approach for plant protection. Its introduction into agricultural practice could minimize the scope of chemical control, thus contributing to the development of sustainable agriculture. Induced resistance can be defined as an increased expression of natural defence mechanisms of plants against various type of pathogens, provoked by a range of factors: pathogens causing hypersensitive necrotic reaction; avirulent or attenuated pathogenic strains; elicitors of pathogenic origin (glucans, proteins, lipids, etc.); abiotic elicitors, including synthetic harmless chemical products, such as 2,6-dichloroisonicotinic acid (INA), b-aminobutyric acid (BABA), benzothiadiazole (BTH), etc. Induced resistance, being based on the expression of latent genetic information present in plants, is not underlied by genome alterations (mutations, introgression of foreign genetic material), this enhancing its biological safety. Molecular bases of induced resistance, involving receptor-elicitor interactions, signal transducing-pathways and SAR gene expression, are discussed.

Key words: localized acquired resistance (LAR), systemic acquired resistance (SAR), induced systemic resistance (ISR), elicitors, SAR genes

Bitki korumada yapay strateji: Direncin arttırımı

Özet

Birçok bitki koruma metodu günümüzde toksik kimyasalların patojen veya böcek koruma amacı ile çevreye uygulanmasıdır. Ancak bunlara alternatif olarak direnç arttırımı ile bitki koruma yapılmakta ve yaygın olmayan ekolojik bir uygulama olarak doğal savunma sağlanmaktadır. Direnç arttırımı veya teşvik edilmesi; bitkilerin değişik patojenlere karşı savunma mekanizmalarında ekspresyonlarını arttırılması olarak tanımlanabilir. Bu patojenler, hipesensitif nekroz reaksiyonları, avirulent veya güçlenmiş patolojik suşlar olabilir. Gecikmiş genetik bilginin bitkilerde ekspresyonunun antması ile genomik değişiklikler altında olmadan (mutasyon, yabancı genin integrasyonu) biyolojik güvenliği arttırmaktadır. Arttırılmış direncin moleküler temeli, reseptör ve kimyasallar arasındaki etkileşimleri, sinyal iletim yolları ve SAR gen ekspresyonu bu derleme yazısında tartışılmaktadır.

Anahtar sözcükler: lokalize gerekli direnç, sistemik gerekli direnç, indüklenmiş sistemik direnç, elisitör, SAR genleri

Introduction

Presently disease control is largely based on the use of fungicides, bactericides and insecticides – chemical compounds toxic to plant invaders, causative agents or vectors of plant diseases. However, the hazardous effect of these chemicals or their degradation products on the environment and human health strongly necessitates the search for new, harmless means of disease control. Since the late 1950s increasing body of evidence on the natural phenomenon of induced resistance has been accumulated, culminating in its successful practical application in the last decade (Kuc, 2001).

The resistance in plants induced by pathogens was first recognized by Ray (1901) and Beauverie (1901). Chester (1930) confirmed those studies, and, by summarizing field observations, supposed that this phenomenon may play an important role in the preservation of plants in nature. Convincing evidences however were obtained only in the 1960s, when reproducible models using tobacco plant were developed (Cruickshank and Mandryk, 1960; Ross, 1961a; Ross, 1961b; Mandryk, 1963). Greenhouse and field experiments in the laboratory of Kuc and co-workers paved the way to the present comprehension of induced resistance as a tool in plant protection (Kuc, 2001), this being supported by numerous authors from around the world (Schönbeck et al., 1993; Kessman et al., 1994; Schneider et al., 1996; Van Loon et al., 1998; Benhamou and Picard, 1999; Tally et al., 1999; Cohen, 2001; Bokshi et al., 2003; Gozzo, 2003; Soylu et al., 2003). Exploiting uniquely the plant potential to combat pathogens, the induced resistance may diminish the use of toxic chemicals for disease control, and thus could be proposed as an alternative, non-conventional, non-biocidal and ecologically-friendly approach for plant protection and hence for sustainable agriculture.

What is induced resistance? Definition, terminology, general models

When a plant is inoculated with a pathogen ("primary inoculation"), and after a time interval is subjected to a secondary ("challenge") inoculation, reduced disease symptoms develop, i.e. the induced plant becomes more resistant than the normal, non-induced plant. Later, stimuli other than pathogens, such as some non-toxic chemicals, were found to be effective at inducing resistance. Thus, the induced resistance can be defined as an **increased expression of natural defense mechanisms of plants** against **different pathogens** provoked by external factors of various type and manifested upon subsequent inoculation. Hence, the low specificity is an inherent character of induced resistance. A very essential trait is that it is based on expression of latent genetic information present in plant, and is not underlied by genome alterations (mutations or introgression of foreign genetic material)-a feature relevant to an important biological safety (Kuc, 1987; Schönbeck et al., 1993; Schneideret al., 1996; Benhamou and Picard, 1999; Kuc, 2001). The induction of resistance in plants is often compared to immunization or vaccination in animals. Although the term "immunization" has been used to denote treatments that enhance the defensive capacity of plants, the correspondence to vaccination in vertebrates is far-fetched: the induced state is by no means specific (absence of antibody formation). Moreover, it is less efficace and durable, with seldom preventing disease from occurring but generally reducing its extent of severity (Hammerschmidt et al., 2001).

The term "induced resistance" (IR) is used synonymously to "acquired resistance" (AR). Depending on the mode of its expression, induced resistance can be systemic (SAR) or local (LAR). As mentioned before, in the early 1960s Ross as a result of his carefully controlled laboratory experiments with tobacco-TMV system coined the terms LAR (Ross, 1961a) and SAR (Ross, 1961b). He inoculated leaves of the cv. Xanthi nc, hypersensitively reacting to TMV, i.e. forming small necrotic lesions following TMV inoculation. The subsequent, "challenge" inoculation of the same leaf after a few days resulted in development of smaller-sized and less numerous lesions, i.e. the disease severity was reduced. In the same system resistance to TMV was also expressed after secondary inoculation of half-leaf, with the opposite half-leaf being previously inoculated with TMV. These phenomena were referred to as LAR (Ross, 1961a). In this series of experiments Ross succeeded also in inducing resistance to TMV in distant upper leaves of tobacco by primary inoculation of lower leaves with the virus, a phenomenon referred to as SAR (Ross, 1961b).

Cruickshank and Mandryk (1960) were the first to report on SAR in tobacco induced by fungi, much more complex and highly structured pathogens than

viruses. Data were presented that SAR against *P.tabacina* was expressed after "challenge" inoculation of upper leaves when the lower leaves or stems of the plants were inoculated 14-21 days ago with this fungus. It is noteworthy that inoculation with *P.tabacina* induced SAR not only to the fungus, but also to TMV (Mandryk, 1963), this pointing to the low specificity of SAR phenomena.

Recently, the term "induced systemic resistance (ISR) was introduced to designate the resistance induced in leaves of plants by inoculation of roots with non-pathogenic rhizobacteria. This novel type of induced resistance was first described in *Arabidopsis* plants, inoculated with the root-colonizing non-pathogenic bacteria *Pseudomonas fluorescens*; leaves of these plant exhibited resistance against the bacterial leaf pathogen *Pseudomonas syringae* pv. *tomato* (Pieterse et al., 1998). Rhizobacteria-mediated ISR has also been demonstrated against fungi, bacteria and viruses in *Arabidopsis*, bean, carnation, cucumber, radish, tobacco and tomato (Van Loon et al., 1998), this confirming the low specificity proper to IR.

In all cases of IR generation of signals is proposed which transmit information from the site of primary treatment to the adjacent tissues (LAR) or to the distant tissues (SAR, ISR) where IR is expressed upon subsequent, "challenge" inoculation. A time interval (lag period) between the primary and "challenge" inoculation is a prerequisite for effective expression of induced resistance. Span of time is necessary for signals to be translocated to non-inoculated tissues and for triggering and development of defense potential in these tissues (Kuc, 1987; Schneider et al., 1996; Benhamou and Picard, 1999; Kuc, 2001).

Inducers of resistance

A multitude of factors are reported to induce resistance in plants: pathogens (fungi, bacteria, viruses) causing hypersensitive necrotic reaction (HR); avirulent and attenuated pathogenic strains; pests (insects, nematodes); elicitors of biotic origin; abiotic elicitors, i.e. chemical products, such as benzothiadiazole (BTH), β-aminobutyric acid (BABA), 2,6-dichloroisonicotinic acid (INA), salicylic acid, inorganic salts, etc. (Kessman et al., 1994; Lyon et al., 1995; Schneider et al., 1996; Benhamou and Picard, 1999; Cohen, 2001; Kuc, 2001).

Pathogen inducers

Numerous experiments have shown that besides TMV and *P.tabacina*, other pathogens are also able to induce SAR in tobacco, such as TNV, PVY, *Thielaviopsis basicola*, *Pseudomonas syringae*. The SAR response in tobacco gives broad-spectrum disease resistance to fungal, bacterial and viral pathogens, namely *Thielaviopsis basicola*, *Phytophthora parasitica, Peronospora tabacina, Pseudomonas syringae, Pseudomonas tabaci, Erysiphe cichoracearum*, TMV, TNV (Schneider et al., 1996).

The extensive work of Kuc and his coworkers enlarged the knowledge on SAR in tobacco and traced the avenues for its practical application. The importance of inoculation procedure for SAR inoculation was demonstrated. When conidia were injected into the stem cambium, the SAR response was linked to severe dwarfing and premature senescence. However, infection external to the cambium leads to an increase in plant weight and leaf number (Tuzun and Kuc, 1985). Interestingly, regenerant plants obtained via callus from leaves of tobacco plants immunized by stem-injection with *P.tabacina* were highly resistant to this pathogen which was demonstrated by both greenhouse and field tests (Kuc, 1987). Abundant data on other pathosystems are available. Thus, stem inoculation of tomato by an avirulent strain of the bacteria *Clavibacter michiganensis ssp. michiganensis* induced long-lasting, high level-protection against the virulent bacterial strain (Griesbach et al., 2000). An avirulent strain of *Pseudomonas syringae* p.v. *pisi* was shown to induce SAR against the fungus *Mycosphaerella pinodes* in pea (Dann and Deverall, 2000). Successful induction of SAR in cucurbits by *Colletetrichum* sp. was demonstrated by Kuc (1987). The hypersensitive necrotic reaction-causing bacterium *Pseudomonas syringae* pv. *syringae* induced SAR against the fungus *Pyricularia oryzae* in rice (Smith and Metraux, 1991).

Induction of SAR by biotic elicitors

A fascinating area of research is the induction of SAR by biotic elicitors, i.e. by pathogen-derived molecules. Biotic elicitors encompass diverse chemical classes (polysaccharides, lipids, proteins, and complexes between them) and are active on various host plants against different pathogens (Lyon et al., 1995; Benhamou and Picard, 1999; Aziz et al., 2003). In tobacco encouraging results are obtained with biotic elicitors of fungal origin named "elicitins".

Elicitins, a small family of highly conserved 10kD secretory holoproteins bearing this generic name, are the first fully characterized proteinaceous fungal elicitors. They are secreted from various species of *Phytophthora* fungi: acidic a-elicitins (capsicein and parasiticein) - from P. *capsici* and P. *parasitica*, respectively, and basic β-elicitins (cryptogein and cinnamomin) - from *P. cryptogea and P. cinnamomi*, respectively. Elicitins were discovered by the group of Bonnet and Ricci and found to induce resistance in tobacco and other plants (Bonnet et al., 1989; Bonnet et al., 1996). Their structure, biological activity, genetical bases and biochemical mechanisms of action are a subject of intensive research (Blein et al., 1991; Viard et al., 1994; Rusterucci et al., 1996; Simon – Plas et al., 1997; Dahan et al., 2001). β-cryptogein belonging to the group of β-elicitins is secreted from *P. cryptogea*, a non-pathogen on tobacco. It produces necroses on leaves of tobacco when applied on both leaves and stems (systemic effect), and induces resistance in the perinecrotic area which is characterized as follows: may be local or systemic; is not established immediately, i.e. requires a lag period; is more or less durable; is non-specific, i.e. is effective against a range of unrelated pathogens, such as *Phytophthora parasitica* var. *nicotianae*, *Botrytis cinerea, Sclerotinia sclerotiorum, Rhizoctonia solani*, and *Erysiphe cichoracearum* (Bonnet et al., 1996; Blancard et al., 1998). The broad spectrum and long duration of β-cryptogein - induced resistance incited the research on possible exploitment of the phenomenon. Resistant transgenic tobacco plants are developed expressing the gene coding for β-cryptogein (Tepfer et al., 1998). Recently, transgenic plants were obtained harboring a fusion between the pathogen - inducible tobacco hsr203J gene promoter and *P. cryptogea* gene encoding β-cryptogein. In non-induced conditions this transgene is silent, and becomes expressed (β-cryptogein is synthesized) only upon pathogen infection (Keller et al., 1999).

The biochemical mechanisms underlying the induction of protection in β-cryptogein treated tobacco involve early events, such as oxidative burst (Rusterucci et al., 1996; Simon-Plas et al., 1997), K^+ efflux, Ca^{2+} influx, alkalinization and increased conductivity of extracellular medium accompanied by acidification of cytoplasm (Blein et al., 1991; Simon-Plas et al., 1997), lipid peroxidation (Schneider et al., 1996) and protein phosphorylation (Viard et al., 1994). The data point to membranes being a primary target for b-cryptogein interaction with the plant interface (Wendehenne et al., 1995) followed by triggering of signal transducing pathways. Evolution of ethylene and phytoalexin biosynthesis were reported to occur in later stages (Blein et al., 1991; Rusterucci et al., 1996). In our study (Edreva et al., 2002) we established induction of peroxidase, b-1,3-glucanase and PR-proteins in β-cryptogein stem-treated tobacco. Defensive functions could be inferred to these molecules. Thus, PO is implicated in the control of the active oxygen species pool, including H_2O_2 which is thought to have a central role in plant signalling (Overney et al., 1998). Moreover, PO/H_2O_2 system is implicated in the regulation of cell wall plasticity by catalyzing lignin biosynthesis and oxidative polymerisation of ferulate and tyrosine residues in cell wall components; this may contribute to cell wall cross-linking and fortification, i.e. to building up of mechanical barrier at the plant interface against potential pathogens (Gaspar et al., 1986; Overney et al., 1998). Recent findings (Kieffer et al., 2000) lend experimental support to this assumption, showing that β-cryptogein applied to tobacco cell suspension cultures induces lose of digestibility and strengthening of cell walls. β-1,3-glucanase is involved in the hydrolysis of fungal cell wall glucans and the release of active fragments eliciting phytoalexin synthesis in plants (Ham et al., 1991). Thus, the enzyme exerts lytic action on pathogens, and may also conduce to the to the formation of toxic barrier against subsequent fungal attack. An impressive body of evidence points to the importance of PR-proteins in plant protection (Viard et al., 1994; Abad et al., 1996). Taken together, the data imply that peroxidase, b-1,3-glucanase and PR-proteins induced in β-cryptogein treated tobacco, acting cooperatively, could contribute to the development of a hostile plant environment to meet forthcoming pathogen invasion.

Chemical inducers

The use of chemicals as inducers of resistance is an area of extensive work aiming at developing new compounds for disease control meeting the requirements for safe application in greenhouse and fields conditions, namely: no direct toxicity to pathogens; no toxicity to plants and animals; no negative effects on plant growth, development and yield; broad spectrum of defense; low loading amount; long lasting protection; low economical cost for

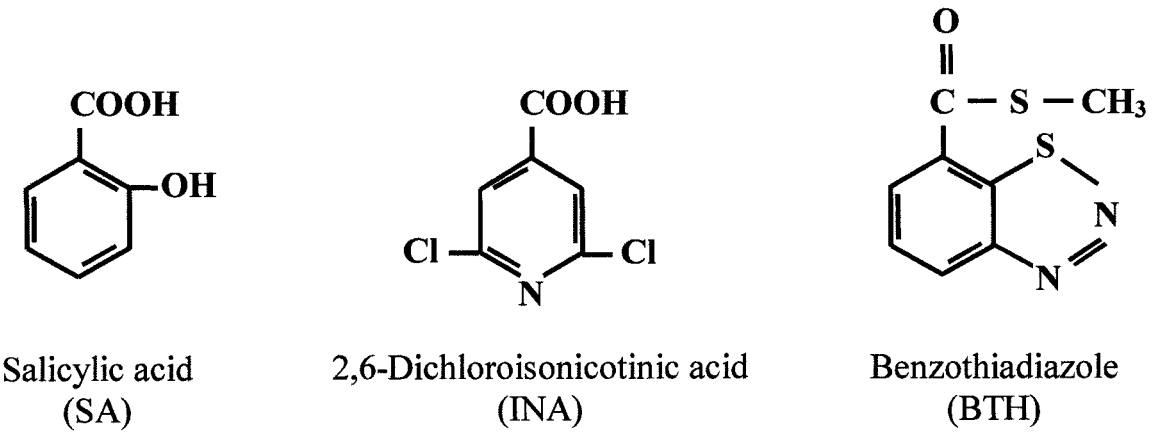

Figure 1: Formulae of commonly used chemical inducers of SAR

farmers; good profit for producers (Kessman et al., 1994; Tally et al., 1999; Kuc, 2001).

Chemical inducers of plant resistance possess quite different mode of action as compared to fungicides and pesticides. The latter products have direct toxic effect on pathogens; are noxious to the environment; have narrow spectrum of defense; ensure shortly lasting protection; are economically costly (Schönbeck et al., 1993; Tally et al., 1999; Kuc, 2001). Thus, the application of chemical inducers of resistance is an exciting new perspective to supplement the classical chemical means of disease control by providing both effective and ecologically-friendly plant protection.

A large array of chemical products are shown to induce SAR in tobacco: salicylic acid, isonicotinic acid (INA), benzothiadiazole (BTH), β-aminobutyric acid (BABA) (Fig. 1), $NaClO_3$, $HgCl_2$, paraquat, polyacrylic acid, SiO_2, etc. Chemically-induced SAR was found to be effective against fungi, bacteria and viruses, namely *Peronospora tabacina, Cercospora nicotianae, Phytophthora parasitica* var. *nicotianae, Pseudomonas syringae* pv. *tabaci*, TMV (Lyon et al., 1995; Strobel and Kuc, 1995; Schneider et al., 1996; Kuc, 2001).

The only commercialized inducer of resistance in tobacco is BTH (full chemical name benzo[1, 2, 3] thiadiazole-7-carbothioic acid S-methyl ester) (Fig. 1). Discovered in 1989 in Ciba-Geigy (Novartis), described by Ryals and coworkers (Friedrich et al., 1996), it was given the trade name BION^{TMV} (in Europe) and Actigard^{TMV} (in USA), and registered and classified as "Reduced Risk Compound" in USA in 1998 (Tally et al., 1999). BTH is the first synthetic non-toxic chemical developed and marketed that functions exclusively by activating the SAR genes. It is supposed to act as a functional analog of salicylic acid entering the signal transducing pathway downstream of it (Friedrich et al., 1996; Wendehenne et al., 1998; Cohen, 2001).

Numerous data are available that BTH is strongly effective against *P. tabacina*, causative agent of blue mold, the most important world-wide distributed tobacco disease. Applied in minimal amounts (around 50 g ha^{-1}), BTH provides field protection lasting until flowering without negative influence on growth, development and yield of tobacco. BTH appears more efficient than metalaxyl, the commonly used blue mold fungicide. It ensures 90% disease reduction on the 17th day after its application versus only 46% for metalaxyl (Tally et al., 1999). It is noteworthy that BTH is an effective inducer of resistance in tobacco not only against fungal pathogens, but also against viruses and bacteria (Tally et al., 1999). Other chemicals, such as BABA and INA, which induced SAR against blue mold, were not commercialized because of side effects, such as low stability and phytotoxicity (Schneider et al., 1996; Tally et al., 1999). BTH was also found to be effective in inducing SAR in wheat (Görlach et al., 1996), pea (Dann and Deverall, 2000), potato (Bokshi et al., 2003), cotton (Colson-Hanks et al., 2000), tomato (Soylu et al., 2003), etc.

BABA was shown to be a unique inducer of plant defense. It is a simple non-protein amino acid which, when sprayed onto the leaf surface or drenched into the soil, induced SAR against various foliar and root pathogens. BABA provided almost complete control

of late blight in tomato plants without being fungitoxic. It has instantaneous action, even when applied post-infection. This feature bears a significant advantage over BTH which has to be applied before the appearance of the disease (Cohen, 2001).

Synergistic effects of BABA and BTH were successfully applied in crop protection. Moreover, synergistic interactions of BABA with fungicides were reported, namely with metalaxyl, controlling blue mold in tobacco, and mancozeb, controlling *Phytophthora infestans* in potato (Baider and Cohen, 2003).

The practical application of chemicals as resistance inducers is mainly based on their systemic effect, i.e. on SAR expression in plants. As mentioned before, an important feature of IR, including SAR, is the low specificity. Thus, SAR is induced by: structurally unrelated compounds (for example, β-aminobutyric acid, isonicotinic acid or phosphates) or unrelated pathogens (fungi, bacteria, viruses); in unrelated plants, i.e. plants belonging to different families; against unrelated pathogens (fungi, bacteria, viruses). The long duration of protection covering the whole vegetation period is a very important feature stimulating the field exploitment of SAR (Kuc, 1987; Schneideret et al., 1996; Kuc, 2001). The successful application of SAR necessitates to determine the exact timing and duration of the lag period between the primary treatment and the secondary ("challenge") inoculation (Schönbeck et al., 1993). In practical terms that assumes a knowledge on the eventual time of invasion of a given pathogen in the concrete situation. Services to warn the appearance of epidemics are a very useful tool to this end applied in many countries; thus, in USA a reliable network for tobacco blue mold warning is functioning (Main et al., 1998).

Mechanisms of SAR

A cascade of molecular and biochemical events underlies the expression of SAR. It is initiated by perception of inducers (pathogens, chemicals) resulting in generation of signal molecules translocated at long distance, and switching on of diverse processes contributing to the development of the defense potential of plants realized upon secondary inoculation. Perception of inducers is effectuated through binding of pathogen-derived molecules (elicitors) or chemical products with receptor sites on plant membranes or cell walls. Generation and nature of signals, the mode of their translocation and interactions are a matter of intensive research. Salicylic acid is commonly recognized as a signal molecule or a prerequisite for signal production in SAR; jasmonic acid and ethylene are involved in signalling upon expression of resistance induced by rhizobacteria (ISR) (Wendehenne et al., 1995, 1998; Schneider et al., 1996; Van Loon et al., 1998; Benhamou and Picard, 1999). Both SAR-mediating signal pathways may act simultaneously, thus providing an additive effect (Van Wees et al., 2000), and enter signal-transducing cascades involving MAP-kinases (Gozzo, 2003). Then interaction with gene promoters or other regulatory factors triggers the expression of the so-called SAR-genes (Ward et al., 1991). The term "SAR-genes" is used to collectively designate this family of nine genes whose expression is correlated with the onset of SAR. For TMV-infected tobacco the SAR-genes code for PR-1 proteins, β-glucanase (PR-2), chitinase (PR-3), hevein-like protein (PR-4), thaumatin-like and osmotin-like proteins (PR-5), PR-1 (basic), basic class III chitinase, acidic class III chitinase, and PR-Q' (Ward et al., 1991). The involvement of PR-proteins in SAR could be related to their characteristic functions. Thus, some PR-proteins exert hydrolytic action (glucanase, chitinase), this suggesting a lytic effect on pathogen cell walls built-up of glucans or chitins (Van Loon et al., 1997; Gozzo, 2003). Members of PR-5 protein family (thaumatin-like, osmotin-like) have membrane-permeabilizing activity due to interaction with membrane components, this leading to conformational changes, dissipation of pH membrane gradient, and formation of pores in membranes (Abad et al., 1996). Systemic induction of lipoxygenase, hydroxyproline rich glycoproteins (HRGP) and callose in non-inoculated leaves may indicate an important role of fatty acid derivatives and cell wall - related structural compounds in SAR. Peroxidase which is also systemically induced is essential for cross-linking and reinforcement of cell walls, the latter being a marker of the induced state. Oxidative burst is proposed to mediate SAR expression (Schneider et al., 1996; Benhamou and Picard, 1999; Kuc, 2001; Gozzo, 2003). It may be assumed that the deployment of SAR-related events allows the plant to respond more rapidly and effectively to a subsequent, "challenge" inoculation.

In addition it is essential to note that application of SAR interferes with the appearance of new strains of pathogens able to overcome the defense of the induced plants. This could be accounted for by the fact that as stated above various components with diverse functions are involved in SAR, i.e. multiple molecular targets for pathogens are available. The situation is opposite to that observed when fungicides or transgenic resistant plants are used. In these cases few targets to be overcome are present which facilitates the arising of new strains pathogenic to plants (Lyon et al., 1995).

Strategies to engineer SAR have been implemented: plants can be manipulated to constitutively express SAR genes (Durand-Tardif and Pelletier, 2003). New concepts however were recently developed (Heil, 2001, 2003), concerning the costs of the constitutive presence of defensive traits in fixed high amounts. Investment in defense is thought to reduce the fitness of plants in enemy-free environment. Phenotypic plasticity, leading to SAR responses, might have evolved mainly to reduce costs, since investments in defense is restricted to situations actually requiring defense. This might have important influences on the evolution of plant defensive traits.

Conclusion

Although not fully understood, induced resistance in plants opens new horizons in plant protection, being a promising tool for ecologically-friendly disease control and sustainable agriculture. It remains a challenge for both fundamental and applied research.

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