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Acquired resistance of rice to the rice blast fungus *Magnaporthe grisea*

P. Schweizer, A. Buchala and J.-P. Métraux

Institut de Biologie Végétale, Fribourg (Suisse)

Abstract. Acquired resistance of rice *Magnaporthe grisea* can be efficiently triggered by different agrochemicals like probenazole or 2,6-dichloroisonicotinic acid (INA), but the molecular basis for this phenomenon is still largely unknown. We are interested in two phases of acquired resistance in INA-treated rice plants: (i) plant conditioning, i.e. built-up of acquired resistance prior to challenge inoculation with *M. grisea* and (ii) plant defence after challenge by the pathogen. Our present approach is focused on evaluating the role of jasmonic acid (JA) as putative product of an activated octadecanoid-signaling pathway in conditioning and defence, and on characterizing gene-expression patterns during the build-up and expression of acquired resistance. For our studies we use a susceptible cultivar (Norin 29) and a race of *M. grisea* highly virulent on this host (race = 283 from Ciba-Geigy).

Several lines of evidence suggest a role of JA in signal transduction in pathogen-attacked rice: (i) JA levels increased in leaves of infected or INA-treated plants; (ii) exogenously applied JA induced accumulation of pathogenesis-related (PR) proteins in rice, and PR-protein induction by INA or crude elicitor from *M. grisea* was blocked by inhibiting JA biosynthesis; (iii) acquired resistance was expressed in new leaves emerging after JA treatment of older leaves; (iv) approximately 80% of pathogen-induced rice mRNAs and 50% of INA-induced mRNAs were also inducible by JA, as determined by 2-dimensional display of *in vitro* translation products.

INA, applied as soil drench, induced resistance in the leaves to *M. grisea*. A combination of INA and JA treatment resulted in almost quantitative resistance of the youngest leaf under severe disease pressure (mean of 30 sporulating lesions per infected control leaf). Overall gene-expression patterns in leaves of INA/JA-treated plants during conditioning and challenge were analysed by 2-dimensional display of *in vitro* translation products of mRNA. The conditioning phase of acquired resistance was characterized by enhanced abundance of 36 mRNAs, which represent approximately 10% of the total number of displayed mRNAs. This suggests a profound change in the physiological state of conditioned leaves. Several conditioning-related mRNAs are not expressed during the compatible interaction with *M. grisea* and therefore represent interesting candidates for novel, defence-related gene products. Challenge inoculation of conditioned plants resulted only in a superimposition of the patterns of conditioning-related and infection-related mRNAs, suggesting that host-gene expression during the conditioning phase might be the resistance-determining factor in rice.

I – General

Inducible defence reactions of plants against pathogen attack has been the object of intensive research efforts in the last twenty to thirty years (see Kessmann *et al.*, 1994, for a recent review). The perspective of new practices in plant protection, using plant immunizing agents rather than conventional pesticides, has stimulated research not only at universities and non-profit research institutions, but also in industrial labs. The advantages of plant immunizing agents over conventional pesticides might be a lower impact on ecosystems, a broader spectrum of target organisms including viral and bacterial diseases, and less problems with pathogen-resistance to these agrochemicals. Although the possible advantages of plant immunizing agents await broad investigation in field practice, the concept of acquired resistance yielded promising results in lab and greenhouse experiments. Moreover, molecular plant-pathogen interactions has become a leading field with respect to signal transduction and gene-regulation in plants.

Since the concept of acquired resistance is based on recognition of chemical signals by plants (naturally-occurring or synthetic molecules), the identification of such biologically active molecules has been a research priority and has led to the identification of a number of extracellular signals called "elicitors" and of a number of endogenous signals or stress metabolites that may play a role in the regulation of defence reactions once a plant has perceived the presence of a pathogen. The most prominent candidates for the latter class of signal molecules are salicylic acid (SA), jasmonic acid (JA) and ethylene (reviewed by Dempsey and Klessig, 1994; Farmer, 1994; Enyedi *et al.*, 1992). SA apparently plays a role in patho-

gen-attacked plants, especially with respect to the manifestation of resistance in plant parts remote from the site of initial attack, referred to as "systemic acquired resistance" (SAR). Ethylene may play a role in both pathogen- and herbivore-attacked (wounded) plants. Originally, JA was thought to play a role exclusively in wounded or herbivore-attacked plants. However, this simple model has recently been questioned because JA can also induce typical responses to pathogen-attack, and JA accumulated in some plant cells in response to fungal elicitor (Gundlach *et al.*, 1992).

While the body of evidence for SAR is mostly derived from a few dicotyledonous model plants, *i.e.* tobacco, cucumber and *Arabidopsis*, the information concerning cereals is more incomplete, and the existence in cereals of SAR, as opposed to local acquired resistance, is still debated. Nevertheless, rice in the field is protected against *Magnaporthe grisea*, the causal agent of the rice blast disease by Probenazole (3-Allyloxy-1,2-benzisothiazol-1,1-dioxide) that is not fungicidal but probably possesses plant immunizing activity (Sekizawa...). With other words, the concept of acquired resistance seems to be applicable to rice in the field. Another agrochemical, 2,6-dichloroisonicotinic acid (INA) was found to improve the resistance of rice against *M. grisea* and *Xanthomonas oryzae* (Métraux *et al.*, 1991). Both compounds seem to transform susceptible rice cultivars into resistant cultivars (referred to as "conditioning" throughout this paper) and the plant reactions to *M. grisea* of these conditioned, formerly susceptible, plants are similar to genetically resistant cultivars (Seguchi *et al.*, 1992; Sekizawa and Mase, 1981).

In this paper, we outline our interest in two aspects of acquired resistance of rice to the rice blast fungus *M. grisea*. Firstly, we are interested in the role of JA in mediating plant defence reactions. Secondly, we are characterizing the phenomenon of conditioning of rice in response to exogenous application of INA and JA with respect to gene-expression patterns.

II – The role of JA in mediating defence reactions in rice

1. Introduction

Since its first description as the fragrant constituent of the essential oil of *Jasminum* (Demole *et al.*, 1962), jasmonic acid (JA) or its methyl ester have been attributed a number of growth-regulating activities. Only very recently, jasmonates or metabolic precursors attracted attention as key molecules of an activated octadecanoid signaling pathway in pathogen- or herbivore-attacked plants (reviewed by Farmer, 1994). The hypothetical role of the octadecanoid signaling pathway in mediating activation of defence reactions is mostly based on results from model systems (*e.g.* Gundlach *et al.*, 1992), and in only a few cases its implication in true pathosystems has been investigated (Cohen *et al.*, 1993; Kogel *et al.*, 1995; Schweizer *et al.*, 1993).

In rice (*Oryza sativa* L.), lipoxygenase induction is correlated with genetically defined as well as acquired resistance to *M. grisea* (Ohta *et al.*, 1991; Babuin and Hofmann, 1993). Moreover, accumulation of anti-fungal, oxygenated C18 fatty acids constitutes one of the defence barriers of rice, these fatty acids being products of a lipoxygenase/ peroxygenase pathway (Kato *et al.*, 1993). Here we provide the first evidence that JA, another product of the lipoxygenase pathway, plays an important role in mediating defence-gene activation and systemic acquired resistance (SAR) in rice.

2. Results

Exogenously applied JA caused coordinate induction of three families of pathogenesis-related (PR-) proteins in rice leaves (Figure 1). The same PR proteins were also accumulating in *M. grisea*-infected leaves, with the exception of a JA-specific, PR2-like protein of approximately 29 kD apparent molecular mass.

To obtain a general picture of gene-expression patterns in rice leaves after treatment of the plants with JA, INA, or after inoculation, mRNA was isolated and translated *in vitro* into ³⁵S-labelled proteins. Two-dimensional display of *in vitro* translation products on polyacrylamide gels revealed a major overlap between the patterns of pathogen-induced and JA-induced rice mRNAs (Table 1). These data might suggest that the majority of pathogenesis-related genes in infected rice are regulated by JA. However, upon

infection with *M. grisea*, no consistent increase in endogenous levels of JA was observed, as determined by combined GC-MS analysis of a methylated fraction of free fatty acids.

Table 1. Acquired resistance in rice to *M. grisea* induced by JA

Treatment	Leaf	Lesions/leaf ^a	% Protection	n ^b
Control ^c	3	11.9 ± 6.1	-	120
JA ^d	3	11.1 ± 8.6	12	128
Control	4	30.3 ± 8.6	-	120
JA	4	16.5 ± 8.5	48	128

a. Two and three d after the treatment leaf 3 (local) and leaf 4 (systemic), respectively, were challenge inoculated with 2×10^5 spores/ml. Five days after challenge, the number of acute, sporulating lesions was counted. Mean ± SD from 3 independent experiments.

b. Number of analysed plants.

c. Leaf 3 was sprayed with ethanol (the solvent for JA).

d. Leaf 3 was sprayed with 100 ppm JA in ethanol.

Exogenously applied JA did not protect the treated leaf 3 but caused SAR in the younger leaf 4 that was emerging after the treatment (Table 2). Although the systemic protection was only partial, it was statistically significant (Wilcoxon test; $p \leq 0.05$). These data may indicate that JA requires the presence of one or several other signals for activation of the full spectrum of defence responses but that JA alone is sufficient for generation of a systemic signal leading to SAR in other parts of the plant.

Table 2. Summary of the *in vitro* translation data with mRNA from inoculated, INA-treated or JA-treated plants

Treatment	induced mRNAs ^a	Overlap with JA spray
<i>M. grisea</i> ^b	27	22
JA spray ^c	75	—

a. Each *in vitro* translation product on the two-dimensional gels was assumed to reflect the abundance, relative to the controls of one mRNA.

b. RNA was isolated 12 and 24 h, respectively, after inoculation with 5×10^5 spores/ml. A compilation of the *in vitro* translation patterns observed at the two time points is shown.

c. RNA was isolated 12 h after spray treatment with 1000 ppm JA.

3. Conclusion

There is circumstantial evidence for a role of JA in mediating defence reactions in pathogen-attacked rice. This is the first report on a major impact of JA on a plant-pathogen interaction. However, conclusive evidence for such a role will have to await results from rice plants with altered endogenous levels of JA or altered sensitivity to JA. Such an approach is currently being undertaken in our lab by using inhibitors of JA biosynthesis.

III – The conditioning phenomenon in rice

1. Introduction

In rice, conditioning by chemical or biological inducers of resistance was not necessarily accompanied by induction of defence reactions prior to challenge inoculation by *M. grisea* (Seguchi *et al.*, 1992; Sekizawa and Mase, 1981; Smith and Métraux, 1991). This means that the acquired resistance was not based on defence reactions already activated in advance of fungal attack. However, challenge inoculation of such conditioned plants led to more pronounced defence reactions, compared to compatible, infected controls. This raises the questions about the mechanism leading to a higher degree of resistance in response to the perception of the inducing molecule or organism.

We therefore asked the question whether conditioning in rice after INA-treatment through the roots and after JA-treatment by foliar spray was characterized by changes in the gene-expression patterns in the non-treated leaf 4.

2. Results

A combined treatment of young rice plants with 10 ppm INA as soil drench and with 100 ppm JA applied as topical spray on leaf 3 induced a high degree of resistance in leaf 3 (sprayed with JA) and in leaf 4 (emerging after JA spray of leaf 3) (Table 3). Protection obtained by the combined treatment was higher than protection obtained by 10 ppm INA alone. The same treatment did not induce proteins belonging to the families of PR1, PR2 or PR3. Upon challenge inoculation of plants conditioned by the above mentioned combination of INA/JA, PR1 proteins accumulated 24 h earlier than in the susceptible control. In contrast, no change in the induction kinetics of PR2 or PR3 was observed. Generally, the correlation of induction of PR proteins with acquired resistance was poor. For instance, no local protection was observed by JA spray on leaf 3 despite induction of PR proteins in this leaf (Figure 1 and data not shown).

Table 3. Acquired resistance in rice to *M. grisea* after a combined treatment with INA and JA

Treatment	Leaf	Lesions/leaf ^a	% Protection	n ^b
Control ^c	3	11.9 ± 6.1	—	120
INA/JA ^d	3	1.1 ± 0.6	91	120
Control	4	30.3 ± 8.6	—	120
INA/JA	4	3.9 ± 1.1	87	120

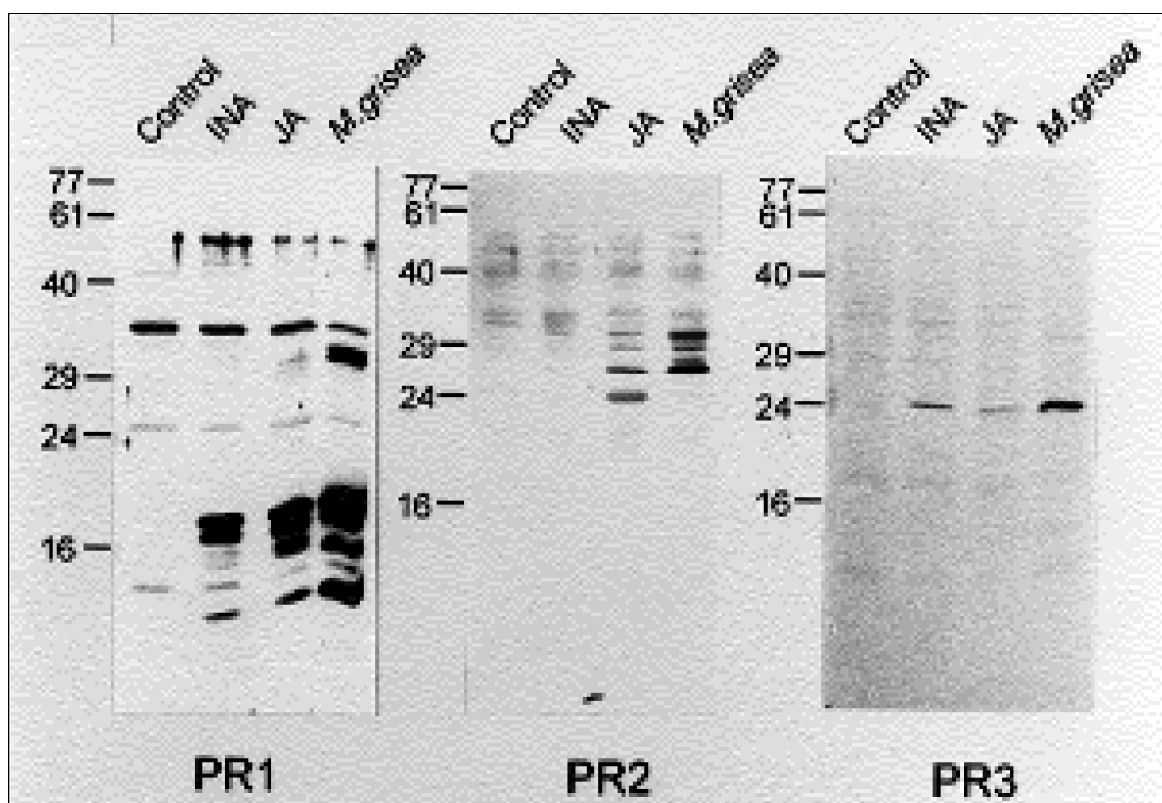
a. Two and three d after the treatment leaf 3 (local) and leaf 4 (systemic), respectively, were challenge inoculated with 2×10^5 spores/ml. Five days after challenge, the number of acute, sporulating lesions was counted. Mean ± SD from 3 independent experiments.

b. Number of analysed plants.

c. Plants were sprayed with ethanol on leaf 3.

d. Plants were treated with 10 ppm INA by soil drench, in combination with spray application of 100 ppm JA in ethanol on leaf 3.

Figure 1. Coordinate induction by JA of three families of pathogenesis-related (PR-) proteins in rice leaves



Acid soluble (pH 2.8) proteins were isolated 3 d after spray treatment with 1 mg/ml JA, drench application of 0.1 mg/ml INA, or inoculation with *M. grisea* (5×10^5 spores/ml). PR1, PR2 and PR3 proteins were detected by using antisera against the major basic PR1 protein from tomato, a PR2 protein (class I) from tobacco, and a PR3 protein from barley, respectively

Two-dimensional display of *in vitro* translation products, each one representing an mRNA species, revealed a considerable number of mRNAs with enhanced abundance in conditioned, non-inoculated leaves as compared to control leaves. The conditioning-related mRNAs accounted for approximately 10% of the

total number of displayed mRNAs (36/400). There was considerable overlap between the patterns of conditioning-related and JA-induced mRNAs, although the mRNA was isolated from leaf 4 that was not treated with JA. This suggests that JA also plays a role in the regulation of conditioning-related rice genes. The overlap between conditioning-related and pathogenesis-related mRNAs from inoculated leaves was partial.

3. Conclusion

The results from analysis of the overall gene-expression patterns suggest a profound change in gene expression of conditioned leaves. Several conditioning-related mRNAs are not expressed during the compatible interaction with *M. grisea* and therefore represent interesting candidates for gene products that may be causally related to establishment of acquired resistance. Challenge inoculation of conditioned plants resulted only in a superimposition of the patterns of conditioning-related and infection-related mRNAs (data not shown), suggesting that hostgene expression during the conditioning phase might be the resistance-determining factor in rice.

Table 4. Summary of the *in vitro* translation data with RNA from conditioned, inoculated or JA-treated plants

Treatment	induced mRNAs	Overlap with	
		Conditioning	JA spray
<i>M. grisea</i> ^a	27	11	22
Conditioning ^b	36	—	25
JA spray ^c	75	—	—

a. RNA was isolated 12 and 24 h, respectively, after inoculation with 5×10^5 spores/ml. A compilation of the *in vitro* translation patterns observed at the two time points is shown.

b. RNA was isolated from leaf 4 48 and 72 h, respectively, after a combined soil-drench application of 1 ppm INA and spray application of 100 ppm JA on leaf 3. A compilation of the *in vitro* translation patterns observed at the two time points is shown.

c. RNA was isolated 12 h after spray treatment with 1000 ppm JA.

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