Vitis **40** (1), 5–10 (2001)

Methyl jasmonate induces a hypersensitive-like response of grapevine in the absence of avirulent pathogens

V. Repka, Irma Fischerová and Katarína Silhárová

Laboratory of Molecular Biology and Virology, Research Institute of Viticulture and Enology (CRIVE), Bratislava, Slovak Republic

Summary

Methyl jasmonate (MeJA), a common plant secondary compound, when applied to the surface of grapevine leaves, caused the formation of lesions that mimic a typical hypersensitive response. Sustained exposure of grapevines to 50 μM MeJA provoked tissue damage, stimulated salicylic acid production, and expression of defense-related genes. Besides these local responses, after several days systemic expression of defense-related genes was induced as well. Thus, grapevine cells that perceived MeJA generated a cascade of events acting at local, short and long distances and causing the coordinated expression of specific defense responses with a timing and magnitude similar to the hypersensitive response against pathogens. MeJA represents a powerful tool to investigate the signals and their respective pathways involved in mechanism of induced disease resistance of grapevine.

K e y w o r d s : Vitis vinifera L., Limberger, elicitor, defense, Western blotting, Northern blotting, salicylic acid, PR-proteins, PR-genes.

Introduction

Jasmonic acid (JA) and its methyl ester (methyl jasmonate, MeJA), collectively named "jasmonates", are lipid-derived compounds widely distributed in the plant kingdom. Based on the biosynthetic pathway elucidated by Vick and Zimmerman (1983), JA and MeJA are formed from linolenic acid (LA) via sequential action of a lipoxygenase (LOX), an allene oxide synthase (AOS), and an allene oxide cyclase (AOC) resulting in the formation of 12-oxophytodienoic acid (OPDA). This octadecanoid (C18) compound is subsequently modified by a reductase and three steps of β-oxidation lead to JA. Jasmonic acid can be catabolized to form MeJA and numerous others conjugates and catabolites that may have a plethora of biological activities (Hamberg and Gardner 1992).

Jasmonates affect a variety of physiological processes, including tuber formation (Koda 1992), root growth (Staswick *et al.* 1992), tendril coiling (Weiler *et al.* 1993), senescence of leaves and stomatal opening (Sembdner and Parthier 1993). Other research described roles for jasmonates in vegetative and fruit development and pollen viability (Creelman and Mullet 1997). The application of JA, MeJA, or various external stimuli such as wounding (O´Donnel *et al.* 1996), burning (Herde *et al*. 1996), UV light (Conconi *et al.* 1996), oligosaccharides (Doares *et al*. 1996), or osmotic stress (Lehmann *et al*. 1995) leads to an endogenous increase of jasmonates. Many of these responses were accompanied by an up-regulation and down-regulation of the expression of specific genes. Therefore, jasmonates can act as a "master switch" (Wasternack and Parthier 1997).

The jasmonates have chemical structures similar to eicosanoids, leukotrienes, and prostaglandins. Since all these compounds mediate localized stress responses in animal cells it was suggested that an additional role for jasmonates might be the mediation of the plant response to stress such as herbivore and pathogen attack (Ryan 1992). Evidence that jasmonate signaling is involved in protection against insects in plants is now well established. Proteinase inhibitor genes are activated by exogenously applied jasmonate (Farmer and Ryan 1992). More directly, mutants affecting jasmonate accumulation exhibit decreased resistance to insect attack (Howe *et al*. 1996). Unfortunately, the evidence that jasmonate signaling is directly involved in the plant defense against microorganisms has been less direct. Exogenous jasmonate induces genes associated with phytoalexin biosynthesis (Choi *et al*. 1994), antifungal thionins (Andresen *et al*. 1992) and osmotin (Xu *et al*. 1994), as well as different enzymes involved in plant defense reactions such as chalcone synthase (CHS) and phenylalanine ammonia lyase (PAL) (Creelman *et al*. 1992, Gundlach *et al*. 1992). The role of JA and MeJA in the induction of systemic acquired resistance (SAR) is also still unclear. However, it has been proposed that these molecules act as secondary messengers in the SAR system, in analogy to the systemic response induced by wounding (Ryan 1992). Moreover, recent studies have demonstrated that jasmonates are important for the induction of nonspecific disease resistance through signaling pathways that are distinct from the classical SAR response pathway regulated by SA (Pieterse *et al*. 1996, Van Wees *et al*. 1997). Other reports on the induction of resistance in different pathosystems by jasmonate are rather contradictory (Schweizer *et al*. 1993, Kogel *et al*. 1994, Mitchell and Walters 1995). It has to be kept in mind that studies on the induction of resistance by JA and MeJA are restricted to herbaceous plants. To our knowledge, there is lack of evidence on the putative role of jasmonates in activating and/or modulating defense responses in woody plants. Thus, for the first time we report here that application of MeJA induces a hypersensitive reaction-like response

Correspondence to: Dr. V. Repka, Laboratory of Molecular Biology and Virology, Research Institute of Viticulture and Enology (CRIVE) Matú skova 25, SK-833 11 Bratislava, Slovakia. Fax: +421-75477-5436. E-mail: repka@crive.axonpro.sk

accompanied by a defense gene expression and SA accumulation in grapevine leaves.

Material and Methods

Plant material and cell cultures: Grapevine (*Vitis vinifera* L. cv. Limberger, covar. *orientalis*) plants were grown in a growth chamber at 28 ± 1 °C (RH 60 %) with a 14 h light period (130 W m^{-2}). Two-month- and/or two-week-old plants were used for experiments.

Preparation of elicitors: Methyl jasmonate (50 μM MeJA, Duchefa, Haarlem, The Netherlands) was prepared from a stock solution in ethanol (10 % final concentration). 10 % ethanol was used as control. The fungal cell wall elicitor was prepared from grey mold (*Botrytis cinerea* PERS. *et* FRIES, a grapevine isolate) according to the protocol of MALOLEPSZA and URBANEK (1994). The elicitor was used at a final concentration of 2.4 μg·ml^{-1} of glucose equivalents (2.2 μg·ml^{-1} of protein).

Preparation and analysis of protein extracts: To prepare extracts from control and treated plant material, the leaves were mixed with 5 volumes (w/v) of ice-cold TRISEPAC extraction buffer (50 mM Tris-HCl, pH 8.0, 500 mM sucrose, 1 mM EDTA, 0.2 % insoluble PVP, 6 mM ascorbic acid and 0.1 % cysteine) and homogenized using a motor-driven Diax 900 homogenizer (Heidolph, Schwabach, Germany) for 5 min at maximum speed. Homogenates were centrifuged at 20,000 *g* for 10 min at 4 °C, concentrated using Centriprep-3 miniconcentrators (Amicon, Witten, Germany) and stored at -20 °C until further use. The protein concentration in the extracts was determined colorimetrically according to BRADFORD (1976), using BSA as standard. Peroxidase activity was assayed in 1-ml volumes at 25 °C in a UV/VIS spectrophotometer (model UV-1601, Shimadzu, Tokyo, Japan). For measurement of guaiacol peroxidase (PRX) activity the previously published protocol of REPKA and SLOVÁKOVÁ (1994) was used.

Analytical SDS-PAGE: SDS-PAGE was performed by the method of LAEMMLI (1970) with a 12.5 % resolving gel and a 4 % stacking gel in a Miniprotean II apparatus (Bio-Rad, Hercules, USA). Each lane of the gel was loaded with equal amounts of protein and the gel was silver-stained by the procedure of BLUM *et al*. (1987). Prestained size markers (Novex, San Diego, USA) were used to calculate the mass of the protein of interest.

Immunoblotting and SIBA-ECL test: For immunodetection proteins were transferred from other gels onto a nitrocellulose membrane (PROTRAN BA-85, Schleicher & Schüll, Dassel, Germany) in a Bio-Rad blotting apparatus. The transfer was carried out at 10 V for 24 h in 40 mM Na-phosphate (pH 6.5). After electrophoretic transfer of the proteins, the blots were immunoprocessed basically according to REPKA (1999). The immunospecific signal was developed with the chemiluminescent substrate from the SuperSignal West Pico kit (Pierce, Rockford, USA) and visualized using the ECL-Hyperfilm (Amersham, Buckinghamshire, UK).

A MeJA-stimulated expression of the PR-2 (β-1,3-glucanase) and PR-9 (peroxidase) gene products was estimated following the SIBA-ECL protocol (REPKA *et al*. 1996). Individual samples equivalent to 2.5 μg of total proteins were slotted onto a PROTRAN BA-85 nitrocellulose membrane using the Slot Blot PR648 apparatus (Hoefer, San Francisco, USA). After loading the samples, the membrane was immunodecorated either with anti-tobacco PR-2a serum or with anti-cucumber peroxidase (PRX) serum, both diluted 1:1000. Serological reactions were detected by chemiluminescence as described for immunoblots.

Total RNA extraction, Northern slot blots: Immediately after the elicitation protocol was completed, leaves were submerged in RNA*later* tissue storage reagent (Ambion, Austin, USA) and stored at -20 °C without jeopardizing the quality and quantity of RNA. For RNA extraction, approximately 1 g of leaves stored in RNA*later* was directly homogenized using an RNA*WIZ* isolation reagent as directed by the manufacturer (Ambion, Austin, USA). Absorbance at 260 and 280 nm was used to determine purity and concentration of RNA. To confirm that RNA had not been degraded and that equivalent samples were loaded in each slot, the Geno*Gold* total nucleic acid staining reagent (Vector Labs, Burlingame, USA) was used following instructions of the manufacturer. For RNA slot blot analysis, aliquots containing 5 μg of RNA were denatured in 2.5 M formaldehyde, 6 x SSPE (6 x SSPE = 900 mM NaCl, 60 mM NaH_2PO_4, 6 mM EDTA, pH 7.7) at 60 °C for 1 h in a total volume of 50 μl. Samples were immediately applied to a prewetted (water then 6 x SSPE) NYTRAN N-13 membrane (Schleicher & Schüll, Dassel, Germany) using a slot blot apparatus PR 648 (Hoefer Scientific, San Francisco, USA). Prehybridization (17 h at 42 °C) was conducted in a nuclease-free BLOTTO-*MF* solution (120 mM Tris-HCl, pH 7.4, 8 mM EDTA, 600 mM NaCl, 1 % non-fat dried milk powder, 50 % deionized formamide, 1 % SDS) according to MONSTEIN *et al*. (1992). A synthetic oligonucleotide (20-mer, MWG Biotech, Ebersberg, Germany) modified at the 5´end with biotin was used as the PR-9 (prx)-RNA complementary probe designed from the nucleotide sequence of the coding strand for the peptide HFHDCFV (5´-CATTTTCACGATTGTTTCGT-3´; HENRISSAT *et al*. 1990). The pCI121 insert (approximately 700 bp) encoding extracellular, acidic β-1,3-glucanase (PR-2, MEMELINK *et al*. 1990) was gel purified and psoralen-biotin-labelled using a BrightStar nonisotopic labeling kit (Ambion, Austin, USA). Hybridization of the probes (100 ng cm^{-3}) to the membrane was carried out in BLOTTO-MF solution at 46 °C for 24 h. Posthybridization stringency washes consisted of three 15 min washes in 6 x SSPE, 0.1 % SDS at 25 °C, one 15 min wash in 6 x SSPE, 0.1 % SDS at 46 °C, and one 15 min wash in 6 x SSPE at 46 °C. Membranes hybridized with biotinylated probes were incubated at 25 °C for 1 h in 5 % BLOTTO-TEN buffer (REPKA and SLOVÁKOVÁ 1994). Membranes were then incubated in a solution of horse radish peroxidase-conjugated avidin D (2.5 μg cm^{-3}, Vector Labs, Burlingame, USA) in TBS buffer (100 mM Tris-HCl, pH 7.5, 100 mM NaCl, 2 mM $MgCl_2$, 0.05 % Tween 20) for 1 h. Finally, the membranes were washed by three 10 min washes in TBS buffer. The signal was visualized using enhanced chemiluminescence as described for Western blots.

Salicylic acid (SA) analysis: Leaf tissues (1 g) from control (untreated) and MeJA-elicited plants were

collected and ground in 2 ml of ice-cold 90 % methanol directly in microfuge tubes. After centrifugation (5 min at 10,000 *g*), the residue was extracted again with 100 % methanol (0.5 ml) and the combined extract was used for quantification of free SA. The sample to be analyzed for total SA (SA plus SA-glucoside) was partitioned into ethyl acetate-cyclopentane-2-propanol (100: 99 : 1, v/v/v) and further processed according to ENYEDI *et al*. (1992). Separation and quantification of SA were carried out at room temperature using an HPLC system (model LC-10A, Shimadzu, Tokyo, Japan) equipped with a UV/VIS detector under the following conditions: column, ChromSphere 5 Poly RP-18, 5 μ (3 x 150 mm, Varian Chrompack Int., Bergen op Zoom, The Netherlands); mobile phase: 23 % methanol in 20 mM sodium acetate, pH 5.0 isocratic; flow rate: 1 ml min^{-1}; detection at 230 nm. The limit of detection for free SA was 40 ng·20 μl^{-1}. Total SA was separated under the same condition except that the column used was μBondasphere 300 RP-18, 5 μ , (3.9 x 150 mm, Waters, Milford, USA), the mobile phase was 90 % acetonitrile in 20 mM sodium acetate, pH 5.0 and SA was detected by fluorescence (excitation 313 nm, emission 405 nm) using a scanning fluorescence detector (Waters, Milford, USA). The limit of detection for total SA was 24 ng·50 μl^{-1}.

Biological assays: Necrosis-inducing activity of MeJA was assayed on 2-month-old grapevine plants grown in a glasshouse under controlled conditions. Routinely, 10 μl drops of MeJA (50 μM in 10 % (v/v) ethanol), 10 % ethanol or sterile distilled water were applied on intact leaves. Necrosis-inducing activity of the elicitor prepared from *Botrytis cinerea* was also tested in similar conditions on 1-month-old plants of *Nicotiana tabacum* L. cv. White Burley. The accumulation of compounds derived from the phenylpropanoid pathway which is highly stimulated during HR was demonstrated using observation of epifluorescence under UV light.

Results and Discussion

Phenotypic consequences of MeJA treatment: Application of drops containing 50 μM MeJA on leaves of grapevines resulted in rapid macroscopic changes. The first symptoms appeared 1-2 h after the treatment. The tissue became necrotic within about 4-8 h. Browning and complete dryness were observed after about 24 h of incubation (Fig. 1 A). The shape of induced necrosis was not found to extend the infiltrated zone suggesting that the elicitor remained at the site of application. A similar observation was documented for elicitors prepared from *Botrytis cinerea* mycelium (Fig. 1 B). Leaves were treated in a similar way either with 10 % ethanol (EtOH) or sterile distilled water as a negative control. No visible symptoms were observed (Fig. 1 C and D).

Further experiments revealed that the appearance and severity of symptoms depend neither on the developmental stage of the leaf (Fig. 2) nor the cultivar used (data not shown).

Observation of grapevine leaves treated with MeJA under UV light revealed a strong epifluorescence located in cells surrounding the observed necrotic lesions (Fig. 3, top).

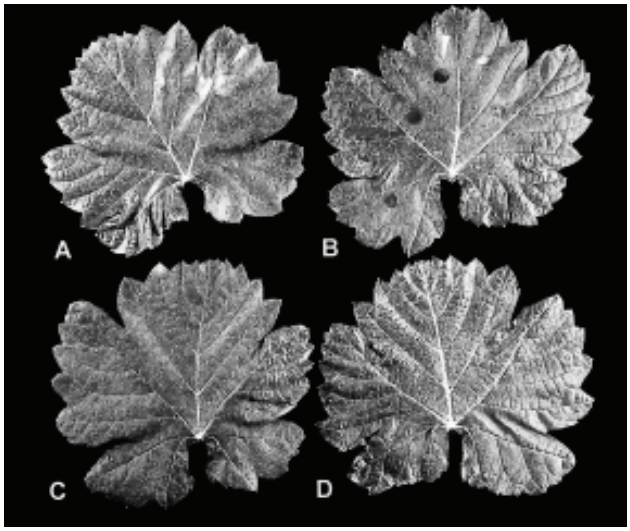

Fig. 1: Phenotypic appearance of *V. vinifera* L. cv. Limberger, 6 d after foliar inoculation of 50 μM MeJA (**A**), *Botrytis cinerea* elicitor (**B**), 10 % EtOH (**C**), and sterile distilled water (**D**) treatment.

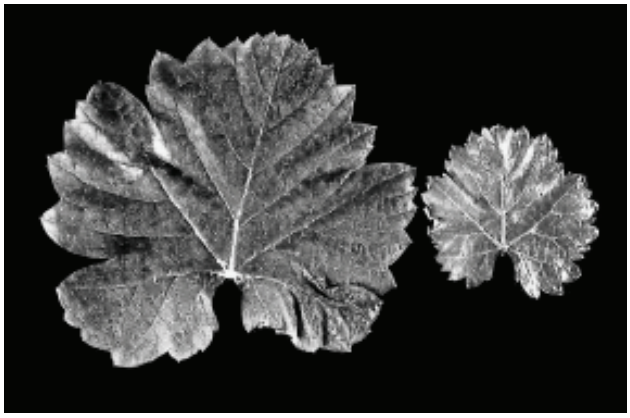

Fig. 2: Symptoms induced in old and young grapevine leaves by MeJA. The left part of 2-month-old (left) and 2-week-old (right) leaves was treated with a few drops of 50 μM MeJA. Water and 10 % EtOH (controls) were applied to the opposite (right) part of the same leaves. Symptoms were photographed 6 d after the onset of treatment.

Similar symptoms occur on tobacco leaves during the hypersensitive response to elicitor from *Botrytis cinerea* (Fig. 3, bottom). Thus in both cases, the epifluorescence is due to the accumulation of compounds derived from the phenylpropanoid pathway which is highly stimulated during HR (FRITIG *et al*. 1972).

Peroxidase activity and PR-9-like PRX accumulation in MeJA-treated grapevines: Prior phenotypic and macroscopic observations revealed that lesions caused by MeJA on grapevine leaves were strikingly similar to the necrotic lesions induced during an HR response caused by inoculation of cucumber with tobacco necrosis virus (TNV; REPKA *et al.* 1993). Consequently, the question arises: does MeJA affect the enzymatic activities known to be induced in response to pathogen attack? Peroxidase (PRX) activities were measured in grapevine leaves after treatment with 50 μM MeJA. The results of these experiments, in which inoculation with sterile distilled water or with 10 % EtOH served as negative controls, are shown in Fig .4. Grapevine leaves were treated with 50 μM MeJA and

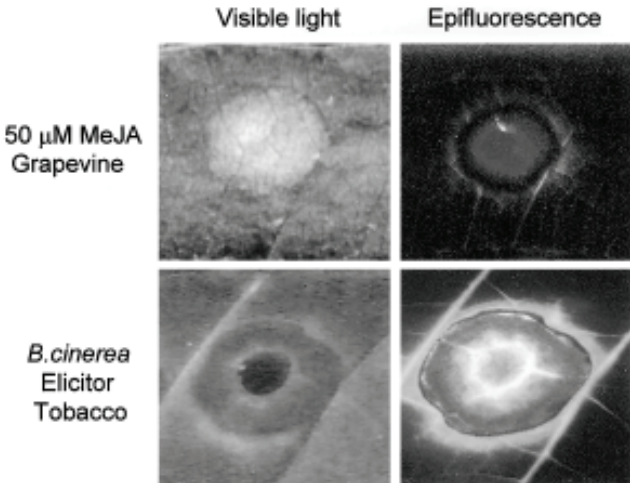

Fig. 3: Macroscopic characterization of the necrotic lesions induced by 50 μM MeJA (top) or by the elicitor isolated from *Botrytis cinerea* mycelium (bottom). The leaf sections were observed under visible light (left row panels) or under UV light (right row panels) 6 d after treatment. Note that the blue fluorescence corresponds to an accumulation of phenylpropanoid-derived compounds.

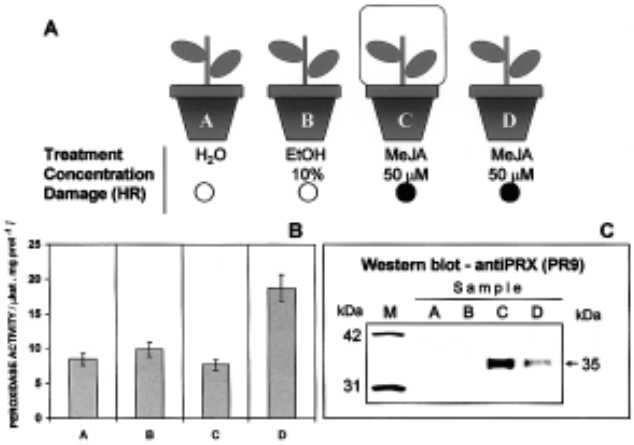

Fig. 4: Accumulation of total soluble PRX (peroxidase) and expression of PR-9 PRX in leaves after MeJA treatment. Two-month-old grapevines were inoculated with a few drops of 50 μM MeJA, then covered with an air-tight plastic chamber or remained uncovered. Control plants were treated either with sterile distilled water or with 10 % EtOH. The presence of leaf damage at the time of harvest is indicated (**A**); 6 d later leaf sap from control and MeJA-treated plants was analysed for total soluble PRX activity (**B**) and PR-9 specific expression (**C**). The immunopositive signal was detected using the chemiluminescence substrate.

then some plants were covered with an air-tight Magenta container or remained uncovered. No difference in symptom appearance and severity was observed in either plants (Fig. 4 A). In comparison with both controls, a nearly 2.5-fold increase in the level of total soluble PRX was detected 6 d after treatment of plants cultivated on air. In contrast to this, PRX activity in covered plants decreased slightly at the same time (Fig. 4 B). We performed Western blot analysis to identify the defense-related PRX gene expressed in control and MeJA-treated plants. Cucumber anti-PR-9 recognizes a specific grapevine homolog (M_r 35,000, Fig. 4 C) shown to be a peroxidase on activity-stained gel (data not shown). A 7-fold increase of immune specific signal could be detected in MeJA-treated plants covered with a Magenta container when compared to uncovered ones. Such a stimulation of grapevine PR-9-like protein could be explained by the fact that MeJA is a highly volatile molecule (FARMER and RYAN 1990) with an almost identical activity in vapor and liquid phase (CREELMAN and MULLET 1997). Thus, the covering of plants treated with MeJA could have assisted to a synergistic effect of both phases. This statement is consistent with earlier results of FARMER and RYAN (1990) who noted that the presence of increasing levels of volatile MeJA in different air-tight chambers resulted in the synthesis and accumulation of proteinase inhibitors I and II in a dose dependent manner.

Local and systemic PR-protein expression: A solution of MeJA was applied on grapevine leaves at a concentration of 0.05 to 50 μM to asses whether PR-proteins are accumulated by a concentration dependent manner. The tobacco anti-PR-2 (acidic β-1,3-glucanase) and cucumber anti-PR-9 (acidic PRX) sera were used to probe the blots. We found that the PR-2 accumulation strongly depends on the concentration of MeJA (Fig. 5 A). On the other hand, the concentration of 0.05 μM was sufficient to induce the accumulation of PR-9 protein as presented in Fig. 5 A, the induced accumulation of this protein was not found to increase, even if concentrations >0.5 μM were applied.

Accumulation of defense-related mRNAs was analyzed by slot-blot hybridization. The tobacco PR-2 and cucumber PR-9 gene specific probes are used as molecular markers for local and systemic expression of their grapevine homologs. Treatment of grapevine leaves with 50 μM MeJA strongly stimulated expression of both PR-genes whereas none of the control plants showed PR-2 or PR-9 mRNA expression (Fig. 5 B, lane L). Interestingly, induction of PR-2 and PR-9 mRNA expression was observed not only in locally treated leaves but also in distal leaves (Fig. 5 B, lane S), indicating that MeJA functions as a trigger for both, local and systemic PR responses. Similar observations were made for other elicitors like H_2O_2 (CHAMNONGPOL *et al.* 1998), a fungal glycoprotein elicitor isolated from *Phytophthora megasperma* (BAILLIEUL *et al*. 1995) or lead nitrate (LUMMERZHEIM *et al*. 1995). Although the actual role of MeJA in signal transduction pathways leading to localized and systemic defense gene expression and perhaps to SAR needs to be further investigated, at the moment there are two possible explanations how MeJA could induce the systemic expression of PR-proteins. First, airborne MeJA molecules may enter the vascular system by way of stomata and then activate the defense-related genes through a receptor-mediated signal transduction pathway. Second, MeJA may diffuse into the leaf cytoplasm where it would be hydrolyzed to JA by intracellular esterases. The free JA, in turn, could be an integral part of a more general signal transduction system that regulates inducible defensive genes in plants. This suggestion is consistent with the model proposed for the MeJA-induced expression of proteinase inhibitor genes in tomato (FARMER and RYAN 1990).

Salicylic acid production: SA accumulation was determined in grapevine leaves treated with 50 μM MeJA. Levels of endogenous SA and salicylate glucoside (SAG) were highly stimulated in leaves 6 d after the onset of

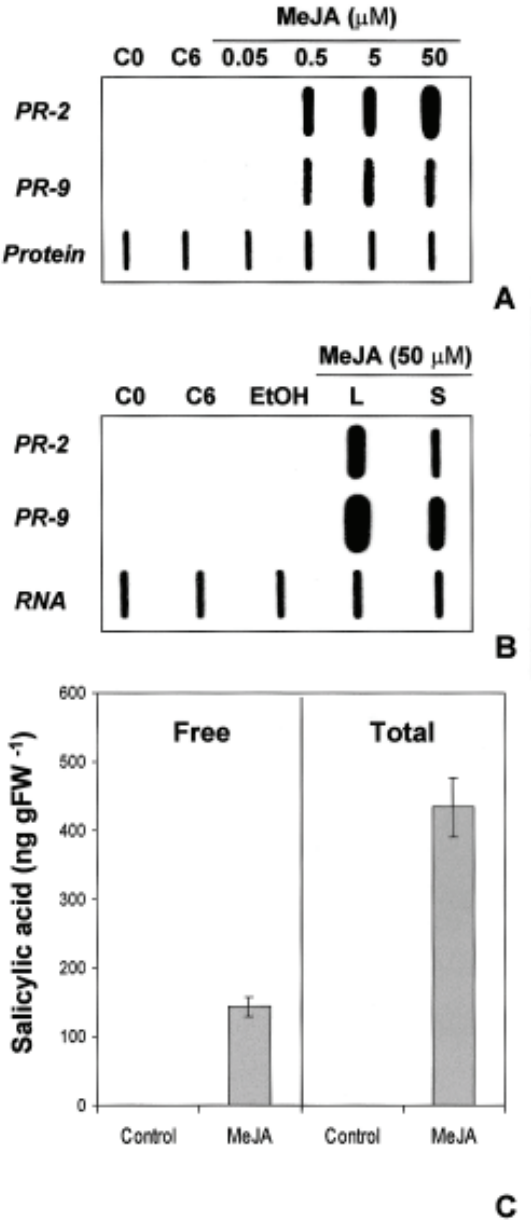

Fig. 5: Expression of pathogenesis-related proteins and salicylic acid production in control and MeJA-treated grapevines. **A**. Western blot analysis of dose dependent accumulation of the PR-2 (glucanase) and PR-9 (PRX) proteins. A 2.5 μg aliquot of protein were loaded per slot. C0 and C6 refer to extracts taken from plants at time of MeJA (50 μM) application or 6 d later, respectively. The total protein stained with amido black being used as a loading control. **B**. PR-2 and PR-9 gene-specific probes were used for slot blot hybridization analysis (5 μg of total RNA per slot). RNA samples were extracted from locally-treated (L) or systemic (S) leaves 6 d after the onset of MeJA application. The same-age leaves were processed from EtOH- or water-treated controls. The total RNA stained with *GenoGold* kit was used as a loading control. **C**. Free and total SA levels in control (water-treated) and MeJA-treated plants. Leaves taken from plants 6 d after treatment were collected and analyzed by HPLC for SA and SA + SAG content. The values presented are the average of two replicates (± SE).

treatment, while neither free SA nor SA + SAG were detected in water-treated control leaves. Thus production of SA, another typical feature of HR, could also be identified as one of the responses of grapevine to the MeJA treatment. Moreover, free and conjugated SA were present in a ratio similar to that described in the HR to TMV (Malamy *et al.* 1992) and in the HR to 32 kDa glycoprotein elicitor from *Phytophthora megasperma* (Baillieul *et al.* 1995). Whether SA produced due to the MeJA treatment is the endogenous signal for grapevine PR-protein gene expression remains, however, to be determined.

In conclusion, there are some striking similarities between the HR induced by the phytopathogen TNV in cucumber and the HR-like lesions induced by MeJA in grapevine: similarities in the detailed macroscopical appearance of lesions, and in the activation of defense responses associated with the HR, such as phenylpropanoid compound accumulation, PR-gene expression and the increase of SA production. These observations suggest the induction of common basic stress responses followed by a downstream diversification of the particular signal transduction pathway(s) leading to the induction of specialized plant responses.

Acknowledgements

We are grateful to Prof. H. J. M. Linthorst (Gorlaeus Lab, Leiden University, Leiden, The Netherlands) for the clone CI121 (PR-2 probe) and to Dr. S. Kauffmann (IBMP-CNRS, Strasbourg, France) for the anti-PR-2 serum. This work was partly supported by Grant no. 27-19/STP from the Slovak Ministry of Agriculture.

References

Andresen, I.; Becker, W.; Schluter, K.; Burges, J.; Parthier, B.; Apel, K.; 1992: The identification of leaf thionin as one of the main jasmonate-induced proteins of barley (*Hordeum vulgare*). Plant Mol.. Biol. **19**, 193-204.

Baillieul, F.; Genetet, I.; Kopp, M.; Saindrenan, P.; Fritig, B.; Kauffmann, S.;1995: A new elicitor of the hypersensitive response in tobacco: A fungal glycoprotein elicits cell death, expression of defence genes, production of salicylic acid, and induction of systemic acquired resistance. Plant J. **8**, 551-560.

Blum, H.; Beier, H.; Gross, H. J.; 1987: Improved silver staining of plant proteins, RNA and DNA in polyacrylamide gels. Electrophoresis **8**, 93-99.

Bradford, M. M.; 1976: A rapid and sensitive method for the quantification of microgram quantities of proteins utilizing the principle of protein-dye binding. Anal. Biochem. **72**, 248-254.

Chamnongpol, S.; Willekens, H.; Moeder, W.; Langerbartels, C.; Sandermann, H.; Van Montagu, M.; Inzé, D.; Van Camp, W.; 1998: Defense activation and enhanced tolerance induced by H_2O_2 in transgenic tobacco. Proc. Natl. Acad. Sci. USA **95**, 5818-5823.

Choi, D.; Bostock, R. M.; Avdiushko, S.; Hildebrand, D. F.; 1994: Lipid-derived signals that discriminate wound- and pathogen-responsive isoprenoid pathways in plants: Methyl jasmonate and the fungal elicitor arachidonic acid induce different 3-hydroxy-3-methylglutaryl-coenzyme. A reductase genes antimicrobial isoprenoids in *Solanum tuberosum* L. Proc. Natl.Acad. Sci. USA **91**, 2329-2333.

Conconi, A.; Smerdon, M. J.; Howe, G. A.; Ryan, C. A.; 1996: The octadecanoid signaling pathway in plants mediates a response to ultraviolet radiation. Nature **383**, 826-829.

Creelman, R. A.; Mullet, J. E.; 1997: Biosynthesis and action of jasmonates in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. **48**, 355-381.

- -; Tierney, M. L.; Mullet, J. E.; 1992: Jasmonic acid/methyl jasmonate accumulate in wounded soybean hypocotyls and modulate wound gene expression. Proc. Natl. Acad. Sci. USA **89**, 4938-4941.

Doares, S. H.; Syrovets, T.; Weiler, E. W.; Ryan, C. A.; 1996: Oligogalacturonides and chitosan activate plant defensive genes

through the octadecanoid pathway. Proc. Natl. Acad. Sci. USA **92**, 4095-4098.

ENYEDI, A. J.; YALPANI, N.; SILVERMAN, P.; RASKIN, I.; 1992: Localization, conjugation, and function of salicylic acid in tobacco during the hypersensitive reaction to tobacco mosaic virus. Proc. Natl. Acad. Sci. USA **89**, 2480-2484.

FARMER, E. E.; RYAN, C. A.; 1990: Interplant communication: Airborne methyl jasmonate induces synthesis of proteinase inhibitors in plant leaves. Proc. Natl. Acad. Sci. USA **87**, 7713-7716.

- -; - -; 1992: Octadecanoid precursors of jasmonic acid activate the synthesis of wound-inducible proteinase inhibitors. Plant Cell **4,** 129-134.

FRITIG, B.; LEGRAND, M.; HIRTH, L.; 1972: Changes in the metabolism of phenolic compounds during the hypersensitive reaction of tobacco to TMV. Virology **47**, 845-848.

GUNDLACH, H.; MÜLLER, M. J.; KUTCHAN, T. M.; ZENK, M. H.; 1992: Jasmonic acid is a signal transducer in elicitor-induced plant cell cultures. Proc. Natl. Acad. Sci. USA **89**, 2389-2393.

HAMBERG, M.; GARDNER, H. W.; 1992: Oxylipin pathway to jasmonates: Biochemistry and biological significance. Biochim. Biophys. Acta **1165**, 1-18.

HENRISSAT, B.; SAHOHEIMO, M.; LAVAITTE, S.; KNOWLES, J. K. C.; 1990: Structural homology among the peroxidase enzyme family revealed by hydrophobic cluster analysis. Prot. Struct. Funct. Genet. **8**, 251-257.

HERDE, O.; ATZORN, R.; FISAHN, J.; WASTERNACK, C.; WILLMITZER, L.; PEÑA-CORTÉS, H.; 1996: Localized wounding by heat initiates the accumulation of proteinase inhibitor II in abscisic acid-deficient plants by triggering jasmonic acid biosynthesis. Plant Physiol. **112**, 853-860.

HOWE, G. A.; LIGHTNER, J.; BROWSE, J.; RYAN, C. A.; 1996: An octadecanoid pathway mutant (JL5) of tomato is compromised in signaling for defense against insect attack. Plant Cell **8**, 2067-2077.

KODA, Y.; 1992: The role of jasmonic acid and related compounds in the regulation of plant development. Int. Rev. Cytol. **135**, 155-199.

KOGEL, K. H.; BECKHOVE, U.; DRESCHERS, J.; MUNCH, S.; 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.

LAEMMLI, U. K.; 1970: Cleavage of structural protein during the assembly of the head of bacteriophage T4. Nature **227**, 680-685.

LEHMANN, J.; ATZORN, R.; BRUCKNER, C.; REINBOTHE, S.; LEOPOLD, J.; WASTERNACK, C.; PARTHIER, B.; 1995: Accumulation of jasmonate, abscisic acid, specific transcripts and proteins in osmotically-stressed barley leaf segments. Planta **197**, 156-162.

LUMMERZHEIM, M.; SANDRONI, M.; CASTRESANA, C.; DE OLIVEIRA, D.; VAN MONTAGU, M.; ROBY, D.; TIMMERMAN, B.; 1995: Comparative microscopic and enzymatic characterization of the leaf necrosis induced in *Arabidopsis thaliana* by lead nitrate and by *Xanthomonas campestris* pv. *campestris* after foliar spray. Plant, Cell Environ. **18**, 499-509.

MALAMY, J.; HENNIG, J.; KLESSIG, D. F.; 1992: Temperature-dependent induction of salicylic acid and its conjugates during the resistance response to tobacco mosaic virus infection. Plant Cell **4**, 359-366.

MALOPESZA, U.; URBANEK, H.; 1994: Changes in peroxidase activity in bean suspension cultures after *B. cinerea* elicitor treatment. J. Phytopathol. **141**, 314-322.

MEMELINK, J.; LINTHORST, H. J. M.; SCHILPEROORT, R. A.; HOGE, J. H.; 1990: Tobacco genes encoding acidic and basic isoforms of pathogenesis-related proteins displays different expression patterns. Plant Mol. Biol. **14**, 119-126.

MITCHELL, A.; WALTERS, D.; 1995: Systemic protection in barley against powdery mildew infection using methyl jasmonate. Aspects Appl. Biol. **42**, 323-326.

MONSTEIN, H. J.; GEIJER, T.; BAKALKIN, G. Y.; 1992: *BLOTTO-MF*, an inexpensive and reliable hybridization solution in northern blot analysis using complementary RNA probes. Biotechniques **13**, 842-844.

O´DONNELL, P. J.; CALVERT, C.; ATZORN, R.; WASTERNACK, C.; LEYSER, H. M. O.; BOWLES, D. J.; 1996: Ethylene as a signal mediating the wound response of tomato Plants. Science **274**, 1914-1917.

PIETERSE, C. M. J.; VAN WEES, S. C. M.; HOFFLAND, E.; VAN PELT, J. A.; VAN LOON, L. C.; 1996: Systemic resistance in *Arabidopsis* induced by biocontrol bacteria is independent of salicylic acid accumulation and pathogenesis-related gene expression. Plant Cell **8**, 1225-1237.

REPKA, V.; 1999: Differential sensitivity of purified isoforms of cucumber anionic virus-inducible peroxidases to exogenous proteases. Acta Virol. **43**, 313-319.

- -; FISCHEROVÁ, I.; CANIGOVÁ, K.; 1996: Expression of cucumber stress-related anionic peroxidases during flower development or a cryptic infective process. Biol. Plant. **38**, 585-596.

- -; KOLLEROVÁ, E.; SLOVÁK, M.; 1993: The extracellular acidic and basic virus-elicited proteins of cucumber cotyledons. Biol. Plant. **35**, 87-93.

- -; SLOVÁKOVÁ, L.; 1994: Purification, characterization and accumulation of three virus-induced cucumber peroxidases. Biol. Plant. **36**, 121-132.

RYAN, C. A.; 1992: The search for the proteinase inhibitor-inducing factor, *PIIF*. Plant Mol. Biol. **19**, 123-133.

SCHWEIZER, P.; GEES, R. E. M.; MOSINGER, E.; 1993: Effect of jasmonic acid on the interaction of barley (*Hordeum vulgare* L.) with the powdery mildew *Erysiphe graminis* f. sp. *hordei*. Plant Physiol. **102**, 503-511.

SEMBDNER, G.; PARTHIER, B.; 1993: The biochemistry and the physiological and molecular actions of jasmonates. Annu. Rev. Plant Physiol. Plant Mol. Biol. **44**, 569-589.

STASWICK, P. E.; SU, W.; HOWELL, S. H.; 1992: Methyl jasmonate inhibition of root growth and induction of a leaf protein are decreased in *Arabidopsis thaliana* mutant. Proc. Natl. Acad. Sci. USA **89**, 6837-6840.

VAN WEES, S. C. M.; PIETERSE, C. J. M.; TRIJSSENAAR, A.; VAN´T WESTENDE, Y.; HARTOG, F.; VAN LOON, L. C.; 1997: Differential induction of systemic resistance in *Arabidopsis* by biocontrol bacteria. Mol. Plant-Microbe Interactions **10**, 716-724.

VICK, B. A.; ZIMMERMAN, D. C.; 1983: The biosynthesis of jasmonic acid: A physiological role for plant lipoxygenase. Biochem. Biophys. Res. Commun. **111**, 470-477.

WASTERNACK,C.; PARTHIER, B.; 1997: Jasmonate-signalled plant gene expression. Trends Plant Sci. **2**, 302-307.

WEILER, E. W.; ALBRECHT, T.; GROTH, B.; XIA, Z. Q.; LUXEM, M.; 1993: Evidence for the involvement of jasmonates and their octadecanoid precursors in the tendril coiling response in *Bryonia dioica*. Phytochemistry **32**, 551-560.

XU, Y.; CHANG, P. F. L.; LIU, D.; NARASIMHAN, M. L.; RAGHOTHAMA, K. G.; 1994: Plant defense genes are synergistically induced by ethylene and methyl jasmonate. Plant Cell **6**, 1077-1085.

Received September 14, 2000