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Research Note

Sequence of a putative *Vitis vinifera* PR-1

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Introduction: Incompatible host-pathogen interactions include localized hypersensitive reaction (HR) and systemic resistances, *e.g.* systemic acquired resistance (SAR) and induced systemic resistance (ISR). Amongst various metabolic alterations associated with HR and SAR, various proteins are induced which are collectively referred to as 'pathogenesis-related proteins' (PRs). These proteins are defined as coded for by the host plant but induced specifically in pathological or related situations (Van Loon and Van Strien 1999). The association of PRs with SAR - but not with ISR - suggests that accumulation of PRs contributes to the induction of resistance but is not a prerequisite for it (Van Loon 1997; Van Wees *et al.* 1999). Fourteen families of PRs have been proposed (Van Loon and Van Strien 1999). The only PR family for which no function is known consists of the PR-1 proteins. Tobacco and tomato PR-1 proteins have antifungal activity, but their mechanism of action is not known.

PR genes have been identified in *Vitis* species coding for chitinases, glucanases, osmotins, a ribonuclease-like protein and a lipid-transfer protein (Busam *et al*. 1997; Salzman *et al*. 1998; Jacobs *et al*. 1999; Robert *et al*. 2001). In addition, peroxydases, glucanases, chitinases and PR-1 like proteins have been serologically detected in grapevines (Deloire *et al.* 1997; Repka *et al*. 2000, 2001). In the present note, we report cloning by RT-PCR of the open reading frame of a putative *Vitis vinifera* cv. Gewürztraminer PR-1.

Material and Methods: Adult grapevines (*Vitis vinifera* cv. Gewürztraminer) were maintained in a growth chamber at 25 ± 2 °C, at 80 % RH and 16 h light. Leaves were sprayed with salicylic acid 300 µM, pH 5.1 (Sigma Chemical) in DMSO (0.5 % final concentration).

E x t r a c t i o n a n d a m p l i f i c a t i o n : Total RNAs were extracted 96 h after spraying from 50 mg of leaves according to a modified method from the RNeasy plant protocol (Qiagen). Based on comparison of various plant PR-1 protein sequences, sense primer and antisense primer corresponding to the consensus peptide motifs SPQDY and HYTQVVW were synthesized. cDNA synthesis and polymerase chain reaction (PCR) were performed using the Superscript One-Step RT-PCR kit with Platinium *Taq* polymerase (Invitrogene). cDNA synthesis was performed at 55 °C for 30 min and PCR amplification was 35 cycles for 30 s at 94 °C, 60 s at 57 °C and 90 s at 72 °C. 5' and 3' cDNA ends were obtained by elongation with the 5'/3' Race kit (Roche Molecular Biochemicals). The different PCR products were separated by electrophoresis on a 1.2 % agarose gel. The expected band was excised and the amplification products extracted and purified by using the nucleospin extract kit (Macherey-Nagel). The product was cloned in the TA cloning vector (Invitrogene) and double strand sequenced (Genome express).

S o u t h e r n b l o t a n a l y s i s : Genomic DNA was isolated from leaves of *V. rupestris*, *V. vinifera* cvs Chardonnay, Gewürztraminer, Merlot, Muscat, Sauvignon, *V. Berlandieri* x *V. rupestris* 110 R and *V. riparia* x *V. rupestris* 3309 C. Samples of 10 µg were digested with the endonuclease *Hind III*. The restriction fragments were separated by electrophoresis on a 1% agarose gel and blotted by downward capillary transfer to a nylon membrane. The blots were hybridized at 42 °C overnight with a digoxigenin-labeled PCR PR-1 fragment, washed subsequently under stringent conditions and developed with a chemiluminescence substrate according to the manufacturer's recommendation (Roche).

Results and Discussion: Like other PR-1 proteins, the primary translation product (161 amino acids) deduced from our nucleotide sequence (EMBL: AJ 536326) contains a hydrophobic signal sequence (Table), which is cleaved off upon entry in the endoplasmic reticulum (Van Loon and Van Strien 1999). The mature protein is 137 amino acids long with a theoretical pI of 6.97 and a calculated molecular weight of 14 999.40 Da (Peptide Mass-Expasy) close to the 15.5 kDa of gPR-1a. GPR-1a is one of the three grapevine PR-1 proteins detected by immunoblotting after fungal infection of *V. vinifera* cv. Limberger (Repka *et al*. 2000).

Southern hybridization analysis shows several bands in the different restriction digests, suggesting a variable genomic organization for the PR-1 gene in the different species and cultivars that were analysed (Figure).

Analysis of the amino acid sequence of our *V. vinifera* PR-1 protein reveals the presence of the structural elements highly conserved in the PR-1 family: 4 α-helices and

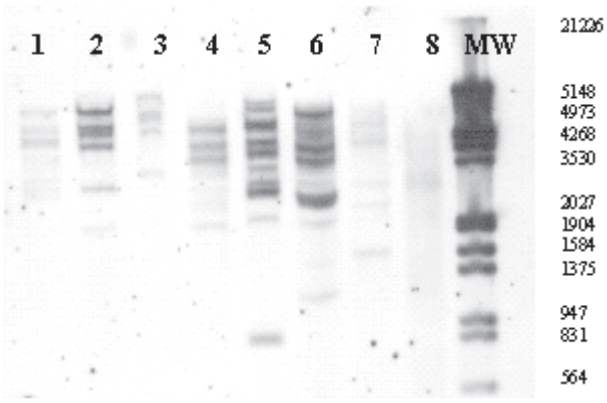

Figure: Leaf DNA of cvs Sauvignon (1), Merlot (2), Chardonnay (3), Muscat (4), *V. rupesris* (5), 3309 C (6), 110 R (7) and Gewürztraminer (8) was used for the restriction with the endonuclease *Hind*III and the Southern hybridization. MW: DNA Molecular weight DIG-labeled (Roche).

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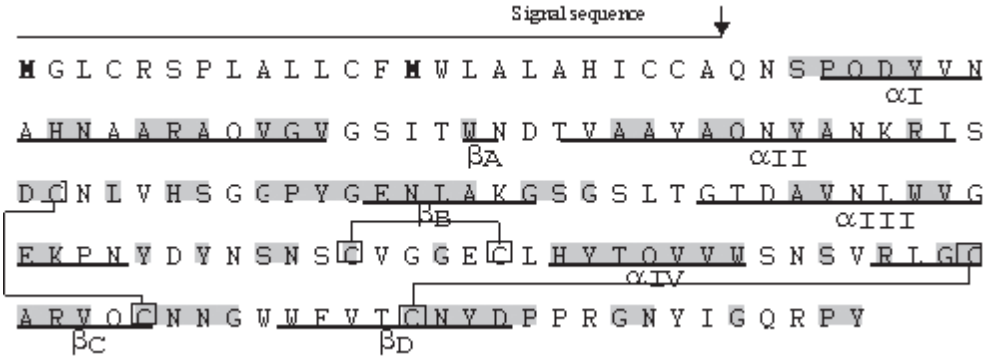
Table

A) Amino acid sequence of *V. vinifera* putative PR-1 including the signal sequence. Amino acid residues identical to the consensus sequences (VAN LOON and VAN STRIEN 1999) are highlighted in gray. Positions of the cysteine residues, α helices and β strands are indicated



B) Homology of PR-1 like protein from *V. vinifera* and other organisms in percentage amino acid identity (upper) and similarity (lower), including the signal sequence: PR-1a *Nicotiana tabacum* (Nt PR1a) (CAA30017), PR-1b *Nicotiana tabacum* (Nt PR-1b) (CAA47374), basic form of PR-1 *Nicotiana tabacum* (Nt basic) (CAA32228), PR-1c *Nicotiana tabacum* (Nt PR-1c) (CAA29023), PR-1 *Capsicum annuum* (C. annu) (AAK30143), PR-1b *Solanum phrija* (Sp PR-1b) (E08876), PR-1b2 *Solanum phrija* (Sp PR-1b2) (CAD38277), PR-1 *Solanum tuberosum* (St PR-1) (CAB58263), PR-1b *Solanum tuberosum* (St PR-1b) (AAL01594), PR-1a *Glycine max* (Gm PR-1a) (AAD33696), PR-1a *Lycopersicon esculentum* (Le PR-1a) (CAA09671), PR-1a *Brassica napus* (Bn PR-1a) (AAB01666), PR-1-1a *Cucumis sativus* (Cs PR-1-1a) (AAL84767), PR-1 *Arabidopsis thaliana* (At PR-1) (AAD24401), PR-1 *Hordeum vulgare* (Hv PR-1) (CAA88618), PR-1b *Hordeum vulgare* (Hv PR-1b) (AQ294250), PR-1 *Oryza sativa* (Oz PR-1) (AAM93438)

	Nt PR-1a	Nt PR-1b	Nt basic	Nt PR-1c	C. annu
V. vinifera PR-1	64 %	63 %	68 %	64 %	66 %
	78 %	74 %	78 %	77 %	79 %
	Sp PR-1b	Sp PR-1b2	St PR-1	St PR-1b	Gm PR-1a
V. vinifera PR-1	66 %	64 %	65 %	64 %	61 %
	75 %	74 %	75 %	75 %	77 %
	Le PR1a	Bn PR1a	Cs PR-1-1a	At PR-1	Hv PR-1
V. vinifera PR-1	63 %	58 %	63 %	58 %	53 %
	75 %	72 %	78 %	75 %	67 %
	Hv PR-1b	Oz PR-1			
V. vinifera PR-1	57 %	55 %			
	72 %	69 %			

4 β-strands, as well as 6 conserved Cys residues were involved in the formation of disulphide bridges (Table). The identity to the type member, tobacco PR-1a, is 64 % and ranges from 53 % to 68 % to different PR-1 proteins (Table). Identity to a partial sequence (99 aa) of a putative *Vitis vinifera* cv. Pinot noir PR-1 (CAA05868) is 81 %.

Homologies and common structural motifs have shown to be between plant PR-1 proteins and proteins from fungi, invertebrate and vertebrate animals and humans suggesting a possible functional link between the human immune system and the defense-related activity in plants (VAN LOON and VAN STRIEN 1999). Our *V. vinifera* PR-1 shows respective identities with proteins from yeasts (29 to 42 % - M81722, M81723, Z28238), insects (17 to 24 % - C44583, L49036, J03601, B37330), and vertebrates (24 to 29 % - X95240, U13619, U16307, S80310, X94323, D45027).

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