BIOLOGIA PLANTARUM 36 (1): 133-138, 1994

Effect of UV-C on peroxidase isoenzymes in axillary bud cultures of *Vitis* species differing in fungal resistance to *Plasmopara viticola*

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Abstract

The effect of shortwave (250 nm) UV radiation (UV-C) on the level of peroxidase activity and peroxidase isoenzyme patterns in leaves of resistant ([*Vitis vinifera* × *Viris riparia*] × *Vitis rupestris* and *Vitis rupestris*) and susceptible (*Vitis vinifera*) grapevine species to *Plasmopara viticola* (downy mildew) was studied. The results show that although UV-C did not produce significant changes in peroxidase activity in susceptible species, and only minor changes in resistant species, treatment with UV-light induces an acidic isoperoxidase (isoperoxidase A_1), capable of oxidising 4-hydroxystilbenes in resistant species. It was named HSPrx 2. Since peroxidase is apparently the enzyme responsible for ε-viniferin synthesis from resveratrol in grapevines, a close relationship between this peroxidase isoenzyme and ε-viniferin synthesis which occurs in grapevine leaves after UV-C treatment must be expected.

Introduction

4-Hydroxystilbenes constitute a group of naturally occurring plant phenolics involved in discowaging diseases caused by fungal pathogens such as *Botrytis cinerea* (grey mould) and *Plasmopara viticola* (downy mildew) in grapevines (Langcake 1981). These compounds are considered to be the precursors of viniferins, a class of stress metabolite with strong antifungal activity (Langcake 1981). The synthesis of viniferins requires a set of enzymes: resveratrol synthase catalyzes the synthesis of the stilbene backbone from phenylpropanoid precursors and malonyl-CoA (Kindl 1986, Schröder and Schröder 1990); and the subsequent oxidative

Received 9 December 1992, *accepted* 10 January 1993.

Acknowledgements: This work was in part supported by a grant from the Comisión Interministerial de Ciencia y Tecnología, Spain, project # AGR 296/89. A.A. Calderón has a fellowship (BPP) from the Ministerio de Educación y Ciencia. Thanks are given to Dr. I. Philippe (Laboratoire de Biotechnologie Amélioration des Plantes, ENSA, Toulouse, France) for her assistance in the growth of vitroplants, and to the Centro Regional de Investigaciones Agrarias of Murcia for providing the grapevine plants used in these studies.

coupling of resveratrol to yield ε-viniferin involves peroxidases (Langcake and Pryce 1977a).

Irradiation of grapevine leaves with shortwave (< 285 nm) ultraviolet radiation (UV) induces the synthesis of resveratrol (Langcake and Pryce 1976 and 1977b, Stein and Hoss 1984, Jeandet *et al.* 1991), and of ε-viniferin (Langcake and Pryce 1977b), in direct relation to disease resistance (Pool *et al.* 1981). This is due to a coordinated induction by UV radiation of phenylalanine ammonia-lyase, cinnamate-4-hydroxylase and stilbene (resveratrol) synthase (Fritzemeier and Kindl 1981), the key enzymes in resveratrol biosynthesis (Schröder and Schröder 1990). Because resveratrol is the precursor of ε-viniferin in a reaction catalyzed by peroxidase (Langcake and Pryce 1977a), it would be of interest to know whether UV treatment induces peroxidase in grapevine leaves in order to explain the increases in the rate of ε-viniferin synthesis.

Materials and methods

Chemicals: 4-Hydroxystilbene (4-HS) was obtained from *Janssen Chimica* (Beerse, Belgium), and its purity checked by elemental analysis and mass spectroscopy. Dioxane and H_2O_2 were purchased from *Merck* (Darmstadt, FRG), 4-methoxy-α-naphtol (4-MN) from *Aldrich-Chemie* (Steinheim, FRG). All other chemicals used in this work were obtained from various commercial suppliers, and were of the highest purity available.

Axillary bud cultures: Shoot cuttings of the following cultivars were taken from greenhouse-grown vines and established as axillary bud cultures in the laboratory: *1*) *Vitis vinifera* cv. Monastrell (clone 35), *V. vinifera* cv. Chardonnay, and *V. vinifera* cv. Grenache, *2*) *Vitis rupestris* cv. de Lot, and *3*) (*Vitis vinifera* × *Vitis rupestris*) × *Vitis riparia*, 1203 C (clone 99), a hybrid resulting from the crossing of cultivars (Monastrell × Martin) × Gloria.

Cultivar Monastrell was chosen for its susceptibility to the downy mildew *Plasmopara viticola*, while cultivar 1203 C represents a hybrid between resistant (*Vitis riparia* cv. Gloria, Langcake 1981), and susceptible (*Vitis vinifera* cv. Monastrell) genotypes.

Axillary bud cultures of these cultivars were established by the method described by Lee and Wetzstein (1988) with modifications (Calderón *et al.* 1992b).

Exposure to UV-radiation: The upper parts of fully expanded leaves from 1-month-old plants cultivated *in vitro* were exposed to 2 W m^{-2} UV-C radiation (*Uvatom TLC* inspection lamp, 254 nm) for 10 min (Fritzemeier and Kindl 1981), and left floating in water in a Petri dish at 25°C for 16 h. Controls were included with leaves not treated with UV-C.

Peroxidase fractions: Leaves were homogenized in 0.2 M Na-phosphate buffer (pH 6.5), containing 6 mM ascorbic acid and 50 mg cm^{-3} polyvinylpolypyrrolidone

(Calderón *et al.* 1992b). The homogenate was centrifuged at 20 000 *g* for 20 min and the supernatant was dialyzed overnight against 50 mM Tris-HCl buffer (pH 7.5). This constituted the peroxidase fraction used in all the corresponding studies.

Spectrophotometric measurements: These were performed using a *Kontron Uvikon-940* spectrophotometer. The assay of peroxidase activity using 4-methoxy-α-naphtol as a substrate was carried out as previously described (Ferrer *et al.* 1990).

Isoelectrofocusing and zymographic assays: Isoelectrofocusing (IEF) of grapevine peroxidases was performed at 4 °C using a *2103 PS LKB* power supply. Polyacrylamide gels for disc-IEF were prepared and polymerized according to Calderón *et al.* (1990). Usually, 100 µl of solution containing the *LKB* ampholines (pH 3.5-10) carrier, 60 % sucrose and enzyme, were layered on each gel, and focusing of protein was done as already described (Calderón *et al.* 1990). Staining of peroxidase isoenzymes with 4-methoxy-α-naphtol and 4-hydroxystilbene was carried out as previously described (Ferrer *et al.* 1990, Calderón *et al.* 1992b). Peroxidase isoenzymes stained with 4-hydroxystilbene were named HSPrx.

Results and discussion

It is generally assumed that the effects of the UV-C component of ultraviolet radiation are the result of the direct absorption of the radiation by DNA, so that, in this case, DNA is both primary chromophore (photosensitizer) and target compound (Peak and Peak 1983). In order to test whether UV-treatment induces a different response of peroxidase activity and/or peroxidase isoenzymes in leaves of grapevine cultivars differing in disease resistance, two grapevine cultivars were chosen for their different susceptibilities to the downy mildew *Plasmopara viticola*: a Monastrell cultivar (*V. vinifera*), of well known susceptibility and a highly resistant hybrid (cv. 1203 C) resulting from the crossing of Monastrell vines with *V. rupestris* and *V. riparia*.

Table 1. Levels of peroxidase activity (nkat g^{-1}(f.m.)) measured using 4-methoxy-α-naphtol in leaves of susceptible *Vitis vinifera* (cv. Monastrell), and resistant (*Vitis vinifera* × *Vitis rupestris*) × *Vitis riparia* (cv. 1203 C) and *Vitis rupestris* (cv. de Lot), grapevines after exposure to UV-C radiation.

Cultivar	Peroxidase activity Control	UV-C treated
Monastrell	107 ± 2[a]	114 ± 19[a]
1203 C	119 ± 5	107 ± 5
De Lot	145 ± 8	103 ± 12

Values followed by the same letter are not significantly different at $P = 0.05$.

Treatment of grapevine leaves with UV-C had no significant effect on soluble peroxidase activity in the Monastrell cultivar, while it slightly reduced peroxidase activity in the cultivar 1203 C (Table 1). UV-radiation treatments of leaves from susceptible *V. vinifera* cultivars, Chardonnay and Grenache, were also accompanied by no significant changes in peroxidase activity. However, treatment with UV-radiation of leaves from the highly resistant cultivar de Lot produced, similarly to that observed on the cultivar 1203 C, a significant decrease in peroxidase activity (Table 1). This suggests that, unlike susceptible cultivars, resistant cultivars respond to UV-radiation treatment by decreasing peroxidase levels, this response being associated with the expression of the resistance character, as it may be seen from comparing the peroxidase responses of the Monastrell cultivar with those found in the cultivar 1203 C.

Similar results were found by Murphy and Huerta (1990) after UV-irradiation of cultured rose cells. These authors found that UV-irradiation produces a significant increase in H_2O_2 production but a weak, hardly significant, decrease in peroxidase levels. In this last case, statistical significance of peroxidase alterations were strongly hampered by the high SE values of peroxidase determinations. A similar high variability was found in the level of peroxidase activity in grapevine leaves after UV-irradiation (Table 1).

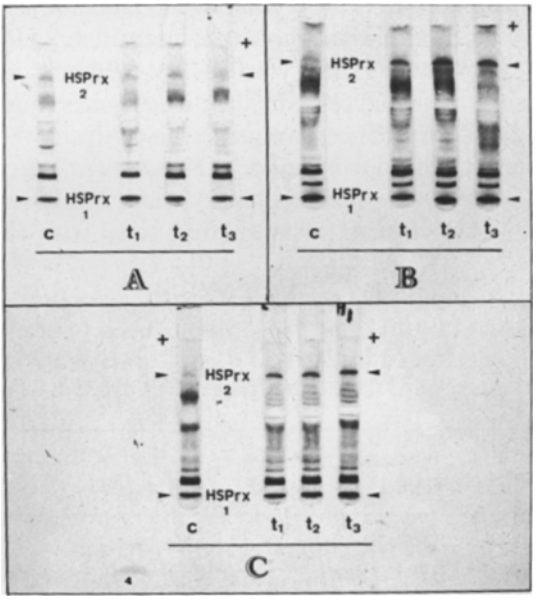

Fig. 1. Peroxidase isoenzyme patterns of *A*) *Vitis vinifera* cv. Monastrell, *B*) (*Vitis vinifera* × *Vitis riparia*) × *Vitis rupestris* (cv. 1203 C, [Monastrell × Martin] × Gloria), and *C*) *Vitis rupestris* cv. de Lot, in control (c) and UV-C treated leaves (t_{1-3}). Subindices 1-3 in treated leaves correspond to independent experiments. HSPrx = 4-hydroxystilbene-oxidizing peroxidases.

It may be concluded from these results that alterations in peroxidase activity after UV-light treatment does not appear to be related to the strong increase in ε-viniferin synthesis which occurs in grapevine leaves after UV-C irradiation (Langcake and Pryce 1977b). From this, it may be suggested that total peroxidase levels, *per se*, did not limit the production of ε-viniferin by grapevine leaves. In order to test possible alterations at the peroxidase isoenzyme level, a screening of grapevine isoperoxidases was carried out in both control and UV-C irradiated leaves.

The treatment of leaves from the cv. Monastrell with UV-C did not modify isoperoxidase patterns significantly (Fig. 1A). Similar results were also found in the susceptible cultivars, Chardonnay and Grenache. As in the measurement of peroxidase activity, high variability was also observed in the isoperoxidase patterns (Fig. 1, t_{1-3}).

However, the treatment of leaves from the resistant cvs. 1203 C and de Lot with UV-C produced a significant increase in the level of an acidic isoenzyme with 4-hydroxystilbene oxidizing properties. This isoenzyme (Figs. 1B and 1C), previously named isoperoxidase A_1 (Ferrer *et al.* 1990), was now denominated HSPrx 2. Isoperoxidase HSPrx 2 was elicited in leaves of the resistant species after UV-light treatment (Figs. 1B and 1C), in contrast to HSPrx 1 which was previously considered to be a marker of the resistance character in healthy grapevine crossings (Calderón *et al.* 1992b). This differential responsiveness of isoperoxidase HSPrx 2 (isoperoxidase A_1) to UV-light treatment is not surprising since this isoenzyme, which was not present in grape berries, was also induced during the induction of grapevine calli from explants of pericarp tissue (Calderón *et al.* 1992a, Zapata *et al.* 1992).

These results reveal that, although the levels of grapevine peroxidase activity in leaves are not directly related to induction of viniferin synthesis after UV-radiation, an isoperoxidase capable of oxidizing 4-hydroxystilbenes, isoperoxidase A_1 (now named HSPrx 2), is elicited after UV-C treatment in *Vitis* species, expressing the resistance to *Plasmopara viticola*. The full importance of this peroxidase isoenzyme in the control of the rate of viniferin synthesis in *Vitaceae* must be further considered.

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Communicated by J. ŠPAK