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Epigenetic control of extracellular auxin catabolism in grapevine cells cultured in suspension

by

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Epigenetische Kontrolle des extrazellulären Auxinabbaus bei suspensionskultivierten Rebzellen

Zusammenfassung: Bei der Rebsorte Gamay (*Vitis vinifera*) wurden die verschiedenen Faktoren untersucht, welche den extrazellulären Enzymabbau kontrollieren; hierbei dienten suspensionskultivierte Zellen als Modellsystem. Aus den Ergebnissen wird gefolgert, daß — im Gegensatz zu früheren Beobachtungen mit kultivierten Zellen — der Auxinabbau im benutzten Kulturmedium von starken Sekretionsschüben sowohl basischer als auch saurer Isoperoxidasen abhängig ist. Er hängt ferner von der Sekretion phenolischer Verbindungen ab — 'Auxinprotektoren', welche die IAA-Oxidasenaktivität der extrazellulären Isoperoxidasen im Verlauf der Kulturperiode abwandeln. Abweichend von Beobachtungen über die Peroxidasenaktivität äußert sich die IAA-Oxidasenaktivität hauptsächlich zu Beginn der postexponentiellen Wachstumsphase der kultivierten Zellen; diesem stark unterteilten Abbauprozeß kommt damit offensichtlich eine zentrale Rolle bei der Absenkung des IAA-Spiegels zu, wenn die kultivierten Zellen ihr Wachstum eingestellt haben.

Key words: tissue culture, cell, growth, growth medium, growth regulator, auxin protector, phenol, enzyme, basic isoperoxidase, IAA-oxidase.

Introduction

Growth of grapevine fruits depends on the synthesis of several enzymes, which results in the increased activity of specific isoenzymes (Dal Bellin Peruffo and Pallavicini 1975). Among these enzymes, peroxidases (E.C. 1.11.1.7) have been implicated in the control of the level of indole-3-acetic acid (IAA), which regulates the growth of grape fruits (Coombe 1976). This enzymatic control takes place through a catabolic reaction which is apparently mediated by the most basic isoenzymes (basic isoperoxidases) (Gaspar *et al.* 1985). However, the specific expression of basic isoperoxidases which occurs in grapevine cells cultured in suspension during vacuolar development (García-Florenciano *et al.* 1990) seems to play no role in the control of auxin catabolism in grapevine cells, since IAA-oxidase activity of cellular basic isoperoxidases is totally inhibited by the presence of endogenous inhibitors of the IAA catabolism (García-Florenciano *et al.* 1990), also known as auxin protectors.

During recent years, extracellular peroxidases have been thought to be involved in cell wall phenolic cross-linking and probably also in IAA catabolism (Gaspar *et al.* 1985). In this case, extracellular peroxidases, which are constitutive enzymes of the cell wall and intercellular spaces, would apparently be regulated in both their catalytic activity and protein level during well-defined stages of plant cell growth (Bolwell 1988; Cuenca *et al.* 1989).

As regards grape peroxidases, there is no information on the apparent changes in the extracellular isoperoxidase patterns during growth of grape fruits, nor on the possible control which extracellular auxin protectors may exert on the IAA oxidizing activity of these extracellular isoenzymes.

Using suspension-cultured grape berry cells as a model system, we show in this paper that a sequential release of both basic and acidic isoperoxidases to the cultured medium, and an epigenetic control by auxin catabolism inhibitors, appear to be the two main factors responsible for the control of extracellular auxin catabolism during the growth of grapevine cells cultured in suspension.

Material and methods

Source of plant material, cell culture and culture samples

Callus cultures derived from growing fruits of grapevines (*Vitis vinifera* L. cv. Gamay), kindly provided by Drs. J. C. PECH and A. LATCHÉ (ENSA, Toulouse, France), were used to establish suspension cell cultures, the culture conditions being similar to that described by GARCÍA-FLORENCIANO *et al.* (1990). Samples (10 ml) for analyses were taken at various days after the beginning of culture and the cells separated by centrifugation (500 g_{max} for 5 min). The supernatants ('spent medium samples') were used without further treatment for routine analysis. Cell culture growth was measured as packed cell volume and expressed as percentage.

Homogenization of grapevine cells, preparation of cell wall fractions, and binding of peroxidases to cell walls

Pelleted grapevine cells were homogenized with a mortar and pestle and cell wall fractions were prepared exactly as described previously (ROS BARCELÓ *et al.* 1987).

Ionically bound peroxidases attached to cell wall fractions were solubilized by treatment with 1 M KCl (ROS BARCELÓ *et al.* 1987), and dialyzed overnight at 4 °C against 50 mM Tris (tris-[hydroxymethyl]aminomethane)-HCl buffer, pH 7.5, containing activated charcoal in order to remove phenolic compounds.

Binding of peroxidases isolated from the culture medium to cell walls was carried out as described (ROS BARCELÓ *et al.* 1988). The total amount of the peroxidase in the binding assay media (buffered to pH 7.2 using 50 mM Tris-HCl) was about 5 nkat.

Enzyme and protein assays

Peroxidase activity was measured by following the increase in absorbance at 470 nm using guaiacol as substrate (ROS BARCELÓ *et al.* 1987). Isoelectrofocusing, zymographic stain and quantification of isoperoxidases were performed as described previously (ROS BARCELÓ 1987).

Indol-3-acetic acid oxidase (IAA-oxidase) activity of both horseradish peroxidase (Sigma, type VI) and grapevine peroxidase were measured according to GARCÍA-FLORENCIANO *et al.* (1990), both in the absence and in the presence (50 mg/ml) of soluble polyvinylpyrrolidone (PVP, BDH) pre-incubating, in the latter case, the grapevine protein fraction with PVP for 15 min at 4 °C before the assay of enzymatic activity.

Protein was determined as previously described (GARCÍA-FLORENCIANO *et al.* 1990) using bovine serum albumin as standard.

Extraction, purification and determination of phenolic compounds

For extraction of the phenolics, 1 vol. of culture medium, from which cells were previously removed by filtration, was incubated overnight with 3 vol. MeOH at 4 °C and

the mixture immediately filtered through filter paper. For phenolic analysis, the MeOH water extract described above was immediately filtered through Celite® (BDH) and reduced to near dryness. The brown residue was taken up in water (1 vol) and partitioned into ethyl acetate (1 vol.). The organic phase was dried on anhydric Na_2SO_4 and reduced to near dryness *in vacuo*. Finally, the residue was dissolved in ethyl acetate (0.05 vol.).

Aliquots (300—400 µl) were applied to cellulose TLC plates (Merck, layer thickness 0.5 mm), which were developed in MeOH : water (1:1, v/v). Detection of phenolics on developed TLC plates was by inspection under short wavelength (254 nm) UV light, and by reaction with diazotized p-nitroaniline reagent (Van Sumere *et al.* 1965).

The concentration of total phenolics was measured as described previously (García-Florenciano *et al.* 1990), using chlorogenic acid as standard.

Measurement of conductivity and pH

After the removal of cell material by centrifugation, conductivity of the spent medium was determined at 25 °C using a conductivity meter Crison 525 and a 109803000 standard conductivity cell. The same solutions were used for the measurement of pH.

Results and discussion

Time-course of the release of guaiacol-peroxidase and IAA-oxidase activities to the medium during growth

The changes in guaiacol-peroxidase activity released to the spent medium during the growth cycle of Gamay grapevine cells cultured in suspension are shown in Fig. 1. It can be seen that during the exponential growth phase the total guaiacol-peroxidase activity present in the medium increased gradually and reached maximum values at

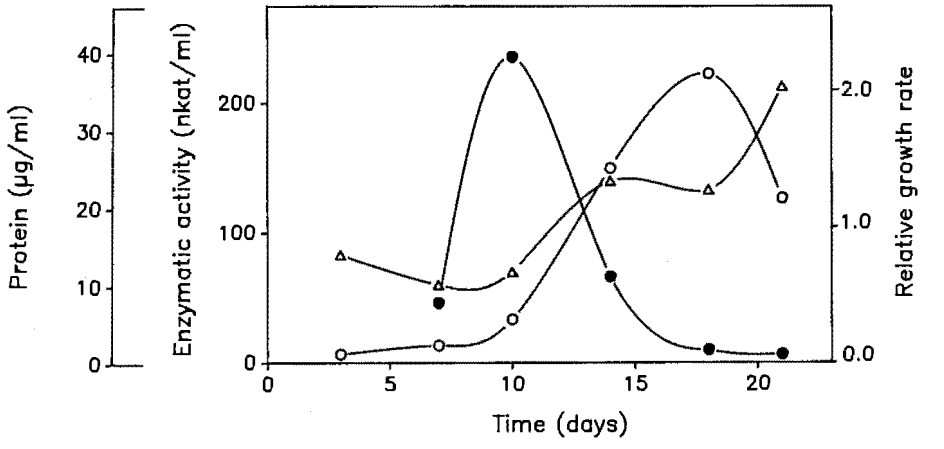

Fig. 1: Changes in the extracellular peroxidase activity (○), protein (△) and relative growth rate (●) during the growth cycle of grapevine cells cultured in suspension. Typical results from five independent experiments are shown.

Veränderungen während des Wachstums suspensionskultivierter Rebenzellen: Aktivität der extrazellulären Peroxidase (○), Protein (△) sowie relative Wachstumsrate (●). Es werden typische Ergebnisse aus fünf unabhängigen Versuchen gezeigt.

the end of the post exponential growth phase. Subsequently, the total guaiacol-peroxidase activity present in the medium decreased. At day 18 of culture, the increase in peroxidase activity was greater than the increase found for the total protein, the increase on a protein basis (specific activity) being 20-fold during this culture period. This suggests that peroxidase is released differentially by the cells to the culture medium, coinciding with the loss of growth potential of the cultured cells.

Like guaiacol-peroxidase activity, IAA-oxidase activity was released to the culture medium during the exponential growth phase of the cultured cells, but maxima levels of IAA-oxidase activity were found at day 15 of the culture (Fig. 2), as opposed to day 18 for peroxidase activity. However, when IAA-oxidase activity released to the spent medium was measured after treatment with PVP, a general increase in the IAA oxidizing activity was found during the whole of the culture period. The maximum for the IAA-oxidase activity now occurred at day 18 of culture similar to guaiacol-peroxidase activity (Fig. 1). This observation suggests that IAA-oxidase activity of peroxidases released to the culture medium is, at least in part, under control of inhibitors of the IAA oxidation, which probably are released to the spent medium during the entire culture period in the same way as peroxidase.

In order to evaluate the extension of the masking of the extracellular IAA-oxidase activity by these inhibitors, *in situ* inhibition of the IAA-oxidase activity was calculated from the ratio of enzymatic activities measured in the absence and in the presence of PVP. The results are shown in Fig. 2 and clearly illustrate that the *in situ* inhibition (in %) of the IAA-oxidase activity varies throughout the culture period and is out of phase with respect to the maximal relative growth rate of the cultured cells. Thus, the smallest degree of inhibition was observed to coincide with the post-exponential growth phase, clearly indicating a smaller inhibition of the enzymatic system responsible for the auxin catabolism during the period in which cell growth begins to cease.

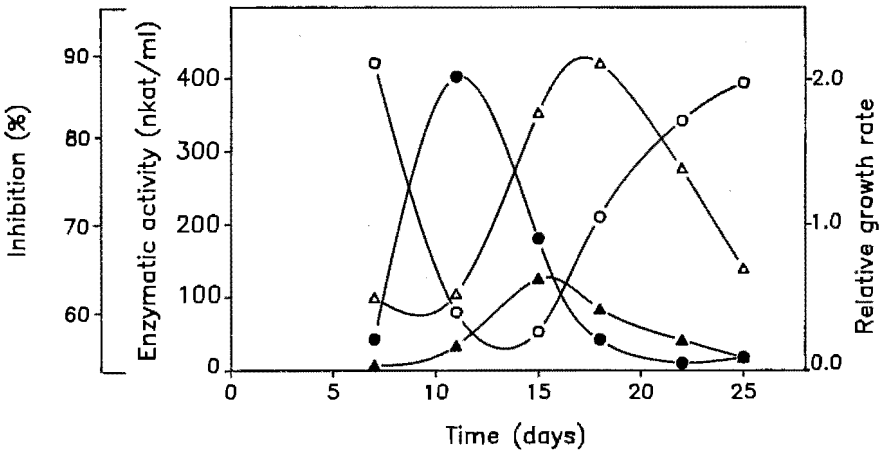

Fig. 2: Changes in the extracellular IAA-oxidase activity measured either in the presence (△) or absence (▲) of polyvinylpyrrolidone, degree of inhibition of extracellular IAA-oxidase activity (○) and relative growth rate (●) during the growth cycle of grapevine cells cultured in suspension. Typical results from five independent experiments are shown.

Veränderungen während des Wachstums suspensionskultivierter Rebenzellen: Aktivität der extrazellulären IAA-Oxidase in Gegenwart (△) und Abwesenheit (▲) von Polyvinylpyrrolidon, Aktivitätshemmung der extrazellulären IAA-Oxidase (○) sowie relative Wachstumsrate (●). Es werden typische Ergebnisse aus fünf unabhängigen Versuchen gezeigt.

This variation in the degree of inhibition of the IAA-oxidase activity associated with the extracellular peroxidases clearly suggests that extracellular auxin catabolism inhibitors are modified as regards their quantity and/or their nature during the whole culture period. Since auxin catabolism inhibitors are regarded as of phenolic nature (STONIER 1972), the heterogeneity of the phenolic fraction released to the medium was

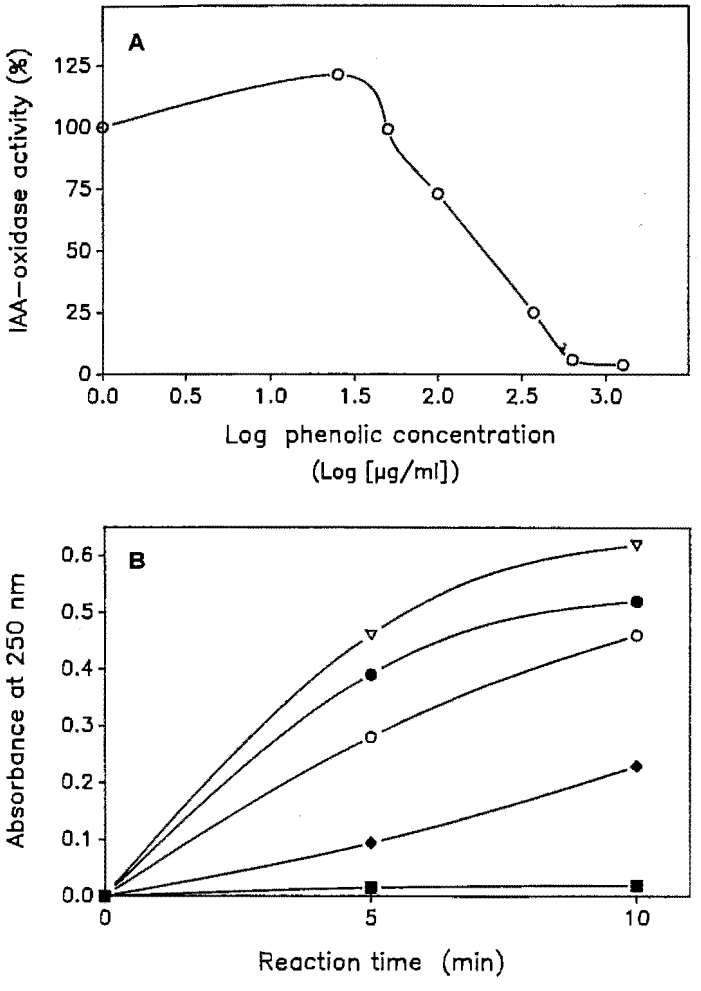

Fig. 3: A) Effect of the concentration of MeOH soluble phenolic compounds extracted from the spent medium of post-exponentially growing grapevine cells on the IAA-oxidase activity of horseradish peroxidase. — B) Time-course of IAA oxidation by horseradish peroxidase in the presence of 25 µg/ml (▽), 100 µg/ml (○), 315 µg/ml (◆) and 1260 µg/ml (■) of total phenolic compounds extracted from the spent medium of post-exponentially growing grapevine cells. Control in the absence of added phenolics (●).

A) Einfluß von MeOH-löslichen Phenolverbindungen auf die IAA-Oxidaseaktivität von Meerrettich-Peroxidase; Extraktion der Phenole aus dem benutzten Medium während der postexponentiellen Wachstumsphase von Rebenzellen. — B) Zeitlicher Ablauf der Oxidation von IAA durch Meerrettich-Peroxidase bei unterschiedlichen Konzentrationen der Gesamtphenole: 25 µg/ml (▽), 100 µg/ml (○), 315 µg/ml (◆) und 1260 µg/ml (■); Extraktion aus dem benutzten Medium während der postexponentiellen Wachstumsphase von Rebenzellen. Kontrolle ohne Phenolzusatz (●).

investigated by TLC analysis. The results illustrate the presence of one phenolic band resolved in chromatograms, with a R_F value of 0.81 in this chromatographic system. Attempts to characterize this phenolic compound were unsuccessful since it was rapidly oxidized during the drying of the plates and lacked a definite UV spectrum, although both the fluorescence at 254 nm and its reaction with diazotized p-nitroaniline support its phenolic nature.

In order to test the ability of this phenolic fraction to inhibit the IAA-oxidase activity, assays were performed as described by STONIER (1972), using a purified horseradish peroxidase fraction. The results are shown in Fig. 3 A, from which can be seen that a concentration as low as 180 µg/ml in the reaction media is capable of reducing the IAA-oxidase activity of horseradish peroxidase in the order of 50 %. This phenolic fraction induces a delay in the time-course of IAA oxidation (Fig. 3 B), characteristic of the compounds called 'auxin protectors' (STONIER 1972). Similar control by phenolic compounds (auxin protectors) of the IAA-oxidase activity of the peroxidases released to the medium of cultured cells has recently been reported in tobacco crown gall suspension cultures (CHIREK 1990). This report as well as our studies support the view that both the release of IAA-oxidizing peroxidases (Fig. 2) and of phenolic compounds are essential elements in the mechanism regulating the auxin level in the spent medium of cultured cells.

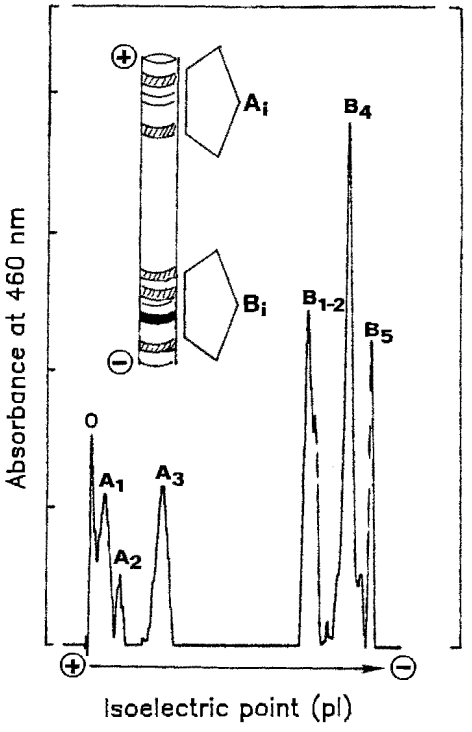

Fig. 4: Isoenzyme pattern of the peroxidase activity present in the spent medium of grapevine cells after 15 d of culture. Zymographic stain of peroxidase isoenzymes was achieved using benzidine as substrate. O: Origin.

Isoenzymmuster der Peroxidaseaktivität im benutzten Medium nach 15tägiger Kultur von Rebenzellen. Die Anfärbung des Enzymogramms erfolgte unter Verwendung von Benzidin als Substrat. O: Start.

Time-course of the expression of peroxidase isoenzymes

In order to check whether the increase in peroxidase activity released to the medium was due to only one isoenzyme or several isoenzymes, the isoenzyme pattern of the peroxidase activity in the medium was investigated. A typical isoperoxidase pattern of the peroxidase present after 15 d of culture is illustrated in Fig. 4. This shows mainly two peroxidase isoenzyme groups, named A_{1-3} and B_{1-5}, partially identical to

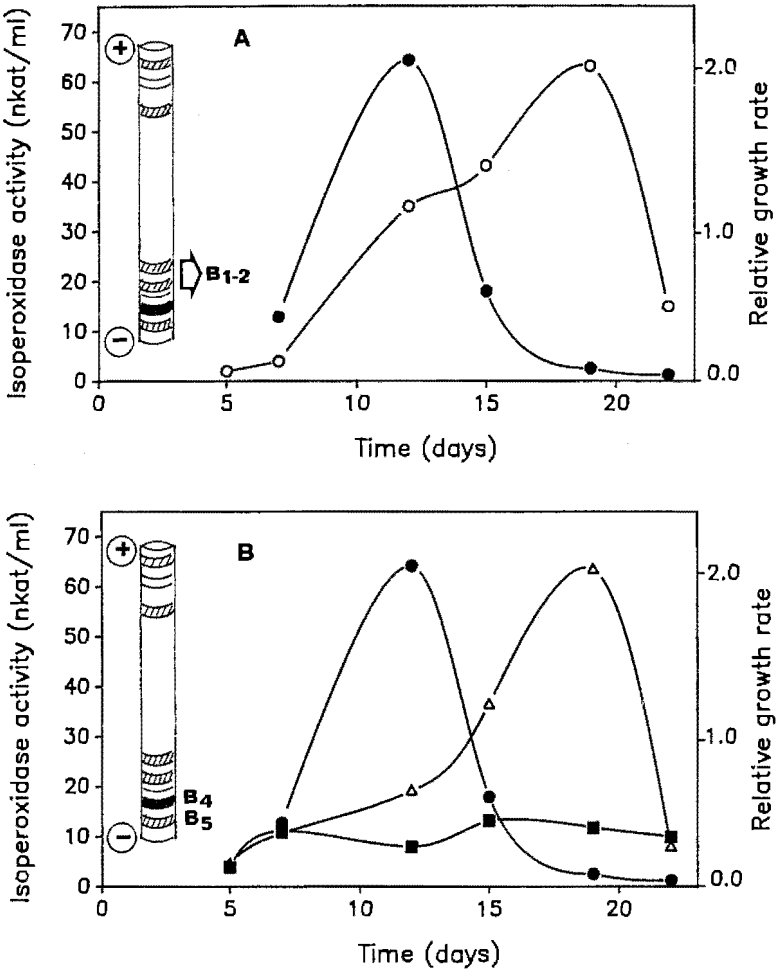

Fig. 5: A) Changes in the level of $B_{1,2}$ (○), and B) changes in the level of B_4 (△) and B_5 (■) basic isoperoxidases located in the spent medium, and changes in the relative growth rate (●) during the growth cycle of grapevine cells cultured in suspension. Inset: Isoenzyme pattern of spent medium peroxidase at day 15 of culture. Typical results from five independent experiments are shown.

Konzentrationsänderungen basischer Isoperoxidasen im benutzten Medium während des Wachstums suspensionskultivierter Rebenzellen sowie Veränderungen der relativen Wachstumsrate (●). — A) Basische Isoperoxidasen $B_{1,2}$ (○), B) Basische Isoperoxidasen B_4 (△) und B_5 (■). Einsatz: Isoenzymmuster im benutzten Medium am 15. Kulturtag. Es werden typische Ergebnisse aus fünf unabhängigen Versuchen gezeigt.

those found in the culture cells (GARCÍA-FLORENCIANO *et al.* 1990). However, some minor difference exists concerning the acidic isoenzyme A_3, which is mainly confined on the culture medium.

Changes in peroxidase isoenzymes released to the spent medium during the growth cycle were analyzed by protein iso-electrofocusing. As occurs with the isoperoxidases located in the cultured cells (GARCÍA-FLORENCIANO *et al.* 1990), the most striking changes in the peroxidase isoenzyme pattern of the spent medium took place during the exponential (7—15 d of culture) and post-exponential (15—23 d of culture) growth phases (Figs. 5 and 6).

Thus, during the exponential growth phase the peroxidase isoenzymes of the B group increased in activity, except for B_5, reaching maximum values at day 18 of culture (Fig. 5). Subsequently, they decreased to the level observed at the beginning of the exponential growth phase. As in the case of the cells (GARCÍA-FLORENCIANO *et al.* 1990), isoperoxidase B_3 was not quantified due to its extremely low level throughout the growth cycle.

In contrast to the general pattern observed for basic isoperoxidases, isoenzymes of the A group, except A_2, are released to the culture medium only during the final stages of the post-exponential growth phase (Fig. 6), and this coincides with the decrease in the level of basic isoperoxidases (Fig. 5). Thus, and in analogy with the findings obtained with suspension-cultured cells of *Capsicum annuum* (CUENCA *et al.* 1989), a sequential release of both basic and acidic isoperoxidases to the media takes place during the late growth phases of the grapevine cells cultured in suspension.

The relative importance of both basic and acidic isoperoxidases released outside the cells in the expression of IAA-oxidizing activity of the spent medium, was investigated by linear regression analysis of the values found for the peroxidase activity associated with each isoenzyme group and the total IAA-oxidase measured in the presence of PVP (Fig. 2). The results show that, although the total level of basic isoenzymes was

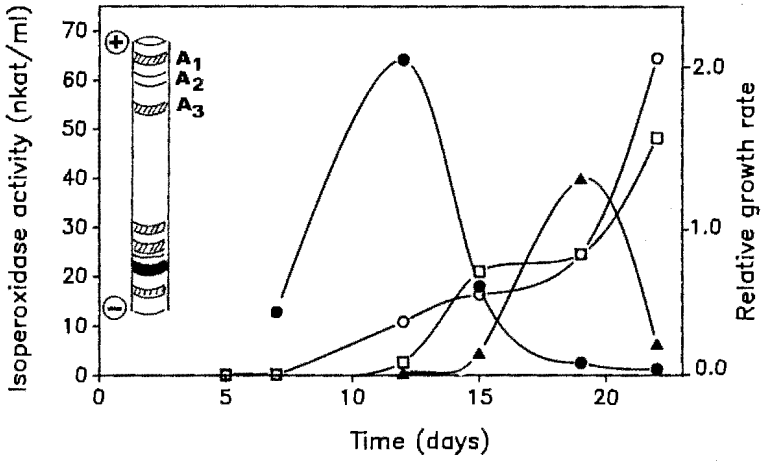

Fig. 6: Changes in the level of A_1 (○), A_2 (▲) and A_3 (□) acidic isoperoxidases located in the spent medium, and changes in the relative growth rate (●) during the growth cycle of grapevine cells cultured in suspension. Inset: Isoenzyme pattern of spent medium peroxidase at day 15 of culture. Typical results from five independent experiments are shown.

Konzentrationsänderungen der sauren Isoperoxidasen A_1 (○), A_2 (▲) und A_3 (□) im benutzten Medium während des Wachstums suspensionskultivierter Rebenzellen sowie Veränderungen der relativen Wachstumsrate (●). Einsatz: Isoenzymmuster im benutzten Medium am 15. Kulturtag. Es werden typische Ergebnisse aus fünf unabhängigen Versuchen gezeigt.

well correlated with the level of IAA-oxidase activity ($r_s = 0.832$, statistically significant at $P < 0.05$) — in contrast to that correlation found for acidic isoenzymes ($r = 0.651$) — the sum of the activity associated with both basic and acidic isoenzymes gives a better correlation ($r = 0.933$, statistically significant at $P < 0.05$ or better). This graphical analysis suggests that although basic isoperoxidases released to the medium are apparently the main responsibles of the IAA-oxidizing activity of the outside peroxidase, a partial involvement of acidic isoperoxidases in this compartmentalized auxin catabolism cannot be discounted.

On the other hand, and due to the clearly understood role of acidic isoperoxidases in the stiffening of the cell wall (LAGRIMINI *et al.* 1990), the abrupt increase in the extracellular acidic isoperoxidases found at the end of the post-exponential growth phase (Fig. 6) seems more related to the irreversible loss of growth potential of the culture cells through the lignification of the cell wall, than to any other physiological role.

Nature of the release of basic isoperoxidases to the culture medium

The release of the putative IAA-oxidases (i.e., basic isoperoxidases) to the culture medium during the exponential growth phase may be the consequence either of i) a detachment from the cell walls of the most basic isoenzymes or ii) an active secretion by the cultured cells (STICHER *et al.* 1981; VAN HUYSTEE and TAM 1988).

In order to test the first hypothesis and taking into account that binding of peroxidase to cell walls is only dependent on both the ionic force and the external pH (ROS BARCELÓ *et al.* 1988), the pH values and the conductivity (indicative of the ionic force) of the spent medium of the cultured cells was followed during the culture period. The results shown in Fig. 7 illustrate that neither the changes in pH nor in conductivity of the medium were enough to explain the massive release of basic isoperoxidases to the culture media during the exponential and post-exponential growth phases. In fact, as has been observed for other cell suspension cultures (HAHLBROCK *et al.* 1974), the conductivity values decreased during the culture period (Fig. 7), which would be in dis-

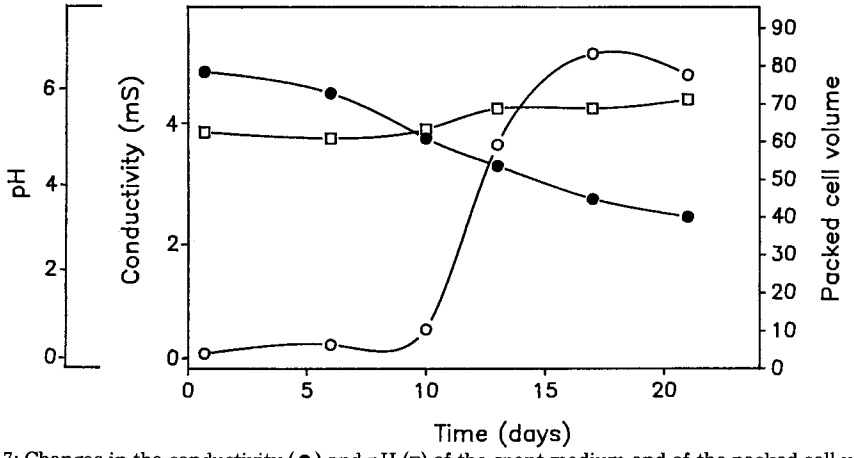

Fig. 7: Changes in the conductivity (●) and pH (□) of the spent medium and of the packed cell volume (○) during the growth cycle of grapevine cells cultured in suspension. Typical results from two independent experiments are shown.

Veränderungen der Leitfähigkeit (●) und des pH (□) im benutzten Medium sowie des Volumens der sedimentierten Zellen (○) während des Wachstums suspensionskultivierter Rebenzellen. Es werden typische Ergebnisse aus zwei unabhängigen Versuchen gezeigt.

agreement with a release of basic isoperoxidases from the cell walls provoked by an increase in the ionic force of the spent medium.

The results obtained from subcellular fractionation studies suggested the same. Thus, the basic isoperoxidase activity bound to cell walls at day 15 of culture (0.95 ± 0.06 nkat/ml cell suspension culture) did not differ significantly from that found at day 18 of culture (1.0 ± 0.04 nkat/ml cell suspension culture), neither did their isoenzyme pattern differ significantly (Fig. 8). This is in contrast with the fact that for the same culture period the basic isoperoxidase activity located in the spent medium increased from 109 to 135 nkat/ml of cell suspension culture.

All these observations and the fact that the increase in the spent-medium basic isoperoxidase takes place subsequently to the decrease in the level of the basic isoperoxidases located in the cultured cells (GARCÍA-FLORENCIANO *et al.* 1990) suggest, as pointed out by other authors (STICHER *et al.* 1981), an active secretion of these isoenzymes by the cultured cells. Identical conclusions can be obtained for the release of acidic isoperoxidases from the cultured cells to the spent medium at the end of the post-exponential growth phase (Fig. 6).

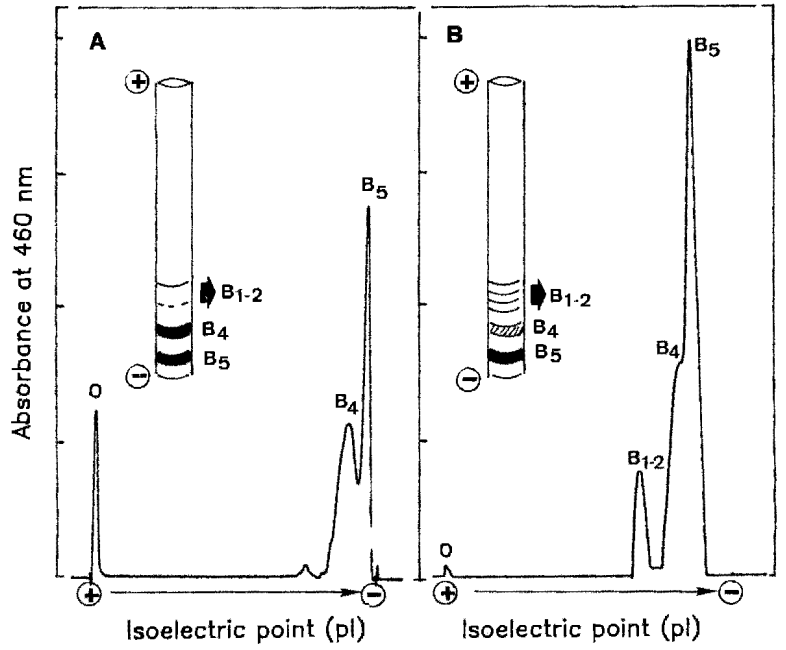

Fig. 8: Isoenzyme pattern of peroxidase activity located in cell walls of 18 d old (A) and 15 d old (B) suspension cultured cells, with their corresponding densitometric tracing. Zymographic stain of peroxidase isoenzymes was achieved using benzidine as substrate. O: Origin.

Isoenzymmuster der Peroxidaseaktivität in den Wänden 18 d alter (A) und 15 d alter (B) suspensionskultivierter Zellen sowie die zugehörigen densitometrischen Aufzeichnungen. Die Anfärbung der Enzymogramme erfolgte unter Verwendung von Benzidin als Substrat. O: Start.

Nature of the decay of basic isoperoxidases in the culture medium

Finally, two possible reasons were studied to explain the decay of basic isoperoxidases in the culture medium after day 18 of culture (Fig. 5): i) the deprivation of Ca^{+2} ions (MORENO *et al.* 1989) and ii) the binding of basic isoperoxidases to cell walls (MADER *et al.* 1981; BREDEMEIJER and BURG 1986).

Ca^{+2} deprivation, which is known to reversibly inactivate extracellular peroxidase activity of suspension cultures (MORENO *et al.* 1989), does not seem to be responsible for the decay of basic isoperoxidases, since the assay of medium peroxidase activity at day 23 of culture in the presence of 5 mM of $CaCl_2$ did not increase the activity (100 nkat) with respect to the control (125 nkat/ml cell suspension culture). Rather, it led to an inhibition of the enzymatic activity, a fact that has also been observed for peroxidases extracted from grapevine cells (data not shown).

Similarly, the binding of basic isoperoxidases to cell walls to explain the decay in their levels at day 23 of culture was also discarded. Although basic isoperoxidases from day 18 of culture can be selectively attached to cell walls of day 23 of culture (Fig. 9), both the binding capacity of 23 d-old cell walls (0.08 nkat/cell wall pellet equivalent to 1.0 ml of cell suspension culture) and the levels of the enzyme *'in muro'* (1.2 nkat/ml of 23 d-old cell suspension culture), obtained by subcellular fractionation studies, were insufficient to explain a decrease in the medium of 98 nkat of basic isoperoxidase activity/ml cell suspension culture.

Thus, an irreversible inactivation of the most basic peroxidase isoenzymes during the oxidation of IAA (NAKAJIMA and YAMAZAKI 1980) seems to be the mechanism which can best explain the decay of the extracellular basic isoenzymes. In this sense, auxin protectors, as participating in the auxin-sparing mechanism, might also protect the extracellular enzyme during the phases of active cell growth.

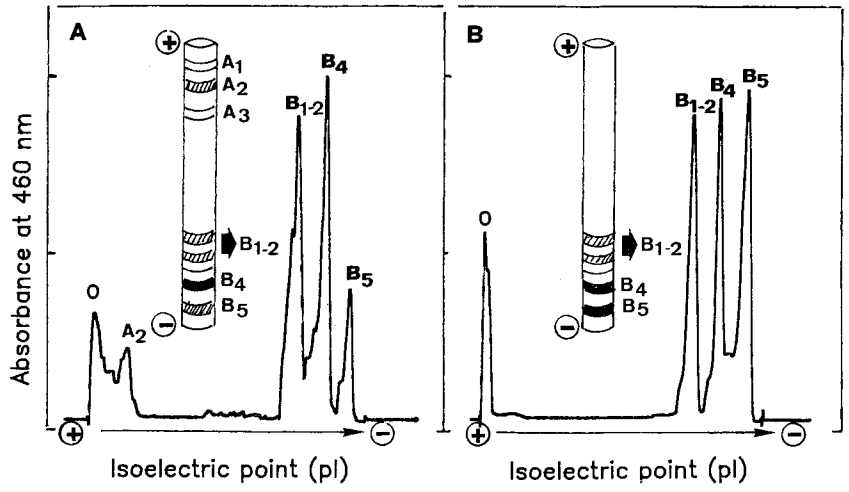

Fig. 9: Isoenzyme pattern and densitometric tracing of spent medium peroxidase harvested at day 18 of culture, found in supernatants (A), and attached to cell walls (B) after the binding assay of spent medium peroxidase to 23 d old cell wall fractions. Zymographic assay of peroxidase isoenzymes was achieved using benzidine as substrate. O: Origin.

Isoenzymmuster und densitometrische Aufzeichnungen der Peroxidase im benutzten Medium am 18. Kulturtag. — A) Im Überstand des Kulturmediums. B) Peroxidase aus dem benutzten Medium an die Zellwandfraktion 23 d alter suspensionskultivierter Zellen gebunden. Die Anfärbung der Enzymogramme erfolgte unter Benutzung von Benzidin als Substrat. O: Start.

Summary

The several factors which control extracellular auxin catabolism were studied in grapevine (*Vitis vinifera* cv. Gamay), using cells cultured in suspension as a model system. From the results, it is concluded that, contrarily to previous observations with cul-

tured cells, auxin catabolism in the spent medium depends on a massive sequential secretion of both basic and acidic isoperoxidases. It also depends on the secretion of phenolic compounds, characterized as of an 'auxin protector' nature, which modulate the IAA-oxidase activity of the extracellular isoperoxidases throughout the culture period. Thus, unlike observations of peroxidase activities, IAA-oxidase activities are expressed mainly during the beginning of the post-exponential growth phase of the cultured cells, giving to this highly compartmentalized catabolic process an apparent central role in the depletion of the IAA content, once the cultured cells have stopped growing.

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