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GIBBERELLIC ACID STIMULATION OF ISOPEROXIDASE FROM PEDICEL OF GRAPE

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Key Word Index—grapes; peroxidase; gibberellic acid; pedicel; Thompson seedless; *Vitis vinifera*; Vitaceae.

Abstract—Soluble peroxidase activity from pedicels of cv. *Thompson* seedless grape was highly stimulated by post-bloom gibberellic acid (GA_3) applications to vines. This stimulation was dependent on the dose employed and increased with berry ripening. In control vines, i.e. non GA_3 treated, the level of peroxidase activity remained constant during berry ripening. Isoelectrofocusing (IEF) analysis of soluble peroxidases from pedicel extracts performed in a 3–10 pH range, showed the presence of a main band that corresponded to an acidic isoperoxidase of (pI 3.5) and the presence of a minor band of (pI 3.9). It is a well-known fact that GA_3 applications to cv. *Thompson seedless* grape beside producing berry enlargement cause berry-drop which has been associated with pedicel thickening. Here we found a positive correlation between pedicel thickness and peroxidase activity within the rates of GA_3 used. The involvement of the acidic isoperoxidases in pedicel thickening and its relation to berry-drop are discussed.

INTRODUCTION

Most seedless table grapes, specially cv. *Thompson seedless*, require gibberellic acid (GA_3) applications for adequate berry size in order to satisfy market requirements [1, 2]. However, GA_3 treatments also produce severe berry-drop from ripe bunches during and especially after harvesting [3, 4]. These combined effects of GA_3 treatments on cv. *Thompson seedless* constitute a great issue for growers, since they must determine the exact concentration and date of GA_3 applications at which the damage—berry drop—begins to overcome the added benefits—larger berries [3].

Berry-drop caused by GA_3 applications has been related to the hardening and thickening of pedicels. Fidan *et al.* [5] observed that cellulose accumulation and lignification of the pedicel are processes associated with berry-drop in *Thompson seedless*. Nakamura and Hori [6], showed that the hardening of the rachis and pedicel were related to pedicel thickening, which in turn, originates from the increase in the number of secondary xylem cells and their lignification. More recently, Botti and Cooper [7], reported that GA_3 generates histological changes in the pedicel and that these changes correspond to increases in the number of cells of the cortex, xylem and pith.

Plant peroxidases have been related to several processes such as cell growth [8] cell differentiation [9] response to pathogens [10] and lignification [11]. Biochemical studies of plant peroxidases are complicated by the large number of similar isoenzymes present normally in vegetable tissues and the even greater number of potential substrates. Peroxidases from grapes have been studied mainly in berries. Thus, the pattern of isoenzymes in berries from *Vitis vinifera* cv. Gamay was reported to be simple, with the presence of a major basic isoenzyme [12]. This basic peroxidase isoenzyme is located in the vacuole and has been postulated to play a role in the anthocyanin degradation pathway [12] and in indole-3-acetic acid catabolism [13]. In this paper, we studied the effect of post-bloom GA_3 applications on soluble peroxidases from the pedicel of c.v. *Thompson seedless* grape and discuss the role of the acidic peroxidases in pedicel thickening, and its relation to berry-drop.

RESULTS AND DISCUSSION

Effect of GA_3 on soluble peroxidase activity from pedicel of Thompson seedless *grape*

Soluble peroxidase activity from pedicels of cv. *Thompson seedless* grapes was highly stimulated by

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post-bloom gibberellic acid (GA_3) application to vines. The activity increased with the dose and with the development stages of the berries (Fig. 1). Thus, during all berry maturation processes the maximum stimulation was reached at a rate of 180 ppm of GA_3 (Fig. 1). However, at earlier berry development stages, 28 days after flowering (d.a.f), the maximum stimulation at this rate was 6.5 fold, while in berries of 60 d.a.f (*véraison*) the stimulus increased to 35 fold, after that time no further increases in activity were detected. But, at lower rates of applied GA_3 30, 60 and 90 ppm, the peroxidase activity rose continuously between fruit set and harvest (Fig. 1). In control plants (untreated with GA_3), no significant changes in activity were observed during berry ripening (Fig. 1). Therefore, pedicel peroxidases from cv. *Thompson seedless* respond to GA_3 in a time dependent way, while their activity remains constant in non GA_3 treated plants.

It is a well known-fact that GA_3 applications to cv. *Thompson seedless* for improving berry size, exacerbate pedicel thickening during berry ripening [3, 4]. Pedicel thickening induced by GA_3 has been associated with an increase in the number of cells from the xylem and with an extension of the lignification processes in this tissue [4, 5]. In this work, we found a positive correlation between pedicel diameter and peroxidase activity within the rates of GA_3 employed (Fig. 2), suggesting that pedicel thickening induced by GA_3 could be mediated by peroxidase. Peroxidase is thought to catalyse the formation of phenolic cross-links between cell wall polymers, and the subsequent deposition of lignin [8, 11]. Furthermore, it has been suggested that peroxidases involved in these processes are of an acidic nature [11]. Since peroxidase activity found in the pedicel of cv. *Thompson seedless* grape responds to GA_3 applications, correlates with pedicel thickening, and that strong evidence supports the involvement of acidic isoenzymes in lignification [11, 15], it was of interest to analyse the pattern of isoenzymes from pedicels of cv. *Thompson seedless* grape by isoelectric focusing (IEF).

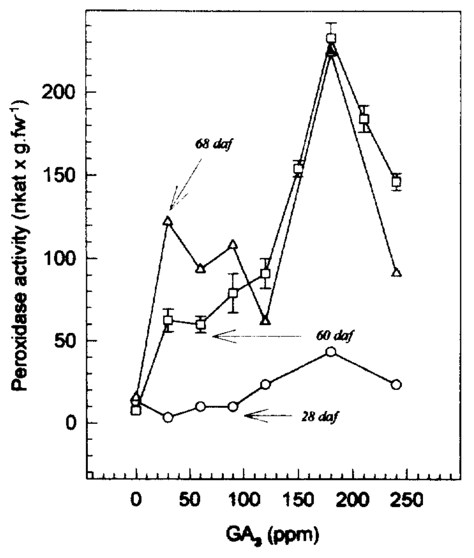

Fig. 1. Effect of different rates of post-bloom GA_3 applications to cv. *Thompson seedless* grape on soluble peroxidase activity from pedicels, measurements were made at 28 (○), 60 (□) and 68 (△) days after flowering.

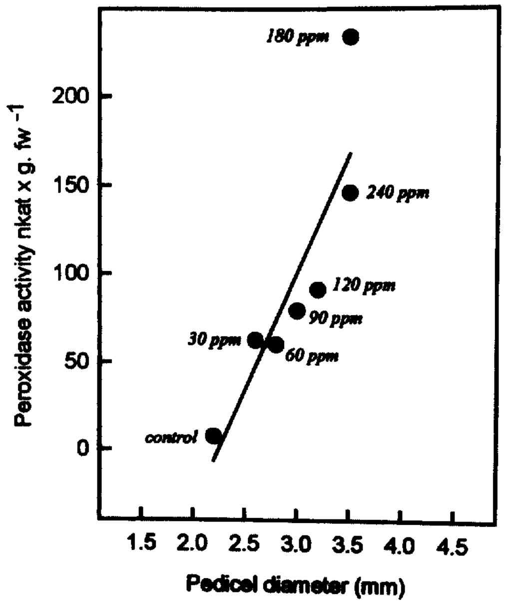

Fig. 2. Correlation between pedicel diameter and peroxidase activity in pedicels of cv. *Thompson seedless* grape submitted to different rates of post-bloom GA_3 applications. The measurements were carried out at harvest and the rates of applied GA_3 were 30, 60, 90, 120, 180 and 240 ppm.

Isoelectric-focusing analysis of soluble peroxidases from pedicel of Thompson seedless *grape*

Soluble fractions of pedicel extracts corresponding to bunches of vines treated with different rates of post-bloom GA_3 and collected at harvest were desalted, concentrated and submitted to isoelectric focusing in the 3–10 pH range. Peroxidase activities were revealed on the gel with a mixture of *o*-PDA and 4-methoxy-naphthol. The coloration with *o*-PDA was strong, but disappeared with time, while with 4-methoxy-naphthol it was weak but permanent, hence both reagents were used simultaneously. The results, showed a simple pattern of isoenzymes with one main band corresponding to an acidic isoenzyme of (pI 3.5) and a second weak band of (pI 3.9). Control samples corresponding to pedicels from untreated GA_3 vines, did not give a reaction, probably because their activity was too small (Fig. 3). The presence of acidic and the absence of basic and neutral isoenzymes in pedicel extracts, supports the hypothesis that acidic peroxidase isoenzymes are related to lignin biosynthesis, and offer a good opportunity to study in detail this enzyme in terms of substrate specificity, subcellular localisation and physiological role.

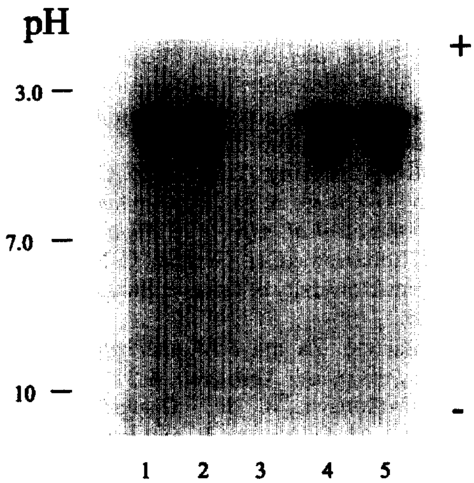

Fig. 3. Isoelectrofocusing (IEF) of soluble extracts from pedicels of cv. *Thompson seedless* grape submitted to different post-bloom GA_3 applications. Lane 1 (60 ppm), lane 2 (120 ppm), lane 3 (control), lane 4 (180 ppm), lane 5 (240 ppm).

A literature survey [1–3] indicated that berry enlargement in cv. *Thompson seedless* grape is controlled by GA_3 treatments, and that this effect is accompanied by increased berry-drop. At high GA_3 rates, berry-drop is severe and is associated with pedicel thickening; in fact under these conditions pedicels lost elasticity and became very rigid, and a light pressure on the berries was enough to break the connection between them and the berries. Therefore, since our results suggest that pedicel thickening induced by GA_3 applications could be mediated by peroxidases, a possible strategy to avoid berry-drop in cv. *Thompson seedless* grape, could be the inhibition of the acidic isoenzyme activity of pedicels by means of specific inhibitors, or the manipulation of the peroxidase isoenzyme gene for the purpose of reducing its expression caused by GA_3 in pedicels of transformed plants.

EXPERIMENTAL

GA_3 applications to vines

The study was performed using plant material from 8-year-old cv. *Thompson seedless* vines, located at the Agricultural Experimental Station La Platina (INIA) Región Metropolitana, Santiago, Chile. All vines received the same pre-bloom handling, i.e. two pre-bloom GA_3 applications, one for bunch elongation (30 ppm $= 2 \times 15$ interval 1 day) applied 2 weeks before bloom and the other for bunch thinning (40 ppm $= 2 \times 20$ interval 1 day) applied at full bloom. Treatments consisted of different rates of post-bloom GA_3 applied, 3 plants were selected for each treatment. Vines were sprayed twice 2 l/plant at 1 day intervals at the stage of 2–5 mm berry dia. The spraying solns contained adequate amounts of GA_3 to achieved the desired final concn; no wetting agents were added to these solns. Between 6 and 8 different GA_3 concns, ranging between 30 and 240 ppm (final concns) were applied, whereas control vines did not receive post-bloom GA_3 applications. Samples were collected at 3 different berry developmental stages 28, 60 and 68 days after bloom that corresponded to 13, 53 and 61 days after the last GA_3 spray. In each sampling date, 50 berries per treatment with pedicel attached were selected randomly from bunches pertaining to different vines.

Pedicel extracts. Pedicels of ca 2 g excised from berries collected at different developmental stages and belonging to different treatments, were homogenised with 2 times their fr. wt in Tris-HCl buffer (50 mM, pH 7.2) containing EDTA (0.2 mM), 2-mercaptoethanol (5 mM) and PVP (2%, w/v). Acid sand was used as an abrasive. The homogenate was filtered through 4 layers of muslin and centrifuged at 2500 *g* for 5 min at 4°. The supernatant was treated with Triton X-100, a white ppt. was obtained and discarded by centrifugation at 5000 *g* for 5 min at 4°. The clear supernatant was used for measuring peroxidase activity.

Measurements of peroxidase activity. Peroxidase (EC 1.11.1.7) activity was assayed in a double-beam spectrophotometer (Shimadzu UV 240) at 25° by a continuous method. All reactions were carried out in a cuvette (1 ml) that contained 2 μl of 2% H_2O_2, 200 μl of 0.22 M *o*-phenylenediamine (*o*-PDA) in citrate buffer 0.1 M pH 4.5., the reaction was started by the addition of 10 μl of pedicel extract. The activity was measured by following the *A* changes at 450 nm. The apparent *Km* for *o*-PDA was 28.2 mM. No substrate oxidation was found in the absence of H_2O_2. Activities were expressed in nkat (nmol of oxidised *o*-PDA per sec) per g of pedicel fr. wt.

Isoelectrofocusing.

IEF was performed in 5% horizontal polyacrylamide gels using the Bio-Rad 111 mini IEF chamber system according to the manufacturer's instructions. The range of pH was 3 to 10 using 2% of commercially available ampholytes (Pharmalytes, Pharmacia LKB). After completion of IEF, peroxidases were detected using a two substrate colour test. Firstly, gels were incubated in a soln containing H_2O_2 0.2% and 4-methoxy-naphthol in buffer 0.1 M citrate pH 4.5 and after 15 min a soln of 2 mM *o*-PDA was added, in this way, the stabilisation of the coloration was achieved. Prior to runs, pedicel extracts were desalted on a PD-10 column (Pharmacia) and concd in (Centricon tubes, Amicon, cut off 10,000).

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