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Extracellular H_2O_2 Induced by Oligogalacturonides Is Not Involved in the Inhibition of the Auxin-Regulated *rolB* Gene Expression in Tobacco Leaf Explants[1]

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α-1,4-Linked oligogalacturonides (OGs) inhibit auxin-regulated transcriptional activation of a *rolB*-β-glucuronidase (*GUS*) gene fusion in tobacco (*Nicotiana tabacum*) leaf explants (D. Bellincampi, M. Cardarelli, D. Zaghi, G. Serino, G. Salvi, C. Gatz, F. Cervone, M.M. Altamura, P. Costantino, G. De Lorenzo [1996] Plant Cell 8: 477–487). In this paper we show that inhibition by OGs is very rapid, with a short lag time, and takes place even after *rolB* promoter activation has initiated. OGs also induce a transient and catalase-sensitive accumulation of H_2O_2 in the leaf explant culture medium. OGs with a degree of polymerization from 12 to 15 are required for both the inhibition of the auxin-induced *rolB*-driven accumulation of GUS and the induction of H_2O_2 accumulation. However, OG concentration for half-maximal induction of H_2O_2 accumulation is approximately 3-fold higher than that for half-maximal inhibition of *rolB* promoter activity. The inhibition of *rolB* promoter activity is not influenced by the addition of catalase or superoxide dismutase, suggesting that H_2O_2 and superoxide are not involved in this effect. A fungal oligo-β-glucan elicitor induces extracellular H_2O_2 accumulation at comparable or higher levels than those observed with OGs, but does not prevent the auxin-induced accumulation of GUS. We conclude that H_2O_2 produced upon treatment with OGs is not involved in the inhibition of the auxin-induced expression of the *rolB* gene.

α-1,4-Linked oligogalacturonides (OGs) are well-known elicitors of plant defense responses derived from the pectic component of the plant cell wall (for review, see Côté and Hahn, 1994; De Lorenzo et al., 1994, 1997; Cervone et al., 1997). OGs also regulate growth and developmental processes in plants: for example, they inhibit auxin-induced cell elongation (Branca et al., 1988), stimulate flower formation (Marfà et al., 1991), regulate morphogenesis in thin-cell-layer explants (Eberhard et al., 1989), inhibit root formation induced by auxin (Bellincampi et al., 1993, 1996), and induce stoma and pericycle cell differentiation in tobacco (*Nicotiana tabacum*) leaf explants (Altamura et al., 1998). Interestingly, in most of these processes OGs antagonize the action of auxin. The interplay between OGs and auxin is very intriguing and may have important implications in both defense and development.

Because, like the majority of the defense-related effects, the developmental effects exerted by OG require a degree of polymerization (DP) between 10 and 15, with shorter OGs being inactive (Bellincampi et al., 1994), the question arises as to whether their capability to counteract the action of auxin is related to their ability to induce defense responses.

The earliest detectable responses induced by OGs are the activation of specific ion channels leading to influx of H^+ and Ca^{2+} and efflux of K^+ (Messiaen and Van Cutsem, 1994; Mathieu et al., 1998; Spiro et al., 1998), and the transient formation of reactive oxygen species (ROS) such as O_2^-, H_2O_2, and ·OH (Svalheim and Robertsen, 1993; Levine et al., 1994). How OGs cause the generation of ROS is not known. The burst of H_2O_2 production at the plant cell surface is known to drive rapid peroxidase-mediated, oxidative cross-linking of structural components of the cell wall, thereby reinforcing this physical barrier with implications in both defense and development (Brisson et al., 1994). While a large and rapid generation of ROS is likely to exert a severe oxidative stress on the plant cell, low doses of ROS may act within the signal transduction pathways downstream of the membrane-associated reactions, with the nucleus as a major target for the modulation of specific gene expression (Lamb and Dixon, 1997; Lander, 1997; Yang et al., 1997). So far, the rapid and transient generation of ROS induced by OGs has not yet been correlated with any of their observed developmental effects.

We have previously reported that OGs prevent the adventitious root formation induced by auxin in leaf explants of transgenic tobacco plants carrying the *rolB* gene of *Agrobacterium rhizogenes*, because they inhibit the auxin-induced activation of the *rolB* promoter (Bellincampi et al., 1996). In this work we have investigated whether, in leaf explants from transgenic tobacco plants carrying a *rolB*-β-glucuronidase (GUS) gene fusion (Capone et al., 1991), production and accumulation of extracellular H_2O_2 induced by OGs is critical for inhibition of the *rolB* gene expression. Our results show that in tobacco leaf explants, extracellular H_2O_2 is not involved in the inhibition of the auxin-regulated expression of the *rolB* gene.

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MATERIALS AND METHODS

Elicitor Preparation

OGs with a DP from 1 to 18 were generated by partial digestion of polygalacturonic acid (Na^+ salt) with homogeneous *Aspergillus niger* endopolygalacturonase, and the mixture of different sized OGs was separated to obtain two preparations of OGs with DPs of 1 to 8 and 9 to 18, respectively, as previously described (Hahn et al., 1991). Size-homogeneous OGs were prepared by high-performance anion-exchange chromatography (Dionex, Sunnyvale, CA) on a semipreparative CarboPac PA1 column (9 × 250 mm) and analyzed by high-performance anion-exchange chromatography-pulsed-amperometric detection, as described by Spiro et al. (1993). The DPs of the individual OGs were determined by comparison of their retention time with those of the standard OGs whose DPs had been determined by fast-atom-bombardment mass spectrometry (Marfà et al., 1991). Heterodispersed oligoguluronides enriched in oligomannuronides (β-1,4-oligo-D-mannosyluronic acid) were prepared as described previously (Marfà et al., 1991). *Phytophthora megasperma* f.sp. *glycinea* void glucan elicitor (Cheong et al., 1991) was a kind gift of Prof. M.G. Hahn (Complex Carbohydrate Research Center, University of Georgia, Athens). OGs, oligomannuronides, and glucan were dissolved in distilled water at a concentration of 1 mg/mL, and sterilized by filtration through membranes (pore size 0.2 μm, Millipore, Bedford, MA).

Plant Material

Tobacco (*Nicotiana tabacum* L. cv Petit Havana SR1) plants harboring the GUS gene fused to the *rolB* promoter (B1185-GUS) (Capone et al., 1991) were a kind gift of Prof. Paolo Costantino (University of Rome "La Sapienza"). Plants were routinely propagated by cuttings and grown aseptically on Murashige and Skoog (1962) medium (Sigma Chemical, St. Louis) supplemented with 20 g/L Suc (basal medium) and 10 g/L Oxoid agar (Unipath, Hampshire, UK) in Magenta boxes at 25°C under a 16-h illumination period as previously described (Bellincampi et al., 1993).

Leaf Explants

Leaves about 5 cm long from plants 4 weeks after propagation were selected. The two apical leaves and the two basal ones were excluded. Explants (rectangles of 2 × 4 mm) with the midrib medially placed were excised from leaves. For each experiment, 10 leaf explants (corresponding to about 100–150 mg of fresh weight) were placed in Petri dishes containing 1 mL of basal medium supplemented with indole-3-acetic acid (IAA). OGs, oligomannuronides, and glucan were added to the explants at the concentrations indicated in "Results"; thymol-free catalase (EC 1.11.1.6) (Sigma) with a specific activity of 25,000 units/mg (416 μkat/mg), and superoxide dismutase (SOD) (EC1.15.1.1) (Sigma) with a specific activity of 3,000 units/mg were used. Upon addition of these compounds or enzymes, explants plunged in the incubation medium were vacuum infiltrated for 30 s, and subsequently incubated for different times at 25°C under slow shaking (25 rpm) in the dark.

GUS Assay

GUS activity was estimated, as described previously (Jefferson, 1987), from a pool of 20 leaf explants and expressed as picomoles of 4-methylumbelliferone per milligram of protein per minute. Protein content was determined by the method of Bradford (1976) using a commercial protein assay kit (Bio-Rad, Milan) using bovine serum albumin as a standard. Each data point is the mean (±SD) of three independent experiments (in each experiment, two replicates of 10 explants were used for each treatment).

Assay for H_2O_2

H_2O_2 concentration in the incubation medium of treated leaf explants was measured by the FOX1 method (Jiang et al., 1990; Wolff, 1994), based on the peroxide-mediated oxidation of Fe^{2+}, followed by the reaction of Fe^{3+} with xylenol orange (*o*-cresolsulfonephthalein 3′,3″-bis[methylimino] diacetic acid, sodium salt; Farmitalia Carlo Erba, Milan). This method is extremely sensitive and used to measure low levels of water-soluble hydroperoxide present in the aqueous phase; the apparent extinction coefficient obtained for H_2O_2 in the reaction conditions described below is 2.2×10^5 M^{-1} cm^{-1}. To determine the H_2O_2 concentration, 500 μL of the incubation medium was added to 500 μL of assay reagent (500 μM ammonium ferrous sulfate, 50 mM H_2SO_4, 200 μM xylenol orange, and 200 mM sorbitol). Absorbance of the Fe^{3+}-xylenol orange complex (A_{560}) was detected after 45 min. The specificity for H_2O_2 was tested by eliminating H_2O_2 in the reaction mixture with catalase. Standard curves of H_2O_2 were obtained for each independent experiment by adding variable amounts of H_2O_2 to 500 μL of basal medium mixed to 500 μL of assay reagent. Data were normalized and expressed as μM H_2O_2 per gram of fresh weight of explants. Each data point was the mean (± SE) of three independent experiments.

Statistics

All statistical tests were performed using ANOVA. Statistical significance of differences was evaluated by *P*-level.

RESULTS

OGs Rapidly Inhibit IAA-Induced Activity of the *rolB* Promoter in Tobacco Leaf Explants

We have previously shown that GUS activity is induced by IAA in leaf explants from transgenic tobacco plants harboring the reporter gene encoding GUS under the control of the *rolB* promoter (B1185-GUS plants). Auxin-induced GUS activity is inhibited by OGs (Bellincampi et al., 1996).

To determine the time by which OGs have to be added in order to inhibit the *rolB* promoter activity, a time course

experiment was carried out. Leaf explants from B1185-GUS plants (*rolB*-GUS explants) were incubated in basal medium containing 0.6 μM IAA, and OGs (DP of 9–18) were added at a final concentration of 10 μg/mL at the beginning of the incubation (time 0) and at the times indicated in Figure 1. The total length of the experiment was 24 h, and GUS activity was measured both at the time of OG addition and at the end of the experiment. GUS activity increased only after 10 h of incubation in the presence of IAA, confirming the requirement of a long lag time for GUS induction (Bellincampi et al., 1996). When OGs were added during the first 16 h of incubation, GUS activity measured at 24 h was not significantly different from that at the time of OG addition. However, when added at 18 h, OGs were not able to totally inhibit auxin-induced GUS expression (Fig. 1). The observation that no significant increase in GUS activity occurs upon OG addition up to 16 h suggests that the effect of OGs is very rapid, with a very short lag time.

OGs Rapidly Elicit a Transient and Catalase-Sensitive Accumulation of H_2O_2

Because OGs have been shown to induce H_2O_2 in several plant tissues and cells (Legendre et al., 1993; Svalheim and Robertsen, 1993; Levine et al., 1994; Mathieu et al., 1998; Spiro et al., 1998; Stennis et al., 1998; Orozco-Cardenas and Ryan, 1999), we determined whether this response also occurred in *rolB*-GUS explants. *Rol*B-GUS explants were cultured in the presence of IAA (0.6 μM), OGs (DP of 9–18; 10 μg/mL), or water (controls) added at time 0 or 8 h later. In both cases, OGs caused a transient extracellular accumulation of H_2O_2. When OGs were added at time 0, accumulation of H_2O_2 was biphasic: levels increased after 15 min, and reached a first maximum (30 μM) by 2 h, followed by a second, more pronounced maximum (48 μM) at 6 to 8 h. Concentration of H_2O_2 declined to basal levels after 8 h (Fig. 2A). When OGs were added at 8 h, the H_2O_2 concentration was 75 μM 1 to 2 h after addition, and about 120 μM after 3 to 4 h; a decline to basal levels occurred after 6 h (Fig. 2B). In both cases, basal levels of H_2O_2 were measured at the end of the experiment (24 h). H_2O_2 accumulation was strongly reduced by catalase (2 μkat/mL) (Fig. 2, A and B). In contrast, short OGs (DP of 1–8; 10 μg/mL) and a mixture of oligomannuronides (DP of 9–20; 10 μg/mL) did not induce H_2O_2 above the levels observed when explants had been incubated with IAA alone.

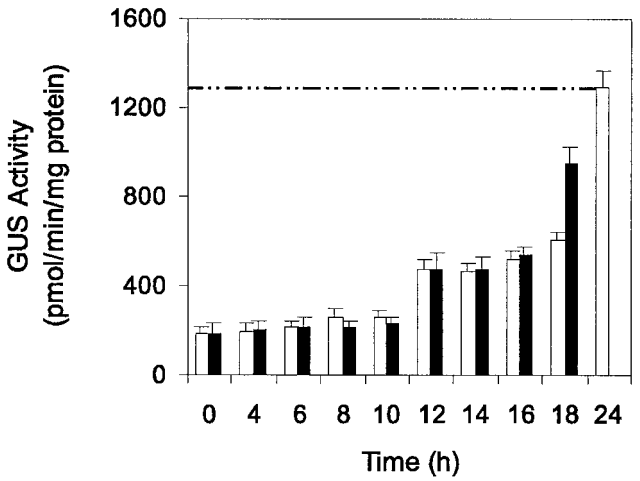

Figure 1. Effect of OGs, added at different times during incubation, on auxin-induced GUS activity. *RolB*-GUS leaf explants were incubated in a medium containing 0.6 μM IAA, and OGs (DP = 9–18) were added at a final concentration of 10 μg/mL at the indicated times after the beginning of the incubation. The total length of the experiment was 24 h. GUS activity was determined at the time of OG addition (white bars) and at the end of the experiment (24 h; black bars). A reference dashed horizontal line indicates GUS activity measured at 24 h in explants incubated in the presence of IAA alone. GUS activity is expressed as pmol 4-methylumbelliferone mg^{-1} protein min^{-1}. Each data point is the mean value (±SD) from three independent experiments.

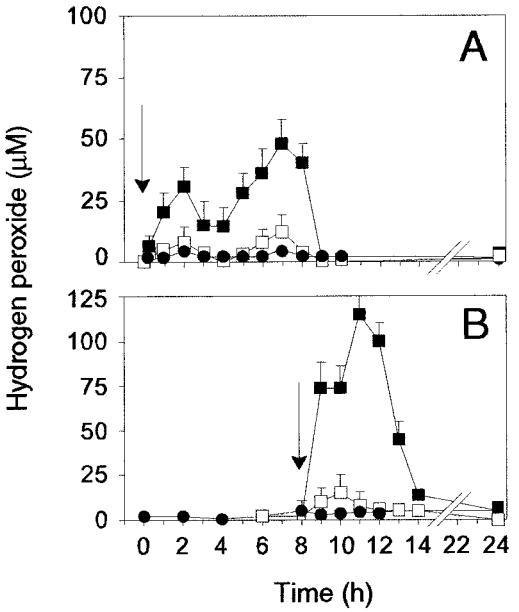

Figure 2. Extracellular H_2O_2 accumulation elicited by OGs. *RolB*-GUS leaf explants were incubated in a medium containing 0.6 μM IAA. Water (●), OGs (DP = 9–18) alone at a final concentration of 10 μg/mL (■), or OGs plus catalase (2 μkat/mL) (□) were added at time 0 (A), or after 8 h (B). Levels of H_2O_2 accumulated in the incubation medium were measured at different times upon addition. Results were normalized and expressed as μM g^{-1} fresh weight explant. Arrows indicate time of OG addition. Each data point is the mean value (±SD) from three independent experiments; when SD < 3.5, bars are not indicated.

Size-homogeneous OGs were tested for their ability to induce H_2O_2 production and to inhibit auxin-induced GUS accumulation. OGs were added separately at time 0 to the explants at a final concentration of 1 μg/mL in the presence of 0.6 μM IAA. Both the ability to induce H_2O_2 accumulation and the ability to inhibit *rolB* expression depended on the size of the oligomers. OGs with DPs of 12 to 15 were the most active in both responses (Fig. 3). In a dose-response experiment, concentrations of OGs (DP of 9–18) ranging from 3 to 10 μg/mL were required to induce maximal levels of extracellular H_2O_2, while lower concentrations of OGs (0.3–1 μg/mL) were effective in inhibiting IAA-induced GUS activity (Fig. 4). In particular, calculated

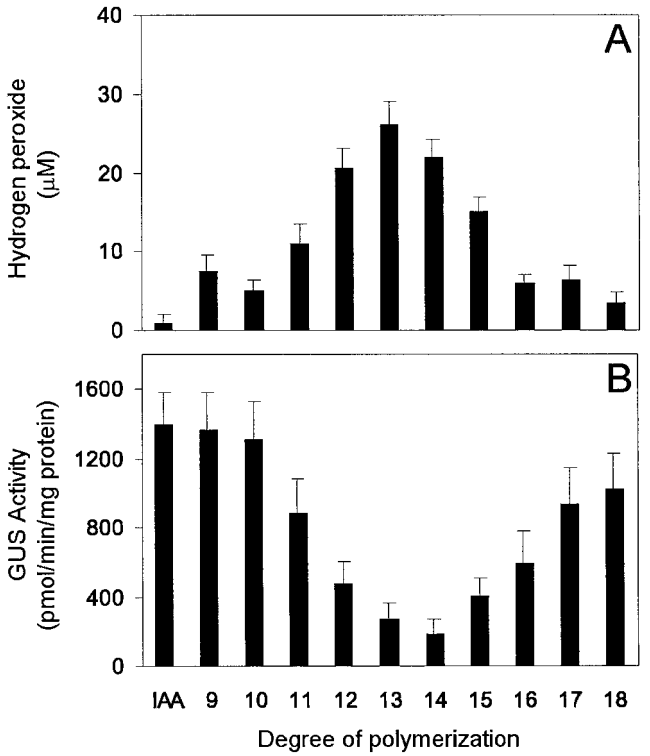

Figure 3. Effect of size-homogeneous OGs on the extracellular H_2O_2 accumulation and the auxin-induced GUS activity. Purified OGs were added separately at a concentration of 1 μg/mL (corresponding to the following molar concentrations: 0.62 μM DP = 9; 0.56 μM DP = 10; 0.51 μM DP = 11; 0.47 μM DP = 12; 0.43 μM DP = 13; 0.40 μM DP = 14; 0.37 μM DP = 15; 0.35 μM DP = 16; 0.33 DP = 17; 0.31 μM DP = 18) to the liquid culture medium containing 0.6 μM IAA at the beginning of the incubation, and concentration of H_2O_2 in the incubation medium (A) and GUS activity (B) were measured. Controls contained IAA alone. H_2O_2 concentration was determined after 2 h and expressed as μM g^{-1} fresh weight explant. GUS activity was determined after 24 h of incubation, and expressed as pmol 4-methylumbelliferone mg^{-1} protein min^{-1}. Each data point is the mean value (±SD) from three independent experiments.

concentrations of OGs required for half-maximal accumulation of H_2O_2 were three times higher than for half-maximal inhibition of auxin-induced *rolB* promoter activity.

While OGs of the same size inhibited the auxin-induced activity of the *rolB* promoter and induced H_2O_2 production, dose requirements for the two responses were different.

H_2O_2 Is Not Involved in the Inhibition of the Auxin-Induced Expression of *rolB*

The ability of catalase and SOD, added with OGs either at time 0 or after 8 h, to influence the inhibition of rolB promoter activity exerted by OGs was tested. In the presence of catalase (2 μkat/mL), levels of extracellular H_2O_2 induced by OGs were decreased by about 80% and were comparable to those induced by the inactive, homogeneous-sized OGs with DPs of 9 and 10 (1 μg/mL); however, the inhibitory effect of OGs on *rolB* promoter activity was not affected (Fig. 5). Similarly, addition of SOD (150 μg/mL)

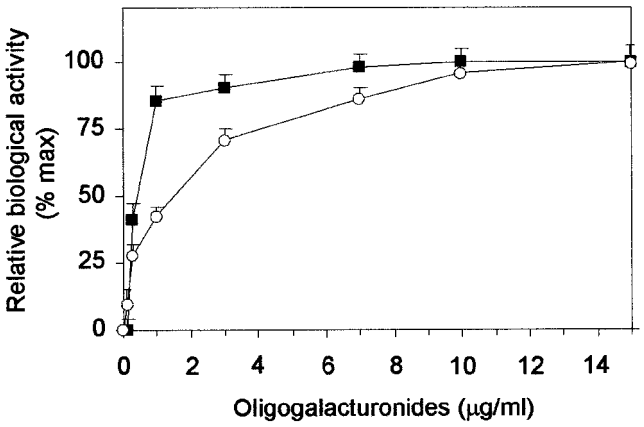

Figure 4. Effect of different concentrations of OGs on extracellular H_2O_2 accumulation and auxin-induced GUS activity. *RolB*-GUS leaf explants were cultured in liquid culture medium in the presence of 0.6 μM IAA and OGs (DP = 9–18) at different concentrations. GUS activity was measured after 24 h. GUS activity measured at 24 h in control explants incubated in IAA alone (1,400 ± 90 pmol 4-methylumbelliferone mg^{-1} protein min^{-1}) was used as a reference for calculating the percent inhibition of GUS activity (■). H_2O_2 levels in the incubation medium were determined after 2 h of incubation. The concentration of H_2O_2 measured in the incubation medium of explants treated with 30 μg/mL OGs (41.3 ± 3 μM g^{-1} fresh weight explants) was used as 100% reference for calculating percent of induction of H_2O_2 accumulation (○). Each data point is the mean value for three independent experiments.

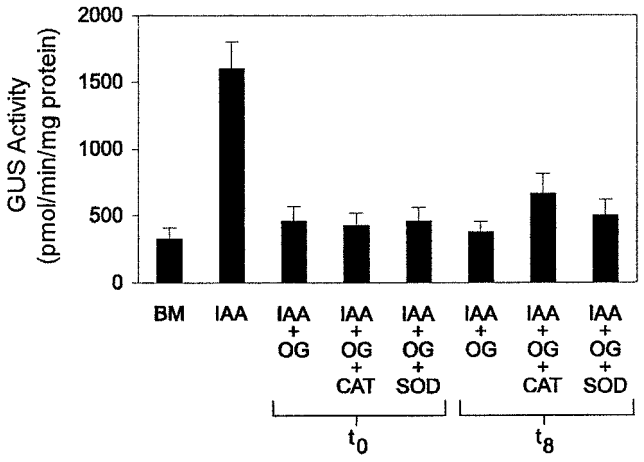

Figure 5. Effect of OGs on auxin-induced GUS activity in the presence of catalase or SOD. *RolB*-GUS leaf explants were cultured in liquid culture medium in the presence of 0.6 μM IAA, and OGs (DP = 9–18) alone at a final concentration of 10 μg/mL, OGs plus catalase (2 μkat; CAT), or OGs plus SOD (150 μg/mL; SOD) were added at the beginning of the incubation (t_0) or after 8 h (t_8) Control explants were incubated in basal medium without IAA (BM). GUS activity, expressed as pmol 4-methylumbelliferone mg^{-1} protein min^{-1}, was determined after 24 h of incubation. Each data point is the mean value (±SD) from three independent experiments.

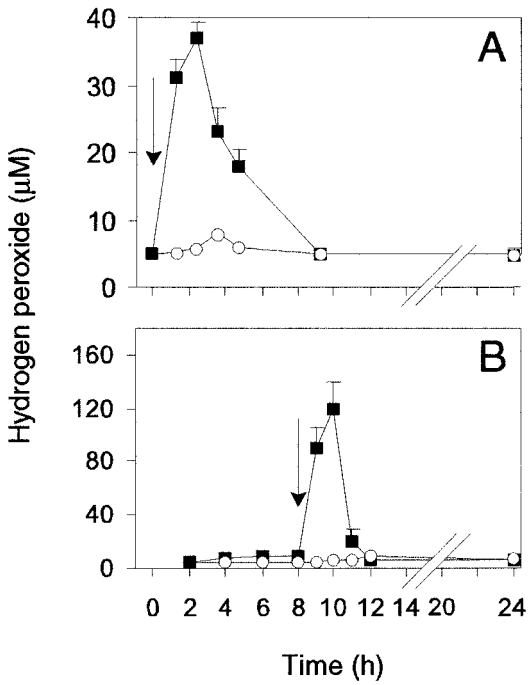

Figure 6. Effect of fungal oligo-β-glucan on the extracellular accumulation of H_2O_2. *RolB*-GUS leaf explants were incubated in a medium containing 0.6 μM IAA, and water (○) or glucan at a final concentration of 50 μg/mL (■) were added at the beginning of the incubation (A), or after 8 h (B). Arrows indicate time of glucan addition. H_2O_2, expressed as μM g^{-1} fresh weight, was measured in the incubation medium at different times upon addition. Each data point is the mean value (±SD) from three independent experiments; when SD < 3.5, bars are not indicated.

had no effect. These results strongly suggest that neither H_2O_2 nor superoxide are involved in the inhibition of auxin-induced GUS expression driven by the *rolB* promoter.

Because the inhibitory effect of OGs on root formation in leaf explants from transgenic plants carrying the *rolB* gene is decreased by increasing concentrations of auxin (Bellincampi et al., 1996), we tested whether the OG-induced accumulation of H_2O_2 is also influenced by auxin. The levels of H_2O_2 induced after 2 h by OGs (DP of 9–18; 10 μg/mL) added at the beginning of incubation in the absence or presence of 0.6 or 6 μM IAA were not significantly different (32 ± 2 μM; 35 ± 4 μM; and 34 ± 2 μM, respectively).

In a confirmatory experiment, we analyzed the ability of an oligo-β-glucan elicitor from *P. megasperma* to induce accumulation of H_2O_2 and to inhibit the auxin-induced expression of GUS driven by the *rolB* promoter. Glucan was added at a concentration of 50 μg/mL to the culture medium containing 0.6 μM IAA, at time 0 and after 8 h. In both cases, H_2O_2 accumulated in the culture medium, reaching maximal levels after about 2 h (40 and 150 μM when glucan was added at time 0 or after 8 h, respectively) (Fig. 6). However, the same concentration of elicitor did not significantly influence the auxin-induced GUS activity (Fig. 7) and the formation of roots induced by 0.6 μM IAA in tobacco leaf explants (results not shown).

These data show that glucan-induced H_2O_2 accumulation at comparable or higher levels than that observed with OGs does not prevent the auxin-induced accumulation of GUS, reinforcing the conclusion that H_2O_2 is not involved in the inhibition of auxin-induced GUS expression driven by the *rolB* promoter.

DISCUSSION

The notion that regulatory pathways that underlie disease resistance also control developmental processes is emerging from recent studies in both plants and animals (Wilson et al., 1997). In plants, OGs are elicitors of defense responses (Hahn et al., 1981; Côté and Hahn, 1994) and regulators of growth and development (Branca et al., 1988; Bellincampi et al., 1993, 1996; Altamura et al., 1998).

A well-characterized effect of OGs is the inhibition of rhizogenesis in *rolB* explants through the inhibition of the auxin-dependent activation of *rolB* expression necessary for root initiation (Bellincampi et al., 1996). The *rolB* gene is a bacterial oncogene; however, we consider the inhibition of its expression by OGs as a development-related effect, not only because *rolB* itself is a potent morphogen in plants, but also because the inhibition is likely due to an interference with processes along the signal transduction pathway leading from the perception of the auxin signal to the activation of the *rolB* promoter. The same signal transduction pathway may be involved in the induction of the expression of endogenous functions necessary for root initiation in normal explants (Bellincampi et al., 1996). The expression of the *rolB* promoter in leaf explants represents a useful tool to study the interplay between OGs and auxin.

Because the induction of the *rolB* promoter is a late response to auxin and requires an 8- to 10-h lag time, we first temporally localized the OG-sensitive event(s). Our results show that OGs can rapidly and totally inhibit the

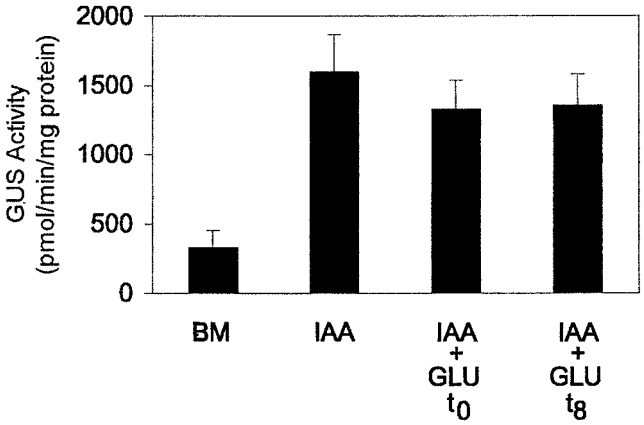

Figure 7. Effect of fungal oligo-β-glucan on auxin-induced GUS activity. *RolB*-GUS leaf explants were cultured in liquid culture medium in the presence of 0.6 μM IAA. Glucan (50 μg/mL) was added at the beginning of the incubation (t_0) or after 8 h (t_8). Control explants were incubated in basal medium without IAA (BM). GUS activity, expressed as pmol 4-methylumbelliferone mg^{-1} protein min^{-1}, was determined after 24 h of incubation. Each data point is the mean value (± SD) from three independent experiments.

IAA-induced *rolB* expression even when added many hours after *rolB* promoter activation has been initiated; only late during induction do OGs become unable to prevent the further accumulation of GUS. The inhibitory effect of OGs is very rapid, with a short lag time, suggesting that auxin-induced processes necessary not only to activate but also to maintain active the *rolB* promoter, are rapidly blocked by OGs. The possibility of uncoupling the OG responses from many of the responses triggered by auxin through the addition of OGs several hours later after the addition of auxin may facilitate a further analysis of the OG action.

It has been reported that, while OG fragmentation occurs in the culture medium of suspension-cultured cells, some of these molecules bind to the cells and are protected from degradation (Mathieu et al., 1998). The presence of cell-bound OGs that continuously convey their signal may explain why inhibition of GUS persists during the many hours of incubation. Alternatively, persistence of the inhibition may not depend on the stability of OGs but, rather, on an OG-induced stable cellular response that prevents the occurrence of the auxin-induced processes necessary to activate and maintain active the *rolB* promoter. The possibility that OG may act by destroying the auxin present in the explant culture medium was ruled out in previous experiments (Bellincampi et al., 1996).

OGs induce accumulation of extracellular H_2O_2 in tobacco leaf explants. The production of reactive oxygen species is one of the earliest events during the defense response, but also occurs in cells undergoing lignification as part of a developmental program (Yang et al., 1997). The main aim of this study was to investigate the possible cause-effect relationship between the induction of H_2O_2 and the ability of OGs to interfere with auxin. A parallel analysis of the production of H_2O_2 and morphogenesis in tobacco has already been reported (Spiro et al., 1998). However, different culture systems were used by the authors: suspension-cultured cells for the H_2O_2 induction and a thin cell-layer explant system for the morphogenetic response.

The kinetics of H_2O_2 accumulation were clearly biphasic when OGs were added at the beginning of the incubation with IAA, with a first maximum after 2 h and a second one, more pronounced, at 7 h. This resembles the kinetics observed for the elicitation of H_2O_2 by avirulent bacteria in suspension-cultured cells (Levine et al., 1994; Baker and Orlandi, 1995). The timing of the first burst observed with the *rolB*-GUS explants coincides with the timing of ion flux activation in response to OGs in tobacco suspension-cultured cells (Mathieu et al., 1991). We measured no significant change in external pH (data not shown); however, we cannot rule out the possibility that ion flux activation underlies both phases of the burst.

H_2O_2 reaches higher concentration when OGs are added after 8 h of incubation of the explants with IAA, suggesting, as reported by other authors, a development of competence for H_2O_2 production in response to the mechanical damage occurring during the preparation of the explants (Fauth et al., 1996). Like many other OG-induced responses, such as plasma membrane depolarization, K^+ efflux, and Ca^{2+} influx (Mathieu et al., 1998; Spiro et al., 1998), the oxidative burst induced by OGs is transient. This may result from a desensitization of the plant cells (Felix et al., 1998) and may involve a scavenging mechanism that prevails as soon as the cells become desensitized. If, on the other hand, desensitization involves the perception system, the difference between the transient nature of the oxidative response and the "persistent" inhibition of the *rolB* promoter implies that different perception systems underlie the two responses. The observation that the concentrations at which OGs regulate developmental processes are usually lower than that required to induce defense responses (Messiaen and Van Cutsem, 1993), suggests the existence of different classes of receptors for defense and developmental responses.

The indication that OG-induced H_2O_2 accumulation and inhibition of *rolB* expression are independent responses derives from the following observations presented in this paper: (a) when OGs are given at the beginning of the incubation in the presence of auxin, H_2O_2 drops to basal levels before the appearance of GUS activity, suggesting that H_2O_2 may not be directly involved in the inhibition of the *rolB* promoter activity; (b) while the inhibition by OGs of auxin-induced *rolB*-mediated root formation is diminished by increasing the concentration of IAA (Bellincampi et al., 1996), H_2O_2 production is not; (c) dose-response curves for the inhibition of auxin-induced activity of the *rolB* promoter and the induction of H_2O_2 production are different, and calculated concentrations of OGs required for half-maximal accumulation of H_2O_2 are three times higher than for half-maximal inhibition of auxin-induced *rolB* promoter activity; (d) prevention of the accumulation of H_2O_2 in the culture medium by catalase has no effect on the OG-dependent inhibition of the *rolB* expression; and (e) conversely, elicitation of H_2O_2 production by a fungal cell wall glucan, a well-known elicitor of defense responses with no involvement in developmental processes, does not interfere with the inhibition of GUS expression driven by the *rolB* promoter.

All in all, our data argue for no cause-effect relationship between H_2O_2 accumulation and the inhibition of *rolB* expression exerted by OGs. It remains to be established whether and where the transduction pathway of the OG signal branches to control developmental processes and to elicit defense responses, and whether any relationship exists between the ability to counteract the action of auxin and the ability to induce defense responses.

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