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Development of β-1,3-glucanase activity in germinated tomato seeds

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Abstract

Laminarin-hydrolysing activity developed in the endosperm of tomato (*Lycopersicon esculentum*) seeds following germination. The enzyme was basic (pI > 10) and the apparent molecular mass was estimated to be 35 kDa by SDS-PAGE. It was specific for linear β-1,3-glucan substrates. Laminarin was hydrolysed by the enzyme to yield a mixture of oligoglucosides, indicating that the enzyme had an endo-action pattern. Thus, the enzyme was identified as β-1,3-endoglucanase (EC 3.2.1.39). The activity of the enzyme developed in the endosperm after radicle protrusion (germination) had occurred and the enzyme activity was localized exclusively in the micropylar region of the endosperm where the radicle had penetrated. When the lateral endosperm region, where no induction of the enzyme occurred, was wounded (cut or punctured), there was a marked enhancement of β-1,3-glucanase activity. Thus the post-germinative β-1,3-glucanase activity in the micropylar endosperm portion might be brought about by wounding resulting from endosperm rupture by radicle penetration.

Key words: Endosperm, β-1,3-glucanase, germination, tomato seed.

Introduction

In seeds of some dicotyledonous plants (e.g. Compositae and Solanaceae), mannose-containing plolysaccharides, such as galactomannans and glucomannans, are the major carbohydrate reserves (Bewley and Black, 1994). They are typically stored in the endosperm cell walls (Halmer *et al*., 1976; Groot *et al*., 1988). Endo-β-mannanase that develops markedly in the endosperm of germinated tomato or lettuce seeds is thought to be involved in the mobilization of reserve mannose polymers (Halmer *et al*., 1976; Nonogaki *et al*., 1992, 1995; Nonogaki and Morohashi, 1999). On the other hand, it has also been observed in tomato that endo-β-mannanase develops prior to germination specifically in the micropylar region of the endosperm where the radicle will penetrate (Nonogaki *et al*., 1992; Nomaguchi *et al*., 1995). Since the endosperm part surrounding the radicle tip mechanically resists radicle protrusion, weakening of this part (micropylar region) of the endosperm is necessary for tomato seeds to germinate (Groot and Karssen, 1987). The endo-β-mannanase that develops prior to germination weakens the micropylar region of the endosperm by helping to hydrolyse the cell wall, thereby allowing the radicle to penetrate (Groot *et al*., 1988; Nonogaki *et al*., 1992; Ni and Bradford, 1993), although it has been pointed out that the presence of endo-β-mannanase is not in itself sufficient to permit tomato seeds to complete germination (Toorop *et al*., 1996; Still and Bradford, 1997; Bewley, 1997). It was reported that the endosperm cell walls of *Datura* species, whose main component is mannan-type polysaccharides, erode prior to germination at the micropylar portion (Sanchez *et al*., 1990). Cell-wall-hydrolysing enzymes other than endo-β-mannanase have also been implicated in micropylar-endosperm weakening. In lettuce, whose endosperm cell walls are also composed primarily of galactomannans (Halmer *et al*., 1975), endo-β-mannanase activity increases only after the emergence of the radicle (Halmer *et al*., 1976; Nonogaki and Morohashi, 1999). The weakening of the cell walls in the micropylar endosperm portion of lettuce seeds may be caused by an as yet unidentified hydrolase(s) (Dutta *et al*., 1994). Cellulase activity is localized in the micropylar region of the endosperm of *Datura ferox* seeds and is induced prior to radicle protrusion (Sanchez *et al*., 1986). Recently it has been shown that β-1,3-glucanase develops prior to germination in the micropylar region

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of the endosperm of tobacco seeds (Vogeli-Lange *et al.*, 1994; Leubner-Metzger *et al.*, 1995), and it was suggested that the enzyme contributes to the weakening of the endosperm and thereby facilitates penetration by the radicle in tobacco seeds.

β-1,3-Glucanases are commonly found throughout the plant kingdom and there is abundant evidence that they are involved in defence mechanism of plants against pathogen infection (Simmons, 1994). It has also been suggested that β-1,3-glucanases are important for diverse physiological processes such as pollen development, stress response, flowering, and mobilization of storage reserves (Simmons, 1994). The proposal that β-1,3-glucanase is critical in triggering seed germination is novel and is of interest in considering the mechanism of seed germination (Vogeli-Lange *et al.*, 1994; Leubner-Metzger *et al.*, 1995).

In the present study, it was also observed that β-1,3-glucanase activity developed specifically in the micropylar endosperm portion of tomato seeds, similarly to tobacco seeds. However, the temporal pattern of the development was completely different in the two seeds; in tomato, in contrast to tobacco, β-1,3-glucanase activity develops only after radicle emergence (germination). These observations suggest that the physiological function of β-1,3-glucanase in tomato seeds is different from that of the enzyme in tobacco seeds. The spatially and temporally regulated pattern of the development of β-1,3-glucanase activity in tomato seeds is reported here in connection with possible physiological significance of the enzyme in germinated seeds.

Materials and methods

Plant material and seed incubation

Tomato (*Lycopersicon esculentum* [L.] Mill. cv. First Up) seeds were obtained from Sakata Seed Corp. (Yokohama, Japan). For germination, they were placed on wet filter paper in Petri dishes and incubated at 28 °C in the dark.

Dissection of seeds

The seed was cut into halves to produce the micropylar half and the lateral half. After cutting, all embryo parts were removed by pushing out with tweezers. The de-embryonated micropylar half and lateral half were denoted as micropylar endosperm half and lateral endosperm half, respectively, although they contained part of the testa. In some experiments, a seed was cut into four consecutive sections approximately the same in width; each section was designated S1, S2, S3, and S4, respectively, from the micropylar end toward the lateral end, as shown in Fig. 1. Embryo parts were removed from the sections as above.

Enzyme extraction

Thirty endosperm parts were homogenized in 0.8–1.2 ml of 15 mM Na-acetate buffer, pH 5.5, in a chilled mortar and pestle. The homogenate was centrifuged at 10 000 *g* for 5 min. The supernatant was used for enzyme assays directly or after dialysis against 15 mM Na-acetate buffer, pH 5.5.

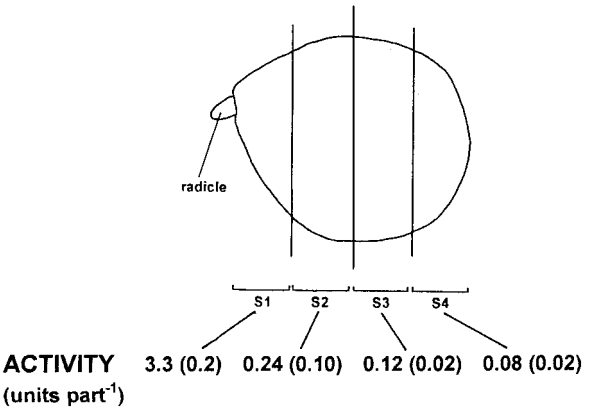

Fig. 1. Schematic presentation of sections through a tomato seed and β-1,3-glucanase activities in the endosperm of each section of seeds of 4-d-old seedlings. Shown is the mean of, and difference between (in parenthesis), two separate experiments. Substrate, laminarin.

Enzyme assays

β-1,3-Glucanase activity was assayed using *Laminaria digitata* laminarin (Sigma) or Azurine-crosslinked pachyman (AZCL-pachyman; Megazyme) as the substrates. In the former case, the assay mixture contained in a total volume of 0.4 ml: 0.5 mg laminarin, 50 μmol of Na-acetate buffer (pH 5.5) and dialysed enzyme solution. After incubation at 35 °C for 1.5 h, the amount of reducing sugars that had been released was determined by Somogyi-Nelson's method (Nelson, 1944; Somogyi, 1952). One unit of activity is defined as the amount of enzyme producing 10 nmol glucose equivalents under the conditions mentioned above. In the latter case, the assay mixture contained in a total volume of 0.4 ml: 20 mg of AZCL-pachyman, 10 μmol K-phosphate (pH 6.5) and undialysed enzyme solution. During incubation at 35 °C for 1.5 h, the mixture was vigorously shaken (120 strokes min^{-1}). The reaction was stopped by adding 1.2 ml ethanol and the amount of soluble dyed fragments released from AZCL-pachyman was determined colorimetrically at 590 nm. One unit of enzyme activity represents an increase in 0.1 absorbance unit under the conditions used.

Chitinase activity was determined using Remazol Brilliant Violet-labelled carboxymethyl-chitin as the substrate by the method of Wirth and Wolf (Wirth and Wolf, 1990).

Purification of β-1,3-glucanase

The enzyme was extracted from the whole endosperm (with the testa attached) which had been manually removed from 3-d-old seedlings. Endosperm (7 g fresh weight) was homogenized in a blender in 40 ml of 50 mM Na-acetate (pH 5.5) containing 3 mM EDTA. The brei was squeezed through cheesecloth and centrifuged at 10 000 *g* for 10 min. Cold ethanol was added to the supernatant to give a concentration of 73% (v/v) and centrifuged. The precipitate was dissolved in 15 mM K-phosphate (pH 7.5) and dialysed against the same buffer. The clarified solution was applied to a DEAE-cellulofine column (1.6 × 8 cm) equilibrated with the dialysis buffer. Most activity was recovered in the fraction that passed through the column. The unbound fractions from the DEAE-cellulofine column were dialysed against 20 mM Na-acetate (pH 5.0) (buffer A) and applied to a CM-Toyopearl column (1.6 × 4 cm) equilibrated with buffer A. The enzyme was eluted with a linear gradient of NaCl concentration (0–0.75 M) in buffer A. β-1,3-Glucanase was eluted as a single peak from the column at approximately 0.5 M NaCl. The active fractions from the CM-Toyopearl

column were dialysed against buffer A and applied to an S HyperD 10 column (Beckman) equilibrated with buffer A on an FPLC system. β-1,3-Glucanase was eluted from the column with a linear gradient of NaCl concentration (0–1.0 M) in buffer A.

Chromatofocusing

The active fraction from CM-Toyopearl column was equilibrated in 25 mM triethylamine-HCl buffer (pH 11) and applied to chromatofocusing of PBE 118 column (Pharmacia) (1 × 20 cm) equilibrated with the same buffer. The column was eluted with Pharmalyte 8–10.5 (Pharmacia), which had been diluted 45-fold with water and adjusted to pH 8.

Substrate specificity and action pattern

The substrate specificity of the enzyme was examined on *Laminaria digitata* (Sigma) and *Eisenia bicyclis* laminarin (Tokyo Kasei, Tokyo), *Cetralia islandica* lichenan (Sigma) and CM-pachyman (Megazyme).

Action patterns were determined by TLC of the products released from *L. digitata* laminarin by the enzyme. This was performed on a silica gel plate which was developed twice with isopropanol : *n*-butanol : H_2O (12 : 3 : 4, by vol.). Spots were made visible by charring (5% [v/v] H_2SO_4 in ethanol).

PAGE

SDS-PAGE was performed in 12% gels according to Laemmli (Laemmli, 1970). The gels were silver-stained.

Immunoblotting

The antiserum raised against soybean β-1,3-endoglucanase (Takeuchi *et al.*, 1990) was used as a probe. Immunodetection was done using horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin. The bands developed on X-ray film after the reaction with a chemiluminescence reagent, Renaissance (DuPont NEN Products).

Protein determination

Protein was determined by the method of Lowry *et al.* (Lowry *et al.*, 1951) using BSA as the standard.

Results

Changes in β-1,3-glucanase activity after the start of imbibition

Activity of β-1,3-glucanase was detected in the endosperm, but not in the embryo, of tomato seeds. Figure 2 shows changes in β-1,3-glucanase activity (with *L. digitata* laminarin as the substrate) in the micropylar and the lateral endosperm half of tomato seeds after the start of imbibition. The activity (on per endosperm-part basis) in the micropylar endosperm half began to increase after the second day of imbibition, while that in the lateral endosperm half remained at low levels through the experimental period (Fig. 2A). When AZCL-pachyman was used as the substrate, the same pattern of the development of the enzyme activity was observed (data not shown). In tomato seeds used in the present study, the radicle began to protrude approximately 28 h after the start of imbibition (arrow in Fig. 2) and more than 80% of the seeds completed germination during the following 30 h. On day 2, some seeds germinated, but some did not yet. Then, on day 2, activities of the enzyme in germinated and ungerminated seeds were separately determined. In ungerminated seeds at this time, the activity of β-1,3-glucanase in the micropylar endosperm half showed no enhancement and was almost the same as that of the enzyme in the lateral endosperm half (open symbols in Fig. 2A).

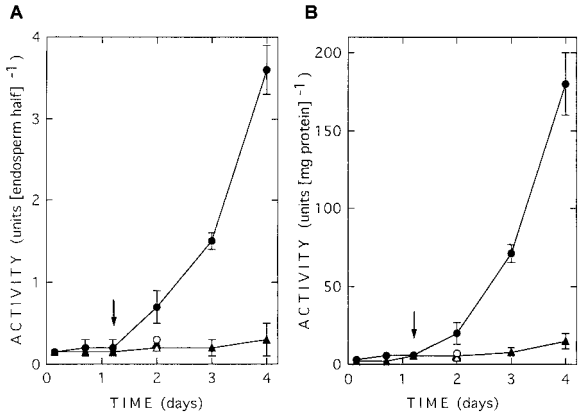

Fig. 2. Changes in β-1,3-glucanase activities in the micropylar endosperm half (●) and the lateral endosperm half (▲) of tomato seeds during and following germination. Activities are expressed on per endosperm basis (A) and per protein basis (B). On day 2, some seeds germinated and some had not yet. Activities were assayed with these two kinds of seeds separately. (○, △), Micropylar and lateral endosperm half of ungerminated seeds, respectively, on day 2. Arrow indicates the time when radicle protrusion begins. The means and standard errors (vertical bars) of three replicates are shown.

A similar pattern of the development of the enzyme was observed when the data were expressed on per protein basis (Fig. 2B). However, the increase in the activity in the micropylar endosperm half was greater on per protein basis than on per tissue part basis; the activity on per endosperm basis increased about 18-fold during the first 4 d after the start of imbibition, while the activity on per protein basis increased about 35-fold during the same period. This is probably due to the fact that the content of protein reserves in the endosperm declined after germination.

Reserves in the endosperm were mostly digested by the 4th to 5th day after the beginning of imbibition and the endosperm remnants (with the testa attached) had been shed from growing seedlings around this time. Even at this stage, β-1,3-glucanase activity in the lateral endosperm half remained low (Fig. 2). Thus, β-1,3-glucanase activity developed only in the micropylar endosperm half, and in the lateral endosperm half the activity did not develop at all during and following germination.

To study in detail in which part of the endosperm the activity developed, the seed of 3-d-old seedlings was divided into four successive parts, S1, S2, S3, and S4 (see Fig. 1 for designation) and the activity in the endosperm

of each part was determined. As seen from Fig. 1, high activity was detected exclusively in the part of the micropylar end (S1).

Effects of wounding on activity development

The effects of wounding on the development of β-1,3-glucanase activity in the endosperm were studied. Seeds of 2-d-old seedlings were cut into halves (micropylar and lateral half) and incubated on wet filter paper for 1 d, and then the enzyme activities in the endosperm part of those halves were determined (Table 1). The enzyme activities were much higher in the micropylar and the lateral endosperm of dissected and incubated half seeds, as compared with those in the respective endosperm part of intact seeds. This indicates that cutting (wounding) brought about the enhancement of β-1,3-glucanase activity in the endosperm. To examine this further, the lateral part of seeds of 2-d-old seedlings was punctured at 3–4 points with a pin. The treated seedlings were incubated for 1 d and then β-1,3-glucanase activity in the endosperm of the punctured lateral part of the seed was determined. As seen from Table 2, the activity was greatly increased by puncturing.

Table 1. *Cutting effects on the development of β-1,3-glucanase activities in the endosperm*

Seeds of 2-d-old seedlings were cut into halves (micropylar and lateral half) and incubated for 1 d. Then the enzyme activities in the endosperm of those halves were determined. For comparison, activities in the micropylar and the lateral endosperm half of intact seeds of 3-d-old seedlings are shown. Substrate, AZCL-pachyman. Shown is the mean of, and difference between (in parenthesis), two replicates.

Endosperm	Activity (units part^{-1})
Intact micropylar half	1.03 (0.01)
Intact lateral half	0.11 (0.00)
Cut and incubated micropylar half	1.80 (0.11)
Cut and incubated lateral half	1.38 (0.14)

Table 2. *Puncturing effects on the development of β-1,3-glucanase activities in the endosperm*

Lateral part of seeds of 2-d-old seedlings were punctured with a needle at 3–4 points and the seedlings were incubated for 1 d. Then the enzyme activities in the micropylar and the lateral endosperm half of the treated seeds were assayed. For comparison, the activities in the micropylar and the lateral endosperm half of intact (unpunctured) seeds of 3-d-old seedlings are shown. Substrate, AZCL-pachyman. Shown is the mean of, and difference between (in parenthesis), two replicates.

Endosperm	Activity (units part^{-1})
Intact (unpunctured) seed	
Micropylar half	0.98 (0.03)
Lateral half	0.13 (0.01)
Seed punctured at lateral part	
Micropylar half	1.13 (0.10)
Lateral half	1.03 (0.05)

Purification of the enzyme

β-1,3-Glucanase was eluted as a single peak from the CM-Toyopearl column at approximately 0.5 M NaCl. No enzyme activity was detected in the fraction passed through the column. β-1,3-Glucanase-active fractions eluted from the CM-Toyopearl column were subjected to SDS-PAGE followed by silver-staining (Fig. 3A). Although several signals were detected, only a band (arrowhead) at 35 kDa exhibited the changing pattern of stain intensity closely correlated with that of β-1,3-glucanase activity. The active fraction in CM-Toyopearl column chromatography was subjected to SDS-PAGE followed by immunoblotting using anti-soybean-β-1,3-endoglucanase antiserum (Akiyama *et al.*, 1990) as a probe. The polypeptide at the position of 35 kDa was recognized by the antiserum (Fig. 3B, arrowhead). Thus, this protein was tentatively identified as β-1,3-glucanase. This was confirmed by further purification of the enzyme. The active fraction from CM-Toyopearl column was applied to an S HyperD 10 column (Beckman) on an FPLC system. An active fraction eluted from the latter column was subjected to SDS-PAGE followed by silver-staining. A single band of 35 kDa was detected (Fig. 3C, arrowhead). Because the protein content in the preparation of the purified enzyme was too low to be assayed, the degree of purification could not be calculated. Since the amount of the purified enzyme was also very small, the enzyme preparation purified at the step of the CM-Toyopearl column chromatography was used in order to characterize the enzyme.

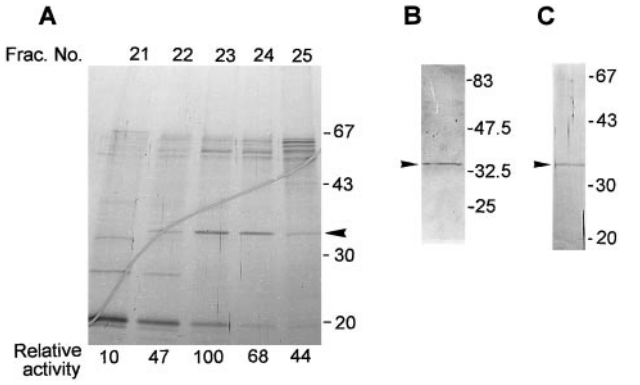

Fig. 3. (A) SDS-PAGE of fractions eluted from the CM-Toyopearl column. Fraction numbers and relative β-1,3-glucanase activities in each fraction are shown at the top and the bottom, respectively, of the panel. Proteins were stained with silver. (B) Immunoblot of SDS-PAGE gel of the active fraction in CM-Toyopearl column chromatography. The antibodies against soybean β-1,3-glucanase were used as a probe for immunoblotting. (C) SDS-PAGE of the active fraction eluted from S HyperD 10 column; stained with silver. The position of β-1,3-glucanase is shown by an arrowhead in each panel. Molecular masses (kDa) are shown on the right of each panel.

Some properties of the enzyme

pH optimum: The pH optimum of the enzyme was different depending on its substrate; optimum pHs for *L. digitata* laminarin, CM- and AZCL-pachyman were approximately 5.5, 6 and 6.5, respectively. Similar situations have been reported with the barley (Ballance and Meredith, 1976) and rye enzyme (Ballance and Manners, 1978). The difference in pH otpima for these 1,3-β-glucans is probably due to the carboxymethyl or azurin groups introduced into the pachyman chain (as indicated by Ballance and Manners, 1978).

Substrate specificity: The substrate specificities of the glucanase were examined on β-1,3-glucans that differ in linkage types and the ratios of linkage types (Table 3). The enzyme preferentially hydrolysed essentially linear 1,3-β-glucans such as *L. digitata* laminarin and CM-pachyman, while it showed low activities against highly side-branched 1,3-β-glucans such as *E. bicyclis* laminarin and *C. islandica* lichenan. A similar substrate specificity has been reported for 1,3-β-endoglucanases from tobacco (Moore and Stone, 1972), barley (Hrmova and Fincher, 1993) and rice (Akiyama *et al.*, 1996).

Action pattern: The products of hydrolysis of *L. digitata* laminarin by the enzyme were determined by TLC (Fig. 4). A series of oligoglucosides, but no glucose, were found to be formed from the 1,3-β-glucan. Thus, the hydrolase has an endo-action pattern, and is identified as 1,3-β-endoglucanase (EC 3.2.1.39).

pI: The glucanase was eluted from the chromatofocusing column (PBE 118) at the top region of pH gradient where accurate determination of pI was not possible. This indicates that the pI of the enzyme is higher than 10.

Table 3. *Substrate specificity of β-1,3-glucanase in tomato endosperm*

The relative hydrolytic activity of the enzyme on *L. digitata* laminarin was set at 100 (corresponding to 4.2 units [reaction mixture]$^{-1}$).

Substrate	Major linkage type[a]	Relative activity
Laminarin (*Laminaria digitata*)	1,3;1,6-β-(7:1)	100
CM-pachyman	1,3-β-	86
Laminarin (*Eisenia bicyclis*)	1,3;1,6-β-(3:2)	9
Lichenin (*Cetraria Islandica*)	1,4;1,3-β-(2:1)	6

[a]Ratios of linkage types are from Hrmova and Fincher (1993).

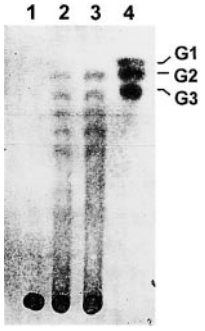

Fig. 4. TLC of the hydrolysis products of laminarin by tomato β-1,3-glucanase. Lanes 1, 2 and 3, hydrolysis for 0, 1 and 3 h, respectively; lane 4, standards (G1, glucose; G2, laminaribiose; G3, laminaritriose).

Discussion

In seeds (caryopses) of cereals, such as barley and rice, one of the main components of the endosperm cell walls is β-1,3-glucan and it is rapidly degraded by glucanase in germinated seeds (Simmons, 1994). The presence of β-1,3-glucan has not been reported in cell walls of tomato endosperms. If substrates for β-1,3-glucanase are present in tomato endosperm, they should be localized in the micropylar endosperm, in light of the spatial pattern of the development of the enzyme activity. It is, however, difficult to read the physiological significance of localized distribution of β-1,3-glucans (if any) and of their degradation after radicle emergence.

The present study shows that wounding (cutting and puncturing) induces the development of β-1,3-glucanase activity in the endosperm of tomato seeds. It has been reported that wounding induces β-1,3-glucanase in tobacco leaves (Neale *et al.*, 1990), pea pods (Mauch *et al.*, 1988) and grape berries (Derckel *et al.*, 1998). In intact tomato seeds, the enzyme activity is detected exclusively in the micropylar endosperm part after the radicle has protruded there. The embryo of tomato seeds is surrounded by the endosperm and, when the radicle of germinating seeds penetrates the micropylar endosperm, that endosperm part will be ruptured and, as a result, will be wounded. It is likely, therefore, that the appearance of enzyme activity at the micropylar endosperm of germinated seeds is due to the wound caused by the radicle penetration.

A similar spatial pattern of the development of β-1,3-glucanase in tobacco seeds has been reported (Vogeli-Lange *et al.*, 1994; Leubner-Metzger *et al.*, 1995); the development occurs exclusively at the micropylar endosperm portion. In contrast to the enzyme in tomato seeds, however, the enzyme in tobacco seeds develops only prior to radicle protrusion (germination). In the light of the results on the temporal and spatial developmental pattern, it has been suggested that, although the presence of β-glucans in tobacco endosperm is not known, tobacco β-1,3-glucanase contributes to the weakening of the cell walls of the micropylar endosperm part by helping to hydrolyse cell wall polysaccharides (β-1,3-glucans) and thereby contributes to facilitating penetration of the radicle (Leubner-Metzger *et al.*, 1995). A similar function has been assigned to endo-β-mannanase in tomato seeds

(Groot *et al.*, 1988; Nonogaki *et al.*, 1992; Ni and Bradford, 1993) and to cellulase in *Datura ferox* (Sanchez *et al.*, 1986). In tomato seeds, however, β-1,3-glucanase activity does not develop before radicle protrusion; it begins to develop only after radicle penetration has occurred (Fig. 2). Thus the completion of germination and β-1,3-glucanase development are causally related in tomato seeds. What then is the physiological function of β-1,3-glucanase in the micropylar endosperm of germinated tomato seeds?

β-1,3-Glucanases are known to be induced as part of the defence reaction of plants to pathogen attack (Simmons, 1994). Taking this into consideration, the possibility is suggested that β-1,3-glucanase accumulating at the site of rupture in the endosperm protects germinated seeds from pathogen invasion. A strong argument against this hypothesis may be that β-1,3-glucanases are induced co-ordinately with chitinases after pathogen infection and act in synergy with chitinases in protecting plants (Simmons, 1994). The induction of both glucanases and chitinases by wounding has been reported with various plants (Mauch *et al.*, 1988; Neale *et al.*, 1990; Derckel *et al.*, 1998). Chitinase activity was already present in the endosperm of ungerminated (20-h-imbibed) tomato seeds, but stimulation of its activity after radicle penetration was not observed (data not shown). Furthermore, no difference in chitinase activity was detected between the micropylar and the lateral endosperm half of seeds of 3-d-old seedlings (data not shown). Thus, co-ordinative induction of β-1,3-glucanase and chitinase does not seem to occur in tomato seeds. Lozovaya *et al.* mentioned in their paper that, although *Aspergillus*-infected maize calli had higher β-1,3-glucanase activity than uninfected calli, there was no difference in chitinase activity between the two calli, and that elevated β-1,3-glucanase activity was correlated with the resistance of maize calli to the infection (Lozovaya *et al.*, 1998). Taking this into consideration, the absence of the co-ordinative induction of chitinase may not rule out the working hypothesis that the β-1,3-glucanase induced in the micropylar endosperm plays a role in preventing pathogen infection.

It has been reported that four β-1,3-glucanases are induced in tomato leaves infected with pathogens (Jooston and DeWitt, 1989; van Kan *et al.* 1992; Domingo *et al.* 1994); two basic ($pI > 10$) glucanases whose molecular masses are 33 and 35 kDa, respectively, and two acidic ($pI = 5–6$) glucanases whose molecular masses are 35 kDa. The β-1,3-glucanase that develops in the micropylar endosperm of germinated tomato seeds is a basic protein ($pI > 10$) with an apparent molecular mass of 35 kDa. Thus, the enzyme in the endosperm of germinated tomato seeds is similar to the pathogen-infection-inducible, basic β-1,3-glucanases. However, it is not known whether or not the glucanase in the endosperm is identical to either of the two basic glucanases induced by pathogen infection. Acidic glucanases were not detected in the endosperm.

The increase in glucanase activity was also observed in the micropylar endosperm of seeds germinated under sterile conditions (data not shown). Therefore, the development of the enzyme activity in the micropylar part of tomato seeds does not seem to be due to the invasion of pathogens through wounded parts.

Wounding causes β-1,3-glucan (callose) deposition that can provide mechanical protection by plugging wounded parts and thereby limit pathogen invasion (Bell, 1981). It is possible that callose deposition occurs at the site (micropylar portion) where the endosperm has been ruptured by radicle penetration. Then, another possibility for the physiological function of β-1,3-glucanase in tomato seeds is that the enzyme may be involved in mobilizing wound-deposited callose. Further experiments are necessary to elucidate the physiological significance of the temporally and spatially regulated development of β-1,3-glucanase activity in tomato seeds.

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